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Novel 7-methoxy-6-oxazol-5-yl-2,3-dihydro-1*H*-quinazolin-4-ones as IMPDH inhibitors

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Abstract—The synthesis and biological activity of a novel series of 7-methoxy-6-oxazol-5-yl-2,3-dihydro-1*H*-quinazolin-4-ones are described. Some of these compounds were found to be potent inhibitors of inosine 5′-monophosphate dehydrogenase type II (IMPDH II).

Inosine 5′-monophosphate dehydrogenase (IMPDH) is an enzyme that catalyses the NAD dependent oxidation of inosine 5′-monophosphate (IMP) to xanthosine 5′-monophosphate (XMP). This is a key step in the de novo synthesis pathway of guanine nucleotides, which is heavily utilized by proliferating cells.[1–4] Two isoforms of the enzyme have been identified and designated type I and type II.[5] Of these it is IMPDH type II that is upregulated in actively proliferating cell type such as cancers and activated peripheral blood lymphocytes.[6,7] Thus, inhibition of IMPDH II will result in inhibition of guanine production and hence inhibition of cell proliferation in the immune system. IMPDH II has therefore become an interesting target for the treatment of autoimmune diseases such as psoriasis, systemic lupus erythematosus and rheumatoid arthritis as well as for transplant rejection.

An IMPDH inhibitor on the market for transplant rejection is Cellcept® (mycophenolate mofetil, MMF), an ester pro-drug of the active component mycophenolic acid (MPA). MPA is a very potent, uncompetitive, reversible inhibitor of both IMPDH I and II[8] showing an IC_{50} of 15 nM in our IMPDH II enzyme assay.[9] The reported gastrointestinal toxicity of this compound has resulted in a search for alternative IMPDH inhibitors with improved therapeutic window.[10] The forerunner of these was the urea VX-497 (in our assay, IC_{50} 21 nM) that reached Phase II clinical trials (Fig. 1).[11]

In an earlier publication, we reported the discovery of a novel series of 7-methoxy-6-oxazol-5-yI-1*H*-quinazoline-2,4-diones exemplified by compound **1** as IMPDH II inhibitors.[12] Here, we describe how that template was modified in order to improve its poor physical

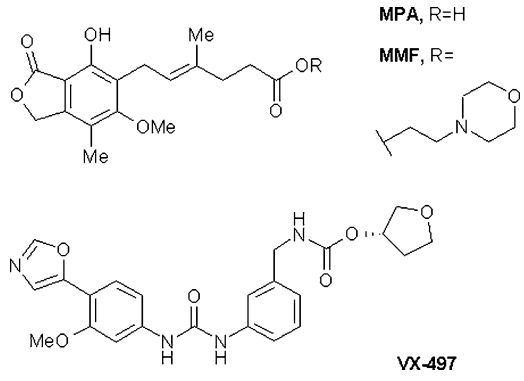

Figure 1. Structures of MPA, MMF and VX-497.

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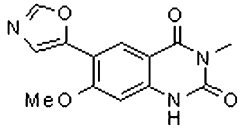
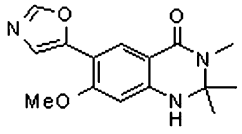
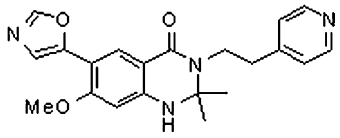
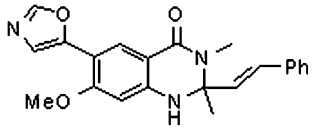
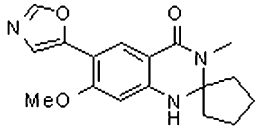
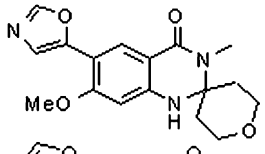
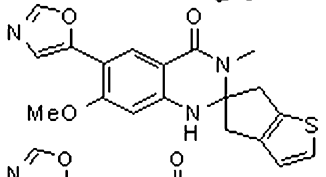
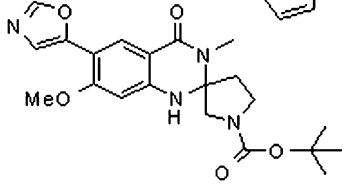
properties resulting in a series of novel 7-methoxy-6-oxazol-5-yl-2,3-dihydro-1*H*-quinazolin-4-ones that were also potent IMPDH II inhibitors.

