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Recent Advances in the Discovery of Competitive Protein Tyrosine Phosphatase 1B Inhibitors for the Treatment of Diabetes, Obesity, and Cancer

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Introduction

Many eukaryote cellular functions are regulated by precise control of the phosphorylation levels of proteins involved in a variety of signal transduction processes. Protein tyrosine kinases (PTKs[a]) (enzymes that catalyze the phosphorylation of phosphotyrosine residues on proteins) and protein tyrosine phosphatases (PTPs) (enzymes that catalyze the dephosphorylation of phosphotyrosine residues on proteins) are the two key enzyme classes that, when functioning properly, work in concert to provide dynamic control of cellular responses to external stimuli. The tremendous diversity of kinases (> 500 members) and phosphatases (> 100 members) that have been identified in the human genome speaks to the essential role these proteins play in our everyday biochemical processes. The aberrant activity of individual kinases and phosphatases has been associated with the pathogenesis of a wide variety of acquired or inherited human diseases.[1]

The recent approval of several small molecule kinase inhibitors, including imatinib, gefitinib, sorafenib, sunitinib, erlotinib, nilotinib, lapatinib, and dasatinib, for the treatment of cancers has validated the approach of modulating the tyrosine phosphorylation levels of specific proteins to treat human disease. The pharmaceutical industry has invested heavily in the kinase field of research with over a thousand clinical trials involving kinase inhibitors ongoing. It has been estimated that nearly half of today's drug discovery efforts are focused on the identification of novel kinase inhibitors as drug candidates. Given the extraordinary pharmaceutical efforts to identify kinase inhibitors, one might find it surprising that phosphatases are currently undervalued as a therapeutic target class. In fact, no competitive small molecule protein tyrosine phosphatase inhibitors are currently reported in human clinical trials. Why is this so?

First, it is true that the field of phosphatase research compared to kinase research is still in its infancy. In 1988, PTP1B was the first protein tyrosine phosphatase (PTP) cloned and fully characterized and at this time it was believed that only a few nonspecific phosphatases existed in the human genome.[2,3] In contrast, kinases had been discovered decades earlier and were believed to play the dominant role in regulating the phosphorylation status of proteins involved in signal transduction processes. Over the next decade it was established that phosphatases also play an essential regulatory role in the intracellular phosphorylation state of proteins. The previously unrecognized number (107) and ample diversity of phosphatases in the human genome suggest there is substrate specificity and fine-tuned regulation of many cellular processes in concert with the kinases.[4]

Second, there was a lack of strong biological validation for any phosphatase as a drug target until nearly a decade later. Growing biochemical evidence was accumulating for the involvement of the prototypical phosphatase, PTP1B, in the intracellular dephosphorylation of the insulin receptor and insulin receptor proteins and thus its involvement in the down-regulation of insulin signaling.[5,6] It was not until 1999 and 2000 that two independent research teams disclosed that PTP1B knockout mice displayed a phenotype strongly suggestive of a role in insulin and leptin signaling.[7,8] The PTP1B null mice showed enhanced insulin sensitivity, lower plasma glucose and insulin levels, and resistance to weight gain compared to control mice when fed high fat diets. The PTP1B deficient mice also have normal development and longevity which is in stark contrast to the typical adverse immune and neuronal development effects observed with most other phosphatase KO mice. For instance, T-cell protein tyrosine phosphatase (TC-PTP) is the most homologous phosphatase to PTP1B with 74% sequence identity in the catalytic domain and identical active sites, but the TC-PTP KO mice die at 3−5 weeks of age because of impaired B cell and T cell functions.[9] Further biochemical validation of PTP1B as a therapeutic target for diabetes and obesity has been from a variety of sources, including antisense oligionucleotide studies, overexpression in vitro, human single nucleotide polymorphisms, and observations of mutations within the human PTP1B gene sequence.[10,11] On the basis of these data, PTP1B is currently considered one of the best validated biological targets for non-insulin dependent diabetes and obesity. In addition, several groups have established a role for PTP1B in cancer.[12−14] For example, Tremblay has demonstrated that overexpression of PTP1B is sufficient to drive tumorigenesis in mice, providing additional support for the use of PTP1B inhibitors for cancer therapy.[15,16] Merck Frosst has provided further support for this hypothesis

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[a] Abbreviations: PTK, protein tyrosine kinase; PTP, protein tyrosine phosphatase; TC-PTP, T-cell protein tyrosine phosphatase; NR-PTP, nonreceptor protein tyrosine phosphatase; HTS, high throughput screening; pTyr, phosphotyrosine; SBDD, structure-based drug design; DFMP, difluoromethylene phosphonate; oGTT, oral glucose tolerance test; DIO, diet-induced obese; OMT, *O*-malonyltyrosine; OBA, 2-oxalylaminobenzoic acid; 2-DOG, 2-deoxy-D-glucose; TDZ, 1,2,5-thiadiazolidin-3-one 1,1-dioxide; (*S*)-IZD, (*S*)-isothiazolidin-3-one 1,1-dioxide; CMS, carboxymethyl salicylate; DFMS, difluoromethylenesulfonic acid.

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by demonstrating an anticancer effect in NDL2 *Ptpn1* transgenic mice with a small molecule inhibitor, **5**, of PTP1B.[17]

So why have we seen a dearth of small molecule PTP1B inhibitors entering clinical trials from the pharmaceutical industry? Simply put, the discovery of cell permeable, orally bioavailable, small molecule phosphatase inhibitors has been a much more difficult endeavor for medicinal chemists than the discovery of clinically viable kinase inhibitors. This is largely due to the highly polar phosphatase active site and the relatively shallow nature of the surrounding protein surface which does not allow for productive binding of typical lipophilic membrane permeable inhibitors. Significant medicinal chemistry efforts since the late 1990s by nearly every major pharmaceutical company have afforded a paucity of cell permeable inhibitors, and fewer still yet have had sufficient potency to demonstrate in vivo activity. These early findings led many to believe that phosphatases are intractable drug targets for small molecule drug discovery programs due to the fundamental nature of the phosphatases' active site. Nevertheless, a few research groups have continued the search for PTP1B clinical candidates. Recent structure-based design and fragment-based approaches aided by crystallographic and protein−ligand NMR data have led to exciting advances in the discovery of novel pTyr mimetics with more druglike structures. While there still remains significant progress yet to be made, these new pharmacophores have provided renewed hope that orally bioavailable small molecule PTP inhibitors suitable for human clinical trials are an obtainable, yet formidable, objective.

This Perspective highlights the most recent medicinal chemistry advances in the design and optimization of new chemical classes of ligand competitive inhibitors of PTP1B. Particular emphasis was given to potent competitive enzyme inhibitors with demonstrated cellular and/or in vivo activity reported since the Perspective written by Hooft van Huijsduijnen in 2004.[18] Noncompetitive inhibitors such as those that function via oxidation of the catalytic cysteine, Cys215, or allosteric inhibitors have been reviewed elsewhere[19] and are not discussed in this Perspective.

PTP1B Classification and Biochemistry

Completion of the sequence of the human genome in 2001 led to the identification of 107 human PTP genes that code for 81 catalytically active PTPs. The "classical PTPome" comprises the 61 dual specificity phosphatases and 38 PTPs. The PTPs are a diverse class of enzymes that have been further subdivided into 17 subfamilies based on their degree of homology between functional domains and architectural domains. PTP1B is a member of the class I cysteine-based PTPs in the classical nonreceptor PTP (NR-PTP) subfamily. The native protein consists of 435 amino acid residues. Residues 30−278 correspond to the catalytic domain, while the 35 C-terminal residues target the enzyme to the cytosolic face of the endoplasmic reticulum. It is here that PTP1B catalyzes dephosphorylation of the insulin receptor and insulin receptor substrates involved in insulin signaling and Jak2 involved in leptin signaling. Consequently, PTP1B acts to negatively regulate the actions of the metabolic hormones insulin and leptin.

