A Novel Class of Inhibitors of Peptide Deformylase Discovered through High-Throughput Screening and Virtual Ligand Screening

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Abstract: Peptide deformylase (PDF) has been identified as a promising antibacterial and herbicide target. A structurally novel class of inhibitors containing a 2-thioxo-thiazolidin-4-one heterocycle substituted by an arylidene group at the 5-position and a hexanoic acid side chain at the 3-position was discovered independently via high-throughput screening and virtual ligand screening. Data mining and analogue synthesis established a structure−activity relationship for the side chain region that is consistent with the docked structure.

High-throughput screening (HTS) of large compound libraries has become a central component of modern drug discovery programs. Such broad-based screening of collections of low molecular weight molecules has indeed resulted in the discovery of compounds that modulate the activity of targets associated with many different therapeutic areas, but this generally requires considerable resources especially when a large number of compounds need to be acquired and screened. The concept of target-biased libraries as alternatives to large, maximally diverse compound libraries is one that has generated considerable interest in the field of lead discovery. This relatively new approach, in which knowledge of the ligand specificity of the biological target (or target family) is used to design or select compounds with enhanced likelihood of activity, offers the possibility of discovering leads more efficiently by screening fewer compounds.[1] While this increase in screening efficiency should enable conservation of valuable research resources, a drawback is that the novelty of the resulting leads will necessarily be limited by the current state of understanding of the structural requirements for ligand binding to the target in question. Virtual screening has therefore emerged as an alternative approach that enables the evaluation of very large numbers of compounds in a fast and resource efficient manner.[2]

Peptide deformylase (PDF), a key enzyme in bacterial and plant protein translation, has been identified as a promising antibacterial and herbicide target.[3] PDF is a metalloprotease that catalyzes the cleavage of the formyl group at the N-terminal methionine residue of nascent polypeptides. It requires a divalent metal ion for catalytic activity. For the native enzyme, an active site Fe^{2+} cofactor is essential for hydrolysis of the formamide bond.

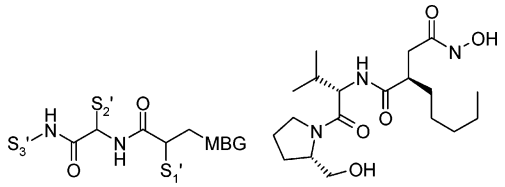

Figure 1. (a) PDF pharmacophore and (b) actinonin.

Accumulated experience has shown that HTS has not been as effective in the discovery of new antibacterial agents as it has in other therapeutic areas, but this is not true for peptide deformylase.[4] PDF inhibitors representing over a dozen distinct chemical classes have been discovered by means of HTS of diverse compounds from private chemical databases and collections of known metalloprotease inhibitors, and mechanism-based design.[5] Virtual ligand screening, an in silico variation on HTS that utilizes the structure of an enzyme active site as a filter for selecting and prioritizing small molecule inhibitor candidates for enzyme assay, has not been applied to PDF until this report.

Comparison of the various PDF inhibitor classes reveals a pharmacophore containing a metal binding group (MBG) separated by a methylene group from lipophilic moieties in the S_1' and S_2' regions (see Figure 1a).[5g,6] The most potent inhibitors, such as the pseudopeptide actinonin (Figure 1b), have a hydroxamic acid as the MBG.[7] The bioisosteric *N*-hydroxyformamide,[8] found in the antibacterial PDF inhibitor BB-3497, is also an effective MBG.[9] Given the metabolic liabilities of these two functional groups in plants, we sought PDF inhibitors containing alternative metal chelating residues.

The source of chemistry for our in vitro HTS against plant PDF[10] was a 10 000-member informer library composed of molecules selected from our corporate collection and designed to represent the broad diversity of biologically relevant chemical space encompassed by the larger collection. An initial cutoff of $IC_{50} < 10\ \mu M$ was set as a threshold for further evaluation of the informer library actives. Among the several in vitro hits meeting this criterion, the 2-thioxo-4-thiazolidinone **1** ($IC_{50} = 4.6\ \mu M$) was selected for further evaluation due to its unique structure relative to known PDF inhibitor chemotypes and its lack of a hydroxamic acid MBG. Data mining of the corporate collection based on the heterocyclic substructure of **1** identified several other related structures that were then assayed to provide insight into the scope of the PDF activity. The results are presented in Table 1.

