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Drugging the Undruggable: Therapeutic Potential of Targeting Protein Tyrosine Phosphatases

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CONSPECTUS

Protein tyrosine phosphatases (PTPs) are essential signaling enzymes that, together with protein tyrosine kinases, regulate tyrosine phosphorylation inside the cell. Proper level of tyrosine phosphorylation is important for a diverse array of cellular processes, such as proliferation, metabolism, motility, and survival. Aberrant tyrosine phosphorylation, resulting from alteration of PTP expression, misregulation, and mutation, has been linked to the etiology of many human ailments including cancer, diabetes/obesity, autoimmune disorders and infectious diseases. However, despite the fact that PTPs have been garnering attention as compelling drug targets, they remain a largely under-exploited resource for therapeutic intervention. Indeed, PTPs have been widely dismissed as “undruggable”, due to concerns that 1) the highly conserved active site (i.e. pTyr-binding pocket) makes it difficult to achieve inhibitor selectivity among closely related family members, and 2) the positive-charged active site prefers negatively charged molecules, which usually lack cell permeability. To address the issue of selectivity, we advanced a novel paradigm for the acquisition of highly potent and selective PTP inhibitors through generation of bivalent ligands that interact with both PTP active site and adjacent unique peripheral pockets. To overcome the bioavailability issue, we have identified nonhydrolyzable pTyr mimetics that are sufficiently polar to bind the PTP active site, yet still capable of efficiently penetrating cell membranes. We show that these pTyr mimetics interact in the desired inhibitory fashion with the PTP active site and tethering them to appropriate molecular fragments to engage less conserved interactions outside of PTP active site can increase PTP inhibitor potency and selectivity. We demonstrate through three pTyr mimetics fragment-based approaches that it is completely feasible to obtain highly potent and selective PTP inhibitors with robust *in vivo* efficacy in animal models of oncology, diabetes/obesity, autoimmune disorders, and tuberculosis. We hope that these results will help dispel concerns about the druggability of PTPs and entice further effort in fostering a PTP-based drug discovery enterprise. Well-characterized, potent, selective and bio-active inhibitors are essential tools for functional interrogation of PTPs in disease biology and target validation. They will also play a critical role in illuminating the druggability of PTPs and provide the groundwork for new therapies for the treatment of human diseases.

Graphical Abstract

Introduction

Protein tyrosine phosphorylation is essential for the initiation, propagation, and termination of signal transduction in response to growth factors, hormones, cytokines and cell-cell contacts. The exact magnitude and duration of tyrosine phosphorylation is important for cell proliferation, metabolism, motility, and survival and is tightly regulated by the opposing activities of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs).^{1,2} Not surprisingly, disturbance of the delicate balance between the actions of PTKs and PTPs causes aberrant tyrosine phosphorylation, which is at the root of many pathological conditions including cancer, metabolic diseases and inflammatory disorders.^{3,4} Accordingly, abnormal signaling events driven by faulty operation of tyrosine phosphorylation offer enormous opportunities for targeted intervention. The therapeutic potential for such targeted approach has been well established by the more than two-dozen small molecule PTK inhibitors that are already in the clinic.⁵ However, drug resistance limits the impact of kinase inhibitor-based therapies. Therefore, despite the proven druggability of targeting PTKs, alternative strategies are needed for improved treatment.

Given the reversible and dynamic nature of tyrosine phosphorylation, it is imperative to recognize that the extent of tyrosine phosphorylation is determined by the balanced activity of both PTKs and PTPs. Consequently, *a priori*, one should also be able to manipulate disease biology at the level of PTPs for therapeutic benefit. Encoded by more than 100 genes in humans, the PTPs constitute a large family of signaling enzymes that rival the PTKs in their structural diversity and functional complexity.⁶ The hallmark that defines the PTP superfamily is the active site sequence (I/V)HCXAGXGR(S/T), also called the P-loop, which houses the catalytic Cys for nucleophilic catalysis.⁷ Studies over the last 25 years have revealed that amplification, mutation and other alterations of PTPs can also lead to disease manifestation.^{4,8} Hence, the PTPs have emerged as compelling targets for drug discovery.

