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Isoxazole carboxylic acids as protein tyrosine phosphatase 1B (PTP1B) inhibitors

Hongyu Zhao,[a,*] Gang Liu,[a] Zhili Xin,[a] Michael D. Serby,[a] Zhonghua Pei,[a] Bruce G. Szczepankiewicz,[a] Philip J. Hajduk,[b] Cele Abad-Zapatero,[b] Charles W. Hutchins,[b] Thomas H. Lubben,[a] Stephen J. Ballaron,[a] Deanna L. Haasch,[a] Wiweka Kaszubska,[a] Cristina M. Rondinone,[a] James M. Trevillyan[a] and Michael R. Jirousek[a,†]

[a]*Metabolic Disease Research, Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, IL 60064-6098, USA*
[b]*Advanced Technology, Global Pharmaceutical Research and Development, Abbott Laboratories, Abbott Park, IL 60064-6098, USA*

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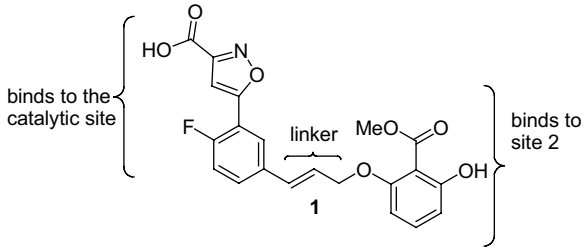
Abstract—Guided by X-ray crystallography, we have extended the structure–activity relationship (SAR) study on an isoxazole carboxylic acid-based PTP1B inhibitor (**1**) and more potent and equally selective (>20-fold selectivity over the highly homologous T-cell PTPase, TCPTP) PTP1B inhibitors were identified. Inhibitor **7** demonstrated good cellular activity against PTP1B in COS 7 cells.

Protein tyrosine phosphatase 1B (PTP1B), an intracellular PTPase, has been implicated in negative regulation of the insulin and leptin signal transduction pathways among other biological functions.[1] Studies from two laboratories have shown that PTP1B knockout mice exhibit the phenotypes of increased insulin sensitivity, improved glucose tolerance, and resistance to diet-induced obesity.[2,3] In a more therapeutically relevant study, Zinker et al. have demonstrated that a PTP1B antisense oligonucleotide normalizes blood glucose and improves insulin sensitivity in diabetic mice via a mechanism of lowering PTP1B protein expression.[4] A synthetic small molecule that selectively inhibits PTP1B action is expected to have similar beneficial effects in type 2 diabetic and obesity patients.

Two major challenges in developing potential therapeutics targeting PTP1B are selectivity and cell permeability. The X-ray crystal structure revealed that the PTP1B catalytic site and vicinal binding sites are highly hydrophilic and homologous to other PTPases.[5,6] These structural features were further substantiated by that fact that most known PTP1B inhibitors are large (MW > 500) and hydrophilic molecules containing multiple acid and peptide groups.[7] Nonetheless, cell permeable PTP1B inhibitors have been reported in several laboratories.[8] High selectivity over PTPases other than TCPTP[9] has been routinely achieved, but only a few compounds showed moderate selectivity over TCPTP (~10-fold).[10]



K_i 6.9 μM (PTP1B), K_i 164 μM (TCPTP)

We have previously reported the discovery of isoxazole carboxylic acid-based PTP1B inhibitors such as **1**.[11]

Keywords: PTP1B; Selective; Cell permeable.
* Corresponding author. Tel.: +1 847 935 4566; fax: +1 847 938 1674; e-mail: hongyu.zhao@abbott.com
† Present address: Pfizer Global R&D, La Jolla Laboratories, 10770 Science Center Drive, San Diego, CA 92121-1187, USA.

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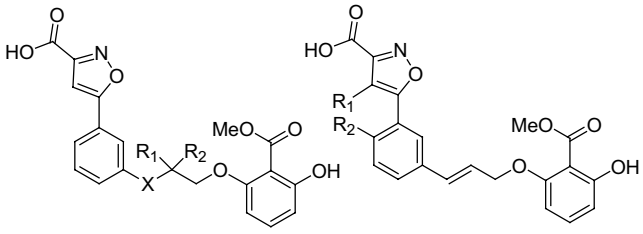
Here we describe our efforts to extend the SAR studies leading to more potent PTP1B inhibitors with similarly high selectivity and cell permeability.

Our SAR plan was directed by the X-ray crystal structure of PTP1B in complex with **1**.[11] It appeared a hydrogen bond donor on or attached to any of the three-carbon atom in the linker would have a chance to interact with the carboxyl group of Asp48.[12] Unfortunately, all efforts to introduce hydrogen bond donors on the carbon linker to interact with Asp 48 resulted in less active compounds. For example, replacing the CH=CH group of the linker in **1** with an NHCO group only produced a weaker inhibitor (**2**, K_i 216 μM).[13] A hydroxy group on the linker (**3**, K_i 122 μM) did not seem to interact with Asp48 favorably. Changing the nature of H-bond donor by removal of the carbonyl oxygen in **2** led to a more potent inhibitor **4** (K_i 60.3 μM), but weaker than **1**. Evidently, these hydrogen bond donors were either not appropriately positioned or their introduction altered the linker conformation too much. Cyclopropanation of the linker double bond to fine tune the linker conformation led to a less potent inhibitor (**5**, K_i 23 μM).