The general catalytic mechanism for dephosphorylation of substrate proteins by PTPs has been delineated in detail. Recognition of the substrate peptide sequence by PTP1B and binding of the phosphotyrosine deep in the catalytic site of the phosphate-binding loop (P-loop) are mediated by residues 214−221 (His, Cys, Ser, Ala, Gly, Ile, Gly, Arg). The WPD (tryptophan, proline, aspartic acid) loop closes down onto the substrate and thereby positions the thiolate of Cys215 for nucleophilic attack upon the phoshotyrosine. Asp181 acts as a general acid catalyst. The phosphate is cleaved from the phosphotryosine residue, allowing the dephoshorylated substrate to diffuse from the active site and water to replace it. General base catalyzed hydrolysis of the resultant phosphocysteine by Asp181 regenerates the active form of the phosphatase and completes the catalytic cycle.

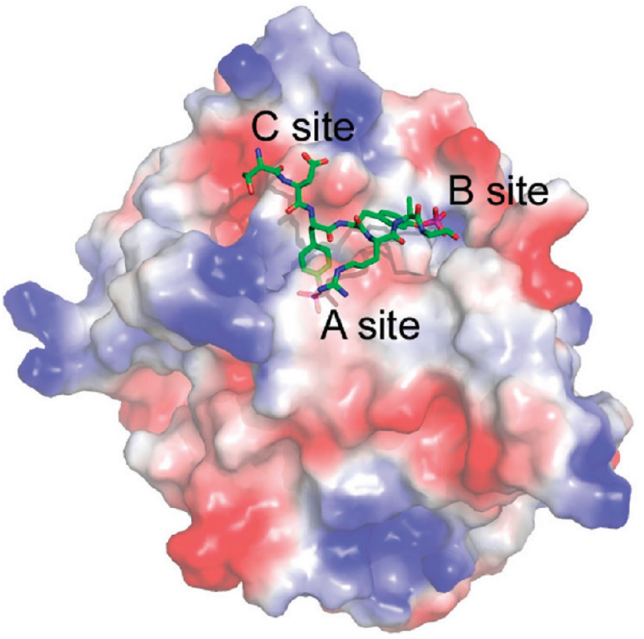


Figure 1. X-ray structure of an insulin fragment/PTP1B.

In 1994, the first crystal structure of PTP1B was disclosed (Figure 1).[20] Recently, Barr published a compendium of 22 new phosphatase X-ray crystal structures comprising at least one member of every PTP subgroup.[21] The wealth of high resolution structural data across the entire classical protein tyrosine phosphatase genome (PTPome) within this article will undoubtedly find utility by chemical biologists and medicinal chemists seeking potent and selective phosphatase inhibitors as biological probes and drug candidates.

Chemical Classes of PTP1B Inhibitors

The discovery of novel pharmacophores and chemotypes for the inhibition of PTP1B has been a difficult task for both biologists screening compound libraries and medicinal chemists designing novel phosphotyrosine mimetics and scaffolds. High throughput screening (HTS) of large corporate collections has uncovered only a handful of novel competitive inhibitors. A large number of false positives have plagued screening efforts because of the sensitivity of PTP1B toward nonspecific inhibition by extremely hydrophobic compounds and oxidation of the catalytic Cys215 residue.[22] The scarcity of lead structures from HTS has required medicinal chemists to design isosteric pTyr mimetics based on the natural phosphopeptide substrates and available PTP1B crystal structures. The pTyr moiety was initially targeted, since it binds deep into the phosphatase active site and is responsible for the majority of the binding energy to PTP1B. The success of these early

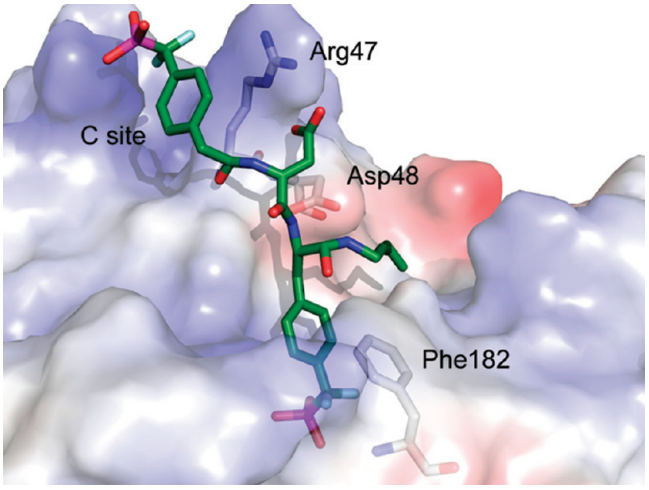


Figure 2. X-ray structure of Zhang's bis-DFMP inhibitor **1**/PTP1B.

structure-based drug design (SBDD) programs led to the identification of a small collection of nonhydrolyzable phosphonate and carboxylic acid pTyr mimetics. Numerous X-ray cocrystal structures of inhibitors bearing these pTyr mimetics bound to PTP1B provided tools necessary for subsequent SBDD and fragment based methods to derive other novel heterocyclic pTyr mimetics. An overview is presented of the most potent chemical structural classes associated with each class of pTyr mimetic.

Bioisosteric Nonhydrolyzable DFMP pTyr Mimetics

The first major medicinal chemistry breakthrough in phosphatase inhibitor design was the discovery of the isosteric difluoromethylene phosphonate (DFMP) pTyr mimetic by Burke et al. in 1994.[23] Original substrate-based peptides substituted with the noncleavable DFMP pTyr mimetic afforded potent inhibition of PTP1B but were nonselective over other phosphatases and devoid of cellular activity. In 1997, an additional noncatalytic aryl phosphate binding site, the so-called B-site, was identified in relatively close proximity to the catalytic phosphate-binding site. Zhang postulated that dual-binding inhibitors that accessed both the active site and B-site would significantly improve the potency of inhibitors due to additive effects. In addition, the B-site of PTP1B differs from TC-PTP by several amino acids and thus offers the opportunity for improved selectivity between these two highly homologous phosphatases for inhibitors that bind in this site. Subsequently, Zhang derived a potent peptide ligand **1** containing a second DFMP group that was determined by cocrystallography not to bind at the B-site but instead interacted with Arg47 in the so-called C-site of PTP1B (Figure 2).[24] Compound **1** demonstrated dramatically improved enzyme potency (PTP1b K_i = 2.4 nM) and specificity over the highly homologous TC-PTP (10-fold selectivity). Unfortunately, the highly polar nature of the bis-anionic DFMPs and peptide scaffold provided compounds with poor physiochemical properties and thus poor cell permeability and low oral bioavailability.

