Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 1887–1890

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Potent, Selective Inhibitors of Protein Tyrosine Phosphatase 1B

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Received 27 December 2002; accepted 24 February 2003

Abstract—We have previously reported a novel series of oxalyl-aryl-amino benzoic acid-based, catalytic site-directed, competitive, reversible protein tyrosine phosphatase 1B (PTP1B) inhibitors. With readily access to key intermediates, we utilized a solution phase parallel synthesis approach and rapidly identified a highly potent PTP1B inhibitor (**19**, $K_i = 76$ nM) with moderate selectivity (5-fold) over T-cell PTPase (TCPTP) through interacting with a second phosphotyrosine binding site (site 2) in the close proximity to the catalytic site.
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Protein tyrosine phosphatases (PTPases) that function as negative regulators of the insulin signaling cascade have been identified as novel targets for the therapeutic enhancement of insulin action in insulin-resistant disease states.[1] Recent studies have provided compelling evidence that one of the main functions of the intracellular enzyme PTPase 1B (PTP1B) is to suppress insulin action.[2] Reducing PTP1B abundance in mice not only enhances insulin sensitivity and improves glucose metabolism, but also protects against obesity induced by high-fat feeding.[3] Inhibition of PTP1B in insulin sensitive tissues using novel antisense oligonucleotides has shown enhanced insulin signaling and glucose tolerance in preclinical models.[4] Small molecule PTP1B inhibitors may find an important clinical role as novel insulin sensitizers in the treatment of type II diabetes.[5]

T-cell PTPase (TCPTP), the most homologous phosphatase to PTP1B known to date, is implicated in regulating T-cell activation.[6] Therefore, selective inhibition of PTP1B without antagonizing TCPTP is highly desirable for an anti-diabetic agent. With one exception, most of the small molecule PTP1B inhibitors reported so far have not addressed the issue of achieving selectivity over TCPTP. Zhang and co-workers have proposed a new paradigm for designing highly potent and selective PTP1B inhibitors, based on the presence of a second phosphotyrosine-binding site (site 2) positioned adjacent to the active site, which lies within a region that is not conserved among PTPases.[7] Recently published results from the same group have confirmed the feasibility of such approach.[8]

Compound **1**, an oxalyl-aryl-amino benzoic acid, was discovered by a NMR-based fragment screening as a novel PTP1B inhibitor with a K_i value of 93 μM from this laboratory.[9,10] Subsequent structure-based drug design approach facilitated the discovery of a relatively potent inhibitors of PTP1B, as represented by compound **2** ($K_i = 0.99$ μM).[9] X-ray crystal structure of the complex between PTP1B and **2** demonstrated that the di-carboxylic acid portion of the molecule binds in the catalytic site of PTP1B as a novel phosphotyrosine mimetic.[9] These compounds exhibited good selectivity for PTP1B over most of the phosphatases in our selectivity panel, such as SHP-2, LAR and CD45. However, they do not discriminate between PTP1B and TCPTP. Analogous to Zhang's approach, we have expanded beyond the di-carboxylic acid pharmacophore anchored in the catalytic site to interact with site 2, and identified PTP1B inhibitors with greater affinity and moderate selectivity over TCPTP.

