

# *Protein Tyrosine Phosphatase 1B Inhibitors: A Molecular Level Legitimate Approach for the Management of Diabetes Mellitus*

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**Abstract:** Diabetes mellitus is a systemic disease responsible for morbidity in the western world and is gradually becoming prevalent in developing countries too. The prevalence of diabetes is rapidly increasing in industrialized countries and type 2 diabetes accounts for 90% of the disease. Insulin resistance is a major pathophysiological factor in the development of type 2 diabetes, occurring mainly in muscle, adipose tissues, and liver leading to reduced glucose uptake and utilization and increased glucose production. The prevalence and rising incidence of diabetes emphasized the need to explore new molecular targets and strategies to develop novel antihyperglycemic agents. Protein Tyrosine Phosphatase 1B (PTP 1B) has recently emerged as a promising molecular level legitimate therapeutic target in the effective management of type 2 diabetes. PTP 1B, a cytosolic nonreceptor PTPase, has been implicated as a negative regulator of insulin signal transduction. Therefore, PTP 1B inhibitors would increase insulin sensitivity by blocking the PTP 1B-mediated negative insulin signaling pathway and might be an attractive target for type 2 diabetes mellitus and obesity. With X-ray crystallography and NMR-based fragment screening, the binding interactions of several classes of inhibitors have been elucidated, which could help the design of future PTP 1B inhibitors. The drug discovery research in PTP 1B is a challenging area to work with and many pharmaceutical organizations and academic research laboratories are focusing their research toward the development of potential PTP 1B inhibitors which would prove to be a milestone for the management of diabetes. © 2010 Wiley Periodicals, Inc. *Med Res Rev*, 32, No. 3, 459–517, 2012

**Key words:** diabetes; protein tyrosine phosphatase 1B (PTP 1B); metabolism; insulin; obesity

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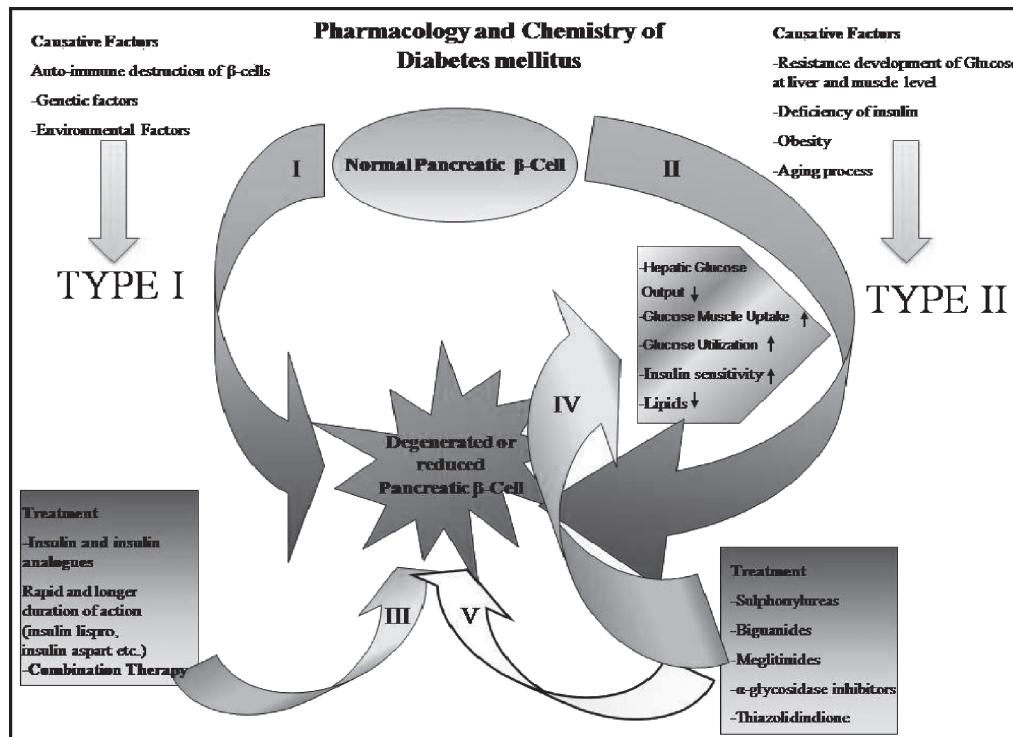
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## **1. INTRODUCTION**

Diabetes mellitus is a chronic multifactorial metabolic disease resulting from insulin deficiency or insulin resistance.<sup>1,2</sup> It is a systemic disease responsible for morbidity in the western world and is gradually becoming prevalent in developing countries too.<sup>3</sup> Diabetes mellitus is considered to be one of the main threats to human health in the 21st century.<sup>4,5</sup> Diabetes is threatening on account of the development of many severe secondary complications, which includes atherosclerosis, microangiopathy, renal dysfunction and failure, cardiac abnormalities, diabetic retinopathy, and ocular disorders.<sup>6,7</sup> With the increased prevalence of obesity in the general population, especially in young adults, the prevalence of diabetes is also on the rise, hence diabetes has been redefined as “diabesity” or “obesity” dependent diabetes mellitus.<sup>8,9</sup> The incidences of the disease are increasing day by day and are estimated to reach 210 million by the year 2010 and 300 million by the year 2025.<sup>10,11</sup> Traditionally, diabetes mellitus is classified as either type 1 or type 2 diabetes. Type 1 diabetes, also known as insulin-dependent diabetes mellitus (IDDM), is an autoimmune genetic disease resulting from an absolute deficiency of insulin due to destruction of insulin-producing pancreatic  $\beta$  cells, has only been controlled by daily subcutaneous injections of insulin, which cause pain and stress to the patients. Type 2 diabetes also known as noninsulin-dependent diabetes mellitus (NIDDM) is characterized by high level of blood glucose, insulin, and impaired insulin action. It is characterized by insulin resistance in peripheral tissue and an insulin secretory defect of the  $\beta$  cell. This is the most common form of diabetes mellitus and is associated predominantly with a family history of diabetes, older age, obesity, and lack of exercise. It is more common in women, especially those with a history of gestational diabetes. More than 90% cases reported are of type 2 diabetes. Causes attributed to type 2 diabetes include: abnormality in glucose receptor in  $\beta$ -cells so that they respond at higher glucose concentration, reduced sensitivity of peripheral tissue to insulin, reduction in insulin receptors (IRs), “down regulation” of IR, and excess of hyperglycemic hormones (Fig. 1).<sup>12</sup>

Insulin resistance is a major pathophysiological factor in the development of type 2 diabetes, occurring mainly in muscle, adipose tissues, and liver, leading to reduced glucose uptake and utilization and increased glucose production, respectively.<sup>13,14</sup> Insulin resistance is associated not only with hyperinsulinemia and hyperglycemia but also with other disorders, such as atherosclerosis, hypertension, and abnormal lipid profile, which are now collectively referred to as Metabolic Syndrome or Insulin Resistance Associated Disorders.<sup>15,16</sup> Amelioration of insulin resistance in both the peripheral tissues and liver is considered to be a reasonable treatment of type 2 diabetes. Several factors have been attributed for generation of insulin resistance in humans. Circulating insulin antagonists were once thought to be a major cause of insulin resistance.<sup>17</sup> The presence of various counter-regulatory hormones, such as glucagon, epinephrine, cortisol, and growth hormone, are also considered to be associated with insulin resistance state.<sup>18</sup> Amylin, a peptide secreted with insulin by the  $\beta$ -cells, may be of greater relevance to type 2 diabetes, having both an acute antagonistic action toward insulin and a long-term deleterious effect, leading to deposition of amyloid around the islets and to progressively decreased islet cell function over a period of years after the development of diabetes. Although it was once common to find circulating insulin antagonists, these insulin antibodies are now an infrequent cause of insulin resistance because of the shift to treatment using human recombinant insulin, less immunogenic forms of exogenous insulin.<sup>19</sup> The prevalence and rising incidence of diabetes emphasized the need to explore newer molecular targets and strategies for development of novel antihyperglycemic agents.<sup>20</sup>



**Figure 1.** Schematic representation of Diabetes mellitus.

## 2. DEVELOPMENT PERSPECTIVES AND CURRENT STATUS

The remedies available in modern system of medicine for the treatment of type 2 diabetic patients have been focused on exercise and dietary management of obesity to improve insulin sensitivity; to increase insulin secretion, and to inhibit or reduce the rate of glucose absorption from the gut.<sup>21,22</sup> In current scenario, the treatment of type 2 diabetes has been revolutionized with the advent of insulin sensitizers like rosiglitazone and pioglitazone that ameliorate insulin resistance and thereby normalize elevated blood glucose levels but are also associated with hepatotoxicity, weight gain, and edema (Table I).<sup>23,24</sup> The alarming situation emphasized the need to discover new antihyperglycemic agents with reduced or no side effect.

The development pipeline for new oral therapeutic agents for type 2 diabetes is encouraging and continues to expand.<sup>34</sup> These intensive research and developmental efforts are in response to: increasing prevalence of the disease and related co-morbidities, realization by care givers that successful glycemic control will likely require combination therapy, a growing understanding of the pathophysiology of the disease, and the identification and validation of new pharmacological targets.<sup>35</sup> These targets include various receptors and enzymes which exhibit mechanisms of action distinct from current therapies thus could be beneficial in the treatment of diabetes without having any major side effects.<sup>35</sup> Some of the new and emerging approaches due to their promise for future clinical success and different mechanisms of action from existing therapies are depicted in Table II.

As the present review mainly pertains to Protein Tyrosine Phosphatase 1B (PTP 1B) inhibitors, the enzyme and its inhibitors are discussed in detail in the following section.

**Table I.** Drug Therapies Used for the Treatment of Type 2 Diabetes

Class of drug	Drugs	Role in blood glucose management
Sulfonylureas <sup>25</sup>		
1st generation	Acetohexamide, Chlorpromazine, Tolbutamide, Tolazamide	Insulin secretagogues
2nd generation	Glibenclamide, Glyburide, Glipizide	Insulin secretagogues
3rd generation	Glimepiride	Insulin secretagogues
Meglitinides <sup>26</sup> (glinides)	Repaglinide, Nateglinide	Insulin secretagogues
Biguanides <sup>27</sup>	Metformin, Phenformin	Insulin sensitizers
Thiazolidinediones <sup>22,23</sup> (PPAR $\gamma$ agonists; glitazones)	Rosiglitazone, Pioglitazone	Insulin sensitizers and decrease insulin resistance
Gliptins <sup>28,29</sup> (DPP-1V inhibitors)	Sitagliptin, Saxagliptin	Insulin secretagogues
Glucagon like peptide-1 <sup>30,31</sup> (GLP-1)	Exenatide, Liraglutide	Insulin secretagogues
Alpha-glucosidase inhibitors <sup>32,33</sup>	Acarbose, Miglitol, Voglibose	Inhibitors of glucose uptake

**Table II.** New Approaches for the Management of Type 2 Diabetes

Targets	Role in blood glucose management
Glycogen synthase kinase-3 (GSK-3) inhibitors <sup>36</sup>	Activation of glycogen synthase
PPAR $\alpha/\gamma$ dual agonist <sup>37</sup>	Insulin sensitizers
Na <sup>+</sup> glucose co-transporter (SGLT) inhibitors <sup>38</sup>	Inhibits renal glucose reabsorption from urine
HGO inhibitors <sup>39</sup>	Insulin sensitizers and decrease insulin resistance
$\beta_3$ -Adrenoreceptor agonist <sup>40</sup>	Decreases food consumption
Retinoid X receptor <sup>41</sup>	Controls lipid and carbohydrate metabolism
Protein tyrosine phosphatase 1B (PTP 1B) inhibitors <sup>42</sup>	Prevents dephosphorylation of activated insulin receptor

### 3. PROTEIN TYROSINE PHOSPHATASES (PTPS)

Phosphatases are the enzymes which remove phosphate group from the substrate, a process known as dephosphorylation. PTPs are the family of enzymes that act by dephosphorylation of the tyrosine kinase receptor.<sup>43</sup> Tyrosine phosphorylation of proteins is a fundamental mechanism for the control of cell growth and differentiation.<sup>44</sup> It is reversible and governed by the opposing activities of protein tyrosine kinases (PTKs) and PTPs, which are responsible for phosphorylation and dephosphorylation, respectively.<sup>45,46</sup>

PTPs, PTKs and their substrates weave an elaborate network that maintains cellular signaling.<sup>47</sup> Defective or inappropriate operation of these networks leads to aberrant tyrosine phosphorylation, contributing to the development of many diseases like cancer, inflammatory disorders, and diabetes.<sup>48,49</sup> PTPs are the enzymes that play an important role in cellular signaling by regulating the phosphorylation status and, in turn, the functional

properties of target proteins in various signal transduction pathways.<sup>50,51</sup> Thus, PTPs are considered as viable targets for the design of novel therapeutics that are capable of inhibiting or modulating activities of these crucial enzymes.<sup>52</sup>

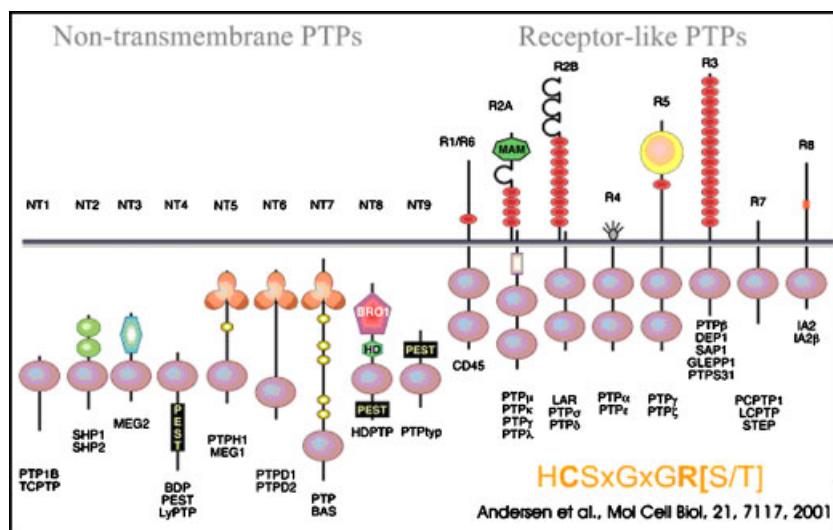
A recent estimation from the nearly completed human genome sequence suggested that humans have 112 PTPs.<sup>53</sup> The hallmark that defines the PTP super-family is the active site sequence (H/V) C (X)<sub>5</sub> R (S/T) [using single letter code for amino acids, where X is any residue], also called the PTP signature motif, in the catalytic domain.<sup>54</sup> PTPs can be broadly classified into three major subfamilies:

1. Tyrosine specific
2. Dual specific
3. Low molecular phosphatases

Tyrosine specific phosphatases can be further divided into two groups: receptor like and intracellular PTPs (Fig. 2). Receptor like PTPs, exemplified by CD45 and PTP $\alpha$ , generally have an extracellular, putative ligand-binding domain, a single transmembrane region, and one or two cytoplasmic PTP domains. Intracellular PTPs, exemplified by PTP 1B and SHP2, contain a single catalytic domain and various carboxy or amino terminal extensions including SHP-2 domains that may have targeting or regulatory functions. The dual-specific phosphatase utilizes the protein substrate that contains pTyr as well as pSer and pThr<sup>55,56</sup>. Several PTPs that are implicated in various disorders/diseases have been listed in Table III.

#### 4. PROTEIN TYROSINE PHOSPHATASE 1B (PTP 1B)

PTP 1B, an intracellular enzyme causes negative regulation of IR as well as leptin signaling system, has emerged as promising legitimate therapeutic target for the treatment of NIDDM and obesity.<sup>58–60</sup> It is a major nontransmembrane phosphotyrosine phosphatase in human tissues and was one of the earliest PTP identified.<sup>61</sup> PTP 1B directly catalyzes the dephosphorylation of cellular substrates of the insulin receptor kinase, resulting in a down regulation of insulin signal transduction.<sup>62</sup> It has been shown recently that PTP 1B negatively regulates leptin receptor signaling in a murine neuronal subline. PTP 1B was subsequently



**Figure 2.** Classification of PTPs.<sup>57</sup>

**Table III.** Role of Various PTPs

PTP(s)	Disorders/Diseases
PTP 1B, LAR	Diabetes, obesity
PTP $\alpha$	Cancer
CD45	Autoimmunity, inflammation, Alzheimer's disease
HePTP	Myelodysplastic syndrome
Cdc25	Cell cycle progression, cancer
PTP $\xi$	Osteoporosis
PTP-SL, LAR	Neuro protection
Yersinia PTP	Plague
MTM-1	X-linked myotubular myopathy
Salmonella PTP	Salmonella infection and typhoid
SHP-1	Inflammation, leukemia
PP1/PP2A	Malignant disorders
VHR	Regulation of MAP-kinases
PP2B/PP2C	Asthma, cardiovascular

shown to bind and dephosphorylate JAK2, which is downstream of leptin receptor.<sup>63,64</sup> Therefore, it seems logical that PTP 1B inhibitors would increase insulin sensitivity by blocking the PTP 1B-mediated negative insulin signaling pathway and might be an attractive target for type 2 diabetes mellitus and obesity.<sup>65</sup> A recent clinical PTP 1B knockout mice study revealed that mice lacking functional PTP 1B exhibited increased sensitivity toward insulin and resistant to obesity.<sup>66</sup> Interestingly, PTP 1B deficient 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. Thus, the resistance to diet-induced obesity observed in PTP 1B deficient mice is likely to be associated with increased energy expenditure owing to enhanced leptin sensitivity.<sup>67,68</sup> Recent tissue-specific knockout results indicate that body weight, adiposity, and leptin action can be regulated by neuronal PTP 1B. Neuronal PTP 1B deficient mice have reduced weight, adiposity, increased activity, and energy expenditure. In contrast, adipose PTP 1B deficiency increases body weight, whereas PTP 1B deletion in muscle or liver does not affect weight.<sup>69</sup> It has also been reported that neuronal PTP 1B inhibition results in decreased hypothalamic AMP-activated protein kinase (AMPK) activity, isoform-specific AMPK activation in peripheral tissues, and downstream gene expression changes that promote leanness and increased energy expenditure. Therefore, the mechanism by which PTP 1B regulates adiposity and leptin sensitivity is likely to involve the coordinated regulation of AMPK in hypothalamus and peripheral tissues.<sup>70,71</sup>

