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Low Molecular Weight Phosphotyrosine Protein Phosphatases as Emerging Targets for the Design of Novel Therapeutic Agents

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INTRODUCTION

Protein phosphorylation represents a key post-translational modification that is critical to the control of many cellular functions. The reversible phosphorylation of tyrosine residues of proteins is a significant regulatory event in eukaryotes compared to other protein phosphorylation processes and is crucially important for the regulation and progression of various cellular signaling cascades, especially those induced by receptor activation mechanisms. The appropriate functioning of these signaling pathways is controlled by the concerted and dynamic activities of protein tyrosine kinases (PTKs) and phosphotyrosine protein phosphatases (PTPs) which play vital roles in numerous fundamental physiological cellular processes, such as growth, differentiation, survival, migration, metabolism, cell−cell communication and adhesion, immune response, and gene transcription.[1−6]

The human genome encodes more than 100 known PTPs, and these include both soluble cytoplasmatic enzymes and transmembrane receptor-like proteins.[3,6] All known PTPs share common structural features in the phosphate-binding active site and act through a similar catalytic mechanism. Their substrate specificity and binding to distinct ligands are mainly determined by the presence of regulatory domains or noncatalytic subpockets in the regions surrounding the conserved catalytic domains.[2,3,6−10]

In recent years, rapid progress in understanding the biological functions of PTPs has uncovered evidence of the importance of the complex roles played by these enzymes as positive or negative modulators of specific cellular signaling pathways. Moreover, it has been ascertained that alterations in the activity or expression of several PTPs are implicated in the pathogenesis of human diseases, including diabetes, obesity, cancer, inflammation, autoimmune, and cardiovascular diseases. As a consequence, like their more extensively explored PTK counterparts, PTPs have aroused interest as strategic targets for the design of novel therapeutic agents to fight several human pathologies by counteracting specific signal transduction aberrations that underlie pathogenetic processes.[1,6,11−19]

However, difficulties were encountered to identify selective and bioavailable PTP inhibitors, mainly due to the polar nature and high degree of homology of PTP catalytic sites, so much so that there was initially some uncertainty about the druggability of these proteins.[20−22] Nevertheless, the successful design of druglike molecules that are active as selective PTP inhibitors is feasible by exploiting specific structural characteristics of the regions surrounding the active sites of different PTPs that represent important areas for the efficient binding of specific ligands. Therefore, the identification of several PTPs as possible drug targets encouraged the search for new PTP inhibitors and further efforts were made to optimize them, which provided interesting results particularly in relation to certain more widely explored PTPs, such as the prototypical PTP1B.[22−32] Indeed, the characterization of PTP1B and its biological validation as a target in the search for novel antidiabetic, antiobesity, and anticancer pharmaceutical agents represented the starting point and the main thrust for the extensive investigation of PTPs, and undoubtedly, this PTP is the most widely studied member of the family, as attested by the wealth of PTP1B inhibitors reported in the past 2 decades by both academic and industrial research groups.[16,26,28−30,33−36]

Besides PTP1B, more recently other PTPs have aroused interest as novel drug targets, stimulating the design of inhibitors as potential therapeutics. Out of these phosphatases, low molecular weight PTPs (LMW-PTPs) have emerged as attractive targets for the pharmacological control of postreceptor events involved in the development of metabolic and neoplastic pathologies as well as for therapeutic intervention in infectious diseases.[37−40]

LMW-PTPs are 18 kDa soluble enzymes, which have been identified and isolated from a wide variety of prokaryotic and eukaryotic organisms, such as bacteria, yeasts, and mammalians.[41−54] LMW-PTPs from different organisms generally display a high degree of homology, especially in their tertiary structure. However, their sequences and three-dimensional folding are rather different from those of higher molecular weight PTPs except for the phosphate-binding loop (P-loop) which contains the typical PTP signature motif $CX_5R(S/T)$ that is conserved in all members of the PTP family. The cysteine and arginine residues that are included in the signature sequence are, in fact, fundamental for the dephosphorylation common to all PTPs.[55,56]

Distinct isoenzymes of the human LMW-PTP are known, i.e., the two active isoforms IF1 and IF2 (named also HCPTPA and HCPTPB, respectively), which have been found to be the most abundant LMW-PTP isoenzymes in many tissues, and two catalytically inactive variants named SV3 and LMPTP-C (or SV4).[38,44,57−62] The active isoforms IF1 and IF2 differ only by a loop that flanks the catalytic site and can determine isoform specificity in the binding to substrates and modulating ligands, thus suggesting distinct cellular roles for the two isoenzymes.[57,59,62,63]

Human LMW-PTPs exert sophisticated control over cell growth and differentiation through the modulation of signaling pathways induced by several growth factors and kinases.[64−68] The enzyme also negatively regulates the metabolic responses

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to insulin,[69] and the sensitivity of specific tissues to the hormone is consequently enhanced as a result of the LMW-PTP suppression.[39] Moreover, the overexpression of LMW-PTP is implicated in tumor onset and progression.[37,38,70] Although the pathophysiological roles of the human LMW-PTP isoenzymes still need to be elucidated in greater detail, current understanding identifies drug design aimed at developing LMW-PTP inhibitors as a useful strategy for developing novel antitumor and antidiabetic agents.

In the past few years, there has been increasing evidence that protein tyrosine phosphorylation can also regulate fundamental functions in prokaryotic organisms. LMW-PTPs have been identified in several pathogenic bacteria, and some of these enzymes have been found to play crucial roles in virulence and bacterial survival in infected hosts and thus represent potential targets for therapeutic intervention.[40,54]

This Perspective focuses on recent research efforts to identify and optimize inhibitors of human LMW-PTP as potential drug candidates. In addition, we discuss inhibitors of MPtpA, a mycobacterial LMW-PTP that has been proposed as a novel antitubercular target. An outline of the current understanding of the pathophysiological roles of human LMW-PTP has also been included in order to give an account of the current state of knowledge about the biological functions of this phosphatase and its involvement in the etiology of human diseases.

STRUCTURE OF THE HUMAN LMW-PTP ISOENZYMES

In humans, LMW-PTP is almost ubiquitous and has been identified in most tissues and organs, such as erythrocytes, endothelial and muscle cells, epithelial, connective and nervous tissues, liver, placenta, and lens.[44,47,48,57,71] In 1998, the crystal structure of the human LMW-PTP isoenzyme IF1 (also known as the electrophoretically fast isoform) was described, whereas the crystal structure of IF2 (the electrophoretically slow isoform) was not reported until 2006.[58,59,62] Both human isoforms IF1 and IF2 consist of a single peptide chain of 157 amino acid residues (Figure 1). The amino acid sequence is identical in the two isoenzymes except for the segment comprising amino acid residues 40−73, called the variable loop, which presents considerable differences and only 41% homology.[44,57] The existence of isoforms IF1 and IF2 is the result of an alternative splicing mechanism in which the mutually exclusive transcription of either exon 3 or exon 4 produces two different mRNA sequences corresponding to the two isoenzymes.[72] Other splicing variants give rise to differently folding proteins, i.e., isoforms SV3 and SV4, which do not appear to possess a functional catalytic site. It has been suggested that eventual shifts toward the transcription of SV3 or SV4 mRNAs, at the expense of IF1 and IF2 variants, might contribute to the regulation of the LMW-PTP activity, resulting in reduced cellular responses to the active isoforms.[60,61]

The three-dimensional structure of the isoenzymes IF1 and IF2 consists of a four-stranded parallel β sheet core surrounded on both sides by α helices.[59,62] The active site P-loop, comprising residues 12−19 that form the sequence CLGNICRS conserved in all mammalian LMW-PTPs, connects the β1 strand to the α1 helix and is situated at the bottom of a central crevice, near the N-terminus of the protein (Figure 1). Like other phosphotyrosine-specific protein phosphatases, this cavity is deep enough to specifically catalyze the dephosphorylation of phosphotyrosine (pTyr) residues, preventing action on phosphoserine or phosphothreonine substrates. The backbone

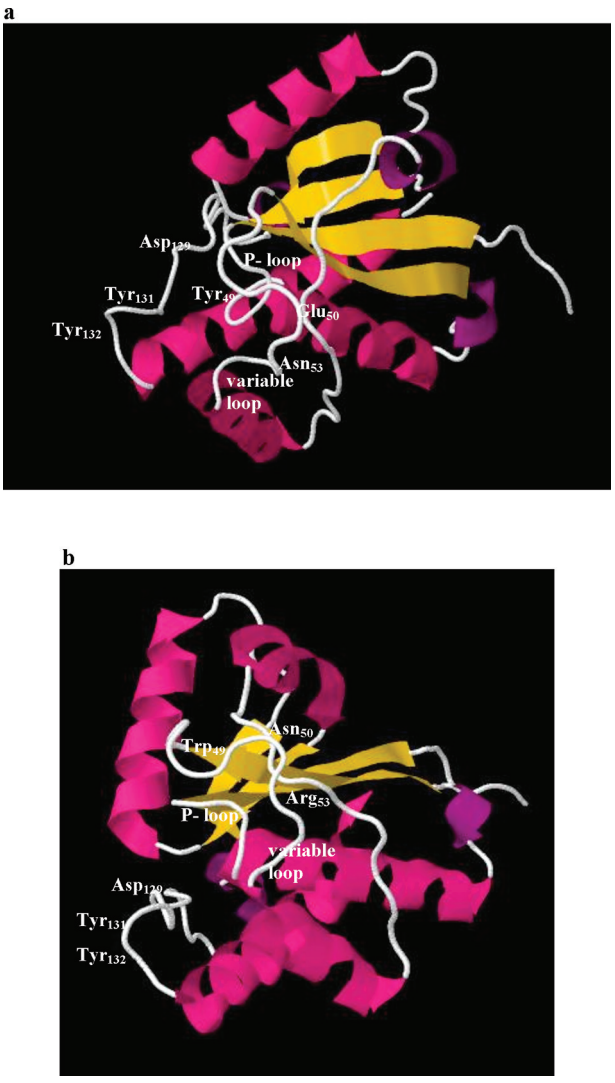

Figure 1. Structures of human LMW-PTP isoforms: (a) IF1, PDB entry 5PNT;[59] (b) IF2, PDB entry 1XWW.[62]

of the P-loop adopts a rigid conformation, which is stabilized by the formation of multiple hydrogen bonds between the active site Asn15 and the conserved residues Ser19, Ser43, His72 and is optimal for binding the substrate phosphate.[59,73] In this arrangement, a network of hydrogen bonds between several nitrogen atoms of the P-loop backbone and the oxygen atoms of the substrate phosphate group is established during the formation of the enzyme/substrate complex. In addition, an interaction between the negatively charged phosphate group and the positively charged guanidium group of Arg18 is critical for the recognition and binding of the phosphorylated substrates. The ionized residue Cys12 of the P-loop functions as the catalytic nucleophile that attacks the phosphorus atom of the substrate to form a phosphoenzyme intermediate (Figure 2). A proximal residue, Ser19, appears to play a key role in this step because it can form a hydrogen bond with the thiolate anion of Cys12 which allows it to adopt an efficient orientation for the nucleophilic attack.[73−75] Release of the dephosphorylated substrate occurs following the transfer of a proton from an adjacent residue, Asp129. In the final and rate-limiting step, this aspartate anion acts as a base that activates a water molecule responsible for the hydrolysis of the cysteinylphosphate thioester bond leading to the release of inorganic phosphate

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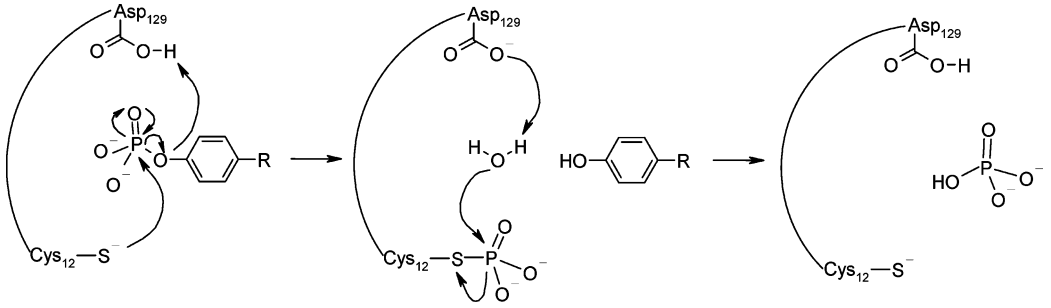