The development of non-peptide scaffolds incorporating the DFMP group has been pursued by several pharmaceutical companies. Merck Frosst's efforts have led to promising PTP1B inhibitor classes: arylketone **3**, benzotriazole **4**, and naphthyl **5** (Figure 3).[17,25,26] The improved potency (IC_{50} = 5 nM) and selectivity (7-fold) of the dual binding bis-DFMP containing benzotriazole **4** compared to initially nonselective mono-DFMP leads **3** (IC_{50} = 120 nM) and **5** (IC_{50} = 120 nM) were designed on the basis of crystallographic data to bind both the catalytic site and B-site. Selectivity for PTP1B over TC-PTP was achieved by taking advantage of the amino acid differences in the B-site (Phe52 and Ala27 are Tyr and Ser, respectively). The proposed binding mode was confirmed by an X-ray cocrystal structure of compound **4** complexed with PTP1B (Figure 4).[27] Each of Merck Frosst's three inhibitor classes demonstrated a modest degree of cell permeability and oral bioavailability in rodents, but the bis-anionic DFMP has demonstrated less desirable pharmacokinetics in higher species. Compounds **3** and **5** demonstrated an antidiabetic effect when tested in an oral glucose tolerance test (oGTT) in diet-induced obese (DIO) mice. Compound **5** also demonstrated an anticancer effect in NDL2 *Ptpn1* transgenic mice when dosed at 30 mg/kg for 21 days. The median tumor-free days (T_{50}) was extended from 28 to 75 days, and the authors also noted that blood glucose levels were lowered to levels comparable to those of control animals. These data demonstrate the antidiabetic activity of the DFMP class of PTP1B inhibitors and provided further validation that pharmacological inhibition of PTP1B has potential as a treatment for diabetes and cancer.

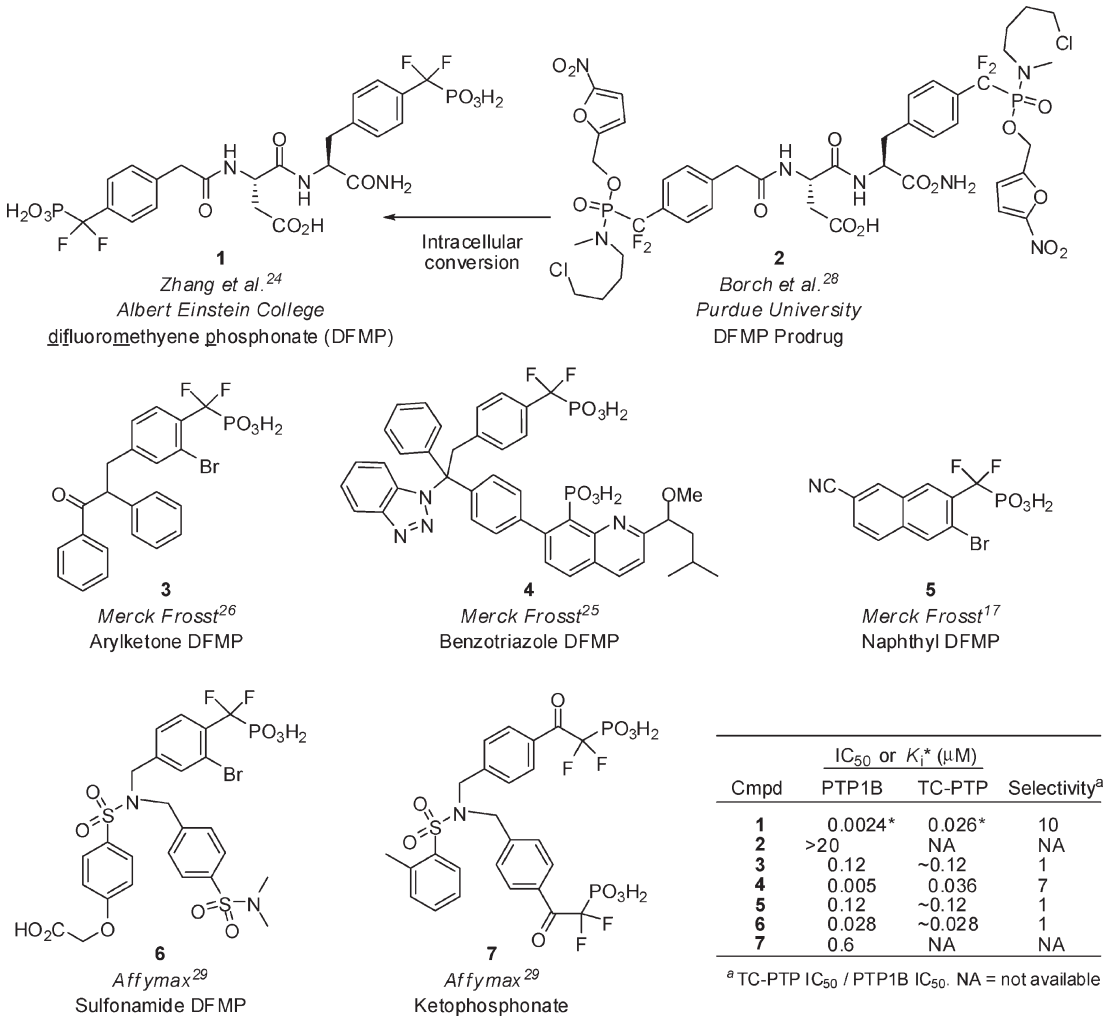
A prodrug approach was recently reported by Borch et al.[28] to improve the permeability of the DFMP containing peptide-based inhibitor **1** originally discovered by Zhang et al.[24] The phosphonamidate prodrug **2** was inactive in the PTP1B enzyme assay as expected but exhibited potent activity in a human hepatoma HepG2 cell-based assay presumably via intracellular delivery of phosphonate **1**. This prodrug strategy has been proposed as a general solution to the intracellular delivery of organophosphonate-based PTP inhibitors.

Affymax has identified a sulfonamide scaffold bearing the DFMP **6** (IC_{50} = 28 nM) and a novel ketophosphonate pTyr mimetic **7** (IC_{50} = 600 nM) as PTP1B inhibitors.[29] Compound **6** is nonselective over TC-PTP, while selectivity of compound **7** has not been reported.

Carboxylic Acid pTyr Mimetics

Replacement of the phosphorus-based mimetics with carboxylate-based pTyr mimetic was sought to improve the membrane permeability of inhibitors bearing these pharmacophores (Figure 5). Dicarboxylic acid-containing *O*-malonyltyrosine (OMT)[30] and *o*-carboxy-(*O*-carboxymethyl)-tyrosine[31] peptide derivatives were the first carboxylic acid containing pTyr mimetics published. Since these early findings, several research groups have made significant advances in peptide and non-peptide carboxylic acid derivatives as potent PTP1B inhibitors. Recent efforts have focused on monocarboxylic acid containing pharmacophores that offer the potential for improved membrane permeability. Overall, these efforts have resulted in the identification of several novel scaffolds with good to excellent enzyme potency (5−1000 nM) and modest selectivity over TC-PTP (< 20), but poor cellular activity (10−100 μM). A lack of membrane permeability remains an issue for this class of compounds.

The Abbott research group used a fragment-based NMR approach to identify novel PTP1B inhibitors. They identified a salicylic acid fragment bound in the B-site of PTP1B, and that moiety could be linked to several scaffolds bearing carboxylic acid pTyr mimetics providing inhibitors with improved enzyme potency and selectivity over TC-PTP.[32,33]



Cmpd	IC_{50} or K_i* (μM) PTP1B	TC-PTP	Selectivity[a]
1	0.0024*	0.026*	10
2	>20	NA	NA
3	0.12	~0.12	1
4	0.005	0.036	7
5	0.12	~0.12	1
6	0.028	~0.028	1
7	0.6	NA	NA

[a] TC-PTP IC_{50} / PTP1B IC_{50}. NA = not available

Figure 3. DFMP containing inhibitors of PTP1B.

