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Novel Indole-Based Inhibitors of IMPDH: Introduction of Hydrogen Bond Acceptors at Indole C-3

Scott H. Watterson,* T. G. Murali Dhar, Shelley K. Ballentine, Zhongqi Shen, Joel C. Barrish, Daniel Cheney, Catherine A. Fleener, Katherine A. Rouleau, Robert Townsend, Diane L. Hollenbaugh and Edwin J. Iwanowicz

Bristol-Myers Squibb PRI, PO Box 4000, Princeton, NJ 08543-4000, USA

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Abstract—The development of a series of novel indole-based inhibitors of 5′-inosine monophosphate dehydrogenase (IMPDH) is described. Various hydrogen bond acceptors at C-3 of the indole were explored. The synthesis and the structure–activity relationships (SARs) derived from in vitro studies are outlined.

Maintaining adequate nucleotide levels is essential for normal cellular function, including the synthesis of RNA and DNA. In mammals, nucleotides may be synthesized through one of two pathways: a de novo synthesis pathway or through a salvage pathway which utilizes existing purines and their nucleotides and nucleosides.[1] The extent to which each pathway is utilized is dependent on the cell type. The de novo synthesis of guanine nucleotides is particularly important in B- and T-lymphocytes. These cells are dependent on the de novo synthesis to generate sufficient levels of nucleotides necessary to initiate a proliferative response to mitogen or antigen.

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).[2–4] 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 up-regulated in actively proliferating cell types including cancers and activated peripheral blood lymphocytes.[8,9] As a result, IMPDH has emerged as an attractive target for inhibiting the immune system without also inhibiting the proliferation of other cells.

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 clinical utility, based on its inhibition of IMPDH, for the treatment of transplant rejection. Dose-limiting gastrointestinal (GI) toxicity exhibited from administration of either CellCept® or

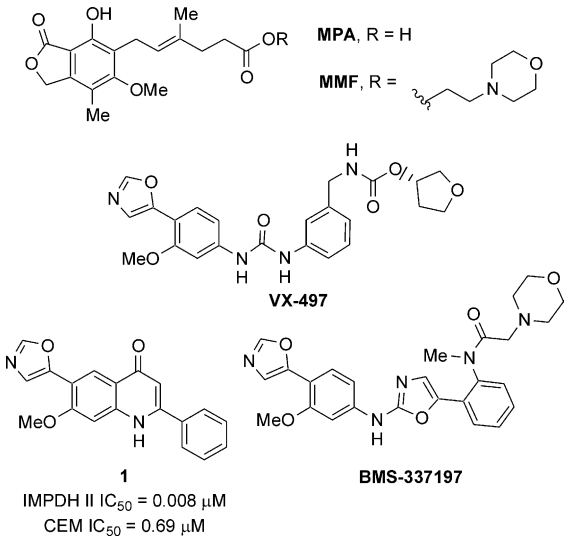

Figure 1. Chemical structures of MPA, MMF, VX-497, quinolone **1**, and amino-oxazole **2**.

*Corresponding author. Tel.: +1-609-252-6778; fax: +1-609-252-6601; e-mail: scott.watterson@bms.com

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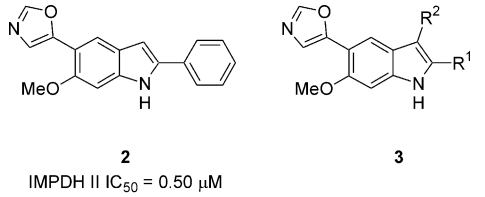
MPA limits its potential for the treatment of other autoimmune disorders, such as psoriasis and rheumatoid arthritis.[12]

Glucuronidation at either the phenolic or carboxylic acid residues of MPA is implicated as a contributing factor in the poor therapeutic index observed for CellCept®.[13,14] Recent, novel inhibitiors of IMPDH, including VX-497 and BMS-337197, are devoid of phenolic and acid functionalities and are reputed to have an improved therapeutic window with regard to dose limiting GI toxicity.[15–17]

Our focus is on the identification and development of potent, selective inhibitors of IMPDH type II with improved pharmacological properties.[18–23] In an effort to find optimal replacements for the urea isostere of VX-497, including amides[18] and diamides,[20] we became increasingly more concerned about the potential liberation of the aniline moiety in vivo. We have recently disclosed an approach which alleviates this concern.[22] This was accomplished through appending a vinylogous amide to the 3-methoxy-5-oxazolylaniline residue to form a quinolone scaffold (**1**). In this paper, we outline an indole scaffold as an alternative bicyclic ring system for the quinolone chemotype (**1**).