Although only modest potency against IMPDH II was achieved with the 7-methoxy-6-oxazol-5-yl-1*H*-quinazoline-2,4-diones, the major flaw with the series was its poor solubility, which was postulated to result from the overall flatness of the structure (compound **1**, pH 6.5: 3 μg/ml). In order to improve this, the 2-oxo moiety was replaced with a *gem*-dimethyl group resulting in compound **2a**. Pleasingly, this compound also showed moderate IMPDH II potency in addition to the predicted improvements in solubility (pH 6.5: 77 μg/ml). Our objective with this series, therefore, was to improve the IMPDH potency and attain favourable DMPK properties.

Replacing the 3-methyl group in compound **2a** with a pyridyl-ethyl substituent (**2b**) gave no increase in activity against IMPDH II, much in the same way that varying this substituent in the quinazoline-2,4-dione series had no dramatic effect on IMPDH II activity. A more fruitful approach was to vary the 2-substituents of the 7-methoxy-6-oxazol-5-yl-2,3-dihydro-1*H*-quinazolin-4-ones. Thus, replacing one of the 2-methyl groups with a styrene moiety gave compound **2c** and an improvement in potency. However, it was found for this acyclic series that both enzyme and cellular activities reached a plateau not acceptable for our programme. It was our

Table 1. SAR of early 7-methoxy-6-oxazol-5-yl-2,3-dihydro-1*H*-quinazolin-4-ones

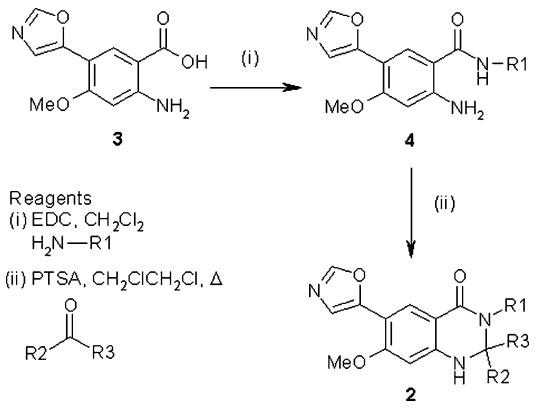
Compound		IMPDH II[9] IC_{50} (nM)	PBMC[15] (μM)
1		104	4.0
2a		192	21
2b		300	37
2c		49	3.0
2d		328	8.5
2e		526	11.6
2f		96	1.9
2g		104	0.97

other strategy of cyclising the 2-*gem*-dimethyl substituents to give a spiro compound that proved to be of greater long-term value. Towards this end, a number of analogues were prepared starting with the cyclopentyl derivative (**2d**), progressing through the tetrahydropyranyl derivative (**2e**) and the thiophene-fused cyclopentyl (**2f**), and culminating in the *tert*-butoxycarbonyl-pyrrolidine compound (**2g**). As can be seen, this compound has enzyme potency similar to dione **1** but with improved activity in our peripheral blood mononuclear cells (PMBCs) proliferation assay (Table 1).[15] In addition, this compound shows the favourable solubility (pH 6.5: 101 μg/ml) seen by early examples in this series.