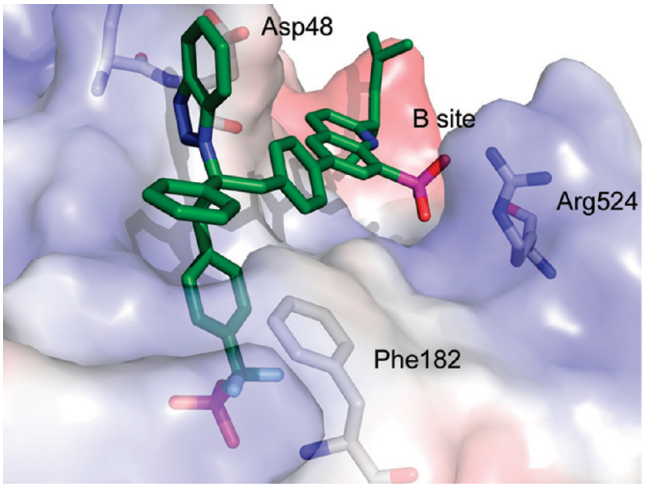
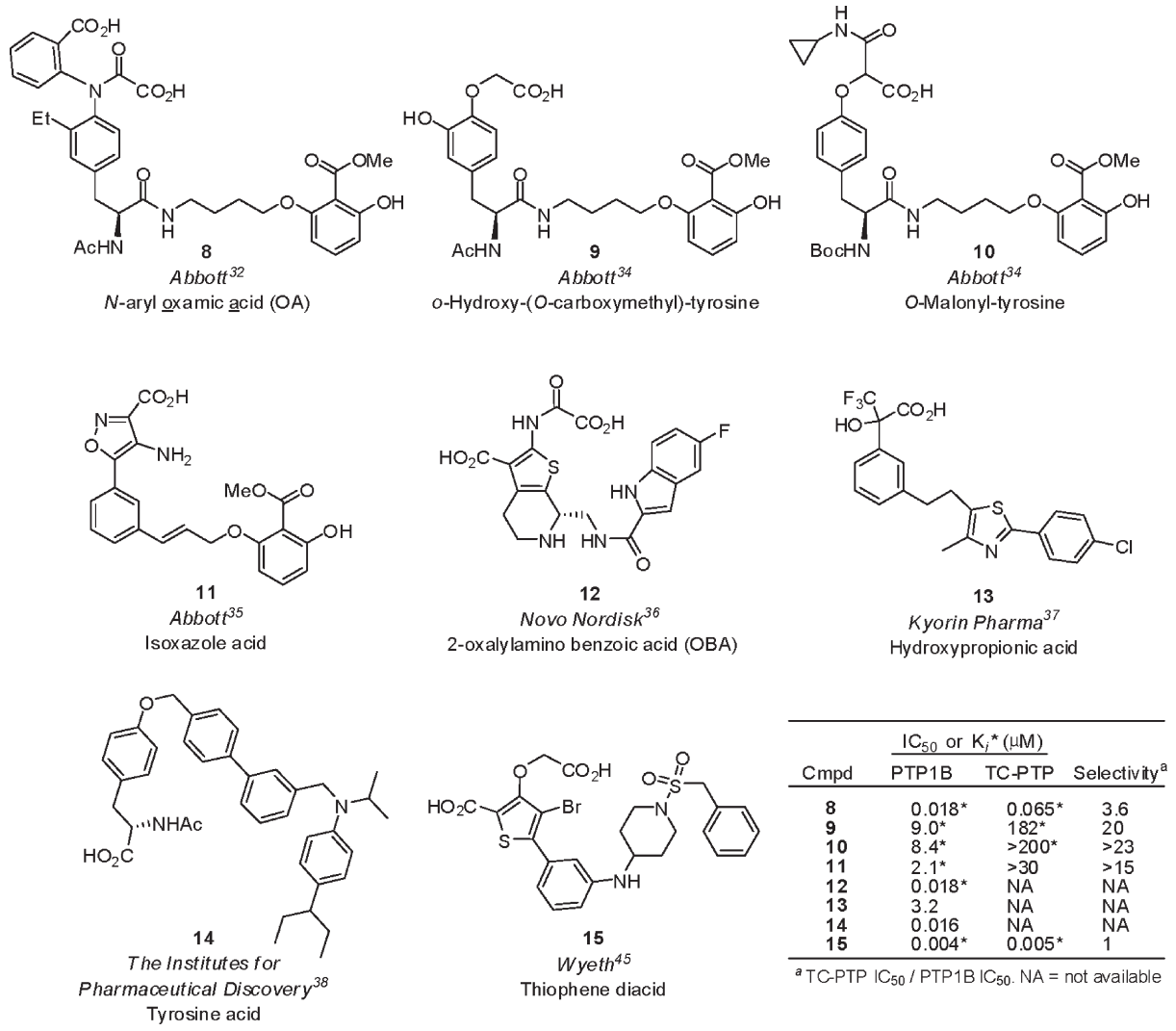


Figure 4. X-ray structure of Merck Frosst's benzotriazole inhibitor **4**/PTP1B.

The 2-oxalylarylaminobenzoic acid derivative **8** demonstrated enhanced potency (IC_{50} = 18 nM) and TC-PTP selectivity (3.6-fold). The X-ray crystal structure of PTP1B/**8** complex revealed a novel binding mode where the *N*-aryl substituent interacts with the WPD loop in an "open" or inactive conformation of the enzyme. Binding to the "closed" or active conformation of the WPD loop is observed for most other small molecule PTP1B structural classes. The diacid class of inhibitors displayed reduced membrane penetration and cellular activity presumably due to their bis-anionic nature.

Replacement of the oxamic acid moiety with monoacids **9** and **10** provided micromolar inhibitors of PTP1B with greater than 20-fold selectivity over TC-PTP.[34] An X-ray cocrystal structure with compound **9** revealed that the monoacid binds PTP1B in the closed conformation with the salicylic acid moiety projecting into the B-site (Figure 6). Interestingly, compound **9** also demonstrated high levels of caco-2 permeability ($> 10 \times 10^{-6}$ cm/s), presumably due to intramolecular aryl-γ-lactone formation of the carboxylic acid and adjacent phenol. The cellular data for this compound were not reported. Subsequently, the Abbott group identified a novel isoxazole carboxylic acid, **11**, as a micromolar inhibitor ($K_i = 2.1\ \mu$M) with good selectivity (>15 fold).[35] Compound **11** demonstrated a dose dependent attenuation of STAT3 phosphorylation levels in a COS 7 cell assay, albeit at high concentrations (10−100 μM).

The 2-oxalylaminobenzoic acid (OBA) containing pTyr mimetic was discovered by researchers at Novo Nordisk. Optimization of a thiophene-based scaffold bearing the OBA provided compound **12** as a potent PTP1B inhibitor.[36] These diacid compounds lacked cellular activity suggested by low permeability in the caco-2 assay ($< 1 \times 10^{-6}$ cm/s).



Cmpd	IC_{50} or K_i* (μM) PTP1B	TC-PTP	Selectivity[a]
8	0.018*	0.065*	3.6
9	9.0*	182*	20
10	8.4*	>200*	>23
11	2.1*	>30	>15
12	0.018*	NA	NA
13	3.2	NA	NA
14	0.016	NA	NA
15	0.004*	0.005*	1

[a] TC-PTP IC_{50} / PTP1B IC_{50}. NA = not available

Figure 5. Carboxylic acid containing inhibitors of PTP1B.