By catalyzing substrate dephosphorylation, PTPs can function as either “on” or “off” switches for signal transduction. For example, PTP1B is a major antagonist of both insulin and leptin signaling (Figure 1). Mice deficient in PTP1B exhibit increased insulin sensitivity

and are resistant to obesity when fed on high-fat diet.⁹ Other PTPs that have also been implicated as negative regulators of insulin and/or leptin mediated pathways include TC-PTP,¹⁰ PTP-MEG2,¹¹ and LMW-PTP¹² (Figure 1). Small molecule inhibitors of these PTPs are expected to increase insulin and leptin sensitivity thereby serving as novel modalities for type 2 diabetes and obesity. The lymphoid-specific tyrosine phosphatase (Lyp), encoded by *Ptpn22*, is a key regulator of immune homeostasis by inhibiting T-cell receptor signaling¹³ (Figure 2). A missense C1858T single-nucleotide polymorphism in *Ptpn22* is found to be associated with an increased risk for the development of type I diabetes, rheumatoid arthritis, Graves disease, and systemic lupus erythematosus.¹³ Consequently, Lyp represents an attractive target for a broad spectrum of autoimmune diseases.

Interestingly, the PTPs can also play a positive role in driving signaling cascades. To this end, SHP2 is required for growth factor induced activation of Ras, a critical node in many signaling pathways that control cell proliferation, migration and survival (Figure 3).¹⁴ In addition, considerable evidence indicate that SHP2 is a *bona fide* oncoprotein.¹⁴ Germline gain-of-function mutations in SHP2 cause 50% of Noonan syndrome, an autosomal dominant developmental disorder with increased risk of malignancy. Activating somatic SHP2 mutations are found in leukemia and solid tumors. Moreover, given the obligatory requirement of SHP2 in growth factor-mediated pathways, thwarting SHP2 activity may also prove effective for cancers caused by abnormal activation of receptor PTKs, some of which respond poorly to kinase inhibitor monotherapy. Indeed, recent studies indicate that SHP2 is a central node in intrinsic and acquired resistance to tyrosine kinase targeted cancer drugs.¹⁵ Thus there is strong interest in developing small molecule SHP2 inhibitors as novel therapeutic agents for cancer.

The significance of PTPs in human health is further emphasized by the fact that they are often exploited and subverted by pathogenic microorganisms to cause infection. For instance, *Mycobacterium tuberculosis* (*Mtb*) encodes two PTPs, termed mPTPA and mPTPB, both of which are secreted by *Mtb* into the macrophage and are required for optimal bacillary survival within the host.^{16,17} mPTPA prevents phagolysosome acidification by exclusion of the macrophage vacuolar-H⁺-ATPase from the vesicle (Figure 4).¹⁸ Once inside the macrophage, mPTPB activates PI3K-Akt pathway and simultaneously blocks ERK1/2 and p38 activation to prevent host macrophage apoptosis and cytokine production (Figure 4).¹⁹ Importantly, deletion of mPTPA or mPTPB decreases *Mtb* survival within interferon- γ activated macrophages and severely reduces the *Mtb* bacterial load in guinea pigs.¹⁶ Thus small molecule inhibitors of mPTPA and mPTPB may augment intrinsic host signaling pathways to eradicate tuberculosis infection.

As exemplified above, dysregulation of PTP activity has been linked to diabetes/obesity, cancer, autoimmunity, and infectious diseases. Consequently, the PTP family offers a rich source of molecular targets for novel therapeutic interventions. However, despite increasing attention to this target class, the PTPs still remain largely an untapped resource. Among the factors that contribute to the still nascent state of PTP-based drug discovery is the lack of detailed understanding of the role of specific PTPs in the etiology of disease. In addition, the PTPs are exceptionally difficult targets for therapeutic development. In this Account, I first discuss technical challenges facing drug discovery programs targeting the PTPs. I then

highlight several successful approaches to overcome the selectivity and bioavailability issues in PTP inhibitor discovery. Potent and specific PTP inhibitors constitute valuable tool compounds for functional interrogation of PTPs in complex cellular signal transduction pathways, proof-of-concept target validation, and therapeutic development for many human diseases.

