



# Chemistry & Biology Interface

An official Journal of ISCB, Journal homepage; [www.cbijournal.com](http://www.cbijournal.com)

## Review Paper

### Covalent Inhibitors of Protein Tyrosine Phosphatases

Dibyendu Dana & Sanjai Kumar\*

*Contribution from the Department of Chemistry & Biochemistry, Queens College – City University of New York, 65-30 Kissena Blvd., Flushing, New York 11367*

*Received 17 December 2011; Accepted 14 February 2012*

---

**Keywords:** PTP inhibitor, Mechanism-based PTP inhibitor, Covalent PTP inhibitory agents, Activity-based probes, PTP drug discovery, Protein tyrosine phosphatase inhibitor

**Abstract:** The importance of a carefully regulated protein dephosphorylation event in cellular signaling by protein tyrosine phosphatases (PTPs) is well recognized. Indeed an anomalous level of PTP activities has been associated with a variety of aberrant cellular signaling and human diseases. As a result of this, potent and selective inhibitory agents of PTP enzymes are sought after by research communities across the globe. While there is considerable interest among academic researchers and pharmaceutical industries to develop non-covalent and reversible inhibitory agents of PTPs, the development of covalent inhibitory agents is plagued with presumption that they are not suitable candidates for drug discovery. This outcome is primarily due to the concern that such molecules could be immunogenic and serve as cellular toxic agents. The current success of covalent therapeutic agents in clinics, however, does not support this notion, and indicates that there is a need to reconsider this hypothesis with regard to the PTP-related drug discovery efforts. This review starts with a discussion of PTPs in general and provides an overview of reported covalent PTP inhibitors, highlighting their modes of interaction with enzymes. Finally, a brief perspective about the potential opportunities for the advancement of covalent inhibitors of PTPs in targeted PTP therapeutics is provided.

---

## Introduction

Protein tyrosine phosphatases (PTPs) are a critically important group of signal transduction enzymes that together with protein tyrosine kinases (PTKs) regulate precisely-orchestrated intracellular protein phosphorylation events.<sup>1, 2</sup> This tightly-regulated process is critical in maintaining

normal cellular physiology, and plays an indispensable role in regulating many important cellular processes, such as cell-cell communication, transmembrane signaling, cell motility, immune response, adhesion, and differentiation.<sup>3-6</sup> Consistent with this finding, recently emerged data strongly suggest that a misguided and promiscuous phosphorylation of cellular targets by aberrant activities of both protein kinases (PKs) and PTPs is linked to a variety of human diseases.<sup>7-9</sup> With the advent of

Corresponding Author\*

E-mail: [Sanjai.Kumar@qc.cuny.edu](mailto:Sanjai.Kumar@qc.cuny.edu). Tel: 718-997-4120;

Fax: 718-997-5531

many PK-targeting drugs in clinics and advanced stage clinical trials, attention has recently been diverted to evaluate PTPs as viable targets of therapeutic intervention in numerous human diseases.<sup>10</sup> This excitement in PTP research is attributed partly due to an important discovery that the PTP1B knock-out mice while remaining viable, are significantly resistant to diet-induced obesity and display enhanced insulin sensitivity.<sup>11, 12</sup>

A favorable interest in PTPs is also spurred by recent findings that a drug molecule can have multiple cellular targets and yet render a favorable clinical outcome. Indeed there are now many reported instances where an effective drug molecule elicits a desired pharmacological response via modulation of multiple protein targets.<sup>13, 14</sup> One such example is Sorafenib, an orally administered kinase inhibitor originally approved for the treatment of patients with advanced renal cell carcinoma, which has been recently approved for the treatment of unresectable hepatocellular carcinoma (HCC).<sup>15</sup> At the molecular level, this multikinase drug simultaneously affects both tumor angiogenesis and tumor proliferation signaling pathways by perturbing the function of both receptor tyrosine kinases such as platelet-derived growth factor receptor- $\beta$  (PDGF- $\beta$ ), KIT and FLT3, and a serine/threonine kinase RAF.<sup>16</sup> Such findings that a 'non- or partially-selective' inhibitory agent with polypharmacological behavior could have a high therapeutic value have reinforced confidence among PTP researchers, many of whom earlier presumed that the highly conserved and polar nature of the PTP active site may prove to be an obstacle for generating potent and selective PTP inhibitory agents of high therapeutic potential. Although currently there are no FDA-approved drugs targeting PTPs in clinics, a few candidate molecules under

investigation at various stages of human clinical trials are anticipated to become clinical agents in the near future. For example, a drug candidate ISIS-PTP1B that targets PTP1B activity is in Phase II stage of clinical trials for the treatment of type 2 diabetes. Similarly CD45, a receptor PTP, is a viable target candidate for the treatment of Alzheimer's disease.<sup>17, 18</sup> This is perhaps not too surprising since it is now well accepted that PTPs are critically important signaling molecules capable of initiating, potentiating, and terminating many important signaling events upon appropriate cellular demand. For example, while in both insulin and leptin signaling pathways PTP1B acts as a terminating agent of signal transduction, in others such as in platelet integrin signaling, it provides a positive regulation.<sup>19-21</sup>