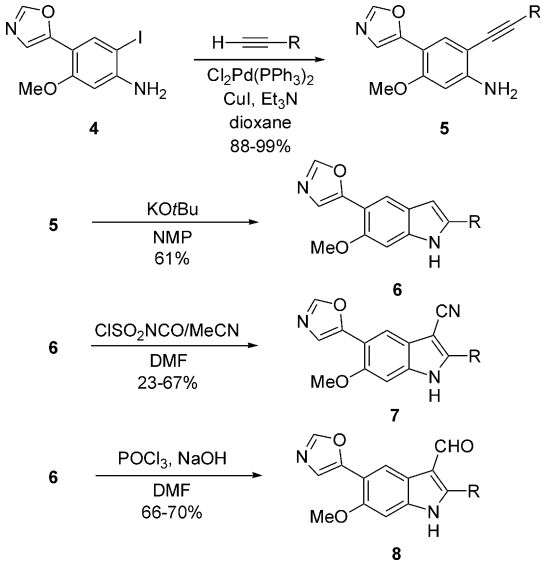
Indole **2** was prepared (Scheme 1, Fig. 2) and was found to have only modest potency against IMPDH type II relative to quinolone **1** ($IC_{50} = 0.50\,\mu M$). Vertex has reported the crystallographic studies on VX-497



2 IMPDH II IC_{50} = 0.50 μM, CEM IC_{50} = >10 μM

3

Figure 2. Indoles **2** and **3**.



Scheme 1.

complexed with Chinese hamster IMPDH type II.[3] The key binding interactions for VX-497 are depicted in Figure 3. The phenyl oxazole moiety of VX-497 binds in the NAD pocket forming hydrogen bonds between the oxazole nitrogen and oxygen and Gly 326 and Thr 333, respectively. Additionally, the methoxy residue binds in a hydrophobic pocket defined by the side chains of Asn 303 and Arg 322. The hydrogen bond interaction between the urea NH and the carboxylate of Asp 274 is a significant contributor to the biological activity of VX-497.[3]

In the quinolone series, we believe that the interactions observed for the methoxy oxazole aniline portion are conserved, including the critical hydrogen bond interaction between the quinolone NH and the carboxylate of Asp 274. More importantly, we believe that the quinolone carbonyl serves as a hydrogen bond acceptor that engages in an interaction with Gln 441 (Fig. 3), contributing to the high potency observed for quinolone **1** ($IC_{50} = 0.008\,\mu M$).[22] For the indole chemotype (**2**), we anticipated that the incorporation of an appropriate hydrogen bond acceptor at the 3-position of the indole ring could potentially improve activity through establishing the key interaction with Gln 441.

The first objective in developing this chemotype was to prepare indoles (**3**) with varying hydrogen bond acceptors at the 3-position of the indole ring. The synthetic pathways utilized in the preparation of these indoles (**3**) are outlined in Schemes 1–4. The synthesis of aniline **4** has been previously described.[22] A Sonogashira[24] palladium-catalyzed coupling of aniline **4** with various substituted acetylenes provided acetylenic anilines **5**, as depicted in Scheme 1. Subsequent heating of anilines **5** in *N*-methylpyrrolidinone in the presence of potassium *t*-butoxide gave indoles **6** in good yield.[25] This sequence was used for the preparation of indoles **2**, **3f**, and **3i**. The acetylenes were either commercially available