Synthesis of these compounds was readily achieved from known amino-acid **3**. An EDC coupling with the appropriate amine (generally methylamine) gave amino-amide 4, which on condensation with a ketone using *para*-toluenesulfonic acid in refluxing dichloroethane afforded the desired quinazolinone **2** (Scheme 1).[13,14]

Clearly, in forming the spiro-centre, in many cases a chiral centre is also generated. Compound **2g** was of sufficient interest for the enantiomers to be separated using chiral HPLC. Encouragingly, most of the activity was found to reside in one of the enantiomers (Enantiomer **1** 56 nM, Enantiomer **2** 4255 nM) Unfortunately all attempts to determine the absolute stereochemistry of this centre using X-ray crystallography failed. However using the docking programme, GOLD,[16] the centre was tentatively assigned an *S* configuration: for this enantiomer the *tert*-butyloxy group fits snugly into a lipophilic pocket in the enzyme active site. X-ray crystal studies of VX-497[3] predict that the oxazole and methoxy groups make key interactions in the active site orientating inhibitors in the manner shown (Fig. 2).

Spiro-pyrrolidine **2g** was also a useful compound from which to start further explorations of the SAR of this series. Removal of the *tert*-butyloxy group proceeds smoothly using hydrogen chloride in diethyl ether to afford the free amine, which was further reacted with a variety of electrophiles. The ethyl carbamate (**5a**)



Scheme 1. Preparation of 7-methoxy-6-oxazol-5-yl-2,3-dihydro-1*H*-quinazolin-4-ones.

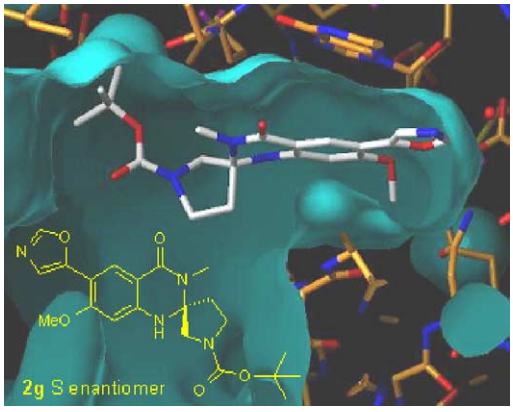


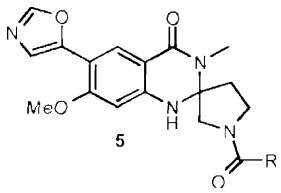
Figure 2. Docking studies on *tert*-butyl 7′-methoxy-6′-(1,3-oxazol-5-yl)-4′-oxo-3′,4′-dihydro-1*H*,1′*H*-spiro[pyrrolidine-3,2′-quinazolinc]-1-carboxylate.

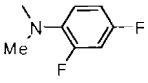
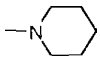
showed enzyme and cellular activities similar to those of its *tert*-butyl analogue (**2g**), as did the *tert*-butyl amide (**5b**). The ethyl urea (**5c**), despite maintaining enzyme activity, was disappointing in the cellular assay. Similarly, a reduction in enzyme and cellular activities was observed for the phenyl urea (**5d**). However, formation of the tetra-substituted urea **5e** not only improved potency against IMPDH II but also more importantly restored cellular activity to that seen for **2g**. Unfortunately, this compound showed a higher human microsomal turnover compared to **2g**. To address this issue, potentially metabolisable sites on the phenyl ring were blocked with fluoro substituents and this, as shown by compound **5f**, was partially successful without impairing potency. Aliphatic cyclic and acyclic ureas, as exemplified by **5g**, **5h** and **5i**, exhibited similar profiles to **5e**. Formation of the oxy-urea (**5j**) reduced microsomal clearance to a level similar to that of **2g** without seriously compromising enzyme and cellular activities. All members of this series showed an improvement in solubility over dione **1** (e.g., **5g**, pH 6.5: 813 μ/ml). Several of these compounds were made chirally pure and, as for example **2g**, most of the activity was associated with one enantiomer (Table 2).