These results taken together, establish a direct role for PTP 1B in down regulating the insulin and leptin functioning. As a result there is a growing interest in the development of potent and specific inhibitors for this enzyme. PTP 1B inhibitors could potentially ameliorate insulin resistance and normalize plasma glucose and insulin without inducing hypoglycemia, and could therefore be a major advancement in the treatment of type 2 diabetes.<sup>72,73</sup>

More recently the endoplasmic reticulum (ER) has been identified as an organelle that is stressed in obesity and could be a molecular link between obesity and impaired insulin action. PTP 1B has an essential function in regulating the unfolded protein response in the ER compartment. It has been demonstrated that absence of PTP 1B caused impaired IRE1-dependent JNK activation as well as XBP-1 splicing and EDEM transcription, which were reduced in immortalized (MEFs) or primary (PMEFs) PTP 1B knock-out mouse embryonic

fibroblasts. Thus ER stress-induced apoptosis was attenuated upon prolonged stress in PTP 1B knock-out cells and it has an important function in potentiating IRE1-mediated ER stress signaling pathways.<sup>74</sup> Liver-specific deletion of PTP 1B protects against high-fat diet-induced ER stress response in vivo, as evidenced by decreased phosphorylation of p38MAPK, JNK, PERK, and eIF2alpha, and lower expression of the transcription factors C/EBP homologous protein and spliced X box-binding protein 1.<sup>75</sup> Various studies assessed the role of PTP 1B in the insulin sensitivity of skeletal muscle under physiological and insulin-resistant conditions. PTP 1B expression has been shown to be increased with high-fat feeding and obesity and associated with an increase in pro-inflammatory cytokine TNF- $\alpha$ .<sup>76</sup> Therefore, genetic ablation of PTP 1B in skeletal muscle confers protection against insulin resistance by this cytokine.<sup>77</sup> Thus, PTP 1B may be a target of anti-inflammatory therapies and pharmacological agents targeted at various components of ER stress in obesity and diabetes.<sup>78</sup>

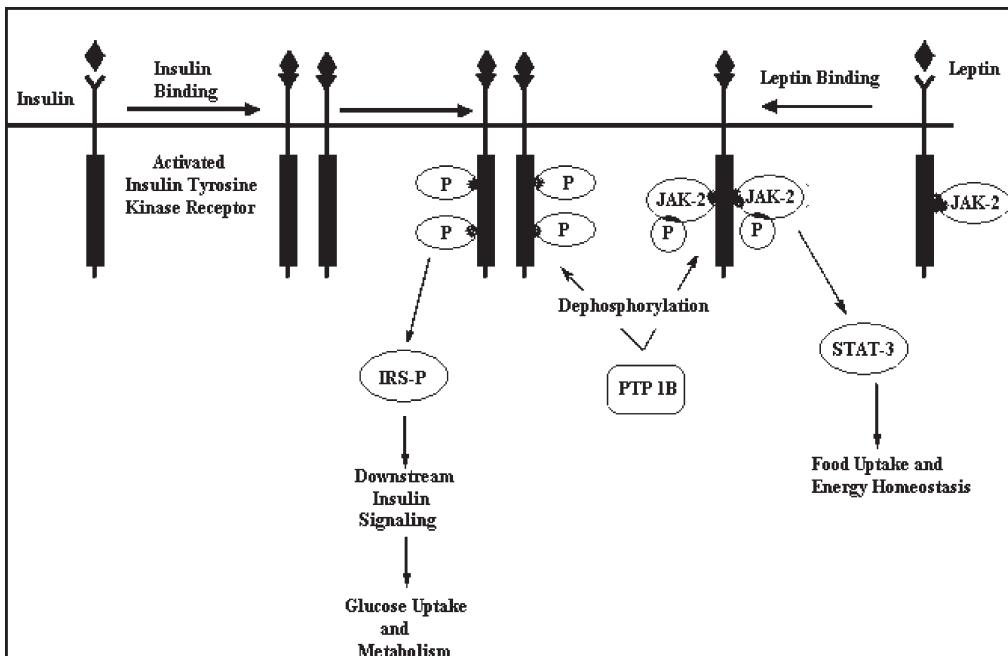
PTP 1B has been implicated as an oncogene in the case of breast cancer. Various studies revealed that PTP 1B is a positive regulator of the Erb2 (HER2/neu) PTK which is over-expressed in about 25% of human breast cancers, where it is associated with a poor prognosis.<sup>79,80</sup> Crossing transgenic mice expressing activated forms of ErbB2 with PTP1B deficient mice caused delayed tumor development and decreased the incidence of lung metastases. Thus, PTP 1B inhibitors may offer a new approach for management of breast cancer.<sup>81,82</sup>

Phosphorylation of protein tyrosyl residues is a controlling event that either activates or attenuates intracellular signaling pathways involved in cell proliferation, differentiation, and metabolism.<sup>83</sup> The insulin receptor is a tyrosine kinase composed of two extra-cellular ligand-binding domains in which  $\alpha$  subunits are linked by disulphide bonds to two trans-membrane  $\beta$  sub units. Insulin initiates its biological actions by binding to the  $\alpha$  subunit, thereby activating the intrinsic tyrosine kinase activity of the  $\beta$  subunits. This results in autophosphorylation of critical tyrosine residues in the regulatory domain to fully activate tyrosine kinase activity of the IR, which is then capable of phosphorylating its various substrates (IRS-P) to propagate the insulin signal transduction. IRS-P is responsible for downstream insulin signaling. The binding of leptin to its receptor leads to phosphorylation of janus kinase 2 (JAK-2), activating the JAK/signal transducer. Activated JAK-2 stimulates STAT-3, which increases food uptake and maintains homeostasis (Fig. 3).<sup>84,85</sup>

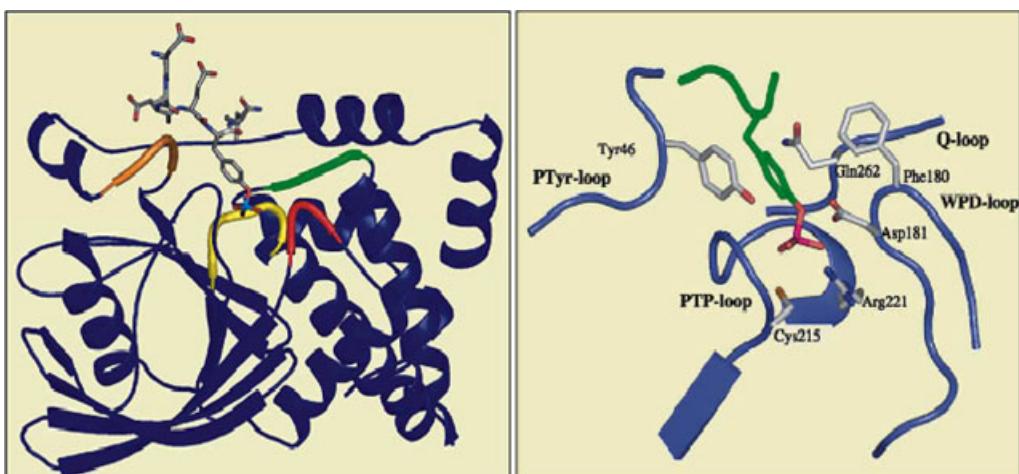
It has been observed that PTP 1B causes dephosphorylation, and thus inactivating insulin and JAK/STAT signal transduction cascade.<sup>85</sup> It has also been hypothesized that disequilibrium in enzyme activity between the IR and PTP 1B could be a contributing factor to the insulin resistance observed in type 2 diabetes. Inhibition of the PTP 1B is thus seen as legitimate approach for sustaining insulin and JAK/STAT signal transduction cascade associated with “diabesity”.<sup>86,87</sup>

The first crystal structure of PTP 1B bound to a tungstate ion was described in 1994. The native protein consists of 435 amino acid residues and amino acid 30–278 comprises the catalytic domain. The 35-carboxy terminal amino acid residues are rich in proline and are involved in targeting the enzyme to the cytoplasmic face of the ER.<sup>55,57,87</sup> The main structural features has been represented using different colors in Figure 4. The left part of Figure 4 illustrates a ribbon representation of the complex between PTP 1B and a hexapeptide substrate. PTP 1B catalytic domain are shown in blue, with the critical elements that comprise the catalytic site highlighted as the signature motif yellow (His214-Arg221), Red WPD loop (Asp181 and Phe162), Orange pTyr loop(Tyr46-Arg47-Asp48) and green Q loop(Gln262). The right part of Figure 4 illustrates a more detailed view of the active site. The pTyr substrate peptide is shown in green and the other structural elements and critical residues are labeled.<sup>88</sup>

The catalytic active site of PTP 1B contains a signature motif from His214- Arg221 (His-Cys-Ser-Ala-Gly-Ile-Gly-Arg), a loop of eight amino acids that forms a rigid cradle



**Figure 3.** Insulin and JAK/STAT signal transduction cascade.



**Figure 4.** Structure of active site of PTP 1B in complex with a hexapeptide substrate showing signature motifs and other characteristic features of PTP 1B.<sup>88</sup>

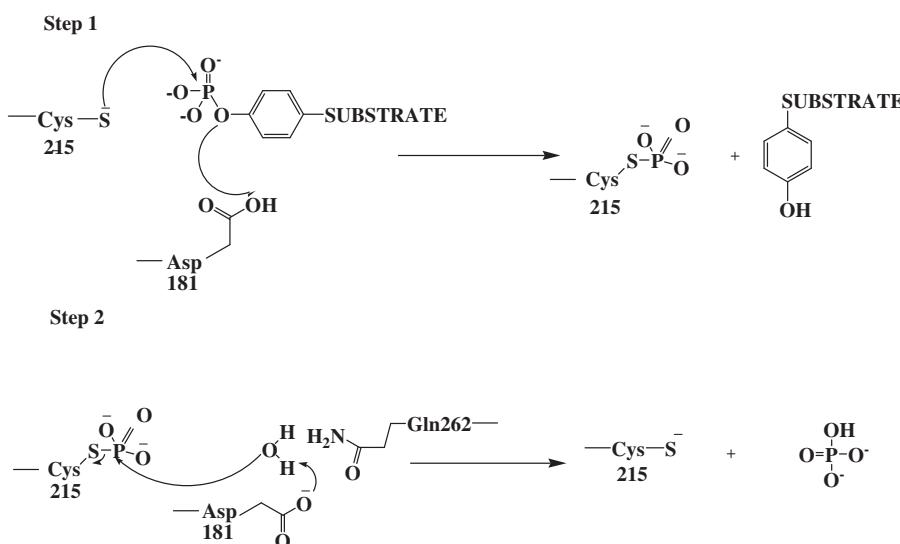
like structure that co-ordinates to the aryl-phosphate moiety of the substrate. This loop also contains the active site nucleophile Cys215.<sup>55,57,87</sup> Four other loops bearing invariant residues from the sides of the catalytic cleft and contribute to the catalysis and substrate recognition. The depth of the catalytic cleft (8–9 Å) imparts substrate selectivity, as serine and threonine are not long enough to reach the Cys215 of PTP 1B. PTP 1B undergoes conformational changes that are associated with the catalytic activity of the enzyme. Large conformational changes occur on substrate binding.<sup>55,89,90</sup> The WPD loop (amino acids

79–187) move up to 12 Å to close down on the phenyl ring of the substrate, which maximizes hydrophobic interactions, Asp181 moves into a position in which it can act as a general acid to protonate the tyrosyl leaving group.<sup>55,91</sup> Arg221 also reorients into a position to optimize salt bridge interactions with the substrate phosphate. Crystallographic studies on PTP 1B indicated the presence of secondary aryl phosphate-binding site (Arg24 and Arg254) adjacent to the catalytic site. This secondary site is catalytically inactive, and provides weaker interactions compared with the primary site, owing to its more open structure to the solvent.<sup>57,91</sup> Nevertheless, it has important implications in the design of inhibitors because it opens a possibility of using the strategy of independently finding molecules that bind to each site, then linking them together to get a much potent inhibitor. YRD motif (Tyr46-Arg47-Asp48) provides features for the phosphatase selectivity.<sup>57,92</sup>

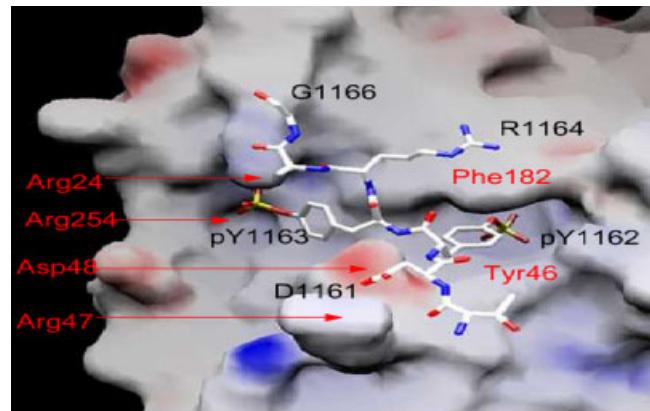
## 5. MECHANISM OF PTP 1B

PTP 1B is characterized by presence of a signature motif containing the Cys215 residue that is essential for catalysis. PTP 1B-mediated catalysis proceeds via a two-step mechanism. In the first step, there occurs a nucleophilic attack on the substrate phosphate by the sulfur atom of thiolate side chain of the Cys215, coupled with protonation of tyrosyl leaving group of the substrate by the side chain of a conserved acidic residue via Asp181 acting as a general acid. This leads to the formation of cysteinyl phosphate intermediate. In the second step mediated by Gln262, which coordinates a water molecule, and Asp181, which now functions as a conjugate base, there is hydrolysis of the catalytic intermediate and release of phosphate (Fig. 5).<sup>57,88</sup>

PTP 1B forms a physical complex with the activated IR. This enzyme also has unique structural features that promote its interaction with the receptor, in particular a second phosphotyrosine-binding site in the PTP 1B catalytic region that strongly enhances its association with the tandem phosphotyrosine residues of the activated insulin receptor kinase domain.<sup>93</sup> It causes simultaneous dephosphorylation of bis-phosphorylated 1162 and 1163 residue of IR (Fig. 6), thereby selectively inhibiting insulin signal transduction while other PTPs are unable to do so.<sup>57,94</sup>



**Figure 5.** Catalytic mechanism of PTP 1B.<sup>88</sup>



**Figure 6.** PTP 1B showing simultaneous dephosphorylation of insulin receptor.<sup>57</sup>

## 6. PTP 1B INHIBITORS

PTP 1B has been inhibited experimentally using a variety of mechanisms and chemical entities. Phosphatase LAR, CD45, SHP-2, cdc25c, and T-cell PTP (TC-PTP) share 50–80% homology in the catalytic domain with PTP 1B, which presents a challenging task of achieving selectivity, especially over TC-PTP.<sup>1</sup> Thus, it was necessary for the inhibitors to interact with the regions outside the catalytic site in order to be selective, i.e. secondary noncatalytic phosphotyrosine-binding site. Hence, targeting both the sites simultaneously may show good activity and selectivity against PTP 1B. The electrostatic properties of the PTP 1B catalytic site are optimal for binding phosphotyrosine, which bears two negative charges at physiological pH. Charged ligands are therefore preferred for potent binding but they lack cell permeability. As a consequence, it has been highly challenging to discover and develop potent, selective, permeable, and orally bioavailable small molecule PTP 1B inhibitors. ISIS-113715 is the only PTP 1B inhibitor which is currently in clinical trials. It is a 20-mer antisense oligonucleotide that has been developed by ISIS Pharmaceuticals Inc. for the management of type 2 diabetes. Interestingly, this oligonucleotide directed against PTP 1B has been shown to reduce PTP 1B mRNA expression in liver and adipose tissue (but not skeletal muscle) by about 50% and to produce significant glucose-lowering effects in hyperglycemic, insulin-resistant *ob/ob* and *db/db* mice.<sup>95,96</sup>

In the following section various classes of synthetic as well as bioactive agents from natural products as inhibitors of PTP 1B have been discussed.