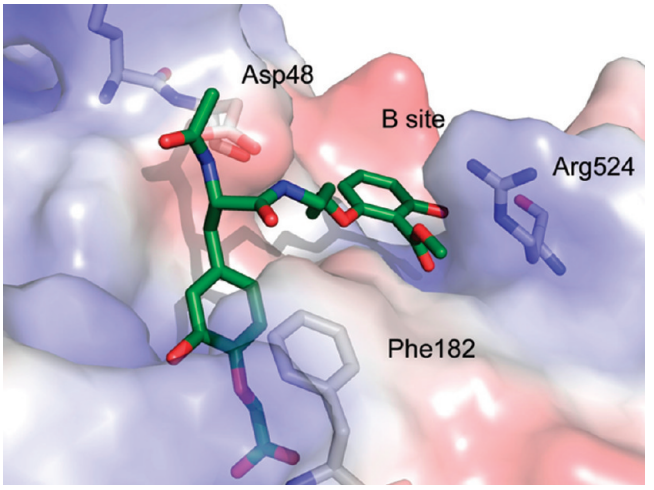


Figure 6. X-ray structure of Abbott's monocarboxylic acid inhibitor **9**/PTP1B.

Interestingly, the diethyl ester prodrug of **12** showed augmentation of insulin-stimulated 2-deoxy-D-glucose (2-DOG) uptake in C2C12 cells at 70% of maximum insulin response when dosed at 100 μM. Attempts to improve the membrane permeability of the OBA series by eliminating either one of the carboxylic acid groups resulted in nearly complete loss of enzyme potency.

Kyorin Pharmaceuticals has reported inhibitors bearing a novel hydroxypropionic acid pTyr mimetic **13** with micromolar potency.[37] The Institutes for Pharmaceutical Discovery published five patent application that disclosed monoacid containing PTP1B inhibitors, such as **14** (IC_{50} = 16 nM).[38−44] While these compounds are potent enzyme inhibitors of PTP1B, it is not clear whether they are competitive inhibitors or nonspecific promiscuous inhibitors due to their hydrophobic nature. As noted earlier, several screening groups have reported that PTP1B is particularly sensitive to nonspecific inhibition by extremely hydrophobic compounds which result in the identification of many false positives. The high molecular weight and hydrophobicity of compound **14** (MW = 617 and clogP = 9.6) renders this compound distinctly outside the desired range of druglike properties.

The Wyeth group identified a dicarboxylic acid containing thiophene scaffold that after extensive optimization led to the potent inhibitor **15** (K_i = 5 nM).[45] Unfortunately, **15** lacked cellular activity, so an effort was made to eliminate or replace one or more of the carboxylic acid substituents with a variety of functional groups, including tetrazole. All derivatives suffered a loss of several orders of magnitude in potency except for the 1,2,5-thiadiazolidin-3-one 1,1-dioxide (TDZ)

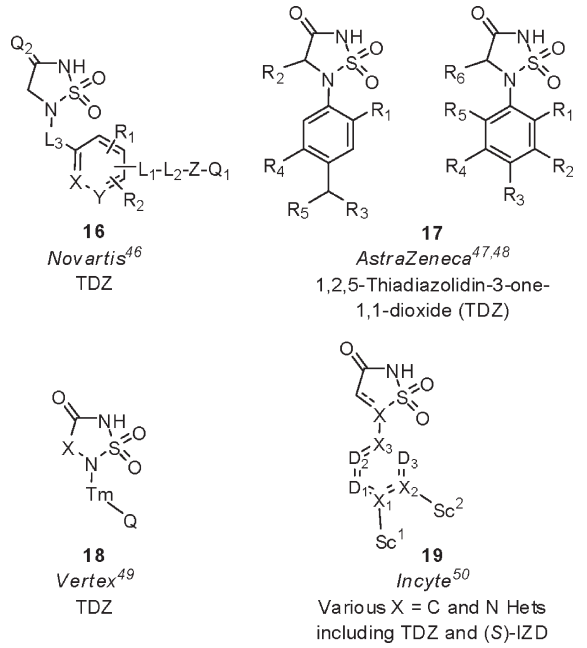
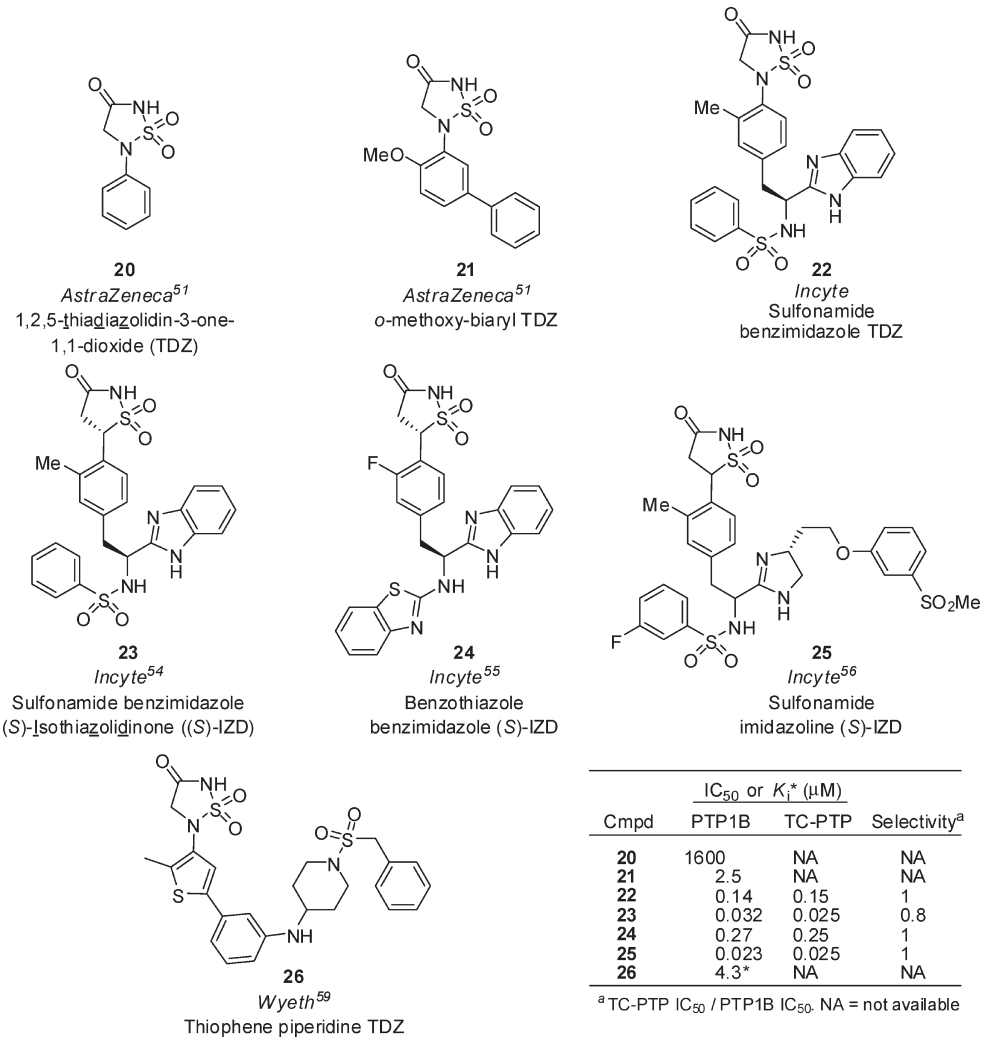


Figure 7. Markush structures from 2003 to 2005 patent applications claiming the heterocyclic pTyr mimetics.

pTyr heterocyclic **26** replacement for the phenoxyacetic acid. The TDZ pTyr mimetic was originally reported by an AstraZeneca group, and the details of this discovery will be discussed in the following section.