Human genome encodes for 107 PTPs compared to only 90 PTKs, perhaps indicating that the signaling events mediated by PTPs are at least as diverse, specific and complex as PTKs. In addition, recent discovery that the catalytic activities of PTPs are further regulated by a carefully controlled redox mechanism have added another tier of complexity in PTP-mediated signaling. This complexity in PTP-derived signaling has led to a flurry of activities among PTP researchers that is geared towards functional annotation of PTPs in both normal and diseased biology. Progress in this direction has been hampered due to the paucity of appropriate tools needed for functional assignments. In recent years, usage of selective small molecule inhibitors for functional perturbation of a protein target have gained considerable popularity.<sup>22, 23</sup> While significant efforts, both by the pharmaceutical industries and academic researchers, have been directed towards developing fast-acting and reversible inhibitory agents of PTPs, little attention has been paid towards invention of covalent

inhibitory agents. This is primarily because of the associated anxieties with the notion that (i) a covalently modified intracellular protein target may have higher probability of eliciting an undesired immunogenic response in a host organism, and (ii) there exists a potential for off-target reactivities, thereby leading to unpredictable drug-related toxicity. Recent data, however, suggest that this safety concern is clearly overblown, since three of the ten top selling drugs in the United States, namely clopidogrel – an inhibitory agent of P2Y<sub>12</sub> receptor, and esomeprazole and lansoprazole – proton pump inhibitors, impart their clinical efficacies by covalent modification of their molecular targets.<sup>24</sup> In fact, there are about thirty covalent drugs that are currently being used in clinics worldwide, with the  $\beta$ -lactam antibiotics being the most notable ones in the treatment of bacterial infection.

In this review, we focus our attention on the covalent types of reported PTP inhibitors that exploit the conserved active site chemistry to modulate PTP functions. First, we discuss the catalytic mechanism employed by the PTP family of enzymes and their regulation by redox mechanism. Subsequently we provide a current account of the reported covalent inhibitors of PTPs, elaborate their possible mechanism of inhibition if demonstrated, and highlight their potential impact on our current understanding of PTP biology. Finally, we conclude our discussion with future prospects of novel covalent PTP inhibitors that could render desirable biological and therapeutic efficacies.

### Mechanism of PTP Action

Overall, the PTP superfamily is broadly classified into three main categories: (1) Classical types (2) Dual-specific types, and (3) Low molecular Weight (LMW)-PTP. While the classical type PTPs (~37 genes in

human), which includes both non-transmembrane (e.g. PTP1B, TCPTP, SHPs, and HePTP) and membrane-bound PTPs (e.g. CD45, LAR, DEP1, and PTP $\alpha$ ), preferentially catalyze the dephosphorylation of only phosphotyrosine (pTyr) residues, the dual-specific types (~64 genes in human; some examples include MKPs, PRLs, CDC14s, Cdc25s) can dephosphorylate pTyr as well as phospho-Ser/Thr (pS/T) residues, and in a few cases phosphoinositides (e.g. PTENs).<sup>8, 25-27</sup> The LMW-PTP (18 kDa) is a lone member in its class, ubiquitously expressed in most human cell types, and is closely related to a bacterial gene across several species. For example, YfkJ protein in the gram-positive bacterium *Basillus subtilis* is 39% identical to human LMW-PTP.<sup>27</sup> While some progress has been made in delineating the role of LMW-PTP in human biology, much still remains to be discovered about its function.<sup>28-30</sup> Although the three classes of PTPs differ considerably in their substrate specificities and primary sequences, all employ a common catalytic mechanism to carry out the dephosphorylation of their putative substrates. The PTP enzymes harbor a key active site signature motif HCX<sub>5</sub>R in which a well-conserved cysteine residue participates as a nucleophile during the substrate turnover (Figure 1).

The catalytic process starts with the binding of the negatively charged pTyr substrate into a positively charged pocket harboring an invariant Arg residue. This binding is followed by nucleophilic attack of the low pK<sub>a</sub> Cys (~pK<sub>a</sub> = 4.2) residue on the electrophilic phosphorus of the phosphotyrosine substrate, leading to the concomitant departure of the Tyr residue with help from the general acid Asp of the flexible WPD loop. This reaction process ultimately results in the formation of the thiophosphoryl enzyme intermediate. In the



