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Discovery of Novel Low Molecular Weight Inhibitors of IMPDH Via Virtual Needle Screening

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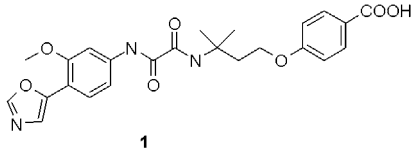
Abstract—Novel, low molecular weight inhibitors of IMPDH have been discovered through the application of a validated virtual screening protocol. A series of 21 IMPDH inhibitors were used to validate the docking procedure. Application of this procedure to the selection of compounds for screening from an in-house database resulted in a 50-fold reduction in the size of the screening set (3425 to 74 compounds) and gave a hit-rate of 10% on biological evaluation.

Inosine 5′-monophosphate dehydrogenase (IMPDH) is a NAD dependent enzyme which catalyses the oxidation of inosine monophosphate to xanthosine monophosphate, a key rate limiting step in the pathway for de novo purine biosynthesis. Inhibition of this enzyme results in depletion of cellular pools of guanine nucleotides and this results in a blockade of replication processes which place a high demand on the de novo purine biosynthetic pathway.[1] Such processes include proliferation of T and B lymphocytes and virus replication. Thus IMPDH inhibitors may be useful for the treatment of Hepatitis C Virus (HCV) infections for two reasons: suppression of T-cell responses could be beneficial in decreasing hepatocyte damage and a direct antiviral effect through inhibition of virus replication.

Several classes of IMPDH inhibitors are in use in the clinic. Ribavirin is a substrate mimic of IMP ($K_i = 250$ nM) with broad spectrum antiviral activity.[2] It enhances the sustained response rate in HCV patients when co-administered with interferon.[3,4] Mycophenolic acid (MPA) is the active metabolite of CellCept™ (mycophenolate mofetil), approved for use following kidney[5–7] or heart[8] transplantation because of its immunosuppressive properties. MPA is non-competitive ($IC_{50} = 20$ nM) with respect to both IMP and NAD and traps the covalent intermediate within the active site. Recently, Vertex have developed a novel series of non-competitive inhibitors, guided in part by protein structural information,[9] and one of these, VX-497, is in Phase II trials.[10]

In a companion paper[11] we report the discovery of a novel series of potent oxamide based inhibitors of IMPDH, represented by **1**. These compounds are non-competitive inhibitors and bind in the NAD pocket. The phenyl oxazole warhead has a stacking interaction with the xanthine ring system of the covalent intermediate of the enzyme reaction, as revealed by in-house crystallographic studies[12] (Fig. 1a). However, medicinal chemistry efforts to modify the warhead met with only limited success (Table 1).

Interestingly, the phenyl oxazole aniline, **2**, has an IC_{50} of 19 μM and thus we reasoned that it should be possible to find novel low molecular weight inhibitors of IMPDH. We embarked upon a strategy to discover alternative warheads through the screening of low molecular weight compounds, selected by a computational docking procedure.



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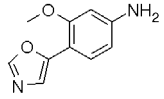

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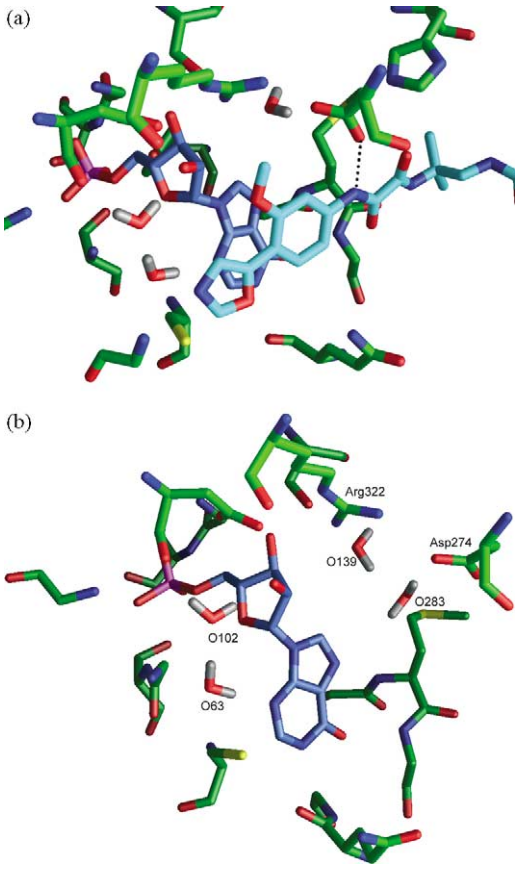
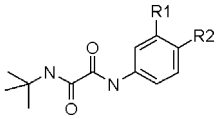


Figure 1. Active site of IMPDH from crystal structures with different structural classes of inhibitor. A). Active site of IMPDH with a phenyl oxazole inhibitor (cyan carbon atoms). Note that O283 is not present in this structure and the oxamide NH H-bonds to Asp274 instead, as shown. B) Active site of IMPDH (green carbon atoms) with IMP (grey/blue carbon atoms), the MPA analogue inhibitor is not shown for clarity. Two water molecules interact with IMP, two other waters mediate a salt-bridge between Arg322 and Asp274.

