Structure-based Design of a Low Molecular Weight, Nonphosphorus, Nonpeptide, and Highly Selective Inhibitor of Protein-tyrosine Phosphatase 1B*

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Several protein-tyrosine phosphatases (PTPs) have been proposed to act as negative regulators of insulin signaling. Recent studies have shown increased insulin sensitivity and resistance to obesity in PTP1B knockout mice, thus pointing to this enzyme as a potential drug target in diabetes. Structure-based design, guided by PTP mutants and x-ray protein crystallography, was used to optimize a relatively weak, nonphosphorus, nonpeptide general PTP inhibitor (2-oxalyl-amino)-benzoic acid into a highly selective PTP1B inhibitor. This was achieved by addressing residue 48 as a selectivity determining residue. By introducing a basic nitrogen in the core structure of the inhibitor, a salt bridge was formed to Asp-48 in PTP1B. In contrast, the basic nitrogen causes repulsion in other PTPs containing an asparagine in the equivalent position resulting in a remarkable selectivity for PTP1B. Importantly, this was accomplished while retaining the molecular weight of the inhibitor below 300 g/mol.

Reversible tyrosine phosphorylation reactions controlled by protein-tyrosine kinases and protein-tyrosine phosphatases (PTPs) are critically involved in the regulation of metabolic and mitogenic signal transduction processes (for reviews see Refs. 1–3). It is generally believed that selective PTP inhibitors could be useful in the treatment of various diseases such as cancer, autoimmunity, and diabetes (4). The aim of the present study was to develop tools that could be used to assess the role of specific PTPs in insulin signaling and in the development of diabetes.

Several PTPases have been proposed as important negative regulators of the insulin signaling pathway, including PTPα (5–8), PTP-LAR (9), and PTP1B (10–12). Recently, studies on PTP1B knockout mice provided significant support for the view that PTP1B is a key regulator of insulin signaling. The lack of PTP1B resulted both in increased insulin sensitivity and resistance to obesity in PTP1B knockout mice, thus pointing to this enzyme as a potential drug target in type 2 diabetes and obesity.

A number of structural and enzyme kinetic studies on PTP1B have given unique insight into the structural requirements for substrate and inhibitor recognition and thus makes this enzyme an ideal target for structure based inhibitor design (14–18). Employing a second aryl phosphate-binding site, significant progress was made toward producing highly selective phosphonate-based PTP1B inhibitors (19). We have recently identified 2-oxalyl-amino benzoxic acid (OBA, Fig. 1, compound 1) as a general inhibitor of PTPs (20). Importantly, OBA seems to be the most potent “minimal unit” phenyl phosphate mimic identified so far (17). X-ray protein crystallography of PTP1B co-crystallized with OBA revealed that it binds to the highly conserved phosphate-binding loop (the PTP loop), thus mimicking part of the binding pattern of the natural substrate (21, 22). In addition, OBA shows a novel binding pattern, interacting with other residues surrounding the active site, which are not directly involved in substrate binding. Because of the unique potency of OBA, its low molecular weight and its enzyme kinetic behavior as classical, time-independent competitive inhibitor, this compound was used as a starting point for structure-based lead optimization with the aim of making potent and selective PTP1B inhibitors.

**EXPERIMENTAL PROCEDURES**

Cloning, Expression, and Purification—cDNAs encoding the catalytic domains of PTP1B (23), SHP-1 (24), PTPα domain 1 (25), PTPζ domain 1 (25), PTPγ2 (25), and CD45 domain 1–2 (26) were obtained by polymerase chain reaction using primers with convenient cloning sites and appropriate cDNA templates. The PTP-LAR expression vector was a kind gift from M. Streuli, Boston. The following PTP mutants were made by overlap extension polymerase chain reaction using appropriate restriction sites for cloning purposes (27): PTP1B to PTPα; (i) R47V, D48N; (ii) R47V; and (iii) D48N. PTPα to PTP1B (PTP1B numbering): (iv) V47R, N48D, L50S; (v) V47R; and (vi) N48D. All constructs were inserted into pGE expression vectors (Amersham Pharmacia Biotech). In addition, for x-ray protein crystallography the cDNA encoding the first 321 amino acids of PTP1B and the PTP1B (R47V, D48N) mutant were inserted in the pET11a expression vector. All coding sequences were confirmed by DNA sequencing. Escherichia coli BL21(DE3) were transformed with the pET11a expression plasmids. An overnight culture was diluted 1:160 into a total volume of 2 liters of SOB medium (2% (w/v) Bacto-tryptone, 0.5% (w/v) Bacto-yeast extract, 10 mM NaCl, 10 mM MgCl₂, and 10 mM MgSO₄) and grown at 37 °C until A₅₅₀ was 0.6. Isopropyl-β-D-thiogalactoside was added to a final concentration of 0.1 mM, and the incubation was continued at room temperature for 3 h.

