Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3947–3950

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Identification of a Monoacid-Based, Cell Permeable, Selective Inhibitor of Protein Tyrosine Phosphatase 1B

Zhili Xin,[a,*] Gang Liu,[a] Cele Abad-Zapatero,[b] Zhonghua Pei,[a] Bruce G. Szczepankiewicz,[a] Xiaofeng Li,[a] Tianyuan Zhang,[a] Charles W. Hutchins,[b] Philip J. Hajduk,[b] Stephen J. Ballaron,[a] Michael A. Stashko,[a] Thomas H. Lubben,[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*

Received 4 June 2003; revised 28 August 2003; accepted 31 August 2003

Abstract—Monoacid-based PTP1B inhibitors with improved physiochemical properties have been investigated. A (2-hydroxyphenoxy) acetic acid-based phosphotyrosyl mimetic has been linked with an optimized second arylphosphate binding site ligand to produce compound **20** with low micromolar potency against PTP1B, good selectivity over TCPTP (20-fold) and high cell permeability in the Caco-2 system.

Protein tyrosine phosphatase 1B (PTP1B) plays an important role in insulin receptor signaling.[1] There is compelling evidence suggesting that PTP1B is primarily responsible for the dephosphorylation of the insulin receptor, and therefore acts to negatively regulate insulin signaling.[2] Inhibitors of this enzyme would be predicted to enhance insulin-stimulated glucose transport and have potential for the treatment of type II diabetes.[3]

The enthusiasm for the development of PTP1B inhibitors as therapeutic agents has led to intensive research in recent years.[4,5] Many efforts were focused on the design of phosphotyrosyl (pTyr) mimetics. Included among these pTyr mimetics (Fig. 1) are difluoromethylene phosphonate **1**,[6] 2-oxalylamino-benzoic acid **2**[7] and *O*-carboxymethyl salicylic acid analogues **3**.[8] Unfortunately, most of these potent pTyr mimetics contain at least two acids and led to inhibitors with poor cellular permeability.

PTP1B inhibitors need to be cell permeable to reach the intracellular target, and also to achieve desirable oral bioavailability. It becomes a significant challenge to develop both potent and cell permeable inhibitors because of the nature of the enzyme. The electrostatic properties of the PTP1B catalytic site are optimal for binding phosphotyrosine, which bears two negative charges at physiological pH. Charged ligands are therefore preferred for potent binding. There are a limited number of reports on cell permeable PTP1B inhibitors.

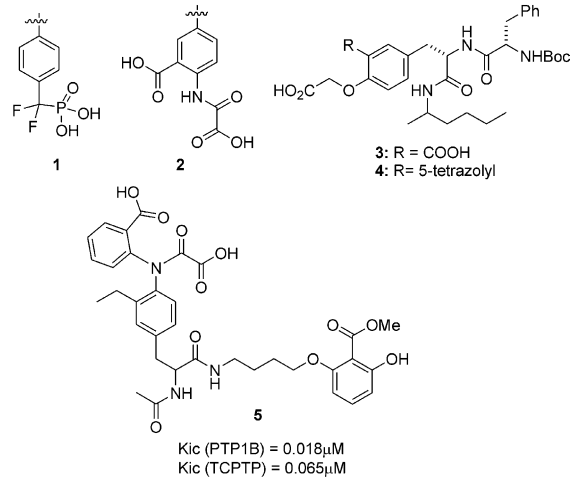


Figure 1.

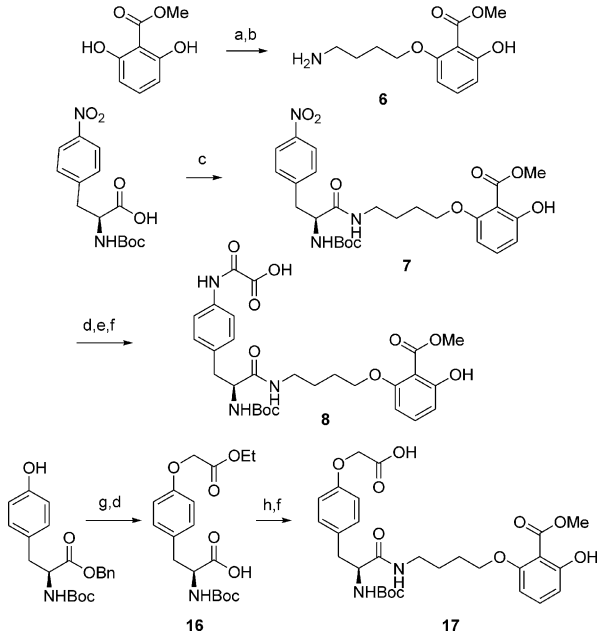
*Corresponding author: Tel.: +1-847-935-7307; fax: +1-847-938-1674; e-mail: zhili.xin@abbott.com
†Current address: Pfizer Global R&D, La Jolla Laboratories, 10770 Science Center Drive, San Diego, CA 92121-1187, USA.