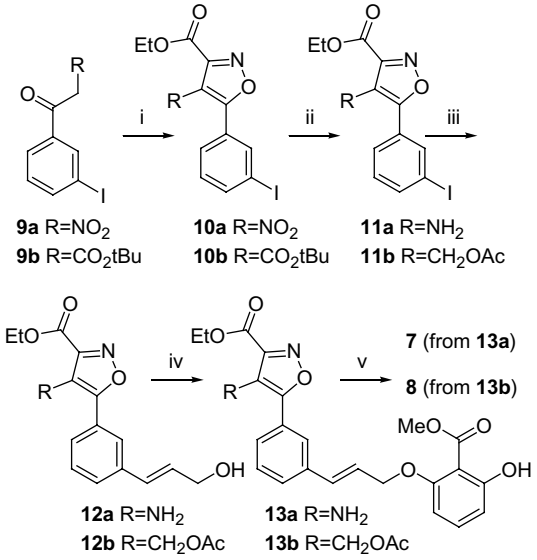


2 X=NH, R_1, R_2=O K_i 216 μM
3 X=CH_2, R_1=H, R_2=OH K_i 122 μM
4 X=NH, R_1=H, R_2=H K_i 60.3 μM
5 X,R_1=$CHCH_2$, R_2=H K_i 23 μM
6 R_1, R_2=CH_2CH_2 K_i 11.5 μM
7 R_1=NH_2, R_2=H K_i 2.1 μM
8 R_1=CH_2OH, R_2=H K_i 0.92 μM

Another observation from the crystal structure of the PTP1B-inhibitor **1** complex was that the isoxazole ring and the benzene ring were nearly co-planar. The unfavorable interactions between C–H's (or C–F) of the benzene and isoxazole rings would be eliminated by chemically forming a ring system between them. However, one molecule of this kind, tricyclic inhibitor **6**, did not show any improvement (K_i 11.5 μM), even though the crystal structure revealed no apparent interference between the added two methylene groups and the protein surface.

The crystal structure of the PTP1B-inhibitor **1** complex suggested that only small additional groups could be accommodated in the catalytic site. Due to the hydrophilic nature of PTP1B catalytic site, polar groups are likely to provide more favorable interactions, but they are also likely to have negative impact on cell permeability. One way to possibly solve this dilemma is to introduce polar groups in such a way that they could increase the pK_a value of the isoxazole carboxylic acid and therefore increase the concentration of the neutral form of the acid in physiological environment. Thus, introduction of an amino group to the only chemically modifiable position 4 of the isoxazole ring provided a more active inhibitor **7** with good selectivity over TCPTP (K_i 2.1 μM, PTP1B; K_i > 30 μM, TCPTP). The calculated pK_a values using ACD/pK_a DB Version 7.0 for the carboxylic acid in **7** was 4.05 in comparison to 3.31 if the amino group was replaced by a hydrogen. We think the increased pK_a value of the acid in **7** was at least partially responsible for its cell permeability (vide infra). Alkylation or acylation of the amino group in **7** provided only weaker inhibitors due to the limited size of the catalytic site. Replacing the amino group with a hydroxy methyl group led to a submicromolar inhibitor **8** (K_i 0.92 μM, PTP1B; K_i 19.2 μM, TCPTP). However, the calculated pK_a for the carboxylic acid in **8** was 2.95 and inhibitor **8** was inactive in COS7 cellular assay.

The syntheses of **7** and **8** are described in Scheme 1. Coupling of β-keto nitro compound (**9a**) or β-keto ester (**9b**) with ethyl chlorooximido acetate followed by an intramolecular dehydration process gave iodo compounds **10a** and **10b**.[14] Starting materials **9a**[15] and **9b**[16] were synthesized using known protocols. Reduction of the nitro group in **10a** gave amine **11a**. Removal of the *tert*-butyl group in **10b** followed by mixed anhydride formation and subsequent $NaBH_4$ reduction, then protection of the resulting alcohol with an acetyl group produced **11b**. Alcohol **12a** and **12b** could then be obtained via a Stille coupling reaction between iodides **11a** and **11b** and 3-tributylstannyl-1-propenol.[17] Finally, introduction of the salicylate via a Mitsunobu reaction with methyl 2,6-dihydroxylbenzoate produced **13a** and **13b**. Selective hydrolysis of **13a** and **13b** led to inhibitors **7** and **8**, respectively.



Scheme 1. Reagents and conditions: (i) ethyl chlorooximido acetate, Et_3N; (ii) **10a**–**11a**: Fe, NH_4Cl; **10b**–**11b**: (a) TFA; (b) methylchloroformate, Et_3N; (c) $NaBH_4$; (d) AcCl, pyridine; (iii) 3-tributylstannyl-1-propenol, $Pd_2(dba)_3$, $(2\text{-furyl})_3P$, CuI, DMF; (iv) Ph_3P, DEAD, methyl 2,6-dihydroxylbenzoate; (v) NaOH.

