Discovery of *N*-[2-[2-[[3-Methoxy-4-(5-oxazolyl)phenyl]-amino]-5-oxazolyl]phenyl]-*N*-methyl-4-morpholineacetamide as a Novel and Potent Inhibitor of Inosine Monophosphate Dehydrogenase with Excellent in Vivo Activity

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Abstract: Inosine monophosphate dehydrogenase (IMPDH) is a key enzyme that is involved in the de novo synthesis of purine nucleotides. Novel 2-aminooxazoles were synthesized and tested for inhibition of IMPDH catalytic activity. Multiple analogues based on this chemotype were found to inhibit IMPDH with low nanomolar potency. One of the analogues (compound **23**) showed excellent in vivo activity in the inhibition of antibody production in mice and in the adjuvant induced arthritis model in rats.

Introduction. Inosine monophosphate dehydrogenase (IMPDH) is an enzyme that catalyzes the nicotinamide adenosine dinucleotide (NAD) dependent conversion of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP).[1] The reaction is irreversible and is the first step in the de novo synthesis of guanine nucleotides. Rapidly proliferating cells such as lymphocytes are dependent on the availability of the nucleotide pool to meet their metabolic requirement, and it is known that the activity of IMPDH is higher in proliferating cells.[2] Because of these cell requirements, IMPDH is an attractive target for immunosuppressive, anticancer, and antiviral therapies.[3]

Two isoforms of the IMPDH enzyme are known to exist: type I and type II. Human types I and II IMPDH cDNAs encode the same-size proteins (514 amino acids) and show 84% sequence identity.[4] It was initially thought that because type II expression is upregulated in neoplastic and replicating cells, it is this isoform that is responsible for cell differentiation and neoplastic transformation.[5] However, more recent work has indicated that induction of types I and II isoforms contributes significantly to the T-cell proliferation response.[6]

The mechanism of IMPDH reaction has been studied in detail (Figure 1).[7] The oxidation of IMP to XMP is an irreversible reaction and utilizes NAD as the cofactor. The reaction involves nucleophilic addition of a cysteine residue (Cys 331 in human type II) to the 2-position of IMP. The resulting enzyme−IMP intermediate (E−IMP) transfers a hydride to NAD, resulting in an enzyme−XMP* (E−XMP*) intermediate, which hydrolyzes to XMP.[8,9] Recent isotope labeling studies suggest that the substrates (IMP and NAD) bind randomly to the enzyme with an ordered release of products (NADH release preceding hydrolysis of E−XMP*).[10] Crystal structures of apo IMPDH and IMPDH complexes with inhibitors bound have been disclosed recently.[11]

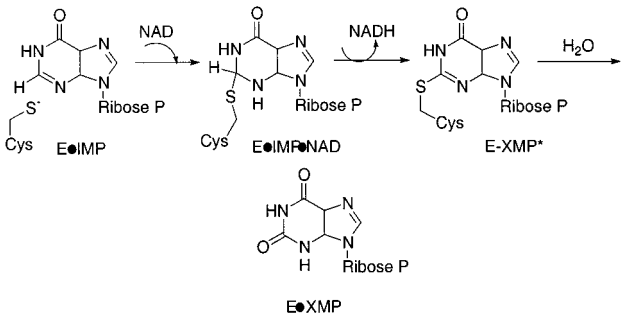

Figure 1. Mechanism of IMPDH inhibition.

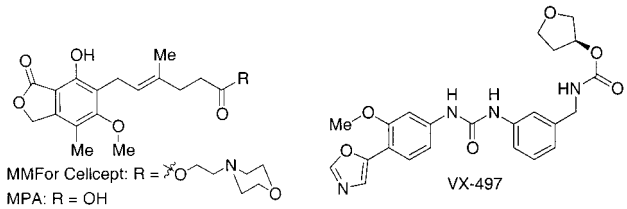

Figure 2. IMPDH inhibitors.

