Prospects for Inhibitors of Protein Tyrosine Phosphatase 1B as Antidiabetic Drugs

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Introduction

Protein tyrosine phosphatase 1B (PTP1B) emerged only 4 years ago as a new drug target for the treatment of diabetes and, possibly, obesity. The enzyme belongs to the family of tyrosine phosphatases, which consists of ∼90 members.[1−3] Interestingly, this target had a very long gestation period, starting with 19th century observations that vanadium salts are of therapeutic utility in diabetes, followed by the biochemical discovery that vanadate is a potent, nonselective inhibitor of phosphatases. By the mid-1980s, it was understood that blocking one or more phosphatases could enhance the phosphorylation state of the insulin receptor kinase β subunit and/or its downstream signaling partners and revert insulin resistance, which is a characteristic of type 2 diabetes. In 1999 the discovery was announced that PTP1B knockout mice represent a phenotype that closely mimics the useful effects of vanadate treatment,[4] and this was independently confirmed a year later.[5] These findings generated a vivid interest in PTP1B and other PTPs[6,7] as drug targets for diabetes and, possibly, obesity. Here, we will briefly review the biology of PTP1B relevant to its identification as a drug target and discuss current prospects and hurdles for the development of PTP1B-selective inhibitors.

What the PTP1B Knockout Mice Have Taught Us

Nearly all members of the protein tyrosine phosphatase members were discovered before the publication of the first drafts of the human genome in 2001.[2] Given the link, as it was understood, among vanadate, phosphatases, and insulin signaling, great efforts were devoted to understanding which of the newly discovered phosphatases had the phosphorylated insulin receptor as its substrate (Figure 1). Although intracellular overexpression of dominant negative mutant or wild-type enzymes has implicated at least nine PTPs in insulin receptor signaling,[8,9] only a single phosphatase gene knockout mouse line shows a phenotype that convincingly suggests that the missing gene is involved in insulin signaling.[4,5]

PTP1B knockout mice were made by two independent labs. A side-by-side analysis of the "Montreal" and "Boston" PTP1B knockout mice shows that animals from both lines undergo increased glucose disposal in oral glucose or insulin tolerance tests.[10] Also, the animals have significantly lower plasma insulin levels and are resistant to weight gain when maintained on a high-fat diet. The animals are low in body fat and yet have normal fertility and life span. The Montreal mice also show prolonged insulin receptor phosphorylation in skeletal muscle upon insulin treatment.

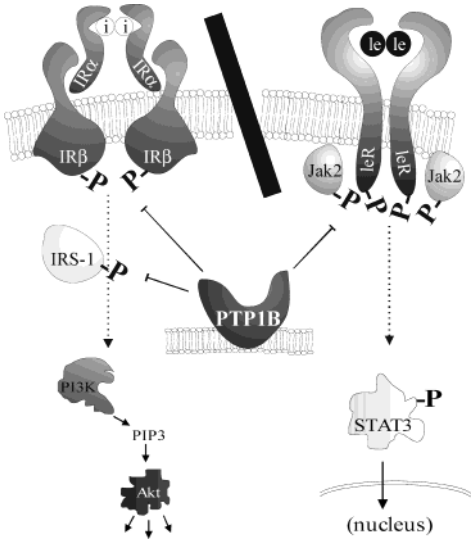

Figure 1. Proposed mode of action of PTP1B: IR, insulin receptor α and β subunits; IRS-1, insulin receptor substrate-1; le(R), leptin(receptor); Jak, Janus kinase; STAT signal transducer and activator of transcription; PI3K, phosphatidylinositol-3-kinase; Akt, protein kinase B.

When these phenotypes were described, it was unclear how genetic ablation of PTP1B could result in weight loss. Insulin is an anabolic hormone, and insulin administration is generally associated with weight gain rather than weight loss. However, consistent with the observations of knockout mice, studies with bis(maltolato)oxovanadium(IV) (Figure 2, **1**) had shown earlier that this compound suppresses food intake and decreased body weight in two obese rat disease models.[11] A number of recent studies have concluded that PTP1B is involved in the dephosphorylation of Jak2, which is an important second messenger of the leptin receptor (Figure 1).[12,13] However, Jak2 is also involved in signaling of receptors for many other cytokines, and it is at present not clear why (apparently) only leptin signaling is affected in the knockout animals. Recently it was shown that PTP1B is a regulator of SREBP, whose overexpression may also result in hyperinsulinemia.[14] There is also literature on PTP1B negatively regulating signaling through EGF and PDG receptor kinases, and uncontrolled activation of both is associated with cancer. Recent data indicate that although these receptors are indeed hyperphosphorylated in cells from PTP1B knockout animals, other phosphatases apparently compensate

