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Targeting Tyrosine Phosphatases: Time to End the Stigma

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Abstract

Protein tyrosine phosphatases (PTPs) are a family of enzymes essential for numerous cellular processes, and several PTPs have been validated as therapeutic targets for human diseases. Historically, development of drugs targeting PTPs has been highly challenging, leading to stigmatization of these enzymes as undruggable targets. Despite these difficulties, efforts to drug PTPs have persisted, and recent years have seen an influx of new probes, providing opportunities for biological examination of old and new PTP targets. Here we will discuss progress towards drugging PTPs, with special emphasis on development of selective probes with biological activity. We will describe development of new small-molecule orthosteric, allosteric and oligomerization PTP inhibitors, and discuss new studies targeting the receptor PTP subfamily with biologics.

Keywords

Protein tyrosine phosphatase; drug target; inhibitor; small-molecule; allosteric; biologic

Protein tyrosine phosphatases

Tyrosine phosphorylation of intracellular proteins is a post-translational modification used to control cell signaling in nearly every biological context[1]. Tyrosine phosphorylation is controlled by the opposing actions of protein tyrosine kinases (**PTKs, see Glossary**), which catalyze phosphorylation of proteins on tyrosine residues, and protein tyrosine phosphatases (**PTPs**), which remove the phosphate[1]. This dynamic regulates a range of cellular processes including survival, growth, migration, differentiation and energy metabolism; consequently, anomalous tyrosine phosphorylation is implicated in numerous human diseases[2,3]. Agents targeting PTKs and PTPs have been heavily pursued for therapeutic interventions, and although several drugs targeting PTKs are in clinical use[3], PTP-targeted drugs are not yet available.

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Conflict of Interest

The authors have no conflicts of interest to declare.

More than 100 PTPs are encoded in the human genome, and are organized into three major classes (Box 1)[2,4,5]. As shown in Table 1, all three classes are represented among PTPs under consideration as drug targets[6].

During the last 15 years, extensive data validating protein tyrosine phosphatase 1B (PTP1B) – an inhibitor of insulin signaling and SH2 domain-containing PTP 2 (SHP-2) –an oncogene and promoter of growth factor signaling- as therapeutic targets for type 2 diabetes/obesity and cancer sparked considerable excitement –and use of resources- in drugging these enzymes[2,6]. Major programs in industry and academic laboratories were dedicated to development of small-molecule PTP inhibitors. These programs largely focused on orthosteric inhibitors; however, efforts were frustrated by the highly charged and highly conserved nature of the PTP active-site. While the charged active-site allows for high affinity accommodation of negatively charged pTyr residues, potent orthosteric PTP inhibitors tend also to be highly charged, which can limit their cell-permeability, bioavailability, and potential for drug development. The high level of active-site conservation among PTPs adds another layer of difficulty, as potent inhibitors often target multiple PTPs. Ultimately, the generation of potent, selective, bioavailable PTP inhibitors suitable for therapeutic use was largely unsuccessful, and PTPs acquired a reputation as “challenging”, “intractable” and “undruggable” targets[2,6].

Despite these setbacks, efforts to drug PTPs continued, and recent years experienced a resurgent global interest in these enzymes. In addition to noteworthy progress in competitive, orthosteric PTP inhibitor development, an influx of new strategies to attack these enzymes has occurred. Moreover, an increasing number of PTPs are being proposed as clinically relevant targets. Here we will describe recent progress towards drugging PTPs, calling particular attention to approaches –orthosteric, allosteric and oligomerization-inhibiting small-molecule, and biologic-(Figure 1, Key Figure) yielding selective agents with biological activity.

Trends in small-molecule PTP inhibitor development

Orthosteric small-molecule inhibitors

While an orthosteric, or active-site, small-molecule approach (Figure 1a) must face the difficulties of the PTP active-site head-on, this area of inhibitor development has seen tremendous persistence. Remarkably, the traditional approach of reversible competitive inhibition is still being sought and yielding some excellent probes. Additionally, alternative methods, such as uncompetitive inhibition and irreversible inhibition are also being explored. As a result of these efforts, high-quality **orthosteric inhibitors** are emerging (Table 2), providing new opportunities for biological examination of old and new targets.

Reversible competitive inhibitors

Reversible inhibitors bind to enzymes, typically through noncovalent interactions, with rapid association and dissociation rates[7]. Competitive inhibitors bind to an enzyme at the site of substrate binding, hence competing with substrate for binding to the enzyme. In competitive inhibition, binding of either the substrate or inhibitor to the enzyme is mutually exclusive[8].

Here we will discuss the selective, reversible competitive inhibitors that are being used to validate several PTPs as drug targets.

CPT-157633, a difluoromethylphosphonic acid PTP1B inhibitor, was used to explore Rett syndrome (RTT) as a new indication for PTP1B [9]. RTT is an X-linked neurodevelopmental disorder often caused by mutations in the transcriptional regulator methyl CpG-binding protein 2 (MECP2)[10]. This study showed that PTP1B expression was suppressed by MECP2, and that PTP1B was upregulated in RTT patients and in the heterozygous *Mecp2*-null RTT mouse model. CPT-157633 is a potent active-site inhibitor with selectivity over several PTPs, albeit moderate for the structurally similar T cell protein tyrosine phosphatase (TCPTP). Daily administration of 5 mg/kg CPT-157633 intraperitoneally (i.p.) or subcutaneously (s.c.) improved behavior and motor skills in *Mecp2*$^{-/+}$ female mice and increased survival in *Mecp2*$^{-/y}$ male mice. PTP1B was previously proposed to inhibit tyrosine phosphorylation of the tropomyosin receptor kinase B (TRKB) -the receptor for brain derived neurotrophic factor-, which is downregulated during RTT[11]. CPT-157633 administration increased brain TRKB Tyr phosphorylation in wild-type (WT) and *Mecp2*$^{-/+}$ female mice, and a substrate-trapping PTP1B mutant precipitated TRKB from mouse brain lysates, suggesting TRKB is a PTP1B substrate in the brain and that augmenting this pathway through PTP1B inhibition could be an RTT therapeutic strategy[9].

Multiple lines of evidence suggest inhibition of PTPN22 –encoded by a major autoimmunity gene- as a strategy for eliminating autoreactive lymphocytes in carriers of an autoimmune-predisposing *PTPN22* variant (C1858T)[12]. PTPN22 inhibitor LTV-1 was identified by screening with small-molecule substrate 3-O-methylfluorescein phosphate (OMFP)[13]. Molecular docking and structure-activity relationship (SAR) studies suggested that LTV-1 interacts with the active-site phosphate-binding loop and a nearby hydrophobic pocket when PTPN22 is in an open conformation. LTV-1 shows moderate selectivity for PTPN22 over PTP1B and TCPTP; however, it is highly selective over other PTPs, including the closely-related PTP-PEST. Since PTPN22 is an inhibitor of T cell receptor (TCR) signaling, LTV-1 activity in cells was confirmed in PTPN22-dependent TCR-signaling assays in the Jurkat-TAg T cell line and in primary human T cells. LTV-1 was used in NOD-scid-common γ chain knockout (NSG) mice engrafted with human hematopoietic stem cells (HSCs) to examine the role of PTPN22 in central B cell tolerance[14]. Engraftment of HSCs from *PTPN22*-C1858T carriers led to increased development of autoreactive B cells compared to engraftment of cells from non-carriers; this phenotype was significantly reduced by treatment of engrafted mice with LTV-1. A similar reduction in autoreactive B cell frequency was obtained when HSCs from *PTPN22*-C1858T carriers subjected to PTPN22 knockdown were engrafted. These findings suggest potential for PTPN22 inhibition in resetting impaired B cell tolerance in autoimmune patients carrying the *PTPN22*-C1858T allele.