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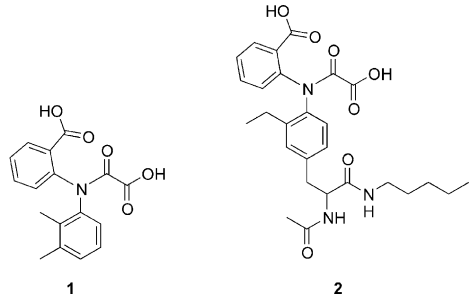
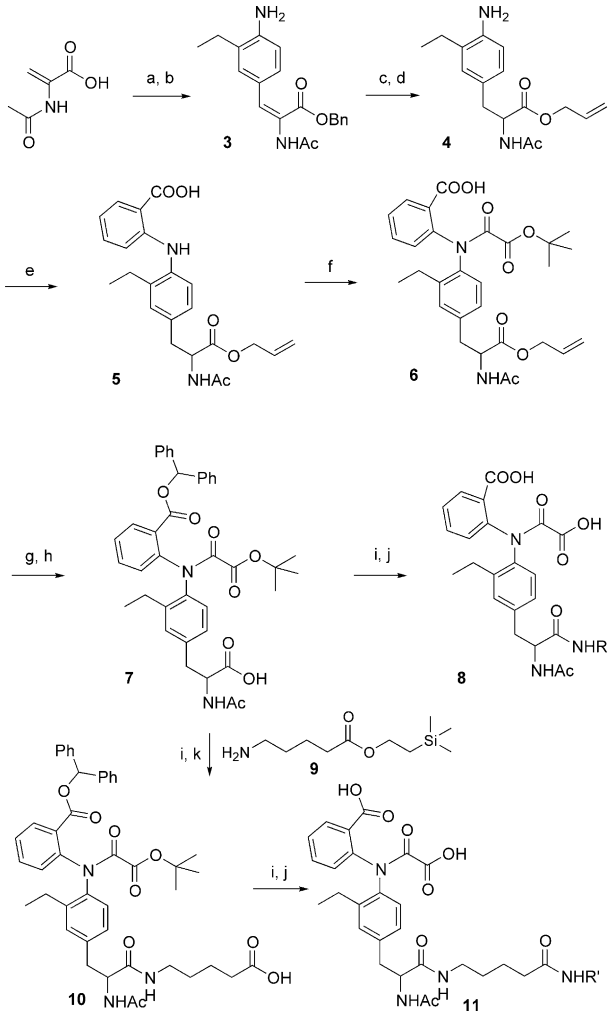
0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00302-0



1 2

X-ray crystal structure **2** also revealed two hydrogen-bonding interactions with Asp48 of PTP1B that are critical for the enhanced affinity.[9] These interactions also orientated the terminus of the pentyl amide of **2** within site 2. Therefore, we developed a solution phase parallel synthesis route to rapidly screen ligands for site 2 of PTP1B using amide-coupling chemistry. Benzyl 2-acetamidoacrylate was coupled via Heck reaction with 4-bromo-2-ethylaniline to give dehydroamino ester **3** (Scheme 1). The double bond was saturated and the benzyl ester of **3** was cleaved via hydrogenation to give the *p*-aminophenylalanine derivative, which was then protected as its allyl ester **4**. Condensation of **4** with DPIC generated benzoic acid **5**.[11] The diphenylaniline was then acylated to provide the *t*-butyloxalyl ester **6**, and the benzoic acid moiety of **6** was protected as its benzhydryl ester.[12] The allyl ester was then removed to liberate the acid core **7** for coupling with different amines. Final deprotection of the two acid-sensitive esters with trifluoroacetic acid provided diacid **8**.[13] Coupling of acid **7** with amino pentanoic acid derivative **9** followed by TBAF treatment of the resulting amide generated acid **10** for the library synthesis.

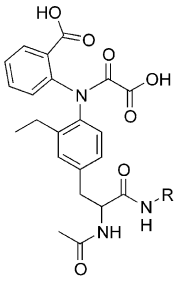


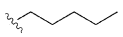
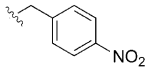
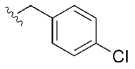
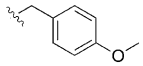
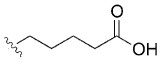
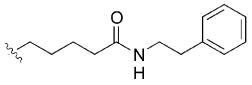
Scheme 1. Reagents and conditions: (a) BnBr, K_2CO_3, DMF, rt, 100%; (b) 4-bromo-2-ethylaniline, $Pd(OAc)_2$, (*o*-Tol)$_3$P, Et_3N, MeCN, reflux, 58%; (c) 10% Pd/C, 4 atm H_2, rt, 100%; (d) allyl bromide, Cs_2CO_3, rt, 62%; (e) diphenyliodonium carboxylate, $Cu(OAc)_2$, *i*-PrOH, 80 °C, 85%; (f) ClCOCO*t*-Bu, *i*-Pr_2NEt, CH_2Cl_2, 0 °C–rt, 72%; (g) diphenyldiazomethane, acetone, rt, 92%; (h) $Pd(Ph_3P)_4$, morpholine, CH_2Cl_2, 100%; (i) TBTU, H_2NR, *i*-Pr_2NEt, DMF, 72–94%; (j) TFA, anisole, CH_2Cl_2, 58–88%; (k) TBAF, THF, 94%.

