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Structure-based design of protein tyrosine phosphatase-1B inhibitors

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Abstract—Using structure-based design, a new class of inhibitors of protein tyrosine phosphatase-1B (PTP1B) has been identified, which incorporate the 1,2,5-thiadiazolidin-3-one-1,1-dioxide template.

1. Introduction

Reversible phosphorylation of tyrosine residues is a universal feature of eukaryotic intracellular signalling and provides an important mechanism for enzymatic and cellular regulation.[1] Tyrosine phosphorylation is controlled by protein tyrosine kinases (PTKs) and phosphatases[2,3] (PTPs), both of which are attractive target classes for pharmaceutical intervention. Although PTPs have been less extensively exploited than PTKs as drug targets, inhibition of PTP1B, the prototypical member of this enzyme class, has emerged as a potential approach to treatment of type II diabetes and obesity.[4–6]

Most approaches to PTP1B inhibition have focused on competition with substrate,[5,6] although allosteric inhibitors have recently been characterised.[7] A combination of charge and shape makes the phosphomonoester group a challenging target for mimicry. Such characteristics may be beneficial in the context of signalling and could be a factor in the evolution of protein phosphorylation as a regulatory mechanism. However, these same characteristics have frustrated the search for inhibitors, and it has proved difficult to achieve potency without compromising physical properties.

At the beginning of this programme, no suitable small molecule inhibitors were available as starting points, although the very acidic phenyldifluorophosphonate group was well known as a phosphotyrosine bioisostere.[8] Public domain[9] crystal structures were available for complexes of PTP1B with both phosphotyrosine peptides (in an inactive C215S mutant of the enzyme)[25] and non-hydrolysable difluorophosphonates.[10] The anionic group sits at the N-terminus of a helix with phosphate oxygen atoms interacting with R221 and a number of backbone amide hydrogen atoms. The ligand aromatic system interacts with both F182 and Y46. Rational, structure-based ligand design approaches resulted in the synthesis of a number of templates that potentially mimic these interactions. Among the most interesting of these was the 1,2,5-thiadiazolidin-3-one-1,1-dioxide template **1**.

2. Synthesis

The 1,2,5-thiadiazolidin-3-one-1,1-dioxide scaffold is synthesised according to Scheme 1.[11] Anilines **2**–**6** were readily alkylated with bromoacetate in the presence of diisopropylamine. The resultant secondary amines reacted cleanly with *tert*-butyl(chlorosulfonyl)carbamate

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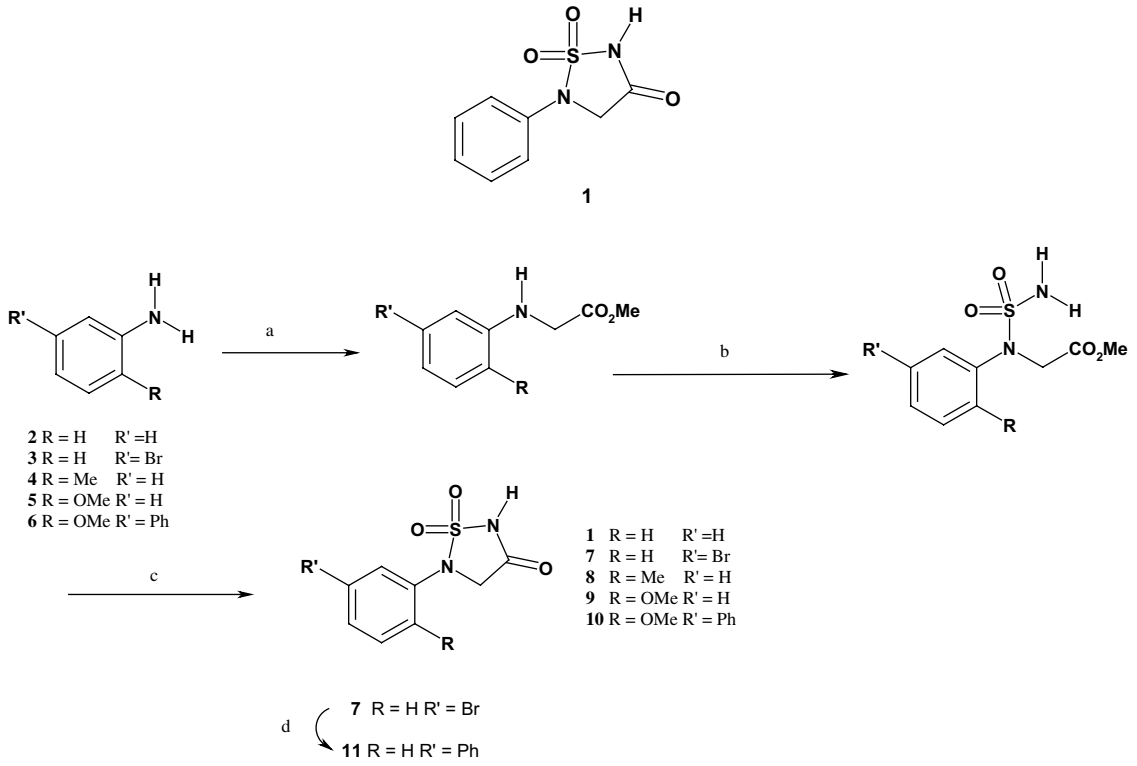