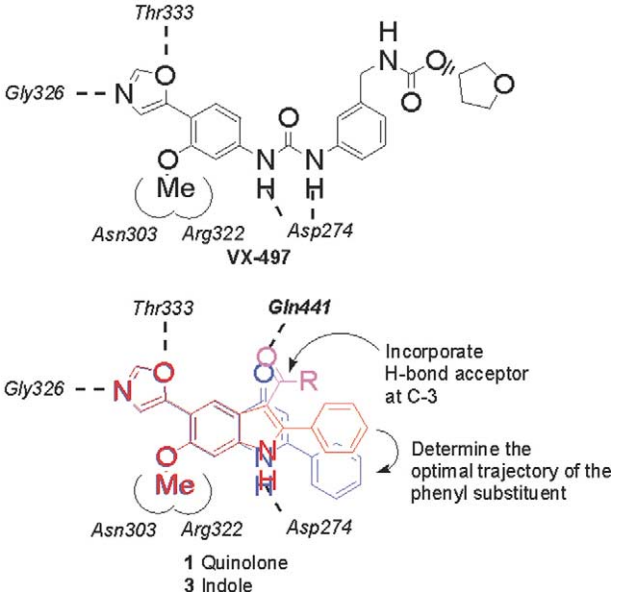
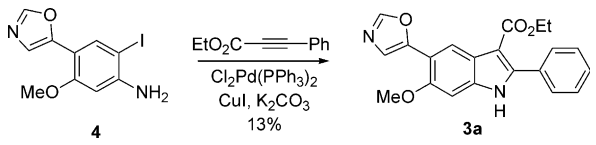
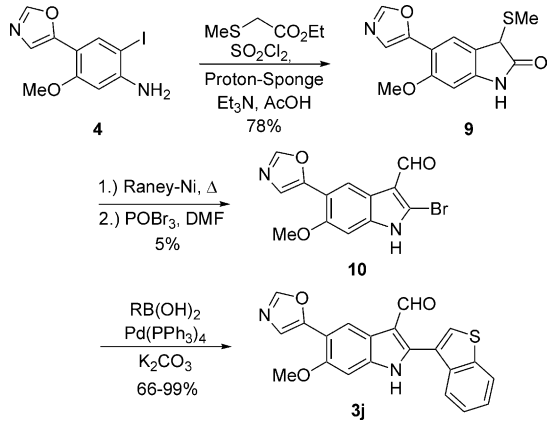


Figure 3. Proposed binding model for the Vertex urea **VX-497**, and a potential binding model for quinolone **1** and indole **3**.



Scheme 2.

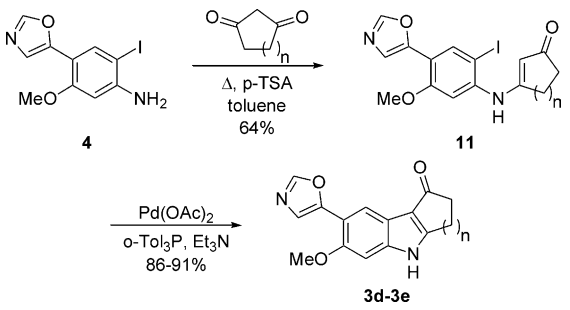


Scheme 3.

or were prepared through a Sonogashira coupling.[26] Additionally, indoles **7** were substituted with a nitrile at C-3 by treatment with chlorosulfonyl isocyanate. Indoles containing an aldehyde at C-3 (**8**) were prepared utilizing a Vilsmeier–Haack reaction as outlined in Scheme 1. The preparation of indoles substituted with an ester group is shown in Scheme 2 and involves a one-pot coupling/cyclization sequence with aniline **4** to give indole **3a**. An alternative approach to 3-formyl-indoles is outlined in Scheme 3. This sequence was used for the preparation of indole **3j**.