The rationale behind this approach can be found in recent work comparing the properties of good leads to those of drugs. Hann et al.[13] have shown on statistical grounds that low complexity (low molecular weight) compounds have an increased probability of being found as hits in screening. Teague and colleagues have noted that leads tend to have lower molecular weight and lower logP than drugs[14,15]—the lead optimisation process tends to add molecular weight and lipophilicity. Thus a suitable starting point for a lead discovery program would be an appropriate low molecular weight compound, with suitable functionality to allow for rapid chemistry follow-up. However, the lower intrinsic activity of lower molecular weight compounds means that they need to be screened at a higher concentration.

Several alternative strategies have been explored in the spirit of these observations. For example, the MULBITS approach[13] where low molecular weight fragments are screened at high concentration, SAR by NMR[16] which uses NMR to detect binding events and the high concentration screening of capped monomers suitable for combinatorial chemistry.[17] The use of structure-biased needle screening, combining several computational techniques such as pharmacophore searching and docking, was recently proposed as an alternative to random screening for lead discovery and exemplified through application to the discovery of novel inhibitors of DNA Gyrase.[18]

Table 1. IMPDH inhibition values for the 21 compound training set



Compds	R1	R2	IMPDH Inhibition IC_{50}, μM[a]
3	OMe	5-Oxazolyl	0.04
4	Cl	5-Oxazolyl	0.18
5	Me	5-Oxazolyl	0.20
6	OMe	5-(4-Me-oxazolyl)	0.43
7	OMe	4-Oxazolyl	0.47
8	F	5-Oxazolyl	0.73
9	OMe	5-Thiazoyl	0.91
10	Et	5-Oxazolyl	1.2
11	OEt	5-Oxazolyl	1.3
12	OMe	2-Pyrroyl	2.4
13	OH	5-Oxazolyl	4.4
14	OMe	2-Furanyl	8.1
15	H	5-(4-Me-Oxazolyl)	14
16	OMe	5-Triazoyl	15
17	5-oxazoyl	OMe	16
18	CF_3	5-Oxazolyl	18
19	H	5-Oxazolyl	20
20	OMe	OMe	21
21	OMe	5-(4-nbutyl-Oxazolyl)	27
22	OMe	5-(4-benzyl-Oxazolyl)	35
23	OMe	Cl	47

[a]Values are means of three experiments.

We decided to employ a structure-based strategy to the identification of low molecular weight inhibitors of IMPDH, based around the docking program FlexX[19–22] to select structures for high concentration screening. A three-step procedure was adopted: (1) Validation of the virtual screening protocol using the structures in Table 1. (2) Virtual screening of a set of readily available low molecular weight, chemically tractable structures suitable for screening at high concentration ('needles'). (3) Biological evaluation in an in vitro assay capable of screening accurately up to several hundred μM.

Prior to performing the virtual screen on a large set of compounds, a number of issues concerning the system set-up had to be addressed; which of the in-house crystal structures to use, which scoring function and whether or not to include crystallographic waters. In order to answer these questions it was decided to use the data for 21 *t*-butyl oxamides[23] with a variety of modifications to the phenyl oxazole to validate the virtual screening protocol (see Table 1). Two in-house crystal structures of IMPDH were available, one complexed with an MPA analogue (XM) and the other with an oxamide analogue (XO). This latter structure was crystallised in the presence of α-NAD rather than β-NAD and hence contained unreacted IMP rather than the covalent complex.[12] Figure 1b shows the IMP binding site in the MPA structure (inhibitor removed for clarity). Three of the four crystallographic waters shown were conserved

between the two structures and have hydrogen bonding interactions with the substrate and/or protein rather than the inhibitor. Water 283 is not present in the oxamide-derived structure, presumably because the oxamide NH hydrogen bonds to Asp274.