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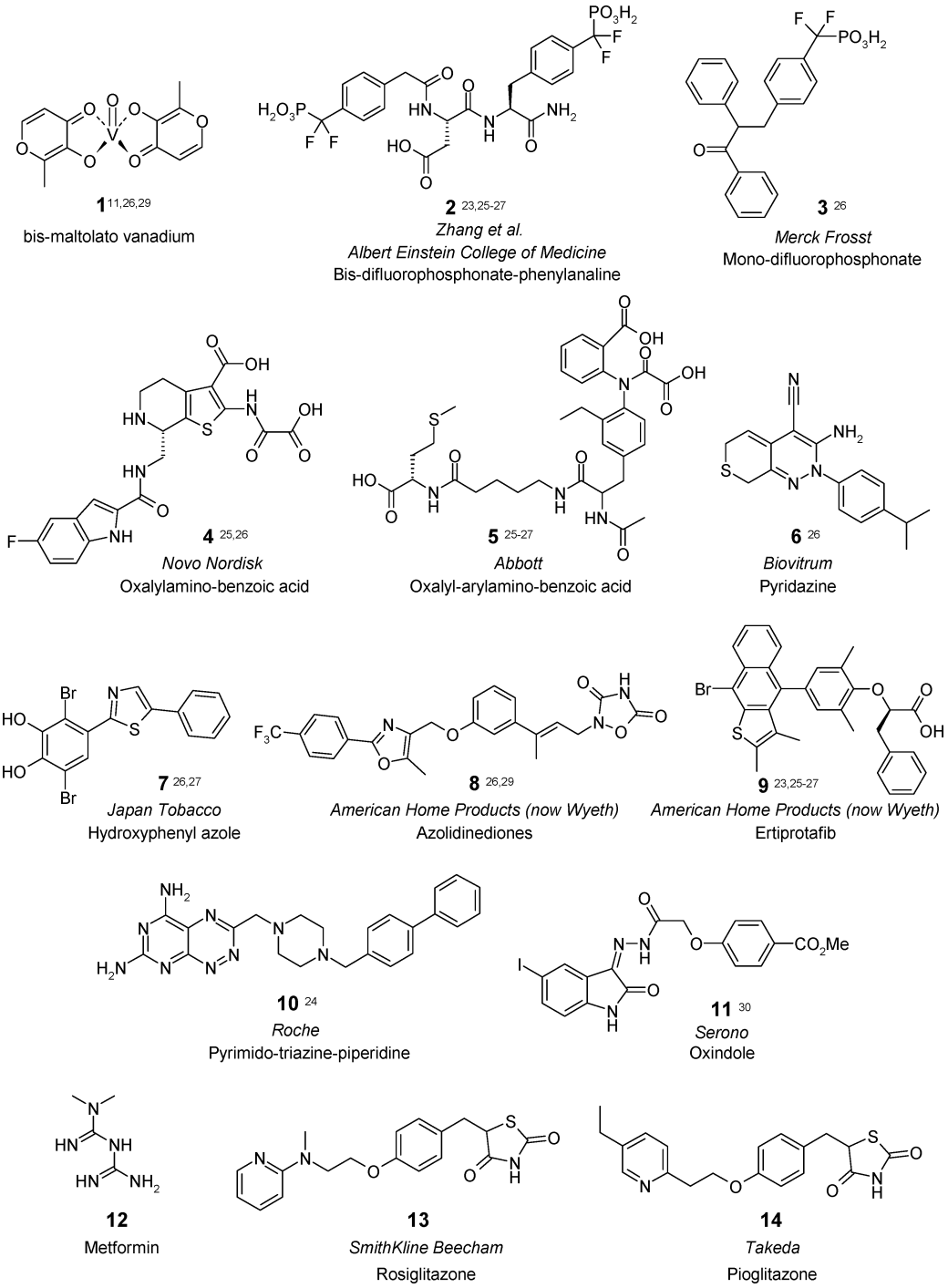

Figure 2. Chemical structures for PTP inhibitors and other antidiabetic compounds.

by quenching downstream Erk-Akt activation.[15] Another mitogenic pathway that PTP1B could be expected to stimulate is IGFR1 signaling, since the insulin and IGFR1 receptors have very similar sequences, especially in their autophosphorylation domains. However, while injection of insulin in PTP1B mutant mice resulted in enhanced insulin receptor phosphorylation, no increased phosphorylation of IGF1R is seen after IGF injection.[16]

From a "rational target discovery" perspective, it is noted that purely molecular studies have struggled to predict which PTP knockout would produce an "antidiabetic" phenotype and to fully explain at the molecular level the effects of PTP1B blocking on signaling. However, analysis of the knockout mice and studies with vanadate and other phosphatase inhibitors (such as antisense) taken together now provide overwhelming evidence that PTP1B is a bona fide target for diabetes.

