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Letter

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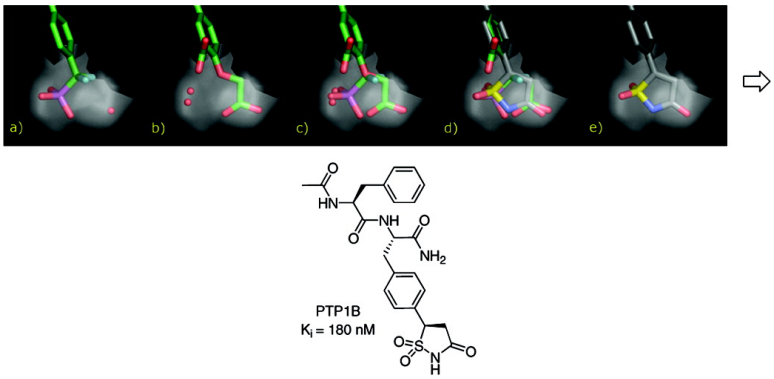

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Letters

Structure-Based Design and Discovery of Protein Tyrosine Phosphatase Inhibitors Incorporating Novel Isothiazolidinone Heterocyclic Phosphotyrosine Mimetics

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Discovery Chemistry, Incyte Corporation, Experimental Station, E336/132A, Route 141 and Henry Clay Road, Wilmington, Delaware 19880

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Abstract: Structure-based design led to the discovery of novel (*S*)-isothiazolidinone ((*S*)-IZD) heterocyclic phosphotyrosine (pTyr) mimetics that when incorporated into dipeptides are exceptionally potent, competitive, and reversible inhibitors of protein tyrosine phosphatase 1B (PTP1B). The crystal structure of PTP1B in complex with our most potent inhibitor **12** revealed that the (*S*)-IZD heterocycle interacts extensively with the phosphate binding loop precisely as designed in silico. Our data provide strong evidence that the (*S*)-IZD is the most potent pTyr mimetic reported to date.

Protein tyrosine phosphatases (PTPs) and protein tyrosine kinases (PTKs) in concert tightly control the phosphorylation state of many proteins involved in signal transduction pathways. The aberrant regulation of these signaling pathways is known to be involved in numerous disease states, including diabetes, cancer, and rheumatoid arthritis.[1] The recent FDA approvals of PTK inhibitors, such as Iressa, Gleevec, and Tarceva, have demonstrated that drug modulation of the phosphorylation state of proteins involved in specific signal transduction pathways is an effective means of treating disease.[2] In contrast to the success of PTK inhibitors, the discovery of drug candidates for PTP drug targets has proven less tractable. While kinases contain a deep hydrophobic pocket in the ATP binding site suitable for discovery of orally bioavailable small-molecule drugs, the active closed form of the catalytic site of PTPs contain a highly polar phosphotyrosine (pTyr) binding site contiguous to a shallow peptide substrate binding region. To date, all potent and competitive inhibitors of PTPs have incorporated various charged pTyr mimetics to achieve strong binding to the phosphonate binding site. Unfortunately, these same polar pTyr mimetics necessary for strong binding have limited the cell permeability and oral bioavailability of inhibitors that contain them.

Protein tyrosine phosphatase 1B (PTP1B) is a paradigm for protein phosphatase research and drug discovery. PTP1B was the first PTP purified[3,4] in the 1980s and has been extensively studied within academia and the pharmaceutical industry. It is regarded by many as one of the best validated drug targets for intervention in type 2 diabetes and obesity. PTP1B knockout and antisense studies have shown lower blood glucose levels and improved insulin responsiveness in normal and diabetic mice through enhanced IR signaling in peripheral tissues.[5−7] These mice are also protected from high-fat-diet-induced obesity. Disappointingly, nearly all medicinal chemistry efforts to date have been severely hindered because of the lack of suitable druglike lead compounds for optimization to drug candidates. High-throughput screening efforts have been ineffective, typically yielding numerous very weak noncompetitive inhibitors presumably due to the oxidation or alkylation of the active site Cys215[8] or to compound aggregation effects.[9] In contrast, the designed inhibitors incorporating pTyr mimetics have provided potent competitive PTP inhibitors. However, none of these compounds containing highly charged nonhydrolyzable phosphonate or carboxylic acid pTyr mimetics have shown significant cell membrane permeability and oral bioavailability. In our initial efforts to identify lead compounds that address these key issues, we utilized structure-based design methods to derive novel heterocyclic pTyr mimetics that strongly bind and effectively replace the undesired highly charged phosphonate and carboxylic acid moieties in known inhibitors of PTP1B.