We explored extensively the SAR of the pentyl amide of **2** with the objective to find better linkers for accessing site 2. Among over 300 compounds synthesized, only benzyl amines with *para* electron withdrawing substituents, such as nitro (**8a**) or chlorine (**8b**), appeared to be a good replacement of the amyl amine, while electron donating methoxy group yielded less potent analogue **8c** (Table 1). However, further SAR on benzyl amides failed to provide any improvement on PTP1B inhibitory potency and selectivity against TCPTP (data not shown). In the ensuing efforts of searching for site 2 ligands, it was decided to use the more flexible aminopentanoic acid (**8d**) as the linker, since the initial phenethyl amide **8e** demonstrated reasonable potency against PTP1B. More importantly, a key hydrogen-bond between the carbonyl of the phenethyl amide of **8e** and Gln262 of PTP1B was observed by X-ray crystallography.

In order to rapidly identify suitable site 2 ligands, different amines were coupled to the readily available acid core **10** in a parallel fashion. The presence of two arginine residues (Arg24 and Arg254) in the vicinity of the phenethyl amide portion of **8e** suggested that a negatively charged acid functionality might be preferred as part of site 2 ligands. Such preference is clearly reflected by the analogues listed in Table 2. Analogues with neutral groups, such as **8e** and **8f**, exhibited slightly decreased potency than compound **2**. Phenylalanine-based **12** with an extra acid functionality gained 24-fold in potency compared to **8e**. This critical observation prompted us to screen a library of amino acids. With different L-amino acids coupled to the core template, most of the compounds retained submicromolar potency, suggesting a rather tolerant site 2 (**13**–**20**). It appears that amino acids with the more lipophilic side chains (**17**–**19**) were slightly more favored than the ones with hydrophilic side chains (**15**–**16**, **20**). *Remarkably, compound **18** and **19** not only showed improved binding affinity, but also for the first time, exhibited some moderate selectivity against TCPTP (5-fold)*. Again, the importance of the acid functionality for site 2 ligands is confirmed with ester **21**, which is 7 times less potent than the corresponding acid **19**. Additionally, the stereochemistry of the amino acid played an important role in anchoring the acid to the right position: the *R*-isomer (**22**) is about 20-fold less potent than its *S*-isomer. Neither **21** nor **22** is selective for PTP1B over TCPTP.

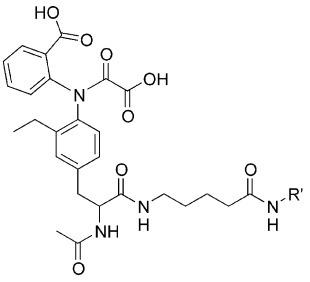
Table 1. SAR on modification of the pentyl amide of **2**

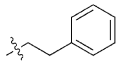
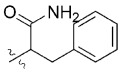
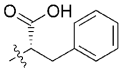
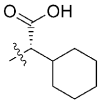
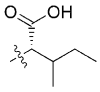
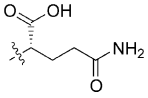
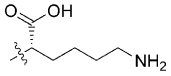
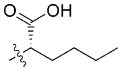
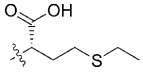
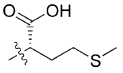
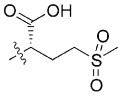
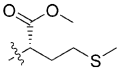
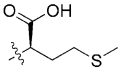


Compd	R	K_i (μM[b]) (PTP1B)	K_i (μM[b]) (TCPTP)
2[a]		1.2 (±0.3)	0.9 (±0.1)
8a[a]		0.54 (±0.14)	0.45 (±0.14)
8b[a]		1.2	1.5
8c[a]		4.7 (±1.6)	3.6
8d[a]		2.5 (±0.2)	2.2 (±0.3)
8e[a]		3.4 (±0.9)	1.4 (±0.3)

[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.

