Drugs of the Future 2004, 29(12): 1245-1259

CCC: 0377-8282/2004

Review Article

Recent advances in protein-tyrosine-phosphatase 1B (PTP1B) inhibitors for the treatment of type 2 diabetes and obesity

Gang Liu

Metabolic Disease Research, Global Pharmaceutical Discovery and Development, Abbott Laboratories, Abbott Park, IL 60064-6098, USA. Correspondence: gang.liu@abbott.com

CONTENTS

Abstract

The rising prevalence of type 2 diabetes and obesity in the worldwide population has fueled an intensified search for new therapeutic treatment options. Protein-tyrosine-phosphatase 1B (PTP1B) has been implicated as a key negative regulator of both insulin and leptin signaling pathways. Potent and highly selective PTP1B inhibitors with superior drug-like properties could therefore provide novel therapeutic agents for the treatment of these metabolic disorders. This review will summarize the recent advances in various series of PTP1B inhibitors, with a focus on structure-based drug design and the efforts in improving cellular and *in vivo* activities of these inhibitors.

Introduction

Diabetes mellitus is the most prevalent and serious metabolic disease, and 1 in 10 adults in industrialized countries will develop type 2 diabetes in their lifetime (1). The total number of cases of diabetes worldwide among adults 20 years of age or over is projected to rise from 171 million in 2000 to 366 million in 2030 (2). Meanwhile, obesity is an increasing problem in most Western countries, and many developing countries as well. More than 64% of U.S. adults are overweight or obese, with nearly 31% of adults –over 61 million people– meeting the criteria for obesity. Obesity is a strong risk factor for such serious diseases as type 2 diabetes, heart disease, depression and certain cancers (3). The economic impact of diabetes and obesity is enormous, representing about 20% of the overall healthcare expenditure in the U.S. (4, 5).

Chronic insulin resistance coupled with eventual β-cell dysfunction leads to type 2 diabetes (6). Insulin resistance is usually defined as resistance to the actions of insulin on metabolism, glucose uptake and/or storage. The common link between obesity and type 2 diabetes is insulin resistance. Insulin resistance can lead to obesity, among other complications, while obesity, present in about 80% of cases of type 2 diabetes, contributes directly to the severity of insulin resistance (7). In man, most cases of obesity are characterized by the presence of high circulating levels of leptin, indicating that resistance to leptin action may underlie most cases of human obesity (8). Therefore, drugs that augment the action of either insulin or leptin have the potential to reverse obesity associated with defective insulin and leptin signaling. Protein-tyrosine-phosphatase 1B (PTP1B), which negatively regulates both insulin and leptin signaling, is a promising drug discovery target for the treatment of type 2 diabetes and obesity.

Target validation

Modification of proteins by tyrosine phosphorylation is a major mechanism to control their functions, and plays a key role in transmembrane and intracellular signaling (9). The tyrosine phosphorylation state of cellular proteins is controlled by the reciprocal actions of protein-tyrosine kinases (PTKs) and protein-tyrosine-phosphatases (PTPs). PTKs transfer the γ-phosphate from ATP to tyrosine residues within their specific protein substrates. This

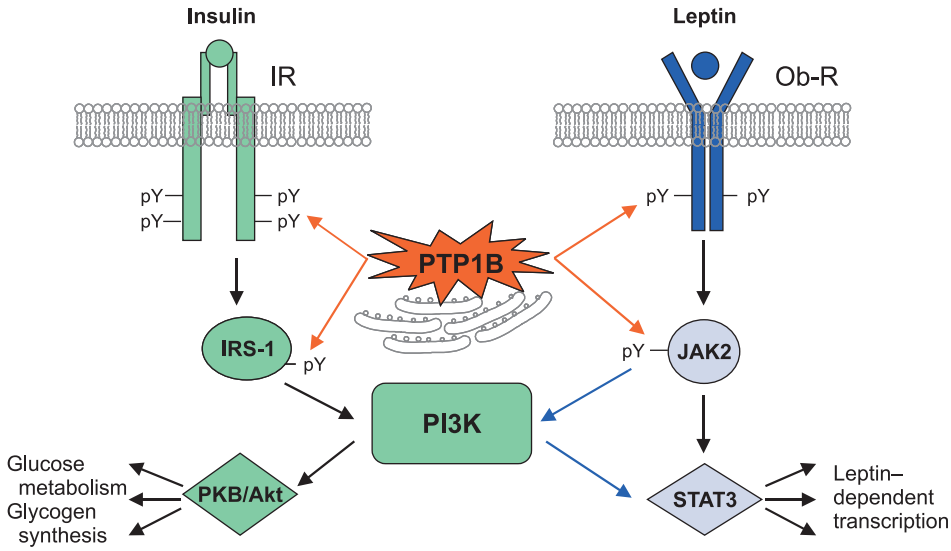


Fig. 1. Mode of action of PTP1B on insulin and leptin signaling pathways.

process is reversed by specific PTPs, which cleave the phosphate from the phosphotyrosine residues (10). The PTP family consists of a large group of enzymes (> 100 in humans) that participate in various signaling pathways and are important for the regulation of cell proliferation, differentiation, metabolism, migration and survival (11).

PTP1B is the prototype for the superfamily of PTPs, and has been the most extensively studied within the group. PTP1B was initially characterized as an inhibitor of growth factor pathways, in particular insulin signaling (Fig. 1). For example, transgenic overexpression of PTP1B in muscle causes insulin resistance in mice (12), whereas inhibition of this phosphatase enhances insulin signaling (13). The most compelling data supporting an important role for PTP1B in regulating insulin signaling come from elegant genetic studies in mice. Generation of PTP1B-deficient mice by two independent labs has shown that this enzyme is dispensable for embryonic development, since homozygous mice are both healthy and fertile (14, 15). PTP1B-deficient mice maintain lower nonfasting blood glucose and insulin levels compared to their wild-type littermates. They also demonstrate greater insulin sensitivity and prolonged insulin receptor (IR) autophosphorylation. PTP1B deficiency does not induce hypoglycemia in the fasted state.

In both sets of PTP1B knockout mice there was an unexpected protective effect against high-fat diet-induced obesity (DIO). Both basal metabolic rate and total energy expenditure are enhanced in PTP1B-deficient mice. These mice are reported to have increased sensitivity to leptin (16, 17). Leptin, an adipocyte-derived hormone, plays an important regulatory role in maintaining body fat by inhibiting food intake. The leptin receptor (Ob-R) is a cytokine receptor that associates with Janus kinase 2 (JAK2) to transduce signals to downstream molecules, including signal transducer and activator of transcription 3 (STAT3) (18). Increased leptin sensitivity arises as a result of hyperphosphorylation and activation of JAK2, followed by its target STAT3 (Fig. 1).

It has long been known that insulin is an afferent signal to the brain that links changes in body adiposity to compensatory changes in food intake (19). The recent finding that neuron-specific deletion of the IR causes weight gain in mice provides further support for the role of brain insulin signaling in the control of energy homeostasis and food intake (20). It was recently demonstrated that leptin administration to rodents *in vivo* activates the insulin receptor substrate (IRS)-phosphatidylinositol 3-kinase (PI3K) pathway in the hypothalamus, and that intracerebroventricular (i.c.v.) infusion of a PI3K inhibitor blocks the ability of leptin to reduce food intake for up to 24 hours (21, 22). The possible involvement of PI3K in the crosstalk between both insulin and leptin signaling pathways (Fig. 1) strongly suggests that PTP1B inhibition could potentially provide an effective means to attenuate or even reverse the brain resistance to both insulin and leptin in obesity.

Disruption of PTP1B with antisense oligonucleotides (ASOs) also confirmed that PTP1B inhibition promotes insulin sensitivity and improves glycemic control in *ob/ob* and *db/db* diabetic mice (23). PTP1B ASOs also improve insulin action and delay the onset of diabetes in Zucker diabetic fatty (ZDF) rats (24). ISIS-113715, a PTP1B ASO, is currently being investigated by Isis Pharmaceuticals as an injectable agent in phase II clinical trials for the treatment of type 2 diabetes (25). The success of this agent could provide the first human clinical validation of the PTP1B inhibition approach.

Biochemical studies have implicated PTP1B in additional signaling pathways, through dephosphorylation of a variety of growth factor receptors, including the epidermal growth factor receptor (EGFR), platelet-derived growth

factor receptor (PDGFR) and insulin-like growth factor type I receptor (IGF-IR), as well as cytosolic tyrosine kinases such as Src, TYK2, STAT5 and p210Bcr-Abl (26). Despite the multiple functions of PTP1B, PTP1B-deficient mice lack any obvious signs of increased activity of the EGFR or PDGFR, such as increased tumor incidence or fibrosis. It has been shown in PTP1B-deficient fibroblasts that loss of PTP1B leads to decreased Ras signaling in the cells, which can largely compensate for loss of PTP1B regulation of EGFR or PDGFR, and which may partly explain the absence of increased tumor incidence in PTP1B-deficient mice (27). Thus, PTP1B appears to be a biologically limiting regulator only for insulin and leptin signaling, but not for other pathways. Hence, specific inhibition of PTP1B could affect two essential features of the metabolic syndrome –insulin resistance and obesity–, making it an exciting target for potential drug discovery.

Implication of PTP1B protein structures for inhibitor design

All PTPs have a highly conserved catalytic pocket characterized by the presence of a signature motif, (H/V)**CX$_5$R**(S/T), a loop of 8 amino acid residues that forms a rigid, cradle-like structure that coordinates to the phosphotyrosine phosphate. The invariant cysteine residue (PTP1B Cys215) is critical for catalytic activity. The cysteine sulfhydryl (pK_a approximately 4.7-5.5) exists as a thiolate ion that acts as a nucleophile to attack the phosphotyrosine phosphate to form a covalent thiol phosphate intermediate (28). The hydrogen bond between the catalytic pocket invariant arginine (PTP1B Arg221) and the phosphotyrosine (pTyr) phosphate moiety aids in substrate binding and stabilization of the transition state (29). PTP1B undergoes a large conformational change upon substrate binding to facilitate the catalytic activity (30). The flexible WPD loop (amino acids 79-187) closes down on the phenyl ring of the substrate to maximize the hydrophobic interaction. The conformational change also brings Asp181 into proximity with the pTyr substrate. Formation of the thiol phosphoryl enzyme intermediate is facilitated by Asp181, which acts as a general acid by providing a proton to the leaving phenolic group, and as a general base by accepting a proton from water during the hydrolysis of the thiol phosphoryl enzyme intermediate.