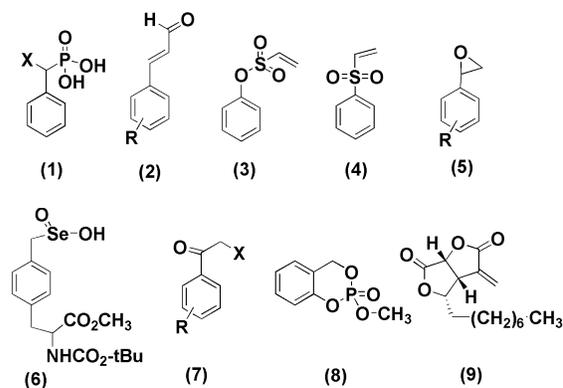
intracellular actions of PTPs.<sup>35-37</sup> For example, a recently published report indicated that in cancer cells, only the catalytic Cys residue of PTP1B was found to be selectively reversibly modified.<sup>38</sup> This added complexity in PTP regulation indicates that an efficient and rapid detection of reversible oxidation states of PTPs in both normal and aberrant living cells is critical to understanding their precise role in cellular signaling events. Indeed, efforts are being made to develop novel chemical tools that would directly report on the activity profile of PTPs in various cellular contexts.

### Covalent Inhibitors of PTPs

The covalent inhibitors of PTPs are needed so that they can be utilized to develop (a) lead inhibitory candidates to expedite PTP-based drug discovery efforts, and (b) effective chemical biology tools, such as activity-based probes for functional analysis of PTPs at proteome level in various cellular contexts. The later application is especially important since activity-based enzyme profiling is an emerging area of great interest to PTP researchers interested in functional assignments of enzymes on a system-wide proteome level in both normal and diseased states.<sup>39</sup> In the following section, we outline the distinct types of reported covalent inhibitors of PTPs (Figure 2), and describe their mode of interaction with PTP enzyme, if known with some degree of certainty.

*$\alpha$ -Halobenzylphosphonates (1, Figure 2):* This class of molecules has been shown to be a quiescent irreversible inactivator of PTPs.<sup>40</sup> A detailed enzyme kinetics analysis reveals that these agents inhibited PTPs in both time- and concentration-dependent manners. Further competition-based experiment suggested that these agents were active site bound, relatively stable in

aqueous and nucleophilic environments, and displayed covalent reactivity only upon binding to the PTP active site. Such desirable properties of  $\alpha$ -halobenzylphosphonates have led towards the development of the first activity-based PTP probe.<sup>41</sup> A comparative labeling experiment with the wild-type and Cys-mutant PTP enzymes indicated that the mechanism of PTP labeling required the presence of the conserved active site Cys nucleophilic residue. Further, the labeling reaction of PTP with probe in presence of hydrogen peroxide revealed that the reduced form of the sulfhydryl group of Cys residue was essential for effective PTP labeling.<sup>42</sup> These data taken together collectively demonstrated that  $\alpha$ -halobenzylphosphonate-derived PTP probes were specific, mechanism-based, and most importantly reacted in an activity-dependent manner with many distinct members of the PTP superfamily enzymes. The impressive level of selectivity by this class of compounds with PTPs suggests that  $\alpha$ -halobenzylphosphonate-derived PTP probes can be put to use in understanding unknown PTP biology in numerous cellular contexts. Indeed a few successful biological applications of these PTP probes have begun to emerge, such as those demonstrated in differential profiling of PTP activities in

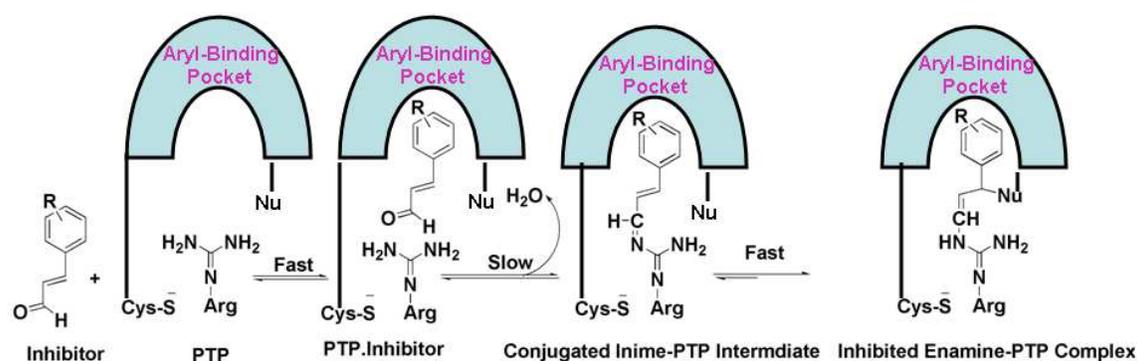


**Figure 2.** Chemical structures of covalent PTP inhibitors (1-9).

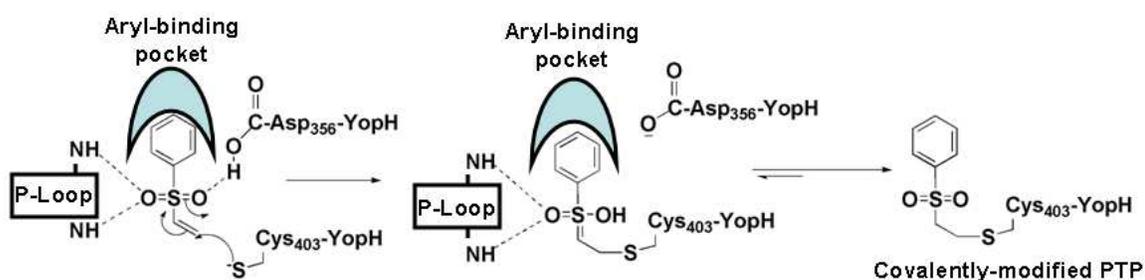
cancerous proteomes, and more recently in the development of a cysteinyl-labeling assay to monitor reversible oxidation of PTPs in angiomyolipoma cells.<sup>37, 42</sup>