Figure 2. Mechanism of the dephosphorylation reaction catalyzed by LMW-PTP.

and restoring the enzyme (Figure 2).[55,56,73,76−78] The active site residue Cys17 contributes to the hydrolysis mechanism by coordinating the water molecule in an appropriate orientation for hydrolysis, which is analogous to the role played by a glutamine residue in high molecular weight PTPs (e.g., Gln262 in PTP1B).[56]

In PTPs, the aspartate residue serving as proton donor/acceptor is generally included in a flexible loop that folds over the active site upon substrate binding. In the human LMW-PTP, the catalytic Asp129 is contained in a mobile loop that extends between the β4 strand and the α5 helix (D-loop, residues 116−134) and flanks the active site, thereby forming one wall of the catalytic crevice (Figure 1). This loop also contains residues Tyr131 and Tyr132, which are important for the substrate recognition because they establish π-stacking interactions with the aromatic pTyr residue ring.[59,62]

The opposite wall of the LMW-PTP active site cavity is lined with the amino acid residues belonging to the variable loop (residues 40−73). This comprises the amino acid sequence from the β2 strand to part of the loop following the α2 helix forming a furrow on the enzyme surface that embraces the active site (Figure 1). This variable region includes the sequence 46−56, which forms a short loop between the β2 strand to the α2 helix and contains amino acid residues that are the main structural determinants for the specific binding of IF1 and IF2 to substrates or small-molecule modulators.[9,62,79] In particular, the residues Tyr49, Glu50, and Asn53 in isoform IF1 and Trp49, Asn50, and Arg53 in isoform IF2 are oriented in strategic poses that are critical for isoenzyme binding to specific ligands.[59,62,63] The residue in position 49 can assume different orientations in the two isoenzymes. In IF1, the side chain of Tyr49 is positioned above the active site cleft, and in the cocrystallized complex with the buffer 2-(*N*-morpholino)-ethanesulfonic acid (MES), the morpholino ring of the ligand is sandwiched between Tyr49 and the tyrosine residues in positions 131 and 132. In the cocrystallized complex of the bovine LMW-PTP (an enzyme that shares a high degree of homology with human IF2)[80] with the *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid (HEPES), the side chain of the corresponding residue Trp49 was directed toward the outer side of the active site without interacting with the ligand.[59] The side chain of Trp49 displays conformational variability, and it is likely that it can adopt different selected conformations depending on ligands.[79] The residue Arg53 of IF2 also presents high flexibility, which may be important for interaction with IF2 selective ligands.[62] In addition, the amino acid residues in positions 49, 50, and 53 determine significant differences in the surface charges near the entrance of the active site crevice of the two isoenzymes. In fact, in isoform IF1, the acidic Glu50 and the neutral Asn53 residues give a predominantly negative surface charge, whereas the neutral Asn50 and the basic Arg53 of IF2 form a positively charged surface on one wall of the active site crevice.[59,62] Therefore, the few residues of the variable loop located near the entrance of the active site crevice are sufficient to determine the specificity of isoenzymes IF1 and IF2 toward different ligands, as also demonstrated for other mammalian LMW-PTP isoforms.[43,59,62,63,81,82] This is of particular interest from a medicinal chemistry perspective, as the differences in the amino acid residues and in the surface charge distribution for this region can be exploited in the structure-based design of molecules that are specifically targeted to modulate the activity of human LMW-PTP. This should achieve selective effects on the two active isoforms.

The aromatic residues Tyr131, Tyr132, and Tyr49 or Trp49 form a hydrophobic edge around the catalytic pocket, which in turn contains other hydrophobic residues, such as Leu13 and Ile16. This may explain the generally higher affinity and catalytic efficiency of IF1 and IF2 toward substrates containing hydrophobic residues adjacent to pTyr as well as the greater effectiveness of inhibitors containing hydrophobic moieties.[58,62,81]

MODULATION OF LMW-PTP ACTIVITY

The activity of LMW-PTP isoenzymes can be modulated by several molecules, such as purine derivatives (Figure 3). In

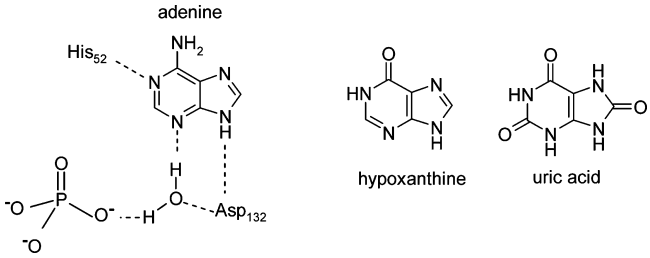

Figure 3. Purine modulators of LMW-PTP activity and schematic representation of the interactions between adenine and the LMW-PTP from *Saccharomyces cerevisiae* (Ltp1).

particular, isoform IF1 is inhibited by adenine and activated by hypoxanthine. In contrast, adenine activates IF2, whereas hypoxanthine does not affect this isoform.[83] Adenine produces an even more powerful activation of LMW-PTP from *Saccharomyces cerevisiae* (Ltp1), which was subsequently used as a simple model to rationalize this effect.[83] The cocrystallized complex of Ltp1 with adenine showed that the ligand is located near the top of the catalytic pocket, with the aromatic system stacked between the residues Trp134 and Tyr51 and with N1 and N9 atoms respectively bound to His52 and Asp132 by

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hydrogen bonds. In this position, the adenine N3 atom can bind a nucleophilic water molecule, which thus can assume a suitable orientation to hydrolyze the phosphoenzyme thioester bond in the rate-limiting step of the enzymatic catalysis, thereby promoting Ltp1 activity (Figure 3). The Ltp1 residues Tyr51 and His52 belong to the variable loop and correspond to Tyr49 and Glu50 in IF1 and Trp49 and Asn50 in IF2. This may determine distinct binding modes of the purine ligand to the two human isoenzymes, resulting in differential effects.[83] Docking simulations to human LMW-PTP indicated that, in addition to the active site, the adenine could also initially bind to a nearby shallow cavity situated in front of Tyr131 and Tyr132, named the Y131 dish.[84] The explanation for the effect of hypoxanthine activating exclusively the IF1 isoenzyme was that the only difference between the structures of these purines is the presence of an amine group at the C6 position of adenine which is replaced by a carbonyl group in hypoxanthine. This may significantly influence the interactions of the protonated N1 atom of hypoxanthine with the amino acid residues present in the various LMW-PTPs (Glu50 in IF1, Asn50 in IF2, His52 in Ltp1).[83] The findings relating to the modulation of LMW-PTP activity by adenine were exploited as a starting point to develop small molecule inhibitors of the enzyme (see below).[84]

Modulating effects are also exerted by purine derivatives toward electrophoretically fast and slow isoenzymes of different origins. cGMP induced a strong increase in the activity of the slow isoform AcP2 from rat liver, whereas the fast isoenzyme AcP1 was only moderately activated, suggesting that variations in the cellular cGMP concentration could result in the modulation of the LMW-PTP activity in vivo.[85] In addition, uric acid was shown to inhibit bovine LMW-PTP at concentrations in the low millimolar range.[86]

Investigating the regulatory mechanisms of LMW-PTP activity can provide useful further knowledge for our understanding of LMW-PTP functions as well as for the rational design of active compounds directed to modulate the activity of the enzyme.

Specific amino acid residues are critically involved in the regulation of LMW-PTP activity. The thiol groups of the neighboring Cys12 and Cys17 are oxidized by both H_2O_2 and NO to form a disulfide bond. The consequent enzyme inactivation is reversible, and the recovery of the LMW-PTP functionality was observed in the presence of reducing agents, such as dithiothreitol or reduced glutathione, or after removal of oxidative conditions.[49,87−90] In vivo, endogenous H_2O_2 is generated in numerous conditions of oxidative stress, such as during growth factor signal transduction, and can lead to transient LMW-PTP inactivation which is reversed by glutathione-dependent systems. A similar control over the activity of several other PTPs in oxidative conditions is well-known. Therefore, the cellular redox balance can strongly influence PTP activity. This is considered to be a significant factor in the regulation of protein tyrosine phosphorylation.[91−93] In LMW-PTP and a few other PTPs, such as PTEN and Cdc25, the formation of the disulfide bridge is a peculiar event that protects the initially formed cysteine sulfenic acid (Cys-SOH) from further irreversible oxidation to sulfinic and sulfonic acids. However, in other PTPs, such as PTP1B, the irreversible oxidation of Cys-SOH is prevented by the formation of a five-membered sulfenamide ring derived from the reaction of the sulfenic group with an adjacent backbone NH group.[92,93]

Two other crucial amino acid residues involved in key regulatory mechanisms are Tyr131 and Tyr132.[94−96] In vivo, the independent phosphorylation of these tyrosine residues can elicit different effects on the enzyme activity. The phosphorylation of Tyr131, the main phosphorylation site, is essential for several functions of LMW-PTP particularly to control cell growth and adhesion, whereas intriguingly, the phosphorylation in position 132 could result in negative regulation of the enzyme. Consequently, the alternative phosphorylation of Tyr131 and Tyr132 can provide a fine-tuned regulation of LMW-PTP activity in different physiological conditions.[95] This tyrosine residue pair also contributes to the self-association of LMW-PTP molecules to form inactive oligomers that are in equilibrium with the active monomers.[79,97,98] This enzyme oligomerization is an alternative and independent event with respect to tyrosine phosphorylation, and it has been suggested that it represents a self-regulatory way to control the phosphorylation at positions 131 and 132.[97,98] In fact, crystallographic and NMR studies have demonstrated that in the dimeric structure the unphosphorylated residues Tyr131 and Tyr132 of one enzyme molecule occupy the catalytic pocket of the other molecule, forming hydrogen bonds with residues flanking the active site in front of them.[79,97,98] Substrate binding can compete with the enzyme dimerization restoring the functional enzyme, leading LMW-PTP oligomers to be defined as a reserve of the enzyme or a proenzyme form.[98]

Further control of LMW-PTP activity is derived from the cellular localization of the enzyme. Similar to other PTPs, LMW-PTP can interact with caveolin-1, a structural protein of the plasma membrane invaginations called caveolae which is involved in the regulation of many signaling molecules, such as G-proteins, kinases, and growth factors. Interestingly, this binding leads to a differential modulation of isoenzymes IF1 and IF2 because it produces a marked inhibition of IF1 while it hardly affects the IF2 activity.[99,100] It has been suggested that this different behavior could be derived from the interaction of caveolin-1 with distinct amino acid residues of the variable loop of each isoform.[100]

BIOLOGICAL FUNCTIONS OF LMW-PTP AND IMPLICATIONS IN HUMAN PATHOLOGIES

The understanding of the biological functions of human LMW-PTP has been significantly extended in the past 2 decades. In the past few years, the results of these biological and biochemical studies have contributed greatly to stimulating the search for active compounds able to modulate LMW-PTP activity, by highlighting not only the main physiological roles of the enzyme but also how its malfunctioning is involved in the development of pathogenetic processes.