As has been seen repeatedly for this series, high microsomal turnover is a major issue. In an attempt to understand this, in vivo PK experiments were performed on compound **2g** in which the metabolites were identified. It was found that a major site of metabolism was the pyrrolidine core of the molecule. One way of preventing this oxidation was to block the labile site with a substituent and towards this end a series of proline-derived compounds was synthesized. The required enantiomerically pure ketones were readily prepared in a three-step synthesis from commercially available starting materials.

Condensation with amino-amide **4** in the manner described above produced the desired quinazolinones as pairs of diastereomers (**6a**–**d**) that could be separated by IIPLC. Some chiral induction was observed with an approximately 4:1 ratio of diastereomers.

Table 2. SAR of 7′-methoxy-3′-methyl-6′-(1,3-oxazol-5-yl)-1′*H*-spiro[pyrrolidine-3,2′-quinazolin]-4′(3′*H*)-ones



Compound	R	IMPDH II[9] IC_{50} (μM)	PBMC[15] (nM)	Clint[15] (μL/min/mg)
2g	O*t*-Bu	104	0.97	42
5a	OEt	80	1.2	24
5b	*t*-Bu	94	0.54	46
5c	NHEt	119	7.2	11
5d	NHPh	393	16	19
5c	NMePh	64	0.86	144
5f		51	1.2	97
5g	NEt_2	45	0.54	92
5h		68	1.8	152
5i	N*i*Pr_2	71	0.07	612
5j	N(Me)OMe	79	1.9	38

Note. All compounds shown are racemic.

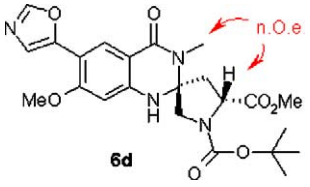
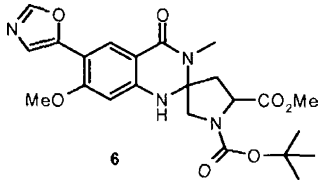


Figure 3. NOE experiments on proline-derived quinazolinones.

Using the known stereochemistry within the molecule, persistence with elegant NMR experiments looking at long-range NOE effects between the α-proton on the pyrrolidine ring and the 3′*N*-methyl protons resulted in determination of the absolute stereochemistry of the spiro-centre (Fig. 3). Testing these compounds in our enzyme assay also produced some interesting results. As can be seen only one of the four diastereomers (the major product from the condensation with the ketone derived from D-proline) shows the desired levels of activity against IMPDH II and hence was the only one tested in our cellular assay. Compound **6d** was deduced to have *S* stereochemistry at the spiro-centre and showed an improvement in enzyme potency as well as the predicted improvement in metabolic stability over its unsubstituted analogue (**2g**) (Table 3). In view of its increased enzyme potency over compound **2g**, the activity in the cell proliferation assay was a little disappointing although still acceptable. However, as can be seen in Table 2, compounds with similar enzyme potencies can give rise to quite different cellular activities (e.g., **5i** and **5j**).

In summary, we have identified a series of novel 7-methoxy-6-oxazol-5-yl-2,3-dihydro-1*H*-quinazolin-4-ones as

Table 3. SAR of proline-derived 7′-methoxy-3′-methyl-6′-(1,3-oxazol-5-yl)-1*H*-spiro[pyrrolidine-3,2′-quinazolin]-4′ (3′*H*)-ones



Compound	Stereochemistry (spiro-centre, α-centre)	IMPDH II[15] IC_{50} (nM)	PBMC[15] (μM)	Clint[17] (μL/min/mg)
6a	*R*,*S*	948	n.t.	n.t.
6b	*S*,*S*	369	n.t.	n.t.
6c	*R*,*R*	26% at 10 μM	n.t.	n.t.
6d	*S*,*R*	35	1.5	18

n.t., not tested.

potent IMPDH II inhibitors that also have physical properties superior to those of our earlier series. The in vivo PK profiles and pharmacological results of some of these compounds will be reported elsewhere.

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