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PTP1B as a drug target: recent developments in PTP1B inhibitor discovery

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Protein tyrosine phosphatase 1B (PTP1B) is an effective target for the treatment of both type 2 diabetes and obesity; however, targeting PTP1B for drug discovery is challenging because of the highly conserved and positively charged active-site pocket. Tremendous progress has been made in the development of potent and selective PTP1B inhibitors that engage both the active site and no catalytic sites. Several strategies are being pursued to improve the pharmacological properties of PTP1B inhibitors. These new developments suggest that it is feasible to acquire PTP1B-based, small-molecule therapeutics with the requisite potency and selectivity. Future efforts will probably transform the potent and selective PTP1B inhibitors into orally available drugs with desirable physicochemical properties and *in vivo* efficacies.

Introduction

Reversible protein tyrosine phosphorylation catalyzed by the coordinated actions of protein tyrosine kinases (PTKs) and phosphatases (PTPs) is of paramount importance to the regulation of the signalling events that underlie such fundamental processes as growth and proliferation, differentiation and survival or apoptosis, as well as adhesion and motility [1]. Consequently, cellular pathways regulated by tyrosine phosphorylation offer a rich source of drug targets for developing novel therapeutics [2–4]. The potential of such targeted therapeutics has been well demonstrated by the successful treatment of human chronic myelogenous leukaemia and gastrointestinal stromal tumours with the PTK inhibitor STI-571 (Gleevec) [5,6], which targets Bcr/Abl or c-kit, aberrantly activated in the malignancies.

PTPs are enzymes that catalyze protein tyrosine dephosphorylation. In humans, more than a hundred PTPs exist that can function either as negative or positive modulators in various signal transduction pathways [7]. As expected, several PTPs are found to antagonize PTK-mediated signalling. For example, mutations in the SH2-containing SHP1 tyrosine phosphatase lead to severe immune dysfunction, giving rise to the *moth-eaten* phenotype in mice [8]. Thus, SHP1 is an important negative regulator of cytokine signalling and its loss results in sustained tyrosine phosphorylation with consequent enhanced proliferation. Loss of SHP1 is frequently observed in myelodysplastic syndrome [9] and lymphomas [10]. A more recent analysis implicates several PTPs as tumour suppressors that are frequently mutated in colon cancer [11].

Interestingly, there is also mounting evidence that PTPs can also potentiate, rather than antagonize, the actions of PTKs. This mode of synergy enhances mitogenic signalling, leading to cell transformation. Thus CD45, through its capacity to dephosphorylate and activate *src* family PTKs, is essential for initiating downstream signalling processes in stimulated T and B cells [12]. SHP2 and its *Drosophila* homolog *corkscrew* are positive mediators of growth factor signalling [13,14]. Several activating (gain of function) mutations in human SHP2 have been identified as the cause of the inherited disorder Noonan syndrome [15] and some forms of leukaemia and solid tumours [16,17]. Most recently, the phosphatase of regenerating liver (PRL) phosphatases have been implicated as potential oncogenes that promote cell growth and tumour invasion [18].

As discussed above, deregulation of PTP activity contributes to the pathogenesis of several human diseases, including cancer, diabetes and immune disorders [19–21]. The importance of the PTPs in diverse pathophysiology has made them the focus of intense interest as a new class of drug targets. Thus, inhibitors of the PTPs are also expected to have therapeutic value with novel modes of action [22,23]. Among various members of the PTP superfamily, PTP1B has emerged as the best-validated drug target

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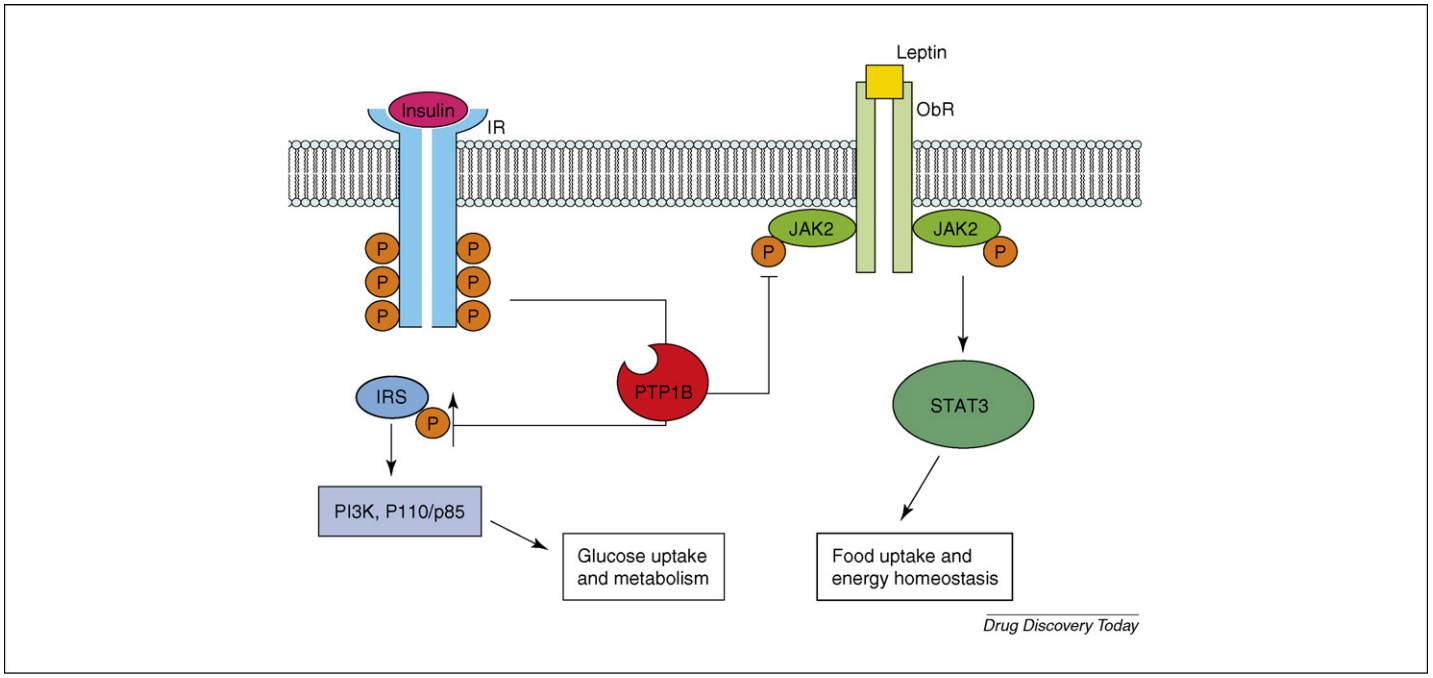

FIGURE 1

The role of PTP1B in insulin and leptin signalling. In the insulin signalling pathway, PTP1B can associate with and dephosphorylate activated insulin receptor (IR) or insulin receptor substrates (IRS) [26–30]. In the leptin pathway, PTP1B binds and dephosphorylateS JAK2, which is downstream of the leptin receptor, ObR [40,41]. STAT3 and P110/p85 are downstream targets of JAK2 and IRS1, respectively. P110/p85 is a specific form of PI3K responsive to insulin signalling.