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.08.064

Liljebris et al. investigated bioisosteric replacements for the carboxyl group of *O*-carboxymethyl salicyclic acid, and discovered that an *ortho* tetrazole analogue **4** ($K_i = 2.0$ μM) was equipotent to the corresponding dicarboxylic acid derivative and this compound achieved a low level of cell membrane permeability.[9] Considering minimization of charge as a means of enhancing cell membrane diffusion, Burke and coworkers examined monocarboxy-based pTyr mimetics and reported poor PTP1B inhibitory potency for these analogues.[10] The need for more potent and selective PTP1B inhibitors with improved physiochemical properties remains a critical issue.

PTP1B inhibitor **5** has been previously reported from this laboratory.[11] Compound **5** exhibited good inhibitory potency against PTP1B ($K_i = 18$ nM) and good selectivity over other phosphatases, including the most homologous TCPTP (4-fold). It contains a diaryl oxamic acid benzoic acid, a catalytic site binding pharmacophore, and a salicylate-based second arylphosphate binding site (site 2)[12] binder. However, both Caco-2 permeability and rat pharmacokinetic studies suggest a major obstacle for future development with this series of compounds. The low cell permeability of **5** as indicated by *P*app less than 1×10^{-6} cm/s is most likely due to the presence of the two carboxylic acids, which both retain negative charges at physiological pH (7.4).

In our efforts to identify PTP1B inhibitors with improved permeability, many phosphotyrosine replacements bearing either one or no charge were linked with a site 2 ligand. The pTyr mimetics generally have little measurable inhibitory activity on their own, but linking these potential active site ligands with our optimized salicylate-based, neutral site 2 ligand[11] offered opportunities to identify cell permeable, potent and selective PTP1B inhibitors.

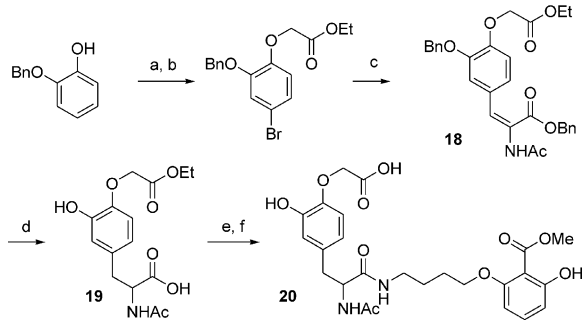


Scheme 1. Reagents and conditions: (a) *N*-Boc-butanol, Ph_3P, DEAD, THF, 54%; (b) 4 N HCl in dioxane, 100%; (c) amine **6**, TBTU, *i*-Pr_2NEt, DMF, 79%; (d) 10% Pd/C, MeOH, H_2, rt, 100%; (e) ClCOCOOEt, *i*-Pr_2NEt, CH_2Cl_2, 0 °C–rt, 84%; (f) NaOH, EtOH, H_2O, 98%; (g) ethyl bromoacetate, K_2CO_3, CH_3CN, reflux, 94%; (h) isobutyllchloroformate, Et_3N, amine **6**, 95%.