Table 2. SAR on site 2 ligand exploration



Compd	R′	K_i (μM) (PTP1B)	K_i (μM) (TCPTP)
8e		3.4 (±0.9)	1.4 (±0.3)
8f		1.3 (±0.4)	0.7 (±0.2)
12[a]		0.14 (±0.02)	0.16 (±0.02)
13[a]		0.25 (±0.10)	0.29 (±0.03)
14[a]		0.43 (±0.2)	0.40 (±0.1)
15[a]		0.33 (±0.16)	0.27 (±0.09)
16[a]		0.71 (±0.28)	0.82 (±0.22)
17[a]		0.12 (±0.07)	0.24 (±0.07)
18[a]		0.13 (±0.01)	0.51 (±0.15)
19[a]		0.076 (±0.015)	0.38 (±0.078)
20[a]		0.47 (±0.19)	0.37 (±0.08)
21[a]		0.54 (±0.19)	0.67
22[a]		1.7 (±0.7)	1.0 (±0.3)

[a]1:1 mixture of diastereomers.

The selectivity of **19** against other phosphatases in our selectivity panel was also confirmed, including SHP-2, LAR, CD45, and cdc25C (Table 3). In general, **19** is at least 100-fold selective for these less homologous phosphatases.

The X-ray crystal structure of PTP1B complexed with **19**[14] demonstrated unambiguously that extends the full span of catalytic site and site 2 (Fig. 1). With **19** bound to PTP1B, the conformation of the WPD loop of PTP1B remains open, unlike most of the other complex structures reported.[15] The oxamic acid and benzoic acid of **19** bind in the catalytic site as a novel phosphotyrosine mimetic.,The diamides of the alanine linker hydrogen bond with Asp48, leading the methionine to site 2, where the carboxylic acid of methionine in compound **19** interacts with Arg24 and the carbonyl group of adjacent amide interacts with Gln262. The lipophilic

Table 3. Phosphatase selectivity profile of compound **19**

K_i (μM) (PTP1B)	K_i (μM) (TCPTP)	K_i (μM) (SHP-2)	K_i (μM) (LAR)	K_i (μM) (CD45)	K_i (μM) (cdc25C)
0.076 (±0.015)	0.38 (±0.078)	73.17	8.75 (±8.84)	>300	>300

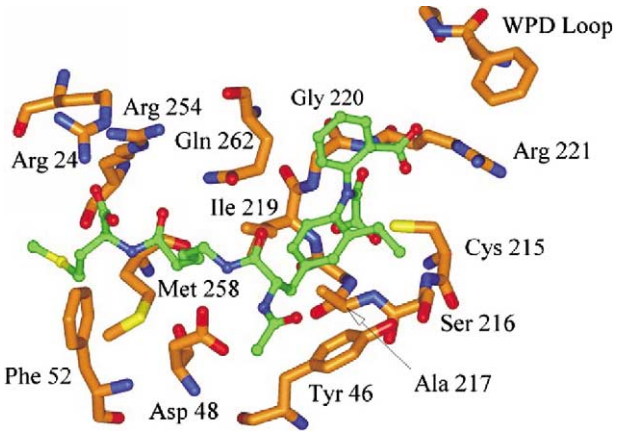


Figure 1. X-ray structure (2.4 Å resolution) of PTP1B complexed with **19**. Color scheme: carbons in orange and green for compound **19**, oxygen in red, nitrogen in blue, and sulfur in yellow.

side chain of the methionine lies on top of a largely hydrophobic region of the protein, consisted of Met258, Phe52. The origin of TCPTP selectivity for **19** is not very clear, since **19** did not interact with any residues that are different between PTP1B and TCPTP, although the interaction with Arg24 and Met258 appear to be critical for achieving such selectivity.

In summary, we have discovered the first series of potent, non-phosphonic acid-containing PTP1B inhibitors with moderate specificity for PTP1B over TCPTP, the most homologous phosphatase to PTP1B. We utilized the strategy of occupying both the catalytic site and the nearby, less homologous, non-catalytic phosphotyrosyl binding site. This series of PTP1B inhibitors identified the residues important for achieving TCPTP specificity in the absence of any structural information,[16] and provided us with opportunities for designing more potent and selective PTP1B inhibitors with improved physical properties.