Structure-Based Design of Heterocyclic pTyr Mimetics

The lack of adequate membrane permeability with the bis-anionic DFMP and dicarboxylic acid containing inhibitors as well as the poor inhibition observed with monoacid containing inhibitors provided impetus to search for novel pTyr mimetics that exhibit potent enzyme inhibition with improved physicochemical properties. While an uncharged pTyr mimetic might be considered ideal, the highly cationic active site on the enzyme appears to prefer binding ligands with at least a monoanionic charge. Therefore, efforts to improve cell permeability and oral bioavailability have focused on reducing the acidity of the pTry mimetic (approaching neutral pK_a) and/or replacing the pTyr mimetic with a diffusely anionic heterocycle, such as the well-known carboxylic acid isostere tetrazole. Independent discoveries by four pharmaceutical companies, Novartis **16**, AstraZeneca **17**, Vertex **18**, and Incyte **19**, of closely related five-membered heterocyclic pTyr mimetics speak to the convergent evolution of medicinal chemistry toward this novel phosphatase pharmacophore and



	IC_{50} or K_i* (μM)		
Cmpd	PTP1B	TC-PTP	Selectivity[a]
20	1600	NA	NA
21	2.5	NA	NA
22	0.14	0.15	1
23	0.032	0.025	0.8
24	0.27	0.25	1
25	0.023	0.025	1
26	4.3*	NA	NA

[a] TC-PTP IC_{50} / PTP1B IC_{50}. NA = not available

Figure 8. PTP1B inhibitors with the TDZ and IZD groups.

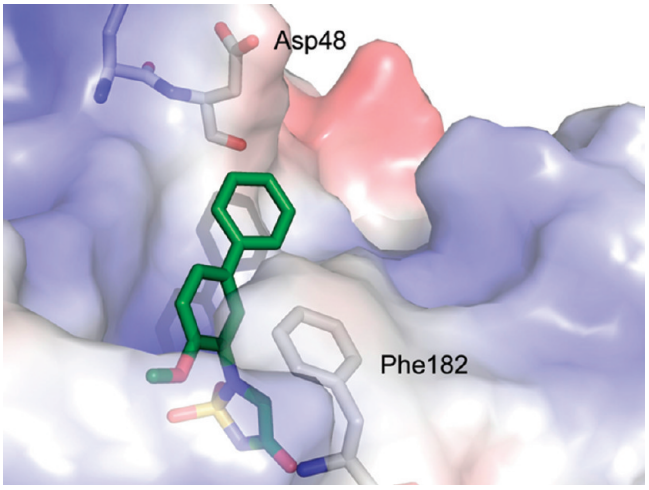


Figure 9. X-ray structure of AstraZenneca's TDZ inhibitor **21**/PTP1B.

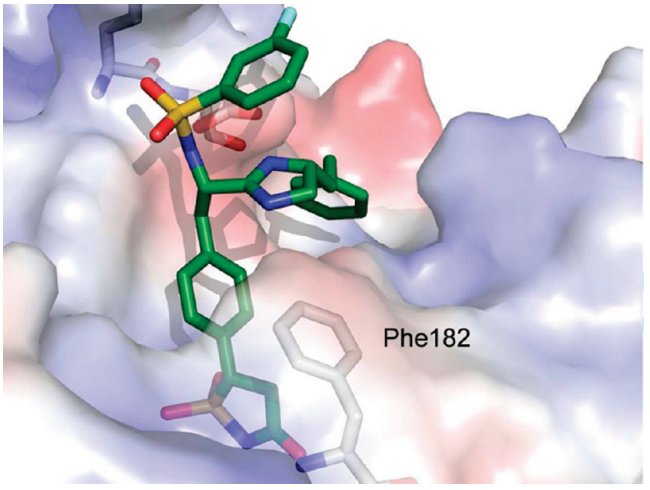


Figure 10. X-ray structure of Incyte's (*S*)-IZD inhibitor/PTP1B processes.

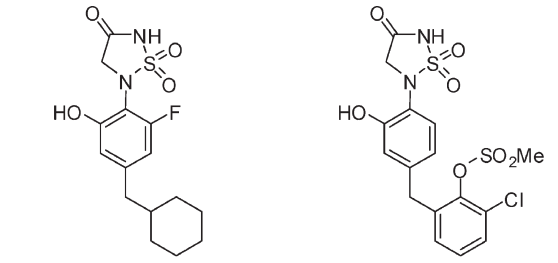
confirm its utility (Figure 7).[46−50] These heterocycles provide potent PTP1B inhibitors, occupy the entire catalytic site in a highly complementary manner, and improve membrane permeability.

In 2005, Black et al. published AstraZeneca group's discovery of the 1,2,5-thiadiazolidin-3-one 1,1-dioxide (TDZ) **20** (IC_{50} = 1.6 mM) identified by using a fragment-based NMR screening approach of pTyr mimetics designed with the aid of PTP1B crystallographic data (Figure 8).[51] A crystal structure of a compound bearing the TDZ in complex with PTP1B revealed that the TDZ occupies the active site in the expected conformation. Ortho substitution on the aryl ring was predicted by quantum mechanical models to force the TZD group to twist out of plane of the aryl ring and produce a low energy orthogonal conformation similar to that observed in the cocrystal structure of PTP1B/**20**. Design of new ligands based on this hypothesis led to the identification of the *o*-methoxylbiphenyl analogue **21** and a cocrystal structure (Figure 9). The *o*-methoxy and 5-aryl substituents each contributed an order of magnitude increase in potency for the scaffold providing nearly a 100-fold improvement in activity (IC_{50} = 2.5 μM). Subsequent medicinal chemistry efforts have not been published, though lectures by AstraZeneca have been presented describing TDZ-based compounds as nanomolar inhibitors of PTP1B. Novartis and Vertex have also not yet published their TDZ research; limited biological data are available from their published patent applications.

Later in 2005, Incyte published a unique structure-based design of five-membered heterocyclic pTyr mimetics, which included the TDZ group and the novel unsaturated isothiazolidin-3(2*H*)-one 1,1-dioxide and saturated isothiazolidin-3-one 1,1-dioxide (IZD) pTyr mimetics.[52] Close inspection of literature crystal structures of carboxymethyl salicylate (CMS) ligands and DFMP ligands bound to PTP1B revealed that these two pTyr mimetics occupy unique, yet complementary, space in the active site of PTP1B. The carbonyl oxygens of the CMS ligand displace a single conserved water molecule, while the phosphonate oxygens of the DFMP ligand displace two different conserved water molecules deep in the active site. Overlay of the two pTyr mimetics demonstrated that a five-membered heterocycle, such as a TDZ or IZD, could effectively occupy the entire active site and displace all three conserved water molecules. Incyte has published that peptides bearing the (*S*)-isomer of the isothiazolidinone ((*S*)-IZD) could inhibit PTP1B approximately 10-fold more potently than the analogous peptide TDZ derivative.[53] We disclose herein that sulfonamidebenzimidazoles bearing the IZD group **23** (IC_{50} = 32 nM) are approximately 5-fold more potent than the analogous TDZ derivatives, such as **22** (IC_{50} = 140 nM), further substantiating these claims. These (*S*)-IZD derivatives were also shown to be 10-fold more potent than the corresponding DFMP analogues and thus are purported to be the most potent pTyr mimetic known to date.[52] In addition, the sulfonamide **23** demonstrates modest caco-2 permeability (0.4×10^{-6} cm/s) and only the (*S*)-IZD diastereomer of **23**, not the (*R*)-IZD diastereomer, displayed dose dependent activity in a cellular insulin receptor (IR) phosphorylation assay plus a cellular assay measuring Akt phospohorylation at 10−80 μM.[54] Incyte has since published several articles describing the optimization of unique scaffolds bearing the (*S*)-IZD by structure-based design methods. Benzothiazolebenzimidazole **24** exhibited weaker potency (IC_{50} = 270 nM) but improved caco-2 permeability (1.1×10^{-6} cm/s) presumably due to the lower polar surface area of the scaffold.[55] Sulfonamideimidazoles and sulfonamideimidazolines were designed to access the B-site and improve potency and selectivity over TC-PTP.[56] Cocrystal structures of several compounds in this series demonstrate that these inhibitors bind in the B-site as predicted (Figure 10).[57,58] Sulfonamideimidazoline **25** was synthesized as a mixture of four diastereomers and demonstrated excellent potency (IC_{50} = 23 nM), albeit without selectivity.