X-ray crystallographic studies of the complex between bis-(*p*-phosphophenyl)methane and PTP1B Cys215Ala mutant led to the discovery of a shallow groove as the second phosphotyrosine binding site (site 2) adjacent to the catalytic site (31). Site 2 is a noncatalytic cleft-like binding pocket which is not conserved among all PTPs and may participate in substrate recognition. The crystallographic structure of PTP1B bound to the IR peptide D-pY-pY-R shows the *N*-terminal pTyr bound to the catalytic pocket while the *C*-terminal pTyr is bound to site 2 (32). This structural feature provides the possibility of targeting both the catalytic site and site 2 simultaneously in inhibitor design to achieve high potency and specificity.

Besides the two phosphotyrosine recognition sites, the interaction with Arg47 and Asp48, which are close to the catalytic site, has proven to be important in achieving inhibitor selectivity over most of the other PTPs. Arg47 and Asp48 along with Tyr46 form a charged region at the bottom of the catalytic site, which is known as the YRD motif (33). Interaction with Arg47 can result in a significant increase in inhibitory potency and may be just as effective a means of increasing inhibitory potency as interaction with site 2 (34). Gly259 and Gln262 are part of the gateway which has been shown to control both substrate and inhibitor access to site 2 of PTPs that are closely related to PTP1B (35). Many other PTPs have bulkier residues at these positions that can be used for designing specific inhibitors.

Increasing evidence suggests that the cellular redox state is involved in regulating PTP activity through the reversible oxidization of the catalytic cysteine to sulfenic acid (Cys-SOH) *in vivo*. Recently, the crystal structures of the regulatory sulfenic and irreversible sulfinic and sulfonic acids of PTP1B have been reported. The sulfenic acid intermediate produced in response to PTP1B oxidation is rapidly converted to a previously unknown sulfenylamide species, in which the sulfur atom of the catalytic cysteine is covalently linked to the main-chain nitrogen of an adjacent Ser216 residue. Formation of the sulfenylamide causes large reversible conformational changes in the PTP1B active site that inhibit substrate binding. This unusual protein modification has been proposed to both protect the active-site cysteine residue of PTP1B from irreversible oxidation and to permit redox regulation of the enzyme by promoting its reversible reduction by thiols (36, 37). The potential utility of the sulfenylamide conformation in inhibitor design remains to be explored.

T-cell PTP (TC-PTP) has the highest homology to PTP1B, with 74% sequence identity to PTP1B in the catalytic domain and the catalytic site being identical. Additionally, the YRD region and Gly259 and Gln262 gateway in TC-PTP and PTP1B contain identical residues. TC-PTP is widely distributed throughout the body. Homozygous TC-PTP knockout mice die at 3-5 weeks of age, although heterozygous mice have only a mild phenotype (38). Therefore, the consequences of a PTP1B inhibitor that also inhibits TC-PTP as a therapeutic agent are not clear. The recently published TC-PTP crystal structure (39) revealed major structural differences between PTP1B and TC-PTP in two areas: 1) a region near the catalytic site where **Ala17** (PTP1B residues are in bold, TC-PTP residues in italics) is changed to *Gln19*, **Gln21** to *Leu23* and **Ala264** to *Pro262*; and 2) a region near site 2 where **Cys32** is changed to *His34*, **Lys39** to *Glu41* and **Phe52** to *Tyr54*. In addition to these obvious amino acid differences to be targeted, it has been demonstrated recently that the subtle difference in the flexibility of PTP1B structure can also be exploited to identify inhibitors with TC-PTP selectivity (40).

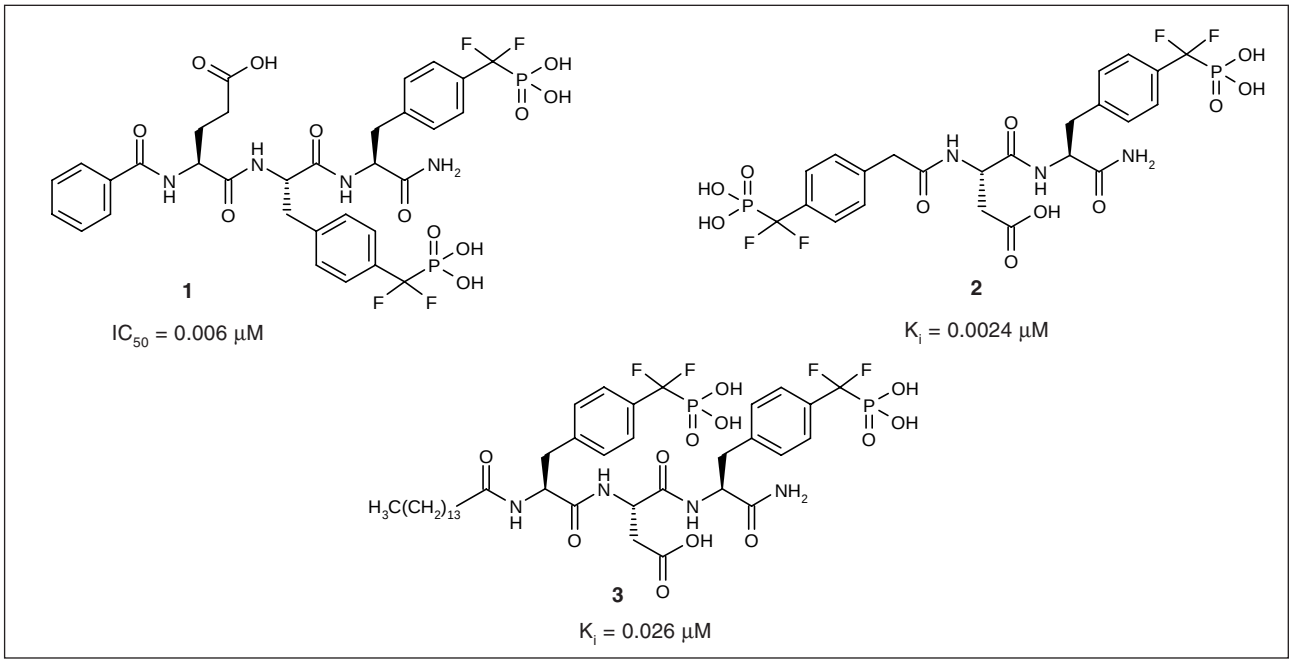


Fig. 2. Peptidic bis(difluorophosphonate)-based PTP1B inhibitors.

Recent advances in small-molecule PTP1B inhibitors

PTP1B inhibitors have been extensively reviewed during the last several years (41). This review will summarize the recent advances in various series of PTP1B inhibitors, with an emphasis on the structure-based drug design and the medicinal chemistry efforts in improving cellular and *in vivo* activities of those inhibitors.

Highly charged PTP1B inhibitors with superior potency and selectivity

Peptide difluorophosphonate PTP1B inhibitors

Mimetics of pTyr are useful general starting points for designing competitive, reversible phosphatase inhibitors. The most effective early pTyr mimetic is 4-phosphono-(α,α-difluoromethyl)-L-phenylalanine (F_2Pmp). Recently, this pTyr mimetic has seen a renaissance in terms of being incorporated in both peptide- and non-peptide-based PTP1B inhibitors, despite its highly charged nature. The synthesis of a peptide library at Merck Frosst led to the discovery of the potent tripeptide-based PTP1B inhibitor **1** (Fig. 2), with an IC_{50} of 0.006 μM and high selectivity over CD45 (IC_{50} = 48 μM). It was designed based on a tyrosine-phosphorylated fragment of the EGFR and the importance of two adjacent pTyr residues for PTP1B substrate recognition. Despite the multiple charges of the molecule at neutral pH, compound **1** showed surprising cellular activity in an S*f*9 cell-based assay, with an IC_{50} of 200 nM for blocking the conversion of exogenous *p*-nitrophenyl phosphate to *p*-nitrophenol (42).

From a directed library of 184 compounds, a group led by Lawrence and Zhang identified the bis(difluorophosphonate) **2** (Fig. 2) as a potent PTP1B inhibitor (K_i = 0.0024 μM). This compound showed > 500-fold selectivity over a panel of 12 phosphatases that included SHP1 (SH2 domain-containing phosphotyrosine phosphatase 1), SHP2 and leukocyte antigen-related tyrosine phosphatase (LAR). It also showed 10-fold selectivity over TC-PTP (43). An extension of this work led to the incorporation of a fatty acid tail in order to facilitate cell permeability. Tripeptide **3** (Fig. 2) was less potent (K_i = 0.026 μM) than **2** but maintained the good PTP1B selectivity profile. It exhibited profound cellular activity in a number of cell lines. When tested at concentrations ranging from 50 nM to 1.25 μM either alone or with submaximal insulin stimulation, peptide **3** increased IR and IRS-1 phosphorylation, and led to phosphotyrosine B (PKB/Akt) and extracellular-regulated kinase ERK1/2 activation in CHO cells expressing the human IR (CHO/hIR) (13). This observation suggests that PTP1B inhibitors can act as both insulin mimetics and insulin sensitizers. It also enhanced insulin-stimulated glucose uptake in both CHO/hIR cells and L6 myotubes. Interestingly, experiments with a potent, cell-permeable derivative of **2** containing rhodamine B and a fatty acid indicated that the inhibitor binds to and localizes with PTP1B on the surface of the endoplasmic reticulum.

The X-ray structures of PTP1B/inhibitor complexes have been solved for several of these molecules. In each case, the *N*-terminal F_2Pmp residue occupies the active site exactly as a pTyr residue occupies the PTP1B C215S

mutant. The *C*-terminal F_2Pmp group interacts with Arg47 of the YRD motif (44, 45). A lysine residue (Lys41) is present in this region and thus can also interact with the acidic residues.

Nonpeptide difluorophosphonate PTP1B inhibitors

Encouraged by the *in vitro* potency and cellular activity exhibited by the peptide difluorophosphonate-based inhibitor **1**, the same group at Merck Frosst designed nonpeptide inhibitors based on the peptide SAR and X-ray structural information. Dufresne *et al.* used two different scaffolds to prepare a series of submicromolar inhibitors. The benzylmalonate **4** (IC_{50} = 0.04 μM) and deoxybenzoin **5** (IC_{50} = 0.06 μM) were selective relative to CD45 (IC_{50} > 50 μM) (Fig. 3). Similar to peptide **1**, compound **5** showed cellular activity in S*f*9 cells, with an IC_{50} of 0.58 μM. It also demonstrated glucose-lowering effects during an oral glucose tolerance test (OGTT) in *ob/ob* mice (43% AUC reduction at 10 mg/kg i.p.) and Zucker *fa/fa* rats (63% AUC reduction at 20 mg/kg i.p.) (46). The monophosphonate analogue **6** (Fig. 3; IC_{50} = 0.12 μM; IC_{50} = 1.22 μM in S*f*9 cells) showed oral bioavailability (F = 13%) in rats, and blood glucose-lowering activity during an OGTT in both Zucker *fa/fa* rats (50% AUC reduction at 30 mg/kg p.o.) and DIO mice (50% AUC reduction at 10 mg/kg p.o.).