PTP1B and the PTP1B (R47V, D48N) mutant were purified essentially as described previously (28). Similar procedures were used for expression of the glutathione S-transferase fusion proteins, with the exception that the overnight cultures were diluted 1:25 and grown for 3 h at 37 °C before the addition of isopropyl-1-thio-β-D-galactopyranoside. The glu-
Highly Selective Nonphosphorus PTP1B Inhibitor

### Statistics of x-ray data and structure refinements

| Protein | Ligand | PTP1B | PTP1B | PTP1B(R47V,D48N) |
|---------|--------|-------|-------|------------------|
| Space group | | P3121 | P3121 | P3121 |
| Unit cell parameters | | a = b = 88.3 c = 103.8 Å | a = b = 87.9 c = 103.21 Å | a = b = 88.3 c = 104.5 Å |
| Completeness (high resolution) | | 99.6% (1.83–1.80 Å) | 96.6% (2.14–2.10 Å) | 97.2% (2.34–2.30 Å) |
| Multiplicity (all data) | | 4.0 (20–1.8 Å) | 4.5 (20–2.1 Å) | 4.8 (20–2.3 Å) |
| Rmerge (all data) | | 12.0% (20–1.8 Å) | 5.5% (20–2.1 Å) | 8.7% (20–2.3 Å) |
| (Rmerge) 5–30 data | | 25.5% (1.83–1.80 Å) | 32.9% (2.14–2.10 Å) | 41.8% (2.34–2.30 Å) |
| (Rmerge) high resolution | | 12.4 (20–1.8 Å) | 16.2 (20–2.1 Å) | 21.8 (20–2.3 Å) |
| Unique reflections | | 43,222 | 26,928 | 21,105 |
| Atoms in structure | | 2,669 | 2,628 | 2,560 |
| R-factor* | | 20.0% | 19.4% | 19.4% |
| Rfree | | 22.8% | 27.4% | 26.2% |
| Root mean square deviations from idealized geometry | | | | |
| Bond lengths (Å) | | 0.019 | 0.018 | 0.017 |
| Bond angles (°) | | 3.68 | 3.51 | 3.54 |
| Dihedral angles (°) | | 3.85 | 2.68 | 2.84 |

*Crystallographic R-factor, data from 6 Å to the high resolution limit were used for each data set in the R-factor calculation.

RESULTS AND DISCUSSION

Unique Structural Elements in PTP1B—To identify unique residues or combinations of residues of PTP1B that could be utilized as points of interaction by selective inhibitors, we first aligned the primary sequences of the catalytic domains of 106 known vertebrate PTPs. Using the crystal structure of PTP1B (33), we next identified unique combinations of residues in the active site pocket or in its vicinity, i.e. at a distance that would allow simultaneous binding to the active site and these residues, while still retaining a low molecular weight. In particu...

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lar, a combination of four residues seems to be unique for the PTP1B family: Arg-47, Asp-48, Met-258, and Gly-259. Arg-47 and Asp-48 contribute significantly to the binding of peptide substrates in PTP1B (14). A comparison of these regions in representative members of 14 PTP families is shown in Table II. Residue 48 seems a particularly attractive binding element for selective PTP1B ligands because this is an Asp in PTP1B and an Asn in many other PTPs. Asp-48 is well defined, as judged by B-factors in the published PTP1B structures (16, 18), and it is believed to play an important role in positioning substrates relative to the active site (34).

**Optimization for Potency**—The key structural features of OBA are the two carboxy groups bound, directly and through a carbonylamino group, to an aromatic ring (Fig. 1, compound 1). In the course of analyzing this system, we have recently shown that the phenyl ring of OBA can be substituted by thiophene, resulting in compounds that are readily synthesized with little difference in potency between 3-(oxalyl-amino)-thiophene-2-carboxylic acid (Fig. 1, compound 2) and 2-(oxalyl-amino)-thiophene-3-carboxylic acid (Fig. 1, compound 3) (Table III, (20)).