The α-sulfophenylacetic amide (SPAA) pharmacophore from Cefsulodin, a β-lactam antibiotic that inhibits several PTPs, inspired generation of selective competitive inhibitors of low-molecular weight PTP (LMPTP or LMW-PTP)[15], an inhibitor of insulin receptor (IR) phosphorylation[16]. Compound 28 was identified from a library generated by reacting α-sulfophenylacetic acid with varying amines, followed by structure-guided medicinal chemistry[15]. X-ray co-crystallization revealed an “induced-fit mechanism”, in which

Compound 28 induces an active-site conformational change, generating a hydrophobic cavity that accommodates the inhibitor α-phenyl ring. The extremely high selectivity for LMPTP over other PTPs is likely explained by differences in the active-site signature motif residues between LMPTP and Class I PTPs, which contribute to the active-site electrostatic charge and shape. Additionally, low-μM concentrations of Compound 28 increased insulin-induced protein kinase B (PKB/AKT) phosphorylation in human HepG2 hepatocytes[15].

Fragment-based optimization of SPAA also led to discovery of L335-M34, a selective inhibitor of *Mycobacterium tuberculosis* (*Mtb*) LMPTP (mPTPA), an *Mtb* virulence factor[17]. L335-M34 does not inhibit human LMPTP, and decreased bacterial load in *Mtb*-infected macrophages (IC_{90}=1.38 μM), without displaying *in vitro* anti-*Mtb* activity[17]. Importantly, L335-M34 displayed oral bioavailability when administered to guinea pigs without causing weight loss or overt toxicity. When administered to guinea pigs orally together with the anti-tuberculosis combination treatment isoniazid, rifampin, pyrazinamide (HRZ) 4 weeks after *Mtb* infection, lung inflammation was substantially lower compared to guinea pigs treated with HRZ alone after 6 weeks. Additionally, treatment with HRZ+L335-M34 combined with the non-competitive inhibitor of mPTPB –also an *Mtb* virulence factor-L01-Z08[18] for 2 weeks significantly reduced lung bacillary burden compared to HRZ treatment alone. These findings suggest targeting mPTPA or mPTPA+mPTPB as a potential combination therapy strategy for tuberculosis infection.

An exciting development in the PTP field is the progression of orthosteric small-molecule vascular endothelial PTP (VE-PTP) inhibitor AKB-9778 into clinical trials for diabetic macular edema (DME)[19,20] (Box 2). *In vivo* VE-PTP inhibition is hypothesized to activate the VE-PTP substrate angiopoietin 1 receptor 2 (Tie2), leading to reduced vascular leakage and ocular neovascularization[21,22]. AKB-9778 was developed from a phenylsulfamic acid core identified in Proctor and Gamble Pharmaceutical's corporate repository as a PTP-inhibiting pTyr mimetic[23]. Structure-based drug design led to AKB-9778, which displays remarkable potency on VE-PTP (IC_{50}=17 pM) and selectivity over most other phosphatases, with notable exceptions being receptor PTPs (**RPTPs**) density enhanced phosphatase 1 (DEP-1) and RPTPγ[21]. AKB-9778 administration in mice promoted Tie2 tyrosine phosphorylation in retinal endothelial cells and reduced ocular neovascularization and VEGF-induced vascular permeability[21,22]. Evidence supporting specific VE-PTP targeting *in vivo* includes: 1) AKB-9778 treatment phenocopied the effect of inducible VE-PTP deletion in adult mice -causing increased Tie2 phosphorylation and reduced vascularization- yet had no effect in mice lacking VE-PTP; 2) AKB-9778 inhibited vascular permeability equally in WT and DEP-1 knockout (KO) mice; 3) administration of anti-VE-PTP blocking antibody to mice led to similar phenotypic results as AKB-9778, reducing VEGF-induced vascular permeability and reduced ocular neovascularization[21,22].

Reversible bidentate inhibitors

Zhong-Yin Zhang's laboratory pioneered the development of PTP **bidentate inhibitors**, which are small-molecules containing a core group for interaction with a PTP active-site and peripheral group for interaction with a proximal –ideally non-conserved- secondary site[24].

The SHP-2 inhibitor 11a-1 resulted from an attempt to generate bidentate SHP-2 inhibitors, and was recently employed to demonstrate that SHP-2 –a known target for cancer–is also a promising target for rheumatoid arthritis[25]. A precursor inhibitor, II-B08, was generated from screening a combinatorial library containing a pTyr mimetic salicylic acid core for binding the PTP active-site and a structurally diverse set of amines and hydrazines for additional interactions[26]. II-B08 inhibits non-competitively; co-crystallization revealed the salicylic acid core interacts with the phosphate-binding loop of the SHP-2 active-site, and the distal phenyl ring interacts within the SHP-2 active-site with the β5–β6 loop (aa 362–365), or E-loop. The **E-loop** is a component of the PTP active-site named after a conserved glutamate residue in classical PTPs that in many structures forms a β-hairpin[5]. Pairing an oxalic linker to a biaryl substituent led to 11a-1, which displays ≥5-fold selectivity for other PTPs[27]. Daily administration of 15 mg/kg i.p. significantly reduced growth of established melanoma tumors in a mouse xenograft model without affecting body weight[28]. We demonstrated that SHP-2 expression is increased in fibroblast-like synoviocytes (FLS) – joint-lining cells that become invasive and contribute to joint destruction during rheumatoid arthritis (RA)- from RA patients, and that SHP-2 promotes platelet-derived growth factor and tumor necrosis factor (TNF) signaling in these cells[29]. Heterozygous deletion of SHP-2 in radioresistant cells (which include FLS) or acute heterozygous deletion in myeloid cells significantly reduced arthritis development in the K/BxN serum transfer mouse model, which is dependent on actions of FLS and myeloid cells[25]. 11a-1 treatment reduced RA FLS migration and expression of mediators of invasiveness in response to TNF and interleukin (IL)-1, and daily administration substantially decreased K/BxN arthritis[25].

Reversible uncompetitive inhibitors

We recently discovered an orthosteric **uncompetitive inhibitor** of human LMPTP[30]. Uncompetitive inhibition occurs when an inhibitor binds to an enzyme-substrate complex[8]. Consistent with the above-mentioned role of LMPTP in dephosphorylating the IR, we found that global and liver-specific LMPTP KO protects mice from high-fat diet induced diabetes without affecting body weight[30]. To validate LMPTP as a new type 2 diabetes target, we developed an LMPTP inhibitor[30]. This series was identified by compound screening using a high concentration of OMFP substrate, such that the enzyme was at V_{max}. Counterscreening and medicinal chemistry led to a quinoline core-based series of LMPTP inhibitors, exemplified by compound (Compd.) 23. Through isothermal titration calorimetry (ITC), nuclear magenetic resonance (NMR) spectroscopy, X-ray crystallography, hydroxyl radical footprinting, and mutagenesis, we determined that during catalysis, these inhibitors bind to the entrance of the active-site of the LMPTP phospho-Cys intermediate, excluding water from the active-site and preventing the release of phosphate required for the final step in catalysis. Other features include exquisite selectivity for LMPTP over other PTPs, oral bioavailability, and efficacy at increasing liver IR tyrosine phosphorylation and reversing high-fat diet-induced diabetes when administered to mice as a food admixture. Compd. 23 did not cause weight loss in mice and had no effect on diabetes or liver IR tyrosine phosphorylation levels in mice lacking liver-specific expression of LMPTP, confirming the specificity of the inhibitor *in vivo* and the action of LMPTP in the liver[30]. These findings suggest LMPTP as a key promoter of insulin resistance and that LMPTP inhibitors could have potential for ameliorating type 2 diabetes.