[24]. However, it has become apparent that the conserved structural and mechanistic features of the PTP active site present substantial challenges to drug development. Nevertheless, great progress has been made to address the inherent potency, selectivity and bioavailability problems associated with targeting PTPs for therapeutic development. In the following review, we summarize the major findings that establish PTP1B as an outstanding target for the treatment of diabetes and obesity, and highlight recent developments in PTP1B inhibitor discovery.

Validation of PTP1B as a drug target for diabetes and obesity

PTP1B is localized to the cytoplasmic face of the endoplasmic reticulum and is expressed ubiquitously, including in the classical insulin-targeted tissues such as liver, muscle and fat [25]. Mounting evidence from biochemical, genetic and pharmacological studies support a role for PTP1B as a negative regulator in both insulin and leptin signalling (Figure 1). PTP1B can associate with and dephosphorylate activated insulin receptor (IR) or insulin receptor substrates (IRS) [26–30]. Overexpression of PTP1B in cell cultures decreases insulin-stimulated phosphorylation of IR and/or IRS-1, whereas reduction in the level of PTP1B, by antisense oligonucleotides or neutralizing antibodies, augments insulin-initiated signalling [31–34]. Analyses of quantitative trait loci and mutations in the gene encoding PTP1B in humans support the notion that aberrant expression of PTP1B can contribute to diabetes and obesity [35–37]. Mice that lack PTP1B display enhanced sensitivity to insulin, with increased or prolonged tyrosine phosphorylation of IR in muscle and liver [38,39]. Interestingly, *PTP1B*$^{-/-}$ mice are protected against weight gain and have significantly lower triglyceride levels when placed on a high-fat diet. This is unexpected because insulin is also an anabolic factor, and increased insulin sensitivity can result in increased weight gain. PTP1B was subsequently shown to bind and dephosphorylate JAK2, which is downstream of leptin receptor [40,41]. Thus, the resistance to diet-induced obesity observed in *PTP1B*$^{-/-}$ mice is likely to be associated with increased energy expenditure owing to enhanced leptin sensitivity. Recent tissue-specific knockout results indicate that body weight, adiposity and leptin action can be regulated by neuronal PTP1B [42]. Inhibiting neuronal PTP1B would require drugs that penetrate the blood–brain barrier. Consistent with the above results, antisense-based oligonucleotides that target PTP1B have shown efficacy in type 2 diabetes and have entered phase 2 clinical trials [32,43]. In addition, small-molecule inhibitors of PTP1B can work synergistically with insulin to increase insulin signalling and augment insulin-stimulated glucose uptake [44]. Moreover, pretreatment of leptin-resistant rats with a potent and selective PTP1B inhibitor results in a marked improvement in leptin-dependent suppression of food intake [45]. Collectively, these biochemical, genetic and pharmacological studies provide strong proof-of-concept, validating the notion that inhibition of PTP1B could address both diabetes and obesity and making PTP1B an exciting target for drug development.

Challenges in developing PTP1B-based small-molecule therapeutics

Selectivity is one of the major issues in the development of PTP1B inhibitors as drugs. Because all PTPs share a high degree of structural conservation in the active site, the pTyr (phosphotyrosine)-binding pocket, designing inhibitors with both high affinity and selectivity for PTP1B poses a challenge. Fortunately, PTP substrate specificity studies have shown that pTyr alone is not sufficient for

high-affinity binding, and residues flanking the pTyr are important for PTP substrate recognition [46]. The results indicate that there are subpockets adjacent to the PTP active site that can also be targeted for inhibitor development. These studies also provide a molecular basis for addressing and manipulating PTP inhibitor potency and specificity, and suggest a novel paradigm for the design of potent and specific PTP inhibitors; namely bidentate ligands that bind to both the active site and a unique adjacent peripheral site. Consequently, unique PTP subpockets that border the active site can be targeted to enhance inhibitor affinity and selectivity. The rationale for the enhanced affinity of bidentate inhibitors is based on the principle of additivity of free energy of binding. The interaction of an inhibitor with two independent sites (e.g. a pTyr site and a unique peripheral site) in one PTP would confer exquisite specificity because other PTPs might not possess an identical second-site interaction. Based on this paradigm, several potent and selective PTP1B inhibitors have been developed; these will be discussed in the following section.

Bioavailability is another important issue in the development of PTP1B-based small-molecule therapeutics. The active sites of PTPs have evolved to accommodate pTyr, which contains two negative charges at physiological pH. Consequently, most active-site-directed PTP inhibitors (non-hydrolyzable pTyr mimetics) reported to date possess a high charge density to serve as competitive inhibitors. Such molecules are generally not drug-like, with limited cell membrane permeability. Several strategies have been applied to improve the cell permeability and/or bioavailability of PTP1B inhibitors that will be highlighted in this review.

Development of potent and selective PTP1B inhibitors

The library approach

A focused library approach was used to identify highly potent and selective PTP1B inhibitors that are capable of bridging and simultaneously associating with both the active site and an adjacent peripheral site [47]. The library contains (i) a biasing pTyr to ensure association with the active site and (ii) a structurally diverse set of 23 linkers that tether the pTyr moiety to (iii) a structurally diverse set of eight aryl acids, which were designed to associate with the peripheral subsite, positioned near the active site. Because the library contains hydrolyzable pTyr residues, the screen used a catalytically inactive PTP1B/C215S mutant that retains its wild-type binding ability for pTyr-bearing library components. The compound with the highest affinity to PTP1B/C215S was identified, and the corresponding non-hydrolyzable bisdifluorophosphonate (compound **1**) was subsequently synthesized (Figure 2). Compound **1** is the most potent and selective PTP1B inhibitor identified to date (K_i = 2.4 nM) – it exhibits, with one exception, a 1000- to 10 000-fold selectivity against a panel of other PTPs [47]. The sole exception is TC-PTP, which is 77% identical to PTP1B. Tenfold selectivity in favour of PTP1B was observed against TC-PTP. Mutagenesis and structural analysis of the interactions between PTP1B and **2** (a derivative of **1**) revealed that the nonhydrolyzable pTyr surrogate phosphonodifluoromethyl phenylalanine (F_2Pmp) occupies the active site, whereas the distal 4-phosphonodifluoromethyl phenylacetyl group makes both van der Waals and ionic contacts with a proximal non-catalytic site formed by Lys41, Arg47 and Asp48 [48]. The results show that, although many of the residues in contact with compound **2** are not unique to PTP1B, the combinations of all contact residues differ between PTPs, which suggest that the binding surface defined by these residues determines inhibitor selectivity. This, in turn, indicates that the library approach can be a general and effective method to acquire potent and selective PTP1B inhibitors.