The PTP1B inhibitors disclosed here demonstrated excellent selectivity over other PTPases. For example, inhibitor **8** showed greater than 20-fold selectivity and inhibitor **7** was inactive at 30 μM (highest concentration tested) toward TCPTP, the most homologous PTPase to PTP1B. The earlier SAR results revealed that the second site binder (methyl salicylate) contributed to 5–10-fold[12] and the catalytic site binding moiety (isoxazole carboxylic acid) provided another 2–4-fold[11] to the selectivity over TCPTP. These compounds showed no inhibitory activity against LAR, CD45, cdc25, and SHP-2 at highest concentration tested (300 μM). To the best of our knowledge, this series of inhibitors represent the most selective PTP1B inhibitors over TCPTP reported to date.[10]

Although these inhibitors possess a reasonable structural profile (MW < 450, contain only one carboxylic acid, and few rotatable bonds), only inhibitors **7** showed PTP1B inhibitory activity in COS7 cells as described below. The beneficial effect of the amino group in **7** most likely derived from its electron donating and hydrogen bonding ability that reduce the acidity of the adjacent carboxylic acid as discussed before.

PTP1B has been shown to negatively regulate leptin pathways by dephosphorylating (inactivating) Janus kinase (JAK)2 both in vitro[18] and in vivo.[19] Inhibitor **7** was analyzed in COS7 cells that were transiently transfected with exogenous PTP1B for its ability to reverse PTP1B-induced signal transducer and activator of transcription (STAT)3 dephosphorylation. The decreased STAT3 phosphorylation caused by PTP1B transfection (25 ng) was attenuated by inhibitor **7** in a dose-dependent manner (Fig. 1), similar to the effect exerted by a nonspecific phosphatase inhibitor BpV(pic).[20,11] The more potent inhibitor **8** showed no activity in the COS7 cellular assays, presumably due to the increased polarity and decreased pK_a value of the acid group compared to **7**.

The X-ray crystal structures of PTP1B (residues 1–322) in complex with inhibitors **4**–**8** were determined and they shared an almost identical binding mode. A representative structure (PTP1B/**8**) is shown in Figure 2.[21] As we expected, these compounds bind both catalytic site and

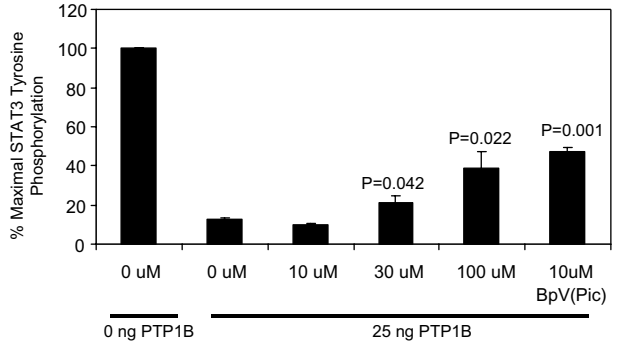


Figure 1. Reversal of PTP1B-dependent dephosphorylation of STAT3 in COS 7 cells by PTP1B inhibitor **7**.

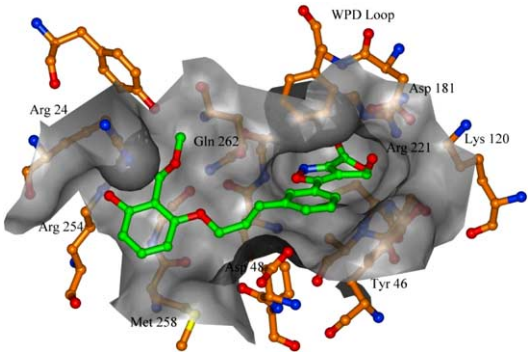


Figure 2. X-ray crystal structure of PTP1B in complex with inhibitor **8** (carbon is in orange and green for inhibitor **8**, oxygen is in red, nitrogen is in blue, and sulfur is in yellow).

site 2 of PTP1B.[22] The isoxazole carboxylic acid binds to the active site of PTP1B with WPD loop in the closed conformation. A salt bridge between the carboxylate and Arg221 provides a critical interaction. The methylenehydroxyl group on the isoxazole ring forms a hydrogen bond with Asp181 carboxyl group. The isoxazole and phenyl rings interact with a hydrophobic pocket normally occupied by the phenyl ring of pTyr. The ether oxygen of the salicylate forms a hydrogen bond with Gln262. The salicylate carboxylate group adapts an out of plane conformation, and is within hydrogen bonding distance to Arg254, Tyr20, and Gln262. The hydroxyl group of the salicylate hydrogen bonds with Arg24 and Arg254. The aromatic portion of the salicylate lies on top of the hydrophobic side chain of Met258, providing a complimentary van der Waals interaction.

In conclusion, guided by X-ray crystallography, we have designed and synthesized several potent and highly selective PTP1B inhibitors. These inhibitors contain only one carboxylic acid and have relatively low molecular weight and polarity. Inhibitor **7** showed good cell permeability in COS7 cells.

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