Previous studies have shown that (difluoro-phenyl-methyl)-phosphonic acid has relatively little affinity for PTP1B, whereas the addition of a second phenyl ring (i.e. (difluoro-naphthalen-1-yl)-methyl)-phosphonic acid) significantly increased the potency (15). The enhanced potency of the naphthalene ring system is because of extensive hydrophobic interactions with the side chains of Tyr-46, Phe-182, Ala-217, and Ile-219. The hydrophobic role of the second aromatic ring might also be served by a saturated ring, which would allow the regioselective introduction of small substituents in this satu-

Consequently, compound 4 was synthesized and shown to be about 8-fold more potent against PTP1B than compounds 2 and 3 and 3-fold more potent than OBA when tested at pH 5.5 (the pH optimum for PTP1B using pNPP as substrate)(Table III). Importantly, the inhibitory profile against this set of PTPs is almost the same as that of the naphthalene derivative of OBA (not shown). Thus, although compound 4 retains the features of a general PTP inhibitor, it shows some selectivity for PTP1B.

**Optimization for Selectivity**—As indicated above, the combination of Arg-47 and Asp-48 offers a rather unique, selective ligand binding region in PTP1B. The side chains of both residues are charged at neutral pH and could therefore be utilized for salt bridge formation. We decided to address Asp-48 for optimization purposes for the following reasons. First, because OBA and its derivatives already contain two negatively charged carboxy groups, we did not want to introduce additional negative charges. Second, the arginine side chain (Arg-47) has high B-factors in the known x-ray structures of PTP1B, indicating substantial flexibility. Thus, energy penalties could be expected when constraining the arginine side chain for ligand binding. Third, and probably most importantly, residue 48 offers a unique possibility for obtaining selectivity because many PTPs contain an uncharged asparagine at this position. By introducing a positive charge in compound 4 at the appropriate position, we expected the formation of a salt bridge with Asp-48, and in addition because of repulsive forces between the
positive ligand charge and the asparagine side chain found in many other PTPs, a decrease in affinity for these PTPs (see below).

Three side chain rotamer conformations are normally defined for an Asp residue (rotamer 1, 47.7%; rotamer 2, 33.6%; and rotamer 3, 15.9%, percentages according to Quanta rotamer library (Molecular Simulations Inc.). In the published x-ray structures of PTP1B, two rotamers for Asp-48 have been described, rotamer 1 and 3. The rotamer 3 conformation is stabilized by an internal hydrogen bond between the side chain of Asp-48 and the main chain amide with the side chain bending toward the active site pocket (as examples, see Figs. 4B, 5A, and 6 below). This rotamer seems to be the preferred one for Asp-48. The rotamer 1 conformation has only been found in four of the 11 published x-ray structures, and in three of these cases the rotamer 1 position is required because of ligand occupancy forcing the side chain of Asp-48 to point away from the active site pocket. We have recently co-crystallized PTP1B with OBA, as well as three derivatives, and found Asp-48 also in the rotamer 3 position in all structures (20). Based on these observations, it seemed likely that introduction of a basic nitrogen in the saturated ring in compound 4 would be sufficiently close to Asp-48 to allow the formation of a salt bridge and thus increase the affinity. A recent survey of 322 unrelated proteins has shown that Asp and Asn residues have a strong tendency to form hydrogen bonds with neighboring backbone amides and in both cases with a significant preference for internal hydrogen bonds (35). Assuming that the equivalent Asn-48 of other PTPs forms an internal hydrogen bond similar to that observed for Asp-48 in PTP1B, the side chain amide of the asparagine with its positive dipole would be in an unfavorable position relative to the proposed basic nitrogen and thus cause repulsion. Importantly, such internal hydrogen bonds can be demonstrated for Asn-48 (PTP1B numbering) in the published x-ray structures of PTPβ (36), PTPμ (37), and SHP-1 (38).

In agreement with the above predictions, compound 5 was found to have an increased affinity for PTP1B of about 20-fold without any significant increase in molecular weight (compared with compound 4, Table III). Further, this compound showed remarkable selectivity for PTP1B versus all other PTPs tested. This is in agreement with the hypothesis that repulsive forces between the basic nitrogen in compound 5 and the positive dipole of the asparagine side chain decrease the potency against other PTPs. CD45, which also contains an aspartic acid in position 48, is a noticeable exception showing only a minor decrease in affinity. We speculate that the preferred rotamer of Asp-48 in CD45 might be rotamer 1, which is too far away for salt bridge formation with compound 5. In addition, CD45 contains a valine in position 47, which may not have the same influence on Asp-48 as an arginine. Further studies will be needed to clarify the role and positioning of Asp-48 in CD45 and other PTPs with an asparagine in this position. In particular, PTPH1, which has a positively charged lysine in position 47, should be included. We have previously found the interaction between PTPs and OBA (as well as its derivatives) to be pH-dependent. For some inhibitor/PTP combinations, the $K_i$ value can be about 10-fold lower at pH 5.5 (the pH optimum for PTP1B) than at pH 7.0 (20). To analyze if the observed selectivity was retained at neutral pH, we also analyzed the compounds against the same set of enzymes at pH 7. Importantly, although some differences could be noted, compound 5 shows the same significant selectivity at this pH (Table IV). Similar to OBA, the kinetic profile of compound 5 shows it to be a classical competitive inhibitor at neutral pH (Fig. 2).