Irreversible inhibitors

PTPs can be irreversibly inhibited by covalent binding or oxidation of the active-site Cys, and this method has been used for inhibition of striatal-enriched PTP (STEP)[31] and cell division cycle 25 (CDC25)[32]. Deletion of central nervous system-expressed STEP improves cognition in Alzheimer's disease (AD) mouse models, pointing to STEP as a promising target for AD[33]. Identification of STEP inhibitor TC-2153 resulted from serendipitous contamination of elemental sulfur in a library used to screen for STEP inhibitors[31]. The cyclicpolysulfide-containing TC-2153 -a reported anti-anxiolytic and anti-convulsant in mice- was subsequently sought after because of its similar chemistry. The inhibition mechanism of TC-2153 likely involves a covalent interaction between the STEP catalytic Cys472 and a TC-2153 sulfur atom. TC-2153 displays moderate selectivity for STEP against the closely-related hematopoietic PTP (HePTP) and STEP-like PTP (PTP-SL), and greater selectivity towards other PTPs. TC-2153 increased tyrosine phosphorylation of STEP substrates glutamate ionotropic receptor NMDA type subunit 2B, protein tyrosine kinase 2 beta (Pyk2) and extracellular signal-related kinase (ERK) 1/2 in cortical neuron cultures from WT but not STEP KO mice. *In vivo* TC-2153 administration caused increased ERK1/2 and Pyk2 phosphorylation only in brain tissues expressing STEP, and improved novel object recognition and reference memory in the 3xTg-AD model. TC-2153 treatment also improved cognitive and motor function in the phencyclidine-induced mouse model of schizophrenia[34].

CDC25 enzymes are overexpressed is different types of cancer, and though most available inhibitors are not selective among the 3 CDC25A/B/C proteins, collective inhibition is viewed as a cancer therapy strategy[35]. The most potent reported CDC25 inhibitor IRC-083864 (IC_{50}~20–50 nM) was developed by improvement of quinone-based BN82685[32]. IRC-083864 is a heterocyclic bis-quinone with high selectivity over other phosphatases. Its inhibitory mechanism was not reported, but BN82865 irreversibly inhibits CDC25 *in vitro*, and these compounds were suggested to deactivate CDC25 enzymes by binding covalently to or oxidizing the active-site Cys. Oral administration of BN82865 and IRC-083864 inhibited growth of pancreatic and prostate carcinoma tumors, respectively, in nude mouse xenograft models, and intravenous (i.v.) administration of IRC-083864 inhibited pancreatic carcinoma tumor growth[35]. Recently, IRC-083864 was shown to inhibit clonogenic capacity of primary acute myeloid leukemia (AML) cells expressing the transforming Fms-like tyrosine kinase 3 internal tandem duplication[36]. IRC-083864 was licensed by Debiopharm Group as Debio 0931 for clinical development.

Allosteric small-molecule inhibitors

A trend surfacing in PTP inhibitor development is the appearance of allosteric small-molecules. In theory, allostery offers occasions for selective and cell-permeable inhibition by avoiding the charged PTP active-site. The first report occurred when Sunesis Pharmaceuticals published a crystal structure of PTP1B in complex with a benzofuran sulfonamide derivative of benzbromarone[37], revealing an allosteric site that can be exploited to inhibit PTP1B by blocking closure of the **WPD loop**[38]. However, while initial **allosteric inhibitors** targeting PTP1B[38] and PTPN22[39] achieved cell-permeability, these early compounds still lacked potency and selectivity. Persistent attempts resulted in

several improved PTP inhibitors (Table 3). These compounds bind outside the active-site, and most exploit unique structural features to lock the enzyme in a catalytically unfavorable conformation. Since crystallization of PTP regions outside the catalytic domain can prove difficult, these studies illuminate the importance of biophysical methods for identifying compound binding sites when co-crystallization may not be feasible.

An allosteric dual-specific phosphatase (DUSP) inhibitor was found by compound screening using transgenic zebrafish embryos expressing GFP as a reporter for fibroblast growth factor (FGF) signaling. (E)-2-benzylidene-3-(cyclohexylamino)-2,3-dihydro-1H-inden-1-one (BCI) showed EC_{50}=10.6 μM in this assay, and was subsequently identified as a DUSP6 inhibitor[40]. BCI also inhibits DUSP1, and is selective over vaccinia H1-related phosphatase (VHR) and DUSP5. DUSP6 is catalytically activated by binding to ERK substrate[41], and *in vitro*, BCI inhibited DUSP6 dephosphorylation of OMFP only in the presence of ERK[40]. Although biophysical data to explain the mechanism of action of BCI on DUSP6 is not yet available, BCI was predicted by molecular docking to allosterically inhibit ERK-induced DUSP6 activation by binding the crevice between the DUSP6 general acid loop and helix α7, preventing the positioning needed for Asp262 to act as an acid during catalysis[40,42]. BCI was used to implicate DUSP6 in acute lymphoblastic leukemia (ALL) cell transformation through negative regulation of ERK[43]. BCI treatment of patient-derived ALL cells caused ERK hyperactivation and induced cell death, suggesting potential for DUSP6 as an ALL therapeutic target[43].

Compound 211 is a selective inhibitor of CD45 –a PTP that promotes antigen receptor signaling in lymphocytes and is considered a drug target for autoimmunity[44]- identified by *in silico* screening for compounds predicted to bind the interface between CD45-D1 and -D2 domains[45]. Compound 211 is an irreversible, non-competitive inhibitor. Circular dichroism analysis indicated that Compound 211 produces dramatic changes in D1–D2 secondary structure suggestive of protein unfolding in CD45 but not leukocyte antigen-related PTP (LAR). This compound increased phosphorylation of CD45 substrate lymphocyte-specific protein tyrosine kinase (LCK) Y394 in Jurkat, but not CD45-null (J.45) cells, and blocked early and proximal TCR signaling. A 3 mg/kg i.p. dose substantially reduced inflammation in the delayed-type hypersensitivity mouse model[45].

Exploiting an auto-inhibitory mechanism of SHP-2, an allosteric approach led to a major progression in SHP-2 inhibitor development[46,47]. Compounds were screened for inhibition of full-length SHP-2 using the small-molecule substrate 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) in the presence of a bis-phosphorylated peptide that released SHP-2 from its autoinhibited form, while counterscreening assays eliminated hits acting only on the PTP domain. Multiple rounds of medicinal chemistry improved inhibitor potency, resulting in SHP099. Co-crystallization revealed SHP099 binds the pocket created by the active-site and SH2 domains in the closed SHP-2 conformation, stabilizing the auto-inhibited form. Other features of SHP099 include its excellent selectivity, efficacy in cell-based assays dependent upon SHP-2 expression, oral bioavailability, and tumor growth inhibition in mouse xenograft models without affecting body weight[46].

The discovery of PTP1B as the target of the aminosterol MSI-1436 (Trodusquemine)[48], which acts as an appetite suppressant in mice[49], raised interest in defining MSI-1436's inhibitory mechanism. The Tonks and Peti groups found that MSI-1436 allosterically inhibited PTP1B by targeting its disordered C-terminal region[50]. MSI-1436 induced a PTP1B conformational change detected by trypsin sensitivity and fluorescence resonance energy transfer (FRET). ITC revealed MSI-1436 bound PTP1B within the C-terminal region (K_d=0.3 μM) and catalytic domain (K_d=2 μM). Biomolecular NMR spectroscopy demonstrated that PTP1B C-terminal residues (300–393) were flexible, predominantly disordered, and contained 2 α-helices. Residues in the C-terminal helix α9′ were most perturbed by MSI-1436 binding; helix-destabilizing mutations confirmed that this helix was crucial for inhibition. NMR spectroscopy followed by mutagenesis localized the second binding site, which was within the PTP1B catalytic domain close to the catalytic pocket and was partly overlapping with the previously described allosteric PTP1B site[38]. Administration of MSI-1436 markedly inhibited tumor growth and metastasis in mouse models of breast cancer[50]. MSI-1436 is currently in a Phase I Clinical Trial for metastatic breast cancer.