### A. Thiazolidinediones

During last decade, a new class of drugs called “glitazones,” such as Rosiglitazone (RSG) and Pioglitazone, were approved by the FDA for the treatment of type 2 diabetes. These agents share a common molecular scaffold: 2, 4-thiazolidinediones (TZDs).<sup>97</sup> TZDs correct hyperglycemia by enhancing tissues’ sensitivity to insulin. TZDs exert their antidiabetic effect through a mechanism that involve activation of gamma isoform of peroxisome proliferator-activated receptor (PPAR- $\gamma$ ), which stimulates certain transcriptional events of lipid and carbohydrate metabolism.<sup>98,99</sup> TZDs treatment is not associated with dangerous hypoglycemic incidents that have been observed with conventional sulfonylurea agents and insulin therapy. These were shown to improve glycemic control by ameliorating insulin resistance both in peripheral tissues and liver in type 2 diabetic patients.<sup>100</sup>

Various studies have suggested that existing TZDs could inhibit PTP 1B. It was postulated that high-fat feeding increases the levels of muscle PTP 1B in mice, increasing the possibility that the antidiabetic effects of RSG involve decreasing muscle PTP 1B levels. The inhibitory effect of RSG on PTP 1B activity is more likely mediated by reducing PTP 1B protein expression in skeletal muscle and liver.<sup>101</sup> It has also been demonstrated that RSG treatment has dramatic effects on glucose homeostasis in mice with a muscle-specific deletion of PTP 1B, thus suggesting that rosiglitazone could be added to PTP 1B inhibitors in order to alleviate the diabetic phenotype caused by high-fat feeding.<sup>102</sup>

Maccari and co-workers demonstrated TZD scaffolds<sup>103</sup> containing arylidene moiety at position-5 exhibited significant inhibitory effects against *h*-PTP 1B enzymes. Benzoic acid could act as nonphosphorus containing pTyr mimic, therefore it was considered of interest to insert the p-methyl benzoic acid residue at N-3 of the TZD scaffold. It has also been reported that carboxylic group mimics the interactions of the phosphate group of pTyr with Arg221 in the PTP 1B catalytic site while the benzene ring might interact with Phe182 and Tyr46. Among the series compounds (**1**) and (**2**), proved to be most effective at micromolar level against *h*-PTP 1B. The investigation of SAR relevant to this new class of PTP 1B inhibitors has so far been limited to a small library of analogs. However, it seems that 5-arylidene moiety markedly influenced on their potency and selectivity. A larger lipophilic arylidene moiety containing two aromatic rings appeared to be more favorable than a smaller one composed of a benzylidene ring with hydrophilic substituents. This may be due to hydrophobic interactions with the lipophilic residues which surround the pTyr-binding site.<sup>103</sup>

Further Maccari et al. performed optimization by introducing second carboxyl group at benzyloxybenzylidene moiety of TZD scaffold. It is considered that this second pTyr mimic group could enhance the affinity of these bidentate inhibitors for the secondary non catalytic pocket of PTP 1B. Compound (**3**) and (**4**) proved to be the most potent PTP 1B inhibitors, with sub-micromolar IC<sub>50</sub> values against *h*-PTP 1B. Molecular docking studies also indicated that these compounds interact with amino acid residues involved in the affinity and selectivity of inhibitors toward this enzyme.<sup>104</sup>

Bhattarai et al. identified TZD scaffolds as PTP 1B inhibitors by means of a computer-aided drug design protocol involving virtual screening. A series of benzylidene-2, 4-thiazolidinedione derivatives with substitutions on the phenyl ring at the *ortho* or *para* positions of the thiazolidinedione (TZD) group were reported with IC<sub>50</sub> values in a low micromolar range. Two compounds (**5**) and (**6**) (Table IV) were the most potent inhibitors of PTP 1B with an IC<sub>50</sub> value of 5.0 μM. Compound (**5**) demonstrated 2.2, >20, and 3.7-fold selectivity for PTP 1B over TC-PTP, LAR-D1, and YPTP1, but little selectivity over SHP-1cat and YOP.<sup>105</sup>

Recently, we have reported 3D-QSAR studies for the further optimization of structural features of 2, 4-thiazolidinediones. The model was developed using electrostatic and shape master grids. Thus, from the results of SOMFA electrostatic and shape potential maps it has been suggested that presence of bulky electronegative group such as benzoic acid at "R2" (N-3) of TZDs skeleton, which behaves as phosphotyrosine-mimetic portion, could result in improvement of potency of compounds against *h*-PTP1B.<sup>106</sup>

A further significant modification was reported by Ottanà et al. where replacement of carbonyl group at position-2 of the 2, 4-thiazolidinedione scaffold with a phenylimino moiety resulted in the development of 5-arylidene-2-phenylimino-4-thiazolidinones as potent inhibitors of *h*-PTP 1B. Enhancement of the inhibitor/enzyme affinity is attributed due to further favorable interactions with residues of the active site and the surrounding loops particularly with the WPD loop. All compounds exhibited good reversible inhibition of *h*-PTP 1B and LMW-PTP with IC<sub>50</sub> values in the micromolar range. Among the series compounds (**7–11**) were found to be most active (Table V).<sup>107</sup>

**Table IV.** Structures and In Vitro *h*-PTP 1B Inhibitory Activities of Compounds (1–6)

Compound number	Ar	<i>h</i> -PTP 1B IC <sub>50</sub> (μM)	
		X	
<b>1</b>			1.6±0.2
<b>2</b>			1.1±0.1



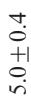
0.24 ± 0.07



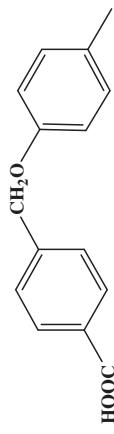
0.63 ± 0.15



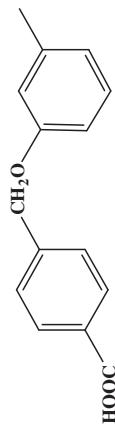
5.0 ± 0.1



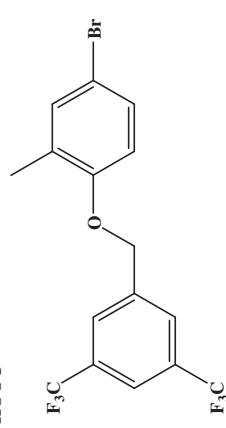
5.0 ± 0.4



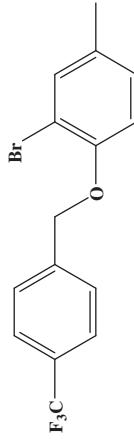
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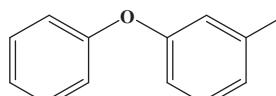
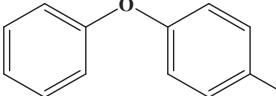
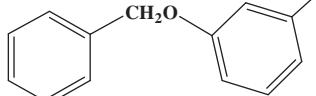
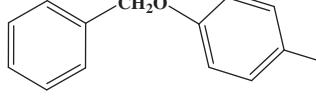
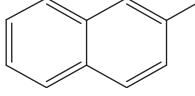


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**Table V.** Structures and In Vitro *h*-PTP 1B Inhibitory Activities of Compounds (7–11)

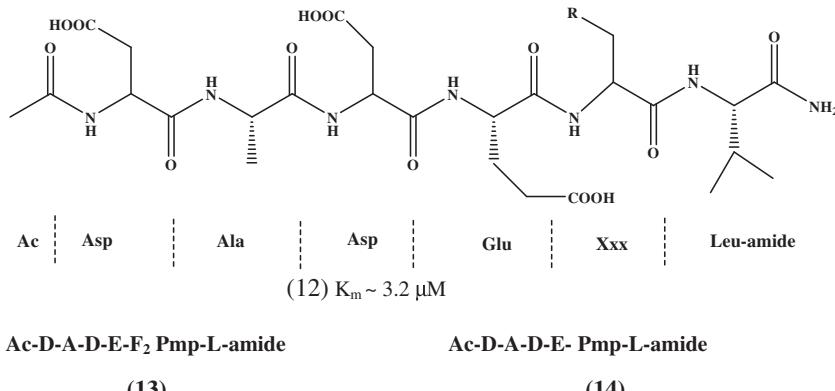
Compound number	Ar	<i>h</i> -PTP 1B IC <sub>50</sub> (μM)
7		1.9±1.0
8		1.1±0.1
9		3.8±0.1
10		1.1±0.1
11		1.9±0.1

### B. Phosphorus-containing Phosphotyrosyl Mimetics

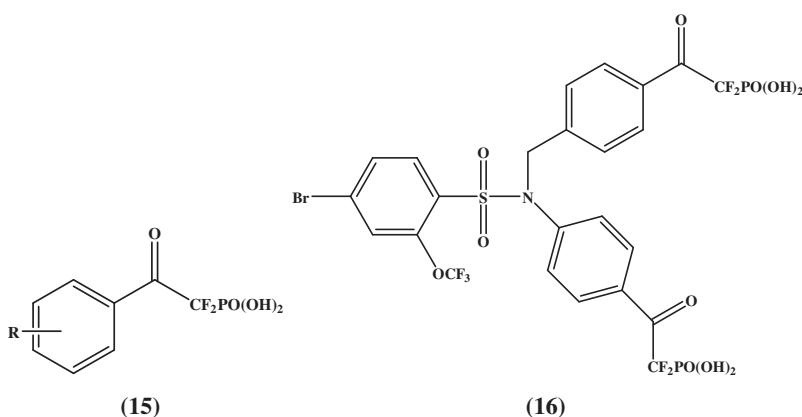
Early PTP 1B inhibitors were phosphate-based and difluorophosphonates (DFMP) analogs were the most extensively studied phosphate-based PTP 1B inhibitors. The difluorophosphonate group was introduced as a nonhydrolyzable phosphotyrosine mimetic in 1992 by Burke and coworkers.<sup>108</sup> The most potent phosphorus-containing PTP 1B inhibitors bear the difluorophosphonate group and exhibit superior potency to all other phosphorus-based analogs such as phosphonates, monofluoromethyl phosphonates, hydroxymethyl phosphonates, and azaphosphonates.<sup>109–111</sup>

Peptidyl phosphotyrosyl (pTyr)-containing substrate binds to PTP 1B *via* critical recognition of the pTyr phenyl phosphate moiety in highly conserved signature motif within the catalytic pocket and secondary interactions of amino acids in the substrate neighboring the pTyr residue, with features outside the catalytic pocket. The phosphopeptide Ac–Asp–Ala–Asp–Glu–Xxx–Leu–NH<sub>2</sub> (**12**) where Xxx is pTyr which is an excellent substrate for PTP 1B.<sup>112</sup> The phosphate group is crucial for PTP substrate binding. Design of effective, nonhydrolysable phosphate mimetic is an important consideration for PTP 1B inhibition. The decamer peptides having phosphonomethylphenylalanine (Pmp) moiety in the place of pTyr residue has been reported as competitive inhibitors with IC<sub>50</sub> values ranging from 10 to 30 μM against PTP 1B.<sup>113</sup>

The related difluorophosphonomethyl phenylalanine (F2Pmp)-containing hexameric peptide (**13**) was 1000-fold more potent ( $IC_{50} = 0.2 \mu M$ ) than a Pmp-containing analog (**14**). Peptide-bearing phosphono (di-fluoromethyl) phenylalanine (F2Pmp) binds better than the analog peptide substrates and can be up to three orders of magnitude more effective than the non-fluorinated analogs.<sup>114</sup>



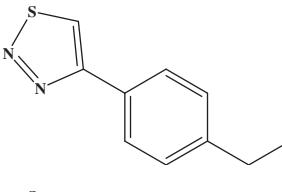
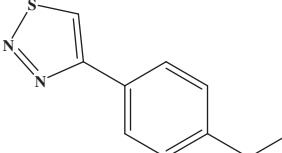
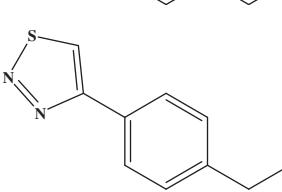
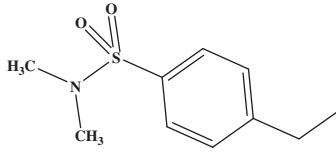
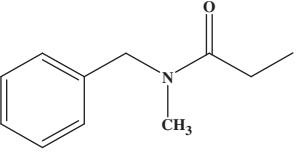
Li et al. at Affymax reported a series of nonpeptidyl phosphorus-containing pTyr mimetics ( $\alpha$ ,  $\alpha$ -difluoro- $\beta$ -ketophosphonates (**15**)). These stimulate a variety of specific interactions with residues in the active site of the phosphatase. The DFMP moiety targets the phosphate-binding site through electrostatic interactions, whereas the carbonyl group adjacent to the aromatic would be able to form additional hydrogen bonds with residues within the pTyr-binding pocket. The carbonyl group in these compounds is highly activated and electrophilic, which can easily form hydrates in aqueous solution. It is also possible that the hydrated form can effectively mimic the water molecule in the active site and therefore exhibit high binding ability to the enzyme. In addition, there is a potential for these compounds to react with the active site Cys or nitrogen nucleophiles of PTP 1B in a reversible covalent fashion to form hemithioketals or enamines. But the dianionic nature of the DFMP is again a limiting factor due to poor cell permeability. Among the series compound (**16**) containing bis-DFMP emerged as the most potent inhibitor with IC<sub>50</sub> and K<sub>i</sub> value in submicro molar level (0.50 and 0.28  $\mu$ M, respectively).<sup>115</sup>



Taking into consideration that sulfonamides are known to function as good hydrogen bond acceptors in many biological systems, and more importantly, they are common features of many known drugs. Holmes et al. at Affymax reported a series of novel sulfonamides

containing a single DFMP group as potent inhibitors of PTP 1B. SAR around the scaffold was also investigated, leading to the identification of compounds with  $IC_{50}$  or  $K_i$  values in the low nanomolar range (**17–21**) (Table VI). These sulfonamide-based inhibitors exhibit 100 and 30 times higher inhibitory activity than the corresponding tertiary amines and carboxamides,

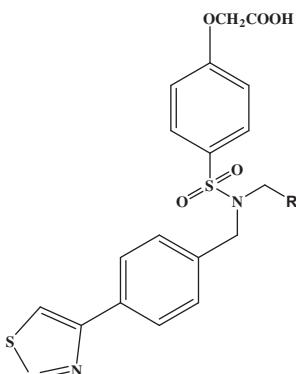
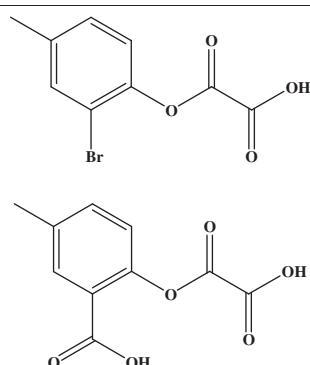
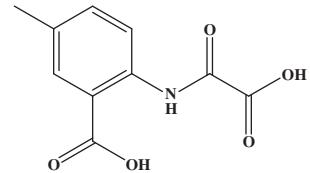
**Table VI.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (**17–21**)

Compound number	R <sub>1</sub>	R <sub>2</sub>	PTP 1B IC <sub>50</sub> (μM)	PTP 1B K <sub>i</sub> (μM)
<b>17</b>	–H		0.074	0.056
<b>18</b>	–Br		0.035	0.053
<b>19</b>	–OCH <sub>3</sub>		0.060	—
<b>20</b>	–Br		0.028	0.013
<b>21</b>	–Br		0.031	0.014

respectively. The presence of a thiadiazole ring and a DFMP group is sufficient to afford potent inhibitors of PTP 1B, and even more potent inhibitors result from inclusion of an additional oxyacetic group into the molecules. Compound (21) emerged as the most potent compound of the series with IC<sub>50</sub> and K<sub>i</sub> at submicromolar level.<sup>116</sup>

Holmes et al. further reported a series of triaryl sulfonamide-based PTP 1B inhibitors in which a DFMP group of a potent lead has been replaced by potential bioisosteric replacements. Although most of monocharged bioisosteres are not as active as those of dianionic pTyr mimetics, o-bromophenoxyacetic acid appears to compare favorably with dianionic o-carboxymethyl salicylic acid and 2-(oxylamino) benzoic acid. Several mono- or di-charged compounds (22–24) were shown to inhibit PTP 1B in the low micromolar range (Table VII), demonstrating the feasibility of using this systematic approach in identifying nonphosphonate pTyr mimetics in a small molecular scaffold.<sup>117</sup>

**Table VII.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (22–24)

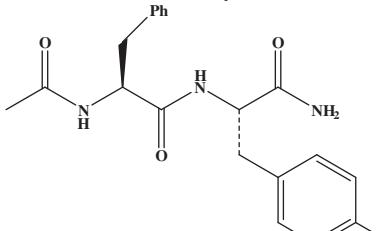
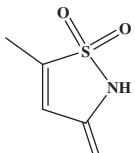
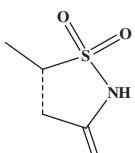
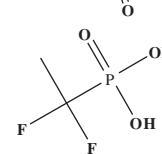
Compound number	R	PTP 1B IC <sub>50</sub> (μM)
22		4.1
23		4.4
24		1.7

### C. Isothiazolidinones

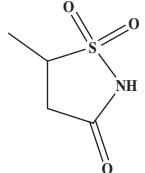
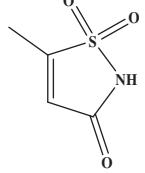
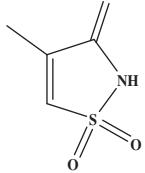
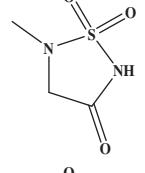
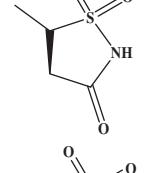
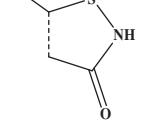
Combs et al. reported the discovery of novel heterocyclic (*S*)-isothiazolidinone ((*S*)-IZD) as phosphotyrosine (pTyr) mimetics using structure-based drug design approach. It was observed that incorporation of heterocycles into dipeptides resulted in exceptionally potent, competitive, and reversible inhibitors of PTP 1B (25–27) (Table VIII). The isothiazolidinone heterocycle was chosen because it allowed the two sulfonyl oxygens to effectively mimic the oxygens of the DFMP inhibitor, while the carbonyl mimicked the carboxymethyl salicylic acid (CMS) inhibitor carbonyl and the ionized NH mimics the carboxylic anion or DFMP anion. Among the series (*S*)-IZD dipeptide (27) was reported as the most potent inhibitor with  $K_i$  value 180 nM. The X-ray crystal structure of PTP 1B in complex with (27) revealed that the (*S*)-IZD heterocycle interacts extensively with the phosphate-binding loop. Furthermore, the (*S*)-IZD pTyr mimetic was found to be a 10-fold more potent inhibitor of PTP 1B than an analogous peptidic compound bearing a DFMP (28).<sup>118</sup>

Later Yue et al. reported the synthesis and SAR studies on a series of IZD heterocycle and peptide scaffold (Table IX). The SAR revealed the saturated IZD heterocycle (29) is the

**Table VIII.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (25–28)

Compound number	“R”Acronym	R	PTP 1B IC <sub>50</sub> (nM)
25	IZD		3000
26	(R)-IZD		16000
27	(S)-IZD		190
28	DFMP		1750

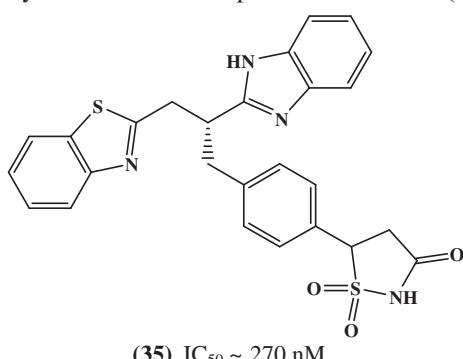
**Table IX.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (29–34)

Compound number	“R”Acronym	R	PTP 1B IC <sub>50</sub> (nM)
29	Saturated-IZD		80
30	Unsaturated-IZD		1200
31	Regioisomeric unsaturated-IZD		6000
32	Thiadiazolidinone (TDZ)		1500
33	Saturated ( <i>S</i> )-IZD		40
34	Saturated ( <i>R</i> )-IZD		15000

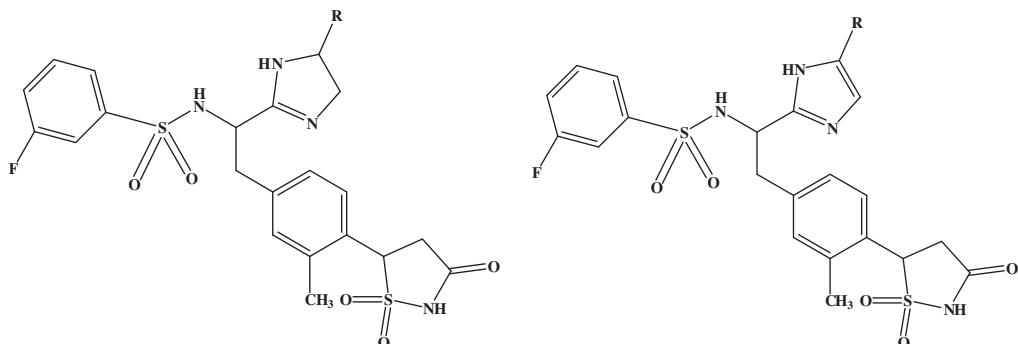
most potent heterocyclic pTyr mimetic compared with the unsaturated IZD (30), regiosomeric unsaturated IZD (31), and the thiadiazolidinone (TDZ) (32). Further Ab initio calculations effectively explained the strong binding of the (*S*)-IZD (33) as compared with

(R)-IZD (**34**) due to the pre-organized binding of the (S)-IZD in low-energy conformation.<sup>119</sup> While the peptidic inhibitors described herein are not cell-permeable or orally bioavailable, these diffusely anionic IZD heterocyclic pTyr mimetics provide new opportunities for the discovery of such PTP 1B inhibitors when incorporated into suitable nonpeptidic scaffolds.