Compounds 5 and 6 were examined for their ability to inhibit the hydrolysis of a peptide substrate that better mimics physiological substrates. The phosphotyrosine (pTyr)-peptide corresponding to the C-terminal 14 residues of p56Lck was 32P-labeled using csk (39) and analyzed in a scintillation proximity assay (Amersham Pharmacia Biotech) with PTP1B. In this assay compound 5 showed an IC$_{50}$ value of 7 µM and compound 6 showed an IC$_{50}$ value of 77 µM. Thus, consistent with the results obtained with pNPP, the selectivity profile for the two compounds was retained when using the peptide substrate. It should be emphasized that the nonphosphorus compound 5 displays an affinity for PTP1B in the same range as the most selective nonpeptide (phosphonate-based) PTP1B inhibitors described so far (19). In agreement with our findings, these

### Table IV

| Compound | $K_i$ (µM) | pH 7.0 | Comp 4 | Comp 5 | Comp 6 |
|----------|------------|--------|--------|--------|--------|
| PTP1B    | 39         | 5.1    | 63     |        |        |
| SHP-1    | 130        | 1500   | 470    |        |        |
| PTPα D1  | 1300       | >2000  | 1400   |        |        |
| PTPε D1  | 590        | >2000  | 290    |        |        |
| PTPδ     | 46         | 1900   | 17     |        |        |
| CD45 D1D2| 510        | 840    | 960    |        |        |
| LAR D1D2 | 1800       | >2000  | 1100   |        |        |

3 C. B. Jeppesen, unpublished data.
inhibitors displayed similar $K_i$ values when using pNPP and a pTyr peptide as substrates.

Recently, the development of nonpeptide, nonphosphorus inhibitors of PTP1B with $K_i$ values in the nanomolar range was reported (40). These compounds had molecular weights close to 700 g/mol and showed different degrees of selectivity against all PTPs analyzed, ranging from 5–6-fold (PTP-LAR and PTP-PEST) to 75- (CD45) and 175-fold (PTPa). In contrast, whereas still retaining the molecular weight below 300 g/mol, we have achieved a considerably higher degree of selectivity against a broad range of PTPs with compound 5. Further, preliminary studies indicate that compound 5 potentiates insulin-mediated inhibition of glucagon-induced glycogenolysis in primary hepatocytes. In comparison with control cells, several proteins show increased phosphorylation levels after treatment with compound 5.

| pH 5.5 | Comp 4 | Comp 5 | Comp 6 |
|-------|--------|--------|--------|
| PTP1B wt | 8.1 | 0.29 | 14 |
| PTP1B R47V D48N | 9.7 | 5.8 | 6.3 |
| PTP1B R47V | 8.2 | 0.25 | 12 |
| PTP1B D48N | 8.7 | 8.5 | 5.2 |
| PTPa wt | 290 | >2000 | 170 |
| PTPa V47R N48D L50S | 760 | 120 | 1400 |
| PTPa V47R | 300 | >2000 | 160 |
| PTPa N48D | 820 | 100 | 550 |

| pH 7.0 | Comp 4 | Comp 5 | Comp 6 |
|-------|--------|--------|--------|
| PTP1B wt | 39 | 5.1 | 63 |
| PTP1B R47V D48N | 42 | 490 | 20 |
| PTP1B R47V | 48 | 4.7 | 110 |
| PTP1B D48N | 37 | 450 | 21 |
| PTPa wt | 1300 | >2000 | 1400 |
| PTPa V47R N48D L50S | >2000 | 480 | >2000 |
| PTPa V47R | 1700 | 1800 | 810 |
| PTPa N48D | 700 | 440 | >2000 |

PTP Mutants—The obtained selectivity for PTP1B is important if compound 5 should be developed further into a drug candidate as well as for target identification/verification. In particular, this selectivity should be against other PTPs that have been claimed as candidates for negative regulation of the insulin receptor, including PTPa (7) and PTP-LAR (41). As seen in Tables III and IV, a significant selectivity was observed for compound 5 for PTP1B versus PTPa (in comparison with the 35-fold preference of OBA for PTP1B over PTPa). In accordance with the theory described above, repulsive forces between compound 5 and PTPa should account for the increased

$^4$ B. Andersen et al., personal communication.