The representative syntheses of monoacids **8** and **17** are outlined in Scheme 1. Amine **6** was prepared by monoalkylation of methyl 2,6-dihydroxybenzoate with *N*-Boc-4-aminobutanol and subsequent deprotection. 4-nitro-*N*-Boc-phenylalanine was coupled with amine **6** to generate amide **7**. The nitro group was then reduced to the corresponding aniline by catalytic hydrogenation. This aniline was acylated and the resulting ethyl ester was selectively hydrolyzed in the presence of a methyl salicylate under basic conditions to provide oxamic acid analogue **8**. Due to steric hindrance and deprotonation of the phenolic hydroxy group, the methyl ester of **8** remained intact. For the synthesis of monoacid **17**, *N*-Boc-tyrosine benzyl ester was *O*-alkylated with ethyl bromoacetate, and the benzyl group was removed by hydrogenation to afford acid **16**. The acid was coupled with amine **6** and subsequent saponification yielded phenoxy acetic acid **17**.

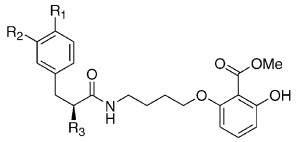
2-Hydroxy substituted analogue **20** was prepared as illustrated in Scheme 2. Bromination of 2-(benzyloxy) phenol was followed by alkylation of the bromophenol with ethyl bromoacetate. It was then coupled via Heck reaction with benzyl 2-acetamidoacrylate to provide ester **18**. Hydrogenation of **18** reduced the double bond and released the acid and the phenol at the same time. Acid **19** was coupled with amine **6**, and saponification of the ethyl ester then afforded (2-hydroxy-phenoxy) acetic acid **20**.

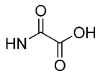
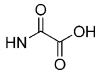
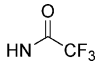
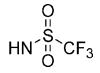
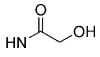
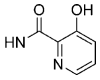
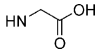
Early SAR studies showed that monoacid analogues having a phenylalanine backbone showed no preference for *N*-Boc or *N*-acetyl substitution group (**8** vs **8a** in Table 1). These groups were assumed to be equivalent for our subsequent SAR investigation. Compound **8** was identified as a low micromolar PTP1B inhibitor ($K_i = 8.8$ μM). Oxamic acids have been previously reported as effective phosphate replacements for SH2 domain ligands.[13] They were also found to be useful for PTP1B inhibition. Compound **8** was a reasonably potent and selective PTP1B inhibitor (16-fold over



Scheme 2. Reagents and conditions: (a) Br_2, HOAc, CH_2Cl_2, rt, 78%; (b) Ethyl bromoacetate, K_2CO_3, DMF, 90 °C, 100%; (c) 2-acetamidoacrylic acid benzyl ester, $Pd(OAc)_2$, (*o*-Tol)$_3$P, Et_3N, MeCN, reflux, 53%; (d) 10% Pd/C, H_2, rt, 100%; (e) amine **6**, TBTU, *i*-Pr_2NEt, DMF, 84%; (f) NaOH, MeOH, H_2O, 91%.

Table 1. SAR on modification of acid as pTyr mimetics



#	R_1	R_2	R_3	K_i[b] (μM) (PTP1B)
8		H	NHBoc	8.8 (±1.4)
8a[a]		H	NHAc	6.1 (±0.2)
9[a]		H	NHAc	>200
10[a]		H	NHAc	>200
11[a]		H	NHAc	>200
12[a]		H	NHAc	>200
13		H	NHBoc	>200
14		H	NHBoc	>200
15		H	NHBoc	>200
17		H	NHBoc	220 (±30)
20[a]		OH	NHAc	9.0 (±1.2)
21[a]		COOH	NHAc	2.2(±0.4)
22[a]		NH_2	NHAc	>200

[a]1:1 racemic mixture.
[b]The kinetic analysis was conducted using *p*NPP as the small molecule substrate in a continuously-monitored colorimetry assay. Values are means of more than two experiments with the number in the parentheses being the standard deviation.

TCPTP, Table 2) bearing only one negative charge at physiological pH. Unfortunately, this monoacid-based inhibitor still has low cell permeability, presumably due to the oxamic acid, which has a calculated pK_a of 2.1. This concern was further confirmed by the fact that *N*-phenyl oxamic acid fails to cross the cell membrane in a Caco-2 permeability assay. The poor permeability of oxamic acids limits their potential to be developed into orally active PTP1B inhibitors. It was decided that the new pTyr mimetic should be designed with no more than one carboxylic acid, and the acid should have a higher pK_a than the oxamic acid of compound **8**.