*Peptidyl cinnamaldehyde derivative (2, Figure 2):* Pei and colleagues have developed peptide-linked cinnamaldehyde moiety that serves as covalent and reversible inhibitor of PTPs, namely PTP1B, SHP-1, VH1 and VHR.<sup>43, 44</sup> This class of inhibitory

agents are slow-binding and reversible in nature, and display a kinetic profile consistent with a sequential two-step inactivation event. After a rapid non-covalent binding of inhibitory agent to the PTP active site, a conjugated imine adduct slowly forms upon reaction with the guanidine group of an Arg residue of PTP (Figure 3). In the second step, an unidentified nucleophilic group in the PTP active site adds to the benzylic position to



**Figure 3.** Proposed mechanism of SHP-1 (a SH2-domain containing PTP) inhibition by a peptidyl cinnamaldehyde derivative (2). Notable distinction here is that the mechanism of SHP-1 inhibition by this class of agent involves formation of a covalent imine/enamine adduct between the aldehyde group of inhibitory moiety and the guanidine group of Arg from the pTyr binding pocket of SH2 domain.



**Figure 4.** Mechanism of covalent PTP inactivation by aryl vinyl sulfon(at)es. This process involves a nucleophilic attack of invariant active site Cys residue onto the terminal vinyl carbon of inhibitor. The crystallographic data of the inhibited complex clearly shows formation of a carbon-sulfur band between the inhibitor moiety and Cys residue, and attests to the importance of sulfonyl oxygen in binding and subsequent inactivation chemistry.

yield the final enamine-PTP inhibitory complex. It is worth noting here that the mechanism of PTP inactivation by this class of compounds does not involve the invariant active site Cys residue.

*Aryl vinyl sulfon(at)es (3 & 4, Figure 2):* A simple strategy to develop specific PTP inhibitory agents with covalent reactivity is to understand and exploit the unique architecture and chemical reactivities of the PTP active site. This could mean designing molecules that, at minimum, include an aryl moiety with a suitably placed electrophilic center (e.g. Michael acceptor) capable of reacting with the conserved nucleophilic reactivity of low pKa Cys residue of PTP enzymes. Irreversible inhibition of a panel of PTPs by both aryl vinyl sulfones, and aryl vinylsulfonates, as shown by Liu et al, seem to strongly support this hypothesis.<sup>45</sup> The mass spectrometric and crystallographic evidence of the inactivated PTP complex by these agents clearly indicate a covalent bond formation between the nucleophilic Cys residue of PTP and the terminal vinylic carbon of the inhibitory agent. The proposed mechanism of inhibition involves a direct 1,4-conjugate addition of Cys sulfhydryl group onto the terminal vinylic carbon, assisted by a proton donation from the conserved Asp of WPD loop. This process results in the formation of a stable thioether-based inhibitory complex. (Figure 4).<sup>45</sup> Using phenyl vinylsulfonate moiety and employing click chemistry-based protocol, the authors have developed selective activity-based PTP probes, and demonstrated that such probes are exquisitely selective towards labeling PTPs from a complex *E. coli* proteome.

*Aryloxiranes (5, Figure 2):* The well conserved active site Cys residue is a good target residue for development of covalent inactivators of PTPs. Because of its

unusually low pKa (~4), this residue exists as a thiolate ion bearing enhanced nucleophilicity. Keeping this in mind, Cho and coworker investigated the possibility of aryloxiranes (**5**; Figure 2) acting as covalent inactivators of PTPs.<sup>46</sup> In this study, the authors synthesized 2-phenyloxirane, 2-(3-chlorophenyl)oxirane, 2-(4-nitrophenyl)oxirane, and 2-(naphthalen-4-yl)oxirane compounds and studied their inhibitory behavior with PTPs. Thus in separate experiments, the incubation of 2-phenyloxirane (20 mM) for 10 minutes with a panel of PTPs under a pseudo-first order condition ( $[I] \gg [E]$ ) resulted into a loss of various levels of PTP activity: PTP1B: 80%, SHP1-D: 85%, YopH: 25%, YOPT1 and CD45 (cytosolic): <10%. Further, the relative inhibitory potencies of 2-phenyloxirane, 2-(3-chlorophenyl)oxirane, 2-(4-nitrophenyl)oxirane, and 2-(naphthalen-4-yl)oxirane compounds were investigated against PTP1B at 2 mM concentration. The data indicated that 2-(4-nitrophenyl)oxirane was the best inhibitor for PTP1B with 32% inhibition. Finally, the authors alluded that poor aqueous solubility of 2-(naphthalen-4-yl)oxirane) was an impediment in obtaining accurate estimate of its covalent reactivity with PTP1B enzyme.