The 'linked-fragment' approach

In addition to the proximal non-catalytic site defined by Lys41, Arg47 and Asp48, a second aryl phosphate-binding site, adjacent to the PTP1B active site, was identified from crystal structures of the protein in complex with pTyr and a small aryl phosphate [49]. This second aryl phosphate-binding site lies within a region (Arg24 and Arg254) that is not conserved among the PTPs. A 'linked-fragment' approach (also referred to as SAR by NMR) was employed to develop potent and selective PTP1B inhibitors that can engage both the active site and the second aryl phosphate-binding site [50–52]. In this approach, NMR was used to identify small molecules that bind to the active site. The identified hits were optimized, based on crystal structures of the complexes. Using a separate NMR screen, small molecules that occupy the second aryl phosphate-binding site were also identified. An appropriate linker was then installed to connect the two binding fragments. Compounds **3** (K_i = 22 nM; twofold selectivity compared with TC-PTP) [50] and **4** (K_i = 18 nM; fourfold selectivity compared with TC-PTP) [51] were both obtained using this approach. Compound **4** is not cell permeable owing to the presence of two negative charges. Prodrug **5** was synthesized, and the carboxylic acids were replaced by their esters, to demonstrate cellular activities [51]. Compound **6**, with a less charged pTyr mimetic and a more rigid linker, displayed a 30-fold selectivity compared with TC-PTP [52]. In addition to its excellent selectivity, compound **6** also exhibited moderate cell permeability, probably because of the decreased charge. The fluorine at the ortho-position can also enhance cell permeability because compound **7**, with a proton at this position, showed significantly lower cell permeability. These examples demonstrate that the linked-fragment approach can be an effective strategy to obtain potent and selective PTP1B inhibitors. Besides the NMR-based screening, the 'tethering' method [53] and high-throughput X-ray crystallography-based screening [54] have also been used to identify small-molecule fragments that target the active site and adjacent peripheral sites.

The conformation-assisted approach

Structure-based modelling has been used to target unique PTP1B conformations for inhibitor development with both high affinity and selectivity [55]. A series of benzotriazole phenyldifluoromethylphosphonic acids were synthesized as non-peptidic PTP1B inhibitors. Many of these compounds showed good inhibitory activity, at the sub-μM level, for PTP1B but none of them had selectivity compared with TC-PTP. One of these inhibitors, compound **8**, was co-crystallized with PTP1B. The central carbon of **8** has four functional substituents. In the crystal structure, one of the phenyldifluoromethylphosphonic acids binds the PTP1B active site; the benzotriazole group interacts with the Arg47 and adjacent residues; the benzene ring is important for the overall rigidity of whole molecule, which was found to be crucial for the inhibitory activity; and the other phenyldifluoromethylphosphonic acid

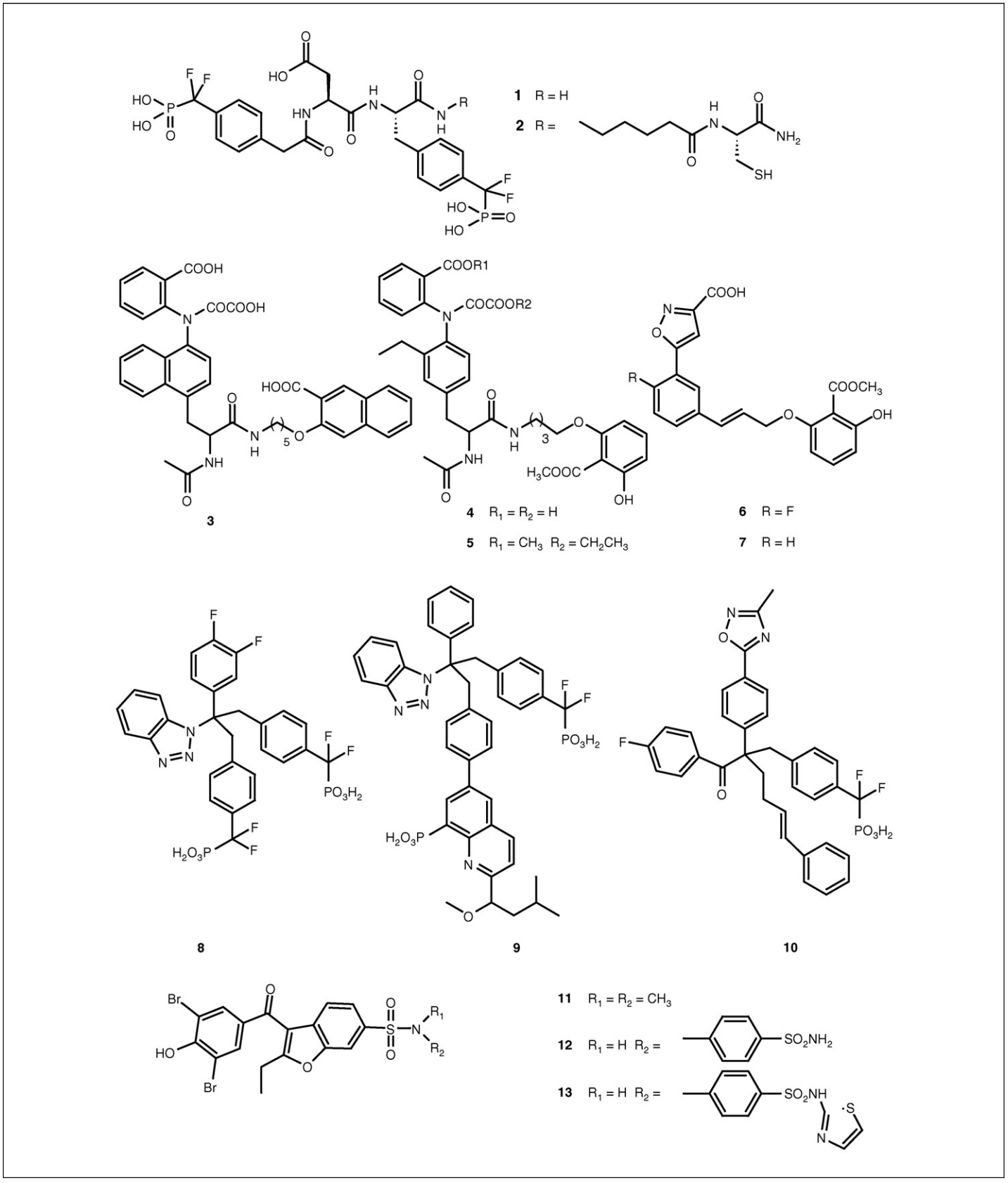

FIGURE 2

Potent and selective PTP1B inhibitors. Compounds **1** and **2** are from [47] and [48], respectively. Compound **3** is from [50]. Compounds **4** and **5** are from [51]. Compounds **6** and **7** are from [52]. Compounds **8** and **9** are from [55], and compound **10** from [57]. Compounds **11**, **12** and **13** are from [59].