LMW-PTP is critically involved in cell growth, differentiation, adhesion, and metabolism by regulating specific signaling pathways induced by several growth factors and receptor tyrosine kinases. A major event resulting from the binding of growth factors to their transmembrane receptors is receptor autophosphorylation on specific tyrosine residues, which in turn determines the recruitment and phosphorylation of proteins responsible for downstream signal transduction. These protein tyrosine phosphorylation reactions are reversed by the regulatory intervention of PTPs. In particular, LMW-PTP plays complex crucial roles by negatively regulating the cellular responses elicited by platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), focal adhesion kinase (FAK), and insulin,

whereas in other cases it acts as a positive modulator, such as in the ephrin A2 (EphA2) and ephrin B1 (EphB1) signaling pathways.[47,49,66,67,69,101−106]

The role of LMW-PTP in the regulation of PDGF signaling has been widely investigated. Initial studies revealed that LMW-PTP overexpression can inhibit the growth of both normal and oncogene-transformed fibroblasts, this effect being more marked in normal cells than in trasformants.[107,108] The increased LMW-PTP levels were found to be linked to conditions of cell growth arrest, such as cell confluence and differentiation, like other enzymes that play a role in the control of mitogenic signals.[66] In particular, the growth inhibition mediated by LMW-PTP was shown to be correlated to a reduced cellular response to PDGF.[102] The activated PDGF receptor (PDGF-R) is a specific substrate of LMW-PTP, and the enzyme causes the receptor inactivation by dephosphorylating PDGF-R pTyr residues, such as the essential pTyr857.[64,109,110] However, the role of LMW-PTP in the transduction of PDGF mitogenic signaling does not consist only of the regulation of PDGF-R phosphorylation, but the enzyme also plays multiple roles in downstream events subsequent to PDGF-R activation (Figure 4).

LMW-PTP specifically influences the Src kinase and some signal transducers and activators of transcription (STATs) pathways that are activated by phosphorylated PDGF-R. The negative regulation of the Src pathway results in decreased mitogenic response and increased cell motility and chemotaxis and, in turn, could affect STAT signaling by causing a down-regulation of STAT1 and STAT3 pathways.[65,111] Recently, LMW-PTP has also been shown to dephosphorylate STAT5, which is involved in cell differentiation during hematopoietic development (Figure 4).[112] In addition, in vascular smooth muscle cells, LMW-PTP negatively controls PDGF signaling by acting on different substrates but not by dephosphorylating PDGF-R as occurs in fibroblasts, suggesting that LMW-PTP could play different roles depending on cell types.[113]

In normal cells, LMW-PTP exists constitutively in both cytosolic and cytoskeleton-associated forms. Only the cytoplasmic LMW-PTP, in its unphosphorylated form, acts on the activated PDGF-R, whereas the cytoskeleton-associated enzyme is phosphorylated by Src kinase upon PDGF stimulation, resulting in a significant increase in LMW-PTP activity and in the consequent dephosphorylation of cytoskeleton-associated proteins.[114]

The cytoskeleton-associated LMW-PTP fraction is critically involved in the integrin-mediated cell adhesion to the underlying extracellular matrix (ECM), mainly through the dephosphorylation of two distinct proteins, p190Rho-GAP and FAK (Figure 4). Through the inactivation of the GTPase-activating protein p190Rho-GAP, LMW-PTP potentiates the action of the GTP-coupled protein Rho, thus increasing cell adhesion and migration. The protein Rho induces integrin clustering, which is essential for the formation of focal adhesions during the process of cell adhesion to ECM, and also controls actin assembly and cell motility.[104,115] However, LMW-PTP can increase cell motility and lead to a reduction in

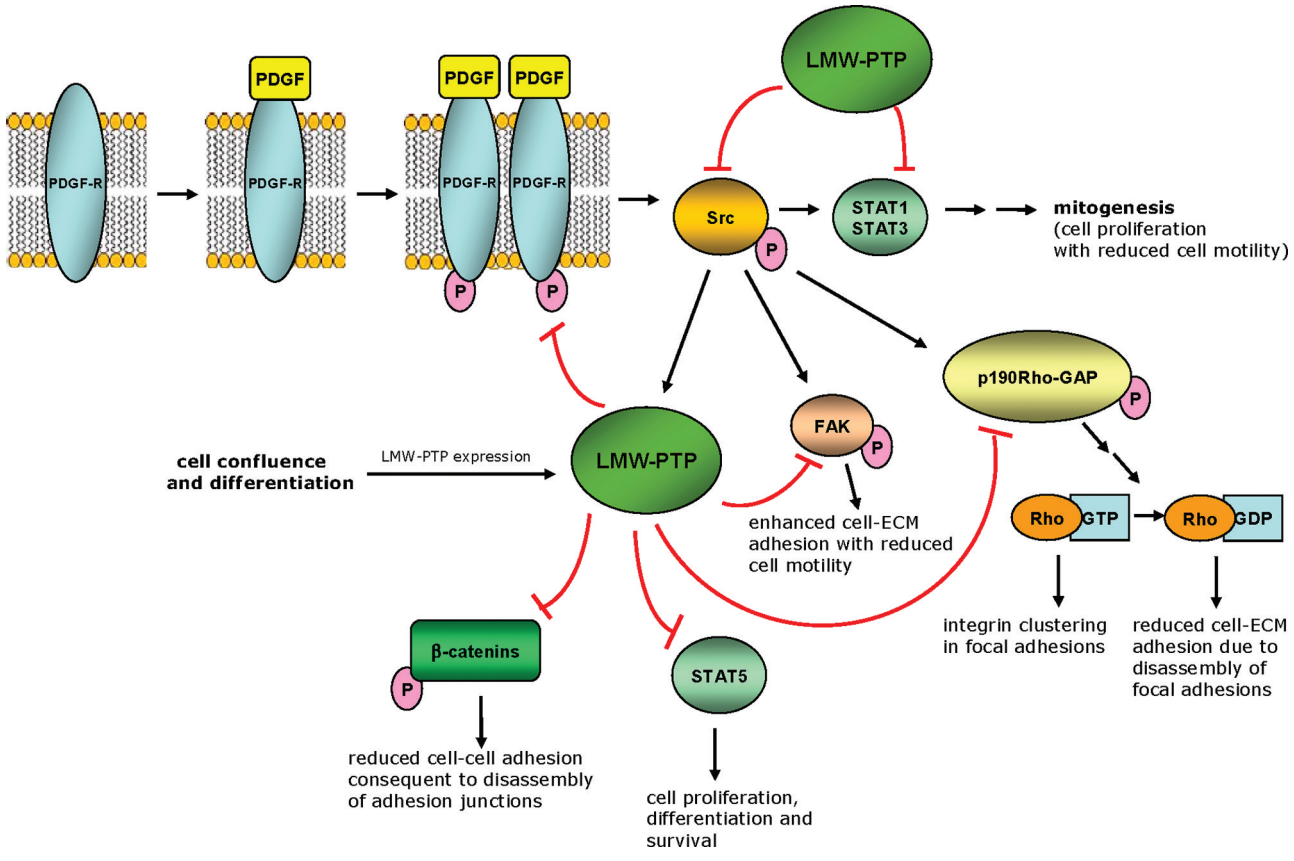

Figure 4. Roles of LMW-PTP in the regulation of cell growth and adhesion in normal cells. LMW-PTP functions as a negative regulator of cell growth and increases integrin-mediated and cadherin-mediated cell adhesion by acting on distinct substrates involved in different cellular signaling pathways. In transformed cells, LMW-PTP overexpression induces overexpression of EphA2 kinase, which is the predominant substrate of this phosphatase in neoplastic cells (for details, see text).

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the number of focal adhesions by inactivating FAK, a key component of the integrin-mediated signaling which modulates the turnover of focal adhesions.[90,105]

The key regulatory role played by LMW-PTP in controlling cell-matrix adhesion and migration can critically influence the modulation of T lymphocyte functions. The FAK dephosphorylation catalyzed by LMW-PTP exerts a negative control over T cell activation and adhesion.[116] On this basis, it has been suggested that LMW-PTP may contribute to the pharmacological effect of teriflunomide, the active metabolite of the immunosuppressant drug leflunomide.[116] On the other hand, LMW-PTP can improve T cell functioning through the dephosphorylation of a regulatory site of the tyrosine kinase ZAP-70. This suggests that LMW-PTP can modulate T lymphocyte functions through distinct mechanisms in different conditions.[116,117]

LMW-PTP controls not only cell-ECM contacts but also cell−cell adhesion, another dynamic crucial event that influences the course of several processes, including embryonic development and tumor invasion. Catenins are a group of proteins that contribute to the formation of adhesion junctions between cells by functioning as linkers between the cytoplasmatic domains of the transmembrane protein cadherins and the actin cytoskeleton.[118] LMW-PTP can enhance the stability of cell−cell contacts through the dephosphorylation of β-catenin complexes (Figure 4), and it has been suggested that this action might fight tumor progression and invasiveness.[119]

On the basis of these findings overall, up until a few years ago, LMW-PTP was thought to function mainly as a negative regulator of cell proliferation that might potentially counteract tumor growth. However, this PTP can also function as a positive growth modulator, as observed in v-Ha-Ras-transformed cells, and can also act as a positive regulator of specific PTK signals.[47,48,103,106] Indeed, the idea initially accepted, according to which PTPs generally function as tumor suppressors by opposing the oncogenic potential of PTKs, proved to be partial, since in the past decade it has been discovered that PTPs play more complex roles as either tumor-suppressors or oncogenic enzymes. In fact, it was ascertained that several PTPs, including LMW-PTP, can act as oncogenic proteins, even by potentiating the transforming effects of some PTKs.[15,19,37,38,70]

The role of LMW-PTP as a positive regulator of tumor onset and progression has been demonstrated in both animal models and in different types of human cancer.[37,38,70] The LMW-PTP overexpression found in oncogene-transformed or tumor-derived cells is a sufficient condition to induce neoplastic transformation in nontransformed cells.[70] Cultured LMW-PTP overexpressing epithelial cells displayed the ability to colonize soft agar and acquire a transformed phenotype.[70] The overexpression of the enzyme was found to positively influence cell motility and invasiveness, thus favoring the tumor colonization of connective tissues and accelerating tumor onset and progression.[37,120]

The oncogenic activity of LMW-PTP is closely related to changes in the expression and function of its substrate EphA2 observed in tumor cells both in vitro and in vivo.[37,70] The EphA2 receptor tyrosine kinase is overexpressed and functionally altered in many human carcinomas and melanomas, especially in aggressive and metastatic types of cancers.[70] EphA2 tyrosine phosphorylation is a crucial feature that strongly influences the oncogenic potential of this kinase. The tyrosine phosphorylated EphA2 receptor, found mainly in normal cells, activates downstream events that lead to inhibition of cell growth and migration.[70,121] In contrast, unphosphorylated EphA2 exerts strong oncogenic actions. In tumor cells, the overexpressed LMW-PTP, which acts as a critical regulator of EphA2 tyrosine phosphorylation, induces EphA2 overexpression and, in addition, determines functional alterations of the kinase by dephosphorylating it.[70] Therefore, LMW-PTP triggers cellular neoplastic transformation and promotes cancer progression through high levels of the unphosphorylated EphA2 tyrosine kinase, which functions as an essential downstream component of this oncogenic signaling pathway.

It was suggested that the recruitment of LMW-PTP by EphA2 could reduce the action of the phosphatase on p190Rho-GAP, thus leading to weaker cell-matrix adhesion and to more invasive malignant cells.[116] Moreover, neither the PDGF-R nor β-catenin were found to be preferential LMW-PTP substrates in developing sarcomas. In fact, EphA2 is the main LMW-PTP substrate in tumors, confirming that the oncogenic potential of this phosphatase is linked to EphA2 dephosphorylation.[37]

LMW-PTP expression levels were found to be significantly increased not only in cell cultures and animal models but also in several human tumor tissues, particularly in breast and colon cancers as well as in neuroblastoma, whereas the enzyme is not overexpressed in lung cancer.[38] Moreover, a close link was observed between high LMW-PTP expression and unfavorable outcome, in terms of a markedly reduced probability of survival, confirming that LMW-PTP overexpression is generally typical of aggressive cancers.[38]

In view of possible therapeutic intervention targeted at LMW-PTP, it is also particularly interesting to consider the expression of the different LMW-PTP isoforms in tumor tissues. The most abundant isoform found is IF1, followed by IF2 and SV3, in both normal and tumor cells.[38] Evaluation of the correlation between the genetic polymorphism of the human LMW-PTP encoding gene *ACP1* and susceptibility to cancer development highlighted that patients affected by various types of cancer presented genotypes characterized by higher IF1 levels compared with healthy subjects, strongly suggesting that this isoform could be associated with increased cancer invasiveness and metastasis development.[122]

All this evidence supports the validity of LMW-PTP as a target for novel anticancer agents. Although the search for drugs targeted at LMW-PTP has only recently begun, efforts have been made to target the enzyme in the search for new methods of treatment of hyperproliferative cell disorders.[123] In addition, the potent antineoplastic effect of aplidin (dehydrodidemnin B, Figure 5), a depsipeptide isolated from the

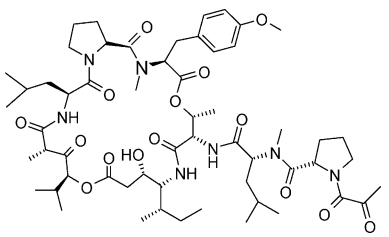

Figure 5. Structure of aplidin.

