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Novel Inhibitors of IMPDH: A Highly Potent and Selective Quinolone-Based Series

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Abstract—A series of novel quinolone-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.
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Inosine 5′-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).[1–3] Two distinct cDNA's encoding IMPDH have been identified and isolated. These transcripts, labeled type I and type II, possess 84% sequence identity.[4–6] IMPDH type II activity is markedly up-regulated in actively proliferating cell types including cancers and activated peripheral blood lymphocytes.[7,8] IMPDH type I, on the other hand, is the predominant, constitutively expressed isoform in normal, non-proliferating cells. Selective inhibitors of the up-regulated IMPDH type II isoform may mitigate toxicity caused by the inhibition of the constitutively expressed IMPDH type I isoform.[8] IMPDH type II has emerged as a target for therapies in immunology, virology, and oncology.[7,9]

Mycophenolic acid (MPA, Fig. 1) and some of its derivatives have been shown to be potent, uncompetitive, reversible inhibitors of human IMPDH type I and type II.[10,11] CellCept® (mycophenolate mofetil, MMF), a prodrug of MPA, has been used clinically for the treatment of transplant rejection, due to its inhibition of IMPDH. Dose-limiting gastrointestinal (GI) toxicity exhibited from administration of either CellCept® or MPA limits its potential for the treatment of other autoimmune disorders, such as psoriasis and rheumatoid arthritis.[12]

The formation of conjugates at either the carboxylic acid or phenolic residues is implicated as a contributing factor in the poor therapeutic index observed for CellCept®.[13,14] Structurally related analogues, devoid of acid and phenolic functionalities, are reputed to have an improved therapeutic window with regard to dose limiting GI toxicity.[15–17] An alternative approach to IMPDH inhibition has been described by Pankiewicz.[18]

Our focus is on the identification and development of potent inhibitors of IMPDH with selectivity for IMPDH type II and with improved pharmacological properties. Recently, we have disclosed several new classes of inhibitors of IMPDH,[15,19–22] of which three are exemplified by structures **2**, **3**, and **4** (Fig. 1). As we examined urea isosteres in an effort to define the optimal linkage between the two aryl groups of urea **1**, we became increasingly more concerned about the potential liberation of an aniline moiety in vivo. In order to alleviate this concern, we directed our efforts toward structures in which the amide isostere of structure **3** may be additionally appended to the 3-methoxy-5-oxazolylaniline residue. In this paper, we have outlined the synthesis and biological evaluation of a new class of potent inhibitors in which a vinylogous amide moiety is constrained

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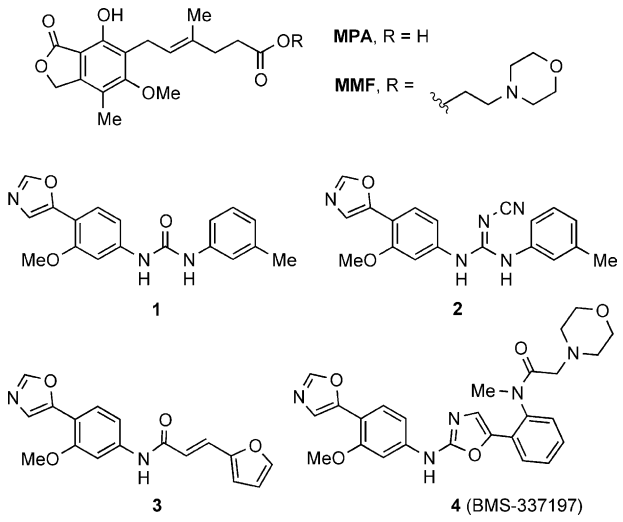


Figure 1. Chemical structures of MPA, MMF, urea **1**, cyano-guanidine **2**, amide **3**, and amino-oxazole **4** (BMS-337197).

to form a quinolone scaffold, as depicted in structure **5** (Fig. 2).

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 (Fig. 2) in the active site of the enzyme.[2] In developing the quinolone chemotype (**5**), we anticipated that the quinolone carbonyl would serve as a hydrogen bond acceptor and engage in an interaction with Gln 441 (Fig. 2). Additionally, the critical hydrogen bond interaction between the quinolone NH and the carboxylate of Asp 274[23] would be maintained.