Evidence for the Role of PTP1B in Glucose Metabolism and Obesity from Human Genetics Studies

Early genetics studies have implicated chromosomal region 20q13.1, where the PTP1B gene is located, as a risk factor for obesity[17] and as a predisposing factor for type 2 diabetes.[18] More directly, overexpression of a PTP1B splice variant has been correlated with increased plasma insulin levels.[19] A large population study recently discovered a rare mutation that may involve

phosphorylation and regulation of PTP1B and that is associated with a nearly 4-fold increased risk for type 2 diabetes.[20] In another study, a single-nucleotide polymorphism (SNP) in the PTP1B gene was associated with protection from diabetes,[21] and another genetic variation in a noncoding region of the gene was associated with skeletal overexpression of PTP1B mRNA and insulin resistance.[22] Although none of these genetic variations can account for the high prevalence of diabetes in the general human population, they do tend to confirm that variations in PTP1B activity can lead to increased or reduced susceptibility to the disease.

Chemical Classes of PTP1B Inhibitors

PTP1B inhibitors have been the subject of extensive recent reviews.[10,23−30] Here, we will concentrate on the discussion and comparison of representative PTP1B inhibitors for structurally different classes presented in the literature.[10,23−30]

One of the earliest strategies for designing PTP1B inhibitors has focused on the incorporation of tyrosine-phosphate mimetics into peptidomimetic backbones. Among these, difluoromethylene phosphonates have been shown to yield potent phosphatase inhibitors. The bis-difluorophosphonate-phenylanaline (**2**, Figure 2), one of the most potent inhibitor series of the target to date (K_i = 2.4 nM), has good selectivity over most phosphatases, including T-cell PTP (TC-PTP). However, the dianionic functionality of the bis-phosphonic acid impedes cell membrane permeability of this series of compounds. Merck Frosst has described a monodifluorophosphonate derivative (**3**) with poor bioavailability (F_z = 13%) and in vivo efficacy in a diet-induced obesity model (with oral glucose tolerance as readout). Unfortunately, no biological data were disclosed in the patent application for these compounds.

The combination of high-throughput screening, X-ray crystallography studies, and rational drug design allowed Novo Nordisk to discover the 2-(oxalylamino)-thiophenecarboxylic acids series as another group of nonhydrolyzable phosphotyrosine mimetics. The PTP1B inhibitor **4** has a reported K_i of 18 nM (at pH 5.5). Close analogues were identified by NMR-based screening at Abbott. An optimization program led to oxalylarylaminobenzoic acids such as **5** with a K_i of 77 nM and good selectivity against a panel of phosphatases but with only 5-fold selectivity over TC-PTP.

High-throughput screening has allowed the identification of several more PTP1B inhibitor classes having various mechanisms of action. Pyridazine derivatives such as **6** were identified at Biovitrum with potencies in the low micromolar range (5.6 μM for **6**) and over 20-fold selectivity over TC-PTP. Hydroxyphenylazole derivatives such as **7**, with IC_{50} values in the micromolar range, were claimed by Japan Tobacco. A series of azolidinediones (e.g., **8**) and phenoxyacetic acid based PTP1B inhibitors (e.g., **9**) have been reported by American Home Products. More recently, a group at Hoffmann-LaRoche described novel pyrimidotriazinepiperidine analogues (e.g., **10**) with oral glucose lowering effects in ob/ob mice. The inhibition of PTP1B by this class of compounds presumably involves the oxidation of the active site cysteine of PTP1B to the corresponding sulfenic acid.

Conclusions and Prospects

Physicochemical properties of compounds, like molecular mass, polar surface area (PSA), and molecular flexibility, have been correlated with oral bioavailability. It has been shown that there are significantly fewer orally available drugs with molecular mass of >500 Da. As molecules become larger, problems with permeability, aqueous solubility, and metabolism likely arise. Polar surface areas above 160 Å^2 have been shown to be detrimental to good oral absorption.