Sparks et al. reported a unique combination of benzothiazole benzimidazole (S)-iso-thiazolidinone derivatives through a peptidomimetic modification of the peptidic (S)-IZD inhibitors. These derivatives are potent, competitive, and reversible inhibitors of PTP 1B. X-ray co-crystal structure of PTP 1B with compound (**35**) demonstrated that the benzothiazole benzimidazole forms bidentate H-bonds to Asp48, and the benzothiazole interacts with the surface of the protein in a solvent exposed region toward the C-site. The benzothiazole binds toward the C-site above Tyr46. The nitrogen lone pair of the benzothiazole forms a hydrogen bond to a water molecule, but is otherwise solvent exposed on the surface of the enzyme. Caco-2 permeability was significantly enhanced for this series of nonpeptidic benzothiazole benzimidazole IZD inhibitors presumably due to the lower polar surface area (PSA).<sup>120</sup>

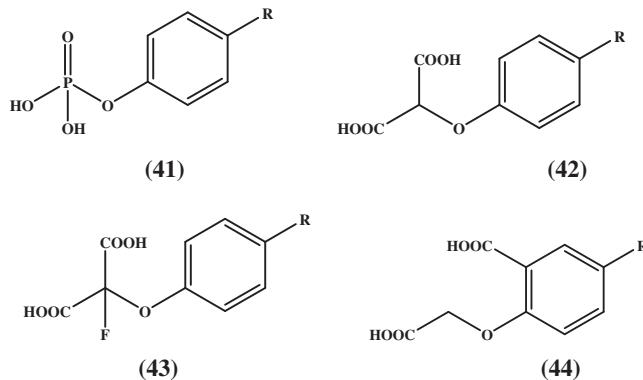
(35) IC<sub>50</sub> ~ 270 nM

Douty et al. further reported isosteric replacements of the benzimidazole with imidazoles and imidazolines as potent inhibitors of PTP 1B. Both heterocycles maintained the critical hydrogen bonding interactions with Asp48 of PTP 1B through the nitrogen atoms which was found to be necessary for high-affinity binding. The unsubstituted imidazoline (**36**) and imidazole (**37**) showed less affinity compared with the benzimidazole (**38**) (Table X). Further functionalization resulted in two highly potent PTP 1B inhibitors (**39**) and (**40**) having IC<sub>50</sub> value 32 and 22 nM, respectively. These interact with the B site which results in increases of the affinity by 32-fold (**39** vs. **36**) in the imidazoline series and by 7-fold (**40** vs. **37**) in the imidazole series. X-ray crystal structures of the imidazolines and imidazoles bound to PTP 1B showed that phenyl substituent on the end of the methylene chain optimally interacting with the hydrophobic residues of the B site when the chain length was sufficiently long enough to extend deep into the B site.<sup>121</sup>

(39) (R)-(CH<sub>2</sub>)<sub>4</sub>O (2-CO<sub>2</sub>H, 3-OH-Ph)(40) (CH<sub>2</sub>)<sub>4</sub>O (2-CO<sub>2</sub>H, 3-OH-Ph)

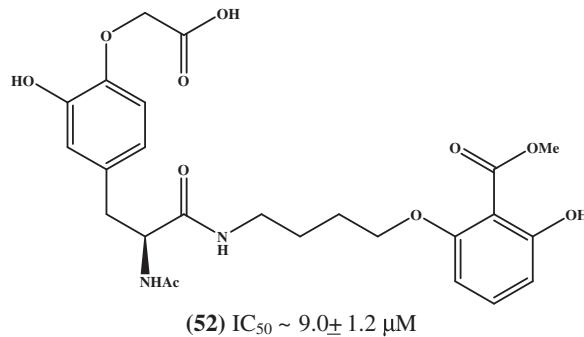
## **D. Acid Derivatives/Nonphosphorus-Containing Phosphotyrosyl Mimetics**

The pTyr phenyl phosphate group (**41**) has been an important component for structure-based designing of PTP 1B inhibitors. As the efficacy of the phosphates is hampered by their inability to penetrate into cells, there is considerable interest in the development of nonphosphorus-containing pTyr mimetics. The analogs that utilize the dicarboxylic acid-containing malonate structure as phosphate isosteres are the most successful nonphosphorus-containing pTyr mimetics. These include *O*-malonyltyrosine (OMT)<sup>122</sup> (**42**), fluoro-*O*-malonyltyrosine (FOMT)<sup>123</sup> (**43**), and 3-carboxy-*O*-carboxymethyl tyrosine<sup>124</sup> (**44**) which in the context of peptides are among the most potent PTP 1B inhibitors. They were designed to potentially afford prodrug protection strategies. It was envisioned that the charged malonyl carboxyl groups could be masked in their ester form, and then liberated once inside the cells to the free carboxyls via the action of cytoplasmic esterase.<sup>125</sup>

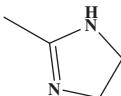
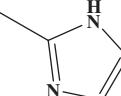
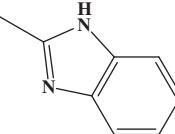


Gao et al. reported PTP 1B inhibitory potency of peptides containing mono as well as dicarboxylic acid-based pTyr mimetics. Among these analogs (**45–51**) (Table XI) which utilize biscarboxy phenylphosphate replacements (**45, 46**) showed good inhibitory potencies depending on the arrangement of the carboxyl groups. The monocarboxy analogs have shown to exhibited lesser affinity than the dicarboxy-based compounds. The overall reduced potency of monocarboxy analogs (**47–51**) is consistent with the bidentate mode of native pTyr binding, in which two positively charged Arg residues form ionic bonds to the doubly charged tyrosyl 4-*O*-phosphate group. It also demonstrates that a minimum of two anionic groups on the phosphate mimicking group would be required to take full advantage of the range of binding interactions displayed by the parent phenyl phosphate moiety.<sup>125</sup>

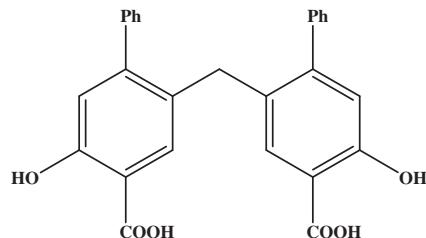
Xin et al. reported monoacid-based, cell permeable, selective inhibitor of PTP 1B having improved physiochemical properties. A (2-hydroxyphenoxy) acetic acid-based phosphotyrosyl mimetic has been linked with an optimized second arylphosphate-binding site ligand to produce compound (**52**) with low micromolar potency against PTP 1B, good selectivity over TC-PTP (20-fold) and high cell permeability in the Caco-2 system.<sup>126</sup>



**Table X.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (36–38)

Compound number	“R”Acronym	R	PTP 1B IC <sub>50</sub> (nM)
36	Imidazoline		700
37	Imidazole		240
38	Benzimidazole		67

Cho and co-workers reported a series of methylenedisalicylic acid derivatives as PTP 1B inhibitors. Among the series, compounds (53) and (54), showed good inhibitory activity against PTP 1B (Table XII) and four- and seven-fold lower values, respectively, compared with those against TC-PTP. These inhibitors exhibited reversible and slow-binding kinetics against PTP 1B.<sup>127</sup> Further, more diverse compounds containing one or two salicylic acid moieties have also been reported, and their inhibitory potency against PTP 1B have been evaluated. Among the series compound (55), was proved to be most potent inhibitor of PTP 1B, and demonstrated a 14-fold greater selectivity over TC-PTP.<sup>128</sup>

(55) IC<sub>50</sub> ~ 6.5 μM

Further Cho and co-workers reported a series of 2-*O*-carboxymethylpyrogallol derivatives in an attempt to improve the inhibitory potency of (2-hydroxyphenoxy) acetic acid

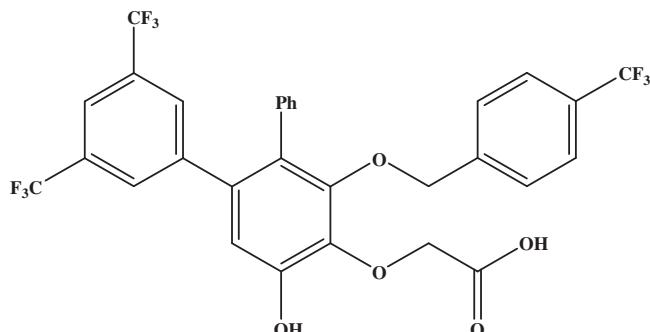
**Table XI.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (45–51)

Compound number	R	PTP 1B IC <sub>50</sub> (μM)
45		0.07
46		0.17
47		0.6
48		2
49		15
50		50
51		>> 100

**Table XII.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (**53–54**)

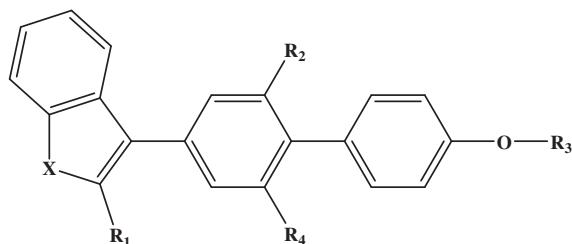
Compound number	R	PTP 1B IC <sub>50</sub> (μM)	PTP 1B K <sub>i</sub> (μM)
<b>53</b>	Ph	20	9.4
<b>54</b>	Bn	15	6.3

derivatives against PTP 1B. Compound (**56**) emerged as the most potent inhibitor against PTP 1B and also significantly lowered the fasting glucose level and improved the glucose tolerance in an obesity-induced diabetic mouse model.<sup>129</sup>

(56) IC<sub>50</sub> ~ 2.0±0.1 μM

#### E. Benzofuran and Benzothiophene Biphenyls

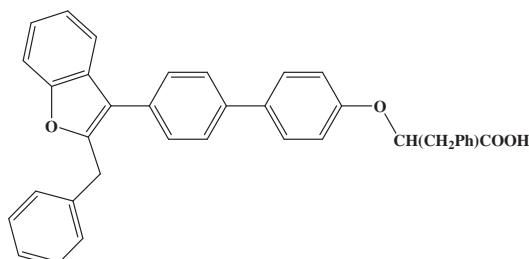
Malamas et al. at Wyeth identified two novel series of benzofuran/benzothiophene biphenyl (**57**, **58**) oxo-acetic acids and sulfonyl-salicylic acids as potent PTP 1B inhibitors with good oral antihyperglycemic activity. Crystallographic studies indicated that the inhibitors bind to the enzyme active site and are held in place through weak hydrogen bonding and van der Waals interactions formed within two hydrophobic conformations.



(57)X=O :Benzofuran biphenyl

(58)X=S: Benzothiophene biphenyl

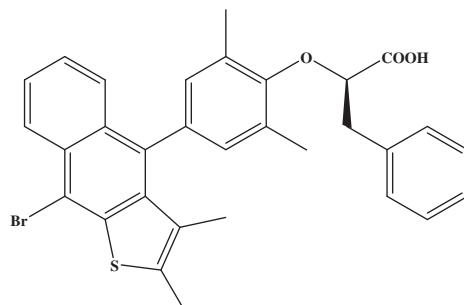
Benzofuran/benzothiophene biphenyl oxo-acetic acids with hydrophobic substitutents at position-2 of the biphenyl framework interacted with Phe182 of the enzyme catalytic site and were very critical for intrinsic activity of the molecule. The hydrophobic region of the catalytic-site pocket was exploited and taken advantage by hydrophobic substituents at the *ortho* aromatic positions of the oxo-acetic acid moiety. *Ortho*-aromatic substitutions on the benzofuran/benzothiophene biphenyl sulfonyl-salicylic acids type inhibitors had no effect due to different orientation of these inhibitors in the PTP 1B catalytic site. The most active inhibitors of both the series showed IC<sub>50</sub> values in the range of 20–50 nM against recombinant human PTP 1B. (2S)-2-[4'-(2-Benzyl-benzofuran-3-yl)-biphenyl-4-yloxy]-3-phenyl-propionic acid tromethamine salt (**59**) emerged as the most potent compound in *in vivo* studies and normalizing plasma glucose levels at the 25 mg/kg dose (p.o.) and the 1 mg/kg dose (i.p.). It also demonstrated 10 to 100 fold selectivity against other homologous PTPases and was least selective (10-fold) over LAR.<sup>130</sup>



(**59**) IC<sub>50</sub> = 0.32 μM

Further Murthy and Kulkarni performed 3D-QSAR study using CoMFA and CoMSIA of the above series. Comparison of 3D-QSAR contour maps with steric, electrostatic and hydrophobic properties of the PTP 1B enzyme showed a high level of compatibility.<sup>131</sup> Further Patankar and Jurs performed QSAR study on the same series and classified PTP 1B inhibitors using molecular structure-based descriptors. The classification rates achieved indicate that these models could serve as a screening mechanism to identify potentially useful PTP 1B inhibitors.<sup>132</sup>

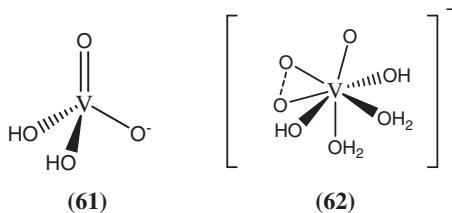
Ertiprotafib (**60**), a mono carboxylic acid benzothiophene phosphotyrosine mimetic discovered at Wyeth as a potent inhibitor of PTP 1B, reached to the clinical trial.<sup>133</sup> The complete mechanism of action for ertiprotafib *in vivo* involved multiple independent mechanisms, including PTP1B inhibition, dual PPAR $\alpha$ /PPAR $\gamma$  agonism, and IKK- $\beta$  inhibition. In the light of this complexity of multiple mechanisms, which could complicate its preclinical efficacy and potentially contribute to its side effects, it was discontinued in Phase II clinical trials.<sup>134,135</sup>



(**60**) IC<sub>50</sub> ~ 1.4+0.1μM

## *F. Vanadium Compounds*

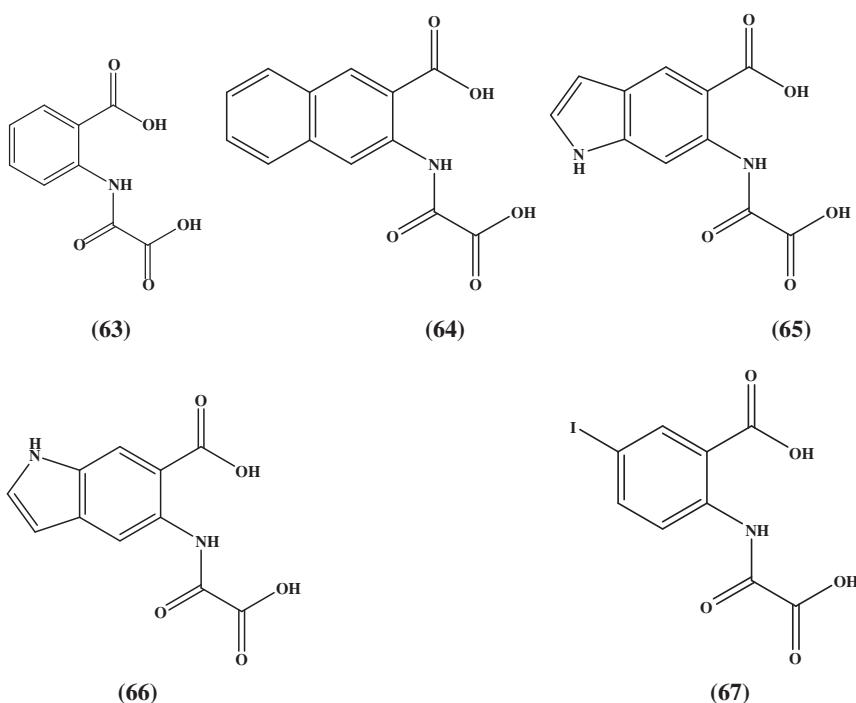
Vanadium compounds have a potential therapeutic value in human diabetes. Insulin like properties of vanadium salts have attracted much interest during the last few years for two main reasons: they are likely to help in elucidating the molecular mechanisms underlying certain perturbations of insulin action and they open new therapeutic perspectives in various states of insulin resistance.<sup>136</sup> Vanadate and pervanadate (the complexes of vanadate with hydrogen peroxide) are two commonly used general PTP inhibitors. These compounds also have insulin-mimetic properties, an observation that has generated a great deal of interest. Vanadate is a phosphate analog and is generally thought to bind as a transition state analog to the phosphoryl transfer enzymes that it inhibits, as it can easily adopt a trigonal bipyramidal structure. Kinetic studies by Ramachandran and co-worker at Merck Frosst indicated that vanadate (**61**) is a competitive inhibitor against PTP 1B, with a  $K_i$  of  $0.38 \pm 0.02 \mu\text{M}$ . Pervanadate (**62**) is also insulin-mimetic and appears to be more effective than vanadate in increasing the level of cellular tyrosine phosphorylation.<sup>137</sup> Pervanadate inhibits by irreversibly oxidizing the catalytic cysteine of PTP 1B. Peroxovanadium complexes containing one or more chelating ligands in addition to the oxo and peroxy ligands are generally more stable and exhibit even more potent insulin-mimetic effects.<sup>138</sup> Complete evaluation of the long-term efficacy and side-effects of the different forms of vanadium remains necessary, however, before their use in clinical practice can be considered.<sup>139</sup>