*Seleninate (6, Figure 2):* The anionic seleninate ( $RSeO_2^-$ ) has been proposed as a potentially bioisosteric match for a phosphate group.<sup>47</sup> In addition, it has been shown that seleninic acids ( $RSeO_2H$ ) can react with thiols ( $R'SH$ ) to give a selenosulfide ( $RSeSR'$ ) as a redox product.<sup>48</sup> These two desirable properties of seleninates have led towards the development of seleninates as a novel class of covalent and irreversible inhibitors of PTPs.<sup>49</sup> This class of inhibitory agent inhibited YopH, PTP1B, VHR and VHX enzymes in a time-dependent manner. A detailed mass

spectrometric-based investigation, coupled with structural and kinetic analysis clearly indicated that the mode of inhibition was active-site directed and resulted from a covalent bond formation between the active site Cys residue and selenium atom of the inhibitor.

*$\alpha$ -Haloacetophenones (7, Figure 2):* This class of compounds is another example where the nucleophilic reactivity of the conserved active site Cys residue was utilized in designing a covalent and photoreversible inactivator of PTP enzymes.<sup>50</sup> Pie and coworkers elegantly showed that four  $\alpha$ -haloacetophenone derivatives displayed a time-dependent inhibition pattern when incubated with both intracellular classical PTPs (PTP1B and SHP-1) and a dual specific PTP (VHR). The mechanism of inhibition presumably involved a direct  $S_N2$  type of attack by the active site Cys residue onto the electrophilic  $\alpha$ -carbon of inhibitory moiety, resulting into the formation of a stable thioether linkage. Further, inhibitor-treated B cells displayed enhanced pTyr level as demonstrated using western-blot analysis. Notably, when 2-bromo-1-phenylethanone – an effective time-dependent inhibitor of PTP1B and SHP-1 – was incubated with alkaline and acid phosphatases, no significant loss in enzyme activity was observed for either enzyme, thereby indicating that these agents employed specific Cys-based PTP chemistry for inactivation. This was further supported using mass spectrometry-based experiments. Most interestingly, the covalent inhibition by these reagents was photoreversible in nature. For example, 80% of the original activity could be quickly recovered upon photolysis of a completely inactivated SHP-1 enzyme. Such photoreversible inhibitory agents of PTPs could prove to be extremely valuable tools in controlling the temporal

activities of intracellular PTPs in cell signaling events.

*2-Methoxy-4H-1,3,2-benzodioxaphosphor-2-one (8, Figure 2):* Ham and coworkers reported that 2-methoxy-4H-1,3,2-benzodioxaphosphor-2-one, also known as salioxon – a known covalent irreversible inhibitor of esterases – inhibited PTP1B, yeast PTP1 (YPTP1), and hSHP-1 $\Delta$  in a time-dependent manner.<sup>51</sup> Preincubation of salioxon (2.0 mM) with PTP1B, YPTP1, and hSHP-1 $\Delta$  in separate experiments resulted into loss of 70%, 70%, and 40% PTP activities respectively. Interestingly this compound did not show any loss in YopH activity under identical conditions. Unfortunately no kinetic parameters and mechanistic inhibition studies were reported, so any conclusions about the inhibitory interactions with the PTP active site at best remain speculative.

*4-Isoavenaciolide (9, Figure 2):* A microbial screening of fungal metabolite led to the discovery of 4-Isoavenaciolide as an inhibitor of primarily dual-specific phosphatases (VHR:  $IC_{50}=1.2 \mu M$ ); Cdc25B:  $IC_{50} = 9.4 \mu M$ ; and Laforin:  $IC_{50} = 1.8 \mu M$ ).<sup>52</sup> This molecule also inhibited classical intracellular enzyme, PTP1B ( $IC_{50} = 10.7 \mu M$ ) but with about ten-fold weaker potency than VHR. Interestingly but not surprisingly, it did not inhibit PP1 and PP2A, two prominent members of serine/threonine phosphatase enzymes, and receptor tyrosine phosphatase CD45. A tandem mass spectrometry-based experiment revealed that the mode of VHR inhibition by **9** involved covalent modification of two Cys residues (active site Cys124 and surface Cys171). This suggests that the mechanism of inhibition by **9** perhaps involves a simple 1, 4-addition of Cys nucleophiles onto the electrophilic exo-

methylene group of inhibitor, thereby yielding an inert thioether enzyme-inhibitor complex.

