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Quinazolinethiones and quinazolinediones, novel inhibitors of inosine monophosphate dehydrogenase: synthesis and initial structure–activity relationships

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Abstract—The development of a series of novel quinazolinethiones and quinazolinediones as inhibitors of inosine monophosphate dehydrogenase (IMPDH) is described. The synthesis, in vitro inhibitory values for IMPDH II and in vitro inhibitory value for PBMC proliferation are discussed.

A key enzyme involved in the de novo synthesis of guanosine nucleotides is inosine monophosphate dehydrogenase (IMPDH) which catalyses the irreversible NAD dependent oxidation of inosine-5′-monophosphate (IMP) to xanthosine-5′-monophosphate (XMP).[1] Two isoforms of the enzyme have been identified and designated type I and type II.[2,3] IMPDH II activity is markedly up-regulated in actively proliferating cell types including cancers and activated peripheral blood lymphocytes.[4] Thus, inhibition of IMPDH II results in inhibition of cell proliferation in the immune system via inhibition of guanosine nucleotide production. This has led to a number of companies[5] identifying IMPDH as a potential target for medical intervention in diseases such as systemic lupus erythematosus, psoriasis and rheumatoid arthritis as well as organ transplant rejection.

Cellcept® (mycophenolate mofetil, MMF), which is a prodrug of mycophenolic acid (MPA) currently has clinical utility, as an IMPDH inhibitor, in organ transplant rejection.[6] Other end points in autoimmune diseases have been, and are currently being, sought but they may well be restricted by reported dose limiting gastrointestinal toxicity of this compound, which is believed to be due to glucuronidation and excretion of MPA via bile and is not mechanism based.[7]

The search for alternative IMPDH inhibitors with an improved therapeutic window has been led by Vertex with VX-497 and VX-148[8,9] as well as BMS with BMS-337197[10] and other structural classes.[5] Many of these have been designed using knowledge of binding interactions aided by crystal structures (Fig. 1).[11]

At Celltech our focus has been on the identification and development of potent, selective inhibitors of IMPDH II with improved pharmacological properties. Our efforts to find optimal replacements for the urea of VX-497 led us to the cyclised quinazolinethione (**1**) (Fig. 2).

With this initial positive result in hand we developed and utilised parallel solution phase chemistry to scope out the SAR around the 3-position with 60 analogues (Scheme 1). Known amino ester (**2**) was readily converted to isothiocyanate (**3**), which was reacted with a number of primary amines in parallel to give quinazolinethiones (**4**).

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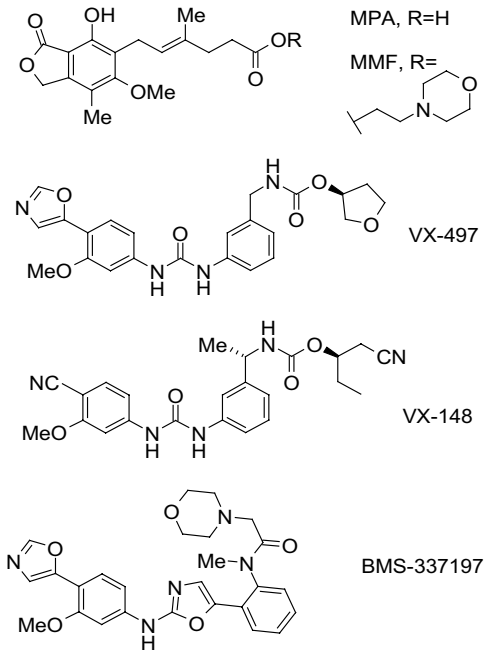
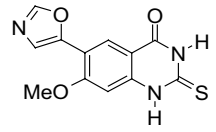


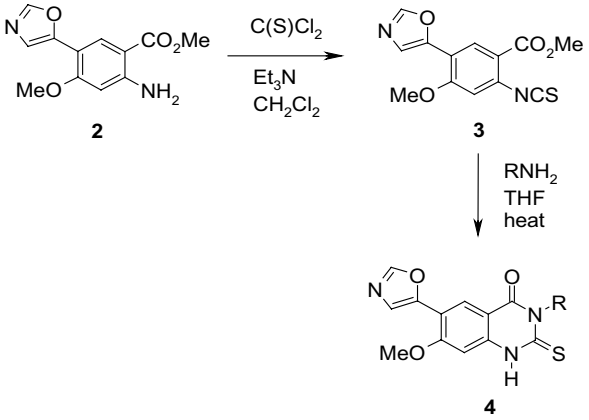
Figure 1. Structures of MPA, MMF, VX-497, VX-148 and BMS-337197.



1

IMPDH II (IC_{50}) = 30nM

Figure 2. Quinazolinethione **1**.



