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Structure-Based Optimization of Protein Tyrosine Phosphatase-1 B Inhibitors: Capturing Interactions with Arginine 24

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Approximately 150 million people worldwide are estimated to be affected by type 2 diabetes,[1] a disease characterized by elevated plasma glucose levels and insulin desensitization. The disease often results in serious vascular complications and high mortality, as well as substantial expense to patients, their families, and society.[2] Current therapies, such as increasing the concentration of circulating insulin, often show undesirable side effects such as hypoglycemia and weight gain.[3] Therefore, there is a need to develop novel and more effective treatments.

Protein tyrosine phosphatase 1B (PTP-1B) is a prototypical intracellular PTPase found in a wide variety of human tissues, and is implicated in the negative regulation of insulin and leptin signaling transduction pathways.[4,5] Numerous studies indicate that PTP-1B plays an important role in insulin insensitivity[6–9] and suggests that PTP-1B inhibitors might be suitable for the treatment of type 2 diabetes and obesity.[10] Therefore, a number of companies and academic institutions have established programs on the discovery of small-molecule inhibitors of PTP-1B.[11]

In our PTP-1B discovery efforts, we have identified a series of potent and selective PTP-1B inhibitors with general structure **1** (Figure 1).[12] For example, analogue **1a** demonstrates excellent potency against PTP-1B ($K_i = 4$ nM). Further improvement in the physiochemical properties of such inhibitors was needed to achieve oral efficacy. Several approaches have been investigated, including: 1) the use of prodrugs to improve cell-membrane permeability; 2) removing one of the two acid moieties; 3) reduction in molecular weight to improve their drug-like properties. In this paper, we describe our work concerning the last approach.

Noting that molecules such as **1** have molecular weights >600 Da, and cleavage of the sulfonamide bond was observed as the major metabolic pathway with certain compounds in this series, we planned to remove the large arylsulfonamide moiety. X-ray co-crystal structures have indicated that the *N*-sulfonylpiperidine moiety plays an important role through binding in the second aryl-phosphate binding site (B-pocket) of the enzyme,[12a] with one of the two sulfonyl oxygen atoms forming a hydrogen bond with the backbone NH of Gly 259, while the other makes a water-mediated hydrogen bond with Arg 24. Thus, eliminating this group was expected to result in a significant loss in potency. As predicted, compound **2**, derived from the removal of the sulfonamide moiety of **1a**, is nearly 1000-fold less potent.[12a] However, replacing the basic piperidine group with a neutral group such as cyclohexane resulted in a near 20-fold increase in potency ($K_i = 210$ nM for **3** vs. 3800 nM for **2**). Therefore, it appears that of the 1000-fold loss of potency from compounds **1a** to **2**, ~50-fold could be attributed to the absence of the arylsulfonyl group, and a further 20-fold loss was due to the presence of the basic nitrogen of the piperidine.

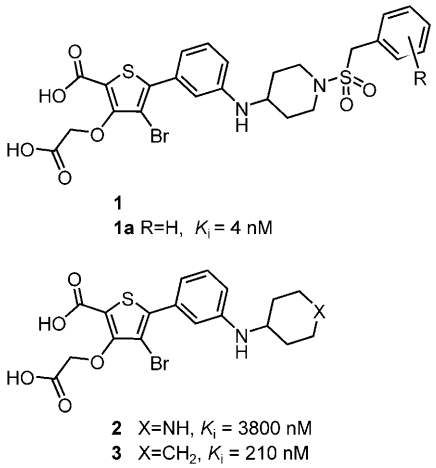

Figure 1. Previously reported PTP-1B inhibitors (Wyeth).[12]

In order to regain potency, further interactions between the inhibitor and PTP-1B were sought. Specifically, hydrogen bonding interactions with Arg 24 were targeted. A hydrogen bond acceptor group on the aniline nitrogen was expected to serve this purpose. Molecular modeling studies suggested that the carbonyl of a urea, carbamate, or amide group stemming from the aniline nitrogen could interact with Arg 24, possibly via water-mediated hydrogen bonds. Figure 2 shows the predicted binding mode of a compound with an isopropylcarboxamido group attached to the aniline nitrogen with PTP-1B. The figure shows that the cyclohexane group of the compound is sitting in the second phosphotyrosine binding pocket of PTP-1B, and the amide carbonyl group is directed towards Arg 24.