Challenges for PTP Inhibitor Development

The PTP-catalyzed reactions encompass recognition and hydrolysis of pTyr within a protein substrate in a highly context specific manner. Kinetic and structural studies reveal that pTyr is essential for peptide/protein substrate binding.⁷ Therefore, earlier drug discovery efforts were primarily directed at the PTP active site, namely the pTyr binding pocket. However, there are technical challenges associated with targeting PTP active site. First, residues involved in pTyr binding and hydrolysis are conserved among PTPs. Consequently it is difficult to develop active site directed inhibitors for one PTP without impeding the activity of closely related family members. Second, the PTP active site is positively charged in order to accommodate the phosphoryl group in pTyr. Thus screening campaigns targeting the PTP active site generally identify negatively charged phosphate analogs, which usually have difficulty entering cells. Third, the side chain of the catalytic Cys within the positively charged active site environment exists as a thiolate anion ($pK_a \sim 5$),⁷ rendering it prime target for 1) various electrophiles, such as iodoacetamides, maleimides, α -halocarbonyls, and Michael acceptors, 2) oxidizing agents, including hydrogen peroxide, quinones, and phenanthrenediones, and 3) heavy metals such as Zn^{2+} and Cu^{2+} .^{20,21} These thiolate reactive molecules often surface either as hits or false positives from high throughput screening but they tend to have limited specificity, decreasing their usefulness as tool compounds or leads for drug discovery. Because of the aforementioned technical challenges in PTP-based drug discovery, the PTPs as a target class have earned the reputation as “undruggable”. In the following, I describe our strategies to address inhibitor selectivity and bioavailability issues and demonstrate that it is feasible to develop high quality potent and selective PTP inhibitors with efficacious *in vivo* activity.

Fragment-based Approach to Target both PTP Active Site and Adjacent Less Conserved Pockets for Enhanced Inhibitor Potency, Selectivity, and Bioavailability

Since PTPs are hydrolytic enzymes, they do not bind endogenous small molecule ligands that could lend leads for drug discovery. To provide starting points for inhibitor development, we investigated PTP substrate specificity using both pTyr-containing peptides as well as small molecule aryl phosphates. We reasoned that by identifying the most optimal substrates^{22,23} we can convert them into potent inhibitors.^{24,25} Indeed, PTP1B inhibitor **1** (Figure 5) was developed by converting the most efficient substrate identified from a focused substrate library screen into a nonhydrolyzable analog.²⁶ **1** inhibits PTP1B with a K_i of 2.4 nM and displays several orders of magnitude selectivity against a large panel of PTPs. Importantly, **1** also exhibits >10-fold selectivity for PTP1B over its closest related family member TC-PTP.

Given the importance of pTyr in PTP substrate recognition, a logical strategy is to identify nonhydrolyzable pTyr mimetics that can efficiently occupy the active site pocket. In addition, we found that although pTyr plays an important role in substrate recognition, amino acid residues flanking pTyr are also important for efficient PTP substrate turnover.⁷ These findings suggest that binding pockets bordering the active site that interact with pTyr proximal residues may also be targeted for inhibitor design. This notion was further solidified by the discovery of a second aryl phosphate binding site in the vicinity of PTP1B active site, which lies within a region that is not conserved among PTPs.²⁷ We thus proposed a novel paradigm for the acquisition of highly potent and selective PTP inhibitors through generation of bivalent ligands that interact with both the PTP active site and distinct peripheral pockets (Figure 6).^{7,27} By targeting less conserved interactions in addition to the active site cleft, increased inhibitor affinity and selectivity can be obtained. To overcome the issue of inhibitor bioavailability, we discovered several novel nonhydrolyzable pTyr mimetics that are sufficiently polar to bind the PTP active site, yet still capable of efficiently penetrating cell membranes.^{28–31} These pTyr mimetics interact in the desired inhibitory fashion with the PTP active site and tethering them to diverse molecular fragments render the resulting inhibitors PTP isozyme-selective. We discuss several successful examples of using nonhydrolyzable pTyr mimetic fragment-based approach to target both PTP active site and adjacent less conserved pockets and demonstrate that it is feasible to obtain PTP inhibitors with high affinity, selectivity, and robust *in vivo* efficacy in animal models of oncology, diabetes/obesity, autoimmunity, and tuberculosis.