The synthetic pathways utilized in the preparation of the quinolones (**5**) are shown in Schemes 1 and 2. The synthesis of 3-methoxy-4-(5-oxazolyl)-aniline **6** has been previously described.[16] The condensation of aniline **6** with various substituted acetoacetates[24] was carried out in toluene at reflux in the presence of catalytic *p*-toluenesulfonic acid to give vinylogous urethane derivatives

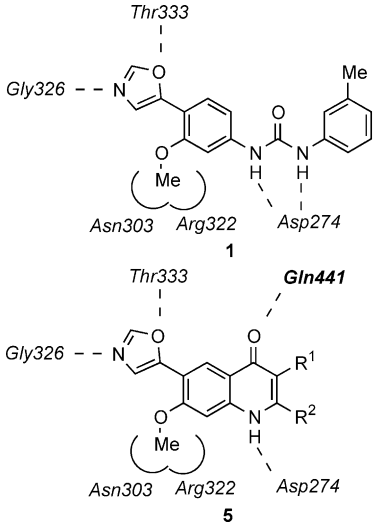
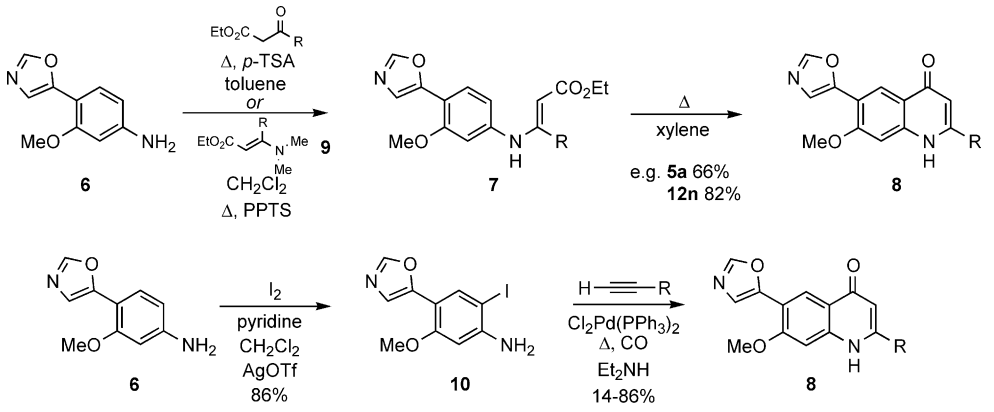


Figure 2. Proposed binding model for the Vertex urea **1**, and a potential binding model for quinolone **5**.

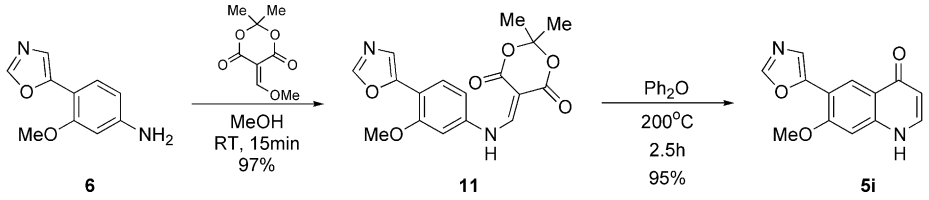
7. The subsequent cyclization to form the quinolone ring system (**8**) occurred during heating in xylene at 250 °C. Alternatively, urethane **7** could be prepared through a *N-N exchange* reaction[25] with the *N*-methyl enamino ester **9**. Quinolone inhibitors were also prepared utilizing a palladium-catalyzed carbonylation sequence,[26] as outlined in Scheme 1.

The in vitro inhibitory activity against IMPDH type II for this series of quinolone inhibitors is summarized in Tables 1 and 2. The phenyl substituted quinolone **5a** very effectively inhibited IMPDH type II with an IC_{50} of 0.008 μM.[27] This compound exemplified an increase in potency relative to the Vertex urea **1**. With this high binding affinity compound in hand, a study of various simple residues for R^1 and R^2 was undertaken in order to optimize the core structure. Replacement of the phenyl ring with various heterocycles was explored. Overall, substitution of the quinolone core with both five- and six-membered heterocycles resulted in modest losses in potency, as demonstrated by compounds **5b** and **5d–5h**. When the quinolone was substituted with the phenyl-like 3-thiophene substituent, activity was maintained. When the phenyl group of **5a** was removed (**5i**), a loss in enzymatic activity of ~37-fold was observed ($IC_{50} = 0.30$ μM). Alkyl groups such as methyl (**5j**) and *t*-butyl (**5k**) also resulted in significant reduction in potency. Extension of the phenyl ring by one methylene group was not as well tolerated (**5l**, $IC_{50} = 0.18$) relative to **5a**. Amino-quinolones **5m** and **5n** showed moderate inhibitory activity against IMPDH type II, although significantly less potent than **5a**, with IC_{50} values of 0.22 and 0.20 μM, respectively. Introduction of a methyl group at the 3-position of the quinolone (**5o**) possibly perturbed the orientation of the phenyl residue enough to result in an 8-fold decrease in potency. Additionally, analogue **5a** was determined to be an uncompetitive, reversible inhibitor of IMPDH type II with respect to IMP and NAD^+ with a $K_i = 7 \pm 2$ nM.[19]