Allosteric oxidation-stabilizing inhibitors

An up-and-coming approach is PTP-targeting with **oxidation-stabilizing inhibitors**. While such small-molecules are not yet reported, Nicholas Tonks' group provided proof-of-principle that intracellular oxidized PTP1B (PTP1B-OX) can be stabilized with antibodies[51,52]. PTPs are oxidized and inactivated by reactive oxygen species (ROS) produced in response to a variety of stimuli[53]. Mild oxidation is reversible and leads to conformational changes in PTP structure. In the presence of ROS, the PTP1B catalytic Cys undergoes oxidation-induced sulfenylamide formation, which inactivates the enzyme[54,55]. Single-chain variable antibody fragments against PTP1B-OX were generated by leveraging active-site double mutant PTP1B-C215A/S216A (CASA), which adopts a conformation similar to PTP1B-OX, as an epitope[51,52]. Clone scFv45 reacted with PTP1B-OX and PTP1B-CASA, but not with reduced PTP1B, and inhibited PTP1B-OX reactivation (IC_{50}=19 nM) by reducing agent. When expressed in mammalian cells, scFv45 immunoprecipitated PTP1B-OX after H_2O_2 or insulin stimulation and colocalized with PTP1B-OX. scFv45 showed notable selectivity for PTP1B-OX over oxidized TCPTP[53]. Expression of scFv45, but not a non-targeting intrabody, enhanced IR phosphorylation and downstream signaling, suggesting PTP1B-OX can be stabilized intracellularly. Given the numerous PTPs whose activities are affected by reversible oxidization and nitrosylation[53], this study indicates the untapped potential that underlies various molecular PTP conformations.

Oligomerization small-molecule inhibitors

The Zhang group developed a small-molecule **oligomerization inhibitor** to inhibit the function of phosphatase of regenerating liver (PRL) PTPs, which are targets for tumor growth and metastasis [56,57], by disrupting their trimerization[58]. This compound series, exemplified by Compound 43, was identified from *in silico* library screening for PRL-1 trimerization disruptors, inhibited PRL trimerization without affecting its catalytic activity, and reduced viability and migration of cancer cell lines at low-μM concentrations[58]. Daily

treatment of mice with 30 mg/kg Compound 43 i.p. blocked tumor growth in a melanoma xenograft model. This series disrupts trimerization of all three PRL proteins, however was well-tolerated in mice after 3 weeks of treatment[58], suggesting collective inhibition of PRL trimerization might be a viable option for cancer.

Trends in PTP-targeted biologics development

Here we will discuss recent progress in the development of **biologic agents** targeting RPTPs.

Radioimmunotherapy-delivering antibodies

As a transmembrane PTP highly expressed on hematopoietic cells, for decades CD45 has been the object of **radioimmunotherapy** strategies[59,60]. Studies are currently ongoing to employ anti-CD45 mAbs to improve outcome during hematopoietic cell transplantation (HCT). These include efforts to reduce the conditioning regimen for patients undergoing allogeneic transplantation[61], minimizing graft-versus-host disease without need of total-body irradiation[62], and delivering α-emitting radionuclides to hematopoietic tissues[63,64].

RPTP decoy biologics

The RPTP subfamily is unique among PTPs in their potential to be targeted through their intracellular wedge motifs or extracellular regions. Recent studies show that administration of an RPTPσ **cell-penetrating wedge peptide mimetic** (ISP) promoted innervation and functional restoration in mice following spinal cord injury[65,66]. RPTPσ is a receptor for chondroitin sulphate proteoglycans (CSPGs), an abundant extracellular matrix (ECM) component in the scar tissue that is generated after CNS injury. Activation of RPTPσ by CSPG transduces an inhibitory signal that inhibits axon regeneration. ISP treatment of cultured adult sensory neurons reduced CSPG-mediated inhibition of these cells -but not neurons lacking RPTPσ-, allowing their axonic extension through a CSPG gradient. ISP s.c. administration to rats following contusive spinal cord injury promoted axon regrowth within the CSPG-rich scar, restoring innervation to the spinal cord and recovery of locomotor and urinary systems[65]. ISP administration also improved motoneuron axon regeneration and motor function in rats subjected to spinal root avulsion injury, in which spinal nerves are disconnected from the spinal cord[66]. ISP administration also promoted cardiac innervation in a mouse model of myocardial infarction[67]. Daily i.p. injection in mice subjected to ischaemia-reperfusion restored sympathetic innervation to the CSPG-rich cardiac scar and the cardiac infarct and reduced cardiac arrhythmias[67]. The mode of action of ISP is not yet clear. ISP pulled-down RPTPσ from rodent brain and spinal cord lysates, and ISP likely inhibited RPTPσ function given that ISP treatment phenocopied RPTPσ KO in allowing axons to penetrate CSPG-rich glial scars[67,68]. It remains to be determined whether ISP acts by inhibiting RPTPσ catalytic activity.

RPTPσ also binds transmembrane heparan-sulfate proteoglycans (HSPGs), which inhibit RPTPσ by inducing oligomerization. The opposing effects of CSPGs and HSPGs on RPTPσ function is termed the “**proteoglycan switch**”[69]. RPTPσ binds proteoglycans through its

N-terminal extracellular immunoglobulin-like domains Ig1&Ig2. RPTPσ is expressed in joint FLS, and the joint ECM comprises abundant proteoglycans. We found that RPTPσ was constitutively bound to HSPG syndecan-4 on the surface of FLS. Disruption of the RPTPσ/syndecan-4 interaction by treatment with 20 nM of RPTPσ-Ig1&Ig2 decoy protein impaired FLS invasiveness and cartilage attachment in a manner requiring RPTPσ catalytic activity. Treatment with RPTPσ-Ig1&Ig2 i.v. also blocked human RA FLS invasion into cartilage in a mouse xenograft model and reversed established arthritis in the K/BxN serum transfer model. Importantly, RPTPσ-Ig1&Ig2 did not affect the invasiveness of RPTPσ-null FLS or arthritis severity in RPTPσ-null mice[70]. These findings indicate the RPTPσ/syndecan-4 interaction as a potential novel therapeutic target for RA.

Concluding Remarks

The past few years have witnessed substantial progress in the development of PTP inhibitors. These advances signify that enzymes deemed undruggable may actually provide unique solutions for treating human disease. The continued generation of high-quality, selective probes to modulate PTP activity is paramount for successful growth of the PTP field. Persistent efforts to generate chemical inhibitors are providing opportunities to re-examine historical targets such as PTP1B and SHP-2, substantiate suspected targets in cases like STEP and PTPN22, and foster the emergence of new targets such as VE-PTP and LMPTP. Novel studies of RPTPσ are revealing potential opportunities for manipulating the function of PTPs within the receptor subfamily through the use of biologics. New approaches to inhibit PTPs, for example through small-molecule oxidation stabilizers, may provide additional options for attacking current targets. Furthermore, for cases where enhancing PTP enzymatic activity would be of therapeutic benefit, development of small-molecule PTP activators would expand our repertoire of PTP drug targets.

How can PTP-targeted probes be more rapidly and effectively developed (see Outstanding Questions)? These recent studies impart that there is no single formula for attacking PTPs. The successful tactic will likely be unique for each enzyme and involve exploiting distinctive active-site features, surrounding determinants, or auto-inhibition mechanisms. Thus molecular knowledge of each target will be of critical importance. We anticipate that the renewed interest in drugging PTPs will soon be followed by a resolve to more deeply understand the fundamental biochemistry of PTPs and result in a rise in studies on the structures and post-translational modifications of these enzymes, as well as their intramolecular and intermolecular interactions. This insight will be key to the intentional design of strategies to drug PTPs that take advantage of the unique biochemical traits of each member of the family.

PTPs remain an unconquered territory, but their exploration has expanded dramatically in the last few years. Persistent efforts have reinvigorated the field, providing fresh expectation that while harnessing PTPs for therapy will be difficult, it is still within our reach.