### Final Thoughts

The studies of PTPs and their involvement in human diseases are beginning to emerge at the forefront of cutting edge biomedical research. Despite this the progress in delineating the role of PTPs in both normal and diseased cell remain slow. To expedite the functional assignment of individual PTPs and validate the hypothesis that small molecule inhibition of PTPs is a viable strategy for undertaking PTP-based therapeutics, selective and potent inhibitory agents are needed. Although some progress has been made towards the development of fast and reversible inhibitory agents of selected PTP enzymes, the progress in the development of fast-reacting covalent inhibitors remain very sluggish. The rich active site chemistry and the uniqueness of surrounding PTP active site pocket(s) offer tremendous opportunities for designing tight-binding and rapidly-reacting (e.g. closer to the inactivation bimolecular rate constant of  $10^4$ - $10^7$   $M^{-1} s^{-1}$ ) covalent inhibitory agents of PTPs with high therapeutic potential. We remain optimistic that advent of such inhibitory agents could eventually lead towards the successful development of PTP-targeting covalent drugs in the near future.

### References:

- [1] Fischer, E. H.; Charbonneau, H.; Cool, D. E.; Tonks, N. K., Tyrosine phosphatases and their possible interplay with tyrosine kinases. *Ciba Found Symp* **1992**, 164, 132-40; discussion 140-4.
- [2] Hunter, T., Protein-tyrosine phosphatases: the other side of the coin. *Cell* **1989**, 58, (6), 1013-6.
- [3] Fischer, E. H.; Tonks, N. K.; Charbonneau, H.; Cicirelli, M. F.; Cool, D. E.; Diltz, C. D.; Krebs, E. G.; Walsh, K. A., Protein tyrosine phosphatases: a novel family of enzymes involved in transmembrane signalling. *Adv Second Messenger Phosphoprotein Res* **1990**, 24, 273-9.
- [4] Fischer, E. H.; Charbonneau, H.; Tonks, N. K., Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes. *Science* **1991**, 253, (5018), 401-6.
- [5] Walton, K. M.; Dixon, J. E., Protein tyrosine phosphatases. *Annu Rev Biochem* **1993**, 62, 101-20.
- [6] Mustelin, T.; Alonso, A.; Bottini, N.; Huynh, H.; Rahmouni, S.; Nika, K.; Louis-dit-Sully, C.; Tautz, L.; Togo, S. H.; Bruckner, S.; Mena-Duran, A. V.; al-Khouri, A. M., Protein tyrosine phosphatases in T cell physiology. *Mol Immunol* **2004**, 41, (6-7), 687-700.
- [7] Hunter, T., Tyrosine phosphorylation in cell signaling and disease. *Keio J Med* **2002**, 51, (2), 61-71.
- [8] Tonks, N. K., Protein tyrosine phosphatases: from genes, to function, to disease. *Nat Rev Mol Cell Biol* **2006**, 7, (11), 833-46.
- [9] Jiang, Z. X.; Zhang, Z. Y., Targeting PTPs with small molecule inhibitors in cancer treatment. *Cancer Metastasis Rev* **2008**, 27, (2), 263-72.
- [10] Zhang, Z. Y., Protein tyrosine phosphatases: prospects for therapeutics. *Curr Opin Chem Biol* **2001**, 5, (4), 416-23.
- [11] Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C. C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P., Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* **1999**, 283, (5407), 1544-8.
- [12] Klamann, L. D.; Boss, O.; Peroni, O. D.; Kim, J. K.; Martino, J. L.; Zabolotny, J. M.; Moghal, N.; Lubkin, M.; Kim, Y. B.; Sharpe, A. H.; Stricker-Krongrad, A.; Shulman, G. I.; Neel, B. G.; Kahn, B. B., Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol Cell Biol* **2000**, 20, (15), 5479-89.
- [13] Hopkins, A. L., Network pharmacology: the next paradigm in drug discovery. *Nat Chem Biol* **2008**, 4, (11), 682-90.
- [14] Knight, Z. A.; Lin, H.; Shokat, K. M., Targeting the cancer kinome through polypharmacology. *Nat Rev Cancer* **10**, (2), 130-7.
- [15] Escudier, B.; Eisen, T.; Stadler, W. M.; Szczylik, C.; Oudard, S.; Siebels, M.; Negrier, S.; Chevreau, C.; Solska, E.; Desai, A. A.; Rolland, F.; Demkow, T.; Hutson, T. E.; Gore, M.; Freeman, S.; Schwartz, B.; Shan, M.; Simantov, R.; Bukowski, R. M., Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med* **2007**, 356, (2), 125-34.