As indicated in Table 1, our efforts to optimize the quinolone core indicated that a phenyl substituent (**5a**) at the 2-position was optimal. We subsequently focused our attention on developing the basic SAR around the phenyl ring, as outlined in Table 2. Exploration of the *ortho*-, *meta*-, and *para*-tolyl analogues **12a**, **12b**, and **12g** demonstrated that *para*- and *meta*-substitution is favored over *ortho*-substitution with both **12b** and **12g** showing an enhancement in potency relative to **5a**. Interestingly, the *para*-bromo analogue **12h** ($IC_{50} = 5$ nM) was more potent than the corresponding meta-derivative **12c**. In general, highly polar residues such as carboxylic acids **12e** and **12k** and methylsulfone **12f** were not as well tolerated, resulting in significant loss in potency. Phenolic residues **12d** and **12i** provided a modest loss in activity. A notable compound in this series was the 4-methoxyphenyl quinolone **12j** which had a IC_{50} of < 0.005 μM against IMPDH type II. Additionally, the synthetic studies were expanded to include di-substitution (**12l–12n**). The 3,4-dimethylphenyl quinolone (**12l**) was as potent as the monomethyl substituted analogues (**12b** and **12g**).

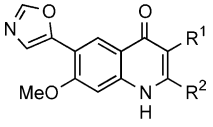


Scheme 1. Methods for the preparation of the quinolones.



Scheme 2. Preparation of quinolone **5i**.

Table 1. SAR of quinolone-based inhibitors of structure **5**

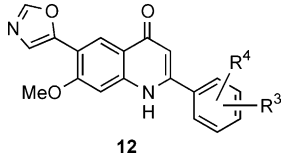


Compd	R^1	R^2	IMPDH II IC_{50} (μM)
MPA	NA	NA	0.014
1	NA	NA	0.019
5a	H	Phenyl	0.008[a]
5b	H	3-Faranyl	0.032
5c	H	3-Thiophenyl	0.009
5d	H	2-Thiophenyl	0.063
5e	H	4-Thiazolyl	0.034
5f	H	2-Pyridyl	0.043
5g	H	3-Pyridyl	0.070
5h	H	4-Pyridyl	0.046
5i	H	H	0.30
5j	H	Me	0.11
5k	H	*t*-Bu	>10
5l	H	Benzyl	0.18
5m	H	NHMe	0.22
5n	H	NMe_2	0.20
5o	Me	Phenyl	0.065

[a] $K_i = 7 \pm 2$ nM (IMPDH-II).

Several of the compounds which were potent inhibitors of IMPDH type II were examined against IMPDH type I in order to determine the relative selectivity.[27] The results are presented in Table 3. The parent phenyl quinolone provided 12-fold selectivity for type II, compared with urea **1** which was only ~3-fold selective. Compounds **12b**, **12j**, and **12l** demonstrated higher selectivity with ratios of type I to type II of 30, 18, and 17, respectively.

Table 2. SAR of phenyl quinolone-based inhibitors of structure **12**



Compd	R^3	R^4	IMPDH II IC_{50} (μM)
5a	H	H	0.008
12a	2-Me	H	0.065
12b	3-Me	H	0.005
12c	3-Br	H	0.022
12d	3-OH	H	0.018
12e	3-CO_2H	H	0.16
12f	3-SO_2Me	H	0.16
12g	4-Me	H	<0.005
12h	4-Br	H	0.005
12i	4-OH	H	0.043
12j	4-OMe	H	<0.005
12k	4-CO_2H	H	>1
12l	3-Me	4-Me	<0.005
12m	2-Me	5-Me	0.029
12n	3-Br	4-Me	0.019

Table 3. IMPDH type I versus type II and T-cell proliferation results

Compd	Type I IC_{50} (μM)	Type II IC_{50} (μM)	Ratio TypeI/II	IC_{50} (μM)(CEM)
MPA	0.055	0.014	3.9	0.39
1	0.055	0.019	2.9	0.32
5a	0.099	0.008	12	0.69
5c	0.055	0.009	6.0	0.34
12b	0.15	0.005	30	1.1
12g	0.046	<0.005	>9.0	0.56
12j	0.090	<0.005	>18	0.37
12l	0.083	<0.005	>17	0.41

These compounds were also examined in a T-cell proliferation assay (CEM) (Table 3).[29] In this assay, MPA inhibited T-cell proliferation with an IC_{50} of 0.39 μM, whereas the phenyl quinolone (**5a**) had an IC_{50} of 0.69 μM. Three compounds, **5c**, **12j**, and **12l**, inhibited T-cell proliferation comparable to MPA with IC_{50} values of 0.34, 0.37, and 0.41 μM, respectively.

In summary, we have identified a novel quinolone-based series of highly potent, selective inhibitors of IMPDH type II. These compounds demonstrate that the urea and amide isosteres can be effectively constrained to the 3-methoxy-5-oxazolylaniline through a vinylogous amide to form a bicyclic quinolone scaffold. This series has been the subject of further studies to enhance the physiochemical properties, and the results are outlined in the accompanying article.[30]

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