References and Notes

1. (a) Saltiel, A. R.; Kahn, C. R. *Nature* **2001**, *414*, 799. (b) Ukkola, O.; Santaniemi, M. *J. Intern. Med.* **2002**, *251*, 467.
2. Cheng, A.; Dube, N.; Gu, F.; Tremblay, M. L. *Eur. J. Biochem.* **2002**, *269*, 1050.
3. (a) Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C. C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P. *Science* **1999**, *283*, 1544. (b) Klaman, L. D.; Boss, O.; Peroni, O. D.; Kim, J. K.; Martino, J. L.; Zabotny, J. M.; Moghal, N.; Lubkin, M.; Kim, Y.-B.; Sharpe, A. H.; Stricker-Krongrad, A.; Shulman, G. I.; Neel, B. G.; Kahn, B. B. *Mol. Cell. Biol.* **2000**, *20*, 5479.
4. Zinker, B. A.; Rondinone, C. M.; Trevillyan, J. M.; Gum, R. J.; Clampit, J. E.; Waring, J. F.; Xie, N.; Wilcox, D.; Jacobson, P.; Frost, L.; Kroeger, P. E.; Reilly, R. M.; Koterski, S.; Opgenorth, T. J.; Ulrich, R. G.; Crosby, S.; Butler, M.; Murray, S. F.; Mckay, R. A.; Bhanot, S.; Monia, B. P.; Jirousek, M. R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11357.
5. Blaskovich, M. A.; Kim, H.-O. *Exp. Opin. Ther. Pat.* **2002**, *12*, 871.
6. You-Ten, K. E.; Muise, E. S.; Itié, A.; Michaliszyn, E.; Wagner, J.; Jothy, S.; Lapp, W. S.; Tremblay, M. L. *J. Exp. Med.* **1997**, *186*, 683.
7. 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.
8. Shen, K.; Keng, Y. F.; Wu, L.; Guo, X. L.; Lawrence, D. S.; Zhang, Z.-Y. *J. Biol. Chem.* **2001**, *276*, 47311.
9. Liu, G.; Szczepankiewicz, B. G.; Pei, Z.; Janowick, D.; Xin, Z.; Liang, H.; Hadjuk, P. J.; Abad-Zapatero, C.; Hutchins, C. W.; Fesik, S. W.; Ballaron, S. J.; Stashko, M. A.; Lubben, T.; Mika, A. K.; Zinker, B. A.; Trevillyan, J. M.; Jirousek, M. R. *J. Med. Chem.* In press.
10. For in vitro assay protocols, see: Lubben, T.; Clampit, J.; Stashko, M.; Trevillyan, J.; Jirousek, M.R. In *Current Protocols in Pharmacology*; Enna, S. J., Williams, M., Ferkany, J. W., Kenakin, T., Porsolt, R. D., Sullivan, J. P. Eds.; Wiley: New York, 2001; p 3.8.1.
11. Scherrer, R. A.; Beatty, H. R. *J. Org. Chem.* **1980**, *45*, 2127.
12. Skelakatos, G. C.; Paganou, A.; Zervas, L. *J. Chem. Soc. C* **1966**, 1191.
13. The purity of the compounds tested exceeds 90% as determined by analytical HPLC.
14. Refined crystallographic coordinates for the structure of PTP1B complexed with compounds **19** have been deposited with the Protein Data Bank (www.rcsb.org) with entry codes 1NZ7.
15. (a) Andersen, H. S.; Olsen, O. H.; Iversen, L. F.; Sorensen, A. L. P.; Mortensen, S. B.; Christensen, M. S.; Branner, S.; Hansen, T. K.; Lau, J. F.; Jeppesen, L.; Moran, E. J.; Su, J.; Bakir, F.; Judge, L.; Shahbaz, M.; Collins, T.; Vo, T.; Newman, M. J.; Ripka, W. C.; Moller, N. P. H. *J. Med. Chem.* **2002**, *45*, 4443. (b) 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.
16. Co-crystalization of TCPTP with inhibitor has failed due to a multimerization process. See: Iversen, L. F.; Moller, K. B.; Pedersen, A. K.; Peters, G. H.; Petersen, A. S.; Andersen, H. S.; Branner, S.; Mortensen, S. B.; Moller, N. P. H. *J. Biol. Chem.* **2002**, *277*, 19982.