Scheme 1. Synthesis of 1,2,5-thiadiazolidin-3-one-1,1-dioxide template. Reagents and conditions: (a) methyl bromoacetate, 1 equiv, DIPEA 2.5 equiv, DMF, 60 °C, 14 h ; (70–90%); (b) (i) $BocNHSO_2Cl$ 0.5 M in CH_2Cl_2, Et_3N, 0 °C, 3 h; (ii) TFA/H_2O (9:1), rt, 2 h; (75–85%); (c) NaH, THF, rt, 2 h; (70–90%); (d) $PhB(OH)_2$, $Pd(PPh_3)_4$, Cs_2CO_3, DME/H_2O, microwave, 30 min, 100 °C; (45–50%).

and treatment with aqueous TFA allowed removal of the Boc protecting group, to give the phenylsulfamides in 75–85% yield for the two steps.

Cyclisation with sodium hydride afforded the 1,2,5-thiadiazolidin-3-one-1,1-dioxide template in excellent yield. Modification of the aryl ring substituents can also be undertaken. Analogue **7** reacted smoothly with phenylboronic acid under Suzuki conditions to afford **11** in reasonable yield.

3. Docking studies

The anion of **1** was docked[12,13] into a PTP1B crystal structure. The predicted binding mode is shown overlayed with the crystallographic difluorophosphonate ligand in Figure 1.

Two of the phosphonate oxygen atoms are matched by their sulfonyl counterparts, while the third overlays with the anionic nitrogen of **1**. The carbonyl oxygen (at position 3 of the 1,2,5-thiadiazolidin-3-one-1,1-dioxide template) occupies a similar position to the water molecule (observed in a number of PTP1B X-ray structures) that mediates the interaction between the Q266 side chain and the ligand CF_2 group. This carbonyl was included originally with the intention of modulating pK_a. Fortuitously, it also appears to be able to mimic the water-mediated interaction with the protein. As discussed below, the orthogonal orientation between the two rings

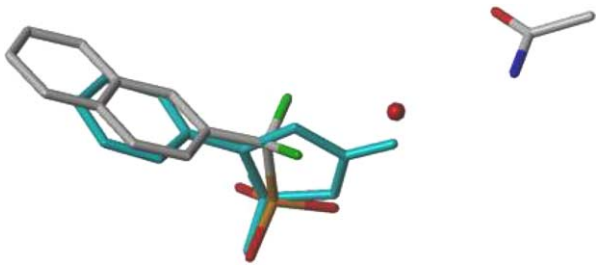

Figure 1. Overlay of docked anion of **1** (in cyan) with a difluorophosphonate ligand (see Ref. 12). Also shown are Q266 side chain amide and the water molecule that mediates the interaction of the difluorophosphonate ligand with this residue. For clarity only a portion of the difluorophosphonate ligand is displayed.

in the docked form of **1** was especially significant. Encouraged by these findings, we decided to explore the scaffold in more detail.