- [16] Ahmad, T.; Eisen, T., Kinase inhibition with BAY 43-9006 in renal cell carcinoma. *Clin Cancer Res* **2004**, 10, (18 Pt 2), 6388S-92S.
- [17] Tan, J.; Town, T.; Mori, T.; Wu, Y.; Saxe, M.; Crawford, F.; Mullan, M., CD45 opposes beta-amyloid peptide-induced microglial activation via inhibition of p44/42 mitogen-activated protein kinase. *J Neurosci* **2000**, 20, (20), 7587-94.
- [18] Masliah, E.; Mallory, M.; Hansen, L.; Alford, M.; Albright, T.; Terry, R.; Shapiro, P.; Sundsmo, M.; Saitoh, T., Immunoreactivity of CD45, a protein phosphotyrosine phosphatase, in Alzheimer's disease. *Acta Neuropathol* **1991**, 83, (1), 12-20.
- [19] Liang, F.; Lee, S. Y.; Liang, J.; Lawrence, D. S.; Zhang, Z. Y., The role of protein-tyrosine phosphatase 1B in integrin signaling. *J Biol Chem* **2005**, 280, (26), 24857-63.
- [20] Zabolotny, J. M.; Bence-Hanulec, K. K.; Stricker-Krongrad, A.; Haj, F.; Wang, Y.; Minokoshi, Y.; Kim, Y. B.; Elmquist, J. K.; Tartaglia, L. A.; Kahn, B. B.; Neel, B. G., PTP1B regulates leptin signal transduction in vivo. *Dev Cell* **2002**, 2, (4), 489-95.
- [21] Kaszubska, W.; Falls, H. D.; Schaefer, V. G.; Haasch, D.; Frost, L.; Hessler, P.; Kroeger, P. E.; White, D. W.; Jirousek, M. R.; Trevillyan, J. M., Protein tyrosine phosphatase 1B negatively regulates leptin signaling in a hypothalamic cell line. *Mol Cell Endocrinol* **2002**, 195, (1-2), 109-18.
- [22] Stockwell, B. R., Exploring biology with small organic molecules. *Nature* **2004**, 432, (7019), 846-54.
- [23] Tan, D. S., Diversity-oriented synthesis: exploring the intersections between chemistry and biology. *Nat Chem Biol* **2005**, 1, (2), 74-84.
- [24] Singh, J.; Petter, R. C.; Baillie, T. A.; Whitty, A., The resurgence of covalent drugs. *Nat Rev Drug Discov* 10, (4), 307-17.
- [25] Andersen, J. N.; Jansen, P. G.; Echwald, S. M.; Mortensen, O. H.; Fukada, T.; Del Vecchio, R.; Tonks, N. K.; Moller, N. P., A genomic perspective on protein tyrosine phosphatases: gene structure, pseudogenes, and genetic disease linkage. *Faseb J* **2004**, 18, (1), 8-30.
- [26] Zhang, Z. Y., Chemical and mechanistic approaches to the study of protein tyrosine phosphatases. *Acc Chem Res* **2003**, 36, (6), 385-92.
- [27] Alonso, A.; Sasin, J.; Bottini, N.; Friedberg, I.; Friedberg, I.; Osterman, A.; Godzik, A.; Hunter, T.; Dixon, J.; Mustelin, T., Protein tyrosine phosphatases in the human genome. *Cell* **2004**, 117, (6), 699-711.
- [28] Bottini, N.; Bottini, E.; Gloria-Bottini, F.; Mustelin, T., Low-molecular-weight protein tyrosine phosphatase and human disease: in search of biochemical mechanisms. *Arch Immunol Ther Exp (Warsz)* **2002**, 50, (2), 95-104.
- [29] Chiarugi, P.; Taddei, M. L.; Schiavone, N.; Papucci, L.; Giannoni, E.; Fiaschi, T.; Capaccioli, S.; Raugei, G.; Ramponi, G., LMW-PTP is a positive regulator of tumor onset and growth. *Oncogene* **2004**, 23, (22), 3905-14.
- [30] Chiarugi, P.; Cirri, P.; Taddei, M. L.; Giannoni, E.; Fiaschi, T.; Buricchi, F.; Camici, G.; Raugei, G.; Ramponi, G., Insight into the role of low molecular weight phosphotyrosine phosphatase (LMW-PTP) on platelet-derived growth factor receptor (PDGF-r) signaling. LMW-PTP controls PDGF-r kinase activity through TYR-857 dephosphorylation. *J Biol Chem* **2002**, 277, (40), 37331-8.
- [31] Hengge, A. C.; Sowa, G. A.; Wu, L.; Zhang, Z. Y., Nature of the transition state of the protein-tyrosine phosphatase-catalyzed reaction. *Biochemistry* **1995**, 34, (43), 13982-7.
- [32] Sundaresan, M.; Yu, Z. X.; Ferrans, V. J.; Irani, K.; Finkel, T., Requirement for generation of H<sub>2</sub>O<sub>2</sub> for platelet-derived growth factor signal transduction. *Science* **1995**, 270, (5234), 296-9.
- [33] Tonks, N. K.; Diltz, C. D.; Fischer, E. H., Characterization of the major protein-tyrosine-phosphatases of human placenta. *J Biol Chem* **1988**, 263, (14), 6731-7.
- [34] Sullivan, S. G.; Chiu, D. T.; Errasfa, M.; Wang, J. M.; Qi, J. S.; Stern, A., Effects of H<sub>2</sub>O<sub>2</sub> on protein tyrosine phosphatase activity in HER14 cells. *Free Radic Biol Med* **1994**, 16, (3), 399-403.
- [35] Rhee, S. G., Cell signaling. H<sub>2</sub>O<sub>2</sub>, a necessary evil for cell signaling. *Science* **2006**, 312, (5782), 1882-3.
- [36] Denu, J. M.; Tanner, K. G., Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. *Biochemistry* **1998**, 37, (16), 5633-42.
- [37] Boivin, B.; Zhang, S.; Arbiser, J. L.; Zhang, Z. Y.; Tonks, N. K., A modified cysteinyl-labeling assay reveals reversible oxidation of protein tyrosine phosphatases in angiomyolipoma cells. *Proc Natl Acad Sci U S A* **2008**, 105, (29), 9959-64.
- [38] Lou, Y. W.; Chen, Y. Y.; Hsu, S. F.; Chen, R. K.; Lee, C. L.; Khoo, K. H.; Tonks, N. K.; Meng, T. C., Redox regulation of the protein tyrosine phosphatase PTP1B in cancer cells. *Febs J* **2008**, 275, (1), 69-88.
- [39] Cravatt, B. F.; Wright, A. T.; Kozarich, J. W., Activity-based protein profiling: from enzyme chemistry to proteomic chemistry. *Annu Rev Biochem* **2008**, 77, 383-414.
- [40] Taylor, W. P.; Zhang, Z. Y.; Widlanski, T. S., Quiescent affinity inactivators of protein tyrosine phosphatases. *Bioorg Med Chem* **1996**, 4, (9), 1515-20.
- [41] Kumar, S.; Zhou, B.; Liang, F.; Wang, W. Q.; Huang, Z.; Zhang, Z. Y., Activity-based probes for