Mediterranean tunicate *Alidium albicans*, has been recognized to be mediated, at least in part, by LMW-PTP inactivation. Aplidin does not exert a direct inhibitory effect on the enzyme;

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rather, LMW-PTP inactivation appears to be caused by the cellular oxidative stress induced following aplidin treatment.[124]

A different LMW-PTP substrate of therapeutic interest is the insulin receptor (IR), a tyrosine kinase that is formed by two extracellular α subunits and two transmembrane β subunits. Insulin binding to the α subunit activates the receptor by inducing β subunit autophosphorylation on several tyrosine residues in the cytoplasmic domain. Subsequently, the phosphorylation of specific IR substrate (IRS) 1−4 proteins occurs. Tyrosine phosphorylated IRS activate other downstream molecules, such as the phosphatidylinositol 3-kinase (PI3K), which in turn promotes the phosphorylation of further signaling components. In particular, the PI3K-dependent activation of downstream proteins, such as Akt (protein kinase B), causes the translocation of glucose transporter 4 (GLUT4) to the plasma membrane, thus enhancing glucose uptake in tissues with insulin-dependent glucose transport, such as skeletal muscle, liver, and fat tissue. This signaling cascade also mediates other insulin actions, such as the increased synthesis of glycogen, fatty acids, and proteins as well as mitogenesis, the latter induced through the activation of the mitogen-activated protein kinase (MAPK) pathway.[125]

Several PTPs (PTP1B, LMW-PTP, PTEN, SHP2, PTPα, LAR-PTP) control insulin signal transduction through the dephosphorylation of pTyr residues on IR or other components of the insulin signaling cascade.[11,12,27,126] In particular, LMW-PTP acts as a key negative regulator of both insulin-mediated mitotic and metabolic signaling pathways by dephosphorylating specific pTyr residues on the IR β subunits.[69] Although the mitogenic responses mediated by insulin and PDGF are similar in part, LMW-PTP affects them differently, exerting a stronger control on the PDGF-induced mitogenesis. This is probably due to the fact that during insulin signaling, LMW-PTP is not able to control cytoskeleton-associated proteins and is therefore not involved in the insulin-mediated cell adhesion and chemotaxis.[127]

The crucial role played by LMW-PTP in insulin signal transduction has been established in both diet-induced obese mice and genetically obese ob/ob mice, in which the condition of insulin resistance typical of type 2 diabetes mellitus (DM2) is reproduced.[39] In this animal model, LMW-PTP gene silencing by means of specific antisense oligonucleotides promoted insulin signaling and improved the cellular response to the hormone, resulting in reduced plasma insulin and glucose levels.[39] In agreement with the previous in vitro investigations by Chiarugi et al.,[69] the in vivo study by Pandey and co-workers confirmed that LMW-PTP negatively regulates insulin signaling mainly through pTyr dephosphorylation on IR, thus reducing the activity of key downstream components of the insulin cascade, such as PI3K and Akt.[39] A possible role of LMW-PTP in the regulation of lipid metabolism has also been suggested but needs to be clarified further. However, in contrast with that of PTP1B, suppression of LMW-PTP was not seen to influence animal body weight. In this pivotal study, the improvement in insulin sensitivity consequent to LMW-PTP silencing was observed mainly in liver and adipose tissue, thus indicating that this phosphatase possesses distinct tissue specificity compared to PTP1B, which in turn controls insulin signaling in liver and skeletal muscle but it does not appear to be appreciably involved in fat tissue.[35,36,128] Moreover, LMW-PTP knockdown did not induce any compensatory increase in the other PTPs involved in the control of insulin response (e.g., PTP1B, SHP2, PTEN), thus indicating that these enzymes exert independent functions in controlling the action of the hormone.[39]

Epidemiological studies provided further evidence that LMW-PTP activity may function as a determining factor in the development of diabetes, contributing to validation of this phosphatase as a potential drug target for the therapeutic treatment of this pathology. Higher glycemic levels were observed in males with high-activity LMW-PTP genotype than in subjects with low-activity genotype, this difference being more marked in diabetic and aged patients than in healthy subjects. However, statistically significant differences were not observed in females. Moreover, in DM2, the isoenzyme IF1 appeared to be the main isoform responsible for the increase in glucose levels, whereas no positive correlation with IF2 was found.[129] In females with type 1 diabetes, high LMW-PTP activity can induce earlier onset of the disease.[130] In diabetic pregnant women glycemic levels also appear to be positively correlated with LMW-PTP activity.[131] In addition, in obese diabetic patients, the enzyme was found to be associated with high levels of plasma triglycerides, suggesting that increased LMW-PTP activity might be a predisposition to metabolic syndrome.[132] In clinical studies, LMW-PTP was also found to be related to the development of diabetic retinopathy and, once again, genotypes with higher concentrations of IF1 isoform showed the highest incidence of this common and severe chronic complication of diabetes.[13]

Relationships have been discovered between the genetic polymorphism of LMW-PTP and several other human pathologies. A positive correlation between myocardial wall thickness and LMW-PTP activity has been established in hypertrophic cardiomyopathy.[13] Genotypes with low activity of this phosphatase exhibited protective effects against some serious cardiovascular risk factors induced by oxidative stress, hypertension, and dyslipidemia.[133] High levels of isoform IF1 also appeared to be a predisposition to inflammatory bowel diseases, whereas high levels of isoform IF2 were correlated to the onset of rheumatoid arthritis and reduced resistance to malaria plasmodium infection. On the other hand, low total LMW-PTP activity appeared to be a predisposing condition for allergies, asthma, Alzheimer's disease, nondiabetic obesity, and repeated spontaneous abortion.[13,116,134,135]

In addition, other biological functions of LMW-PTP have been under study. It was suggested that LMW-PTP may function as a negative regulator of platelet activation through immunoreceptors,[136] and it may be an important signaling molecule in membrane skeleton remodeling[137] and in osteoblast differentiation.[138] The presence of LMW-PTP in nerve endings suggests that the enzyme may also play a role in synaptic functions.[139] These findings stimulate further investigation.

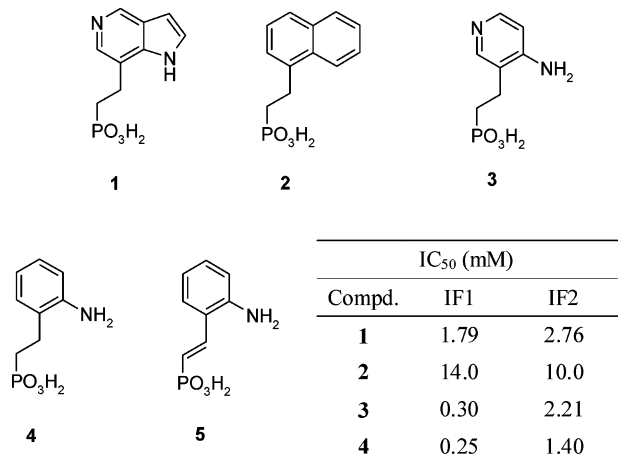
IN SEARCH OF HUMAN LMW-PTP INHIBITORS AS NOVEL DRUG CANDIDATES

Although a wide variety of natural and synthetic compounds are known to be PTP inhibitors, a relatively limited number of human LMW-PTP inhibitors have been identified to date, as the recognition of LMW-PTP as a possible drug target is recent.

Until a few years ago, the only known LMW-PTP inhibitors were the early transition metal oxoanions, including vanadate, molybdate, and tungstate.[140] The vanadate ion acts as a trigonal bipyramidal transition state analogue and inhibits the enzyme by covalently binding the nucleophile Cys12. In contrast,

molybdate does not form covalent bonds with the enzyme but acts as a weaker inhibitor by mimicking the tetrahedral phosphate geometry.[141] In spite of their appreciable inhibitory effects at micromolar concentrations and their ability to function as insulin-mimetic and antitumor agents, vanadate-containing compounds are only used as reference inhibitors in studies relating to PTP functions because their nonselective inhibitory effect toward all the members of the PTP family, as well as toward other phosphomonoesterases and phosphodiesterases, noticeably limits their usefulness for therapeutic purposes.[24,62,141] Ionic surfactants can also inactivate LMW-PTP, probably by establishing electrostatic and hydrophobic interactions with the active site or neighboring regions. Anionic surfactants, such as *n*-alkyl sulfates, produce a more marked inhibitory effect than *n*-alkyl trimethylammonium salts, independently of pH values.[142]

An initial study for the structure-based design of LMW-PTP inhibitors was based on the above-described crystal structure of the yeast enzyme Ltp1 cocrystallized with adenine.[83] This led to the identification of the first rationally designed inhibitors of human LMW-PTP, even though they were endowed with weak millimolar affinity for the enzyme.[84] A phosphonated 5-azaindole (compound **1**, Figure 6) was designed to mimic

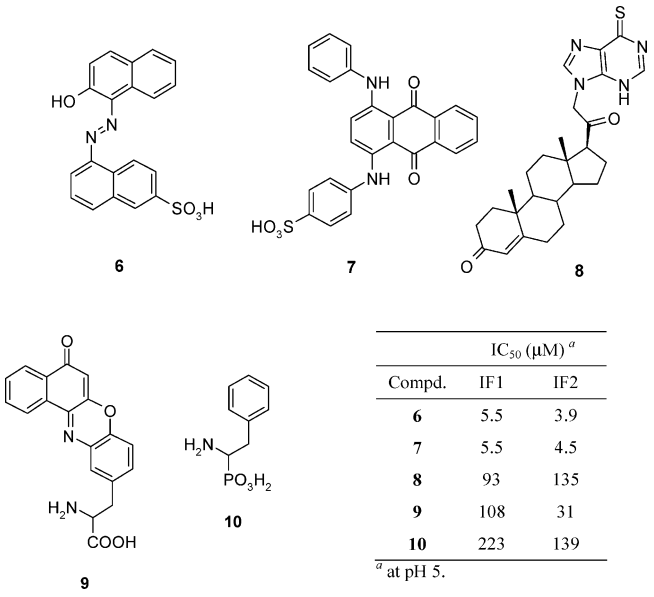


Compd.	IC$_{50}$ (mM) IF1	IF2
1	1.79	2.76
2	14.0	10.0
3	0.30	2.21
4	0.25	1.40
5	5.30	13.0

Figure 6. Phosphonic acid containing inhibitors of human LMW-PTP.

the interactions of adenine with LMW-PTP. The two heterocyclic nitrogen atoms of **1** correspond to the adenine N1 and N9 that are directly involved in hydrogen bonding to the residues His52 and Asp132 of the yeast enzyme, respectively (Figure 3), whereas the phosphonate group was used as a nonhydrolizable phosphate mimic. Molecular dynamics indicated that the molecule could interact with the target enzyme effectively. The phosphonate group can bind residues of the P-loop at the bottom of the catalytic pocket, whereas the azaindole moiety may assume different orientations, thus interacting either with aromatic residues situated at the entrance of the active site or with the catalytic Asp129.[84] Phosphonic acids **2**–**5** were then synthesized as analogues of the lead compound **1** by retaining the phosphonate group as well as the ethyl or ethylene linker and simplifying the aromatic moiety, which varied from a naphthyl system to an amino-pyridyl or phenyl ring (Figure 6). The affinity of compounds **1**–**5** for the two human isoforms IF1 and IF2 were generally in the millimolar range, reaching K_i values of 300 and 250 μM for compounds **3** and **4**, respectively. The latter compounds were the most active LMW-PTP inhibitors of these phosphonic acids, with moderate selectivity toward IF1 over IF2.[84,143] The results of this study highlighted simple structural elements that could be important for the interaction with the enzyme and, therefore, for the subsequent design of more effective LMW-PTP inhibitors. In fact, two molecular portions of inhibitors appeared to be required to effectively bind the enzyme: the first an ionized group that was able to anchor the molecule to the positively charged catalytic pocket and the other a planar and preferably aromatic moiety bearing suitable functional groups that are able to form hydrogen bonds with the enzyme.