Mycophenolate mofetil (MMF or Cellcept, Figure 2), the morpholino ester prodrug of mycophenolic acid (MPA), is an uncompetitive inhibitor of IMPDH and has been approved for the treatment of kidney and heart transplantation in combination with cyclosporin A and corticosteroids.[12] Recently the U.S. FDA has approved Cellcept for use in preventing rejection of organ transplantation in allogeneic liver transplant recipients. Despite the clinical efficacy of Cellcept, its clinical utility is limited because of an unfavorable gastrointestinal (GI) tolerability profile. This has been attributed to the high concentrations of free MPA in the GI tract resulting from enterohepatic circulation of the phenolic glucoronide.[13] Mizoribine and ribavarin, unlike MPA, are competitive inhibitors of IMPDH. Mizoribine is marketed in Japan for lupus nephritis, nephrotic syndrome, rheumatoid arthritis, and the prevention of renal transplant rejection. However, its use is also limited by GI toxicity. Ribavarin (Virazole, Rebetol) has been approved in the U.S. for the treatment of respiratory syncytial virus and in combination with interferon-α for treatment of hepatitis C. The use of ribavarin is limited because of its side effects, which include hemolytic anemia.

To overcome the limitations of current IMPDH inhibitors, Vertex, using a combination of high-throughput screening and structure-based drug design, identified

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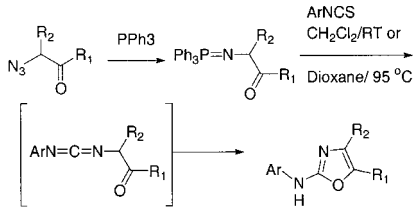
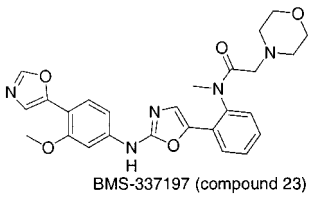
Scheme 1. General Method for the Synthesis of 2-Amino-1,3-oxazoles



VX-497 (merimempodib) (Figure 2) as an orally bioavailable inhibitor of IMPDH.[14,15] VX-497 is currently in phase IIb trials for the treatment of hepatitis C in combination with interferon-α.

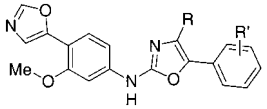
A program to identify novel IMPDH inhibitors was initiated with the goal of identifying compounds with improved physicochemical and pharmacological properties. Our strategy was to retain the aniline moiety of VX-497 and to develop the SAR for a new series featuring various heterocycles as replacements for the central urea linkage.[16] In this communication, we describe the synthesis and SAR of a novel series of 2-aminooxazoles resulting in the identification of compound **23** as a potent IMPDH inhibitor with excellent


BMS-337197 (compound 23)

activity in a mouse model for inhibition of antibody production and in a rat adjuvant induced arthritis model.

Chemistry. The 2-aminooxazoles were synthesized utilizing a modified[17] iminophosphorane/heterocumelene mediated approach[18] as outlined in Scheme 1. In general, reaction of a isothiocyanate with an acyl azide and triphenylphosphine in dioxane at 95−100 °C or in dichloromethane at room temperature yielded the corresponding 2-aminooxazoles.[19] The acyl azides can either be obtained from the corresponding acylbromides or the treatment of an enol ether with *N*-bromosuccinamide and water. As a representative example, the synthesis of compound **23** is outlined in Scheme 2.