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Glossary

Allosteric inhibitor
inhibitor that binds outside of the enzyme active-site and induces or stabilizes a catalytically unfavorable enzyme conformation

Bidentate inhibitor
inhibitor that contains a core group that interacts with the enzyme active-site and a peripheral group that interacts with a proximal –ideally non-conserved- secondary site

Biologic agent
a substance derived from a living organism, used to modulate the function of a protein; examples include antibodies, proteins and peptides

Cell-penetrating wedge peptide mimetic
a peptide consisting of 24 amino acids of the helix-loop-helix found in RPTP wedge motifs, conjugated to a cell-penetrating peptide derived from the sequence of the transactivator of transcription (TAT) protein of the human immunodeficiency virus

Competitive inhibitor
inhibitor that binds to an enzyme at the site of substrate binding

E-loop
a loop in the PTP active-site named after a conserved glutamate residue in classical PTPs

Irreversible inhibitor
inhibitor that modifies the enzyme to render it non-functional

Oligomerization inhibitor
inhibitor that disrupts a complex consisting of several protein monomers; examples include covalent interaction with or oxidation of the active-site

Orthosteric inhibitor
inhibitor that binds at the enzyme active-site

Oxidation-stabilizing inhibitor
inhibitor that binds to and stabilizes the reversible oxidation of a PTP on the catalytic Cys residue, maintaining the enzyme in an inactive state

Proteoglycan switch
the reciprocal regulation of RPTPσ function that occurs when chondroitin-sulfate proteoglycans (CSPGs) or heparan sulfate proteoglycans (HSPGs) bind the RPTPσ extracellular region

PTK
protein tyrosine kinase, enzyme that catalyzes phosphorylation of proteins on tyrosine residues

PTP
protein tyrosine phosphatase, enzyme that catalyzes hydrolytic dephosphorylation of proteins on tyrosine residues

Radioimmunotherapy
targeted therapy involving conjugation of an antibody to a radioactive agent in order to specifically deliver radiation to hematopoietic cells and tissues in patients with leukemias, lymphomas, or myelodysplasias

Reversible inhibitor
inhibitor that binds to an enzyme with rapid association and dissociation rates

RPTP
receptor protein tyrosine phosphatase, transmembrane enzyme that catalyzes hydrolytic dephosphorylation of proteins on tyrosine residues through an intracellular catalytic domain

Uncompetitive inhibitor
inhibitor that binds to an enzyme-substrate complex

WPD loop
a highly conserved loop in PTPs -named after the tryptophan-proline-aspartate residues present in most phosphotyrosine-specific PTPs- that closes around the PTP active-site upon substrate binding to place a catalytic aspartate residue in position for participation in catalysis

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Box 1

The classification of protein tyrosine phosphatases

- The **protein tyrosine phosphatases (PTPs)** are characterized by a conserved amino acid sequence **(V/H)CX$_5$R**, called the “PTP signature motif”. This motif contains a catalytic Cys residue, which acts as a nucleophile during catalysis, and an Arg residue, which assists in substrate binding[2,4,5].
- **Class I** is the largest and includes the “classical” phosphotyrosine-specific PTPs and the classical and atypical dual-specific PTPs (DUSPs).
- **Class II** consists of only the low molecular weight PTP (LMPTP or LMW-PTP).
- **Class III** contains the 3 isoforms of cell division cycle 25 (CDC25) PTPs.

Box 2

Clinical Trials with vascular endothelial protein tyrosine phosphatase inhibitor AKB-9778

- In a **Phase IB** Trial in diabetic macular edema (DME) patients, vascular endothelial protein tyrosine phosphatase (VE-PTP) inhibitor AKB-9778 was safely self-administered subcutaneously twice daily for 4 weeks at doses up to 30 mg[19].
  - At doses ≥22.5 mg, there was a modest transient decrease in blood pressure, presumably an on-target effect as activation of the VE-PTP substrate angiopoietin 1 receptor 2 (Tie2) is expected to stimulate vasodilation.
  - Pharmacokinetics in DME patients showed a dose-dependent increase in AKB-9778 levels that peaked ~1 hr post-administration and decreased to low levels by 4 hr post-injection, and plasma half-life ~1 hr.
- In a **Phase IIA** Trial in DME patients, 15 mg AKB-9778 was self-administered subcutaneously twice daily alone or in combination with monthly intraocular injections of 0.3 mg ranibizumab, a VEGF neutralizing first-line DME treatment[20].
  - AKB-9778 as a monotherapy did not cause improvement of macular edema after 12 weeks.
  - AKB-9778 treatment combined with ranibizumab significantly reduced macular edema compared to ranibizumab treatment alone (29% of study eyes showed resolution in edema vs 17%, respectively).
  - No significant improvement in visual acuity was observed with AKB-9778 treatment. The study authors commented that this should occur after edema resolution, likely requiring a longer and more-powered study.
  - Although mild-to-moderate transient dizziness and fatigue occurred after AKB-9778 dosings, AKB-9778 demonstrated a favorable safety profile at this regimen. No patient discontinued treatment early due to complications of the study drug treatment.

Outstanding Questions Box

- Can small-molecules be developed as oxidation-stabilizing PTP inhibitors?
- How can we develop small-molecule PTP activators to expand our repertoire of PTP drug targets?
- How can we develop methods to more rapidly discover selective, bioavailable probes?
- Can high-throughput methods be developed to discover inhibitors for multiple protein tyrosine phosphatases (PTPs) at a time?
- How can we more efficiently isolate full-length recombinant PTP proteins for allosteric inhibitor development?

Trends Box

- PTPs are critical for numerous cellular processes in health and disease, and several PTPs are validated drug targets.
- Despite historical difficulties in drugging PTPs, efforts have persisted and led to development of new probes that are being used for biological examination of old and new PTP targets.
- Allosteric PTP inhibitors are emerging, most of which exploit catalytically unfavorable conformations of the targeted enzyme.
- New studies of receptor PTPs reveal the unique potential in targeting this PTP subfamily with decoy biologics.
- Small-molecule inhibitors of VE-PTP and PTP1B are currently undergoing clinical trials for diabetic macular edema and metastatic breast cancer, respectively.

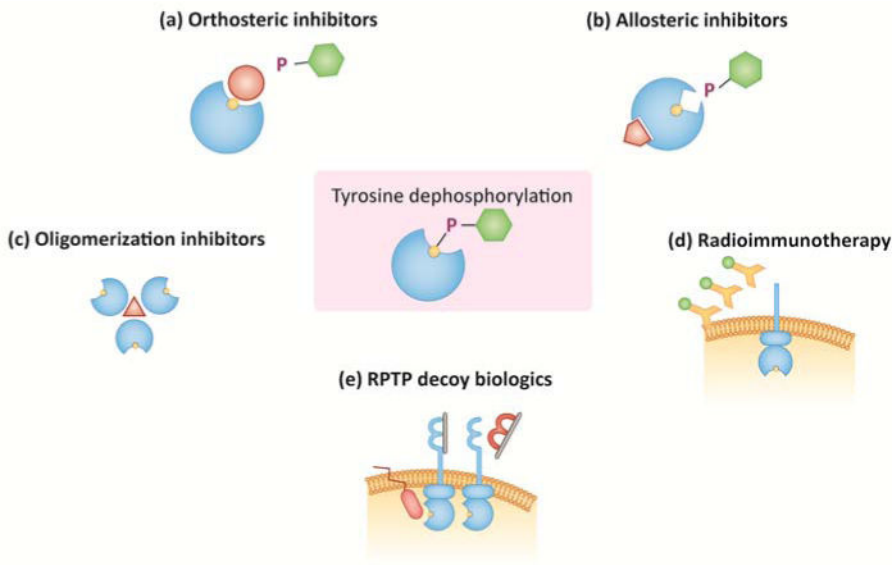


Figure 1. Key Figure. Recent approaches for developing PTP-targeting drugs

Tyrosine phosphorylation occurs when PTPs hydrolytically remove phosphate (P) from Tyr amino acids (depicted as turquoise hexagon). The reaction involves transient covalent interaction with the PTP active-site nucleophile (Cys in Class I, II and III PTPs; Asp in aspartate-based PTPs; depicted in yellow) (**a–c**) Approaches in small-molecule PTP inhibitor development. (**a**) Orthosteric inhibitors bind to the enzyme active-site, and typically compete with substrate for binding. (**b**) Allosteric inhibitors bind outside of the enzyme active-site, inducing or stabilizing a catalytically unfavorable enzyme conformation. (**c**) Oligomerization inhibitors are being used to disrupt trimerization of PRL proteins. (**d–e**) Approaches in RPTP-targeted biologics development. (**d**) The RPTP CD45 has been the object of radioimmunotherapy strategies, which involve conjugation of an antibody to a radioactive agent for specific delivery of radiation to hematopoietic cells and tissues. (**e**) RPTPσ is being targeted with decoy biologics that mimic regions of the protein. A cell-penetrating wedge peptide mimetic targets the RPTPσ intracellular region. A decoy protein of the extracellular RPTPσ Ig1&2 domains targets RPTPσ by disrupting interactions with extracellular matrix proteoglycans (depicted as gray bar).