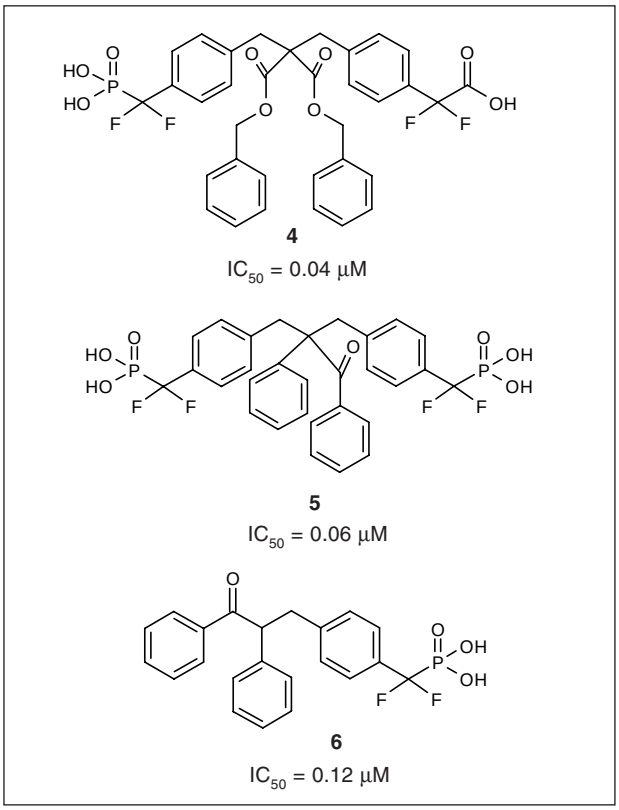


Fig. 3. Nonpeptide difluorophosphonate-based PTP1B inhibitors.

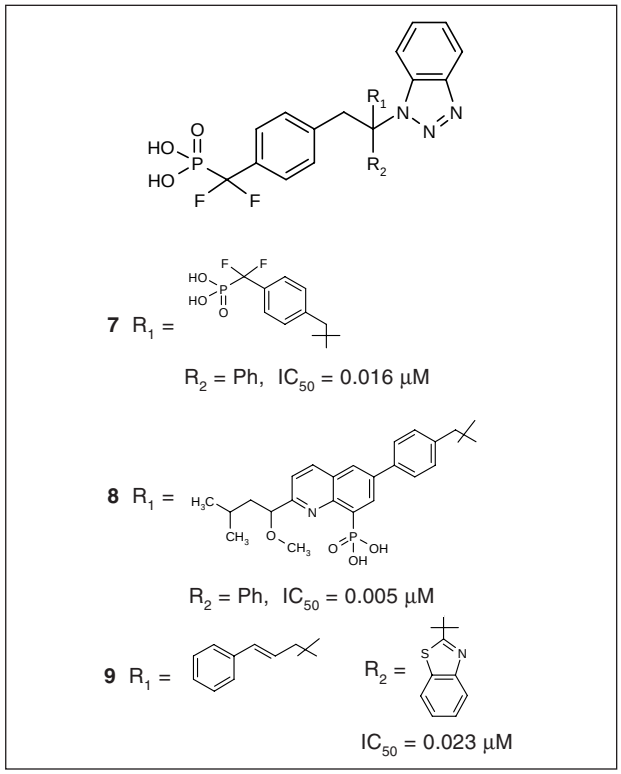
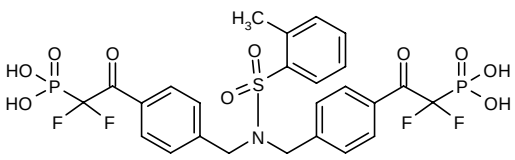


Fig. 4. Highly selective nonpeptide difluorophosphonate-based PTP1B inhibitors.

Lau *et al.* reported the design of a series of inhibitors featuring benzotriazole linked with arylphosphate groups (47). These molecules clearly bind to the active site and site 2 of PTP1B, and show excellent selectivity profiles. The bis-difluorophosphonate **7** (Fig. 4) inhibits PTP1B with an IC_{50} of 0.016 μM and shows good selectivity over SHP1 (IC_{50} = 5.8 μM), SHP2 (IC_{50} = 7.4 μM), LAR (IC_{50} = 8.7 μM), CD45 (IC_{50} > 50 μM), PTPβ (IC_{50} > 50 μM) and Cdc25 (IC_{50} > 50 μM), but no selectivity over TC-PTP. Modifying one of the difluorophosphonate groups gave benzotriazole **8** (Fig. 4), with an IC_{50} of 0.005 μM for PTP1B and 7-fold selectivity over TC-PTP. Interestingly, benzotriazole **9** (Fig. 4) bearing a neutral cinnamyl substituent still exhibits good inhibitory activity (IC_{50} = 0.023 μM) for PTP1B. Based on X-ray crystal structures of several benzotriazoles bound to PTP1B and single point mutations, it was determined that the interaction with the side-chain of Phe52 (Tyr54 in TC-PTP) in site 2 is responsible for most of the observed selectivity over TC-PTP) (48).

More recently, novel phenyl-α,α-difluoro-β-ketophosphonates have been reported as pTyr mimetics by Affymax. Incorporation of two pTyr mimetics into small-molecule scaffolds produced a series of potent PTP1B inhibitors. For example, compound **10** (Fig. 5) has an IC_{50} of 0.6 μM and a K_i of 0.17 μM against PTP1B. This series of aryl-α,α-difluoro-β-ketophosphonate-based PTP1B inhibitors was claimed to be more potent than the



10 IC_{50} = 0.6 μM, K_i = 0.17 μM

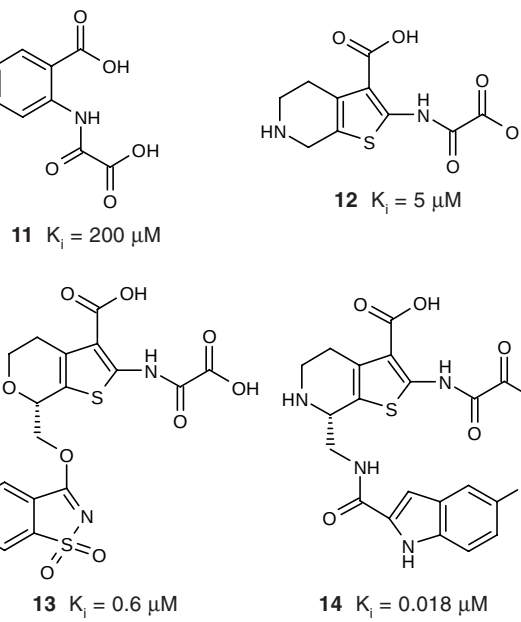
Fig. 5. α,α-Difluoro-β-ketophosphonate-based PTP1B inhibitors.

corresponding α,α-difluoromethylphosphonate-based inhibitors (49).

Inhibitors with non-phosphonate-based pTyr mimetics

Oxamic acid was recently identified as a key component of potent pTyr mimetics in several series of selective PTP1B inhibitors. The 2-(oxalylamino)benzoic acid (OBA) **11** (Fig. 6) was discovered as a general, reversible and competitive inhibitor of several PTPs from a high-throughput screening at Novo Nordisk (50). OBA at neutral pH (K_i ~ 200 μM) is about 10-fold more potent than the non-hydrolyzable α,α-difluorobenzylphosphonic acid (K_i = 2500 μM). X-ray crystallography of PTP1B complexed with OBA revealed that the WPD loop is in the closed conformation, which imparts a favorable aromatic-aromatic interaction between **11** and Phe182 of PTP1B.

A structure-based core modification of **11** to tetrahydrothieno[2,3-*c*]pyridine led to compound **12,** with a K_i of 5 μM and at least 20-fold selectivity over CD45, SHP1



11 K_i = 200 μM

12 K_i = 5 μM

13 K_i = 0.6 μM

14 K_i = 0.018 μM

Fig. 6. 2-(Oxalylamino)benzoic acid-based PTP1B inhibitors.

and other phosphatases (Fig. 6). A salt bridge formed between the basic nitrogen of **12** and Asp48 functioned as a selectivity-determining residue (51), since concomitant repulsion would take place between this basic nitrogen and many other PTPs containing an asparagine in the equivalent position. Despite the fact that OBA is highly charged, good oral bioavailability has been observed in rats for dicarboxylic acid **12** (F = 56%; $t_{1/2}$ i.v. = 168 min) (52). However, augmentation of insulin-stimulated 2-deoxyglucose (2-DOG) uptake into C2C12 cells could only be observed with the diethyl diesters of **12** at 100 μM.

Based on the concept of the "258/259 gateway" (35), a submicromolar PTP1B inhibitor **13** (Fig. 6; K_i = 0.6 μM) was designed through introduction of a saccharin substituent to the tetrahydrothieno[2,3-*c*]pyran core that formed a good steric fit (minimum low-energy conformation) in PTP1B (53). This steric fit imparted good specificity (> 35-fold) over other phosphatases, including PTPβ, PTPH1, LAR and SHP1. However, selectivity relative to TC-PTP was not achieved (K_i = 1.1 μM) with compound **13**. An amide library using the aminomethyl group pendant on the tetrahydrothieno[2,3-*c*]pyridine core resulted in inhibitors with further potency improvement for PTP1B and TC-PTP (54). The most potent analogue was the indoleamide **14** (Fig. 6), with a K_i of 18 nM (pH ~ 5.5). An unexpected feature of analogues represented by indoleamide **14** is the π-π interaction between the indole ring and Phe182 of PTP1B, through a conformation derived from a preorganizing intramolecular hydrogen bond, which might offer potential for achieving specificity over TC-PTP (55).

The oxalylarylaminobenzoic acid **15** (Fig. 7) was identified by an NMR-based screening at Abbott as a general PTP inhibitor with a K_i value of 200 μM. X-ray crystallography revealed that this series of inhibitors bound to the catalytic site of PTP1B, with the WPD loop in the open, presubstrate-binding conformation. Improved potency (K_i = 1.1 μM) and phosphatase selectivity (6-, 30- and > 300-fold selectivity for PTP1B/TC-PTP over LAR, SHP2 and CD45, respectively) were observed with **16** (Fig. 7) through additional sets of H-bond interaction with Asp48, a key residue in controlling access to site 2 of PTP1B (56). Interestingly, the hydroxylethyl analogue **17** (Fig. 7; K_i = 1.9 μM for PTP1B) was found to be orally bioavailable (F = 16.7%) in Sprague-Dawley rats, with an oral half-life of 9 hours. It was found to have a marginal plasma glucose-lowering effect (24% reduction) in *ob/ob* mice when dosed at 120 mg/kg/day s.c. for 5 days (57).