2 **3** **4**

Scheme 1. Solution phase parallel synthesis of quinazolinethiones.

Selection of groups for inclusion in this set was driven by CADD as we not only desired diversity within the set synthesised but also wanted to incorporate groups likely to yield favourable physicochemical properties. We used docked structures to guide our search for additional binding affinity and to trim out groups, which would have putative negative interactions (Fig. 3).[12]

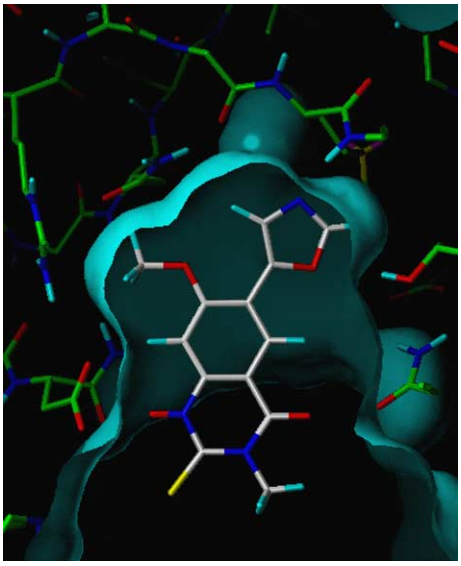
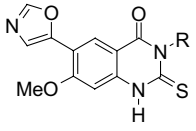


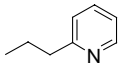
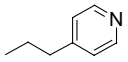
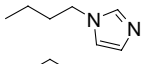
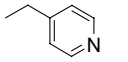
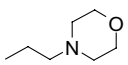
Figure 3. Quinazolinethione **4a** docked into IMPDH.

From the initial set of compounds synthesised (Table 1) it became obvious that branching close to the bicyclic ring of our quinazolinethiones as exemplified by compounds **4e**, **4g** and **4h** gave us a fall in activity. This was consistent with docking of these structures where clashes with protein could be observed. Some of our more potent compounds (e.g., **4c**) pleasingly gave submicromolar IC_{50}s in our peripheral blood monocyte (PBMC) proliferation assay.[13]

Table 1. SAR of quinazolinethiones



4

Compd	R	IMPDH II,[14] nM	PBMC,[13] μM
4a	Me	17	1.1
4b		91	1.0
4c		13	0.78
4d		22	0.98
4e		1710	n.t.
4f		100	4.1
4g		821	n.t.
4h	*i*-Pr	27% @ 1 μM	n.t.
4i		71	2.76

n.t. = not tested.

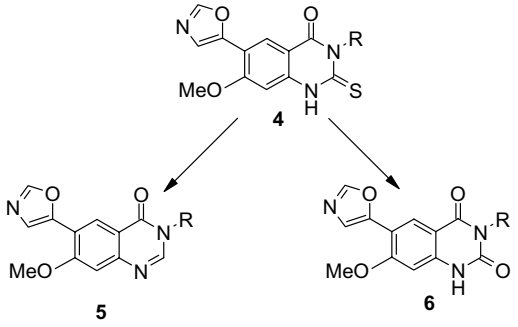
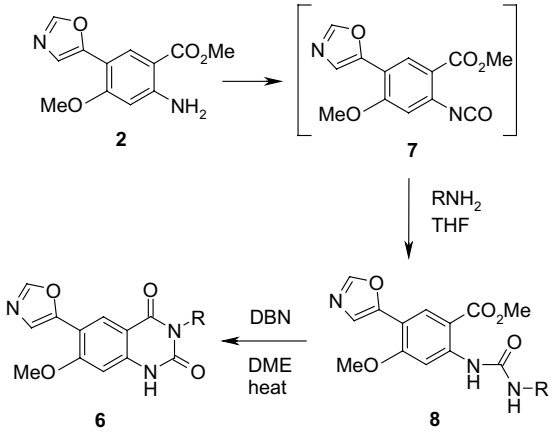


Figure 4. In vitro microsomal turnover of quinazolinethiones by rat liver microsomes at a concentration of 1 mg/mL.