Our efforts toward the design of PTP1B inhibitors started with a comprehensive review of the X-ray crystal structures of PTP1B/inhibitor complexes available in the Protein Data Bank (PDB). As is well documented in the literature, nearly all competitive inhibitors of PTP1B derive the majority of their binding energy through multiple hydrogen-bonding interactions with the catalytic residues in the deep tyrosine phosphate binding pocket. The most potent of these PTP1B inhibitors incorporate highly charged anions that mimic the pTyr substrate **I**, such as difluoromethylphosphonates (DFMP) **II**, carboxymethylsalicyclic acids (CMS) **III**, and oxalylaminobenzoic acids (OBA) **IV** that strongly bind the highly polarized pTyr binding site residues[10] (Figure 1).

Detailed analysis of the crystal structures of apoPTP1B and PTP1B complexed with DFMP, CMS, and OBA containing inhibitors revealed that each pTyr mimetic selectively displaces one or two of the three tightly bound water molecules in the tyrosine phosphate binding site. In the crystal structures of PTP1B with inhibitors bearing a DFMP pTyr mimetic, the bis-anionic phosphorus moiety is stabilized by binding to backbone NH groups of the loop (C215−R221), located at the N-terminus of helix α4 (Figure 2a). Specifically, one

* To whom correspondence should be addressed. Phone: (302) 498-6832. Fax: (302) 425-2708. E-mail: acombs@incyte.com.

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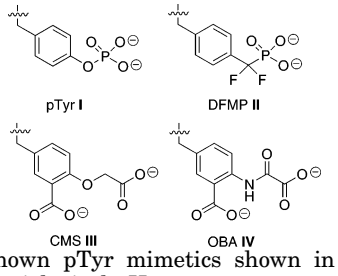


Figure 1. Known pTyr mimetics shown in ionized form expected at physiological pH.

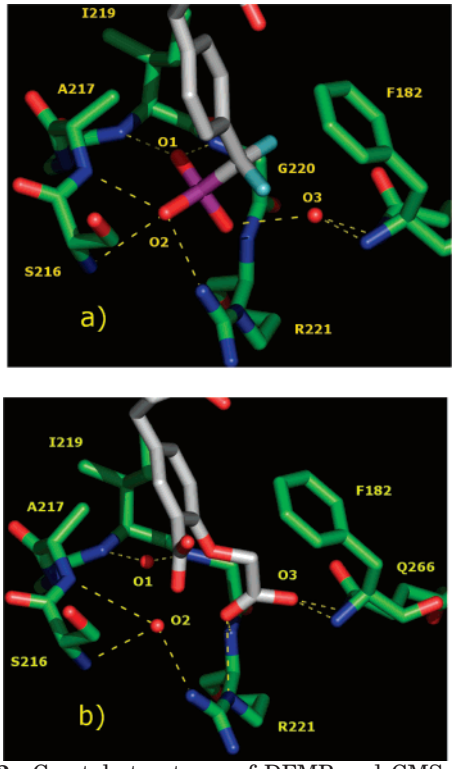


Figure 2. Crystal structures of DFMP and CMS mimetics bound in the PTP1B pTyr active site. The inhibitors are depicted with gray carbon atoms, a purple phosphorus atom, red oxygen atoms, and aqua fluorine atoms. The protein is depicted with green carbon atoms, blue nitrogen atoms, and red oxygen atoms, and the water molecule is depicted as a red sphere. The three water molecule binding sites are labeled O1, O2, and O3. Hydrogen bonds are shown as dashed yellow lines. (a) Two water molecules (O1 and O2) have been replaced by the DFMP oxygen atoms (PDB code 1N6W). (b) The water molecule at O3 has been replaced by the CMS carboxylate oxygen (PDB code 1G7G).