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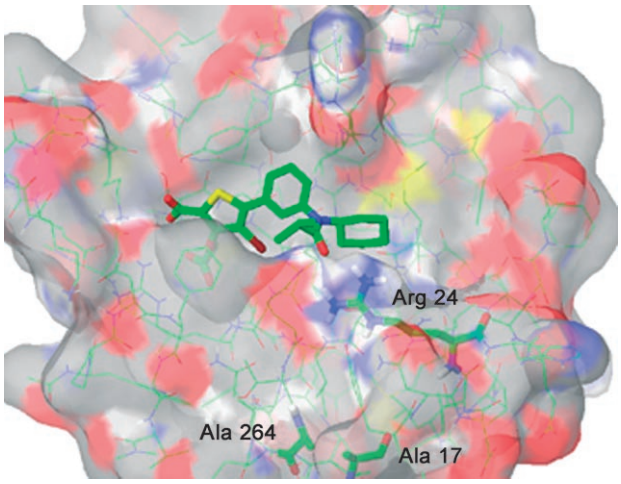
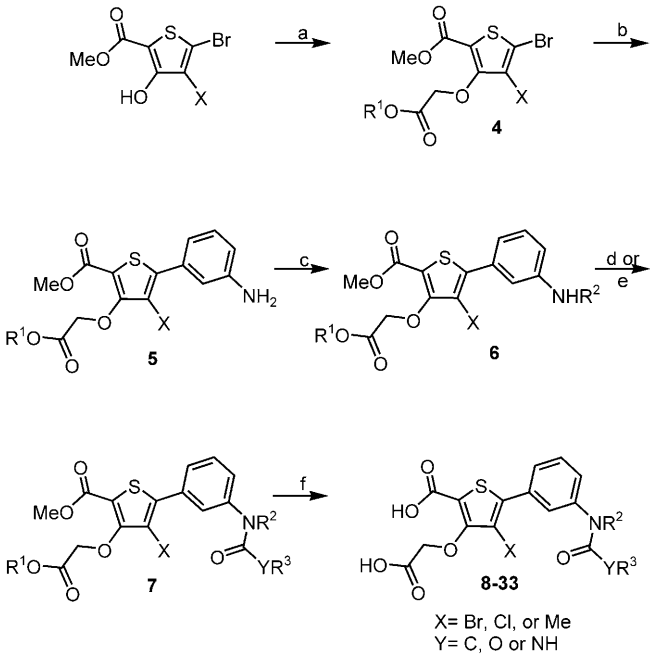


Figure 2. Predicted binding conformation of an inhibitor with an isopropyl carboxamido group at the aniline nitrogen bound to PTP-1B (based on X-ray structure 2qbs.pdb), generated through Monte Carlo docking.[13] The molecular surface of the protein is colored by element, Arg 24 and nearby residue differences with TCPTP (Ala 264–Pro and Ala 17–Gln) are highlighted in thick lines.

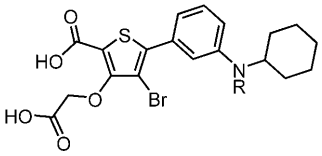
Based on this analysis, compounds **8**–**33** were prepared following the general synthetic route in Scheme 1. Alkylation of 5-bromo-4-substituted-3-hydroxythiophene-2-carboxylic acid with ethyl bromoacetate or *tert*-butyl bromoacetate gave intermediate **4**, which was then coupled to 3-aminophenylboronic acid to provide the extended analogue **5** in 70–80% yield. Reductive amination of compound **5** with a ketone or aldehyde gave the corresponding secondary aniline **6**, which was then treated with an acid chloride, isocyanate, or chloroformate following standard procedures to afford the amides, ureas, or carbamates **7**, respectively. Finally, saponification afforded the final products **8**–**33** in overall yields of 40–60%. These amides, ureas and carbamates were tested for PTP-1B inhibition in our enzymatic assay[12b] and the results are shown in the tables.