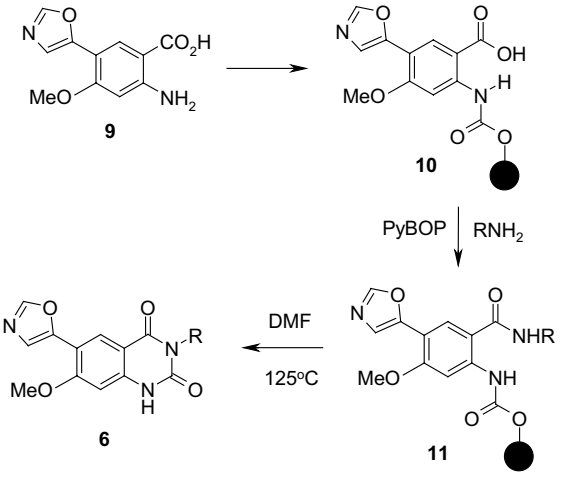
However, many of the quinazolinethiones, with differing 3-substituents, were found to be rapidly turned over in in vitro microsomes assays. Subsequent metabolite identification studies (Fig. 4) indicated that two major metabolites were formed in each case, irrespective of 3-substituent. One route of metabolism resulted in the formation of the corresponding quinazolinediones (conversion of C=S to C=O). Once formed, the diones (**6**), which also inhibited IMPDH II, appeared stable and did not undergo further metabolism. The other route of metabolism led to compounds in which the S was replaced by H and these quinazolinones (**5**) were inactive against IMPDH II. The identity of both of these metabolites were confirmed by comparison with authentic samples. As a consequence of these results no further thiones were produced and instead optimisation of the diones was undertaken.

The synthetic tractability of the quinazolinediones was initially not as good as had been found with the quinazolinethiones. The corresponding isocyanate (**7**) was not as stable as its isothiocyanate analogue (**3**) had been and so was not isolable. This required us to prepare it each time in situ. The intermediate ureas (**8**) also had to be purified and so this lowered the throughput of target molecules (Scheme 2).

We therefore developed and utilised a solid phase 'catch and release' route, which allowed us to generate 80 ana-



Scheme 2. Solution phase synthesis of quinazolinediones.



Scheme 3. Solid phase synthesis of quinazolinediones.

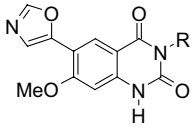
logues in parallel. The known aminoacid (**9**) was loaded onto Merrifield resin as the carbamate.[15] Amines were coupled with PyBOP, then cyclisation and release of product was achieved at 125 °C with DMF as solvent to give diones (**6**) without the need for any purification (Scheme 3).

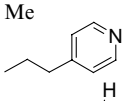
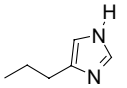
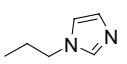
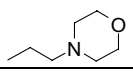
The SAR of the quinazolinediones generally mirrored that of the quinazolinethiones as one would envisage from docking studies, but were intrinsically less potent (Table 2). We postulate that this lower potency is due to a weaker key H-bond interaction between the N–H and Asp274 (Fig. 3).

In conclusion the quinazolinediones seem to hit a plateau of activity beyond which it was difficult to drive activities lower. They routinely gave poor absorbance in vivo and also in in vitro Caco-2 diffusion experiments (data not shown).

We intend to publish further modifications of this template, which resulted in compounds with improved PK profiles and cellular potencies in due course.

Table 2. SAR of quinazolinediones



Compd	R	IMPDH II,[14] nM	PBMC,[13] μM
6a	Me	104	4
6b		188	1.2
6c		47	Inactive
6d		86	2.4
6e		297	9.13

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13. Inhibitors are dissolved in 100% DMSO at a concentration of 20 mM and an initial dilution of 1:71 into RPMI 1640 medium containing penicillin/streptomycin and glutamine 5% human serum. 1:10 serial dilutions are then prepared, these inhibitors are added at $T = 0$. PBMC's are plated out at a density of 2×10^6/mL in RPMI medium containing 5% human serum in the presence and absence of inhibitor and 100 μM guanosine. The plates are incubated at 37 °C/5% CO_2 for 48 h. After 48 h 0.5 μCi of ^3H thymidine is added per well and the plates incubated for a further 18 h. The final assay volume is 150 μL. The contents of the plate are then transferred to a filter plate and the cells washed with saline using the Millipore microtitre plate filtration system. The plates are dried and 100 μL of microscintillation '0' fluid is added to each well and the plate counted in the TopCount.
14. IMPDH activity was determined by monitoring the production of the fluorescent product, NADH. Assays were performed in a final volume of 200 μL containing IMPDH (0.4 μg), NAD (100 μM), IMP (100 μM), 1% DMSO, 30 mM KCl and 100 mM Tris/HCl, pH 7.5. Fluorescence at excitation 340 nm/emmission 465 nm was read continuously at 25 °C for 30 min. From this data, initial rates (i.e., change in fluorescence intensity per minute) were calculated. To determine the IC_{50} values, test compounds were prepared at an initial concentration of 1.0 mM in 100% DMSO, then diluted in assay buffer to 0.2 mM. Further dilutions were made in assay buffer containing 20% DMSO, prior to diluting 20-fold into the assay, to allow testing across the range 1 nM to 10 μM.
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