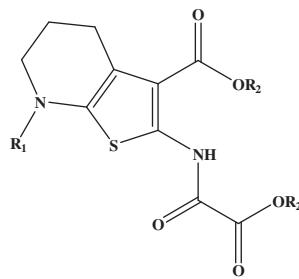
### **G. 2-(Oxallylamino)-Benzoic Acid**

Using a scintillation proximity technology-based high throughput screening assay Novo Nordisk identified several 2-(oxalylamino)-benzoic acid (OBA) derivatives (63–67) as competitive, reversible, active site-directed inhibitors of PTP 1B.<sup>140</sup> X-ray protein crystallography of PTP 1B co-crystallized with OBA revealed that it binds to the highly conserved phosphate-binding loop (the PTP loop), thus mimicking part of the binding pattern of the natural substrate. In addition, it was also reported that OBA exhibited a novel binding pattern, interacting with Lys120 surrounding the active site, which are not directly involved in substrate binding. Because of OBA's low molecular weight and its enzyme kinetic behavior as classical, time-independent competitive inhibitors Novo Nordisk further performed structure-based lead optimization to develop selective small molecules as inhibitors of PTP 1B.<sup>141</sup>

Various aromatic and heteroaromatic substituents, such as indole, naphthalene, and thiophene, were introduced in place of aromatic part of OBA to optimize the structure for improved binding with the hydrophobic part of the tyrosine phosphate. Among the series compound (**68**) and (**69**) emerged as promising inhibitors with a high potency and selectivity against PTP 1B (Table XIII). As cell permeability was a major issue with above compounds, diester prodrugs (**70**, **71**) were also reported with improved accumulation of 2-deoxyglucose (2-DOG) into C2C12 cells.<sup>142</sup>

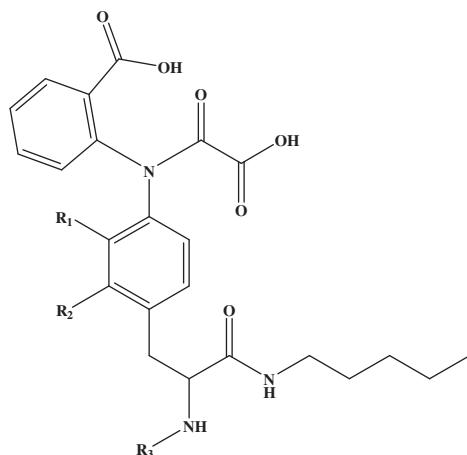


**Table XIII.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (68–71)



Compound number	R <sub>1</sub>	R <sub>2</sub>	PTP 1B IC <sub>50</sub> (μM)
<b>68</b>	-H	H	0.29
<b>69</b>	-(CH <sub>2</sub> ) <sub>2</sub> Ph	H	0.27
<b>70</b>	-H	Et	—
<b>71</b>	-(CH <sub>2</sub> ) <sub>2</sub> Ph	Et	—

Using an NMR-based screening approach with  $^{15}\text{N}$  and  $^{13}\text{C}$  labeled PTP 1B, Abbott reported 2, 3-dimethylphenyloxalylaminobenzoic acid derivatives (**72–80**) as reversible and competitive PTP 1B inhibitors (Table XIV). Among the series naphthyl (**72**), *O*-ethyl (**73**), *O*-isopropyl (**74**), *O*-hydroxyethyl (**75**), and *O*-piperidinyl (**76**) based analogs displayed  $K_{\text{i}}$  values in low micromolar level, with a 10-fold improvement in binding affinity toward PTP 1B and TC-PTP when compared with (**77**, **78**). The enantiomerically pure analog (**79**)

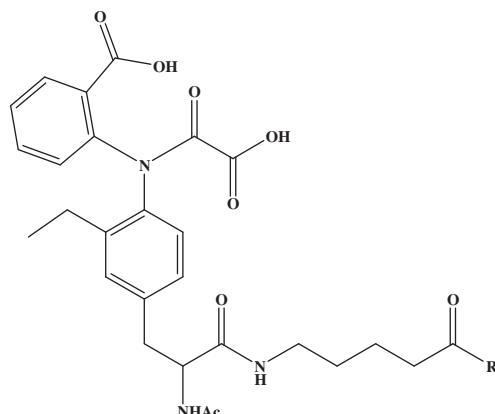
**Table XIV.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (72–80)

Compound number	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	PTP 1B IC <sub>50</sub> (μM)
72			CH <sub>3</sub> CO-	1.1±0.5
73	-CH <sub>2</sub> CH <sub>3</sub>	-H	CH <sub>3</sub> CO-	1.2±0.3
74	-CH <sub>2</sub> (CH <sub>3</sub> ) <sub>2</sub>	-H	CH <sub>3</sub> CO-	1.2±0.1
75	-CH <sub>2</sub> CH <sub>2</sub> OH	-H	CH <sub>3</sub> CO-	1.5±0.7
76	1-Piperidinyl	-H	CH <sub>3</sub> CO-	2.0±1.5
77	-H	-H	t-Bu-CO-	17.3±1.9
78	-H	-H	CH <sub>3</sub> CO-	9.8±2.1
79	-(E) CH <sub>2</sub> = CH <sub>2</sub> CONH <sub>2</sub>	-H	CH <sub>3</sub> SO <sub>2</sub> -	0.17±0.07
80	-H	-H	CH <sub>3</sub> SO <sub>2</sub> -	9.1

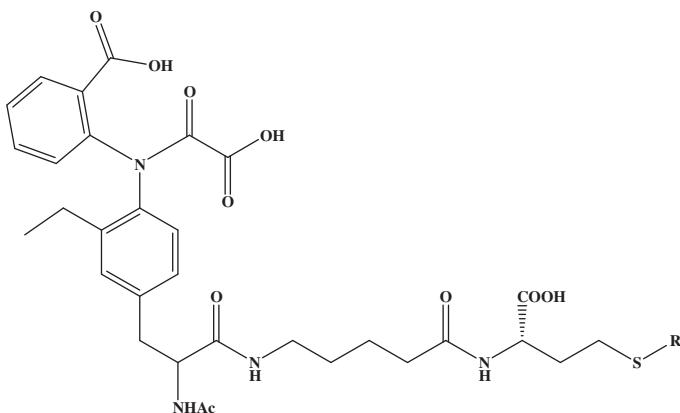
exhibited a submicromolar inhibitory constant ( $K_i = 0.17 \mu\text{M}$ ) for both PTP 1B and TC-PTP, an over-50-fold improvement over compound (80). As the basic structures of these inhibitors are charged, reasonable bioavailability was reported in rats.<sup>143</sup>

From the SAR studies of the above reported series, various structural modifications have been attempted for higher and selective PTP 1B inhibitory activity. Various flexible linkers such as aminopentanoic acid (81) and phenylethylaminopentanoic acid (82) (Table XV) along with chiral amino sulfide such as (83) and (84) (Table XVI) showed increasing inhibitory potency as well as moderate selectivity.<sup>144</sup>

In search for novel phosphotyrosine mimetics, as lead PTP 1B inhibitors, Wyeth screened several hundred compounds at high micromolar concentration. Compound (85) was found to be the most potent, reversible, and competitive inhibitor of PTP 1B with a  $K_i$  of 230 μM at pH 7.4. It was further observed that inhibition of the enzyme by this compound was independent of pH ( $K_i = 200 \mu\text{M}$  at pH 5.5). X-ray crystallography of the compound (85) with PTP 1B revealed that the thiophene ring was sandwiched between Phe182 and Tyr46, which provided pi-interactions and mimicked the phenyl group of phosphotyrosine (pTyr). The acidic side chain at the 3-position was buried deep down in the enzyme active site, mimicking the phosphate group of pTyr. The carboxyl group formed a salt bridge with

**Table XV.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (81–82)

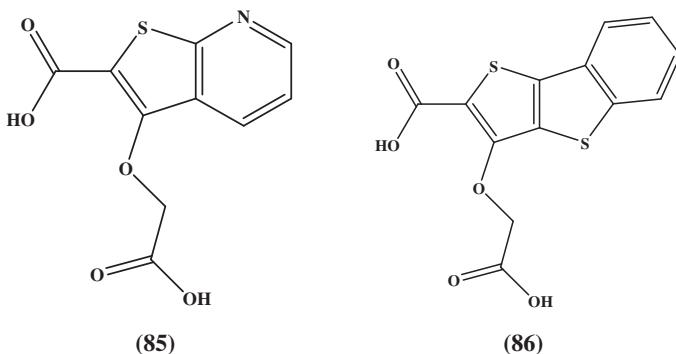
Compound number	R	PTP 1B $K_i(\mu\text{M})$
81	OH	2.5
82	$\text{NH}(\text{CH}_2)_2\text{Ph}$	3.4

**Table XVI.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (83–84)

Compound number	R	PTP 1B $K_i(\mu\text{M})$
83	Methyl	0.076
84	Ethyl	0.13

Arg221 and multiple hydrogen bonds with the backbone amides of Gly218, Ile219, and Gly220 at the bottom of the active site. The ether oxygen formed water-mediated hydrogen bonds with Ala217 and Arg221. The carboxyl group at the 2-position interacted with Lys120 via a salt bridge. The 5-position of (85) packed tightly against the side chain of Ile219. Because of these multiple interactions, the inhibitor positioned itself nicely at the enzyme

active site. Though the potency of **(85)** was weak, the structural information provided guidance for further optimization of various bicyclic scaffolds.



During optimization tricyclic thiophenes were also synthesized, which showed inhibitory activities at micromolar level. The thienobenzothiophene template of (**86**) was chosen as the backbone ( $K_i = 9.2 \mu\text{M}$ ) for further optimization due to its novelty and synthetic flexibility. Compounds (**87–92**) out of 5- and 6-substituted thienobenzothiophenes showed inhibitory activities at submicromolar level (Table XVII). X-ray crystallography of compound (**92**) with PTP 1B revealed that, the acid moieties of (**92**) binds to the active site in a similar binding mode as the compound (**85**). In addition, one of the sulfonamide oxygen forms hydrogen bond to the backbone nitrogen of Gly259 and the other enters into interactions with Arg24 and Arg254 through bridging water molecules. These interactions overcome any unfavorable effect of the proximity of the piperidine nitrogen to the hydrophobic Met258 side chain.

Thienobenzothiophene analogs were very selective against other PTPs such as CD45 and LAR (1000-fold), except the highly homologous TC-PTP. Nevertheless, extending the inhibitor from the active site into the second phosphotyrosine-binding site did shift the relative selectivity of PTP 1B vs. TC-PTP (**86** vs. **92**) slightly. Compound (**86**), only binding to the active site, favored TC-PTP by about twofold. Compound (**92**), spanning the active and the second phosphotyrosine-binding site, was equally potent against both enzymes. This serves as a good starting point for developing low nanomolar PTP 1B inhibitors by further optimizing the occupancy of the active site and the second phosphotyrosine-binding site.<sup>145</sup>

## *H. 1, 2-Naphthoquinones*

The 1, 2-naphthoquinones scaffold was discovered as a hit toward PTP 1B inhibitor through high throughput screening (HTS) using a chemical library of 40,000 compounds from the Korea Chemical Bank. Ahn et al. reported a series of 1, 2-naphthoquinones having different substituents (93). In vitro inhibitory activity of these compounds against recombinant human PTP 1B using fluorescein diphosphate (FDP) as the substrate demonstrated inhibitory activity even at submicromolar levels. Among the series, compounds (94) and (95) were found to be most potent having IC<sub>50</sub> values of 0.27 and 0.32 μM against PTP 1B, respectively. The selectivity of several compounds was also evaluated and compound (96) demonstrated 10- to 60-fold selectivity against the tested phosphatase. Compound (96) was also found to be the most potent in reducing plasma glucose levels in the *ob/ob* mice.<sup>146</sup>

Based on the hypoglycemic potency of compound (96) Cheon et al. selected it for lead development. In vivo and in vitro studies demonstrated that compound (96)

**Table XVII.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (87–92)

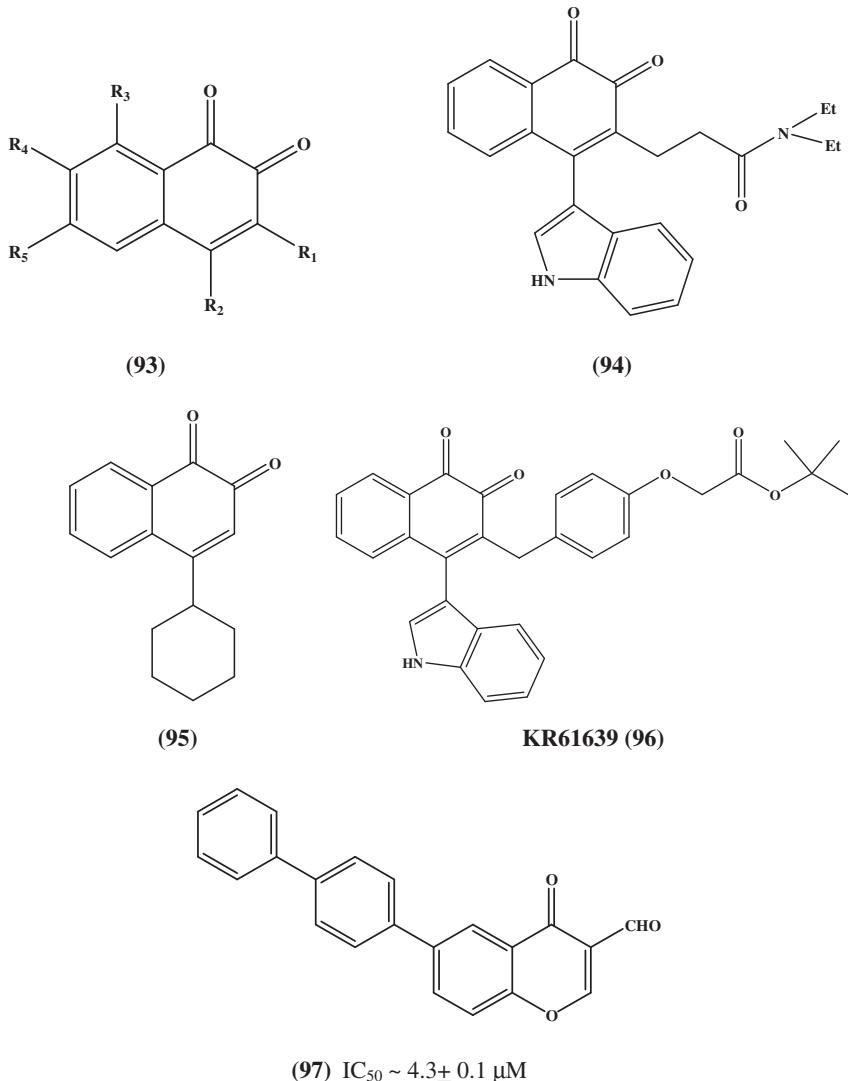
Compound number	R <sub>1</sub>	R <sub>2</sub>	PTP 1B K <sub>i</sub> (μM)
87		H	0.92
88	H		0.68
89		H	1.7
90	H		0.74
91	H		1.6
92	H		0.37

(KR61639){4-[1-(1H-indol-3-yl)-3, 4-dioxo-3, 4-dihydro-naphthalen-2-ylmethyl]-phenoxy}-acetic acid tert-butyl ester} exerted a hypoglycemic action via increased insulin-stimulated glycogen synthesis in HepG2 cells and stimulated 2-deoxyglucose uptake in 3T3/L1 adipocytes and having submicromolar PTP 1B inhibitory activity ( $IC_{50} = 0.65 \mu\text{M}$ ). From the kinetics study, it was observed that (96) inhibited PTP 1B in a noncompetitive manner, indicating that it may bind to the enzyme substrate complex or interacts with the secondary aryl phosphate-binding site near the active site pocket of enzyme.<sup>147</sup>

### I. Formylchromones

Formylchromone derivatives were reported as potent, selective PTP 1B inhibitors. Formylchromone moiety, a neutral pharmacophore without charge and the examination of the structure reveals that it is well suited for derivatization. 6-Biphenyl-3-formylchromone (97) was found to be the most potent inhibitor in the series and possess strong or medium selectivity against other human PTPases, LAR and TC-PTP. This compound, however, was

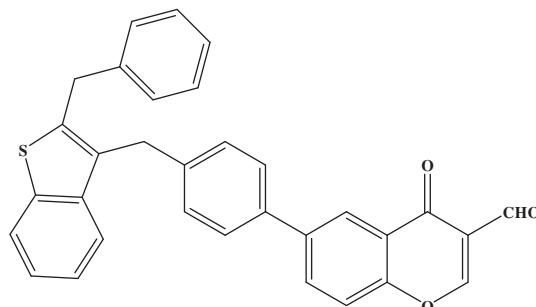
not selective against microbial PTPases, YPTP1 and YOP. It is suggested to have extended interaction of the extra phenyl ring with the surface near the active site of the enzyme.<sup>148</sup>



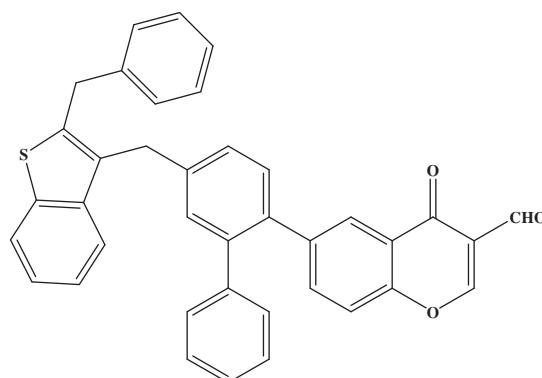
Shim et al. further performed extensive derivatization of 3-formylchromone and identified several potent PTP 1B inhibitors with IC<sub>50</sub> values as low as 1.0 μM. These compounds exhibited remarkable selectivity for PTP 1B over other human PTPases. The most potent inhibitors, (98) and (99), were PTP 1B selective and the IC<sub>50</sub> values were 500-, 30-, and >10-fold lower than those against LAR-D1, TC-PTP, and SHP-1, respectively. Kinetic studies revealed that formylchromones derivatives are irreversible and active site-directed inhibitors likely by forming covalent adduct with the active site Arg221.<sup>149</sup>