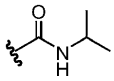
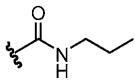
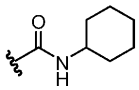
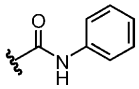
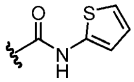
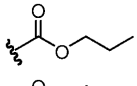
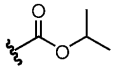
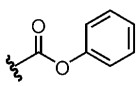
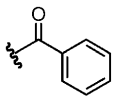


Scheme 1. Synthesis of 2-phenyl-thiophene PTP-1B inhibitors: Reagents and conditions: a) $BrCH_2CO_2Et$ or $BrCH_2CO_2tBu$, K_2CO_3, DMF, 60 °C, overnight, 99% (R^1 = Et or *t*Bu); b) $Pd(PPh_3)_4$, 3-aminophenylboronic acid, KF, THF, microwave, 100 °C, 40 min, 70–80%; c) ketone or aldehyde, $NaBH(OAc)_3$, DCE, RT, overnight, 60–85%; d) R^3YCOCl, DIPEA, DCM, RT, 2 h, 95–100%; e) R^3NCO/EtOH, RT, overnight, 90–99%; f) $LiOH.H_2O$, THF/H_2O, RT, overnight, 75–90%.

Table 1 shows the results of the first compounds synthesized and tested (Table 1). Compound **8**, with a methyl urea group as the branch at the aniline nitrogen, was eightfold more potent than the reference compound **3**. This increase in potency was assumed to be the result of the aforementioned hydrogen bond interaction between the carbonyl group and

Table 1. Inhibition constants of derivatives **8**–**19** against PTP-1B.



Compound	R	PTP-1B Inhibition K_i (nM)
3	H	210
8		25
9		15
10		13
11		13
12		24
13		21
14		42
15		21
16		26
17		18
18		98
19		13

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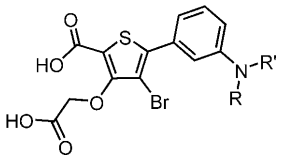
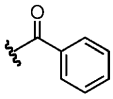
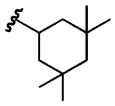
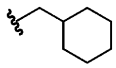
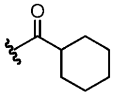
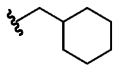
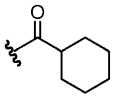
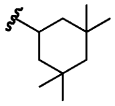
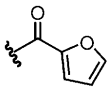
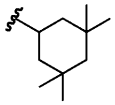
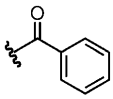
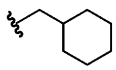
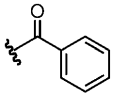
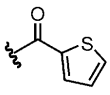
Arg 24; generally, compounds containing urea moieties were consistently potent regardless of other aliphatic or aromatic substituents. Replacing the urea with a carbamate (**14**–**17**) or amide (**18**–**19**) led to similarly enhanced PTP-1B inhibition. These results indicated that the carbonyl oxygen, common to all of these functional groups, is behaving as a hydrogen bond acceptor, despite some electronic differences. In most cases, when the branched substituents were of similar size, regardless of functionality (i.e., amide vs. urea vs. carbamate), compounds were equipotent, for example, compounds **10** and **15** (K_i = 13 and 21 nM, respectively).