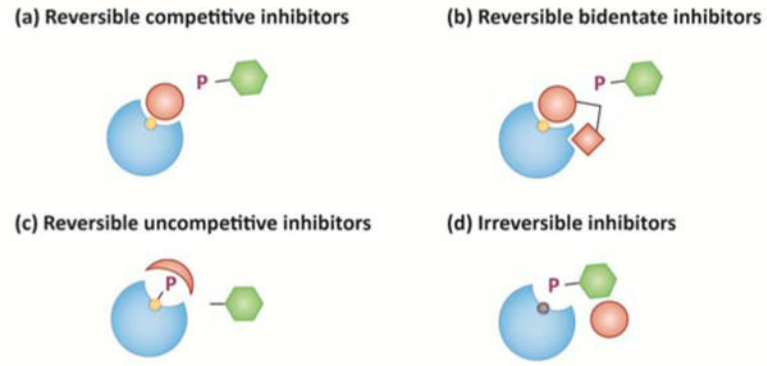


Figure 2. Targeting PTPs with orthosteric small-molecule inhibitors

(**a**) Reversible competitive inhibitors bind to enzymes with rapid association and dissociation rates at the site of substrate binding, and thus compete with substrate for binding to the enzyme. (**b**) Bidentate inhibitors consist of two chemical moieties, and bind to the enzyme active-site and a proximal secondary site (in some cases this can occur within the active-site). (**c**) Uncompetitive inhibitors bind to an enzyme after formation of an enzyme-substrate complex, preventing completion of catalysis. (**d**) Irreversible inhibitors modify the enzyme active-site, rendering the enzyme non-functional.

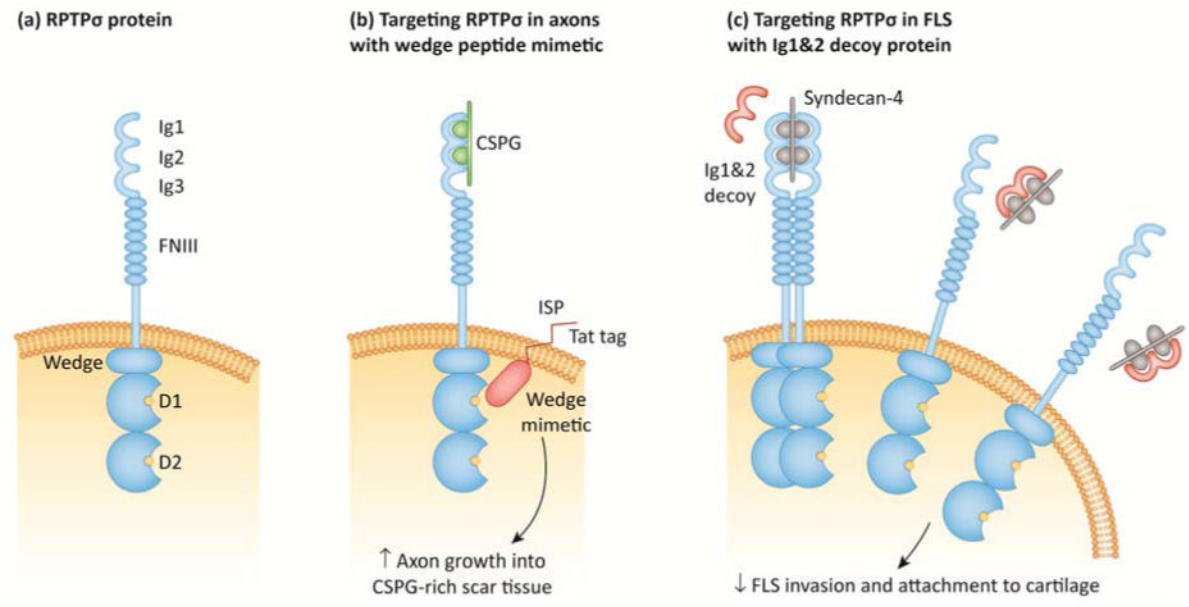


Figure 3. Targeting RPTPσ with biologics

(**a**) Schematic of RPTPσ protein domains. RPTPσ consists of extracellular, transmembrane, and intracellular regions. The extracellular region consists of amino-terminal immunoglobulin-like domains (Ig1–Ig3) and multiple fibronectin type III (FNIII) repeats. As described in (**b–c**), the Ig1&2 domains mediate RPTPσ interactions with proteoglycans in the extracellular matrix (ECM). The intracellular region consists of a juxtamembrane helix-loop-helix "wedge" motif and two PTP domains (D1 and D2). (**b**) Targeting RPTPσ with a cell-penetrating wedge peptide mimetic (ISP). In neuronal cells, RPTPσ Ig1&2 domains act as a receptor for chondroitin sulfate proteoglycans (CSPG), an ECM component that is abundant in scar tissue and inhibits axon regeneration. Treatment with ISP, a wedge peptide mimetic conjugated to a cell-penetrating transactivator of transcription (TAT) peptide, alleviates CSPG-mediated inhibition of axon extension into CSPG-rich scars. The mechanism of action of ISP is currently unknown, although it likely inhibits RPTPσ function. (**c**) Targeting RPTPσ with an Ig1&2 decoy protein. On the surface of fibroblast-like synoviocytes (FLS), RPTPσ Ig1&2 domains bind to the heparan sulfate proteoglycan syndecan-4, which inhibits RPTPσ by inducing oligomerization. Treatment with recombinant Ig1&2 protein disrupts the interaction between RPTPσ and syndecan-4, inhibiting the invasiveness and cartilage attachment of FLS through RPTPσ catalytic activity.

Table 1

PTPs discussed in this review and the indications for which they are being explored.