Two approaches were taken in identifying site 2 ligands for PTP1B based on the core structure of **16**. A parallel synthesis approach yielded **18** (Fig. 7) as a potent (K_i = 0.077 μM) and selective PTP1B inhibitor relative to a panel of phosphatases, including SHP2 (K_i = 17 μM), LAR (K_i = 5.0 μM) and CD45 (K_i > 200 μM). Remarkably, it also exhibited 5-fold selectivity over TC-PTP (K_i = 0.38 μM) (58). The interaction between the methionine moiety of **18** and Arg24 in site 2 appears to be critical in imparting the TC-PTP selectivity, possibly through the subtle

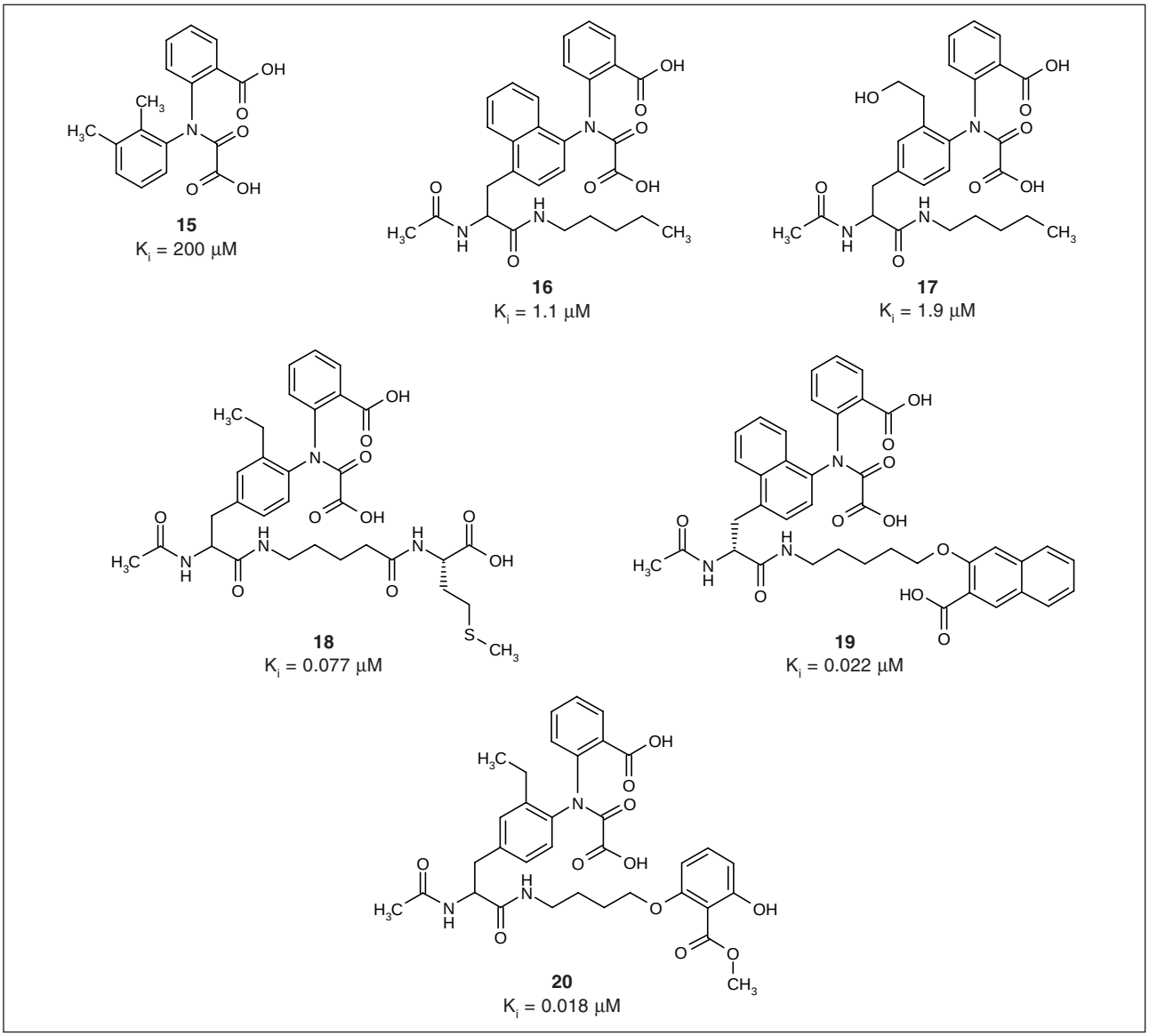


Fig. 7. Oxalylarylaminobenzoic acid-based PTP1B inhibitors.

difference in the overall flexibility difference between PTP1B and TC-PTP structures in site 2.

NMR-based screening was also applied at Abbott to the identification of site 2 ligands for PTP1B. X-ray structure-aided linking of a site 2 ligand with **16** produced the dinaphthalene **19** (Fig. 7) as a potent PTP1B inhibitor (K_i = 0.022 μM) with excellent selectivity over other phosphatases (36-fold to > 10,000-fold for LAR, SHP2 and CD45) (56). The X-ray crystal structure of the complex of **18** bound to PTP1B clearly shows that the inhibitor occupies both the catalytic site and site 2 with the carboxylate group of the naphthoic acid interacting with Arg24 and Arg 254 in site 2. Structure-based assembly of a non-carboxylic acid-containing, salicylate-based site 2 ligand with the core of **18** resulted in the discovery of **20** (Fig. 7) as a potent inhibitor of PTP1B (K_i = 0.018 μM) with moderate selectivity over TC-PTP (K_i = 0.065 μM) but excellent selectivity over other PTPs (> 75-fold for LAR, SHP2 and CD45). A diester prodrug of **20** showed augmentation of submaximal insulin-stimulated PKB phosphorylation in a concentration-dependent manner at concentrations of 100 μM (65% increase) and 300 μM (150% increase) in FAO cells (59).

An *N*-terminal tripeptide of CCK-8 analogues with a sulfonic acid (**21**; Fig. 8) was identified as a PTP1B inhibitor (K_i = 5 μM) from a screening at the former Pharmacia (now Pfizer) (60). Replacement of the sulfonic acid with *O*-malonyltyrosine as the pTyr mimetic and other structural modifications produced the more potent **22** (Fig. 8; K_i = 1.2 μM). Further optimization using

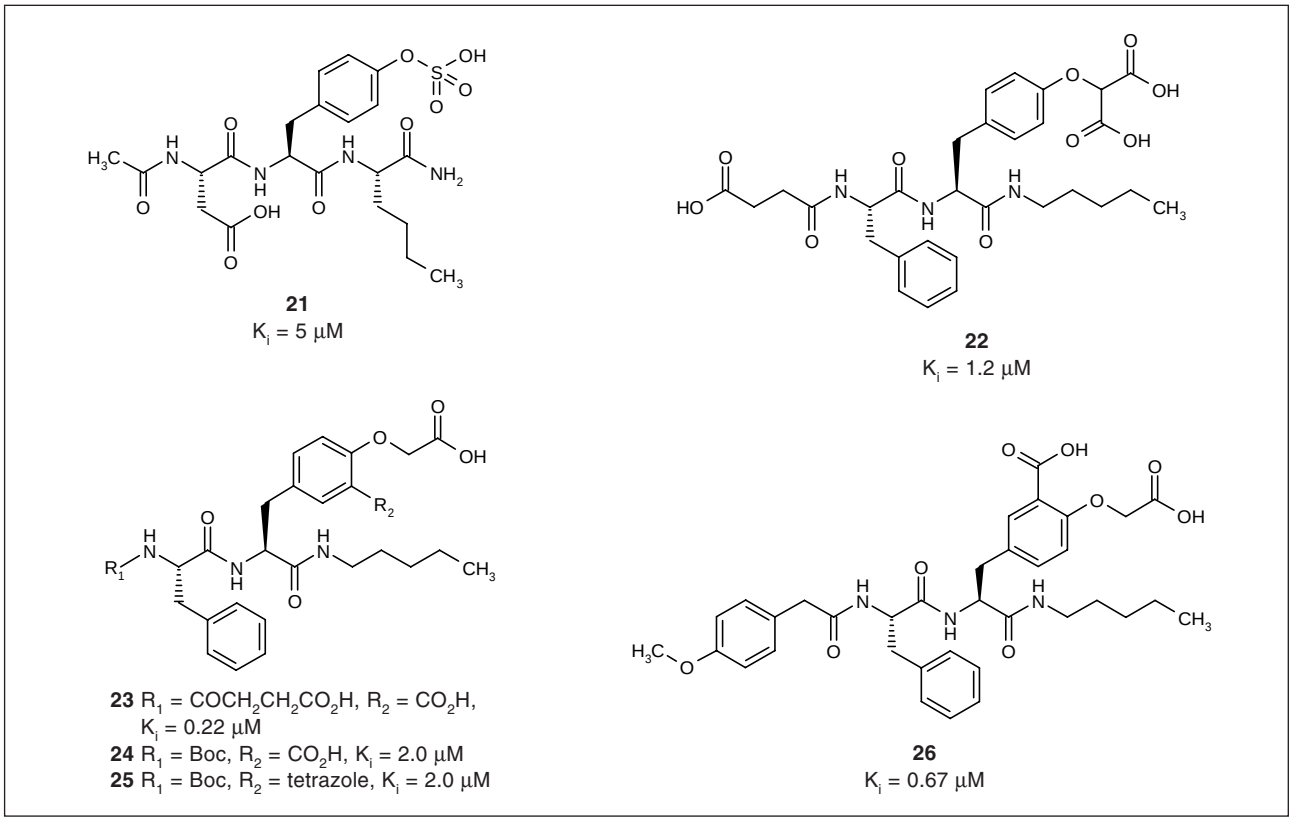


Fig. 8. *O*-Carboxymethylsalicylic acid-based PTP1B inhibitors.

O-carboxymethylsalicylic acid as the pTyr mimetic led to the potent inhibitor **23** (Fig. 8), with a K_i of 0.22 μM and no inhibition of SHP2 or LAR at concentrations up to 100 μM. An X-ray co-crystal structure of **23** bound to PTP1B revealed that the WPD loop is in the closed conformation. Cellular activity was only demonstrated with the trimethyl ester prodrug of **23**. A large enhancement of 2-DOG uptake into L6 myocytes and 3T3-L1 adipocytes, with a concomitant increase in tyrosine phosphorylation levels of the insulin-signaling molecules IR, IRS-1 and IRS-2, was observed. However, no effect on 2-DOG uptake in the presence of insulin was observed with the ester prodrug of **23**, indicating a lack of activity in terms of potentiating insulin signaling (61).