As can been seen from the data presented in Table 1, 2-thioxo-4-thiazolidinone *N*-hexanoic acids are potent inhibitors of plant PDF. A variety of heteroarylidene groups are tolerated at C5. Variously substituted five-membered heterocycles (pyrroles and pyrazoles) show low micromolar levels of inhibition. Interestingly, 2-thioxo-4-thiazolidinones having shorter side chains,

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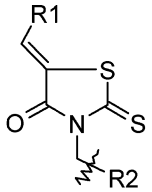
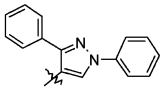
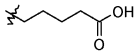
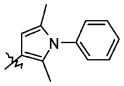
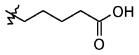
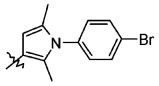
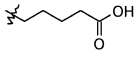
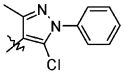
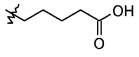
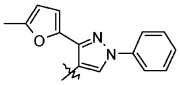
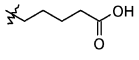
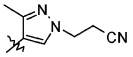
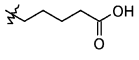
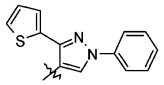
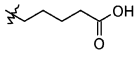
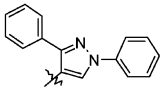
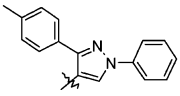
Table 1. Data Mining on HTS in Vitro Hit **1**[a]



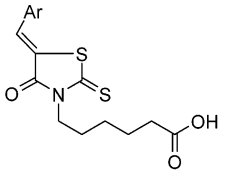
Number	R1	R2	IC50 (μM)
1			4.66
2			1.66
3			1.96
4			2.52
5			4.41
6			7.11
7			7.33
8			IA
9			IA

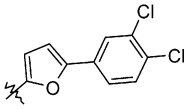
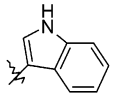
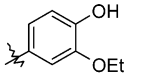
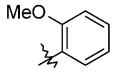
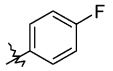
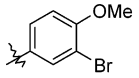
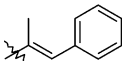
[a] Enzyme inhibitory activities were measured as described in the Supporting Information.

such as acetic acid (**9**) and propionic acid (**8**), were inactive against PDF.

Concurrent with the above HTS effort, we were also exploring VLS for the discovery of new PDF inhibitors using the ICM method,[11] which was recently benchmarked on a set of nuclear receptors and ligands[12] and applied to discover novel thyroid receptor antagonists.[13] The homology model of the matured soybean PDF2 was built in ICM based on the X-ray structure of the homologous enzyme from *Plasmodium falciparum*[14] (pdb code 1JYM). The two proteins have overall sequence identity of 43.0%. Residue conservation in the active site is stronger, although there are several substitutions of the residues delimiting the hydrophobic pocket that binds the methionine side chain of the substrate. Conformational search and energy optimization of these residues was performed in ICM.[11] The substrate molecule was kept in the pocket during model optimization to avoid the pocket collapse. The resultant 3D model of soybean PDF was used to computationally screen a collection of 528 439 vendor-offered compounds.

Table 2. Evaluation of VLS Hits against PDF2[a]



Number	Ar	IC50 (μM)
10		0.89
11		1.17
12		1.25
13		1.59
14		2.88
15		3.61
16		8.61

[a] Enzyme inhibitory activities were measured as described in the Supporting Information.

The virtual library screening (VLS) procedure involved fast flexible-ligand docking using grid representation of the receptor[15] and evaluation of the ICM score[16] of the best docked pose for each compound. Actinonin, a known PDF2 inhibitor was also docked and its ICM score evaluated at −46. This value places actinonin within 0.2% of the top-ranked compounds in the database screened. Subsequently, a cutoff of −41 was used to generate an initial compound selection comprising ∼1% of the top-ranked database compounds. The relaxed cutoff value was chosen to provide a margin for scoring errors and weaker binders.

After removal of structures with undesirable properties and database comparison against our corporate database, a list of 4425 compounds was generated and ordered. Biochemical evaluation of those compounds (3169 received) led to the discovery of several novel chemotypes of PDF inhibitors. Among these were the 2-thioxo-4-thiazolidinone *N*-hexanoic acids shown in Table 2.

The results from the VLS experiment confirmed the activity seen from the HTS effort and expanded the SAR at C5 to include other heterocycles (furan, indole) and substituted benzenes. It also provided the most potent 2-thioxo-4-thiazolidinone found yet, the furan **10** that had an IC_{50} of 0.89 μM.