4. NMR and crystallographic studies

Protein NMR was used to determine if **1** bound to the active site of PTP1B. NMR experiments were conducted using samples (0.3 mM) of uniformly ^{15}N-labelled recombinant human PTP1B (residues 1-321) in a 50 mM sodium phosphate-based buffer, pH 7.0, at 600 MHz and 25 °C. ^{15}N–^{1}H HSQC spectra acquired at increasing concentrations of **1** showed chemical shift perturbations

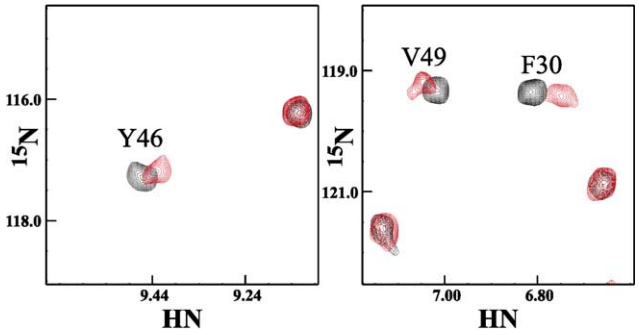

Figure 2. Selected regions of the ^{15}N–^{1}H HSQC spectra of recombinant human PTP1B(1-321) in the absence (black contours) and presence (red contours) of 4.0 mM **1**. Labelled cross-peaks experience characteristic chemical shift perturbations on binding of ligands to the active site of the enzyme.

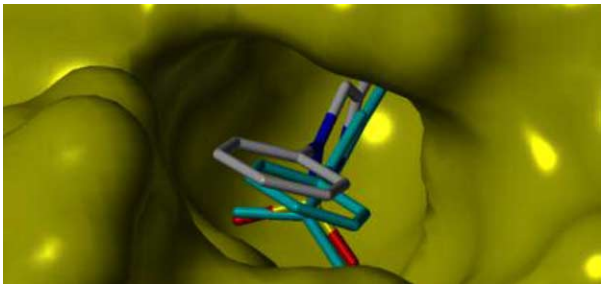

Figure 3. Crystal structure of **1** (coloured by atom type) bound to PTP1B, showing molecular surface of binding site compared with the result from docking (cyan).

in amide resonances of PTP1B known to be sensitive to occupation of the enzyme's active site, confirming that the 1,2,5-thiadiazolidin-3-one-1,1-dioxide motif mimics the native substrate (Fig. 2). The K_d calculated from the shift perturbations was 8 mM. Assuming competitive inhibition and a K_d for inorganic phosphate of around 20 mM,[14] this equates to approximately 2 mM in the absence of buffer phosphate.

The crystal structure of the PTP1B complex with **1** was determined in order to confirm the predicted binding mode.[18,20] Crystallographic and docked ligands are shown in Figure 3.

5. Conformational lock

Protein crystallography confirmed the prediction from docking[12] that **1** would bind with rings in an orthogonal orientation. This anionic system lies outside the parameterisation of empirical force fields so the energetic consequences of adopting this conformation were investigated with three commonly used quantum mechanical models. Two stationary points were found on the potential energy hypersurface and it is the higher energy of these that most closely approximates the bound conformation. Full details of the calculations are provided in the Supplementary material (Table 1).

Table 1. Computed[a] energy differences between orthogonal and coplanar conformations of anion of **1**

Basis set	ΔE(RHF)[b] /kJ mol^{-1}	ΔE(B3LYP)[c] /kJ mol^{-1}	ΔE(MP2)[d] /kJ mol^{-1}
6-31G*	12	24	19
6-31+G*	12	23	17

[a] Calculations carried out with Gaussian 98 program (Ref. 15).
[b] Restricted Hartee Fock calculation (Ref. 16).
[c] Density functional theory using B3LYP functional (Ref. 17).
[d] Second order Møller-Plesset perturbation theory (Ref. 16).

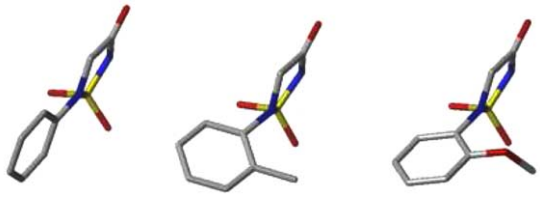

Figure 4. Minimum energy conformations for anionic forms of **1**, **8** and **9** from calculations at the RHF/6-31G* level.

ortho Substitution of the phenyl ring was predicted to destabilise the lower energy conformer, thereby rendering the bound conformation more accessible. Energy minimised structures of **8** and **9** in which the rings are forced out of coplanarity by the introduction of an *ortho* substituent are shown in Figure 4.

6. Ligand binding and enzyme inhibition

Following the observation of binding by NMR, motif **1** was tested in a biochemical assay for its ability to inhibit a C-terminal truncated form of recombinant human PTP1B using a *para*-nitrophenyl phosphate (*p*NPP) assay. Compound **1** showed an IC_{50} = 1.6 mM for the rate of hydrolysis of the *p*NPP substrate. The results for other analogues and their K_d from NMR experiments are summarised in Table 2.