1. Phosphonodifluoromethyl Phenylalanine (F₂Pmp) Fragment Based Approach.

One of the most commonly used nonhydrolyzable pTyr mimetic is phosphonodifluoromethyl phenylalanine (F₂Pmp).²⁴ Interestingly, the binding affinity of F₂Pmp for individual PTPs is not the same. For example, the *IC*₅₀ values of F₂Pmp for PTP1B and PTP-MEG2 are 0.55 and 11.2 mM, respectively. Installation of a 3-bromo-4-methylbenzoic amide group through a Lys linker to F₂Pmp generates **2** (Figure 5) which inhibits PTP1B with an *IC*₅₀ of 120 nM, a nearly 4,600-fold increase in potency (unpublished observations from the author's laboratory). Importantly, **2** exhibits no inhibition toward PTP-MEG2 even at 20 μM concentration (Figure 7A). The co-crystal structure of PTP1B with **2** revealed that the 3-bromo-4-methylbenzoic group is placed within the unique second aryl phosphate binding pocket formed by Arg24, Ala27, Asp29, Phe52, Arg254, and Met258, which likely explains **2**'s potency and specificity for PTP1B (Figure 7B) (unpublished observations from the author's laboratory). Conversely, when the 3-iodobenzoic amide group was attached to F₂Pmp through a ornithine linker, the *IC*₅₀ of the resulting **3** (Figure 5) for PTP-MEG2 was 0.90 μM, which is 8.7-fold lower than that for PTP1B (7.8 μM) and a greater than 12,400-fold improvement in affinity over F₂Pmp (Figure 7A).³² The structure of PTP-MEG2•**3** showed that the 3-iodobenzoic amide moiety sits above a hydrophobic patch created by residues Pro315, Phe319, Pro337 and Phe556, which are unique to PTP-MEG2³² (Figure 7C). The stacking interactions between the 3-iodobenzoic amide and the unique hydrophobic patch are likely responsible for **3**'s impressive potency and selectivity for PTP-MEG2, despite F₂Pmp's 23-fold preference for PTP1B (*IC*₅₀=0.5 mM) over PTP-MEG2 (*IC*₅₀=11.5 mM) (Figure 7A). Overall, these results demonstrate the power of the pTyr

mimetic fragment based approach to transform a common F₂Pmp core into potent and selective PTP inhibitors.

To further generalize the F₂Pmp fragment based approach for PTP inhibitor development, we devised a novel stepwise fluorophore-tagged focused library synthesis and screening strategy to tether F₂Pmp to a large number of carboxylic acids of varying size, shape, polarity, and hydrophobicity in order to increase the number and strength of noncovalent interactions with peripheral binding pockets in the vicinity of PTP active site. We were able to convert the weak and general PTP inhibitor F₂Pmp into extremely potent and selective inhibitors for TC-PTP (compound **4**)³³ and PTP-MEG2 (compound **5**)³² (Figure 5). Indeed, **4** inhibits TC-PTP with a K_i of 4.3 nM and its affinity for TC-PTP is more than 200-fold higher than those for a large panel of PTPs. In addition, **4** also has a 8-fold selectivity for TC-PTP over its closest homologue PTP1B. Kinetic analysis reveals that **5** is a reversible and competitive inhibitor for PTP-MEG2 with a K_i of 34 nM. Compound **5** exhibits >10 fold selectivity for PTP-MEG2 over PTP1B and TC-PTP, and no inhibition at 10 μ M against a large panel of PTPs. X-ray crystallographic studies provide direct evidence that PTP inhibitor potency and selectivity can be achieved by targeting peripheral pockets in addition to the active site.³²