For this review, we have analyzed 63 small and structurally different molecules that have been described in the literature as potent PTP1B inhibitors.[10,23−30] As illustrated in Figure 3, many of these violate the Lipinski rules.[31] It is interesting to note that most of the known PTP1B inhibitors are close to, or exceed, the molecular weight cutoff of 500 Da and/or have PSA values greater than 160 Å^2. For instance, several polyanionic species such as di-difluorophosphonate-phenylanaline (**2**), oxalylarylaminobenzoic acid (**5**), and 2-(oxalylamino)thiophenecarboxylic acid (**4**) are among the most potent and/or selective PTP1B inhibitors described to date but exhibit nondruglike properties that could prevent them from reaching the required level of bioavailability. This is probably due to the nature of the highly charged phosphotyrosine-binding pocket or adjacent binding sites, with little opportunity for potent hydrophobic interactions in the immediate neighborhood. The inhibitor needs to extend beyond the pocket to make large numbers of weak interactions and gain some potency, which results in an increase in its molecular weight. To avoid having multiple negative charges while still showing potency, several inhibitors such as ertiprotafib (**9**) had to increase their lipophilicity (clogP > 5) to such a degree that they would be anticipated to have solubility or metabolic problems. Nevertheless, despite its modest selectivity profile, ertiprotafib (**9**) was tested in clinical trials and reached phase II development.[32]

Among the 63 compounds selected, some obey the Lipinski rules and have a PSA below 160 Å^2 (see white area datapoints in Figure 3). These compounds are the hydroxyphenyl azole (**7**), the pyridazine (**6**), the oxindole (**11**), the monodifluorophosphonate (**3**), the azolidinedione (**8**), and the pyrimidotriazinepiperidine (**10**). It is interesting to note that most of these compounds were initially discovered in high-throughput screening and that their mode of action (if described) varies from the classical competitive time-independent type of inhibitors to noncompetitive binders (e.g., **6** or **11)** or redox agents (like **10**).

If one compares PTP1B inhibitors with existing antidiabetic drugs such as Glucophage (metformin, **12**), whose mode of action is unknown, or more recently developed molecules such as peroxisome proliferator-activated receptor γ (PPAR-γ) agonists Avandia (rosiglitazone, **13**) and Actos (pioglitazone, **14**) as plotted in Figure 3, one can appreciate that despite good biological target validation, designing PTP1B inhibitors as oral agents is challenging because of the highly charged nature of the catalytic domain of the target. Moreover, because of the strong homology between some PTP targets (e.g., PTP1B vs TC-PTP), selectivity is often difficult to achieve. The level of selectivity that is

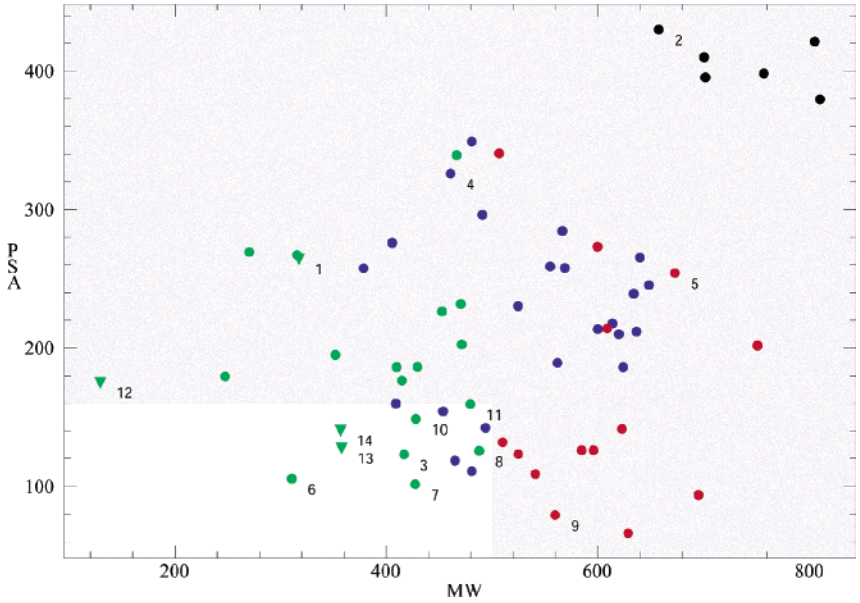

Figure 3. Plot of polar surface area (PSA) vs molecular weight (MW) for 64 antidiabetic compounds (the three triangles are non-PTP1B compounds; see text), color-coded by the number of violations against Lipinski's "Rules of 5" (MW ≤ 500; clogP ≤ 5.0; number of H-bond acceptors, ≤10; number of H-bond donors, ≤5).[31] Color represents the following: green, no violation; blue, one violation; red, two violations; black, three (or more) violations. Compounds that violate no more than one of these rules and have a PSA less than 160 Å^2 (white area) are more likely to be orally bioavailable. Compound numbering is as in Figure 2.