In pursuing the search for human LMW-PTP inhibitors, the same research group recently reported a virtual-screening-based study that led to the identification of structurally diverse inhibitors.[144] Among the molecules that were initially selected to be submitted to an in vitro enzyme inhibition assay, compounds **6**–**9** (Figure 7) were found to have IC$_{50}$ values



Compd.	IC$_{50}$ (μM) [a] IF1	IF2
6	5.5	3.9
7	5.5	4.5
8	93	135
9	108	31
10	223	139

[a] at pH 5.

Figure 7. Some inhibitors of human LMW-PTP isoforms IF1 and IF2.

lower than or around 100 μM, at pH 5, against both isoforms IF1 and IF2. A further six compounds (**11**–**16**, Figure 8), although weak inhibitors at pH 5, produced at least 50% inhibition of isoform IF1 at 100 μM when tested at pH 7.[144] All assayed compounds (Figures 7 and 8) contain structural portions that can be likened to those previously suggested to be critical for LMW-PTP inhibition. In fact, they possess an acidic functionality, mostly a sulfonic or a carboxylic group that can form multiple interactions with amino acid residues of the P-loop, and one or more cyclic systems containing heteroatoms or groups able to establish hydrogen bonds with the target enzyme. Extended cyclic systems, especially aromatic ones, generally appeared to enhance the affinity for the enzyme, probably through further hydrophobic interactions, in agreement with the predominantly hydrophobic nature of the amino acid residues that surround the LMW-PTP active site. Unfortunately, of these compounds only the sulfonic acid derivative **6** did not prove to cause the formation of protein aggregates and, therefore, was selected as a new lead candidate for the design of human LMW-PTP inhibitors.[144] In contrast with the numerous phosphonic acid derivatives that have been

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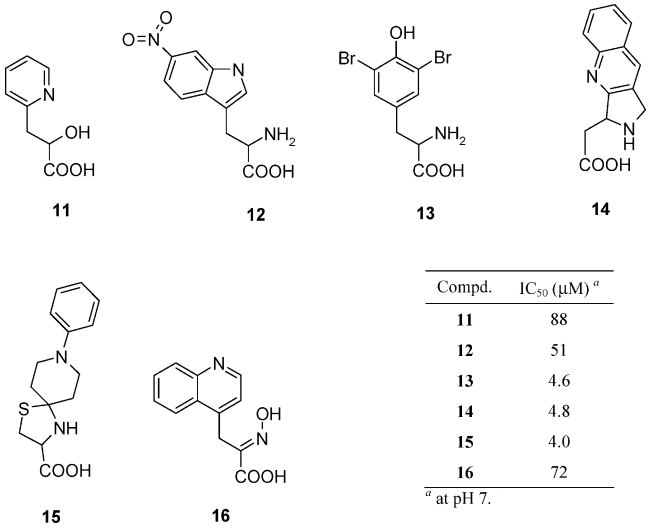


Compd.	IC_{50} (μM) [a]
11	88
12	51
13	4.6
14	4.8
15	4.0
16	72

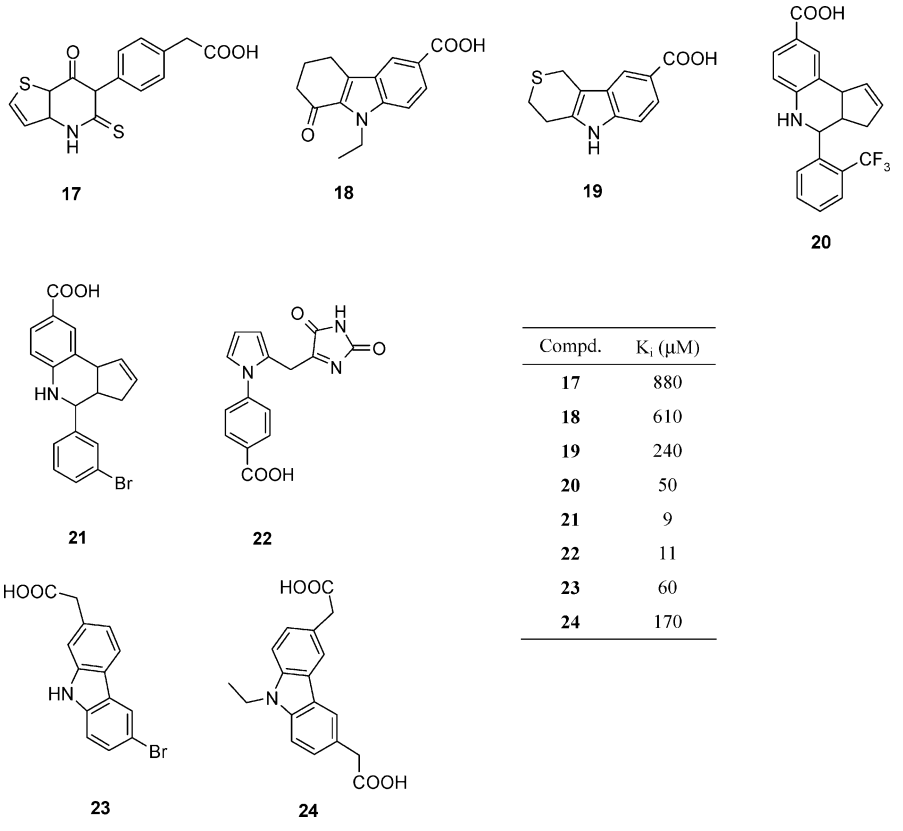
[a] at pH 7.

Figure 8. Inhibitors of human LMW-PTP isoform IF1.

reported so far as potent in vitro inhibitors of other PTPs, especially PTP1B,[24−26] the only phosphonic acid present in the set of selected molecules was compound **10**, which, moreover, was the weakest LMW-PTP inhibitor out of this molecule series, with IC_{50} values of 223 and 139 μM against IF1 and IF2, respectively, at pH 5.[144]

In fact, in the context of the design of PTP inhibitors, the insertion of the double-negatively charged phosphonate group as a phosphate mimic onto various structural frameworks has frequently been exploited. However, this strategy has often produced molecules endowed with high in vitro inhibitory potency but low bioavailability because of their highly ionized nature and consequently poor ability to cross biomembranes, although some phosphonate derivatives endowed with cellular or in vivo activity have been reported.[24,145,146] Therefore, the current search for PTP inhibitors is mainly aimed at identifying nonphosphorous small-molecule inhibitors designed by inserting less charged pTyr-mimetic groups onto different optimal templates in order to obtain inhibitors with better druglike properties than phosphonate-containing molecules.[24,26,30,147] In view of this, the bioisosteric replacement of the pTyr phosphate group with appropriate monoanionic groups, such as the carboxylic one or the isothiazolidinone system, is considered an effective design approach and has already led to PTP inhibitors with appreciable cellular or in vivo activity.[24,26,147−149] It is plausible that this strategy can also be adopted for the rational design of LMW-PTP inhibitors.

Besides acids **9** and **11**−**16** (Figures 7 and 8), other carboxylic acid containing inhibitors of LMW-PTP were selected by means of a subsequent virtual screening of available libraries.[150] In this case, the selected compounds were assayed against the bovine LMW-PTP, which shares a 94% sequence identity and a high degree of structural similarity with the isoform IF2 of the human LMW-PTP, thus adequately predicting the inhibition of this human isoform.[80] The screening of a first subset of compounds led to the selection of four different carboxylic acid derivatives (**17**−**20**, Figure 9) with K_i ranging between 50 and 900 μM, and afterward, additional structurally similar molecules were assayed. This second step resulted in the identification of compounds **21** and **22** (Figure 9) with K_i values of 9 and 11 μM, respectively, and two weaker inhibitors **23** and **24** (Figure 9) with K_i values of 60 and 170 μM, respectively. All these different carboxylic acid derivatives act as competitive LMW-PTP inhibitors.[150] The



Compd.	K_i (μM)
17	880
18	610
19	240
20	50
21	9
22	11
23	60
24	170

Figure 9. LMW-PTP inhibitors assayed against the bovine enzyme.

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replacement of the 2-trifluoromethylphenyl moiety of analogue **20** with the 3-bromophenyl one (compound **21**) on the same cyclopenta[*c*]quinoline-8-carboxylic acid template yielded a 5-fold gain in inhibitory potency. A different tricyclic scaffold is present in the structures of compounds **18**, **19**, **23**, and **24**. The carbazole system with different substituents appeared to be associated with moderate inhibition levels (compounds **23** and **24**), whereas compounds **18** and **19**, in which a fused benzene ring was replaced by different ring systems, exhibited even weaker activity. Molecular docking simulations indicated, once again, that the inhibitors can bind the LMW-PTP active site by means of the ionized carboxylic phosphate mimic, whereas additional interactions with peripheral regions near the catalytic pocket may be responsible for even considerable differences in inhibitory effectiveness.[150]

Recently, in the search for new LMW-PTP and PTP1B inhibitors, novel carboxylic acid containing inhibitors of both these human enzymes have been discovered.[151−153] As mentioned above, PTP1B exerts a crucial negative control of insulin signaling shown to be distinct from that of LMW-PTP.[35,36,39] Furthermore, it acts as a negative regulator of leptin signaling, an antiobesity hormone that reduces appetite and increases the body's energy expenditure by dephosphorylating the Janus 2 kinase (JAK2) that is associated with the leptin receptor and thus preventing the activation of downstream molecules such as STAT3.[154] PTP1B deficiency or silencing enhances insulin sensitivity and resistance to diet-induced obesity without causing abnormalities in growth or other vital functions.[14,35,36] Hence, the inhibition of both PTP1B and LMW-PTP, which have partially concurring biological functions, may represent an attractive approach for the development of novel agents directed toward counteracting insulin resistance. The latter is a condition underlying common metabolic diseases, such as DM2 and metabolic syndrome, and further new therapeutic treatments are desirable to adequately control it. In addition, like LMW-PTP, PTP1B is considered to be a novel therapeutic target in cancer. Its implication in the development of specific tumor types is well documented, such as in the onset of Erb2-induced breast cancers and subsequent metastasis formation that are promoted by PTP1B.[15,16,155]

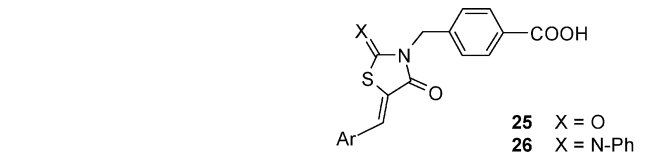
Although there is low sequence homology between the two enzymes, certain structural similarities constitute a basis for the design of possible dual inhibitors. In PTP1B, the catalytic domain is surrounded by the flexible WPD loop, containing the catalytic residue Asp181, and the YRD loop, containing Tyr46 which is critical for pTyr recognition, and this assumes a position similar to position 49 of the aromatic residue in the LMW-PTP isoforms with respect to the catalytic site.[34] A secondary noncatalytic aryl phosphate binding site adjacent to the PTP1B active site is also present, which is lined by poorly conserved residues, such as Arg24, Ala27, Phe52, Arg254, Met258, Gly259, while it is lacking in several other PTPs including LMW-PTP. This secondary pocket represents an important structural part of PTP1B, since it allows the rational design of potent selective bidentate inhibitors that can simultaneously fit both the active site and the nearby noncatalytic site pocket.[7] The smaller molecule of human LMW-PTP is essentially formed by the PTP catalytic domain, and its active site can be structurally superimposed onto that of PTP1B. Suitably functionalized compounds may be able to interact with both the active site and the adjacent loops of both PTPs and be able to function as effective dual inhibitors.