As mentioned in the previous section, Wyeth has published a comparison of thiophene-based inhibitors bearing their original dicarboxylic acid **15**, a monocarboxylic acid, and a TDZ **26**.[59] While the diacids were consistently the most potent, the overall permeability of the series, based on PAMPA data, showed the following trend: TDZ > monoacid > diacid. These data further support the hypothesis that compounds bearing the diffusely anionic heterocyclic pTyr mimetics will have improved membrane permeability compared to compounds bearing carboxylic acids or phosphonates.

The heterocyclic TDZ and IZD pTyr mimetics have been explored by research groups from Novartis, The Institutes for Pharmaceutical Discovery, and TransTech Pharmaceuticals (Figure 11). Novartis published a series of seven patent applications covering a variety of scaffolds (**27**, **28**, **29**, and **30**) bearing an *o*-hydroxyaryl TDZ.[60−66] Biological data for only

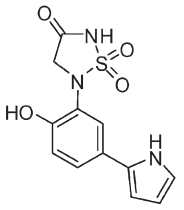


27
Novartis[63]
2-Hydroxy, 6-fluoroaryl TDZ

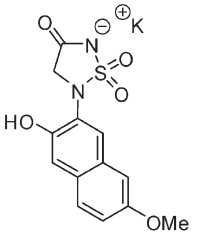
28
Novartis[61]
o-Hydroxyaryl TDZ

Cmpd	IC_{50} (μM) PTP1B	TC-PTP
27	0.104	NA
28	0.126	
29	0.080	
30	< 0.300	
31	0.130	
32	NA	
33	NA	

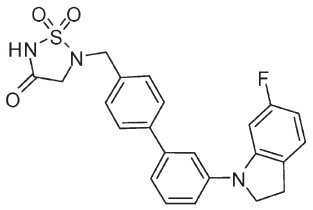
NA = not available



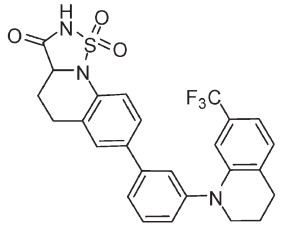
29
Novartis[62]
o-Hydroxyaryl TDZ



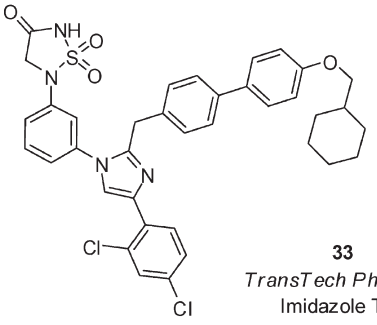
30
Novartis[65]
o-Hydroxyaryl TDZ



31
The Institutes for Pharmaceutical Discovery[38]
Biarylmethylene TDZ



32
The Institutes for Pharmaceutical Discovery[38]
Fused tricyclic TDZ



33
TransTech Pharma[68]
Imidazole TDZ

Figure 11. TDZ containing inhibitors of PTP1B disclosed in recent patent applications.

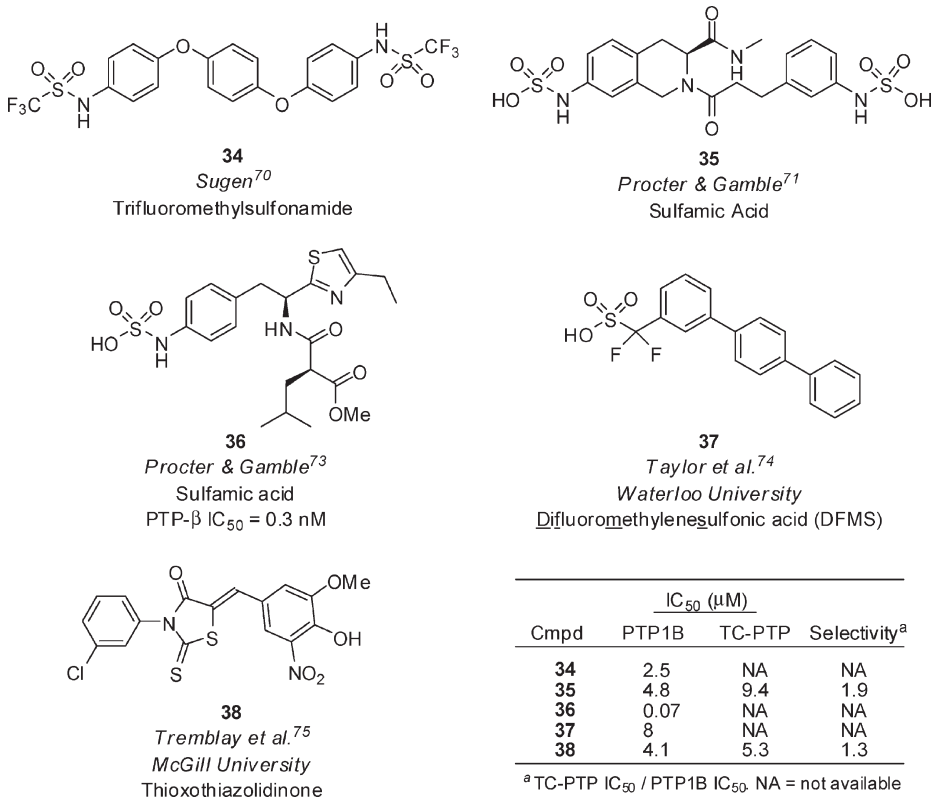
two compounds from each patent provide limited SAR but demonstrates that the *o*-hydroxy substituted TDZs are exceptionally potent inhibitors of PTP1B (IC_{50} = 80−126 nM) for their relatively low molecular weight (MW = 293−446). For instance, compound **29** (MW = 293; IC_{50} = 80 nM) has a good ligand efficiency of 0.36, while AstraZeneca's *o*-methoxy TDZ **20** (MW = 318; IC_{50} = 2500 nM) of similar molecular size has a ligand efficiency of only 0.26. Novartis's most recent patent application presents only four compounds, of which inhibitor **30** is best characterized.[65] Novartis is clearly interested in this compound and likely has chosen it for advanced studies toward possible clinical candidate selection.

The Institutes for Pharmaceutical Discovery and TransTech disclosed three new scaffolds bearing the TDZ, exemplified by the biarylmethylene bridged aryl-TDZ **31** (IC_{50} = 130 nM), the biaryl bicyclic-TDZ **32**, and the 3-imidazolearyl-TDZ **33**.[38,67−69] These unique scaffolds provide new opportunities and potential solutions to optimizing the efficiency of binding and inhibiting PTP1B while maintaining the necessary physiochemical properties to achieve cell permeability and oral bioavailability.