Much to our delight, compounds **4** and **5** also possess highly efficacious cellular and *in vivo* activity.^{32,33} Intracerebroventricular administration of TC-PTP inhibitor **4** enhances leptin-induced phosphorylation on Tyr705 in STAT3, represses food intake and body weight and expands energy expenditure in mice.¹⁰ Interestingly, a cell permeable derivative of PTP1B inhibitor **1** augments insulin signaling by increasing insulin receptor phosphorylation³⁴ and restores leptin's ability to reduce food intake in leptin resistant Sprague Dawley rats through increased JAK2 phosphorylation.³⁵ PTP-MEG2 inhibitor **5** promotes insulin signaling and improves insulin sensitivity and glucose homeostasis in diet-induced obese mice,³² consistent with the observation that hepatic PTP-MEG2 silencing in *db/db* mice leads to a reversal of insulin resistance and hyperglycemia.¹¹ Collectively, these findings serve as proof-of-concept that F₂Pmp-based PTP inhibitory agents can be endowed with excellent *in vivo* efficacy.

2. Bicyclic Salicylic Acid Fragment Based Approach.

Given the success with F₂Pmp based approach in addressing PTP inhibitor potency, selectivity, and bioavailability, we wanted to identify new pTyr mimetics with improved drug-like properties. Since an exceedingly high proportion of FDA-approved drugs have their origin from natural products, we focused our effort on pursuing natural product-like PTP inhibitory agents. We found that salicylic acid can function as a pTyr surrogate,²⁸ and further discovered that naphthyl and polyaromatic salicylic acid derivatives exhibit enhanced affinity for the PTP active site relative to the corresponding single ring counterparts.^{28,36} Consequently, we sought to develop bicyclic salicylic acid-based PTP inhibitors that carry sufficient polar and nonpolar interactions with the active site and yet still capable of penetrating cell membrane. In particular, benzofuran and indole derivatives are of considerable interest because of their widespread occurrence in natural products as well as synthetic compounds with medicinal value.^{37,38} Thus, we explored benzofuran- and indole-

based salicylic acids as potential non-phosphorus-containing pTyr mimetics. We hypothesized that benzofuran- or indole-based salicylic acids may bind and interact with the PTP active site in the desired inhibitory fashion, and installation of molecular diversity to the salicylic core could render the inhibitors PTP isozyme-selective.^{19,29,30}

To target both the active site and adjacent peripheral binding cavities in PTPs, we developed a bicyclic salicylic acid-based focused library approach using the following criteria: (1) each library member will be of a modular, bidentate structure containing both an active site-directed core (e.g., benzofuran or indole salicylic acid derivative) and a diversity element for interaction with adjacent peripheral sites; (2) both the core and the diversity elements will retain favorable pharmacological properties; and (3) each library member will be efficiently assembled *in situ* for facile screening against the PTPs. A number of different strategies, including Click chemistry and carboxylic acid-amine condensation reaction, have been employed to connect the active site targeting core with various diversity elements for library construction. Structure-guided medicinal chemistry optimization of initial hits identified from the focused libraries afforded potent and selective bicyclic salicylic acid-based inhibitors for a number of PTPs with excellent cellular and *in vivo* activities.^{39–42} For example, compound **6** (Figure 5), a potent and selective inhibitor of Lyp with a K_i value of 110 nM and more than 9-fold selectivity over a large panel of PTPs, displays highly efficacious activity in both T- and mast cells and blocks systemic anaphylaxis in a mouse model.³⁹ Consistent with salicylic acid derivatives being effective pTyr mimetics and the observed competitive mode of Lyp inhibition, the 6-hydroxy-benzofuran-5-carboxylic acid moiety is found in the Lyp active-site pocket. The remarkable potency and selectivity of **6** for Lyp are the results of numerous specific interactions with both the active site and its nearby unique peripheral pockets.³⁹