required for chronic dosing in the treatment of type 2 diabetes or obesity is still a matter of debate. From biochemical studies alone it is very difficult to predict how off-target enzyme inhibition translates into undesirable in vivo effects. Although both PTP1B and TC-PTP dephosphorylate the insulin receptor in vitro,[9] mice mutated for TC-PTP all die within weeks after birth,[33] in sharp contrast with PTP1B knockout mice. However, heterozygous TC-PTP animals are healthy and fertile. Overall, TC-PTP expression is significantly higher than PTP1B (our unpublished data on mRNA quantification). Finally, vanadate, which has no PTP substrate selectivity at all, seems to reproduce few, if any, of the runting, splenomegaly, and lymphadenopathy seen in the TC-PTP knockout animals. This would tentatively suggest that a successful PTP1B inhibitor may have a useful therapeutic index even when it has less than perfect selectivity. More evidence that PTP1B is a more "sensitive" target than TC-PTP is that heterozygous PTP1B knockout animals show a "useful" phenotype. As for the human genetics studies, small differences in PTP1B activity seem to translate into a relatively large effect on glucose metabolism. Finally, once-weekly injection of a PTP1B antisense drug candidate shows promising efficacy in animal models,[34,35] which is remarkable for a class of compound that is perceived as having poor bioavailability. This antisense drug, ISIS-113715, showed efficacy (mid-2003) in human phase I trials and has entered phase II trials.[36]

Overall, the quest for oral PTP1B inhibitors with a satisfactory balance between physicochemical properties and selectivity is still in its early stages, and despite recent progress,[37,38] optimal compounds with oral activity remain to be discovered.

Biographies

Rob Hooft van Huijsduijnen is Project Leader Phosphatase Targets and Principal Scientist at the Serono Pharmaceutical Reserarch Institute in Geneva, Switzerland. His team is involved in target discovery and drug development. Prior to joining Serono he was Staff Scientist with Glaxo-Wellcome at the Glaxo Institute for Molecular Biology located in Geneva, Switzerland (1990−1997). He did his postdoctoral training (1986−1990) as an EMBO Fellow at the LGME (Université Louis Pasteur) in Strasbourg, France, which was directed by Prof. P. Chambon. He obtained his doctorate from the Chemical Faculty of the University of Leiden, The Netherlands, in 1986. He has authored over 55 peer-reviewed scientific publications and patents.

Wofgang H. B. Sauer, a born and bred Franconian, studied chemistry at the Universities of Erlangen (Germany) and Strathclyde (Glasgow, Scotland). He received his Dr.re.nat. from the Computer Chemistry Center in Erlangen for work on peptide design and intermolecular interactions. From 1994 to 1999 he worked as a Support & Applications Scientist with Oxford Molecular, where he covered the product areas QSAR, Quantum Mechanics, and Molecular Modelling. In 1999, he joined the Serono Pharmaceutical Research Institute close to Geneva (Switzerland), where he is responsible for computational chemistry and the CADD support of medicinal and combinatorial chemists. His research interests include the prediction of drug properties, peptidomimetics, and molecular recognition.

Agnes Bombrun started her studies in chemistry close to her hometown in Lyon, France, where she graduated as an Engineer of Chemistry. In 1992 she received her Ph.D. from Emory University in Atlanta, GA, under the supervision of Prof. Lanny S. Liebeskind. She is currently Head of Combinatorial Chemistry and Group Leader in Medicinal Chemistry at the Serono Pharmaceutical Research Institute in Geneva, Switzerland. Her previous experience includes research with Prof. Jan Erling Baeckvall in Uppsala, Sweden, and industrial positions in Drug Discovery with GSK (Paris) and Affymax (Palo Alto, CA). Her research focus is on the discovery and optimization of enzyme inhibitors.

Dominique Swinnen graduated from the University of Namur (Belgium), specializing in synthetic organic chemistry. He obtained his Ph.D. in 1998 on the stereoselective synthesis of cyclopropanes under the supervision of Prof. A. Krief. After a postdoctoral fellowship with Prof. D. Hilvert (ETH Zürich, Switzerland) on novel strategies for the preparation of C-terminally modified peptides, he joined the Serono Pharmaceutical Research Institute (Geneva, Switzerland) in 1999,

where he is responsible for the medicinal chemistry program on several targets. His main research activities focus on the chemistry and medicinal chemistry of small-molecule enzyme inhibitors encompassing several therapeutic areas such as metabolic diseases and inflammation.

Supporting Information Available: Molecular structures, Lipinski parameters, PSA values, and literature references for the compounds used in Figure 3. This material is available free of charge via the Internet at http://pubs.acs.org.

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