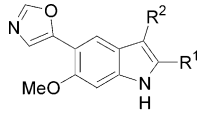
The preparation of cyclic-ketone indoles **3d** and **3e** is outlined in Scheme 4 and relies on a novel intramolecular Heck Reaction for the indole ring construction.[28]

The in vitro inhibitory activity against IMPDH type II for this series of indoles is summarized in Table 1. As mentioned earlier, indole **2** was only a modestly potent inhibitor of IMPDH type II with an IC_{50} value of 0.5 μM.[26] As depicted in Figure 3, we anticipated that the addition of a carbonyl residue at the 3-position of the indole would direct the carbonyl oxygen proximal to Gln 441. In order to establish the viability of our hypothesis, we choose to explore both acyclic and cyclic



Scheme 4.

Table 1. SAR of quinolone-based inhibitors of structure **3**



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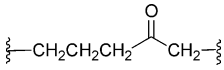
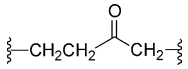
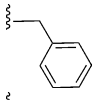
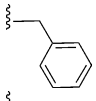
Compd	R^1	R^2	IMPDH II IC_{50} (μM)
2		H	0.50
3a		CO_2Et	>1
3b		CHO	0.23
3c		CN	0.13
3d			0.15
3e			0.29
3f		H	0.60
3g		CHO	0.36
3h		CN	0.22
3i		H	0.50
3j		CHO	0.030
3k		CN	0.064

indoles. Substituting the 3-position of the indole core with an ethyl ester (**3a**) resulted in a loss in activity. Substitution with a smaller hydrogen bond acceptor, a formyl group (**3b**), provided a 2-fold improvement in binding affinity. Interestingly, when the 3-position was appended with a nitrile moiety (**3c**), a 3–4-fold increase in activity was observed (IC_{50} = 0.13 μM). In an effort to decrease the entropy of the core by increasing the rigidity of the R^2 substituent, cyclic indoles **3d** and **3e** were examined. The 6-5-6 tricyclic indole **3d** was considerably more active than indole **2** with an IC_{50} value of 0.15 μM. Contracting the ring to a 6-5-5 ring system (**3e**) resulted in a 2-fold decrease in activity, relative to **3d**. This data

supports our hypothesis that a hydrogen bond acceptor at the 3-position of the indole can interact with Gln 441 in a similar fashion to quinolone **1**.

With improvement in activity with the addition of a hydrogen bond acceptor moiety at the 3-position, we next focused our attention on improving the trajectory of the appended R^1 phenyl group. The structural overlap of indole **3** and quinolone **1** in Figure 3 indicated that the orientation of the indole phenyl ring needed to be optimized to fully mimic the spatial arrangement of the quinolone core. Our first approach in addressing this issue was through a benzyl substituent. Indole **3f** with a benzyl substituent did not offer any improvement over indole **2**. Consequently, the appendage of a formyl or nitrile substituent at the 3-position of the indole core (**3g** and **3h**) did not improve potency relative to **3b** and **3c**, respectfully. In an effort to lower the entropy of the benzyl group and further optimize the orientation relative to the phenyl group of quinolone **1**, a benzothiophene substituent was explored. Although the core indole **3i** was equivalent to indole **2** in the inhibition of IMPDH type II ($IC_{50} = 0.50\,\mu M$), the addition of a hydrogen bond acceptor at the 3-position provided a significant enhancement in binding affinity. The formyl-substituted indole (**3j**) and the nitrile-substitituted indole (**3k**) had IC_{50} values against IMPDH type II of 0.030 and 0.064 μM, respectfully. Indole **3j** also showed good selectivity versus IMPDH type I ($IC_{50} = 0.160\,\mu M$).[26] Furthermore, analogue **3j** had an IC_{50} value of 0.55 μM in a T-cell proliferation assay (CEM).[29]

In summary, we have developed a novel indole-based series of potent inhibitors of IMPDH type II. Through the incorporation of a hydrogen bond acceptor at the 3-position of indole **3**, we have demonstrated that we can significantly improve binding affinity, as seen in indole **3j**, which has an IC_{50} value of 30 nM. Studies to optimize this series of analogues to achieve oral activity in a T-cell mediated pharmacodynamic model are ongoing. The emphasis of future studies is to evaluate this 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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28. The IMPDH enzymatic inhibition protocol is outlined in ref 22 (ref 27).
29. The CEM proliferation protocol is outlined in ref 18 (ref 21).