Kinetic analysis of the initial HTS hit **1**, the most potent VLS hit **10**, and the prototypical PDF inhibitor actinonin revealed a competitive relationship with a model substrate, formyl-Met-Ala-Ser. Using a GraFit analysis package (Erithacus Software, Surrey, UK), K_i

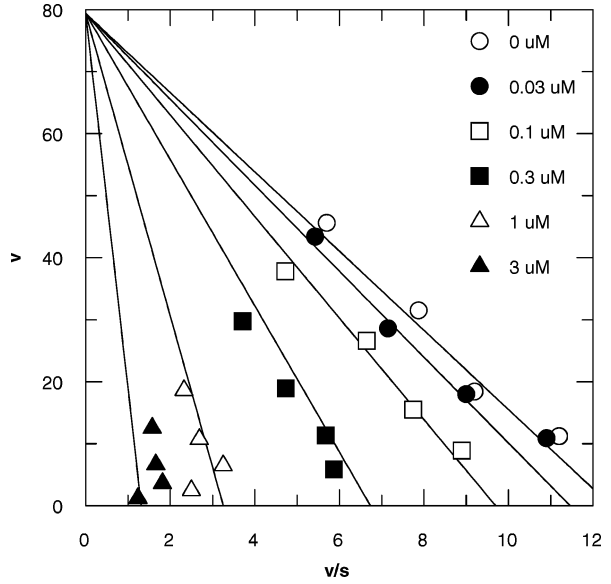


Figure 2. Kinetic analysis of **10**.

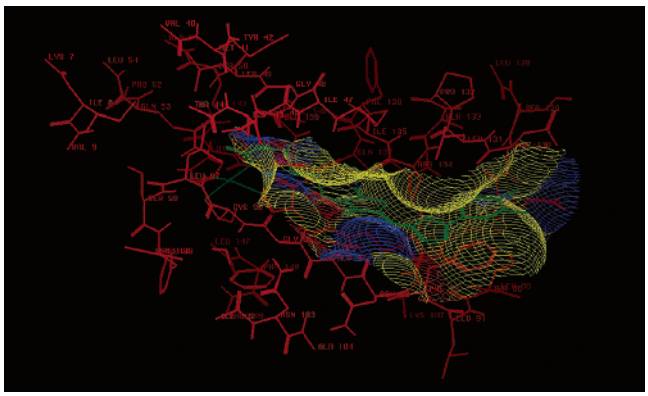


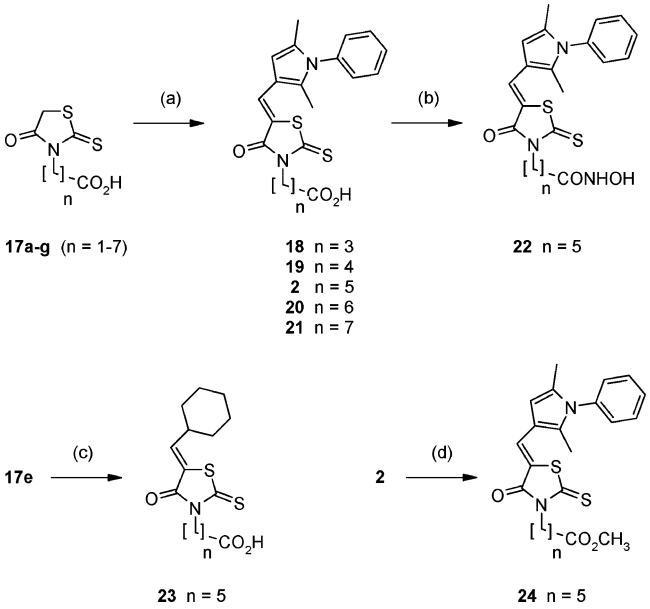
Figure 3. Compound **10** docked into the binding site of PDF. The accessible surface of the binding site is showed as color-coded mesh: yellow (hydrophobic), red (H-bond donor region), and blue (H-bond acceptor region). Generated using the Flo99 software.

values of 0.53, 0.34 and 0.003 μM, respectively, were generated. In Figure 2 the data for **10** plotted as v/S vs v, (where S is the substrate concentration and v, the initial rate of substrate turnover) produced distinctive converging lines indicative of competitive inhibition over the substrate and inhibitor concentration ranges chosen.

Moreover, binding to the enzyme was shown to be reversible. In the presence of 3 μM **10** the enzyme was inhibited 84% but following dialysis, in which the inhibitor concentration was reduced by a factor of 100 within 30 min, 88% of the activity was restored. This indicates there is no covalent bond formed between the inhibitor and enzyme.