It is worth mentioning that in the amide series, the amide branches seem to play a significant role in the PTP-1B binding potency. For example, replacing the acetamido with benzamido led to a seven-fold increase in potency (compounds **19** vs. **18**). Therefore, compounds with a variety of amide branch groups were investigated further. Some representative examples listed in Table 2 indicate that the relative size of the two groups at the aniline nitrogen plays a role in PTP-1B binding affinity. Fine-tuning the size of these two groups provided a means to optimize this class of inhibitors. When the side branch is a small group such as acetamido, increasing the size of the other group at the aniline nitrogen leads to potency enhancement, for example, compound **18** with a cyclohexyl group at the aniline nitrogen showed a K_i = 98 nM; replacement of this group with a larger cyclohexylmethyl group led to a 5-fold increase in potency (compound **21**, K_i = 22 nM). On the other hand, when the size of the side branch group (R) is increased, larger R′ substituents appears not to be favored. For example, compound **26** (R′ = cyclohexylmethyl), is four times less potent than derivative **19**, in which R′ is a smaller cyclohexyl group. These results suggest that the binding pocket has an intrinsic space limitation at this particular site and/or that larger substituents on the aniline nitrogen affect the geometry of the compound such that the R′ sits differently in its binding pocket, affecting the strength of the hydrogen bonding between the carbonyl group and Arg 24. Optimizing the size of these two groups gave compound **27**, which appears to achieve the ideal combination of appropriate size and geometry. Compound **27** has a K_i of 5 nM, which is 20-fold more potent than derivative **18**, and an approximate 40-fold improvement over analogue **3**. Compound **27** is almost as potent as extended analogue **1a**, the lead compound with the bulky benzylsulfonamide group, while its molecular weight was reduced by 45 Da.

Table 2. Inhibition constants of derivatives **19**–**27** against PTP-1B.



Compound	R′	R	PTP-1B inhibition K_i (nM)
19			13
20			61
21			22
22			14
23			22
24			14
25			16
26			51
27			5

X-ray co-crystal structures of both compounds **8** and **19** with PTP-1B were obtained at a resolution of 2.1 Å (pdb codes 2ZMM and 2ZN7) (Figure 3 and 4). These figures clearly indicate that the carbonyl group of the urea or amide moieties interacts with Arg 24 through a water-mediated hydrogen bond. Although the carbonyl functionalities in analogues **8** and **19** bind in a different orientation compared with the sulfonyl

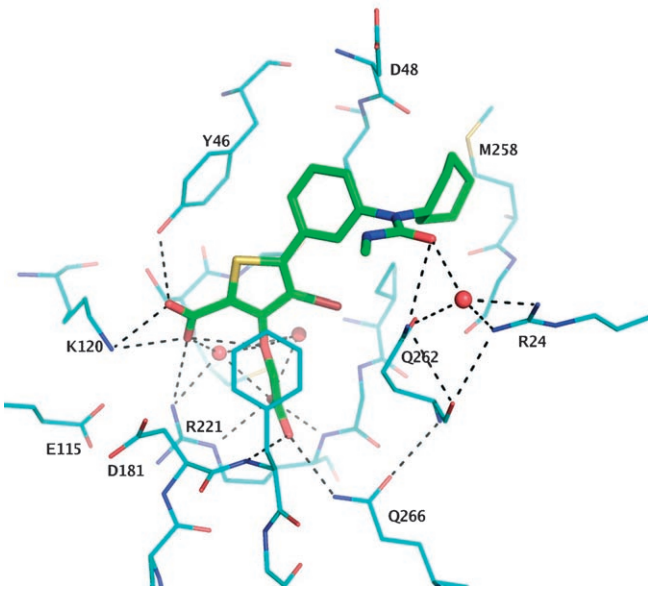


Figure 3. X-ray co-crystal structure of PTP-1B with inhibitor **8**, showing residues within 5 Å of the inhibitor, polar interactions between the inhibitor and PTP-1B (-----), and water molecules involved in hydrogen bonding (●).

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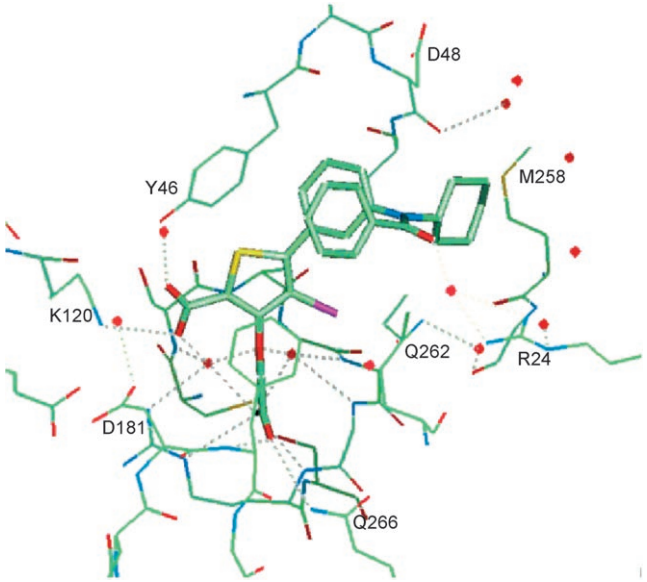
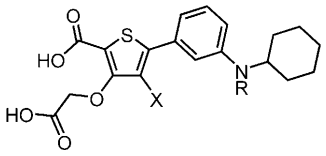
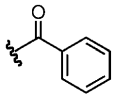
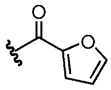
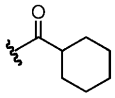
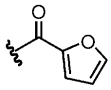
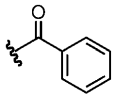