Table 2. Monoacid-based PTP1B inhibitors and their respective cell permeability

Compd	K_i (μM) (PTP1B)	K_i (μM) (TCPTP)	Cell pemeability (Papp×10^{-6} cm/s)
8	8.8 (±1.4)	141	Low (<1)
17	220 (±30)	>200	Moderate (1–10)
20	9.0 (±1.2)	182 (±59)	High (>10)

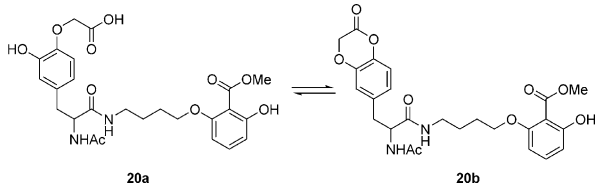


Figure 2.

The design of new pTyr mimetics turned out to be a difficult task. A few analogues with no ionizable group at physiological pH were investigated (Table 1, compounds **9**–**12**). Despite the potential for some of these entities to participate in hydrogen bond interactions within the catalytic pocket, none of the compounds showed any inhibitory activity. It seemed inevitable to include at least a mono-charged functionality to obtain an electrostatic interaction that could anchor a ligand into the catalytic site. Among the acids evaluated, compound **13**, closely related to oxamic acid analogue **8**, showed no inhibitory activity when tested at 200 μM concentration. Phenoxy acetic acid has been evaluated as a pTyr mimetic by other groups, but showed no effective inhibition.[8,10] However, the phenoxy acetic acid containing analogue **17** provided weak inhibitory activity against PTP1B (K_i = 220 μM) when linked with our salicylate-based site 2 ligand. Compound **17** (pK_a ~3) also had improved Caco-2 cell permeability compared to oxamic acid analogue **8** (Table 2).

Further SAR on the phenoxy acetic acid series identified 2-hydroxy substituted compound **20** with low micromolar potency against PTP1B (K_i = 9.0 μM), only a 4-fold decrease from di-acid analogue **21** (K_i = 2.2 μM). 2-Amino substituted analogue **22**, however, did not exhibit any inhibitory activity. Compound **20** also exhibited good selectivity over most of other phopatases and it was 20-fold selective for PTP1B over TCPTP.

Of particular note was that compound **20** demonstrated a high level of membrane penetration (>10×10^{-6} cm/s). The 2-hydroxy group makes formation of a lactone possible with compound **20**. The equilibrium between the lactone and acid forms (Fig. 2, **20a** and **20b**) has potential utility in circumventing the poor cell permeability of the existing PTP1B inhibitors. However, the existence of this equilibrium under various physiological conditions has yet to be determined.

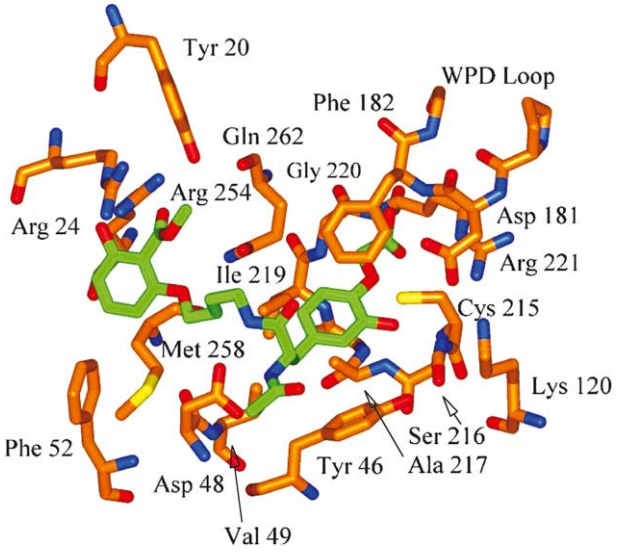


Figure 3. X-ray structure (2.2 Å resolution) of PTP1B soaked with **20**. Color scheme: carbons in orange and green for compound **20**, oxygen in red, nitrogen in blue, and sulfur in yellow.