II-B08, an initial hit for SHP2 ($IC_{50}=5.5\ \mu\text{M}$) from an indole salicylic acid library,³⁰ inhibits SHP2 activity in several cancer cell lines as well as in mast cell leukemia, xenograft lung cancer and orthotopic intracranial glioblastoma xenograft models.^{30,43–45} Given its promising biological activities, structure-based design was used to transform II-B08 into a second-generation SHP2 inhibitor **7** (Figure 5) with an IC_{50} of 200 nM for SHP2. **7** exhibits >5-fold selectivity for SHP2 over a large panel of 20 PTPs.⁴² Of particular note, **7** has a 7-fold preference for SHP2 over its closely related homolog SHP1. Like II-B08, **7** efficaciously blocks growth factor stimulated ERK1/2 and Akt activation, cell proliferation, and exhibits *in vivo* anti-tumor activity melanoma tumor xenograft growth.^{42,46} A recently disclosed allosteric SHP2 inhibitor SHP099 that stabilizes the autoinhibited closed conformation of SHP2 can also suppress Ras–ERK1/2 signaling and inhibit proliferation of receptor PTK-driven cancer cells in mouse tumor xenograft models.⁴⁷ Together, these promising pharmacological results with SHP2 inhibitors of diverse structural scaffolds and different mode of action strongly support the validity of therapeutic targeting of SHP2 for the treatment of cancers. Moreover, upregulation of SHP2 has also been linked to the pathogenesis of lupus and rheumatoid arthritis, and normalization of SHP2 activity with **7** prevents the progression of systemic lupus erythematosus and attenuates K/BxN serum transfer arthritis in mice.^{48,49} Thus, **7** serves as a promising lead molecule for the development of novel therapeutic agents targeting SHP2.

Fragment based and medicinal chemistry optimization of the benzofuran and indole salicylic acid scaffolds also led to highly potent and selective mPTPB inhibitors **8**⁴⁰ and **9**⁴¹ (Figure 5). Compounds **8** and **9** inhibit mPTPB with a K_i of 44 and 50 nM respectively, and display >50 and 100-fold higher affinity for mPTPB over all mammalian PTPs examined.

Importantly, these inhibitors reverse the altered host immune responses induced by the bacterial phosphatase and restore the macrophage's full capacity to secrete IL-6 and undergo apoptosis in response to IFN- γ stimulation, validating the concept that inhibition of mPTPB may be therapeutically useful for novel TB treatment. Collectively, these results further demonstrate that bicyclic salicylic acid pharmacophores can be used to deliver PTP inhibitors with high potency, selectivity, and cellular efficacy.

3. α -Sulfophenylacetic Amide (SPAA) Fragment Based Approach.

To further address the bioavailability issue, we also harnessed the privileged drug space to identify previously unknown PTP inhibitory activities. Since known drugs are already used in humans with acceptable pharmacokinetic properties, such a drug repurposing strategy may supply promising starting points for PTP-based drug development. To this end, we discovered cefsulodin, a third generation β -lactam antibiotic, as an inhibitor of SHP2.³¹ Structural analysis reveals SPAA, a fragment originated from cefsulodin, as a novel pTyr mimetic. Structure-guided and fragment-based optimization of SPAA furnished potent and selective inhibitors for SHP2,³¹ mPTPA,⁵⁰ mPTPB,⁵¹ and LMW-PTP.⁵² Notably, **10** (Figure 5) inhibits mPTPA with a K_i of 56 nM and greater than 20-fold selectivity over all PTPs examined.⁵⁰ Dual inhibition of mPTPA and mPTPB with **10** and **8** enhances the antitubercular activity of the first-line regimen and improves lung pathology in the guinea pig model.⁵⁰ Compound **11** (Figure 5) represents the most potent and selective mPTPB inhibitor, with a K_i of 7.9 nM and more than 10,000-fold preference for mPTPB over a large panel of 25 phosphatases.⁵¹ **11** also exhibits excellent cellular activity and specificity in blocking mPTPB function in macrophage.⁵¹ Finally, **12** (Figure 5) inhibits LMW-PTP with a K_i of 1.0 μ M and exhibits greater than 50-fold preference for LMW-PTP over a large panel of PTPs.⁵² **12** also promotes insulin induced Akt phosphorylation in HepG2 cells through specifically inhibiting LMW-PTP activity.⁵² Crystal structures of LMW-PTP-inhibitor complexes reveal a ligand induced conformational change in LMW-PTP that is responsible for the exquisite inhibitor selectivity.⁵² Together, our results with F₂Pmp, bicyclic salicylic acids, and SPAA pTyr mimetics demonstrate the feasibility of developing highly potent and selective PTP inhibitors with robust *in vivo* activity and offer potential solutions to overcome inhibitor selectivity and bioavailability issues that have plagued the PTP drug discovery field for many years.