On this basis, a series of 4-[(5-arylidene-2,4-dioxothiazolidin-3-yl)methyl]benzoic acids **25** and their 2-phenylimino analogues **26** (Figure 10) were designed and in vitro tested against human LMW-PTP and PTP1B.[151−153] Compounds **25** and **26** act as reversible competitive inhibitors of both enzymes. Most of them displayed IC_{50} values in the low micromolar or submicromolar range and a generally appreciable selectivity toward PTP1B and the isoform IF1 of LMW-PTP, being up to 62-fold more efficacious against isoenzyme IF1 than IF2.[151−153] The preferential inhibition of IF1 over IF2 represents a promising feature from a pharmaceutical point of view because IF1 appears to be the human LMW-PTP isoform that is more implicated both in cancer development and in diabetes, as reported above.

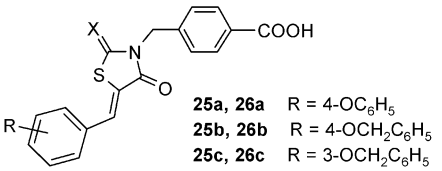
Out of the initially tested members of both series **25** and **26**, the derivatives 4-phenoxy- and 4-benzyloxybenzylidene substituted at position 5 of the thiazolidinone scaffold (**25a**,**b** and **26a**,**b**, Figure 10) were the most active compounds.[151,152] The 2-phenylimino derivative **26a** was about 3-fold less effective against IF1 than its 2,4-thiazolidinedione counterpart **25a**, whereas **25b** and **26b** were equipotent against this isoform. However, compounds **26c−e**, which are 3-benzyloxybenzylidene, 3-phenoxybenzylidene, and naphthalen-2-ylmethylene substituted, respectively, were from twice to about 7-fold more effective IF1 inhibitors than their 2,4-thiazolidinedione counterparts (Figure 10).[152]

In the structures of benzoic acid derivatives **25** and **26**, the molecular portions that appeared to be essential to inhibit both LMW-PTP and PTP1B are the following: (i) the *p*-methylbenzoic acid residue in position 3 of the pentatomic ring, which functions as an efficient nonphosphorus monoanionic pTyr-mimic targeting the catalytic site and (ii) the 5-arylidene moiety, which can enhance inhibitor affinity by establishing favorable interactions with amino acid residues of the regions adjacent to the active site. In fact, the latter moiety proved to markedly influence both inhibitor potency and selectivity.[151−153]

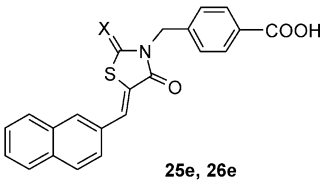
Molecular docking simulations into the LMW-PTP active site indicated that the most active phenoxy- and benzyloxybenzylidene substituted derivatives can act as bidentate inhibitors.[151−153] The *p*-methylbenzoic acid residue attached to N-3 is embedded within the positively charged active site and can establish a strong electrostatic interaction and a network of hydrogen bonds between its ionized carboxylic group and amino acid residues of the P-loop, including the catalytically critical cysteine and arginine residues (Figure 11). Hydrophobic interactions between the phenyl ring of the pTyr-mimic and surrounding lipophilic amino acid residues, such as Ile16 and Tyr131 of LMW-PTP or Phe182 and Tyr46 of PTP1B, were also predicted. The 5-arylidene moiety can favorably interact with Tyr49 or Trp49 of the variable loop of the LMW-PTP isoforms and, on the other hand, can efficaciously fit the noncatalytic aryl phosphate binding site of PTP1B.[151−153] In addition, in the case of **26**, in both isoenzymes IF1 and IF2 the amino acid residue in position 49 is surrounded by the thiazolidinone ring, the 5-arylidene moiety, and the phenylimino moiety, which enclose it almost like a claw (Figure 11). The benzene ring of the phenylimino moiety of **26** can contribute to the affinity toward the target PTPs by means of additional hydrophobic interactions with amino acid residues adjacent to the catalytic sites, such as Tyr/Trp49 of LMW-PTP or Phe182 of PTP1B. In agreement with these computational data, several 2-phenylimino-4-thiazolidinone



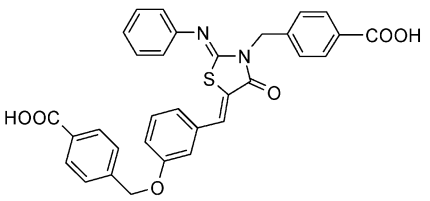
25 X = O
26 X = N-Ph



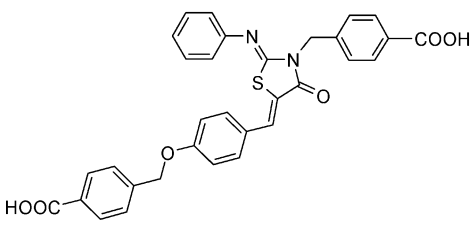
25a, 26a R = 4-OC_6H_5
25b, 26b R = 4-$OCH_2C_6H_5$
25c, 26c R = 3-$OCH_2C_6H_5$
25d, 26d R = 3-OC_6H_5



25e, 26e



26f



26g

Compd.	IC_{50} (μM) IF1	IF2	PTP1B
25a	0.9	13.0	2.8
25b	2.5	5.6	1.6
25c	7.1	18.4	1.1
25d	37.0	130.0	18.0
25e	12.0	92.0	6.5
26a	3.1	8.2	1.1
26b	2.7	21.5	1.1
26c	3.1	12.1	3.8
26d	5.4	26.1	1.9
26e	3.7	13.8	1.9
26f	0.5	27.6	0.22
26g	5.8	14.3	0.55

Figure 10. General formula and structures of selected benzoic acid derivatives active as dual inhibitors of human LMW-PTP and PTP1B.

derivatives **26** were shown to be more potent inhibitors than their 2,4-thiazolidinedione counterparts **25**.[152,153]

The structure-based optimization of benzoic acids **25** and **26** led to the identification of more potent benzyloxybenzylidene derivatives, in which groups able to establish further hydrogen bonds were inserted in the 5-arylidene moiety.[153] Out of these, the 2-phenylimino-4-thiazolidinone derivative **26f** proved to be an excellent IF1 inhibitor, with an IC_{50} of 0.5 μM and a significant 55-fold selectivity over IF2. It is also a potent PTP1B inhibitor, with IC_{50} = 0.22 μM. Its 4-benzyloxy isomer **26g** was shown to be less effective against both IF1 and PTP1B (IC_{50} of 5.8 and 0.55 μM, respectively).[153] Recently, further optimization efforts have led to new 2-arylimino-4-thiazolidinone derivatives active as inhibitors of human LMW-PTP isoform IF1 and PTP1B with appreciable cellular effectiveness.[156]

Structurally different LMW-PTP modulators have been discovered among flavonoids and their analogues. When tested against LMW-PTP from bovine kidney, a series of natural flavonoids proved to modulate enzyme activity differently. In particular, quercetin and morin (Figure 12) displayed opposite effects. In the presence of *p*-nitrophenyl phosphate (*p*-NPP) as substrate, quercetin was able to activate LMW-PTP (about 2.6-fold at 400 μM), whereas in contrast, morin inhibited the enzyme (35% enzyme inhibition at 400 μM). Morin reached an IC_{50} of 10 μM in the presence of phosphotyrosine as substrate. Other tested molecules (catechin, myricetin, phloretin, and rutin) produced lower inhibition levels (from 14% to 25% inhibition at 400 μM with *p*-NPP as substrate), whereas narigin, kaempferol, and taxifolin did not exert any significant effect.[157]

It is still unclear whether the activation mechanism of quercetin is similar to the one proposed by Wang et al. for the activation of the yeast LMW-PTP by adenine.[83] Miranda et al.[157] suggested that, like adenine, the hydroxy groups at positions 3′ and 4′ of quercetin might form hydrogen bonds with a water molecule and keep it in a suitable orientation for the hydrolysis of the phosphoenzyme intermediate, thus promoting the catalytic reaction. However, the presence of these hydroxy groups alone does not appear to be enough to determine the activating effect of quercetin, and other structural elements, such as the hydroxy group in position 3 or the double bond between C2 and C3, might play a role. It was proposed that the displacement of one hydroxy group from the position 3′ of quercetin to position 2′ of morin could prevent the interaction with the water molecule responsible for the hydrolysis of the phosphoenzyme intermediate. Thus, morin could inhibit LMW-PTP by establishing interactions between the hydroxy groups in positions 2′ and 4′ and residues of the catalytic site.[157] The double bond between C2 and C3 and the ketone group in

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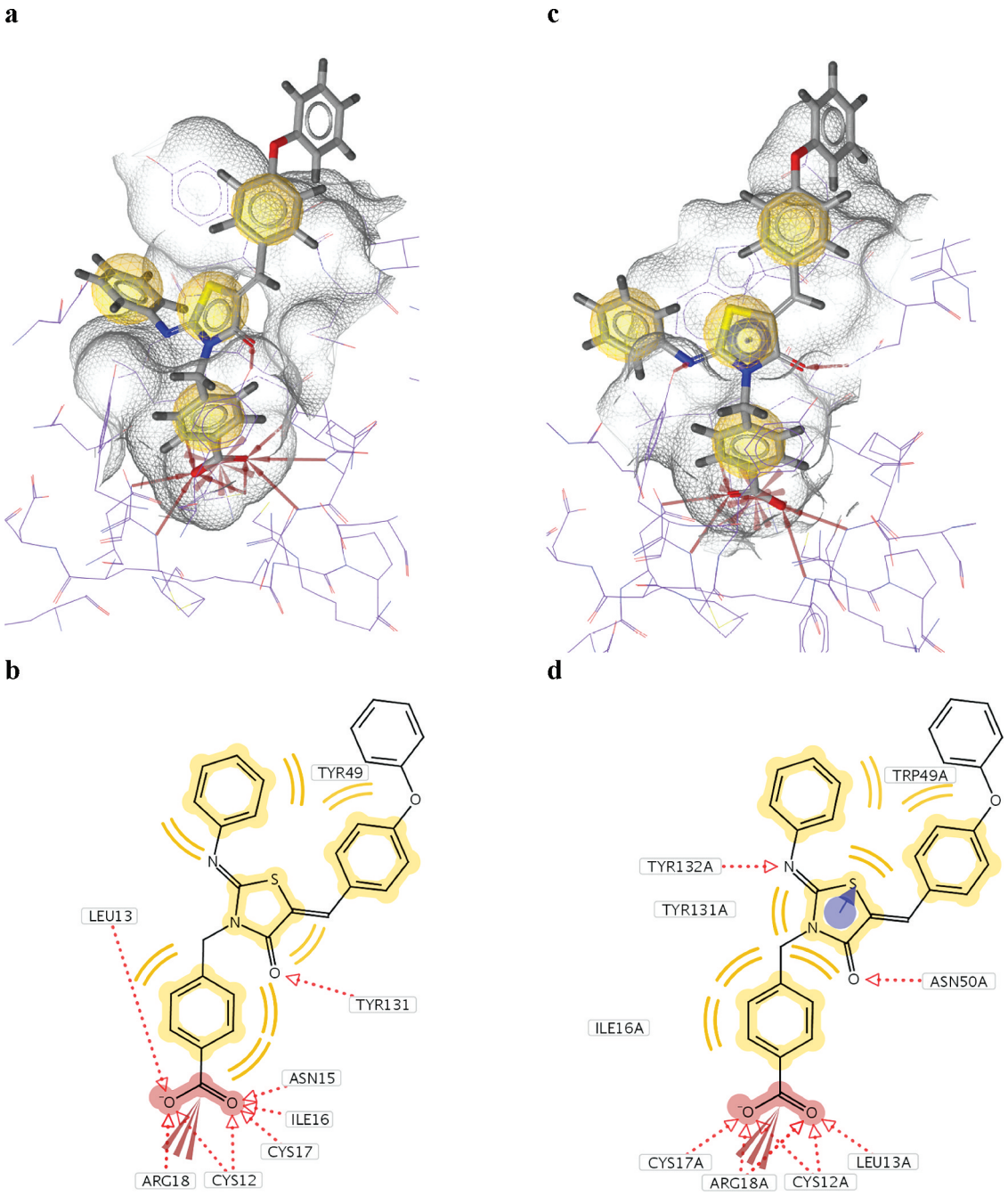