Molecular modeling study identified the orientation of the inhibitor bound at the active site of PTP 1B. Compound (99) extended deep into the active site pocket, making several hydrogen bonds and hydrophobic interactions with key residues of the catalytic site. The two carbonyl groups of compound (99) forms three hydrogen bonds with Gly220 and Arg221 and benzopyran group forms van der Waals interaction with the phenyl ring of Tyr46 and Phe182.<sup>150</sup> 3D-QSAR studies also have been performed on formylchromones using Genetic function

approximation (GFA) technique which demonstrated that shape, electronic and thermodynamic descriptors contribute significantly to the biological activity of these PTP 1B inhibitors.<sup>151</sup>



(98)  $IC_{50} \sim 1.1 \pm 0.3 \mu\text{M}$



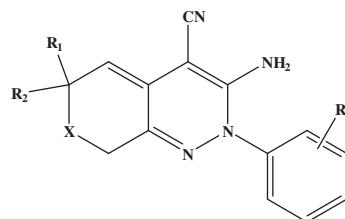
(99)  $IC_{50} \sim 1.0 \pm 0.2 \mu\text{M}$

### J. Pyridazine Analogs

A series of pyridazine analogs have been identified at Biovitrum as noncompetitive and reversible inhibitors of PTP 1B with most of the compounds showing  $IC_{50}$  values in the low micromolar range. These compounds were the first reported noncompetitive binders of PTP 1B, which may suggest that there is an allosteric site through which the enzymatic activity can be inhibited. Among the series, compounds (100–114) were found to be potent having  $IC_{50}$  values in low micro molar range and compound (111) emerged as most potent inhibitor (Table XVIII). These compounds were also tested for their inhibitory activity against other PTPases, and were found to exhibit surprisingly high level of selectivity. For example, compound (114) was 20-fold more selective for PTP 1B than LAR and TCPTP.<sup>152</sup> Due to small molecular weight and nonpolar properties of this class of compound, they can transit the cell membrane easily. Modeling studies using CoMFA and Leap-Frog has also been reported in the literature which would help in development and optimization of lead molecules.<sup>153</sup>

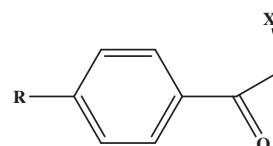
### K. Acetophenones

Various studies revealed that acetophenone derivatives are potent inhibitors of PTP 1B. Initially Pei and co-workers reported that  $\alpha$ -haloacetophenone (115) derivatives as potent neutral PTP inhibitors, which covalently alkylate the conserved catalytic Cys215 residue in the PTP 1B active site.<sup>154</sup> Because they are neutral, these agents readily diffuse into human cells and inhibit the intracellular PTPs. The results show that the bromides are much more potent than the

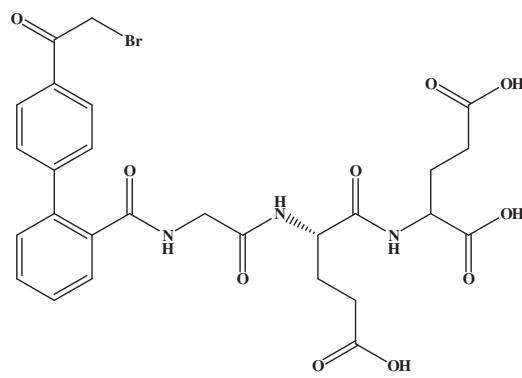
**Table XVIII.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (100–114)

Compound number	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	X	PTP 1B IC <sub>50</sub> (μM)
100	H	H	2-iPropyl	S	2.0
101	H	H	4-F	S	2.0
102	H	H	4-OMe	S	1.3
103	H	H	H	S	1.1
104	CH <sub>3</sub>	CH <sub>3</sub>	2-Butyl	O	1.5
105	CH <sub>3</sub>	CH <sub>3</sub>	2-F	O	2.4
106	CH <sub>3</sub>	CH <sub>3</sub>	4-OCH <sub>3</sub>	O	2.2
107	CH <sub>3</sub>	CH <sub>3</sub>	3-NHCOCH <sub>3</sub>	O	2.3
108	CH <sub>3</sub>	CH <sub>3</sub>	2-CH <sub>2</sub> CH <sub>3</sub>	O	1.6
109	CH <sub>3</sub>	CH <sub>3</sub>	4-CH <sub>2</sub> OH	O	1.7
110	CH <sub>3</sub>	CH <sub>3</sub>	3-CH <sub>2</sub> OH	O	2.4
111	H	H	H	O	0.35
112	CH <sub>3</sub>	CH <sub>3</sub>	3-COOCH <sub>3</sub>	O	2.1
113	CH <sub>3</sub>	CH <sub>3</sub>	4-CH <sub>2</sub> CH <sub>3</sub>	O	2.8
114	H	H	4-iPropyl	S	5.6

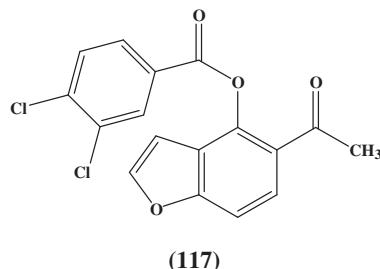
corresponding chlorides, whereas the phenyl ring is remarkably tolerant to modifications. Derivatization of the phenyl ring with a tripeptide Gly-Glu-Glu via a rigid biphenyl linker resulted in a potent, selective inhibitor (**116**) with 54-fold selectivity toward PTP 1B.<sup>155</sup>



(115)

(116) K<sub>i</sub> ~ 9.9 μM

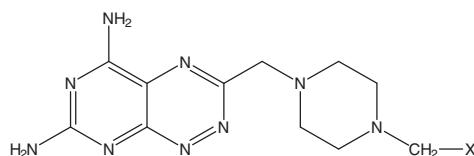
Later Dixit et al. reported various small acetophenones functionalized with alkoxy and aroyloxy moieties as potential PTP 1B inhibitors. One of these compounds, 4-(3, 4-dichlorobenzoyloxy)-5-acetyl-benzofuran (**117**), showed 54% inhibition against PTP 1B at 100 μM concentration. The rest of the compounds were either inactive or possessed poor activity.<sup>156</sup>



#### L. Pyrimido [5, 4-3] [1, 2, 4] triazine-5, 7-diamine

Guertin et al. reported a novel series of orally active pyrimido [5, 4-3] [1, 2, 4] triazine-5, 7-diamine for inhibition of LAR through HTS. Subsequently it was found to have high residual PTP 1B inhibitory activity as well, and served as a starting point for the development of novel PTPase inhibitors. These compounds showed nonselective inhibitory properties against a panel of PTPs including PTP 1B. Triazine ring seems to be responsible for the redox activity. The mechanism of pyrimidotriazine indicated that in presence of Dithiothreitol (DTT) and absence of oxygen, the structurally related pyrimidotriazine rapidly and quantitatively underwent reduction to the corresponding dihydro derivative which was reversible in the presence of atmospheric oxygen. Among the series compound (**118–121**) showed inhibitory activity against PTP 1B in micromolar concentration indicating hydrophobic benzylic side chains (e.g. **118–121**) were favored on the piperazine ring (Table XIX). Kinetic studies revealed that these compounds were reversible and competitive inhibitors of PTP 1B. Compound (**119**) containing a biphenyl moiety emerged as the most potent compound of the series. The pharmacokinetic properties of (**119**) are summarized in Table XX. This compound (**119**) is rapidly and extensively distributed and achieved systemic exposure ( $C_{max}$ ) approximated the measured IC<sub>50</sub> against PTP 1B in vitro (Table XX). It has a favorable  $t_{1/2}$

**Table XIX.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (**118–121**)



Compound number	X	PTP 1B IC <sub>50</sub> (μM)
<b>118</b>	Phenyl	6.2
<b>119</b>	Biphenyl	2.9
<b>120</b>	1-Naphthyl	3.5
<b>121</b>	2-Naphthyl	4.5

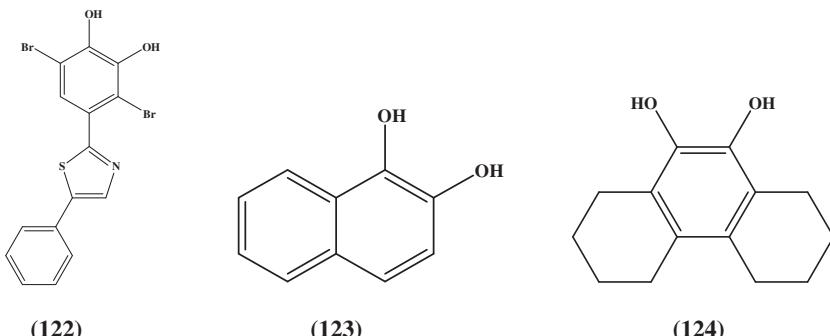
**Table XX.** Pharmacokinetic Properties of Compound (119)

Compound number	$t_{1/2}$ (hr)	CL(mL/kg/min)	$V_{ss}$ (L/kg)	$C_{max}$ ( $\mu$ M)	F (%)
119	8	104	3.1	4.0	97

and excellent oral bioavailability. The large steady state volume of distribution ( $V_{ss}$ ) of (119) is approximately six times the total body water volume of the animal, suggesting deep tissue and cell penetration. On the other hand, the compound suffers from high systemic clearance which exceeds hepatic blood flow of the mouse.<sup>157</sup>

### **M. Catechols**

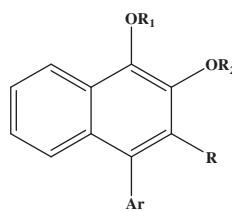
Catechol derivatives were also discovered as hits of PTP 1B inhibitors from HTS of the library of Korea Chemical Bank. Compound (122) and other similar compounds have been reported as PTP 1B inhibitors, thus catechol scaffolds were chosen to be tested as possible inhibitors of PTP 1B. Among the commercially available catechol 1, 2-naphthalenediol (123) and octahydrophenanthrenediol (124) showed strong inhibitions with IC<sub>50</sub> of 1.25 and 3.65  $\mu$ M respectively, indicating 1, 2-naphthalenediol seemed well suited for further optimization.



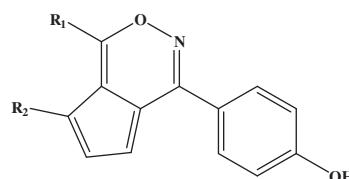
Unexpectedly, diacetoxynaphthalenes (125) and (126) with substituents at 3-position showed remarkable in vitro activity, comparable to their naphthoquinone homologs. Also 1, 2-naphthalenediol (127) substituted with 3-indolyl group at position-4 showed the most potent inhibitory activity among the series (Table XXI). Thus, (127) was chosen as lead for further study. As the selectivity of the inhibitors is important to minimize the undesirable side effects, the selectivity was tested against nine phosphatases and the compound (127) showed good selectivity. Compound (127) also exhibited hypoglycemic activity in the diabetic mice via oral administration.<sup>158</sup>

### **N. Cyclopenta[d] [1, 2]-oxazine**

High-throughput screening led to the identification of cyclopenta[d] [1, 2]-oxazine class of novel compounds as hits against PTP 1B with micromolar IC<sub>50</sub>s. Because of the novelty of cyclopenta [d] [1, 2]-oxazine as a small molecule PTP 1B inhibitor, a series of such compounds were synthesized with introduction and modification of substituents on cyclopenta

**Table XXI.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (125–127)

Compound number	R	R <sub>1</sub>	R <sub>2</sub>	Ar	PTP 1B IC <sub>50</sub> (μM)
125	CH <sub>2</sub> Ph-4-OMe	Ac	Ac	-Ph	3.89
126	CH <sub>2</sub> Ph-4-OCH <sub>2</sub> COO- <i>t</i> -Bu	Ac	Ac	3-Indolyl	1.69
127	H	H	H	3-Indolyl	1.61

**Table XXII.** Structures and In Vitro PTP 1B Inhibitory Activities of Compounds (128–130)

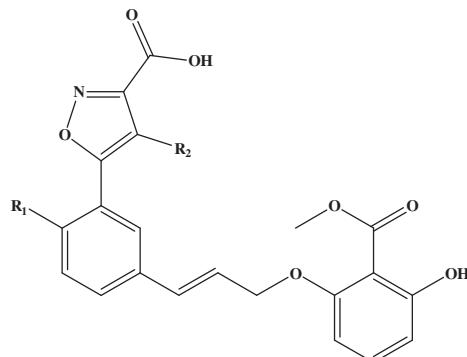
Compound number	R <sub>1</sub>	R <sub>2</sub>	PTP 1B IC <sub>50</sub> (μM)
128	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>13</sub> CO	CH <sub>2</sub> COOH	0.27
129	4-CH <sub>3</sub> (CH <sub>2</sub> ) <sub>13</sub> CO	CH <sub>2</sub> COOH	0.14
130	4-CH <sub>3</sub> OPhCO	CH <sub>2</sub> COOH	0.80

[d] [1, 2]-oxazine skeleton. Most of the compounds were found to be active in in vitro screening, and a few of them (128–130) were found to exhibit activity even at sub micromolar range. Compound (129) emerged as the most active compound in the series (Table XXII). SAR indicated that the introduction of long-alkyl group chain in compound (128) greatly enhanced the activity. When evaluated for selectivity against recombinant phosphatases, compound (130) showed good to reasonable selectivity, while introduction of a long hydrocarbon unit as seen in compound (129) resulted in complete loss of selectivity. Compound (130) normalized plasma glucose level when administered through i.v. route but only was weakly effective on oral administration due to low bioavailability.<sup>159</sup>

### O. Isoxazole Carboxylic Acids

Liu et al. at Abbott laboratories discovered moderately potent, highly selective, and cellular active isoxazole carboxylic acid analogs as PTP 1B inhibitor using NMR-based fragment screening and X-ray crystal structure-based assembly approach. Compounds (131) and (132) demonstrated greater than 30-fold selectivity over TC-PTP and showed no inhibitory activity against LAR, CD45, cdc25, and SHP-2 even at the highest concentration. X-ray crystallography of compound (132) revealed that isoxazole carboxylic acid binds to the active site of

PTP 1B with the WPD loop in the closed conformation. Bidentate-type hydrogen bonds between the carboxylic acid and Arg221 provided the critical interactions. The isoxazole and phenyl rings lie in close proximity to the hydrophobic pocket normally occupied by the phenyl ring of pTyr.<sup>160</sup>

(131)  $R_1 = H$ ;  $R_2 = H$ ;  $K_i = 5.7 \pm 0.9 \mu M$ (132)  $R_1 = F$ ;  $R_2 = H$ ;  $K_i = 6.9 \pm 2.3 \mu M$ (133)  $R_1 = H$ ;  $R_2 = NH_2$ ;  $K_i = 2.1 \mu M$ (134)  $R_1 = F$ ;  $R_2 = CH_2OH$ ;  $K_i = 0.92 \mu M$ 

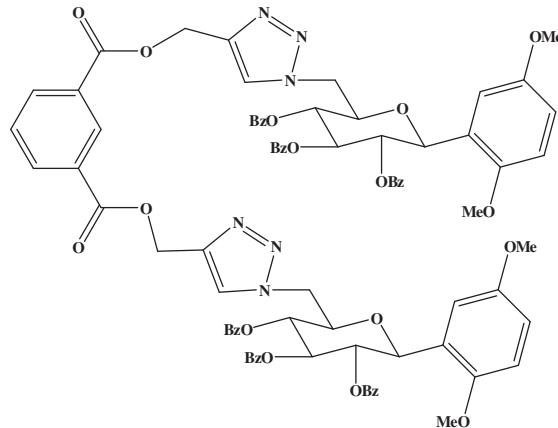
In order to extend the SAR studies, leading to more potent PTP 1B inhibitors, with similarly high selectivity and cell permeability some useful structural modifications were carried out by Zhao et al. introducing linkers as well as substitution on the oxazole ring. Compounds (133) and (134) obtained via substitution on the oxazole ring showed activities in low micromolar range as well as selectivity against other homologous PTP's.<sup>161</sup>

#### P. Miscellaneous

Lin et al. discovered dimeric acetylated and benzoylated  $\beta$ -C-D-glucosyl and  $\beta$ -C-D-galactosyl 1, 4- dimethoxy benzenes or naphthalenes as PTP 1B inhibitors having a triazole moiety. Benzoylated glucosyl and galactosyl dimers containing a 1, 4-dimethoxybenzene or 1, 4-benzoquinone moiety (135–138) showed submicromolar inhibitory activity against PTP 1B probably by binding in a hydrophobic pocket of enzyme, with no significant difference between gluco and galacto derivatives.<sup>162</sup>

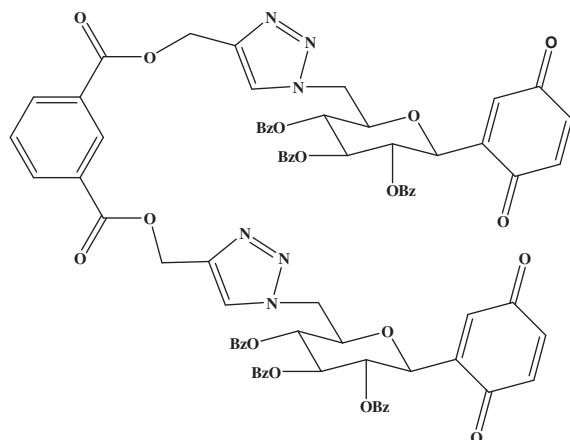
In order to get novel NCEs, Saxena et al. designed novel 2-[(4-methoxyphenyl) ethyl] acetamide derivatives as PTP 1B inhibitors using best CoMFA model. The above designed molecules (139–141) were synthesized and evaluated for their in vitro as well as in vivo activities where these compounds (139–141) showed low PTP 1B inhibitory activities 76.9% ( $IC_{50} = 69 \mu M$ ), 62.5% ( $IC_{50} = 87 \mu M$ ), and 68.2% ( $IC_{50} = 71 \mu M$ ), respectively. Docking studies further indicated 4-methoxyphenyl group of most active (139) showed hydrogen-bonding interaction with the  $-NH_2$  of Arg221 similar to the hydrogen-bonding interaction of carbonyl of reference ligand (sulfamic acid) with Arg221 of catalytic site of PTP 1B. The  $-NH-$  group of compound (139) also showed hydrogen bond interaction with water molecule similar to  $-NH-$  in the sulfamic acid. However unlike the sulfamic acid, it does not show close interactions with the active site amino acids Ser216, Ala217,

Gly220, and Ile219 nor with secondary binding site residues of PTP 1B (Arg24, Arg254, and Gln262).<sup>163</sup>



(135) gluco ( $I_{C_{50}} \sim 0.87 \pm 0.04 \mu M$  )

(136) glacto ( $IC_{50} \sim 0.88 \pm 0.01 \mu M$ )



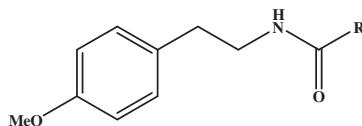
(137) gluco ( $\text{IC}_{50} \sim 0.62 \pm 0.06 \mu\text{M}$ )

(138) glacto ( $IC_{50} \sim 0.72 \pm 0.05 \mu M$ )

## O. Natural PTP 1B Inhibitors

Natural products, containing inherently vast structural diversity than synthetic compounds, have been the major sources of bioactive agents and will continually play as protagonists for discovering new drugs. Phytochemicals are considered privileged structures as they have the diversity space in which chemical scaffolds embody characteristics that promote binding to multiple protein targets. An analysis of the origin of the drugs that were launched in the last 25 years showed that both natural products and semi-synthetic compounds, derived from natural origin, composed 34% of all new chemical entities, while 18% of them were synthetic mimics of natural compounds. Therefore, natural products are considered important sources for new drugs or lead optimization for PTP 1B inhibition for management of diabetes and

obesity.<sup>164</sup> In the following section, PTP IB inhibitory activities of wide variety of compounds derived from natural sources have been reported.