More recently, compound **24** (Fig. 8; K_i = 2.0 μM), bearing a neutral *N*-terminus, and its equipotent tetrazole analogue **25** (Fig. 8) have been reported. The tetrazole analogue **25** has significantly higher Caco-2 cell permeability as compared to all previous compounds in the series and exhibited modest enhancement of insulin-stimulated 2-DOG uptake in L6 myocytes at concentration of 100 μM, without esterification of the carboxylic acid (62). Replacement of the Boc group in **24** led to compound **26** (Fig. 8) with both improved potency (K_i = 0.67 μM) and cellular activity (63).

The propionyloxamic acid **27** (Fig. 9; K_i = 1.7 μM) was discovered as a novel PTP1B inhibitor at Abbott using the same salicylate ligand for PTP1B site 2 binding as **20**.

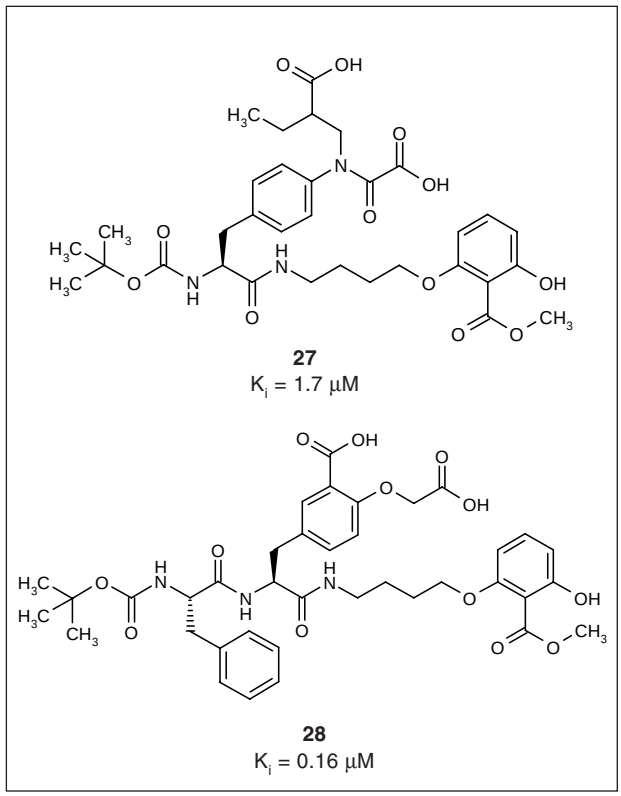


Fig. 9. Salicylate-containing TC-PTP-selective PTP1B inhibitors.

Replacement of the propionyloxamic acid with the *O*-carboxymethylsalicylic acid led to the bis-salicylate **28** as a potent PTP1B inhibitor (Fig. 9; K_i = 0.16 μM). Both of these compounds are selective over a number of phosphatases including TC-PTP (> 15- and 12-fold, respectively) (64). In comparison, the corresponding *O*-carboxymethylsalicylic acid-based inhibitors (**23-26**) without a site 2 ligand attached are not selective over TC-PTP.

PTP1B inhibitors with reduced charge and improved cell permeability

Orally active monocarboxylic acid-based PTP1B inhibitors, exemplified by the phenoxyacetic acid derivatives **29** and **30** (Fig. 10), were first reported by the former American Home Products (now Wyeth) (65). These compounds were later found to have crossactivity as peroxisome proliferator-activated receptor PPARγ agonists (66), which could contribute (at least partially) to their *in vivo* efficacy. Ultimately, inhibitor **31** (Fig. 10; PTP-112, ertiprotafib) was advanced into human clinical trials. However, phase II trials in type 2 diabetes have since been discontinued due to unsatisfactory clinical efficacy, as well as dose-limiting side effects (67).

A series of heterocyclic carboxylic acids with reduced pK_a values were designed as potential oxamic acid mimetics at Abbott, and the isoxazolecarboxylic acid **32** (Fig. 11) was identified as a weak binder to PTP1B

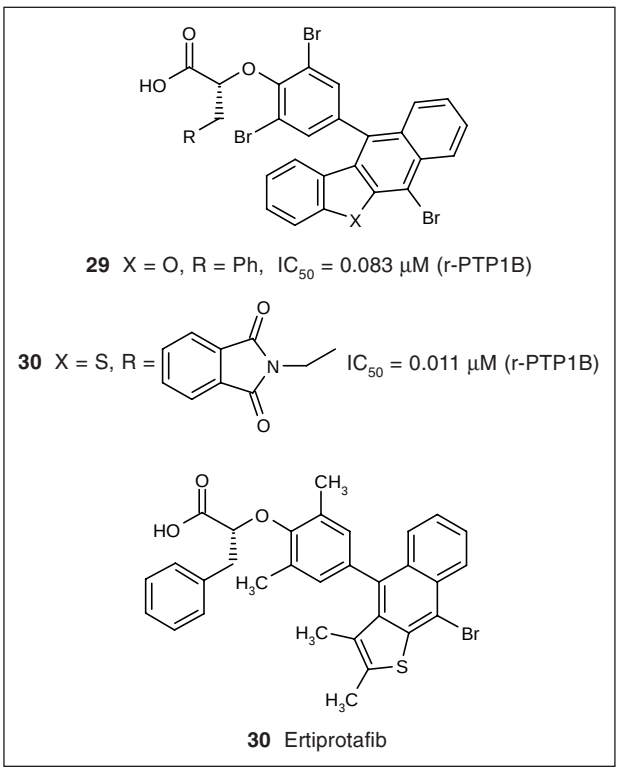


Fig. 10. Phenoxyacetic acid-based PTP1B inhibitors.

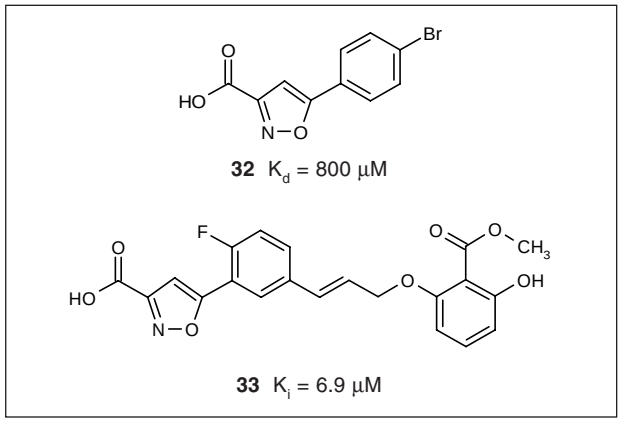


Fig. 11. Isoxazolecarboxylic acid-based selective PTP1B inhibitors.

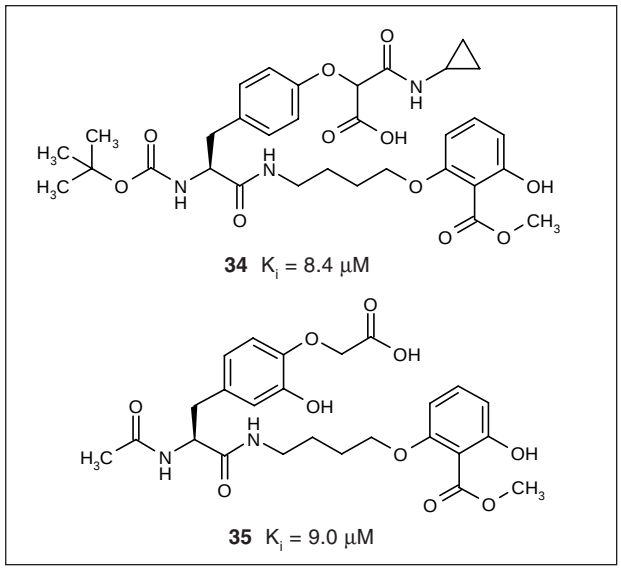


Fig. 12. Monocarboxylic acid-based TC-PTP-selective PTP1B inhibitors.

(K_d = 800 μM) in an NMR-based screening (68). Linking this active site-directed fragment with the methylsalicylate-based site 2 ligand produced the moderately potent (K_i = 6.9 μM), highly selective (K_i = 164 μM for TC-PTP, K_i > 300 μM for LAR, SHP2 and CD45) PTP1B inhibitor **33** (Fig. 11). It reversed PTP1B-dependent inhibition of STAT3 phosphorylation caused by the overexpression of PTP1B in COS-7 cells in a concentration-dependent fashion starting from 10 μM. X-ray crystallography of the PTP1B/**33** complex revealed that the combination of the WPD loop in the closed conformation and site 2 interaction provided the additional boost in terms of selectivity over TC-PTP.

When one of the acid groups in the *O*-malonate-based inhibitor was converted to an amide, the resulting monoacid **34** (Fig. 12) showed a K_i of 8.4 μM and > 24-fold selectivity over TC-PTP (64). If a prodrug approach is needed, this type of monoacid-based inhibitors may have

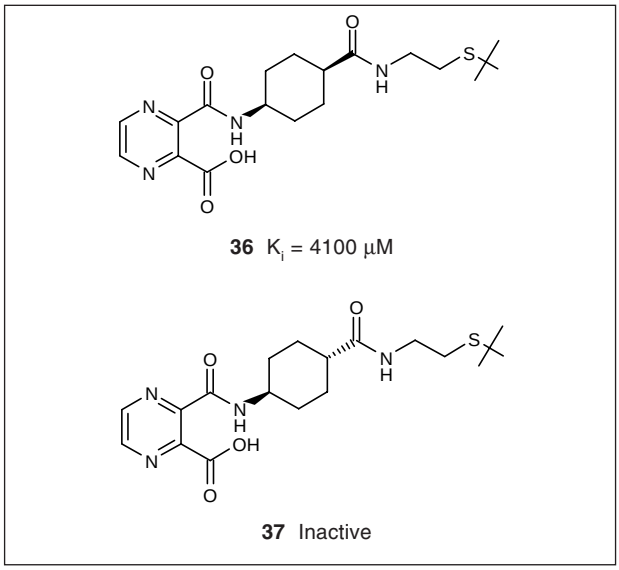


Fig. 13. pTyr mimetic identified by breakaway tethering strategy.

the advantage in that hydrolysis of only one ester is needed to give the parent molecule. Another monoacid-based analogue, **35** (Fig. 12), showed modest potency (K_i = 9.0 μM) and > 20-fold selectivity over TC-PTP (69). It also showed modest Caco-2 cell permeability, which could be partially due to the equilibrium between the acid **35** and its corresponding lactone.

Using a breakaway tethering strategy, the 3-carbamoylpyrazine-2-carboxylic acid **36** (Fig. 13) was discovered as a new pTyr mimetic at Sunesis (70). The approach involves first irreversibly modifying a protein with an extender that contains both a masked thiol and a known pTyr mimetic. The extender is then cleaved to release the pTyr mimetic, thereby unmasking the thiol. The resulting protein is screened against a library of disulfide-containing small-molecule fragments. Any molecules with inherent affinity for the pTyr binding site will preferentially form disulfides with the extender thus identified by mass spectrometry. The potency of **36** is comparable to that of pTyr itself (K_i = 4100 μM). The technique is fairly structurally sensitive since the inactive stereoisomer **37** (Fig. 13) was not selected from the library.