Figure 11. Compound **26a** docked in LMW-PTP IF1 (PDB code 5PNT[59]) (a,b) and IF2 (PDB code 1XWW[62]) (c, d):[152] yellow sphere and arc, hydrophopic feature; red arrow, hydrogen bond acceptor; red star, negative ionizable feature,; purple ring and arrow, aromatic feature. Reprinted from *Bioorganic & Medicinal Chemistry* (http://www.sciencedirect.com/science/journal/09680896); Vol. *17*; Ottanà, R.; Maccari, R.; Ciurleo, R.; Paoli, P.; Jacomelli, M.; Manao, G.; Camici, G.; Laggner, C.; Langer, T.; 5-Arylidene-2-phenylimino-4-thiazolidinones as PTP1B and LMW-PTP Inhibitors; pp 1928−1937; Copyright 2009, with permission from Elsevier.[152]

position 4 were also hypothesized to be important for the inhibitory effect of morin, but the interaction mode of the molecule with LMW-PTP was not definitely clarified.[157,158]

On the basis of the activity of these flavonoids, a series of chromones active as human LMW-PTP inhibitors has recently been reported.[158] The initial screening of an available collection of natural and synthetic flavonoids allowed the selection of compound **27**, which was shown to inhibit isoform IF1 with an IC_{50} of 5.7 μM (Figure 13). This compound, which was employed as a lead for further optimization, possessed the benzopyrone core of flavonoids with a biphenyl moiety in position 3. Evaluating a series of analogues of **27** highlighted that this biphenyl moiety is an important structural portion for enzyme inhibition because its removal led to considerably less active compounds. However, biphenyl derivatives lacking the flavone system also displayed negligible activity, indicating that both the benzopyrone scaffold and its biphenyl substituent are critically involved in the interaction with the target enzyme. In contrast, the length of the chain at position 2 and the displacement of the hydroxy group from position 6 to 7 of the benzopyrone system were found to be of little importance.[158]

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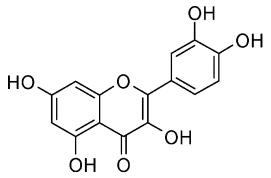
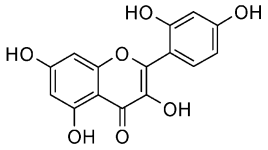
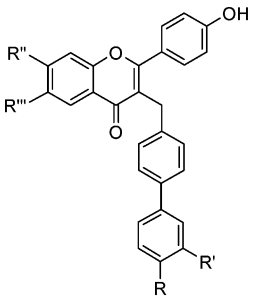


Quercetin



Morin

Figure 12. Structures of quercetin and morin.



27 R = R' = R''' = H; R'' = OH
28 R = COOH; R' = R'' = OH; R''' = H
29 R = R' = H; R'' = R''' = OH
30 R = COOH; R' = OH; R'' = R''' = H
31 R = R' = R'' = OH; R''' = H

	IC_{50} (μM) [a]		
Compd.	IF1	IF2	PTP1B
27	5.7	13.9	4% [a]
28	1.3	2.2	1.0
29	1.1	3.0	2.0
30	0.7	5.7	0.9
31	7.4	12.4	7.7

[a] inhibition at 15 μM dose.

Figure 13. Structures of selected chromones active as human LMW-PTP inhibitors.

Among the tested compounds, derivatives **28** and **29** emerged as the most effective against both isoforms IF1 and IF2 of human LMW-PTP as well as against PTP1B, with IC_{50} values ranging from 1 to 3 μM, whereas analogue **30** was more active toward both IF1 and PTP1B (IC_{50} values of 0.7 and 0.9 μM, respectively) than toward IF2 (Figure 13). Hence, the presence of both a hydroxy group and a carboxylic group on the biphenyl moiety (compounds **28** and **30**) or an additional hydroxy group in position 6 of the benzopyrone system (compound **29**) was found to promote inhibitory efficiency toward both LMW-PTP and PTP1B.[158] Interestingly, compound **30** along with the above-reported compounds **25a** and **26f** (Figure 10)[151,153] represent the most potent inhibitors of the isoform IF1 of human LMW-PTP reported so far, with submicromolar affinity for the isoenzyme, followed by analogues **28** and **29**, with IC_{50} values slightly higher than 1 μM.[158]

In agreement with the inhibition data, docking simulation into the IF1 active site indicated that the flavone system of compound **29** occupies the catalytic pocket where it is embraced by residues of the active site and bordering loops, such as Leu13, Glu15, Ile16, Asp129, and Tyr131 (Figure 14). This pose is stabilized by the formation of several hydrogen bonds between these residues and the hydroxy groups in positions 6 and 7. The deprotonated 7-hydroxy group also forms an electrostatic interaction with the positively charged Arg18 side chain. Hydrophobic interactions between the 2-(4′-hydroxyphenyl) residue and Leu13 and Tyr131 as well as between both phenyl rings of the biphenyl residue and Tyr49 were also predicted. Analogue **28** displayed a different binding mode in which the biphenyl moiety fits the catalytic pocket (Figure 14). This is attributable to the presence of the anionic carboxylic group on the biphenyl moiety that can interact with Ile16, Cys17, and Arg18, whereas the ionized adjacent hydroxy group forms additional hydrogen bonds with Arg18 and Asp129.[158] Interestingly, compounds **28**, **29**, and **31** were shown to enhance the phosphorylation of IR in cultures of human liver carcinoma HepG2 cells, which constitutively express the receptor,[158] thus being promising agents for further developments.

INHIBITORS OF *MYCOBACTERIUM TUBERCULOSIS* LMW-PTP (MPTPA) AS NOVEL ANTITUBERCULAR CANDIDATES

In the past few years, a LMW-PTP identified in *Mycobacterium tuberculosis* (MPtpA) has been proposed as a novel drug target to fight tuberculosis, which has remerged in both developing and industrialized countries as a serious global health threat also due to the worldwide spread of drug-resistant mycobacterial strains.[159] *M. tuberculosis* also possesses a second PTP with higher molecular weight, MPtpB, which can also dephosphorylate phosphoserine/threonine and phosphoinositide substrates, in contrast with MPtpA that is pTyr specific.[40] Both MPtpA and MPtpB are essential virulence factors for *M. tuberculosis*, being involved in host infection and mycobacteria survival mechanisms, particularly in signaling pathways that govern the communication between mycobacteria and host macrophages.[40,160,161] In particular, MPtpA was shown to interfere with phagocytic activity of macrophages by dephosphorylating the protein VPS33B that is involved in the membrane fusion during phagosome formation.[40,160] MPtpA and MPtpB are secreted by *M. tuberculosis* into the cytosol of infected macrophages. This is considered an attractive feature because a drug targeting these PTPs would need to enter macrophages but not mycobacterial cells, thus eluding the permeability barrier to certain drugs represented by the mycobacterial cell wall as well as the efflux mechanisms that are responsible for mycobacterial drug resistance.[162] Nevertheless, inhibitors of MPtpA or MPtpB proposed as antimycobacterial agents have to be sufficiently selective toward the mycobacterial enzymes over human orthologues to avoid possible toxic effects for the host. From this point of view, MPtpB appears to be a less challenging drug target than MPtpA because of the lack of human orthologues of MPtpB.[40,161]

Like other LMW-PTPs from different sources, MPtpA presents a four-stranded twisted β sheet structure surrounded by α helices. The phosphate-binding loop (residues 11−18) is located in a deep crevice, the walls of which are mainly formed by aromatic and hydrophobic amino acid residues, such as Trp48, His49, Tyr128, Tyr129.[163] The residues Cys11, Arg17, and Asp126 are equivalent to Cys12, Arg18, and Asp129 of human LMW-PTP and play the same catalytically essential roles.[163,164] By analogy with human LMW-PTP, the P-loop of MPtpA can adopt a favorable conformation for substrate binding by means of a network of hydrogen bonds between conserved residues, such as Asn14, Ser18, Ser42, and His71. Despite the similarity of the overall structure with that of mammalian LMW-PTPs, significant differences in the variable loop can be observed. The MPtpA variable loop appears to be conformationally less stable than that of mammalian LMW-PTPs because of the absence of two proline residues (at positions 53 and 54 in IF1) that can restrict the conformational variability of the loop. In addition, a different charge distribution around the active site was revealed, which was due to the presence in the MPtpA variable loop of His49 and Ser52 (corresponding to Glu50 and Asn53 in IF1 and Asn50