points to the second aryl phosphate site. Based on this structural information, the second phenyldifluoromethylphosphonic acid group was modified. A longer and more rigid bi-phenyl linker was chosen, so that the phophonic acid could reach the second aryl phosphate-binding site to increase binding affinity. To gain selectivity, the crystal structures of PTP1B and TC-PTP were compared, to identify residues that are unique to PTP1B. One of the residues is Phe52 in PTP1B, whereas the corresponding position in TC-PTP is Tyr54. Methoxylisobutylmethylquinoline was then appended to the bi-phenyl linker (Figure 2; compound **9**) to interact with Phe52. Interestingly, compound **9** has a sevenfold selectivity compared with TC-PTP. The X-ray crystal structure of **9** with PTP1B confirmed the interactions between the methoxylisobutylmethyl group and Phe52. In addition, a PTP1B F52Y mutant displayed similar affinity to compound **9** as TC-PTP did [56]. A similar approach was used to target Leu119 in PTP1B (Val121 in TC-PTP) to generate compound **10** [57]. The oxadiazole group in **10** was responsible for a tenfold selectivity compared with TC-PTP. An analogous structure-based approach was used to transform 2-(oxalyamino)-benzoic acid, a broad-specificity low-affinity inhibitor, into PTP1B inhibitors with greater potency and specificity [58].

Targeting allosteric sites for improved selectivity and bioavailability

A secondary allosteric site has recently been described for PTP1B, and several small-molecule inhibitors that occupy this site stabilize an inactive conformation of PTP1B [59]. Unlike the pTyr-binding active site, the allosteric site is not well conserved and possesses is substantially less polar. Thus, targeting the allosteric site might present an alternative strategy for developing selective inhibitors with acceptable pharmacological properties. Compounds **11**, **12** and **13** (Figure 2) are examples of allosteric inhibitors reported for PTP1B [59]. Compound **11** (IC_{50} = 350 μM) was identified through a screen of a non-pTyr-like compound library. Elaboration at the sulfonyl end of **11,** with additional aromatic rings, afforded compounds **12** (IC_{50} = 22 μM) and **13** (IC_{50} = 8 μM) with improved potency and selectivity. As revealed by the co-crystal structures, the aromatic rings of compound **13** form a pocket around Phe280, whereas compound **12** only partially wraps around Phe280. The interactions between Phe280 and the aromatic rings in these inhibitors are correlated with their binding affinity. Interestingly, compound **12** showed a sixfold selectivity compared with TC-PTP, and compound **13** exhibited cellular activities (increasing the phosphorylation level of IR and IRS-1) at 250 μM.

Improving bioavailability of PTP1B Inhibitors

As mentioned earlier, bioavailability represents another major challenge in transforming PTP1B inhibitors into therapeutics because most of the current active site-directed inhibitors contain substantial negative charges. Various approaches have been used to improve cell permeability of PTP1B inhibitors, and some of the commonly used strategies will now be discussed.

Charge reduction

The most straightforward approach is to reduce the number of negative charges, so that a less-charged derivative might be able to penetrate the cell membrane. One example is compound **14** (Figure 3) [60]. Its analogue, **15**, was first identified as an effective inhibitor of PTP1B, but the two negative charges limit its cell permeability [61]. Interestingly, when one of the carboxylic acids was replaced by a tetrazole group, the resulting compound, **14,** gained cellular activity without loss in inhibitory activity. Another example is compound **16a** (K_i = 9.0 μM), which forms an equilibrium with the lactone form **16b** [62]. The lactone form is non-charged and thus endows the inhibitor with high cell permeability.

Increasing hydrophobicity

Another approach to increase cell permeability is to enhance the hydrophobic character of the compounds. Because most pTyr mimetics have negative charge(s), they usually are hydrophilic. As more hydrophobic components are introduced, the compounds become more lipophilic, with favourable membrane partition coefficients. For example, although compound **9** (Figure 2) contains four negative charges, it is active in a cell-based assay, possibly owing to the presence of multiple aromatic rings [55]. Similarly, compounds **17** and **18** (Figure 3) are orally bioavailable and active in animal models of type 2 diabetes, even although they are highly charged [63]. Thus, increasing hydrophobicity can be an effective strategy to improve PTP1B inhibitor bioavailability.

Prodrug delivery

The prodrug approach has been widely used to deliver compounds containing one or more carboxylic acid group(s). The corresponding methyl or ethyl esters are called prodrugs, and they are much easier to pass through the cell membrane. Once inside the cell, the prodrugs are hydrolyzed to regenerate the original inhibitors. Compound **5** (Figure 2) is an example of a prodrug for a carboxylic acid-based PTP1B inhibitor [51], and so are compounds **19** [64] and **20** [65] (Figure 3). Because several organophosphonate-based prodrugs have advanced to the clinic and beyond [66], a prodrug strategy can also be employed to improve the cellular delivery of difluorophosphonate-based PTP1B inhibitors. A novel prodrug approach that has been developed for intracellular delivery of nucleotides and aryl phosphates [67] has been extended to investigate the synthesis and activation of difluoromethyl phosphonate prodrugs. Studies with the prodrug of compound **1** (compound **21** in Figure 3) in a cell-based system indicate that this prodrug strategy provides an excellent solution to the drug delivery problem associated with organophosphonate-based PTP1B inhibitors [68].

Targeting the allosteric site

Allosteric modulation of PTP1B activity with small molecules might provide a promising approach to overcome the potential challenges of targeting the active site. Allosteric inhibitors, mentioned previously, are more likely to be cell permeable because they normally do not have negative charges. In addition to compounds **11–13**, compound **22** might serve as another example of an allosteric inhibitor (Figure 3). Compound **22**, with an IC_{50} of 1.6 μM, is a reversible non-competitive inhibitor of PTP1B, suggesting that it can bind to a pocket other than the active site. As a non-charged inhibitor, compound **22** shows good cellular activity [69].