Acid **38** (Fig. 14) was reported by Structural Bioinformatics (now Cengent Therapeutics) to have IC_{50} values of 0.8 μM and 1.6 μM against PTP1B and TC-PTP, respectively, and moderate selectivity over PTPβ (IC_{50} = 30 μM) and CD45 (IC_{50} = 14 μM) (71). A series of azole compounds, exemplified by **39** (Fig. 14), were patented as PTP1B inhibitors by Japan Tobacco. Compound **39** was shown to lower blood glucose levels by 32% and 39% in *ob/ob* mice when dosed at 1 and 3 mg/kg orally, respectively (72). No structural information regarding the binding modes of these two series of compounds is available.

Replacement of the acidic hydroxyl group of a sulfonic acid with an electronegative, nonacidic CF_3 group led to the identification of sulfonamides **40** and **41** (Fig. 15) as PTP1B inhibitors at Sugen (Pfizer) (73). Docking models show that these compounds can bind to both the catalytic site and site 2, although neither X-ray crystal structure nor kinetic information is available to confirm this. Unfortunately, these compounds appear to be nonspecific inhibitors of several other PTPs, including SHP2, PTPε, etc.

Noncompetitive/allosteric PTP1B inhibitors with improved drug-like properties

The difficulties associated with identifying pTyr mimetics with suitable potency, selectivity and bioavailability have prompted investigations on other mechanisms of inhibiting PTP1B. A series of pyridazine analogues were identified at Biovitrum as noncompetitive PTP1B inhibitors (74), with **42** being the most potent (Fig. 16; IC_{50} = 0.35 μM). The thiopyridazine **43** (Fig. 16) showed over 20-fold selectivity relative to LAR (IC_{50} > 100 μM) and TC-PTP (IC_{50} > 100 μM). These pyridazine analogues lacking negative charges were initially speculated as binding to an unknown allosteric site. It was later found that they probably cause catalytic oxidation of the

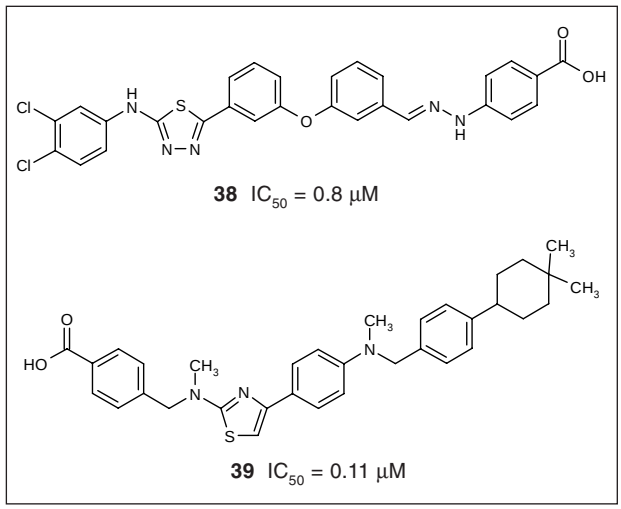


Fig. 14. Monocarboxylic acid-based PTP1B inhibitors.

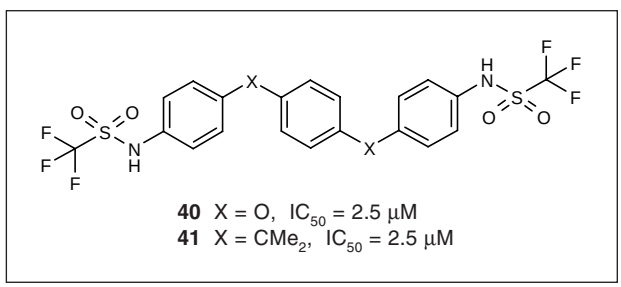
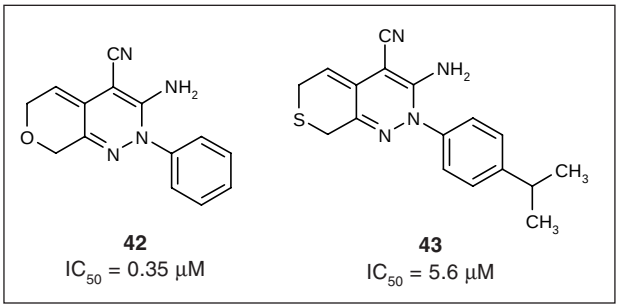


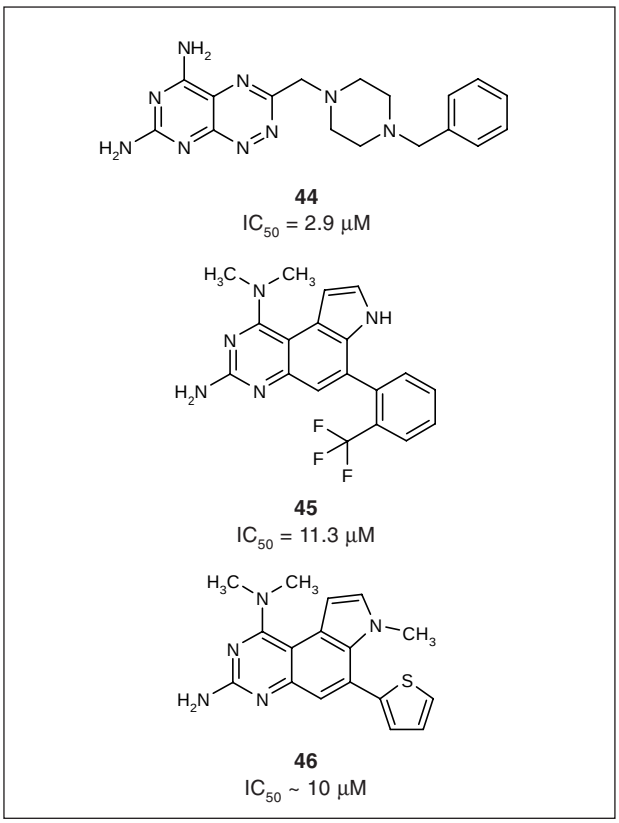
Fig. 15. Bis-sulfonamide-based PTP1B inhibitors.



42
IC_{50} = 0.35 μM

43
IC_{50} = 5.6 μM

Fig. 16. Pyridazine-based oxidative PTP1B inhibitors.



44
IC_{50} = 2.9 μM

45
IC_{50} = 11.3 μM

46
IC_{50} ~ 10 μM

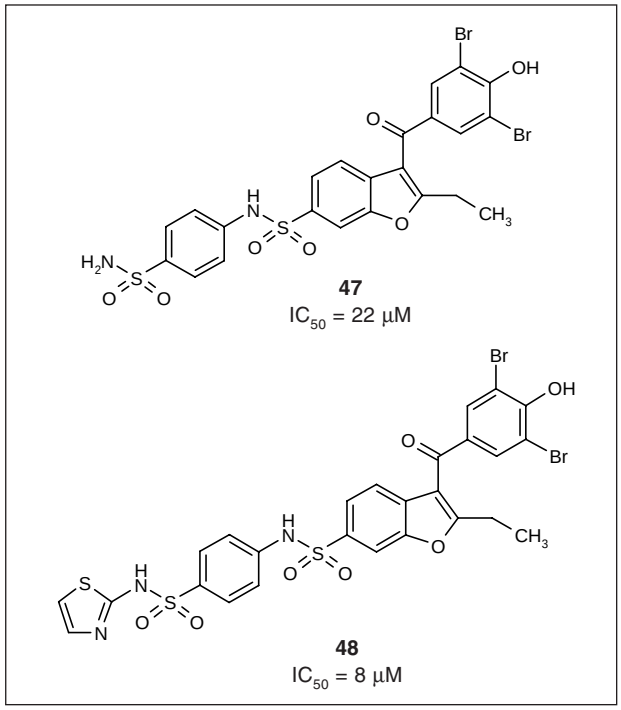
Fig. 17. Diaminopyrimidine-based PTP1B inhibitors.

reducing agents in the assay, generating hydrogen peroxide from O_2, which oxidizes the active-site cysteine (C215) of PTP1B, leading to enzyme inactivation (75). No inhibition by these compounds was observed in the absence of reducing agents such as dithiothreitol.

A series of pyrimidotriazinediamines were discovered at Roche as nonselective PTP1B inhibitors, the most potent being **44** (Fig. 17; IC_{50} = 2.9 μM). It is most likely that the triazine ring is responsible for the redox activity of these inhibitors in a similar fashion as **43** (76). A more stable series of diaminopyrroloquinazolines was recently reported as selective PTP1B inhibitors by the same group (77). Exemplified compound **45** (Fig. 17; IC_{50} = 11.3 μM) concentration-dependently prolonged IR phosphorylation in CHO/hIR cells. It lowered plasma glucose levels in DIO mice following a single oral dose of 50 mg/kg. A subchronic dosing regimen (50 mg/kg once daily for 5 days) of **45** in DIO mice resulted in significantly reduced plasma glucose and insulin levels. It also induced beneficial changes in the levels of free fatty acids (FFA), triglycerides (TG) and high-density lipoproteins (HDL). These changes correlated well with the significantly reduced cumulative food intake and body weight of these mice.

The binding mode of a similar analogue, **46** (Fig. 17; IC_{50} ~ 10 μM), was studied using NMR with ^{15}N- or ^{13}C-labeled PTP1B. The chemical shift perturbation is consistent with a model in which **46** binds near the catalytic site but does not penetrate into the catalytic site, and does not induce closure of the WPD loop (78). Consistently, **46** was found to be a mixed competitive inhibitor and was not sensitive to reducing agents. Interestingly, both **45** and **46** showed very significant crossreactivity against another unidentified enzyme, which imparts ambiguity to the mechanism of action for this series of inhibitors.

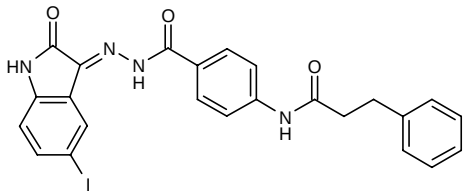
A series of benzbromarone derivatives have been discovered by Sunesis as reversible, noncompetitive PTP1B inhibitors (79). The exemplified compound **47** (Fig. 18) was shown to have an IC_{50} of 22 μM against PTP1B and selectivity over LAR (IC_{50} > 500 μM) and TC-PTP (IC_{50} = 129 μM). X-ray crystallography revealed that **47** binds to a novel allosteric site located ~20 Å away from the catalytic pocket, and stabilizes a conformation that is very similar to the presubstrate-binding, WPD loop open



47
IC_{50} = 22 μM

48
IC_{50} = 8 μM

Fig. 18. Allosteric inhibitors of PTP1B.