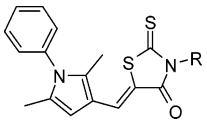
This is consistent with the model of the interaction of inhibitors, and particularly **10** with PDF derived from the VLS experiment where the carboxylic acid is coordinated to the active site metal center as shown in Figure 3. Also consistent with the metal coordination role for these 2-thioxo-4-thiazolidinone carboxylic acids is the fact that the methyl ester **24** is completely inactive, while the corresponding hydroxamic acid **22** was more potent, having an IC_{50} of 0.45 μM (see Scheme 1 for structures).

Scheme 1.[a] Synthesis of 2-Thioxo-4-thiazolidinone *N*-Alkanoic Acids and Related Analogues



[a] Reagents: (a) ethylenediamine diacetate, 2,5-dimethyl-1-phenylpyrrole-3-carboxaldehyde, MeOH, rt, 38−79%, (b) (i) *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine, HOBt, EDC, CH_2Cl_2, 0 °C to room temperature, (ii) p-TsOH, MeOH, rt, 60% (two steps), (c) ethylenediamine diacetate, cyclohexanecarboxaldehyde, MeOH, rt, 42%, (d) iodomethane, K_2CO_3, CH_3CN, 58%.

Table 3. 2-Thioxo-4-thiazolidinone *N*-Alkanoic Acids: Effect of Side Chain Length[a]



number	R	IC_{50} (μM)	side chain
25	CH_2CO_2H	>30	acetic acid
26	$CH_2CH_2CO_2H$	>30	propanoic acid
18	$CH_2(CH_2)_2CO_2H$	>30	butanoic acid
19	$CH_2(CH_2)_3CO_2H$	10.1	pentanoic acid
2	$CH_2(CH_2)_4CO_2H$	1.66	hexanoic acid
20	$CH_2(CH_2)_5CO_2H$	25.3	heptanoic acid
21	$CH_2(CH_2)_6CO_2H$	>30	octanoic acid

[a] Enzyme inhibitory activities were measured as described in the Supporting Information.

To explore the effect of chain length between the carboxylate and 2-thioxo-4-thiazolidinone ring on inhibitor binding affinity, we prepared a series of 2-thioxo-4-thiazolidinone *N*-alkanoic acids containing the pyrrole substituent at C5 as in **2**. The synthetic route to these new 2-thioxo-4-thiazolidinone *N*-alkanoic acids is shown in Scheme 1. The results of their evaluation are shown in Table 3.

Consistent with the VLS-derived binding model, the *N*-hexanoic acid side chain of **2** is the optimum length, with the corresponding pentanoic and heptanoic acids (compounds **19** and **20**, respectively) showing significantly less activity. Side chain acids shorter than five carbons (compounds **18**, **26**, and **25**) and longer than seven carbons (compound **21**) were inactive.

Finally, we have also explored alkylidene substituents at the 2-thioxo-4-thiazolidinone C5 position. A variety of alkyl and cycloalkyl substituents show submicromolar inhibition. For example, the cyclohexyl-substituted de-

rivative, **23** (see Scheme 1 for structure), had an IC_{50} of 0.11 μM against PDF2, making it one of the most potent carboxylic acid inhibitors known for peptide deformylase.

The series of compounds (Tables 1 and 2) were also assessed for their ability to inhibit *E. coli* PDF. In this case their IC_{50} values were all in excess of 30 μM. This was not surprising as the VLS experiment was carried out on a protein model based on the sequence of plant PDF, not the *E. coli* enzyme.

Convergence of HTS and VLS approaches has led to the discovery of a new class of PDF inhibitors, the 2-thioxo-4-thiazolidinones. The length of the alkanoic acid side chain at N3 is critical, with six carbons being optimum. A wide variety of arylidene and heteroarylidine substituents at C5 are tolerated. Kinetic analysis establishes that these new inhibitors bind competitively with substrate, and analogues bearing modifications to the carboxylic acid suggest metal chelation to the active site iron cofactor, consistent with the binding model from the VLS experiment. Our results demonstrate the value of virtual screening for the discovery of novel inhibitors that diverge from the established pharmacophore model for PDF inhibition.

Acknowledgment. We thank Drs. Susanta Samajdar and Shaji George (Biocon, Ltd.) for technical assistance.

Supporting Information Available: Experimental procedures for all synthesized compounds; melting points and spectroscopic data for all commercially procured and archival inhibitors; experimental procedures for cloning, expression, purification, and assay of plant PDF; further details of the VLS experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

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