Three water models were evaluated for each protein; XM: no waters, two waters (W63, W102) or four waters (W63, W102, W139, W283); XO: no waters, two waters (W63, W102) or three waters (W63, W102, W139). Hydrogens were added to the structures and positions optimised using the in-house software MOLOC.[24] In addition, the oxamide structure was modified by rotating torsion $\chi 1$ of Cys331 by analogy to that in XM so that it was in a position to make the covalent bond with carbon C2 of the IMP ring.

The docking program FlexX was used to dock the structures in Table 1 into IMPDH in the presence of the substrate. Three scoring functions were evaluated, the default FlexX function,[19] an implementation of the PLP scoring function[25,26] and the newly developed ScreenScore function.[26] The performance of the FlexX docking algorithm in combination with several scoring functions was evaluated recently by Stahl and Rarey in the context of virtual screening.[26] The default FlexX scoring function is a modified version of the empirical scoring function developed by Boehm[27] and is particularly effective in polar hydrogen bonding sites. PLP has a simpler functional form that is less dependent on the exact orientation of hydrogen bonding interactions and favours good steric fit. The ScreenScore function was developed by Stahl and Rarey to incorporate the best features of the complementary FlexX and PLP functions and was shown to perform well in virtual screening over a broad range of protein targets, from hydrophobic to polar active sites.

In order to evaluate the performance of the different scoring functions and protein set-ups, the Spearman rank correlation coefficient was calculated for the docking scores versus pK_i in each of the systems studied. Results are plotted in Figure 2. Inclusion of waters is necessary to obtain a good correlation though, in general, there is little difference between the different water models. As the waters do not hydrogen bond with the inhibitors, they presumably better define the steric shape of the binding pocket. Interestingly, the ScreenScore function performs particularly well in this application and was chosen for the virtual screening.

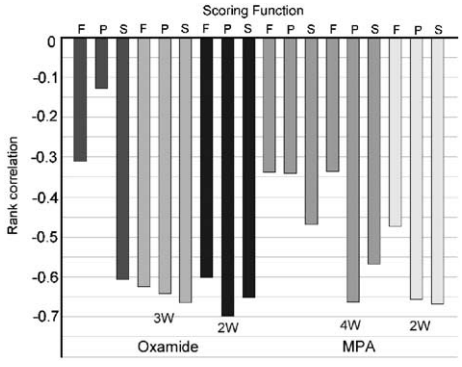
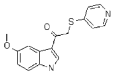


Figure 2. Rank correlation coefficient of pK_i versus FlexX score for the 21 compounds in Table 1. Results for different scoring functions (F: FlexX, P: PLP, S: ScreenScore), X-ray structures and water models are shown.

Compounds for virtual screening were extracted from our in-house reagent inventory system. A molecular weight filter ($80 < M_W < 400$) was applied to give 3425 compounds. The source of the compounds ensured that they possessed functionality suitable for rapid follow-up (acids, amines, aromatic halogens etc.). The compounds were docked using FlexX into two different protein models, one taken from the MPA complex with four water molecules and the other from the oxamide complex with three water molecules. The ScreenScore function was used for docking and scoring. Structures were sorted according to the docking score and one hundred were selected from the top-ranked structures following visual inspection and clustering using topological pharmacophore descriptors.[28]

Seventy four compounds were sent for biological evaluation, having eliminated compounds insoluble at the necessary high concentrations or where there was insufficient sample. Compounds were screened initially at 250 μM and an IC_{50} determined for any compound with >65% inhibition. Eight compounds had an IC_{50} determined and the results are shown in Table 2. Interestingly, three of these compounds have an IC_{50} less than 35 μM, comparable to that of the phenyl oxazole aniline, **2**. Kinetic studies on two of these MFCD00003925 and WISP00000743 showed them to

Table 2. Structures and activities of 8 'hit' compounds from virtual screening

Compd	Structure	IMPDH Inhibition IC_{50} μM
MFCD00003925		31
WISP00000743		32
MFCD00022717		32
MFCD02178385		54
MFCD00191957		88
MFCD00005669		99
MFCD00828822		168
MFCD00002303		620

be non-competitive with respect to IMPDH and NAD, as predicted.

In conclusion, structure-based virtual screening has been used to discover novel low molecular weight inhibitors of IMPDH. An initial set of 3425 compounds was reduced to just 74 (i.e., 50-fold) by a validated docking procedure. Biological evaluation of these selected compounds identified seven compounds with an IC_{50} less than 200 μM, a 10% hit-rate. The active compounds represent novel, low molecular weight inhibitors of IMPDH and are ideal candidates for further optimization.

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