49
IC_{50} = 0.34 μM

Fig. 19. Oxindole hydrazide-based PTP1B inhibitors.

conformation. This allosteric inhibitor blocks several interactions and side-chain movements that are associated with the closure of the WPD loop and the formation of the catalytically competent conformation. A slightly more potent analogue, **48** (Fig. 18; IC_{50} = 8 μM), was shown to stimulate the phosphorylation of IR, IRS-1 and Akt when tested at a concentration of 250 μM in CHO/hIR cells. It also attenuated the suppression of insulin-induced phosphorylation caused by the overexpression of PTP1B in the same cells.

A series of oxindole hydrazide derivatives were reported by Serono as PTP1B inhibitors. For example, compound **49** (Fig. 19) displays IC_{50} values of 0.34 μM for PTP1B and 0.496 μM for glomerular epithelial protein-1 (GLEPP-1), a receptor PTP. Oral treatment of *db/db* mice with 100 mg/kg of **49** resulted in a 30% reduction in blood glucose levels induced by food intake. Additionally, 5-day subchronic once-daily oral treatment of *db/db* mice with 50 mg/kg of **49** caused a 22% reduction in blood glucose levels (80). No information regarding the sites of interaction with PTP1B for this series of inhibitors has been disclosed.

Conclusions

The development of PTP1B inhibitors that help ameliorate insulin and leptin resistance can be potentially important not only for the prevention and treatment of type 2 diabetes and obesity, but also for reducing their associated cardiovascular risk profile. The structure-based design of potent, selective, small-molecule PTP1B inhibitors has shown that it is possible to obtain inhibitors specific for selected members of the PTP family, even by addressing highly conserved regions of the protein. However, the highly charged nature of these molecules will inevitably pose a tremendous challenge in achieving reasonable cellular permeability and oral bioavailability for their use as therapeutic agents.

Amazingly, despite these challenges, tremendous progress in terms of achieving oral bioavailability and *in vivo* activity has been made with selective, nonpeptide phosphonate-based inhibitors. This series of compounds appears to be most advanced in validating the therapeutic approach in diabetic rodent models. It would be interesting to see whether any compounds from this series advance into human clinical trials. Additionally, the identification of an allosteric site for regulating PTP1B conformational change upon substrate binding offers another potential venue for designing potent and selective inhibitors without the liability associated with targeting the catalytic pocket of PTP1B.

PTP1B remains an attractive, but as yet challenging, target for small-molecule drug discovery. In particular, reasonable brain penetration is needed for PTP1B inhibitors to harness the potential benefit of increasing leptin and insulin sensitivities in the hypothalamus. Despite these challenges, after nearly a decade of intensive research, the search for true small-molecule PTP1B inhibitors suitable for clinical development may start to bear fruit. The move of PTP1B inhibitors from the sideline to the frontline as therapeutic agents could be realized in the next decade.

References

1. Zimmet, P., Alberti, K.G.M.M., Shaw, J. *Global and societal implications of the diabetes epidemic.* Nature 2001, 414: 782-7.

2. Wild, S., Roglic, G., Green, A., Sicree, R., King, H. *Global prevalence of diabetes.* Diabetes Care 2004, 27: 1047-53.

3. Must, A., Spadano, J., Coakley, E.H., Field, A.E., Colditz, G., Dietz, W.H. *The disease burden associated with overweight and obesity.* JAMA - J Am Med Assoc 1999, 282: 1523-9.

4. Hogan, P., Dall, T., Nikolov, P. *Economic costs of diabetes in the US in 2002.* Diabetes Care 2003, 26: 917-32.

5. *Statistics related to overweight and obesity.* NIH publication No.03-4158, July 2003.

6. Beck-Nielsen, H., Henriksen, J.E., Vaag, A., Hother-Nielsen, O. *Pathophysiology of non-insulin-dependent diabetes mellitus (NIDDM).* Diabetes Res Clin Pract 1995, 28(Suppl.): S13-25.

7. Kahn, B.B., Flier, J.S. *Obesity and insulin resistance.* J Clin Invest 2000, 106: 473-81.

8. Maffei, M., Halaas, J., Ravussin, E. et al. *Leptin levels in human and rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects.* Nat Med 1995, 1: 1155-61.

9. Hunter, T. *Signaling - 2000 and beyond.* Cell 2000, 100: 113-27.

10. Hunter, T. *Protein kinases and phosphatases: The yin and yang of protein phosphorylation and signaling.* Cell 1995, 80: 225-36.

11. Tonks, N.K., Neel, B.G. *Combinatorial control of the specificity of protein tyrosine phosphatases.* Curr Opin Cell Biol 2001, 13: 182-95.

12. Zabolotny, J.M., Haj, F.G., Kim, Y.B. et al. *Transgenic overexpression of protein-tyrosine phosphatase 1B in muscle causes insulin resistance, but overexpression with leukocyte antigen-related phosphatase does not additively impair insulin action.* J Biol Chem 2004, 279: 24844-51.

13. Xie, L., Lee, S.-Y., Andersen, J.N. et al. *Cellular effects of small molecule PTP1B inhibitors on insulin signaling.* Biochemistry 2003, 42: 12792-804.

14. Echebly, M., Payette, P., Michaliszyn, E. et al. *Increased insulin sensitivity and obesity resistance in mice lacking the protein-tyrosine phosphatase-1B gene.* Science 1999, 283: 1544-8.

15. Klaman, L.D., Boss, O., Peroni, O.D. et al. *Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice.* Mol Cell Biol 2000, 20: 5479-89.

16. Cheng, A., Uetani, N., Simoncic, P.D. et al. *Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B.* Dev Cell 2002, 2: 497-503.

17. Zabolotny, J.M., Bence-Hanulec, K.K., Stricker-Krongrad, A. et al. *PTP1B regulates leptin signal transduction in vivo.* Dev Cell 2002, 2: 489-95.

18. Ahima, R.S., Flier, J.S. *Leptin.* Annu Rev Physiol 2000, 62: 413-37.

19. Woods, S.C., Lotter, E.C., McKay, L.D., Porte, D. Jr. *Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons.* Nature 1979, 282: 503-5.

20. Brüning, J.C., Gautam, D., Burks, D.J. et al. *Role of brain insulin receptor in control of body weight and reproduction.* Science 2000, 289: 2122-5.

21. Niswender, K.D., Schwartz, M.W. *Insulin and leptin revisited: Adiposity signals with overlapping physiological and intracellular signaling capabilities.* Front Neuroendocrinol 2003, 24: 1-10.

22. Niswender, K.D., Morrison, C.D., Clegg, D.J. et al. *Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: A key mediator of insulin-induced anorexia.* Diabetes 2003, 52: 227-31.

23. Zinker, B.A., Rondinone, C.M., Trevillyan, J.M. et al. *PTP1B antisense lowers PTP1B protein, normalizes blood glucose and improves insulin sensitivity in diabetic mice.* Proc Natl Acad Sci USA 2002, 99: 11357-62.

24. Bush, E.N., Shapiro, R., Clampit, J. et al. *Treatment of Zucker diabetic fatty rats with antisense oligonucleotides to protein tyrosine phosphatase 1B for 5 weeks halts development of diabetes.* Diabetes 2001, 50(Suppl. 2): A81.

25. Liu, G. *Technology evaluation: ISIS-113715, ISIS.* Curr Opin Mol Ther 2004, 6: 331-6.

26. Dubé, N., Tremblay, M.L. *Beyond the metabolic function of PTP1B.* Cell Cycle 2004, 3: 550-3.

27. Dubé, N., Cheng, A., Tremblay, M.L. *The role of protein tyrosine phosphatase 1B in Ras signaling.* Proc Natl Acad Sci USA 2004, 101:1834-9.

28. Jackson, M.D., Denu, J.M. *Molecular reactions of protein phosphatases - Insights from structure and chemistry.* Chem Rev 2001, 101: 2313-40.

29. Denu, J.M., Lohse, D.L., Vijayalakshmi, J., Saper, M.A., Dixon, J.E. *Visualization of intermediate and transition-state structures in protein-tyrosine phosphatase catalysis.* Proc Natl Acad Sci USA 1996, 93: 2493-8.

30. Jia, Z., Barford, D., Flint, A., Tonks, N. K. *Structural basis for phosphotyrosine peptide recognition by protein tyrosine phosphatase 1B.* Science 1998, 268: 1754-8.

31. Puius, Y.A., Zhao, Y., Sullivan, M., Lawrence, D.S., Almo, S.C., Zhang, Z.-Y. *Identification of a second aryl phosphate-binding site in protein-tyrosine phosphatase 1B: A paradigm for inhibitor design.* Proc Natl Acad Sci USA 1997, 94: 13420-5.

32. Salmeen, A., Andersen, J.N., Myers, M.P., Tonks, N.K., Barford, D. *Molecular basis for the dephosphorylation of the activation segment of the insulin receptor by protein tyrosine phosphatase 1B.* Mol Cell 2000, 6: 1401-2.

33. Asante-Appiah, E., Ball, K., Bateman, K. et al. *The YRD motif is a major determinant of substrate and inhibitor specificity in T-cell protein-tyrosine phosphatase.* J Biol Chem 2001, 276: 26036-43.

34. Jia, Z., Ye, Q., Dinaut, A.N. et al. *Structure of protein tyrosine phosphatase 1B in complex with inhibitors bearing two phosphotyrosine mimetics.* J Med Chem 2001, 44: 4584-94.

35. Peters, G.H., Iversen, L.F., Branner, S. et al. *Residue 259 is a key determinant of substrate specificity of protein-tyrosine phosphatases 1B and α.* J Biol Chem 2000, 275: 18201-9.

36. Salmeen, A., Andersen, J.N., Myers, M.P. et al. *Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate.* Nature 2003, 423: 769-73.

37. van Montfort, R.L., Congreve, M., Tisi, D., Carr, R., Jhoti, H. *Oxidation state of the active-site cysteine in protein tyrosine phosphatase 1B.* Nature 2003, 423: 773-7.

38. You-Ten, K.E., Muise, E.S., Itié, A. et al. *Impaired bone marrow microenvironment and immune function in T cell protein tyrosine phosphatase-deficient mice.* J Exp Med 1997, 186: 683-93.

39. Iversen, L.F., Møller, K.B., Pedersen, A.K. et al. *Structure determination of T cell protein tyrosine phosphatase.* J Biol Chem 2002, 277: 19982-90.

40. Liu, G., Trevillyan, J.M. *Protein tyrosine phosphatase 1B as a target for the treatment of impaired glucose tolerance and type 2 diabetes*. Curr Opin Invest Drugs 2002, 3: 1608-16.