**Fig. 3.** Binding mode of compound 5 in PTP1B. A, $2F_o-F_c$ omit maps for the PTP1B/compound 5 complex structure. The electron density maps are contoured in blue at 1$\sigma$ level and in red at 3$\sigma$ level. The inhibitor has been omitted from the phasing model and refined several cycles before map calculation. Atoms are colored according to atom type (carbon in white, oxygen in red, sulfur in yellow, and nitrogen in blue). B, stereo picture of compound 5 in the active site pocket of PTP1B (atoms are colored as in A).
selectivity. Therefore, to test this hypothesis, we made a series of PTP1B and PTPα mutants in which the corresponding amino acid residues were exchanged in PTP1B and PTPα: (i) 47 and 48, (ii) 47, and (iii) 48. Table V shows that the change of residue 48 in PTP1B to the corresponding residue in PTPα (Asn) caused a marked reduction in affinity, whereas the reverse change in PTPα significantly increased the affinity for compound 5. In comparison, almost the same affinities were observed for compound 4 in these mutants and the wild-type enzymes.

To further substantiate the residue in position 48 as an important selectivity determining residue in PTPs we next synthesized compound 6 to introduce oxygen instead of the basic nitrogen. Theoretically, when positioned identically to the basic nitrogen of compound 5, the oxygen in compound 6 should show weak repulsion against Asp-48 in PTP1B and attraction toward Asn-48 in PTPα. The measured $K_a$ values (Table V) are in agreement with this prediction. By shuffling either the 48 residue position (Asp/Asn) or the ligand (salt bridge accepting basic nitrogen/hydrogen bonding accepting oxygen) attraction or repulsion can be established. The PTP mutants clearly demonstrate the repulsive and attractive forces around residue 48. Further, these results show that PTP mutants may be powerful tools in lead optimization.

**X-ray Protein Crystallography**—As shown above, based on the kinetic studies with wild-type and mutant PTPs we were able to conclude that the basic nitrogen of the tetrahydropyridine ring of compound 5 would be sufficiently close to Asp-48 to form a salt bridge. However, it was not possible based on biochemical analyses alone to map the exact binding mode of these inhibitors, which is critical for efficient structure-based design and optimization. We therefore undertook x-ray protein crystallographic studies of PTP1B and compounds 5 and 6 as well as the PTP1B (R47V,D48N) mutant complexed with compound 6.

In all structures, well suited electron densities were identified in the active site pocket (see Table I for x-ray data and statistics). No other densities were identified to fit the inhibitors. The $2F_o-F_c$ omit map of compound 5 in the active site pocket is shown in Fig. 3. The structure of compound 5 and OBA have identical binding mode both regarding protein and ligand interaction. The oxalic and the ortho-carboxylic acid have identical binding mode both regarding protein and ligand interaction.
and the tetrahydropyridine ring of compound with a peptide with a favorable internal hydrogen bond be-

of Asp-48 is similar to that observed when PTP1B is complexed (Figs. 3, 4, and 5). The observed rotamer of Asp-48 to allow the formation of an important salt bridge in the PTP1B complex (Figs. 3, 4, and 5). A similar movement of the WPD loop is observed when compound 5 and 6 bind to PTP1B. In addition to the backbone motion of the WPD loop we also observe a change in the side chain torsion angle of Phe-182. This brings the Phe-182 side chain into a more favorable position for aromatic-aromatic interaction with the phenyl ring of pTyr and the side chain of Phe-182. The WPD closure brings the conserved Asp-181 in a position to participate in substrate binding and to serve as a general acid in hydrolysis of substrates (43).

A similar movement of the WPD loop is observed when and ligand binding conformations (not shown).

Binding of pTyr, tyrosine-phosphorylated peptide substrates (14), bis-(para-phosphophenyl) methane (16), and vanadate (42) induces a 8 Å movement of the WPD loop, which closes the active site pocket and in turn traps the substrate (or inhibitor). The closed conformation is stabilized by a number of interactions including aromatic-aromatic interactions between the phenyl ring of pTyr and the side chain of Phe-182. The WPD closure brings the conserved Asp-181 in a position to participate in substrate binding and to serve as a general acid in hydrolysis of substrates (43).