- protein tyrosine phosphatases. *Proc Natl Acad Sci U S A* **2004**, 101, (21), 7943-8.
- [42] Kumar, S.; Zhou, B.; Liang, F.; Yang, H.; Wang, W. Q.; Zhang, Z. Y., Global analysis of protein tyrosine phosphatase activity with ultra-sensitive fluorescent probes. *J Proteome Res* **2006**, 5, (8), 1898-905.
- [43] Fu, H.; Park, J.; Pei, D., Peptidyl aldehydes as reversible covalent inhibitors of protein tyrosine phosphatases. *Biochemistry* **2002**, 41, (34), 10700-9.
- [44] Park, J.; Fu, H.; Pei, D., Peptidyl aldehydes as slow-binding inhibitors of dual-specificity phosphatases. *Bioorg Med Chem Lett* **2004**, 14, (3), 685-7.
- [45] Liu, S.; Zhou, B.; Yang, H.; He, Y.; Jiang, Z. X.; Kumar, S.; Wu, L.; Zhang, Z. Y., Aryl vinyl sulfonates and sulfones as active site-directed and mechanism-based probes for protein tyrosine phosphatases. *J Am Chem Soc* **2008**, 130, (26), 8251-60.
- [46] Park, D.; Shim, Y. S.; Kin, K. C.; Park, J.; Yang, D.; Cho, H., Inactivation of Protein Tyrosine Phosphatases by Aryloxiranes. *J Korean Chem. Soc.* **2002**, 46, (3), 296-300.
- [47] Rye, C. S.; Baell, J. B., Phosphate isosteres in medicinal chemistry. *Curr Med Chem* **2005**, 12, (26), 3127-41.
- [48] Kice, J. L.; Lee, T. W. S., Oxidation-Reduction Reactions of Organoselenium Compounds. 1. Mechanism of the Reaction between Seleninic Acids and Thiols. *J. Am. Chem. Soc.* **1978**, 100, 5094-5102.
- [49] Abdo, M.; Liu, S.; Zhou, B.; Walls, C. D.; Wu, L.; Knapp, S.; Zhang, Z. Y., Seleninate in place of phosphate: irreversible inhibition of protein tyrosine phosphatases. *J Am Chem Soc* **2008**, 130, (40), 13196-7.
- [50] Arabaci, G.; Yi, T.; Fu, H.; Porter, M. E.; Beebe, K. D.; Pei, D., alpha-bromoacetophenone derivatives as neutral protein tyrosine phosphatase inhibitors: structure-Activity relationship. *Bioorg Med Chem Lett* **2002**, 12, (21), 3047-50.
- [51] Cho, H.; Park, J.; Yang, D.; Ham, S. W., Inactivation of Protein Tyrosine Phosphatases by Salioxon. *Bull. Korean Chem. Soc.* **2000**, 21, (5), 515-517.
- [52] Ueda, K.; Usui, T.; Nakayama, H.; Ueki, M.; Takio, K.; Ubukata, M.; Osada, H., 4-isoavenaciolide covalently binds and inhibits VHR, a dual-specificity phosphatase. *FEBS Lett* **2002**, 525, (1-3), 48-52.