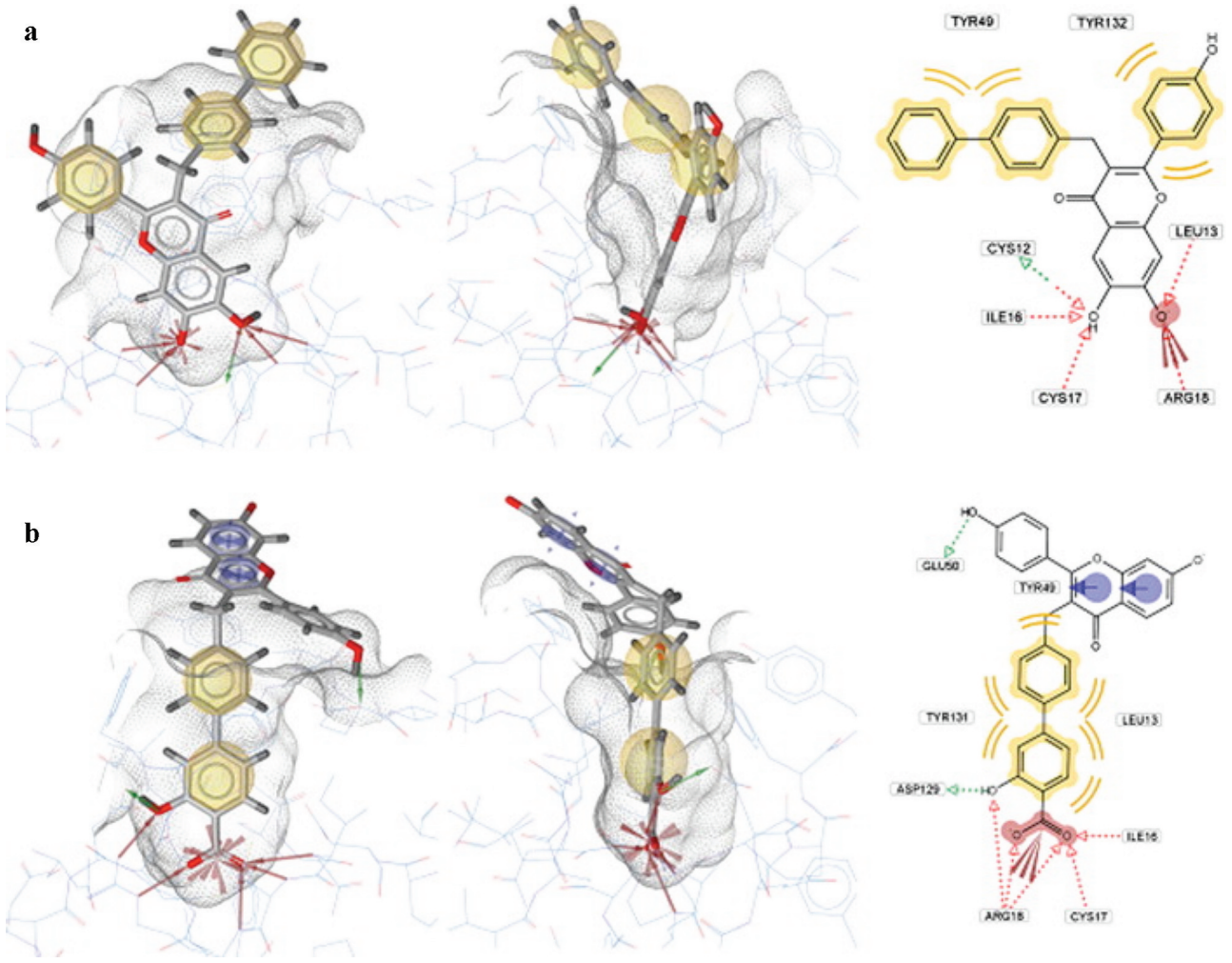


Figure 14. Docking conformations proposed for **29** (a) and **28** (b) in LMW-PTP IF1[158] (PDB structure 5PNT[59]): yellow sphere and arc, hydrophopic feature; green arrow, hydrogen bond donor; red arrow, hydrogen bond acceptor; red star, negative ionizable feature; purple ring and arrow, aromatic feature. Reprinted from *Bioorganic & Medicinal Chemistry* (http://www.sciencedirect.com/science/journal/09680896); Vol. *17*; Forghieri, M.; Laggner, C.; Paoli, P.; Langer, T.; Manao, G.; Camici, G.; Bondioli, L.; Prati, F.; Costantino, L.; Synthesis, Activity and Molecular Modeling of a New Series of Chromones as Low Molecular Weight Protein Tyrosine Phosphatase Inhibitors; pp 2658−2672; Copyright 2009, with permission from Elsevier.[158]

and Arg53 in IF2), and these may be determining residues for the substrate specificity of the enzyme.[163]

MPtpA inhibitors were identified starting from natural product structures as well as by exploiting fragment-based approaches. A cyclodepsipeptide analogue of the microbial metabolites stevastelins (compound **32**, Figure 15) and a derivative of the alkaloid roseophilin (compound **33**, Figure 15) exhibited micromolar affinity toward MPtpA, with IC_{50} values of 8.8 and 9.4 μM, respectively, and also displayed similar activity toward the human phosphatases VHR and PTP1B.[165] On the basis of the inhibitory activity of cyclodepsipeptides, such as compound **32**, more recently a series of synthetic cyclic peptides have been explored, among which compound **34** (Figure 15) stood out with a K_i of 8 μM.[166]

Fragment-based approaches led to the identification of more effective MPtpA inhibitors, such as pyrrol-1-ylbenzoic acids **35** (Figure 15). Hydroxy substituted benzoic acids **35a** and **35b** were the most potent inhibitors of this series (IC_{50} values of 1.9 and 1.6 μM, respectively) and were also effective PTP1B inhibitors (IC_{50} of 1.1 and 3.0 μM, respectively).[165] Another fragment-based screening provided an aryldifluoromethylphosphonic acid (compound **36**, Figure 15), which displayed activity equivalent to that of compounds **35a** and **35b** and an appreciable 11-fold selectivity toward MPtpA over the human IF1 isoenzyme.[167] The trifluoromethyl substituents and the amide function (compounds **36**−**38**) were related to the highest inhibition levels, and the replacement of the amide group with different linkers, such as a sulfonamide or an oxymethylene group, resulted in inactive compounds. The only exception was ureic analogue **37**, which had an affinity very similar to that of its amide counterpart **38**.[167]

Other small-molecule MPtpA inhibitors have been identified within a series of synthetic chalcones **39** and **40**.[168,169] The presence of a methoxy- or hydroxy-substituted phenyl moiety and a naphthyl system appeared to be determinant for the effective inhibition of the target enzyme, as rationalized by molecular docking simulations into the active site of the enzyme, which implied the formation of hydrogen bonds between the methoxy groups and the active site residues Thr12, Arg17, and His49, whereas the 2-naphthyl system established π-stacking interactions with Trp48 near the catalytic pocket.[169] When tested in infected macrophages, compound **40b** (Figure 15) reduced

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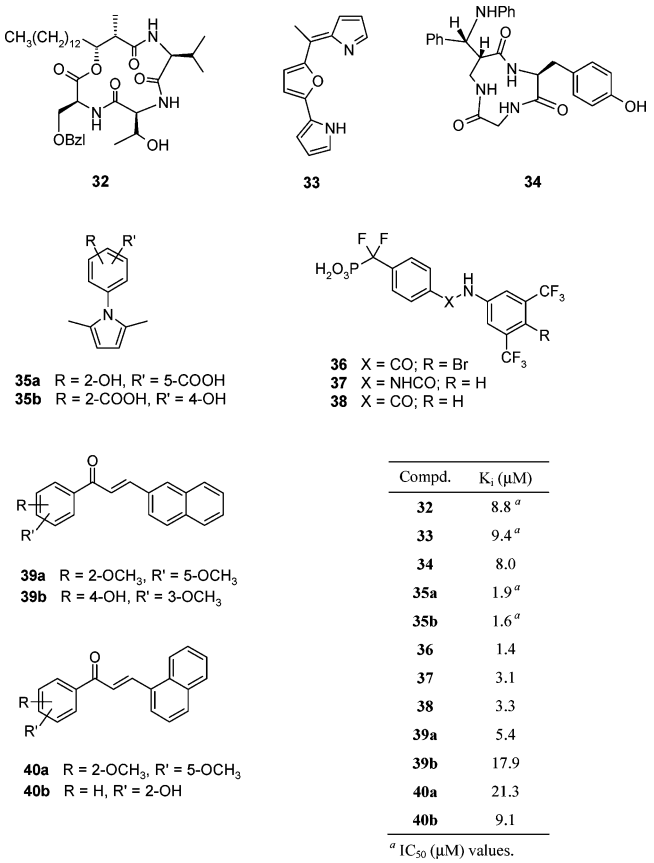

Compd.	K_i (μM)
32	8.8 [a]
33	9.4 [a]
34	8.0
35a	1.9 [a]
35b	1.6 [a]
36	1.4
37	3.1
38	3.3
39a	5.4
39b	17.9
40a	21.3
40b	9.1

[a] IC_{50} (μM) values.

Figure 15. Inhibitors of the *Mycobacterium tuberculosis* MPtpA.

M. tuberculosis growth within the host cells without displaying toxicity against the human host cells.[169]

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The development of selective and orally available PTP inhibitors involves several challenging tasks, mainly deriving from the highly conserved and charged nature of the PTP active sites and from the consequent difficulties of finding druglike molecules that can efficiently bind the enzyme and, at the same time, satisfy appropriate selectivity and bioavailability requirements. Nonetheless, in the attempt to face and solve the major selectivity and pharmacokinetic problems, structure-based design efforts as well as virtual screenings and fragment-based approaches have enabled PTP inhibitors to be identified that can be assumed as suitable lead compounds with druglike properties. In the development of site-directed PTP inhibitors, sufficiently selective inhibition can be achieved by exploiting peculiar structural features in peripheral regions surrounding the PTP catalytic sites, whereas inhibitor bioavailability can be enhanced by improving the hydrolipophilic balance and the druglike character of the active molecules.

Considering the crucial involvement of PTPs in the control of many cellular events as well as the implications of these enzymes in signal transduction aberrations that underlie the development of many human diseases, the value of pursuing new PTP-targeting drugs is being realized. Given the rapid progress in our understanding of PTP functioning and of their interplay with PTKs, it is possible that novel agents will emerge from PTP inhibitors with druglike properties to correct modifications of signaling pathways relevant to pathogenetic events.

In the case of LMW-PTP, the development of targeted inhibitors is still in its infancy, having started less than 10 years ago. Starting from the first small-molecule inhibitors reported in 2004, with millimolar affinities for the isoforms IF1 and IF2 of the human enzyme, subsequent structure-based efforts have provided 1000-fold more active inhibitors displaying potency at low micromolar concentrations with appropriate cellular activity and good selectivity between isoforms IF1 and IF2. The best LMW-PTP inhibitors present different moieties that are able to bind the enzyme at both the catalytic site and neighboring critical loops. In addition, the presence of an extended aromatic moiety is beneficial for enhancing both the affinity and selectivity of inhibitors; this is in line with the higher lipophilicity of the LMW-PTP binding site compared to other members of the PTP family. Thus, the reported inhibition studies offer good blueprints for rationally designing and identifying new and better LMW-PTP inhibitors. At the same time, although the in vivo activity of the reported LMW-PTP inhibitors has still not been evaluated, the removal and replacement of moieties that do not appear to be crucial for binding the enzyme and could be responsible for possible drawbacks need to be carefully considered in order to obtain more selective and safer inhibitors.

Moreover, the different effects of isoenzymes IF1 and IF2 in various human diseases strongly support a major area of research in this field, i.e., the structure-based design of LMW-PTP inhibitors with appreciable selectivity toward one isoform over the other, as this could lead to the development of new drugs targeting the control of specific signal transduction perturbations that occur in certain human diseases. Selective inhibitors of LMW-PTP isoenzymes not only are compounds of considerable medicinal interest but also may be useful as pharmacological tools to further investigate the precise molecular and cellular mechanisms via which these isoenzymes are involved in physiological and pathogenetic processes.

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Biographies

Rosanna Maccari graduated with a M.Sc. degree in Medicinal Chemistry (1992) and earned her Ph.D. degree in Pharmaceutical Sciences (1997) at the University of Messina, Italy. She has been a researcher in Medicinal Chemistry at the Faculty of Pharmacy of the University of Messina since 1999. Her main research interests concern drug design, lead discovery and optimization, and chemical synthesis in medicinal chemistry. For the past few years she has dealt with the design, synthesis, and SAR studies of phosphotyrosine protein phosphatase inhibitors and aldose reductase inhibitors. In recent years she was also engaged in the search for the identification of new antitubercular, anti-inflammatory, antitumoral, and antiviral agents.

Rosaria Ottanà received a M.Sc. degree in Industrial Chemistry (1980) and specialized in "Chemistry and Technology of Catalysis" (1982) at the Messina University, Italy. She has been Associate Professor in Medicinal Chemistry since 2001 and was University Researcher from 1983 to 2001 at the Faculty of Pharmacy of the Messina University. Her research interests have included the design and synthesis of bisthiazolidinones as anti-inflammatory, analgesic agents and lipophilic analogues of isoniazid with activity against AIDS-associated infections and tumoral pathologies.

Currently, her research targets the design, synthesis, SAR studies, and molecular modeling of 2-oxo/2-phenylimino-4-thiazolidinones as inhibitors of (a) aldose reductase for the treatment of diabetic complications and (b) phosphotyrosine protein phosphatases, as agents to fight diabetes, obesity, and cancer.

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■ ABBREVIATIONS USED

ACP, acid phosphatase; Akt, protein kinase B; DM2, type 2 diabetes mellitus; ECM, extracellular matrix; EGF, epidermal growth factor; EphA2, ephrin A2; FAK, focal adhesion kinase; FGF, fibroblast growth factor; GAP, guanosine triphosphatase activating protein; cGMP, cyclic guanosine monophosphate; GTP, guanosine triphosphate; IR, insulin receptor; IRS, insulin receptor substrate; LAR-PTP, leukocyte antigen-related phosphotyrosine protein phosphatase; LMW-PTP, low molecular weight phosphotyrosine protein phosphatase; *p*-NPP, *p*-nitrophenyl phosphate; PDGF, platelet-derived growth factor; PDGF-R, platelet-derived growth factor receptor; PI3K, phosphatidylinositol 3-kinase; PTK, protein tyrosine kinase; PTP, phosphotyrosine protein phosphatase; pTyr, phosphotyrosine; SHP2, Src homology 2 domain-containing phosphotyrosine protein phosphatase; STAT, signal transducer and activator of transcription; SV, splice variant

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