There is a good correlation between activity and ability to adopt an orthogonal conformation. Introduction of a methyl or methoxy substituent at the *ortho* position leads to an order of magnitude increase in potency. Further kinetic analyses with these compounds indicated that they were all competitive and reversible inhibitors of PTP1B, and NMR chemical shift perturbation studies corroborated the improvement in affinity and the preservation of binding mode (data not shown).

Analysis of literature data suggested how the compounds might be elaborated to increase potency. While phenyl difluorophosphonate shows no inhibition of PTP1B at 200 μM,[8] an IC_{50} of 15 μM is observed for its 3-phenyl analogue.[23] Following this approach it was determined that the 5-position of the aromatic ring was the preferred point for substitution. Subsequently, compounds **10** and **11** were synthesised, with biological results shown in Table 2.

Table 2. IC_{50} and NMR K_d values

Compound	NMR K_d/μM[a]	PTP1B Inhibition IC_{50}/μM[f]
1	8000[b]	1608 (425)
8	Not tested	203 (43)
9	183[c]	138 (37)
10	<10[d]	2.47 (0.19)
11	Not determined[e]	132 (43)

[a] Measured in presence of 50 mM inorganic phosphate; for true K_d these numbers should be divided by a factor of 3–4.
[b] Fast exchange.
[c] Fast–intermediate exchange.
[d] Intermediate–slow exchange—assumes diffusion-limited k_{on}.
[e] Protein gel formation.
[f] Compounds were pre-incubated with 27 nM recombinant human PTP1B (1-314) in buffer (50 mM Bis–Tris, 2 mM EDTA, 5 mM DTT, 0.001% Triton X-100) for 10 min at room temperature. *p*NPP was then added to a final concentration of 400 μM and the reaction allowed to run for a further 15 min at room temperature. The reaction was stopped by the addition of 1 M NaOH. Absorbance at 405 nm was measured using a Fluoroskan. Values are means of three experiments; standard deviation is given in parentheses. [*p*NPP] = K_m.

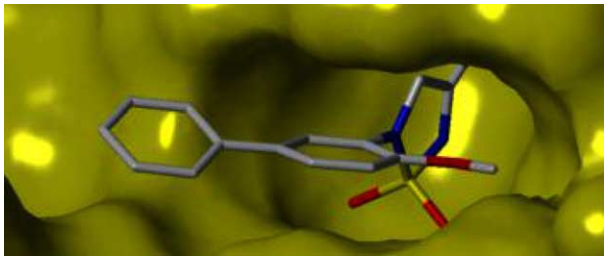

Figure 5. Crystal structure of **10** bound to PTP1B showing molecular surface of protein.

Encouragingly, these compounds show significant improvements in inhibiting PTP1B activity compared with their unsubstituted partners, SAR being additive. The crystal structure of **10** bound into the active site of PTP1B was obtained[18,21] and is shown in Figure 5.

This work shows how joint application of protein NMR and crystallography can allow a millimolar lead to be exploited with a minimum amount of synthetic effort. The approach highlighted above has identified low micromolar PTP1B inhibitors, with molecular weights of ~300 Da and can be regarded as an example of fragment based drug discovery.[24] The 1,2,5-thiadiazolidin-3-one-1,1-dioxide template has been shown to be an excellent warhead for PTP1B inhibition. Compound **10** is a useful low molecular weight starting point for a drug discovery programme.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.03.068.

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99.7% completeness with R_{merge} = 6.6% and $I/\sigma(I)$ of 11.0. The final model containing 2424 protein, 378 water and 14 inhibitor atoms has an *R*-factor of 21.8% (R_{free} using 5% of data is 24.5%). Mean temperature factor for protein is 32.7 Å^2, and for ligand is 31.3 Å^2.

21. Crystallographic parameters and statistics for **10** are: space group $P3_121$, unit cell 88.9, 88.9, 104.5 Å, resolution 2.4 Å, 17,876 unique reflections from 176,071 observations give 98.6% completeness with R_{merge} = 5.5% and $I/\sigma(I)$ of 11.2. The final model containing 2398 protein, 174 water and 31 inhibitor atoms has an *R*-factor of 16.6% (R_{free} using 5% of data is 20.0%). Mean temperature factor for protein is 25.7 Å^2, and for ligand is 34.8 Å^2.
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