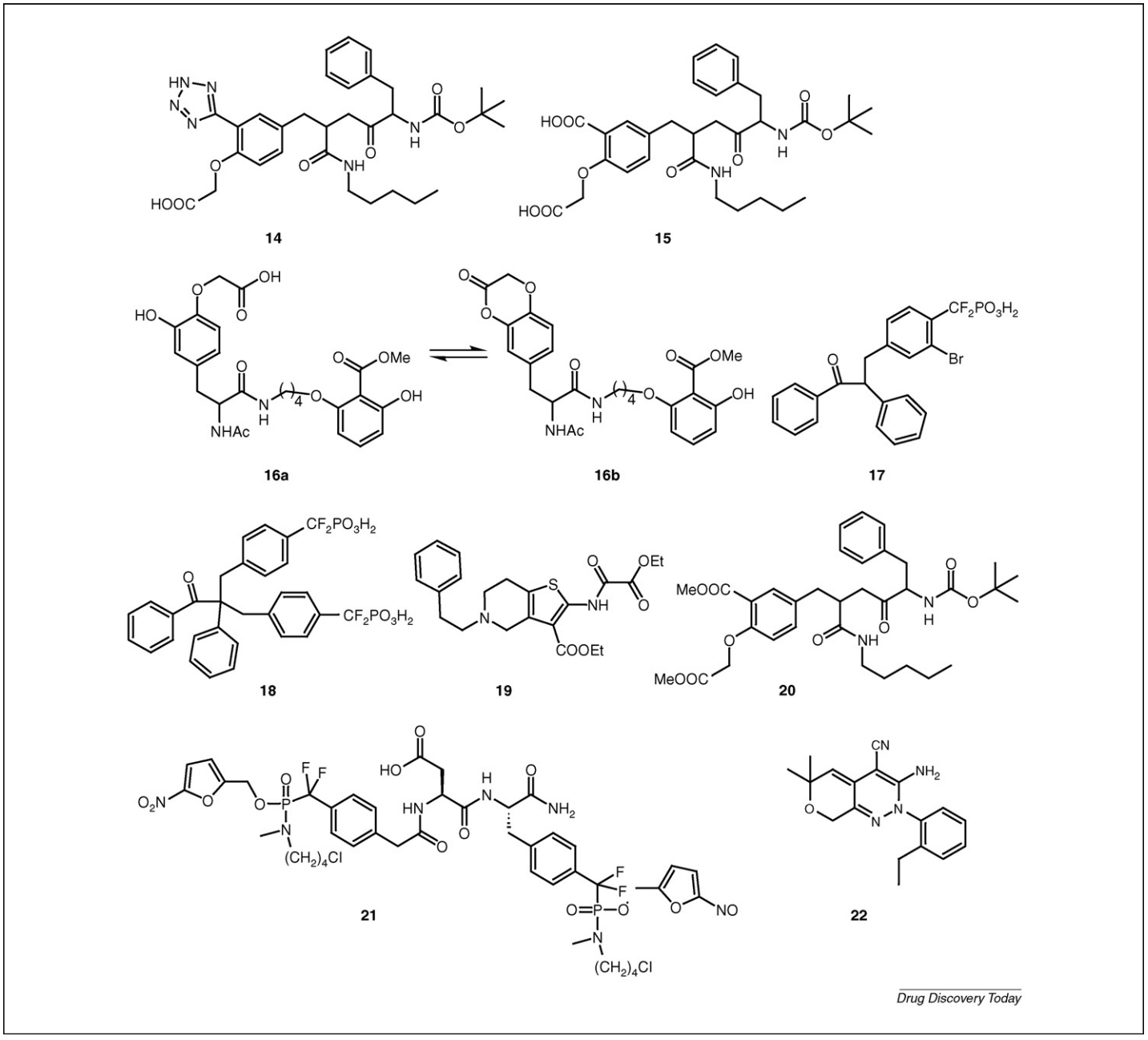

FIGURE 3

PTP1B inhibitors with improved bioavailability. Compound **14** is from [60], **15** from [61], and **16** from [62]. Compounds **17** and **18** are from [63]. Compound **19** is from [64], **20** from [65], **21** from [68], and **22** from [69].

Identification of novel pTyr mimetics

Breakaway tethering

There is continued interest in developing novel pTyr mimetics with more acceptable pharmacological properties. A 'breakaway-tethering' method was applied to search for novel pTyr mimetics [53]. In this approach, a library of small molecules containing a disulfide-bond tail was screened against PTP1B with a free thiol group engineered at position 47, near the active site. Under partially reducing conditions, if a compound in the library has an affinity for a site near the free thiol group, a disulfide bond will be formed between the small molecule and the engineered thiol group, enabling the mass spectrometric identification of the bound ligand. A novel pTyr mimetic (compound **23**; Figure 4) was identified by the breakaway-tethering-based screening. In addition, to identify active-site-binding fragments, the tethering method can also be used to discover fragments that bind to peripheral sites near the pTyr binding pocket.

Structure-based search

An X-ray crystallography-based screening was recently used to search for new pTyr mimetics [54]. Briefly, PTP1B crystals were soaked in a solution of two to eight possible active-site-binding fragments. After soaking, X-ray diffraction data were collected and processed in an automated manner, so that the fragments that bind to the active site can be quickly identified. Out of 264 compounds, compound **24** (K_i = 86 μM) was reported as a novel