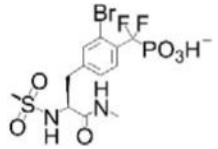
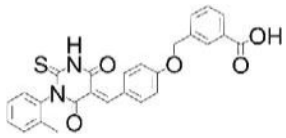
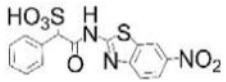
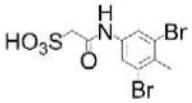
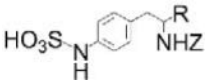
	Refs.
Class I Receptor PTPs	
VE-PTP. Encoded by the *PTPRB* gene, VE-PTP is a transmembrane PTP expressed in endothelial cells. VE-PTP dephosphorylates and inhibits the activation of Tie2, a receptor PTK that suppresses vascular leakage. VE-PTP is being targeted as a therapeutic for retinal and choroidal vascular disease, particularly diabetic macular edema.	[19,20,71]
CD45. Encoded by the *PTPRC* gene, CD45 is a transmembrane PTP expressed on the surface of nearly all hematopoietic cells. CD45 is the target of radioimmunotherapy strategies to deliver radiation to immune cells and tissues in patients with leukemias, lymphomas, or myelodysplasias. Since mutations in *PTPRC* associate with autoimmune diseases and CD45 is critical for signaling in immune cells by dephosphorylation of SFKs, this enzyme has also been explored as a target for immunosuppression. CD45 has also been proposed as a target for Ebola and anthax infections.	[44,59,72,73]
RPTPσ. Encoded by the *PTPRS* gene, RPTPσ is a transmembrane PTP expressed in the nervous system and stromal cells that acts as a receptor for extracellular matrix proteoglycans through its N-terminal immunoglobulin-like domains. RPTPσ dephosphorylates the cytoskeletal-associated protein ezrin. RPTPσ is being considered a target for axon regrowth/regeneration following spinal cord injury or spinal root avulsion injury, for reversing cardiac sympathetic denervation caused by myocardial infarction, and for non-immunosuppressive therapy for rheumatoid arthritis.	[65–67,70]
Class I Non-Receptor PTPs	
PTP1B. Encoded by the *PTPN1* gene, PTP1B was the first PTP identified and the first validated PTP therapeutic target. PTP1B is ubiquitously expressed and contains an N-terminal PTP domain and a C-terminal regulatory region. PTP1B acts as an inhibitor of insulin and leptin signaling. PTP1B has been sought as a drug target for type 2 diabetes, obesity and cancer and was recently proposed as a target for Rett syndrome and stress-induced anxiety.	[6,9,74]
STEP. Encoded by the *PTPN5* gene, STEP is expressed as 2 major isoforms ($STEP_{46}$ and $STEP_{61}$) in the brain. STEP contains KIM region N-terminal to the PTP domain that allows STEP to interact with its MAPK substrates ERK and p38. STEP acts as an inhibitor of synaptic strengthening. High STEP expression was observed in the prefrontal cortex in human postmortem Alzheimer's disease patients and mouse models. STEP is being considered as a target for neurological disorders such as Alzheimer's disease and schizophrenia.	[33,75,76]
SHP-2. Encoded by the *PTPN11* gene, SHP-2 is ubiquitously expressed. SHP-2 contains 2 SH2 domains N-terminal to the catalytic domain, and undergoes an intramolecular autoregulation mechanism in which the SH2 domains bind to the catalytic domain and block its activity. *PTPN11* is a proto-oncogene; gain-of-function mutations in SHP-2 can cause Noonan Syndrome, Leopard syndrome and cancers. SHP-2 has long been considered a drug target for cancer, and recently is being explored as a target for rheumatoid arthritis.	[25,77]
PTPN22. Encoded by the *PTPN22* gene, PTPN22 is expressed in hematopoietic cells. PTPN22 contains an N-terminal PTP domain, an interdomain region, and a C-terminal domain with 4 proline-rich motifs. PTPN22 acts as a negative regulator of early mediators of TCR signaling. A single nucleotide polymorphism (C1858T) in *PTPN22* is associated with autoimmunity, thus PTPN22 is being considered as a target for autoimmune diseases such as rheumatoid arthritis and type 1 diabetes.	[12]
Class I Dual-Specific PTPs	
DUSP6. Encoded by the *DUSP6* gene, DUSP6 is a widely expressed classical DSP that dephosphorylates and inhibits the MAPK ERK. DUSP6 is activated by ERK substrate binding, which induces a conformational change that positions Asp262 to serve as an acid during catalysis. DUSP6 has been suggested as a potential target for elimination of pre-B acute lymphoblastic leukemia cells.	[41,43]
PRL-1/2/3. Encoded by the *PTP4A1/2/3* genes, PRL enzymes are prenylated DSPs. PRL-1 and PRL-2 are nearly ubiquitous, while PRL-3 expression is restricted to the heart, skeletal muscle, vasculature and brain. PRLs contain a PTP domain and a C-terminal prenylation motif that recruits them to the plasma membrane. PRL-1 homotrimerizes in the crystalline state; trimerization is essential for its growth and migration-promoting functions in human epithelial kidney 293 cells. PRL enzymes are being explored as therapeutic targets for cancers, including melanoma and leukemias.	[56,57]
Class II PTPs	
LMPTP. Encoded by the *ACP1* gene, LMPTP is ubiquitously expressed as 2 isoforms, LMPTP-A and LMPTP-B. LMPTP inhibits insulin signaling by IR dephosphorylation. LMPTP is being considered as a target for type 2 diabetes and heart failure.	[16,78]
Class III PTPs	
CDC25A/B/C. Encoded by the *CDC25A/B/C* genes, CDC25 enzymes are expressed in most tissues and dephosphorylate pTyr and pThr residues. CDC25 enzymes regulate cell cycle progression by dephosphorylation and activation of cyclin-dependent kinases within their ATP-binding loops. CDC25A and B are overexpressed in a number of human cancers and are sometimes associated with poor prognosis. Inhibition of all 3 CDC25 isoforms is considered a therapeutic strategy for cancer.	[35]
Bacterial PTPs	

	Refs.
mPTPA. mPTPA from *Mycobacterium tuberculosis* is a low-molecular weight PTP virulence factor secreted by *Mtb* into host macrophages, and thus is considered a potential drug target for TB infection.	[79]

KIM, kinase interaction motif; MAPK, mitogen-activated protein kinase; Refs., references; SFK, SRC family kinase

Table 2

Features of recent orthosteric small-molecule PTP inhibitors

	Chemical structure	Special features	Potency & selectivity	Discovery	MOA	Refs.
CPT-157633: PTP1B inhibitor		Potent; Improved symptoms in Rett syndrome mouse model (5 mg/kg i.p. or s.c. daily)	PTP1B K_i=40 nM; ~2-fold selective vs TCPTP; greater vs SHP-2, LAR, RPTPα & RPTPμ; no inhibition of PEZ, JSP1 or PTEN	Developed by Ceptyr, Inc.	Reversible, competitive	[9]
LTV-1: PTPN22 inhibitor		Reduced frequency of autoreactive B cells in a mouse model of central B cell tolerance (0.15 or 0.75 mg twice daily i.p. for 1 week)	PTPN22 IC_{50}=0.51 μM; 3-fold vs TCPTP & PTP1B; 46-fold vs SHP-1; 59-fold vs CD45; >200-fold vs PTP-PEST	50,000-compound screen using K_M [OMFP substrate]; counterscreen against HePTP and VHR	Reversible, competitive	[13]
Compound 28: LMPTP inhibitor		Highly selective, induced fit mechanism, increased insulin signaling in human HepG2 hepatocytes at [low-μM]	LMPTP IC_{50}=2.1 μM; >50-fold selectivity vs panel of 24 PTPs	Screen of SPAA pharmacophore-based library, followed by structure-guided medicinal chemistry	Reversible, competitive, generates change in conformation of active-site signature motif	[15]
L335-M34: mPTPA inhibitor		Orally bioavailable; reduced TB infection in guinea pig model (50 mg/kg daily for 2 weeks orally) in combination with HRZ	mPTPA IC_{50}=160 nM; >20-fold selectivity vs panel of 18 PTPs, including mPTPB and human LMPTP	Fragment-based optimization of SPAA pharmacophore	Reversible, competitive	[17]
AKB-9778: VE-PTP inhibitor		Remarkable potency; currently in clinical trials for diabetic macular edema	VE-PTP IC_{50}=17 pM; DEP-1 IC_{50}=36 pM; RPTPγ IC_{50} =100 pM; >45-fold selectivity vs PTP1B, greater selectivity vs other PTPs	Screen of Proctor and Gamble Pharmaceutical's corporate repository, followed by structure-guided medicinal chemistry	Reversible, competitive	[19,21,22]