Figure 4. X-ray co-crystal structure of inhibitor **19** bound to PTP-1B, showing residues within 5 Å of the inhibitor, polar interactions between the inhibitor and PTP-1B (-----), and water molecules involved in hydrogen bonding (●).

Table 3. Inhibition constants of derivatives **28**–**33** against PTP-1B.



Compound	X	R	PTP-1B inhibition K_i (nM)	TCPTP inhibition K_i (nM)
28	Me		110	59
29	Me		32	16
30	Me		33	30
31	Me		24	14
32	Cl		8	7
33	Cl		4	4

group of **1a**, they are still able to interact with Arg24 via a similarly positioned water molecule, approached from the opposite side.

In order to further reduce molecular weight, we sought to replace the bromine substituent on the thiophene ring with smaller groups (Table 3). While a methyl substituent in the 4-position resulted in slightly lower potencies (**28**–**31**), replacing the 4-bromo with 4-chloro led to a two- to four-fold improvement in potency (**32**–**33**). For example, derivative **29** (X = Me) is twofold less potent than the bromo analogue (**19**), on the other hand, the analogous chloro derivative (**33**) is threefold more potent than compound **19**, and eightfold more potent than compound **29**.

In terms of selectivity against for PTP-1B over other phosphatases, TCPTP remains the biggest challenge due to close structural homology between the two.[10] As shown in Table 3, all compounds in this series are also potent inhibitors of this phosphatase. This is in line the results observed with our original inhibitor **1a**, which is equipotent against both PTP-1B and TCPTP, despite exquisite selectivity over other phosphatases such as CD45 (19,000 fold) and LAR (>125,000 fold).[12] The result indicated that this new series of compounds conserved the binding mode of the benzenethiophene compounds,[12] which the molecular modeling predicted and the X-ray co-crystal structures of compounds **8** and **19** confirmed (Figure 2).

Compound **33**, with a *N*-benzamido group and a chlorothiophene, showed a strong PTP-1B binding affinity (K_i = 4 nM), equipotent to the original inhibitor **1a**. Additionally, compound **33** has improved molecular properties over inhibitor **1a**, such as a lower molecular weight (514 vs. 609), fewer rotatable bonds (8 vs. 10), smaller topological polar surface area (104 vs. 133), and improved aqueous solubility (>100 vs. 13 μg mL^{-1} at pH 4.5). However, despite the improved properties, these compounds showed only a marginal increase in cellular permeability and improvement in vivo efficacy in subsequent animal studies (results not shown). Nevertheless, this new series of lower molecular weight, potent inhibitors offers a new starting point for the design of analogues with improved membrane permeability.

In summary, in order to improve the drug-like properties of the thiophene based PTP-1B inhibitors, the *N*-sulfonylpiperidine moiety was removed and a new side chain was employed to successfully target a new hydrogen bonding interaction with PTP-1B. X-ray co-crystal structures indicated that the newly installed branches interact with the Arg24 residue of PTP-1B through water-mediated hydrogen bonds. This approach has led to the discovery of a new series of potent inhibitors that have lower molecular weight, fewer rotatable bonds and a smaller polar surface area. By targeting Arg24 in the second aryl-phosphate binding site, we found compounds with activities equal to original lead compound **1a**, although no specific improvement on selectivity over TCPTP was realized. Efforts to further improve membrane permeability of this series will be reported in due course.

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