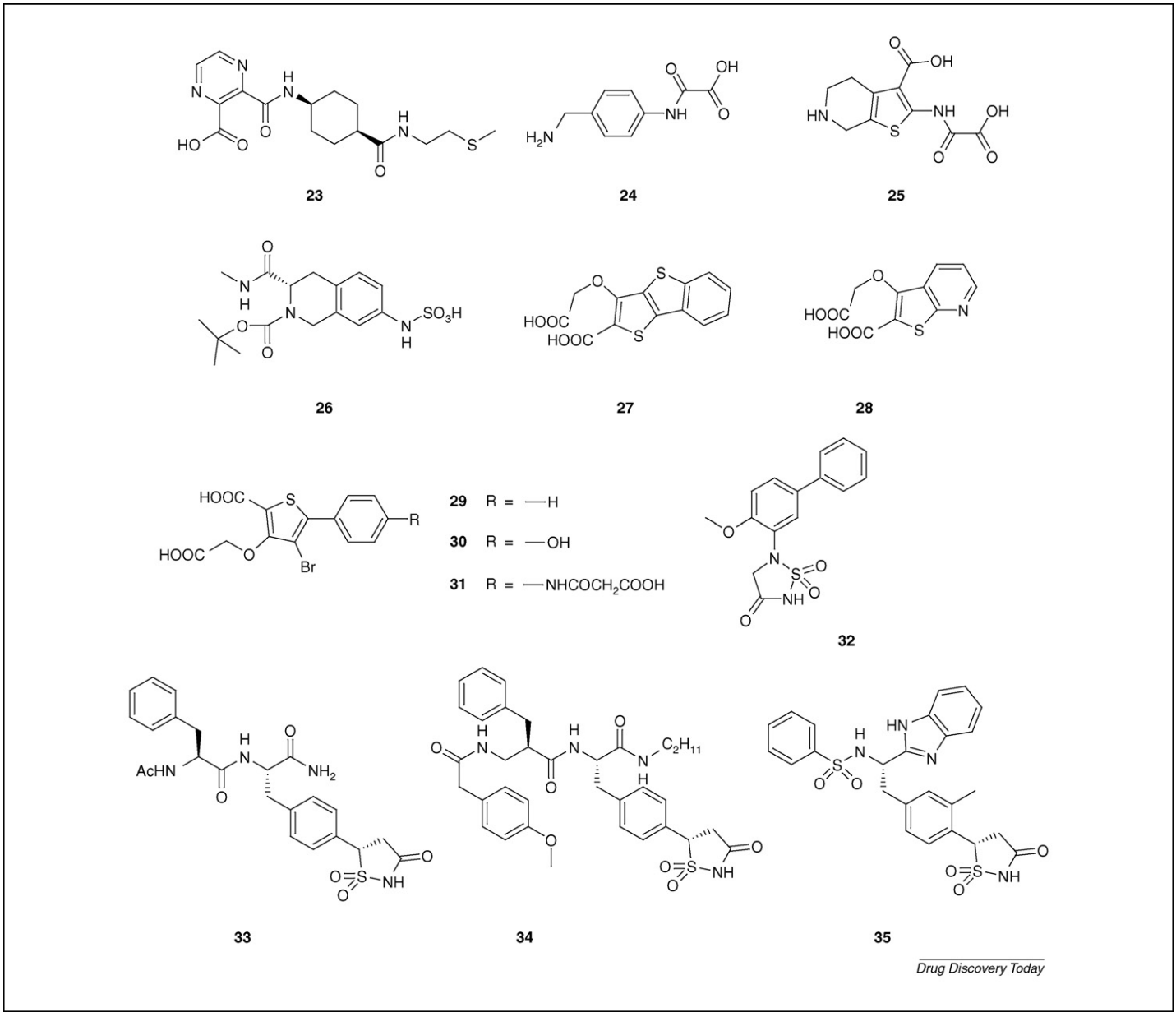

FIGURE 4

Novel pTyr mimetics. Compounds **23** and **24** are from [53] and [54], respectively. Compound **25** is from [58] and **26** from [70]. Compounds **27** and **28** are from [71]. Compounds **29**, **30** and **31** are from [72]. Compound **32** is from [73], **33** from [74], **34** from [75], and **35** from [76].

pTyr mimetic using this approach. Compound **24** is similar to compound **25**, which was developed from a high-throughput screen [58], highlighting the utility of the structure-based screen for the discovery of novel pTyr mimetics.

High-throughput screening

High-throughput screening is also commonly used to identify novel pTyr mimetics. Compound **26** (Figure 4) was developed based on hits identified from a compound collection [70]. With an IC_{50} of 42.5 μM and fourfold selectivity compared with TC-PTP, the single negatively charged compound **26** is a promising pTyr mimetic that is worth further optimization, as is compound **27** [71]. Compound **28** (K_i = 230 μM) was obtained from an initial screen as an active site binding motif. Optimization efforts guided by crystal structures resulted in compound **27**, with a K_i of 9.2 μM. A series of monocylic thiophenes were then synthesized and tested as PTP1B inhibitors [72]. Among them, compound **30** (K_i = 0.3 μM) showed tenfold increase in potency compared with the parental compound, **29** (K_i = 3.2 μM). As shown in the co-crystal structure, the OH group of compound **30** makes contacts with residue Asp48, which explains its higher inhibition activity. Compound **31** was designed to have additional electrostatic interactions with Arg47 and, as a result, it has an even lower K_i (0.14 μM).

Rational design

In addition to various screening methods, rational design continuously serves as an efficient way to develop novel active-site-

binding motifs. For example, a 1,2,5-thiadiazolidin-3-one-1,1-dioxide group was recently reported to mimic the phosphoryl moiety of pTyr, and the corresponding 1,2,5-thiadiazolidin-3-one-1,1-dioxide-containing compound, **32,** (Figure 4) has an IC_{50} of 2.47 μM for PTP1B, indicating that compound **32** is an effective pTyr mimetic [73]. Compound **33**, which contains a similar isothiazolidinone group, is also an excellent pTyr mimetic [74]. When incorporated into a di-peptide structure, the isothiazolidinone-containing inhibitor **33** has a K_i of 0.19 μM. Using the isothiazolidinone group as the pTyr mimetic, a peptide-based inhibitor, **34,** was synthesized that has an IC_{50} of 40 nM. This demonstrates the utility of the isothiazolidinone to serve as a highly efficacious pTyr mimetic [75]. To improve cell permeability and oral bioavailability, a series of non-peptide-based inhibitors using the same isothiazolidinone group as the pTyr mimetic were synthesized. Among them, compound **35** displayed high inhibition potency, with an IC_{50} of 35 nM. It also exhibited considerable cellular activity, increasing the IR phosphorylation level at 80 μM [76].

Conclusion

Mounting evidence from biochemical, genetic and small molecule studies have established PTP1B as an outstanding drug target for the treatment of diabetes and obesity. Recent studies have revealed that it is highly feasible to achieve potency and selectivity in PTP1B inhibitor development. In addition, several strategies are being explored to improve the bioavailability of PTP1B inhibitors. It is probable that potent and selective PTP1B inhibitors with optimal pharmacological properties will emerge in the not too distant future.