terminal oxygen atom is held in place by interactions with the backbone amides of I219 and G220 (position O1) and a second terminal oxygen is bound to the backbone of S216 and A217 and the side chain of R221 (position O2). Interestingly, in these structures the binding site is larger than the substrate and retains a water molecule at position O3, which is bound to the backbone amide of F182 and the side chain of Q266, in a lobe of the binding site directly beneath the phenyl ring of F182. This water molecule is not believed to be the water that hydrolyzes the phosphocysteinyl intermediate in the second step of PTP1B catalysis[11] but instead appears to be involved in positioning and bonding to the third terminal oxygen and the tyrosine phenolic oxygen in the substrate. This water molecule is present in crystal structures of PTP1B in complex with vanadate, DFMP, and pTyr, suggesting that it is tightly bound and integral to the structure of the catalytic site. In contrast, crystal structures in complex with compounds bearing the CMS **III** pTyr mimetic show that the charged carboxylic acid displaces only the water molecule at position O3 while maintaining the two additional water molecules at positions O1 and O2 (Figure 2b). Thus, DFMP pTyr mimetics replace water molecules at positions O1 and O2 while retaining O3, whereas CMS pTyr mimetics replace water molecules at position O3 while retaining O1 and O2.

Graphical overlay of the DFMP and CMS containing inhibitors illustrated their complementary, yet distinctly different modes of binding (Figure 3). Closer inspection and chemical intuition suggested that five-membered heterocycles **V**, such as the thiadiazolidinone (TDZ, **VI**) and isothiazolidinones (IZD, **VII** and **VIII**), could effectively mimic the interactions of DFMP and CMS ligands, thus displacing all three water molecules while realizing all of the corresponding hydrogen bonds (Figure 4).[12] We anticipated that these heterocycles would have significantly increased binding affinity because the entropic gain from displacing a single structural water molecule has been estimated to be as high as 2 kcal/mol.[13] In addition, the rigid heterocycle has significantly less conformational freedom than that of the acyclic pTyr mimetics DFMP and CMS, thus reducing the entropic cost of binding to the protein. Similar design concepts had been utilized in the discovery of cyclic urea HIV protease inhibitors where the urea oxygen was designed to constrain the original acyclic inhibitor while simultaneously replacing a highly conserved structural water molecule in the protein.[14] Since our initial research, several companies have disclosed the discovery of PTP1B inhibitors containing the TDZ heterocycle in the patent literature.[15−18] Just recently, a fragment-based design of compounds containing TDZ pTyr mi-

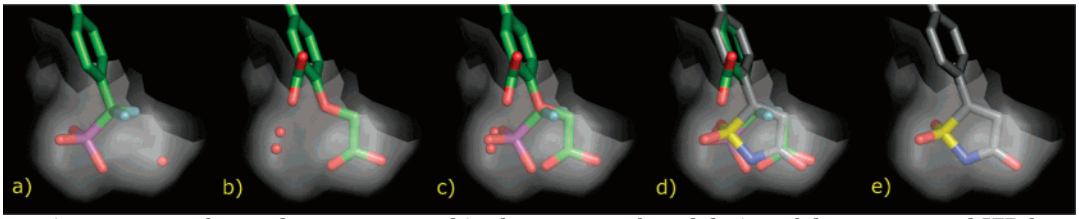


Figure 3. Stepwise sequence of crystal structures used in the structure-based design of the unsaturated IZD heterocyclic pTyr mimetic **VII**. The pTyr mimetics are depicted with green carbon atoms bound in the pTyr binding site depicted as a gray surface. Red spheres indicate water molecules: (a) structure of a DFMP inhibitor and water molecule O3 bound to the active site of PTP1B in structure 1N6W; (b) structure of a CMS inhibitor with water molecules in the O1 and O2 sites in the same reference frame, from structure 1G7G;[9] (c) overlay of the two structures, showing the genesis of the five-membered ring heterocycle; (d) designed IZD heterocycle **VII** overlaying the two original structures; (e) our designed IZD heterocycle **VII** alone.