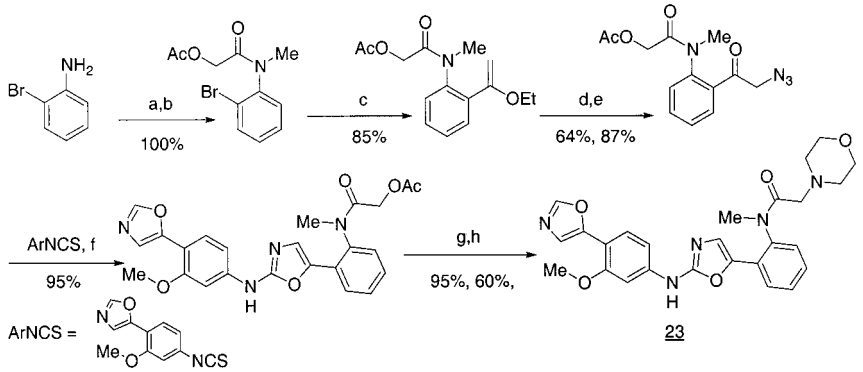
Table 1. In Vitro Activity of 5-Phenyl-2-amino-1,3-oxazole Derivatives



compd	R	R′	IC_{50}(IMPDH II), nM $n = 3$	IC_{50}(CEM), μM $n = 3$
1	H	H	20 ± 4	7 ± 3
2	H	*o*-Me	25 ± 3	>10
3	H	*m*-Me	70 ± 23	>10
4	H	*p*-Me	85 ± 17	>10
5	H	*o*-OMe	23 ± 4	7 ± 2
6	H	*m*-OMe	97 ± 19	>10
7	H	*p*-OMe	115 ± 56	9 ± 1
8	H	*o*-CO_2Et	127 ± 10	>10
9	H	*o*-CO_2H	11% inhib at 20 μM	
10	H	*o*-$CONMe_2$	141 ± 34	9 ± 1
11	H	*o*-CONHMe	104 ± 7	8 ± 3
12	Me	H	27 ± 8	9 ± 0.4
13	Et	H	86 ± 47	>10
14	H	−NHCOMe	30 ± 5	1 ± 0.7
15	H	−NMeCOMe	18 ± 9	0.73 ± 0.2

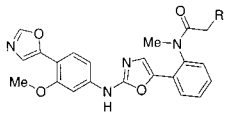
In Vitro SAR. Table 1 summarizes the structure−activity relationships for the inhibition of IMPDH II activity in the 2-amino-5-phenyloxazole series. The parent phenyl oxazole (**1**) is a potent inhibitor of IMPDH II with an IC_{50} of 20 nM. However, in a secondary T-cell (CEM) proliferation assay, the compound had a potency of only 5 μM. Hypothesizing that the poor cell activity might be due to poor cell permeability and/or solubility of the compound, we decided to incorporate solubilizing residues on the phenyl ring to improve cell potency while retaining enzyme activity. The ortho position of the phenyl ring appeared to be optimal for the introduction of solubilizing residues based on the data for compounds **2** and **5**. Compound **2** with an ortho methyl substituent is approximately 2- to 3-fold more potent than the corresponding meta **3** and para-substituted **4** derivatives. Introduction of polar residues **8**−**15** led to mixed results. While ester **8**, acid **9**, and carboxamides **10** and **11** led to a 5- to 6-fold decrease in potency for the enzyme and consequently no improvement in cell activity, the acetamide derivative **14** maintained enzyme potency and inhibited T-cell proliferation with an IC_{50} of 1 μM. On the other hand, the *N*-methylacetamide **15** is a potent inhibitor of IMPDH II (IC_{50} = 26 nM) and was the first compound in this series that exhibited

Scheme 2. Synthesis of Compound **23**[a]



[a] (a) $ClCOCH_2OAc$, pyridine, CH_2Cl_2, 2 h; (b) NaH, MeI, DMF, 1 h; (c) $Pd(PPh_3)_2Cl_2$, $CH_2C(OEt)(Bu_3Sn)$, dioxane, 100 °C, 18 h; (d) NBS, H_2O, THF, 50 °C, 10 min; (e) NaN_3, acetone, H_2O, room temp, 30 min; (f) PPh_3, dioxane, 90 °C, 15 min; (g) LiOH, MeOH, H_2O, room temp, 30 min; (h) MsCl, Et_3N, THF, 1 h; then morpholine, Et_3N, DMF, room. See Supporting Information for full experimental details.