(139) R=1-Methyl naphthyl

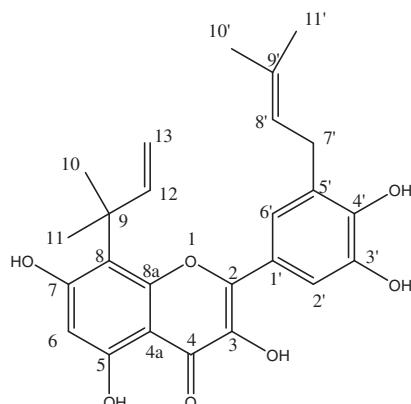


(140) R=1-Methyl-2-nitro-phenyl

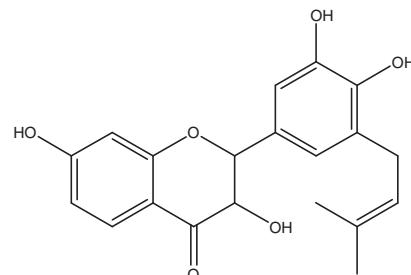


(141) R=1-Methyl phenoxy

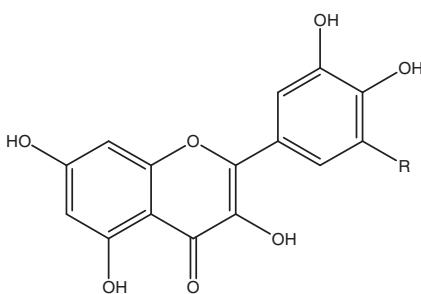
While screening for inhibitors of PTP 1B from an extract bank, a fraction from the ethanol extract of the roots of *Broussonetia papyrifera* (L.) Vent. showed strong inhibitory activity against PTP 1B. Two new compounds, 8-(1,1-dimethylallyl)-5'-(3-methylbut-2-enyl)-3',4',5,7-tetrahydroxyflavonol (**142**), 3'-(3-methylbut-2-enyl)-3',4',7 trihydroxy flavane (**143**) and three known compounds, 3, 3', 4', 5, 7-pentahydroxyflavone (**144**), uralenol (**145**) and broussochalcone A (**146**) were identified from above extract. Compounds (**142**), (**144**), (**145**), and (**146**) were found to be potent inhibitors of PTP 1B. Among them (**142**), (**144**), and (**145**) have the same 3',4', 5, 7-tetrahydroxyflavonol skeleton, and more nonpolar substituents at this skeleton increase their inhibitory activities [from **142** (two isoprenyl substituents) to **146** (one isoprenyl substituent), **144**].<sup>165</sup>



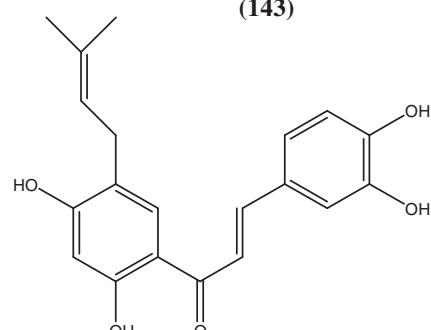
(142)



(143)

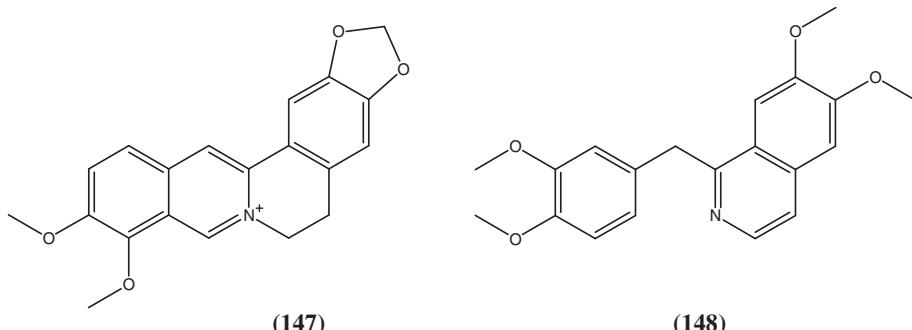


(144) R=H

(145) R=CH<sub>2</sub>CH=C(CH<sub>3</sub>)<sub>2</sub>

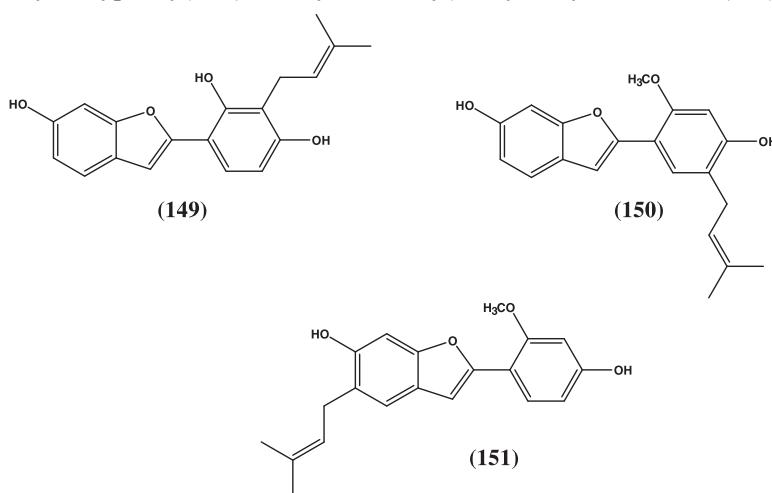
(146)

Berberine (**147**), an isoquinoline alkaloid is widely distributed in nature and used in traditional eastern homeotherapy particularly, in treating gastrointestinal infections. Berberine has been reported to possess potent antidiabetic property.<sup>166,167</sup> The hypoglycemic effects of berberine were accidentally discovered when it was administered to a diabetic patient with diarrhoea.<sup>168</sup> Bustanji et al. further investigated the *h*-PTP 1B inhibitory activity in order to explain the antidiabetic property. It competitively inhibit recombinant PTP 1B in vitro ( $K_i$  value = 91.3 nM). It was reported to readily fit within the binding pocket of PTP 1B in a low-energy orientation characterized with optimal electrostatic attractive interactions bridging the isoquinolinium positively charged nitrogen atom of berberine and the negatively charged acidic residue of Asp48 of PTP 1B.<sup>169</sup>



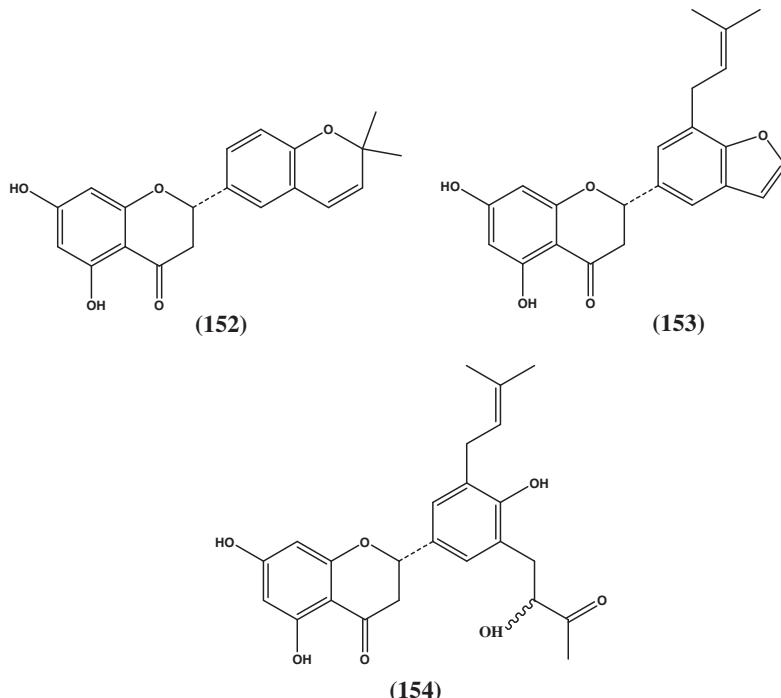
Recently Papaverine (**148**), a prominent member of isoquinoline alkaloids structurally similar to berberine, was found to readily dock within the binding pocket of PTP 1B in a low energy orientation via an optimal set of attractive interactions. Papaverine also exhibited potent in vitro inhibitory effect against recombinant *h*-PTP 1B ( $IC_{50}$ ~1.20  $\mu$ M) and significantly decreased fasting blood glucose level *in vivo*.<sup>170</sup>

The genus Erythrina of the family Leguminosae comprises over 110 species that are widely distributed in tropical and subtropical regions, and representative species have been used in indigenous medicine.<sup>171,172</sup> In a continuing effort to search for natural PTP 1B inhibitors, Na et al. reported three new 2-arylbenzofurans isolated from the stem bark of *Erythrina addisoniae*, which were found to exhibit potent in vitro PTP 1B inhibitory activities. These are 2-[20, 40-dihydroxy-30-(3-methylbut-2-enyl) phenyl]-6-hydroxybenzofuran (**149**), 2-[20-methoxy-40-hydroxy-50-(3-methylbut-2-enyl) phenyl]-6-hydroxybenzofuran (**150**), and 2-(20-methoxy-40-hydroxyphenyl)-5-(3-methylbut-2-enyl)-6-hydroxybenzofuran (**151**).<sup>173</sup>



These new 2-arylbenzofurans (**149–151**) exhibited PTP 1B inhibitory activity with  $IC_{50}$  values ranging from  $13.6 \pm 1.1$  to  $17.5 \pm 1.2 \mu\text{M}$  in in vitro assays. Hence, 2-arylbenzofurans with prenyl group may be considered as a new class of PTP 1B inhibitors.<sup>173</sup>

From a different genus of Erythrina, Oh and his colleagues reported three new prenylated flavanones, abyssinoflavanones V, VI, and VII (**152–154**), together with eight known flavanones and two chalcones from the stem bark of *E. abyssinica*. Most of the compounds strongly inhibited PTP 1B activity with  $IC_{50}$  values ranging from  $14.2 \pm 1.7$  to  $26.7 \pm 1.2 \mu\text{M}$ . These compounds have a common 5, 7-dihydroxyflavanone skeleton or its chalcone analogs and differ with regard to the substitution patterns in the B ring.<sup>174</sup>

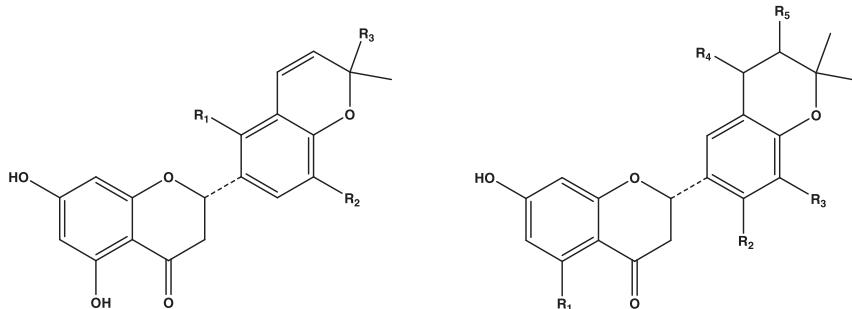
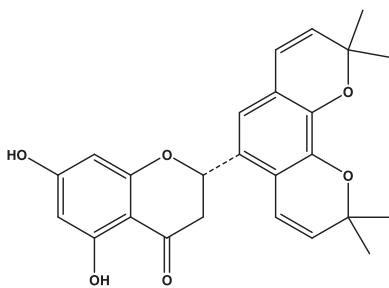


Later on Oh and his colleagues further investigated 12 new flavanones (**155–166**) bearing a 2, 2-dimethylpyrano ring from extract of stem bark of *E. abyssinica*. Compounds (**155**, **157**, **159**, **160**, **162**, and **163**) exhibited inhibitory effects on PTP 1B with  $IC_{50}$  values ranging from  $13.9 \pm 2.1$  to  $19.0 \pm 1.8 \mu\text{M}$ .<sup>175</sup>

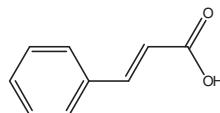
Results from both studies indicated that presence of a lipophilic group (methoxy and/or prenyl residues) on the B ring seemed to increase the inhibitory effect of these flavanoids against PTP 1B, while the presence of a polar moiety (hydroxy group) did not seem to affect their biological activity.

*Cinnamomum cassia* extracts have been reported in the literature to improve fasting glucose, glucose tolerance, and insulin sensitivity in women with insulin resistance associated polycystic ovary syndrome. Cinnamic acid (**167**), a known compound from the bark of *C. cassia* showed dose-dependent PTP 1B inhibitory activity with an  $IC_{50}$  of  $4.4 \mu\text{g}/\text{mL}$ . The time-course studies for PTP 1B inhibition by cinnamic acid were performed over the period 0–200 min indicating time-dependent inhibition and it also appeared that cinnamic acid is a fast-binding inhibitor.<sup>176</sup>

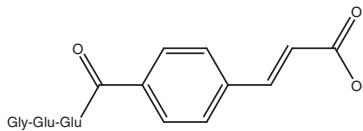
Moran et al. have demonstrated that the attachment of a tripeptide, Gly-Glu-Glu-NH<sub>2</sub>, to the *para* position of cinnamic acid results in a potent inhibitor (**167A**) for PTP1B ( $K_i \sim 0.079 \mu\text{M}$ ).<sup>177</sup> Further Pei and his colleagues reported cinnamaldehyde containing similar tripeptide (**167B**) at *para*-position having good inhibitory activity ( $K_i \sim 5.42 \mu\text{M}$ ) but less as comparable to acid derivative (**167A**).<sup>178</sup>

(155) R<sub>1</sub>=prenyl, R<sub>2</sub>=OH, R<sub>3</sub>=CH<sub>3</sub>(156) R<sub>1</sub>=H, R<sub>2</sub>=OH, R<sub>3</sub>=CH<sub>2</sub>OH(157) R<sub>1</sub>=H, R<sub>2</sub>=OCH<sub>3</sub>, R<sub>3</sub>=CH<sub>2</sub>OH(158) R<sub>1</sub>=R<sub>5</sub>=OH, R<sub>2</sub>=R<sub>4</sub>=H, R<sub>3</sub>=prenyl(159) R<sub>1</sub>=R<sub>5</sub>=OH, R<sub>2</sub>=R<sub>4</sub>=H, R<sub>3</sub>=OCH<sub>3</sub>(160) R<sub>1</sub>=R<sub>4</sub>=R<sub>5</sub>=OH, R<sub>2</sub>=R<sub>3</sub>=H(161) R<sub>1</sub>=R<sub>4</sub>=R<sub>5</sub>=OH, R<sub>2</sub>=H, R<sub>3</sub>=prenyl(162) R<sub>1</sub>=R<sub>4</sub>=R<sub>5</sub>=OH, R<sub>2</sub>=prenyl, R<sub>3</sub>=OH(163) R<sub>1</sub>=R<sub>3</sub>=OH, R<sub>2</sub>=R<sub>5</sub>=H, R<sub>3</sub>=(-O)(164) R<sub>1</sub>=R<sub>2</sub>=R<sub>4</sub>=H, R<sub>3</sub>=R<sub>5</sub>=OH(165) R<sub>1</sub>=R<sub>2</sub>=H, R<sub>3</sub>=R<sub>4</sub>=R<sub>5</sub>=OH

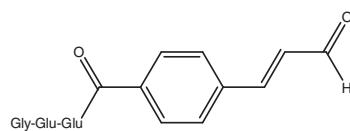
(166)



(167)



(167A)



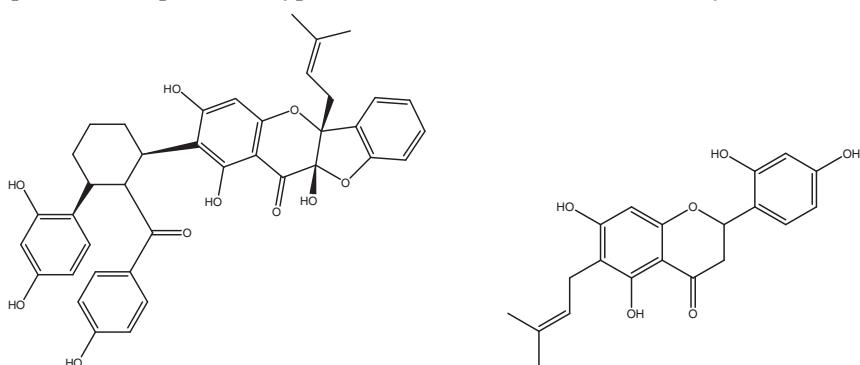
(167B)