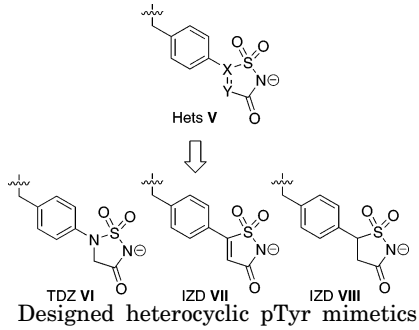
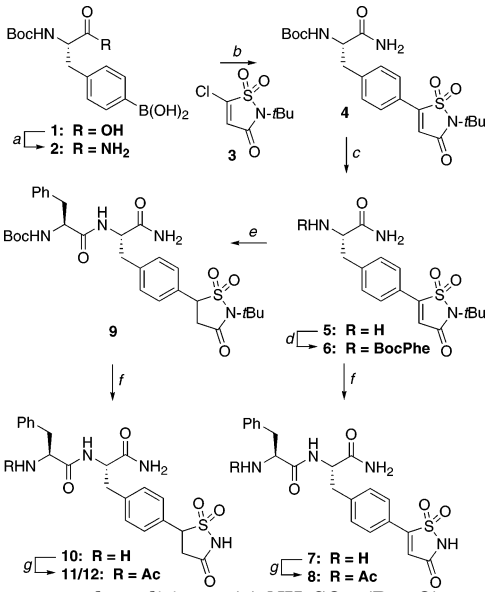


Figure 4. Designed heterocyclic pTyr mimetics shown in ionized form expected at physiological pH.

Scheme 1. Synthesis of Dipeptides Containing IZD Heterocycles[a]



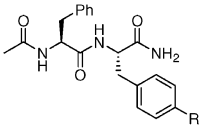
[a] Reagents and conditions: (a) NH_2CO_3, (Boc_2O), 97%; (b) **3**, $Pd(dppf)_2Cl_2DCM$, K_2CO_3, dioxane, 80 °C, 78%; (c) 4 M HCl/dioxane, 98%; (d) BocPhe, EDC, HOAt, DIEA, DMF/DCM, 84%; (e) H_2, Pd/C, MeOH, 96%; (f) TFA/dioxane, $(i\text{Pr})_3SiH$, **7** = 65%, **10** = 79%; (g) AcOH, EDC, HOAt, DIEA, DMF/DCM, **8** = 75%, **11/12** = 82%.

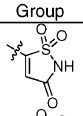
metics that inhibit PTP1B was published, albeit with only micromolar activity.[19]

The isothiazolidinone (IZD) heterocycles (**VII** and **VIII**) were chosen as our initial synthetic target because the IZD heterocycle allows the two sulfonyl oxygens to effectively mimic the oxygens of the DFMP inhibitor, while the carbonyl mimics the CMS inhibitor carbonyl and the ionized NH mimics the carboxylic anion or DFMP anion. We postulated that non-peptide-containing inhibitors containing the highly delocalized anion of the cyclic *N*-acylsulfonamide heterocycle containing inhibitors may provide improved membrane permeability compared to the hard charged phosphonates and carboxylate anion containing inhibitors, since the analogous acyclic acylsulfonamides are known cell-permeable isosteres for carboxylic acids.[20]

Peptides containing the IZD heterocyclic pTyr mimetics were synthesized in 10−11 linear steps from readily available starting materials (Scheme 1). The key synthetic reaction was a novel Suzuki coupling of chloroheterocycle **3**[21,22] with 4-phenylalaneboronic acid **2** to afford the fully protected scaffold **4**. The N-terminus of **4** was subsequently elaborated via peptide couplings and the heterocycle was deprotected to give inhibitor **8**. The heterocycle of common intermediate **9** was reduced, isomers were separated, and each was further elaborated to afford the diastereomers **11** and **12**.

Table 1. Dipeptide Containing IZD Inhibitors of PTP1B



Compd	R Acronym	R Group	PTP1B[a] IC_{50} (nM)
8	IZD		3000
11	(*R*)-IZD		16000
12	(*S*)-IZD		190 (180)[b]
13	DFMP		1750

[a] PNPP enzyme assay. [b] K_i.

Unsaturated IZD heterocycle containing **8** and saturated IZD heterocycle containing **11** and **12** were tested for their enzymatic inhibitor activity toward PTP1B. Gratifyingly, unsaturated IZD heterocyclic **8** and saturated IZD heterocyclic **12** were potent inhibitors of PTP1B with IC_{50} values of 3000 and 190 nM, respectively (Table 1). Mode of inhibition studies unequivocally established that both compounds were competitive and reversible inhibitors of PTP1B (**12**, K_i = 180 nM). Ab initio calculations predicted that the (*S*)-IZD would be the active isomer, based on comparison of calculated conformations of (*S*)- and (*R*)-IZD heterocycle fragments with the crystal structure of an inhibitor bearing the unsaturated IZD (see Supporting Information). Indeed, X-ray crystallography of the PTP1B/**12** complex revealed that the (*S*)-IZD isomer was active. Compound **12** was also tested against the phosphatases TCPTP, SHP-1, and SHP-2. As expected, **12** was shown to be an equipotent inhibitor (IC_{50} = 135 nM) of the highly homologous (74% identity) phosphatase TCPTP while maintaining complete selectivity (IC_{50} > 100 000 nM) over the more distantly related PTPs.