41. a) van Huijsduijnen, R.H., Sauer, W.H.B., Bombrun, A., Swinnen, D. *Prospects for inhibitors of protein tyrosine phosphatase 1B as antidiabetic drugs.* J Med Chem 2004, 47: 4142-6. b) Taylor, S.D., Hill, B. *Recent advances in protein tyrosine phosphatase 1B inhibitors.* Exp Opin Invest Drugs 2004, 13: 199-214. c) Liu, G. *Protein tyrosine phosphatase 1B inhibition: Opportunities and challenges.* Curr Med Chem 2003, 10: 1407-21. d) Harley, E.A., Levens, N. *Protein tyrosine phosphatase 1B inhibitors for the treatment of type 2 diabetes and obesity: Recent advances.* Curr Opin Invest Drugs 2003, 4: 1179-89. e) Johnson, T.O., Ermolieff, J., Jirousek, M.R. *Protein tyrosine phosphatase 1B inhibitors for diabetes.* Nat Rev Drug Discov 2002, 1: 696-709. f) Møller, N.P.H., Iversen, L.F., Andersen, H.S., McCormack, J.G. *Protein tyrosine phosphatases (PTPs) as drug targets: Inhibition of PTP1B for the treatment of diabetes.* Curr Opin Drug Discov Devel 2000, 3: 527-40.

42. Skorey, K.I., Kennedy, B.P., Friesen, R.W., Ramachandran, C. Development of a robust scintillation proximity assay for protein tyrosine phosphatase 1B using the catalytically inactive (C215S) mutant. Anal Biochem 2001, 291: 269-78.

43. Shen, K., Keng, Y.-F., Wu, L., Guo, X.-L., Lawrence, D.S., Zhang, Z.-Y. *Acquisition of a specific and potent PTP1B inhibitor from a novel combinatorial library and screening procedure.* J Biol Chem 2001, 276: 47311-9.

44. Asante-Appiah, E., Patel, S., Dufresne, C. et al. *The structure of PTP-1B in complex with a peptide inhibitor reveals an alternative binding mode for bisphosphonates.* Biochemistry 2002, 41: 9043-51.

45. Sun, J.-P., Fedorov, A.A., Lee, S.-Y. et al. *Crystal structure of PTP1B complexed with a potent and selective bidentate inhibitor.* J Biol Chem 2003, 278: 12406-14.

46. Dufresne, C., Roy, P., Wang, Z. et al. *The development of potent non-peptidic PTP-1B inhibitors.* Bioorg Med Chem Lett 2004, 14: 1039-42.

47. Lau, C.K., Bayly, C.I., Gauthier, J.Y. et al. *Structure based design of a series of potent and selective non peptidic PTP-1B inhibitors.* Bioorg Med Chem Lett 2004, 14: 1043-8.

48. Scapin, G., Patel, S.B., Becker, J.W., Wang, Q., Desponts, C., Waddleton, D. *The structural basis for the selectivity of benzotriazole inhibitors of PTP1B.* Biochemistry 2003, 42: 11451-9.

49. Li, X., Bhandari, A., Holmes, C.P., Szardenings, A.K. *α,α-Difluoro-β-ketophosphonates as potent inhibitors of protein tyrosine phosphatase 1B.* Bioorg Med Chem Lett 2004, 14: 4301-6.

50. Andersen, H.S., Iversen, L.F., Jeppesen, C.B. et al. *2-(Oxalylamino)-benzoic acid is a general, competitive inhibitor of protein-tyrosine phosphatases.* J Biol Chem 2000, 275: 7101-8.

51. Iversen, L.F., Andersen, H.S., Branner, S. et al. *Structure-based design of a low molecular weight, nonphosphorus, nonpeptide, and highly selective inhibitor of protein-tyrosine phosphatase 1B.* J Biol Chem 2000, 275: 10300-7.

52. Andersen, H.S., Olsen, O.H., Iversen, L.F. et al. *Discovery and SAR of a novel selective and orally bioavailable nonpeptide classical competitive inhibitor class of protein-tyrosine phosphatase 1B.* J Med Chem 2002, 45: 4443-59.

53. Iversen, L.F., Andersen, H.S., Møller, K.B. et al. *Steric hindrance as a basis for structure-based design of selective inhibitors of protein-tyrosine phosphatases.* Biochemistry 2001, 40: 14812-20.

54. Andersen, H.S., Hansen, T.K., Lau J. et al. (Novo Nordisk A/S) *Modulators of protein tyrosine phosphatases (PTPs).* WO 0204459.

55. Hansen, T.K., Iversen, L.F., Lau, J. et al. *Potent non-peptide protein-tyrosine phosphatase inhibitors.* 223rd ACS Natl Meet (April, Orlando) 2002, Abst MEDI-003.

56. Szczepankiewicz, B.G., Liu, G., Hajduk, P.J. et al. *Discovery of a potent, selective protein tyrosine phosphatase 1B inhibitor using a linked-fragment strategy.* J Am Chem Soc 2003, 125: 4087-96.

57. Liu, G, Szczepankiewicz, B.G., Pei, Z. et al. *Discovery and structure-activity relationship of oxalylarylaminobenzoic acids as inhibitors of protein tyrosine phosphatase 1B.* J Med Chem 2003, 46: 2093-103.

58. Xin, Z., Oost, T.K., Abad-Zapatero, C. et al. *Potent, selective inhibitors of protein tyrosine phosphatase 1B.* Bioorg Med Chem Lett 2003, 13: 1887-90.

59. Liu, G., Xin, Z., Liang, H. et al. *Selective protein tyrosine phosphatase 1B inhibitors: Targeting the second phosphotyrosine binding site with non-carboxylic acid-containing ligands.* J Med Chem. 2003, 46: 3437-40.

60. Bleasdale, J.E., Ogg, D., Palazuk, B.J. et al. *Small molecule peptidomimetics containing a novel phosphotyrosine bioisostere inhibit protein tyrosine phosphatase 1B (PTP1B) and augment insulin action*. Biochemistry 2001, 40: 5642-54.

61. Larsen, S.D., Barf, T., Liljebris, C. et al. *Synthesis and biological activity of a novel class of small molecular weight peptidomimetic competitive inhibitors of protein tyrosine phosphatase 1B.* J Med Chem 2002, 45: 598-622.

62. Liljebris, C., Larsen, S.D., Ogg, D., Palazuk, B.J., Bleasdale, J.E. *Investigation of potential bioisosteric replacement for the carboxyl groups of peptidomimetic inhibitors of protein tyrosine phosphatase 1B: Identification of a tetrazole-containing inhibitor with cellular activity.* J Med Chem 2002, 45: 1785-98.

63. Larsen, S.D., Stevens, F.C., Lindberg, T.J. et al. *Modification of the N-terminus of peptidomimetic protein tyrosine phosphatase 1B (PTP1B) inhibitors: Identification of analogues with cellular activity.* Bioorg Med Chem Lett 2003, 13: 971-5.

64. Pei, Z., Li, X., Liu, G. et al. *Discovery and SAR of novel, potent and selective protein tyrosine phosphatase 1B inhibitors.* Bioorg Med Chem Lett 2003, 13: 3129-32.

65. Wrobel, J., Sredy, J., Moxham, C. et al. PTP1B inhibition and antihyperglycemic activity of novel 11-arylbenzo[b]naphtho[2,3-d]furans and 11-arylbenzo[b]naphtho[2,3-d]thiophenes. J Med Chem 1999, 42: 3199-202.

66. Pei, Z., Zinker, B.A., Gum, R.J. et al. *(2R)-2-(2',6'-Dichloro-4'-dibenzo(b,d)furan-4''-ylphenoxy)-3-phenylpropanoic acid (A-321842) as a protein tyrosine phosphatase 1B (PTP1B) inhibitor with anti-diabetic effects in ob/ob mice.* Diabetes 2001, 50(S1): 1524-P, A401.

67. PTP-112, Drug Report, Investigational Drugs Database, www. Iddb3.com. (April 9, 2003).

68. Liu, G., Xin, Z., Pei, Z. et al. *Fragment screening and assembly: A highly efficient approach to a selective and cell active protein tyrosine phosphatase 1B inhibitor.* J Med Chem 2003, 46: 4232-5.

69. Xin, Z., Liu, G., Abad-Zapatero, C. et al. *Identification of a monoacid-based, cell permeable, selective inhibitor of protein tyrosine phosphatase 1B.* Bioorg Med Chem Lett 2003, 13: 3947-50.

70. Erlason, D.A., McDowell, R.S., He, M.M. et al. *Discovery of a new phosphotyrosine mimetic for PTP1B using breakaway tethering.* J Am Chem Soc 2003, 125: 5602-3.

71. Wang, J., Rideout, D., Ramnarayan, K. et al. *Organosulfur inhibitors of protein phosphatase.* WO 03032916.

72. Inaba, T., Ikemoto, T., Maegawa, H., Kashiwagi, A. (Japan Tobacco Inc.) *Azole compound and medical use thereof.* WO 03048140.

73. Huang, P., Ramphal, J., Wei, J. et al. *Structure-based design and discovery of novel inhibitors of protein tyrosine phosphatases*. Bioorg Med Chem 2003, 11: 1835-49.

74. Liljebris, C., Marinsson, J., Tedenborg, L. *Synthesis and biological activity of a novel class of pyridazine analogues as non-*

competitive reversible inhibitors of protein tyrosine phosphatase 1B. Bioorg Med Chem 2002, 10: 3197-212.

75. Tjernberg, A., Hallen, D., Schultz, J. et al. *Mechanism of action of pyridazine analogues on protein tyrosine phosphatase 1B (PTP1B).* Bioorg Med Chem Lett 2004, 14: 891-5.

76. Guertin, K.R., Setti, L., Qi, L. et al. *Identification of a novel class of orally active pyrimido[5,4-3]triazine-5,7-diamine-based hypoglycemic agents with protein tyrosine phosphatase 1B inhibitory activity*. Bioorg Med Chem Lett 2003, 13: 2895-8.

77. Thakkar, K.C., Berthel, S., Bose, J. et al. *Diaminopyrroloquinazolines 2: Novel and highly selective protein tyrosine phosphatase 1B inhibitors.* 227th ACS Natl Meet (March 28-April 1, Anaheim) 2004, Abst MEDI-049.

78. Thakkar, K.C., Bose, J., Banner, B. et al. *Diaminopyrroloquinazolines 3: Novel and highly selective protein tyrosine phosphatase 1B inhibitors.* 227th ACS Natl Meet (March 28-April 1, Anaheim) 2004, Abst MEDI-053.

79. Wiesmann, C., Barr, K.J., Kung, J. et al. *Allosteric inhibition of protein tyrosine phosphatase 1B.* Nat Struct Mol Biol 2004, 11: 730-7.

80. Bombrun, A., Gerber, P., Church, D. *Preparation of oxindole hydrazide modulators of protein tyrosine phosphatases (PTPs).* WO 03037328.