Compound **20** has been crystallized with PTP1B. The X-ray crystal structure of **20** bound to PTP1B[14] (Fig. 3) revealed that it exists as the hydroxy acid form. It binds very tightly in the catalytic pocket, with the WPD flap down. The molecule also extends to the second phosphotyrosine binding site. The salicylate-based ligand binds as the same fashion as previously described[11] in site 2. The carboxylic acid moiety in the catalytic site has a similar binding mode to the other acids reported with the WPD loop in the closed conformation.[8] The improved potency and selectivity against TCPTP (20-fold) of compound **20** might be due to the effect of the phenol hydroxyl group, which interacts with Lys120 and Tyr46 via a water molecule (not shown).

In summary, we have discovered monoacid-based, selective PTP1B inhibitors with low micromolar inhibitory activity. Of particular interest, compound **20** achieved significantly higher Caco-2 cell permeability as compared to all previous compounds. This compound also exhibited 20-fold selectivity over TCPTP, the highest degree of selectivity reported to date. These results provided us with opportunities for designing more selective, drug-like PTP1B inhibitors.

Acknowledgements

The authors thank the Abbott Analytical Department for assistance in acquiring ^{1}H NMR and mass spectra for the compounds discussed in this report.

References and Notes

1. (a) Saltiel, A. R.; Kahn, C. R. *Nature* **2001**, *414*, 799. (b) Evans, J. L.; Jallal, B. *Exp. Opin. Invest. Drugs* **1999**, *8*, 139.
2. Cheng, A.; Dube, N.; Gu, F.; Tremblay, M. L. *Eur. J. Biochem.* **2002**, *269*, 1050.
3. Blaskovich, M. A.; Kim, H.-O. *Exp. Opin. Ther. Pat.* **2002**, *12*, 871.
4. Ripka, W. C. In *Annual Reports in Medicinal Chemistry*; Doherty, A. M., Ed.; Academic: San Diego, CA, 2000; Vol. 35, p 231.
5. Johnson, T. O.; Ermolieff, J.; Jirousek, M. *Nat. Rev. Drug Discov.* **2000**, *1*, 696.
6. Burke, T. R., Jr.; Kole, H. K.; Roller, P. P. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 129.
7. Andersen, H. S.; Iversen, L. F.; Jeppesen, C. B.; Branner, S.; Norris, K.; Rasmussen, H. B.; Møller, K. B.; Møller, N. P. H. *J. Biol. Chem.* **2000**, *275*, 7101.
8. Larsen, S. D.; Barf, T.; Liljebris, C.; May, P. D.; Ogg, D.; O'Sullivan, T. J.; Palazuk, B. J.; Schostarez, H. J.; Stevens, F. C.; Bleasdale, J. E. *J. Med. Chem.* **2002**, *45*, 598.
9. Liljebris, C.; Larsen, S. D.; Ogg, D.; Palazuk, B. J.; Bleasdale, J. E. *J. Med. Chem.* **2002**, *45*, 1785.
10. Gao, Y.; Wu, L.; Luo, J. H.; Guo, R.; Yang, D.; Zhang, Z.-Y.; Burke, T. R., Jr. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 923.
11. Liu, G.; Xin, Z.; Liang, H.; Abad-Zapatero, C.; Hadjuk, P. J.; Janowick, D. A.; Szczepankiewicz, B. G.; Pei, Z.; Hutchins, C. W.; Ballaron, S. J.; Stashko, M. A.; Lubben, T.; Berg, C. E.; Rondinone, C. M.; Trevillyan, J. M.; Jirousek, M. R. *J. Med. Chem.* **2003**, *46*, 3437.
12. Puius, Y. A.; Zhao, Y.; Sullivan, M.; Lawrence, D. S.; Almo, S. C.; Zhang, Z.-Y. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 13420.
13. Beaulieu, P. L.; Cameron, D. R.; Ferland, J.-M.; Gauthier, J.; Ghiro, E.; Gillard, J.; Gorys, V.; Poirier, M.; Rancourt, J.; Wernic, D.; Llinas-Brunet, M. *J. Med. Chem.* **1999**, *42*, 1757.
14. Refined crystallographic coordinates for the structure of hPTP1B complexed with compound **20** have been deposited with the Protein Data Bank (www.rcsb.org) with entry code 1QXK.