As a comparator to other pTyr mimetics, an identical dipeptide **13** containing a DFMP, arguably the most potent pTyr mimetic reported to date, was synthesized according to literature methods and assayed against PTP1B. The (*S*)-IZD heterocyclic pTyr mimetic containing inhibitor **12** demonstrated an impressive 10-fold more potent inhibition of PTP1B than the DFMP derivative **13**. As we predicted, the combined entropic gains from eliminating the third structural water molecule from the active site and the rigid conformation of the (*S*)-IZD heterocyclic pTyr mimetic gave an estimated 1.4 kcal/mol lowering in binding energy compared to the DFMP.

The crystal structure of PTP1B in complex with **12** was solved to 2.0 Å resolution and confirmed that the (*S*)-IZD heterocyclic pTyr mimetic binds to the active closed form of the catalytic pocket in precisely the in silico designed conformation (Figure 5). The (*S*)-IZD

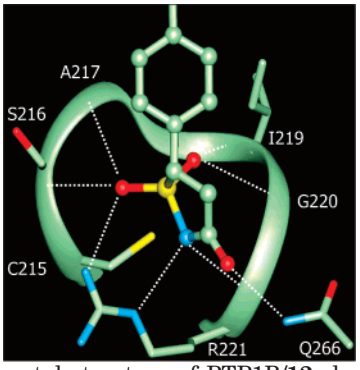


Figure 5. Crystal structure of PTP1B/**12** showing the (*S*)-IZD heterocyclic pTyr mimetic **VIII** bound to the phosphate binding loop of PTP1B. The protein is depicted in ribbon-and-stick style, and the inhibitor is depicted in ball-and-stick style. The (*S*)-IZD heterocycle makes all of the predicted polar contacts and displaces all three active site water molecules. Hydrogen bonds are indicated with dashed lines.

heterocycle binds at the center of the phosphate binding loop (Cys215−Arg221), where each of the three oxygen atoms of the (*S*)-IZD heterocycle displaces a structural water molecule and forms the expected hydrogen-bonding interactions with backbone amides and side chain of Arg221. The aryl ring directly attached to the (*S*)-IZD heterocycle participates in extensive van der Waals interactions with Tyr46, Val49, Ile219, and F182 of the flap. The peptidic portion of **12** binds in an extended conformation, identical to other known peptide-like structures bound to PTP1B (see Supporting Information for full view of inhibitor **12** bound to PTP1B).

Novel heterocyclic pTyr mimetics were designed through meticulous analysis and overlay of structural information available in the PDB. A dipeptide containing the (*S*)-IZD pTyr mimetic was synthesized and demonstrated competitive, reversible, and potent inhibition of PTP1B (**12**, K_i = 180 nM). An X-ray crystal structure of PTP1B/**12** showed that the ligand bound precisely as designed in silico. Furthermore, the (*S*)-IZD pTyr mimetic was found to be a 10-fold more potent inhibitor of PTP1B than an analogous peptidic compound bearing a DFMP. These data support the conclusion that the (*S*)-IZD heterocycle is the most potent pTyr mimetic discovered to date. While the peptidic inhibitors described herein are not cell-permeable or orally bioavailable, these diffusely anionic IZD heterocyclic pTyr mimetics provide new opportunities for the discovery of such PTP1B inhibitors when incorporated into suitable nonpeptidic scaffolds.

Acknowledgment. We thank Rakesh Kohli at the University of Pennsylvania for providing HRMS data.

Supporting Information Available: Synthesis procedures and analytical data for intermediates and final products (^{1}H NMR, ^{13}C NMR, HRMS, HPLC), experimental procedures for the biological assays, ab initio modeling, and crystallographic methods and data. This material is available free of charge via the Internet at http://pubs.acs.org.

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