The Tetrahydropyridine Ring—The phenyl ring of pTyr (14) and the tetrahydropyridine ring of compound 5 show overlapping binding modes and interact with several nonpolar residues, including van der Waals contacts with Ala-217, Ile-219, Val-49, and aromatic-aromatic interaction with Tyr-46. In addition, as suggested by our enzyme kinetic studies with wild-type and mutant PTPs, the basic nitrogen of the tetrahydropyridine ring is positioned sufficiently close to the carboxy group of Asp-48 to allow the formation of an important salt bridge in the PTP1B complex (Figs. 3, 4, and 5A). The observed rotamer of Asp-48 is similar to that observed when PTP1B is complexed with a peptide with a favorable internal hydrogen bond be-

tween the side chain and main chain amide of Asp-48 (rotamer 3). As described above, Asn–48 in other PTPs (Table II) causes repulsion of compound 5.

To get further insight into the observed attractive and repulsive forces that regulate the specificity of compounds 5 and 6, we next cocrystallized compound 6 with PTP1B. As described above, when using PTP1B or the PTPα mutants the oxygen atom in compound 6 introduces a (relatively weak) repulsion. In agreement with this notion, Asp-48 is pushed outward in the rotamer 1 conformation (see Fig. 5B). When, on the other hand, compound 6 is cocrystallized with the PTP1B mutant in which Asp-48 and Arg-47 are substituted for an Asn and a Val, respectively, a hydrogen bond is formed between the Asn residue and the oxygen in compound 6 (Figs. 5C and 6). In accordance with this, compound 6 shows slightly higher affinity for PTP1B mutants with D48N (Table V). Again, this points to Asp-48 as a selectivity-determining residue (Fig. 7).

From the B-factor analysis shown in Table VI, it can be deduced that in both cases of inhibitor binding (compounds 5 and 6) to PTP1B the residues Arg-47 and Asp-48 are stabilized compared with the apoPTP1B structure. Furthermore, it appears that the salt bridge formation between Asp-48 and compound 5 destabilizes the side chain of Arg-47 (see also electron density maps in Fig. 5), because the Arg-47 side chain is relatively stable in the PTP1B-compound 6 complex.

In summary, using a structure-based lead optimization strategy based on PTP mutants and high resolution x-ray pro-

![Fig. 6. Superimposition of PTP1B/compound 5 and PTP1B (R47V, D48N)/compound 6. The structures were superimposed using Quanta (Molecular Simulations Inc.). The active site area is shown. Atoms are colored according to atom type (carbon in white, oxygen in red, sulfur in yellow, and nitrogen in blue).](http://www.jbc.org/)

![Fig. 7. Attraction/repulsion utilizing residue 48. The structures of PTP1B/compound 5 and PTP1B/compound 6 were superimposed by the use of Quanta (Molecular Simulations Inc.). Residue 48 and the inhibitors are shown. Atoms are colored according to atom type (carbon in white, oxygen in red, sulfur in yellow, and nitrogen in blue).](http://www.jbc.org/)

**Table VI**

| Structure | PTP1B/compound 5 | PTP1B/compound 6 | Apo-PTP1B |
|-----------|-----------------|-----------------|-----------|
| Average <span class="math">(\text{Å}^2)</span> | | | |
| Residues Arg-47 | Asp-48 | Arg-47 | Asp-48 | Arg-47 | Asp-48 |
| Main chain <span class="math">(\text{Å}^2)</span> | 29 | 20 | 22 |
| Side chain <span class="math">(\text{Å}^2)</span> | 74 | 20 | 29 | 15 | 57 | 48 |

*All nonhydrogen atoms were used in the average B-factor (atomic displacement factor) calculation.*
tein crystallography, we have succeeded in turning a relatively weak, general PTP inhibitor obtained in a high throughput screening into a highly selective, relatively potent PTP1B inhibitor. Most importantly, this has been accomplished with only a moderate increase in the molecular weight (from 209 to 270 g/mol), leaving room for further optimization for potent orally active drugs.

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Highly Selective Nonphosphorus PTP1B Inhibitor

10307
Structure-based Design of a Low Molecular Weight, Nonphosphorus, Nonpeptide, and Highly Selective Inhibitor of Protein-tyrosine Phosphatase 1B

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