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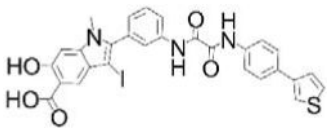
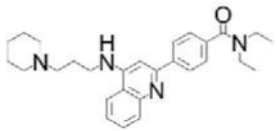
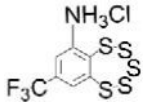
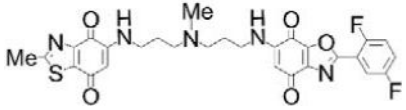
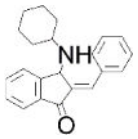
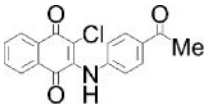
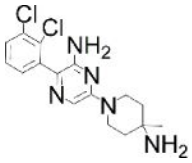
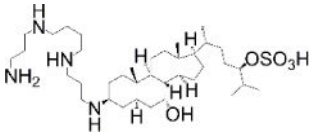
	Chemical structure	Special features	Potency & selectivity	Discovery	MOA	Refs.
11a-1: SHP-2 inhibitor		Daily i.p. administration reduced disease in mouse melanoma (15 mg/kg) and rheumatoid arthritis (7.5 mg/kg) models	SHP-2 IC_{50} =200 nM; >5-fold selectivity over panel of 21 PTPs	Structure-guided combinatorial library based off previous SHP-2 inhibitor II-B08	Reversible, non-competitive; binds SHP-2 active-site and groove formed by β5–β6 E-loop	[25,27,28]
Compd. 23: LMPTP inhibitor		Highly selective, uncompetitive mechanism, orally bioavailable; reversed high-fat diet induced diabetes in mice (0.05% food admixture)	LMPTP IC_{50} =800 nM; no inhibition of panel of 15 PTPs	364,168-compound screen from NIH MLPCN using high [OMFP substrate], followed by medicinal chemistry	Reversible, uncompetitive	[30]
TC-2153:STEP inhibitor		Potent; improved cognition and motor function in mouse models of AD and schizophrenia (10 mg/kg)	$STEP_{61}$ IC_{50}=93 nM; $STEP_{46}$ IC_{50}=57 nM; HePTP IC_{50}=364 nM; PTP- SL IC_{50}=221 nM; greater selectivity vs other PTPs	Elemental sulfur contamination in library used for STEP inhibitor screen; TC-2153 chosen due to similar chemical structure	Irreversible; likely covalent interaction with STEP Cys472	[31,80]
IRC-083864: CDC25 inhibitor		Potent, orally bioavailable, reduced prostate (70 mg/kg p.o. cycles) and pancreatic (10 mg/kg i.v. qwk × 4) carcinoma tumor growth	CDC25 IC_{50}~20–50 nM; selective vs alkaline phosphatase and the CD45, LAR, PTP1B, PTP-PEST, VHR and VHX	Developed based on irreversible quinone CDC25 inhibitor BN82685	Irreversible	[32,35]

JSP1, c-JUN N-terminal kinase (JNK) stimulatory phosphatase 1; PEZ, phosphatase with ezrin domain; PTEN, phosphatase and tensin homolog; VHX, Vaccinia virus-VH1-related MKP X

Table 3

Features of recent small-molecule allosteric PTP inhibitors

	BCI: DUSP6 inhibitor	Compound 211: CD45 inhibitor	SHP099: SHP-2 inhibitor	MSI-1436: PTP1B inhibitor
Chemical structure				
Special features	Inhibits FGF signaling in zebrafish embryo reporter assay; inhibits ERK-mediated DUSP6 activation; induces death of human ALL cancer cells	Potent, selective; a single 3 mg/kg dose i.p. reduced inflammation in mouse delayed-type hypersensitivity model	Potent, highly selective, orally bioavailable; 75–100 mg/kg p.o. for 10 days decreased tumor cell growth in mouse xenograft models	Binds disordered PTP1B C-terminus, 5 mg/kg i.p. every 3 days inhibited breast tumor growth and metastasis in xenograft models; in Phase I Trial for metastatic breast cancer
Potency & selectivity	EC_{50}=10.6 μM in zebrafish assay; inhibits DUSP6 and DUSP1, but not DUSP5, CCDC25B, PTP1B or VHR	CD45 IC_{50} =290 nM; no inhibition of PTPs LAR, PTP1B, RPTPσ, SHP-1 or DUSP22	SHP-2 IC_{50}=71 nM; IC_{50}>100 μM on panel of 21 PTPs; IC_{50}>10 μM on panel of 66 kinases; no reactivity against most targets in preclinical safety pharmacology panel up to 30 μM	PTP1B IC_{50} and K_i=600 nM; 10-fold selective for PTP1B vs TCPTP; 30-fold selective vs CD45, even greater for LAR & PTP-PEST; no activity on RPTPα or RPTPμ
Discovery	BCI identified in screen of 5,000 diverse compounds using live zebrafish embryo reporter for FGF activity; DUSP6 identified as molecular target; analog BCI-215 with similar potency and reduced toxicity identified in SAR analysis using zebrafish reporter assay	120,000-compound *in silico* screen of NCI database for potential binding in groove at interface between CD45 D1 & D2 domains; hits tested for *in vitro* inhibition of intracellular region of CD45 using SRC peptide substrate; hit 37p analog 211 identified by SAR analysis	100,000-compound screen of Novartis archive for inhibitors of full-length SHP-2 (in the presence of 0.5 μM bisphosphorylated IRS-1 peptide) but not SHP-2 PTP domain using DiFMUP substrate; medicinal chemistry improved initial hit SHP836 to SHP099	Identified as appetite suppressant in mice; PTP1B later identified as molecular target
MOA	Inhibits substrate-induced stimulation of DUSP6 activity; predicted to bind crevice between general acid loop and helix α7 in low-activity DUSP6 form, preventing positioning of residue Asp262 for catalysis	Irreversible, non-competitive; causes conformational change of protein; predicted to bind in goove near interface between CD45 D1 & D2 domains	Binds allosteric pocket formed at interface of C-terminal SH2, N-terminal SH2 and PTP domains when enzyme is in closed, inactive conformation; stabilizes enzyme in inactive conformation	Reversible, non-competitive; likely binds to C-terminal helix α9′ and to another site close to catalytic region; induces conformational change in protein
Binding validation	Binding predicted by molecular docking	Circular dichroism suggested dramatic change in secondary structure of CD45 but not LAR; binding supported by mutation	Differential scanning fluorimetry: ΔT_m=3.02°C; Xray co-crystallization with SHP-2 aa 1–525 (PDB 5EHP); mutation	Binding & conformational change shown by ITC, trypsin sensitivity, FRET, NMR spectroscopy and mutation
Cellular efficacy & specificity	Promoted FGF signaling in zebrafish embryos; restored ERK phosphorylation in phorbol ester-stimulated HeLa cells overexpressing DUSP6 (EC_{50}=13.3 μM) or	At 0.5 μM, increased phosphorylation of LCK-Y394 in Jurkat but not CD45-null J.45 T cells, and blocked TCR-induced phosphorylation of LCK-Y394, ZAP-70-Y319 and ERK1/2, IL-2	At [low-μM], slowed growth of hematopoietic cancer cells dependent on RTK or JAK1/2 signaling & colorectal cancer cells sensitive to Lapatinib; did not inhibit growth of cells carrying mutations in RAS	At [low-μM], blocked HER2 activation in MCF10A mammary epithelial cells; reduced migration of HER2 positive cell lines but not in MDF10A-NeuNT cells carrying PTP1B-L192A/S372A mutant; MSI-1436, but not inactive analog,

	BCI: DUSP6 inhibitor	**Compound 211: CD45 inhibitor**	**SHP099: SHP-2 inhibitor**	**MSI-1436: PTP1B inhibitor**
	DUSP1 (EC_{50}=8.0 μM), while inactive analogs did not; induced ALL cancer cell death (IC_{50}=2.1 μM)	release and proliferation of primary mouse splenocytes	or BRAF or KYSE520 cells carrying SHP-2-T253M/Q257L mutant	pulled-down PTP1B from tumor lysates
Refs.	[40,42,43]	[45]	[46,47]	[48,50]

BRAF, B-raf proto-oncogene; HAD, haloacid dehalogenase; HER2, human epidermal growth factor receptor 2; IRS-1, insulin receptor substrate 1; JAK, Janus kinase; NIH, National Institutes of Health; MLPCN, Molecular Libraries Probe Centers Network; NCI, National Cancer Institute; PPM1A, protein phosphatase magnesium-dependent 1A; Refs., references; RTK, receptor tyrosine kinase; SCP1, small C-terminal domain phosphatase 1; SRC, proto-oncogene tyrosine protein kinase; ZAP-70, zeta-chain-associated protein kinase of 70 kDa