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References

1 Hunter, T. (2000) Signalling – 2000 and beyond. *Cell* 100, 113–127
2 Hunter, T. (1998) The phosphorylation of proteins on tyrosine: its role in cell growth and disease. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 353, 583–605
3 Blume-Jensen, P. and Hunter, T. (2001) Oncogenic kinase signalling. *Nature* 411, 355–365
4 Ventura, J.J. and Nebreda, A.R. (2006) Protein kinases and phosphatases as therapeutic targets in cancer. *Clin. Transl. Oncol.* 8, 153–160
5 Goldman, J.M. and Melo, J.V. (2001) Targeting the BCR-ABL tyrosine kinase in chronic myeloid leukaemia. *N. Engl. J. Med.* 344, 1084–1086
6 Berman, J. and O'Leary, T.J. (2001) Gastrointestinal stromal tumour workshop. *Hum. Pathol.* 32, 578–582
7 Alonso, A. *et al.* (2004) Protein tyrosine phosphatases in the human genome. *Cell* 117, 699–711
8 Shultz, L.D. *et al.* (1993) Mutations at the murine motheaten locus are within the hematopoietic cell protein tyrosine phosphatase (*Hcph*) gene. *Cell* 73, 1445–1454
9 Mena-Duran, A.V. *et al.* (2005) SHP1 expression in bone marrow biopsies of myelodysplastic syndrome patients: a new prognostic factor. *Br. J. Haematol.* 129, 791–794
10 Zhang, Q. *et al.* (2005) STAT3- and DNA methyltransferase 1-mediated epigenetic silencing of SHP-1 tyrosine phosphatase tumour suppressor gene in malignant T lymphocytes. *Proc. Natl. Acad. Sci. U. S. A.* 102, 6948–6953
11 Wang, Z. *et al.* (2004) Mutational analysis of the tyrosine phosphatome in colorectal cancers. *Science* 304, 1164–1166
12 Pingel, J.T. and Thomas, M.L. (1989) Evidence that the leukocyte-common antigen is required for antigen-induced T lymphocyte proliferation. *Cell* 58, 1055–1065
13 Perkins, L.A. *et al.* (1992) *corkscrew* encodes a putative protein tyrosine phosphatase that functions to transduce the terminal signal from the receptor tyrosine kinase torso. *Cell* 70, 225–236
14 Noguchi, T. *et al.* (1994) Role of SH-PTP2, a protein-tyrosine phosphatase with src homology 2 domains, in insulin-stimulated Ras activation. *Mol. Cell. Biol.* 14, 6674–6682
15 Tartaglia, M. *et al.* (2001) Mutations in *PTPN11*, encoding the protein tyrosine phosphatase SHP2, cause Noonan syndrome. *Nat. Genet.* 29, 465–468
16 Tartaglia, M. *et al.* (2003) Somatic mutations in *PTPN11* in juvenile myelomonocytic leukaemia, myelodysplastic syndromes and acute myeloid leukaemia. *Nat. Genet.* 34, 148–150
17 Bentires-Alj, M. *et al.* (2004) Activating mutations of the noonan syndrome-associated *SHP2/PTPN11* gene in human solid tumours and adult acute myelogenous leukaemia. *Cancer Res.* 64, 8816–8820
18 Stephens, B.J. *et al.* (2005) PRL phosphatases as potential molecular targets in cancer. *Mol. Cancer Ther.* 4, 1653–1661
19 Zhang, Z.-Y. (2001) Protein tyrosine phosphatases: prospects for therapeutics. *Curr. Opin. Chem. Biol.* 5, 416–423
20 Andersen, J.N. *et al.* (2004) A genomic perspective on protein tyrosine phosphatases: gene structure, pseudogenes, and genetic disease linkage. *FASEB J.* 18, 8–30
21 Arena, S. *et al.* (2005) Genetic analysis of the *kinome* and *phosphatome* in cancer. *Cell. Mol. Life Sci.* 62, 2092–2099
22 van Huijsduijnen, R.H. *et al.* (2002) Selecting protein tyrosine phosphatases as drug targets. *Drug Discov. Today* 7, 1013–1019
23 Bialy, L. and Waldmann, H. (2005) Inhibitors of protein tyrosine phosphatases: next-generation drugs? *Angew. Chem. Int. Ed. Engl.* 44, 3814–3839
24 Zhang, Z.-Y. and Lee, S.-Y. (2003) PTP1B inhibitors as potential therapeutics in the treatment of type 2 diabetes and obesity. *Expert Opin. Investig. Drugs* 12, 223–233
25 Tonks, N.K. (2003) PTP1B: from the sidelines to the front lines! *FEBS Lett.* 546, 140–148
26 Bandyopadhyay, D. *et al.* (1997) Protein-tyrosine phosphatase 1B complexes with the insulin receptor *in vivo* and is tyrosine-phosphorylated in the presence of insulin. *J. Biol. Chem.* 272, 1639–1645
27 Dadke, S. *et al.* (2000) Down-regulation of insulin signalling by protein-tyrosine phosphatase 1B is mediated by an N-terminal binding region. *J. Biol. Chem.* 275, 23642–23647
28 Walchli, S. *et al.* (2000) Identification of tyrosine phosphatases that dephosphorylate the insulin receptor: a brute-force approach based on 'substrate-trapping' mutants. *J. Biol. Chem.* 275, 9792–9796
29 Goldstein, B.J. *et al.* (2000) Tyrosine dephosphorylation and deactivation of insulin receptor substrate-1 by protein-tyrosine phosphatase 1B. Possible facilitation by the formation of a ternary complex with the Grb2 adaptor protein. *J. Biol. Chem.* 275, 4283–4289
30 Calera, M.R. *et al.* (2000) Dynamics of protein-tyrosine phosphatases in rat adipocytes. *J. Biol. Chem.* 275, 6308–6312
31 Ahmad, F. *et al.* (1995) Osmotic loading of neutralizing antibodies demonstrates a role for protein-tyrosine phosphatase 1B in negative regulation of the insulin action pathway. *J. Biol. Chem.* 270, 20503–20508
32 Kenner, K.A. *et al.* (1996) Protein-tyrosine phosphatase 1B is a negative regulator of insulin- and insulin-like growth factor-I-stimulated signalling. *J. Biol. Chem.* 271, 19810–19816
33 Byon, J.C.H. *et al.* (1998) Protein-tyrosine phosphatase-1B acts as a negative regulator of insulin signal transduction. *Mol. Cell. Biochem.* 182, 101–198
34 Zinker, B.A. *et al.* (2002) PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11357–11362
35 Di Paola, R. *et al.* (2002) A variation in 3'UTR of *hPTP1B* increases specific gene expression and associates with insulin resistance. *Am. J. Hum. Genet.* 70, 806–812
36 Echwald, S.M. *et al.* (2002) A P387L variant in protein tyrosine phosphatase-1B (PTP-1B) is associated with type 2 diabetes and impaired serine phosphorylation of PTP-1B *in vitro*. *Diabetes* 51, 1–6
37 Mok, A. *et al.* (2002) A single nucleotide polymorphism in protein tyrosine phosphatase PTP-1B is associated with protection from diabetes or impaired glucose tolerance in Oji-Cree. *J. Clin. Endocrinol. Metab.* 87, 724–727

38 Elchebly, M. *et al.* (1999) Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science* 283, 1544–1548

39 Klaman, L.D. *et al.* (2000) Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. *Mol. Cell. Biol.* 20, 5479–5489

40 Zabolotny, J.M. *et al.* (2002) PTP1B regulates leptin signal transduction *in vivo*. *Dev. Cell* 2, 489–495

41 Cheng, A. *et al.* (2002) Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. *Dev. Cell* 2, 497–503

42 Bence, K.K. *et al.* (2006) Neuronal PTP1B regulates body weight, adiposity and leptin action. *Nat. Med.* 12, 917–924

43 Liu, G. (2004) Technology evaluation: ISIS-113715. *Isis. Curr. Opin. Mol. Ther.* 6, 331–336

44 Xie, L. *et al.* (2003) Cellular effects of small molecule PTP1B inhibitors on insulin signalling. *Biochemistry* 42, 12792–12804

45 Morrison, C.D. *et al.* (2007) Increased hypothalamic PTP1B contributes to leptin resistance with age. *Endocrinology* 148, 433–440