Table 2. In Vitro Activity of 5-Acetamido-2-amino-1,3-oxazole Derivatives



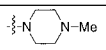
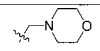
Compound #	R	IC_{50} (IMPDH II) nM n = 3	CEM IC_{50} (μM) n = 3
16	-NHMe	22 ± 2	0.96 ± 0.0
17	$-NMe_2$	15 ± 5	1.0 ± 0.1
18		17 ± 4	0.94 ± 0.1
19		13 ± 6	0.65 ± 0.1
20	-NHt-Bu	20 ± 6	0.7 ± 0.2
21		11 ± 3	0.89 ± 0.2
22		13 ± 6	0.68 ± 0.1
23		12 ± 3.6	0.59 ± 0.07
VX-497		10 ± 2	0.43 ± 0.06
MPA		14 ± 2	0.34 ± 0.04

Table 3. PK Profile of Compound **23** in Rats ($n = 3$)

	dose, μmol/kg	C_{max}, μM	$t_{1/2}$, h	Cl, mL/min/kg	V_{ss}, L/kg	AUC_{0-inf}, μM min	bio-avail, %
IA	10		5.9 ± 1.1	71 ± 8	2.1 ± 0.3	142 ± 15	
PO	20	1.5 ± 0.5	2.5 ±0.8			72 ± 13	25

submicromolar potency (IC_{50} = 530 nM) in inhibiting T-cell proliferation.

To further explore the SAR around acetamide **15** and to address potential formulation issues, compounds having a basic residue were examined (Table 2). As is evident from Table 2, most of the compounds examined maintained excellent enzyme and cell potency. From this set of compounds, compound **23** was identified for further in vivo evaluation. Compound **23** is a potent uncompetitive inhibitor of IMPDH II enzyme (IC_{50} = 16 nM, K_i = 3.2 nM, IMPDH I IC_{50} = 120 nM) and has an IC_{50} of 520 nM in inhibiting the proliferation of the CEM T-cell line. It inhibited the proliferation of normal human PBMCs stimulated with anti-CD3 MAB and anti-CD28 MAB, with an IC_{50} of 130 nM. More importantly, the hydrochloride salt of compound **23** is highly water-soluble, thus enabling it to be dosed using an aqueous vehicle for in vivo studies.

PK Profile of Compound 23. Before the compound was subjected to in vivo efficacy studies, pharmakokinetic characteristics of compound **23** were evaluated in rats. Results are outlined in Table 3. Compound **23** has an estimated oral bioavailability of 25% and an apparent elimination half-life of 5.9 h, suggesting the compound was suitable for further evaluation in the inhibition of antibody production in mice and in the adjuvant induced arthritis model in rats.

In Vivo Evaluation of Compound 23. The ability of the compound to inhibit an antibody response to a soluble antigen was examined using keyhole limpet hemocyanin (KLH) as the immunogen.[20] In this model, BALB/c mice are immunized by intraperitoneal injection of KLH and then treated with compound **23** or control compounds for 4 days. Antibodies to KLH in the serum are measured by ELISA on day 10 (Figure 3). Compound **23** was equally efficacious as CellCept when dosed twice daily. Further studies indicated that twice daily dosing was not necessary to maintain efficacy in this model (data not shown).

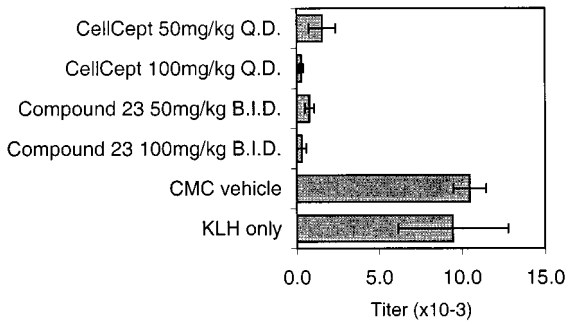


Figure 3. Compound **23** suppressing a humoral immune response to KLH. BALB/c mice were immunized with KLH on day 0 and then were treated by oral gavage, as indicated on days 0−4. Serum titers were measured on day 10 by ELISA.