Cui et al. reported the PTP 1B inhibitory activity of compounds isolated from organic extract of Chinese crude drug “Sang-Bai-Pi” (*Morus* root bark). Bioassay-guided fractionation resulted in isolation of flavanoids sanggenon C (168), sanggenon G (169), mulberrofuran C (170), and kuwanon L (171) as PTP 1B inhibitors, along with moracin O and moracin P. Compounds (168–171) inhibited PTP 1B with IC<sub>50</sub> values ranging from 1.6±0.3 to 16.9±1.1 μM. Kinetic studies revealed that compounds (168–170) inhibited PTP 1B in the mixed-type manner, indicating that they may bind both at the active site and an additional binding site of the PTP 1B enzyme.<sup>179</sup>

The genus Siegesbeckia has been used as a traditional medicine, “Hi-Chum” in Korea (common name: Siegesbeckia herb), to treat inflammatory diseases. Kim et al. reported isolation of two active diterpenes, ent-16βH,17-isobutyryloxy-kauran-19-oic acid (172) and ent-16βH,17-acetoxy-18-isobutyryloxy-kauran-19-oic acid (173), along with ent-16βH, 17-hydroxy-kauran-19-oic acid (174) from methanolic extract of the aerial part of

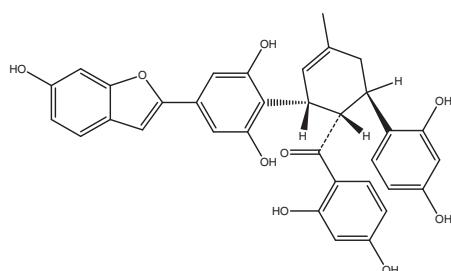
*Siegesbeckia glabrescens*. Study revealed that only compounds (**172**) and (**173**) inhibited the PTP 1B activity with IC<sub>50</sub> values of  $8.7 \pm 0.9$  and  $30.6 \pm 2.1 \mu\text{M}$  in a noncompetitive manner.<sup>180</sup>

Diterpenoids from roots of *Acanthopanax koreanum*, a medicinal plant indigenous to Korea exhibited significant PTP 1B inhibitory activity. Na et al. reported isolation of eight diterpenoids from the active fraction, which were evaluated for their inhibitory effect on PTP 1B. A kaurane-type diterpene, 16 $\alpha$ H, 17-isovaleryloxy-ent-kauran-19-oic acid (**175**), inhibited PTP 1B with an IC<sub>50</sub> value of  $7.1 \pm 0.9 \mu\text{M}$  in a noncompetitive manner while acanthoic acid (**176**) and ent-kaur-16-en-19-oic acid (**177**) also inhibited PTP 1B in dose-dependent manners. SAR of various diterpenoid indicated that introduction of a hydroxyl group or reduction of a carboxyl group at C-19 in pimarane-type to alcohol abolished the inhibitory effects.<sup>181</sup>

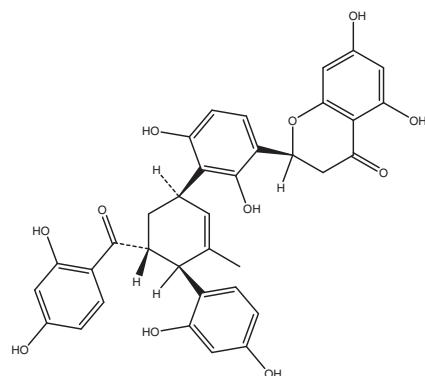


Sanggenon C (168)

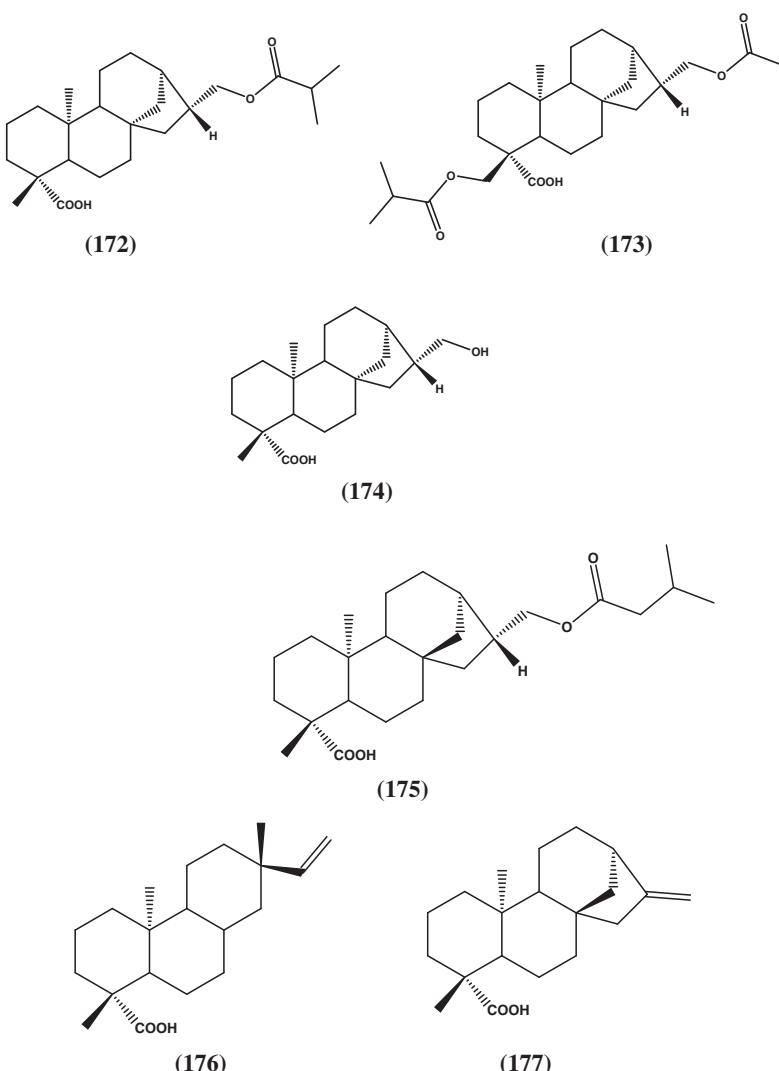
Sanggenon G (169)



Mulberrofuran C (170)



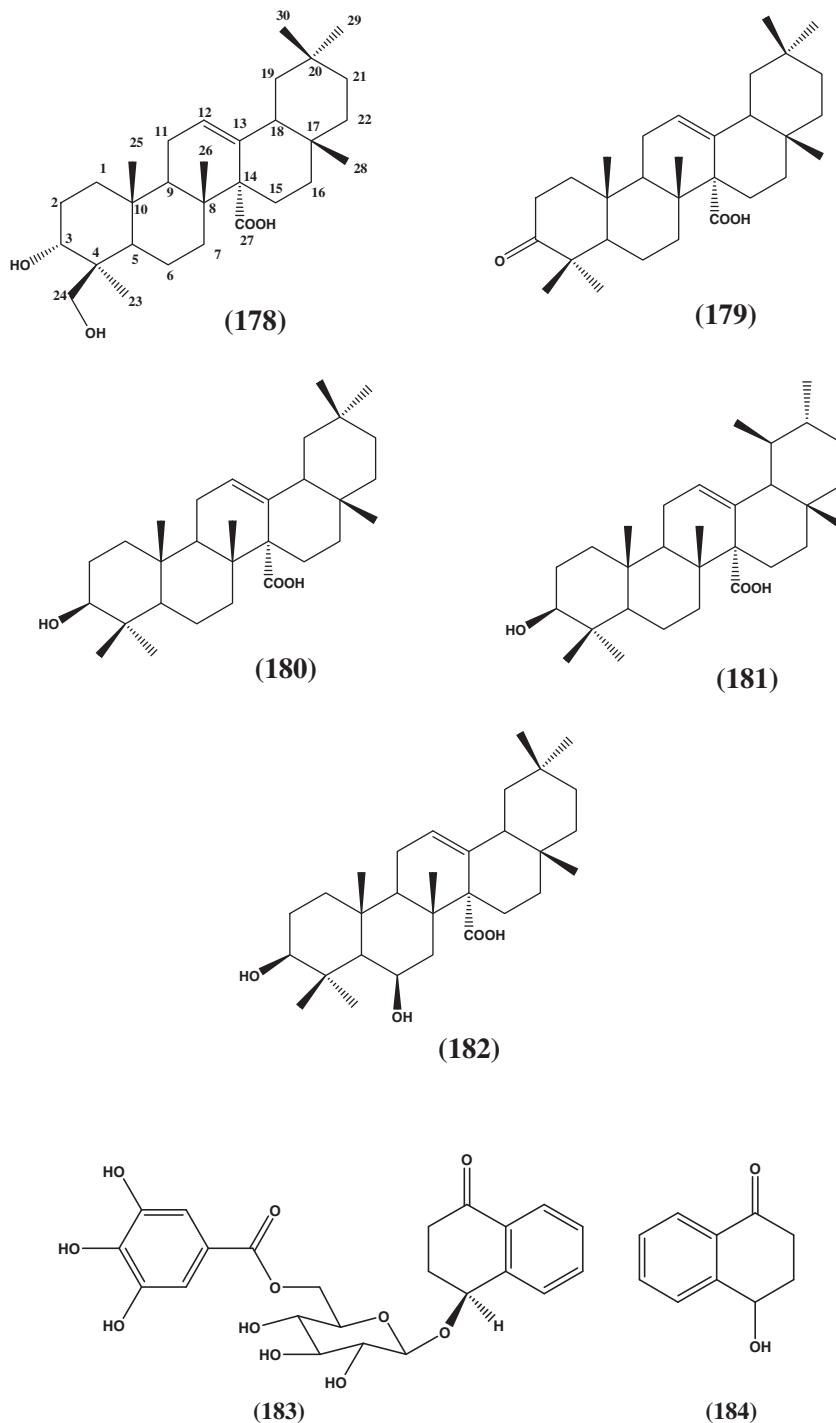
Kuwanon L (171)

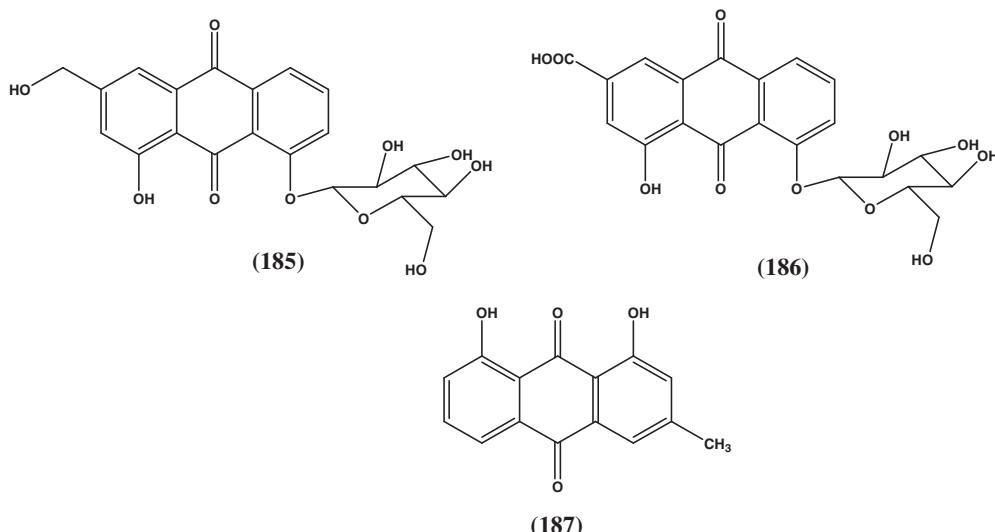


Later on Na et al. further reported isolation of a new triterpene  $3\alpha$ , 24-dihydroxyolean-12-en-27-oic acid (**178**), along with four known triterpenes, 3-oxoolean-12-en-27-oic acid (**179**),  $3\beta$ -hydroxyolean-12-en-27-oic acid ( $\beta$ -pebtoboykinolic acid; **180**),  $3\beta$ -hydroxyurs-12-en-27-oic acid (**181**), and  $3\beta, 6\beta$ -dihydroxyolean-12-en-27-oic acid (astilbic acid; **182**) from the methanolic extract of the rhizomes of *Astilbe koreana*. Compounds (**178–182**) inhibited PTP-1B with  $IC_{50}$  values of  $6.8 \pm 0.5$ ,  $5.2 \pm 0.5$ ,  $4.9 \pm 0.4$ ,  $11.7 \pm 0.9$ , and  $12.8 \pm 1.1 \mu M$ , respectively. SAR indicated that 3-hydroxyl group and a carboxyl group in this type of triterpenes may be required for inhibitory activity, while addition of one more hydroxyl group at C-6 or C-24 may be responsible for a loss of activity.<sup>182</sup>

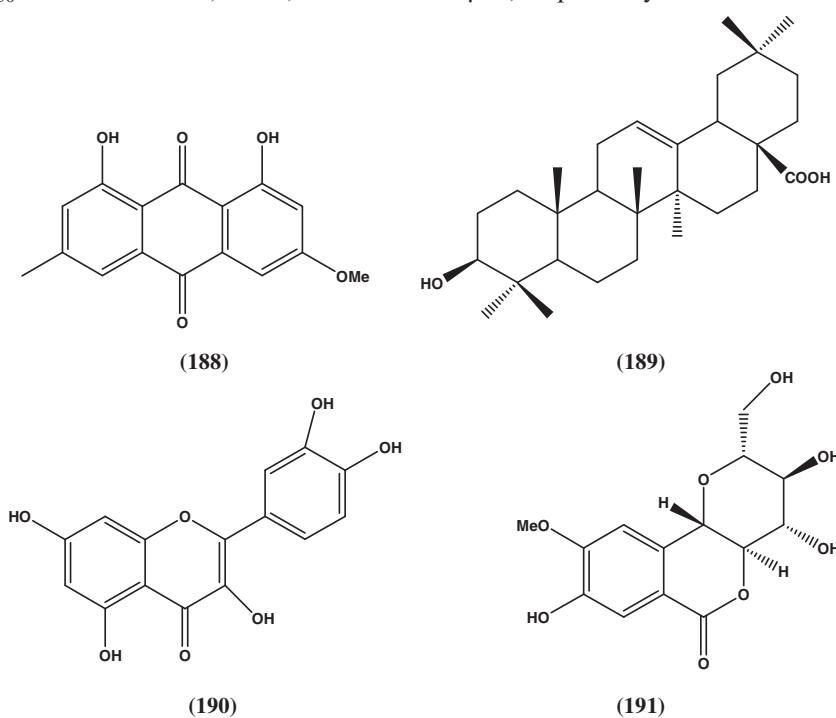
An et al. reported a new compound, 4-hydroxy- $\alpha$ -tetralone-4-O- $\beta$ -D-[6-O-(3'',4'',5''-tri-hydroxybenzoyl) glucopyranoside (**183**), together with a known compound, (**184**) from the ethanolic extracts of roots of *Juglans regia*. 4-Hydroxy- $\alpha$ -tetralone (**184**) showed moderate bioactivity against PTP 1B. [ $IC_{50} = 66.7 \mu\text{mol/L}$ ].<sup>183</sup>

Li et al. studied three anthraquinones (**185–187**) from *Saussurea radix*, the roots of *Saussurea lappa clarke* (Compositae), a Chinese traditional herbal medicine. These showed moderate bioactivity against human h-PTP 1B.<sup>184</sup>



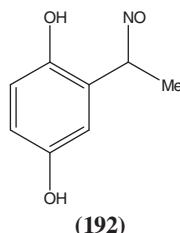


Li et al. investigated sixteen known compounds from the extract of *Ardisia japonica*. Among these isolates, compound (**188–191**) showed moderate bioactivity against PTP 1B in vitro with IC<sub>50</sub> values of 121.50, 23.90, 28.12 and 157 μM, respectively.<sup>185</sup>

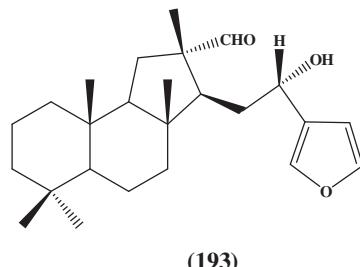


Butanol soluble fraction from *Psidium Guiana* (Myrtaceae) leaves exhibited significant inhibitory effect on PTP 1B with IC<sub>50</sub> 2.6 µg/mL.<sup>186</sup>

Imoto et al. reported a novel PTP 1B inhibitor, Dephostatin (**192**), from the culture filtrate of *Streptomyces* sp. MJ742-NF5. However, sufficient amounts of dephostatin were difficult to obtain from the natural source because of low production by microorganisms and liability to air, heat, light, solvents, and acidic conditions. Later, Watanabe et al. reported the first total synthesis of dephostatin in 1994.<sup>187</sup> Dephostatin inhibited PTP 1B prepared from a human neoplastic T-cell line with an IC<sub>50</sub> at 7.7 μM. The inhibitory pattern of dephostatin was competitive against the substrate. Dephostatin also inhibited the growth of Jurkat cells.<sup>188</sup>



Hyrtiosal (**193**), a marine natural product obtained from the marine sponge *Hyrtios erectus*, has been discovered to act as a PTP 1B inhibitor and to show extensive cellular effects on PI3K/AKT activation, glucose transport, and TGF/Smad2 signaling. This inhibitor was able to inhibit PTP 1B activity in a dose-dependent fashion, with an IC<sub>50</sub> value of 42 μM in a noncompetitive manner.<sup>189</sup>



## 7. CONCLUSION

PTP 1B inhibitors have emerged as a legitimate approach for the management of Diabetes mellitus and obesity, i.e. "diabesity". In fact, the diversity in the mechanisms by which PTP 1B may be inhibited by numerous classes of synthetic chemical compounds as well as natural products has opened a new era in developing therapeutic agents. Despite good biological target validation, designing PTP 1B inhibitors as oral agent is challenging because of the highly charged nature of the catalytic domain of the target enzyme. Moreover, because of the strong homology between PTPs, targets selectivity is often difficult to achieve although sufficient groundwork based on rational drug design using computer-assisted pharmacophore-based methods has been laid out. A principal focus of most research effort to date has centered on design of inhibitors which interact both with the catalytic as well as the secondary binding pockets of PTP 1B. The wide variety of information available till date indicates that this search is worthwhile, and identification of a hit would have a marked impact in combating global epidemic of diabesity.

## **ACKNOWLEDGMENTS**

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