46 Zhang, Z.-Y. (2002) Protein tyrosine phosphatases: structure and function, substrate specificity, and inhibitor development. *Annu. Rev. Pharmacol. Toxicol.* 42, 209–234

47 Shen, K. *et al.* (2001) Acquisition of a specific and potent PTP1B inhibitor from a novel combinatorial library and screening procedure. *J. Biol. Chem.* 276, 47311–47319

48 Sun, J.-P. *et al.* (2003) Crystal structure of PTP1B complexed with a potent and selective bidentate inhibitor. *J. Biol. Chem.* 278, 12406–12414

49 Puius, Y.A. *et al.* (1997) Identification of a second aryl phosphate-binding site in protein-tyrosine phosphatase 1B: a paradigm for inhibitor design. *Proc. Natl. Acad. Sci. U. S. A.* 94, 13420–13425

50 Szczepankiewicz, B.G. *et al.* (2003) Discovery of a potent, selective protein tyrosine phosphatase 1B inhibitor using a linked-fragment strategy. *J. Am. Chem. Soc.* 125, 4087–4096

51 Liu, G. *et al.* (2003) Selective protein tyrosine phosphatase 1B inhibitors: targeting the second phosphotyrosine binding site with non-carboxylic acid-containing ligands. *J. Med. Chem.* 46, 3437–3440

52 Liu, G. *et al.* (2003) Fragment screening and assembly: a highly efficient approach to a selective and cell active protein tyrosine phosphatase 1B inhibitor. *J. Med. Chem.* 46, 4232–4235

53 Erlanson, D.A. *et al.* (2003) Discovery of a new phosphotyrosine mimetic for PTP1B using breakaway tethering. *J. Am. Chem. Soc.* 125, 5602–5603

54 Hartshorn, M.J. *et al.* (2005) Fragment-based lead discovery using X-ray crystallography. *J. Med. Chem.* 48, 403–413

55 Lau, C.K. *et al.* (2004) Structure-based design of a series of potent and selective nonpeptidic PTP-1B inhibitors. *Bioorg. Med. Chem. Lett.* 14, 1043–1048

56 Scapin, G. *et al.* (2003) The structural basis for the selectivity of benzotriazole inhibitors of PTP1B. *Biochemistry* 42, 11451–11459

57 Asante-Appiah, E. *et al.* (2006) Conformation-assisted inhibition of protein-tyrosine phosphatase-1B elicits inhibitor selectivity over T-cell protein-tyrosine phosphatase. *J. Biol. Chem.* 281, 8010–8015

58 Iversen, L.F. *et al.* (2000) Structure-based design of a low molecular weight, nonphosphorus, nonpeptide, and highly selective inhibitor of protein-tyrosine phosphatase 1B. *J. Biol. Chem.* 275, 10300–10307

59 Wiesmann, C. *et al.* (2004) Allosteric inhibition of protein tyrosine phosphatase 1B. *Nat. Struct. Mol. Biol.* 11, 730–737

60 Liljebris, C. *et al.* (2002) Investigation of potential bioisosteric replacements for the carboxyl groups of peptidomimetic inhibitors of protein tyrosine phosphatase 1b: identification of a tetrazole-containing inhibitor with cellular activity. *J. Med. Chem.* 45, 1785–1798

61 Bleasdale, J.E. *et al.* (2001) Small molecule peptidomimetics containing a novel phosphotyrosine bioisostere inhibit protein tyrosine phosphatase 1B and augment insulin action. *Biochemistry* 40, 5642–5654

62 Xin, Z. *et al.* (2003) Identification of a monoacid-based, cell permeable, selective inhibitor of protein tyrosine phosphatase 1B. *Bioorg. Med. Chem. Lett.* 13, 3947–3950

63 Dufresne, C. *et al.* (2004) The development of potent non-peptidic PTP-1B inhibitors. *Bioorg. Med. Chem. Lett.* 14, 1039–1042

64 Andersen, H.S. *et al.* (2002) Discovery and SAR of a novel selective and orally bioavailable nonpeptide classical competitive inhibitor class of protein-tyrosine phosphatase 1B. *J. Med. Chem.* 45, 4443–4459

65 Larsen, S.D. *et al.* (2002) Synthesis and biological activity of a novel class of small molecular weight peptidomimetic competitive inhibitors of protein tyrosine phosphatase 1B. *J. Med. Chem.* 45, 598–622

66 Dang, Q. (2006) Organophosphonic acids as drug candidates. *Expert Opin. Ther. Patents* 16, 343–348

67 Garrido-Hernandez, H. *et al.* (2006) Design and synthesis of phosphotyrosine peptidomimetic prodrugs. *J. Med. Chem.* 49, 3368–3376

68 Boutselis, I.G. *et al.* (2007) Synthesis and cell-based activity of a potent and selective PTP1B inhibitor prodrug. *J. Med. Chem.* 50, 856–864

69 Liljebris, C. *et al.* (2002) Synthesis and biological activity of a novel class of pyridazine analogues as non-competitive reversible inhibitors of protein tyrosine phosphatase 1B (PTP1B). *Bioorg. Med. Chem.* 10, 3197–3212

70 Klopfenstein, S.R. *et al.* (2006) 1,2,3,4-tetrahydroisoquinolinyl sulfamic acids as phosphatase PTP1B inhibitors. *Bioorg. Med. Chem. Lett.* 16, 1574–1578

71 Moretto, A.F. *et al.* (2006) Bicyclic and tricyclic thiophenes as protein tyrosine phosphatase 1B inhibitors. *Bioorg. Med. Chem.* 14, 2162–2177

72 Wan, Z.-K. *et al.* (2006) Monocyclic thiophenes as protein tyrosine phosphatase 1B inhibitors: capturing interactions with Asp48. *Bioorg. Med. Chem. Lett.* 16, 4941–4945

73 Black, E. *et al.* (2005) Structure-based design of protein tyrosine phosphatase-1B inhibitors. *Bioorg. Med. Chem. Lett.* 15, 2503–2507

74 Combs, A.P. *et al.* (2005) Structure-based design and discovery of protein tyrosine phosphatase inhibitors incorporating novel isothiazolidinone heterocyclic phosphotyrosine mimetics. *J. Med. Chem.* 48, 6544–6548

75 Yue, E.W. *et al.* (2006) Isothiazolidinone heterocycles as inhibitors of protein tyrosine phosphatases: synthesis and structure–activity relationships of a peptide scaffold. *Bioorg. Med. Chem.* 14, 5833–5849

76 Combs, A.P. *et al.* (2006) Potent benzimidazole sulfonamide protein tyrosine phosphatase 1B inhibitors containing the heterocyclic (*S*)-isothiazolidinone phosphotyrosine mimetic. *J. Med. Chem.* 49, 3774–3789