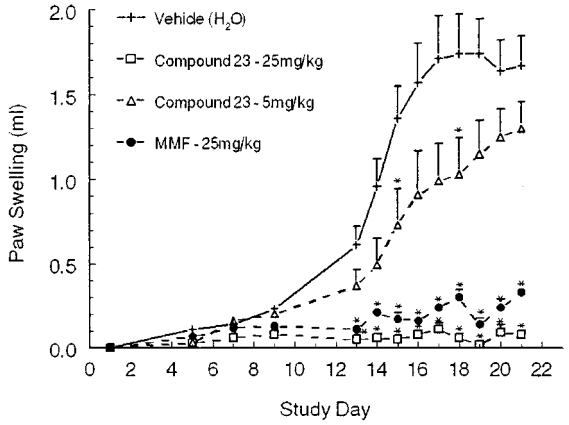


Figure 4. Treatment of adjuvant induced arthritis in rats with compound **23** ($p < 0.05$ vs vehicle, Student's t test). The graph depicts SEM for $n = 8$ rats per treatment.

Adjuvant induced arthritis in Lewis rats is a widely utilized model of human rheumatoid arthritis.[21] This model has been used to evaluate the antiarthritic potential of agents such as methotrexate.[22] Here, we demonstrate the efficacy of the IMPDH inhibitor compound **23** in rat adjuvant induced arthritis.

Arthritis was induced by the subcutaneous injection of Freund's complete adjuvant into the base of the tail of male Lewis rats. Compound **23** (5 or 100 mg/kg) or mycophenolate mofetil (MMF, 25 mg/kg) was administered to rats by daily oral gavage beginning on the day of adjuvant injection. Baseline measurements of hind paw volume were obtained by plethysmometry. Additional paw volume measurements were performed over the next 3 weeks, and the increases in paw volume above baseline were calculated. Hind paws (hocks) were collected at necropsy and were submitted for histopathological examination.

As shown in Figure 4, compound **23** prevented disease onset and progression. At 100 mg/kg daily, it almost completely suppressed disease development, significantly ($p < 0.05$ vs water vehicle, Student's t test)

inhibiting paw swelling from day 13 onward. These rats showed no signs of compound-related toxicity, and they continued to gain weight during the course of the study (data not shown). Specifically we did not see GI toxicity by either gross examination or histopathology. At a dose level of 5 mg/kg daily, compound **23** also delayed the progression of paw swelling compared to vehicle-treated rats, although the reduction in swelling was statistically significant in only 2 days. MMF, when dosed to rats at 25 mg/kg, was highly efficacious in preventing arthritic paw swelling. Higher doses of Cellcept could not be tolerated because of significant GI toxicity. These data demonstrate the efficacy of the novel, orally active 2-amino-1,3-oxazole, compound **23**, in adjuvant arthritis, a rat model of human rheumatoid arthritis.

Conclusion. In summary, novel 2-aminooxazoles were synthesized and tested for inhibition of IMPDH. Multiple analogues based on this chemotype were found to inhibit IMPDH with low nanomolar potency. One of the analogues, compound **23**, showed excellent in vivo activity in the inhibition of antibody production in mice and in the adjuvant induced arthritis model in rats. Further exploration of the 2-amino-1,3-oxazole series and evaluation of compound **23** are underway and will be reported.

Supporting Information Available: Full experimental details for the preparation of compound **23** and the protocols used for rat PK, the enzyme (IMPDH) inhibition assay, inhibition of proliferation of the CEM T-cell line, and PBMCs. This material is available free of charge via the Internet at http://pubs.acs.org.

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