Conclusion

The PTPs comprise a large family of signaling enzymes that are key regulators of cellular signaling. There is ample evidence that alteration of PTP expression, misregulation, and mutation lead to disease manifestations. These connections have made targeting of PTPs attractive as a novel therapeutic strategy. However, the PTPs have historically been widely dismissed as "undruggable" and so they remain a largely under-exploited resource for therapeutic intervention. Major obstacles to successful PTP-based drug discovery are target

validation and attaining inhibitor selectivity and bioavailability. Our work demonstrate that PTP inhibitor potency and selectivity can be addressed by targeting both the active site and unique peripheral binding pockets. Using appropriate pTyr mimetic to anchor the inhibitor to PTP active site, the success of this approach relies on the introduction of secondary binding elements to enhance on-target potency and impart selectivity against off-target phosphatases. Our work also establish that it is feasible to acquire PTP inhibitors that are sufficiently polar to bind the active site and yet still possess favorable pharmacological properties. By taking a fragment-based approach with a number of nonhydrolyzable pTyr mimetics, we have obtained PTP inhibitors with high potency and selectivity as well as excellent *in vivo* efficacy in animal models of oncology, diabetes/obesity, autoimmunity, and tuberculosis. Well-characterized, potent, selective and bio-active inhibitors are essential tools for functional interrogation of PTPs in disease biology and target validation. They will also play a critical role in illuminating the druggability of this under-explored target class and provide the groundwork for new therapies for the treatment of human diseases.

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Zhong-Yin Zhang graduated from Nankai University, China, with a B.S. in Chemistry in 1984. He obtained a Ph.D. in Chemistry from Purdue University in 1990. After a brief stint at Upjohn, he completed his postdoctoral training at the University of Michigan. Zhang established his own laboratory in 1994 at Albert Einstein College of Medicine, where he rose to the rank of Professor in 2002. From 2005-2015, Zhang was Robert A. Harris Professor and Chairman of the Department of Biochemistry and Molecular Biology at Indiana University School of Medicine. In 2016, Zhang moved back to Purdue as Distinguished Profesor of Medicinal Chemistry, Robert C. and Charlotte P. Anderson Chair in Pharmacology, and Head of the Department of Medicinal Chemistry and Molecular Pharmacology. His research spans the disciplines of chemistry and biology with an emphasis on PTP structure and function, and development of PTP inhibitors as novel therapeutics.

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Figure 1.
Roles of PTP1B, TC-PTP, PTP-MEG3, and LMW-PTP in insulin and leptin signaling.

Figure 2.
Role of Lyp in T-cell signaling and homeostasis.

Figure 3. Requirement of SHP2 in growth factor receptor mediated Ras activation. GOF, gain of function.

Figure 4.
Roles of mPTPA and mPTPB in mediating pathogen-host interaction.

Figure 5.
Chemical structures of potent, selective and bioactive PTP inhibitors discussed in this Account.

Figure 6.

A pTyr mimetic fragment-based approach to target both PTP active site and adjacent less conserved pockets for increased inhibitor potency and selectivity.

A

	F ₂ Pmp (IC ₅₀)	Compound 2 (IC ₅₀)	Compound 3 (IC ₅₀)
PTP1B	0.55 mM	120 nM	7.8 μM
PTP-MEG2	11.2 mM	>>20 μM	0.9 μM

B **C**

Figure 7. Capturing interactions with peripheral binding pockets for increased inhibitor potency and selectivity. (A). *IC*₅₀ values of F₂Pmp, compound **2** and compound **3** for PTP1B and PTP-MEG2. (B). Crystal structure of PTP1B in complex with **2**. (C). Crystal structure of PTP-MEG2 in complex with **3**.