Various Other Novel pTyr Mimetics and Chemotypes

Over the past decade, many additional novel pTyr mimetics and unique structural classes of competitive PTP1B inhibitors have been reported. A subset of these is briefly highlighted below (Figure 12). The aryltrifluoromethylsulfone and aryltrifluoromethylsulfonamide pTyr mimetics were discovered by de novo fragment-based searching performed in the program LUDI.[70] Most notably, these compounds were the first uncharged competitive inhibitors reported. Compound **34** is a micromolar inhibitor of PTP1B but also inhibits a variety of other phosphatases. The lack of a cocrystal structure of this class of inhibitors bound to PTP1B and the broad nonselective nature of these ligands make one question whether or not these are viable phosphatase inhibitor leads. Proctor and Gamble (P&G) has reported that sulfamic acids, such as **35**, are also competitive inhibitors of several phosphatases.[71] The arylsulfamic acids were initially identified by high-throughput screening of the P&G corporate collection, and subsequent cocrystal structures allowed for the structure-based design of more potent inhibitors.[72] Remarkably, a recent P&G patent application discloses that incorporation of the arylsulfamic acid pTyr mimetic can result in extremely potent small molecule inhibitors of PTPβ, such as **36** (PTPβ IC_{50} = 0.3 nM).[73] Cellular data were not reported for these sulfamic acids which would give insights into their membrane permeability.

Tayor et al. designed and synthesized the analogous sulfonic acid, difluoromethylenesulfonic acid (DFMS), to the well-known DFMP phosphonate pTyr mimetic.[74] Compounds



	IC_{50} (μM)		
Cmpd	PTP1B	TC-PTP	Selectivity[a]
34	2.5	NA	NA
35	4.8	9.4	1.9
36	0.07	NA	NA
37	8	NA	NA
38	4.1	5.3	1.3

[a] TC-PTP IC_{50} / PTP1B IC_{50}. NA = not available

Figure 12. Most recent novel pTyr mimetics and chemotypes as PTP1B inhibitors.

possessing the DFMS, such as **37**, have been shown to be several orders of magnitude less potent than similar DFMP containing derivatives. Most recently, Tremblay et al. reported that in vitro screening of a chemical library from Kinetek Pharmaceuticals resulted in the identification of a thioxothiazolidinone derivative **38** with low micromolar enzyme inhibition of PTP1B.[75] The uncharged inhibitor **38** demonstrated cell activity in FLT3-ITD and Akt phosphorylation assays at micromolar concentrations (10−25 μM) and displayed competitive inhibition kinetics (K_i = 1.8 μM). Modeling with the program AutoDock revealed a possible binding mode of **38** to PTP1B. The proposed conformation of ligand **38** places the trisubstituted phenyl ring deep into the active site with the nitro group positioned in a similar manner as the phosphonate of other cocrystal structures. The nitro group could be replaced with a carboxylic acid but not a methylene phosphonate, and the thioxothiazolidinone core was an important structural component of inhibitor **38**. Unfortunately, an X-ray cocrystal structure was not obtained to further support this unique ligand binding mode to PTP1B.

Perspectives and Future Directions

The discovery of potent cell permeable and orally bioavailable PTP1B inhibitors is a challenging medicinal chemistry objective. The highly cationic nature of the active site coupled with the lack of adjacent hydrophobic binding sites have thwarted the efforts by many research groups. The Merck Frosst research group has invested significant medicinal chemistry efforts toward the optimization of DFMP containing inhibitors with limited success. Their recent report of an orally bioavailable and in vivo active naphthyl DFMP containing inhibitor **5** is particularly noteworthy, since it is recognized that the physiochemical nature of the bis-anionic mimetic provides a significant barrier to improving cell activity and oral bioavailability. Prodrug approaches to these DFMP containing inhibitors have recently been published, and it will be interesting to see if this approach will prove to be a viable and general strategy. The potent dicarboxylic acid containing PTP1B inhibitors have also been shown to suffer from poor membrane permeability. Attempts to eliminate one of the carboxylic acid moieties have resulted in the 100- to 1000-fold loss in enzyme potency or afford extremely hydrophobic compounds that may result in nonspecific inhibition. Therefore, the carboxylic acid structural class of PTP1B inhibitors may have limited utility as a lead pharmacophore for medicinal chemistry optimization.

Nevertheless, this well validated therapeutic target for diabetes, obesity, and cancer has continued to inspire scientists to utilize new medicinal chemistry approaches. The design of prodrugs, fragment-based screening, and novel structure-based drug design strategies have each proven successful. Zhang's proposed dual binding strategy to develop potent inhibitors that occupy both the active site and B-site has led to significant advances in scaffold design by medicinal chemists. The success of fragment-based methods to identify the salicylic acid moiety by the Abbott group and the identification of selective and potent benzotriazoles by Merck Frosst have unambiguously shown that binding in the B-site provides not only more potent inhibitors but improved selectivity over the highly homologous phosphatase TC-PTP. While the clinical outcome of pharmacological inhibition of TC-PTP continues to be debated, selectivity over TC-PTP remains a desirable profile for a PTP1B drug candidate.

Proctor and Gamble's discovery of the sulfamic acid pTyr mimetic by high throughput screening of their corporate

collection is another significant finding. Not only are these low molecular weight compounds potent phosphatase inhibitors, but they demonstrate that high throughput screening can still be an effective tool in the discovery of novel pTyr mimetics. The optimized low molecular weight, picomolar PTP-β inhibitors exemplified in their recent patent filings show considerable promise for the future development of phosphatase inhibitors bearing this novel pTyr mimetic. Membrane permeability of compounds containing this pharmacophore remains to be demonstrated.

A notable advance in the phosphatase field has been the structure-based design of related five-membered heterocyclic pTyr mimetics by four independent research groups. The discovery of potent small molecule inhibitors that incorporate the TDZ and (*S*)-IZD pTyr mimetics has provided the most potent pTyr mimetics class of PTP1B inhibitors reported to date. More importantly, compounds bearing the diffusely monoanionic heterocyclic pTyr mimetics have shown improved membrane permeability when compared against carboxylic acid and DFMP containing inhibitors. Unfortunately, inhibitors reported to date still lack sufficient cellular penetration to drive robust in vivo efficacy and oral bioavailability. Continued progress by several pharmaceutical research groups attest to the utility of the new heterocyclic pTyr mimetic class of PTP1B inhibitors. Of particular interest are the potent low molecular weight PTP1B inhibitors bearing the TDZ group identified by Novartis. Activity in cellular assays and/or oral bioavailability remains to be demonstrated, but progress has been clearly made toward improving ligand efficiency and reducing polar surface area, properties that are known to be associated with improved pharmacokinetic profiles.

Taken as a whole, these advancements in the field of phosphatase drug discovery have provided direction to medicinal chemists pursuing these challenging drug targets and hope that a pharmacological inhibitor of PTP1B suitable for human clinical trials may emerge.

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Biography

Andrew P. Combs earned B.S. degrees in Chemistry and Molecular Biology at University of Wisconsin−Madison (1988) and a Ph.D. degree in Organic Chemistry at University of California, Los Angeles (1993), and was a HHMI Postdoctoral Fellow with Prof. Schreiber at Harvard University (1994−1996), Cambridge, MA. He started his industrial career at DuPont-Merck where he built a Parallel Synthesis group that discovered and optimized novel inhibitors for a broad range of biological targets. In 2000, Andrew was promoted to Director at DuPont Pharma, which was subsequently acquired BMS, where his responsibilities included leading medicinal chemistry programs in CNS and oncology areas. He joined Incyte in 2003 and is currently Executive Director of Chemistry pursuing clinical candidates for drug targets within metabolic disorders and oncology areas. Andrew also heads the Chemical Synthesis Technologies and Analytical and Computational Chemistry groups.

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