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Examination of Novel Non-Phosphorus-Containing Phosphotyrosyl Mimetics Against Protein-Tyrosine Phosphatase-1B and Demonstration of Differential Affinities Toward Grb2 SH2 Domains

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Abstract—Inhibitory potencies were compared of several mono- and dicarboxy-based *p*Tyr mimetics in Grb2 SH2 domain versus PTP1B assays. Although in both systems *p*Tyr residues provide critical binding elements, significant differences in the manner of recognition exist between the two. This is reflected in the current study, where marked variation in *relative* potencies was observed between the two systems. Of particular note was the poor potency of all monocarboxy-based *p*Tyr mimetics against PTP1B when incorporated into a hexapeptide platform. The recently reported high PTP1B inhibitory potency of similar phenylphosphate mimicking moieties displayed in small molecule, non-peptide structures, raises questions on the limitations of using peptides as platforms for *p*Tyr mimetics in the discovery of small molecule inhibitors.

Introduction

Intracellular generation of phosphotyrosyl residues (*p*Tyr, **1**) transmits cellular information by switching from low to high, the affinity of cytosolic tyrosyl-containing ligands for protein-binding modules such as src homology 2 (SH2) domains. Additional signal transducing enzymes that recognize endogenous ligands in a *p*Tyr-dependent manner are protein-tyrosine phosphatases (PTPs). Because for SH2 domains and PTPs, the *p*Tyr phenyl phosphate group provides key affinity elements, a search for phenyl phosphate mimicking moieties has been an important component of structure-based inhibitor design for both.[1,2] In these efforts several biscarboxy-based *p*Tyr mimetics have been reported that are recognized with high affinity by SH2 domains as well as PTPs, including *O*-malonyl tyrosine[3,4] (OMT, **2**), fluoro-*O*-malonyl tyrosine[5] (FOMT, **3**), and 3-carboxy-*O*-carboxymethyl tyrosine[6,7] **4** (Fig. 1). One disadvantage that these share with *p*Tyr is a formal (−2) charge at physiological pH. Since a consideration in the design of *p*Tyr mimetics is the minimization of charge as a means of enhancing cell membrane transport, a number of monocarboxy-based *p*Tyr mimetics have recently been examined in the context of SH2 domain inhibitors. Included among these are carboxymethyl tyrosine[8] **5**, carboxymethyl phenylalanine[8,9] **6** and carboxydifluoromethyl phenylalanine[8,9] **7**. Although some of these have shown moderately good potencies in SH2 domain systems, little has been reported on their affinities against PTPs. Accordingly, herein is detailed an examination of PTP1B inhibitory potencies of a range of mono and biscarboxy-based *p*Tyr mimetics, including mimetics which have shown high SH2 domain binding affinity.

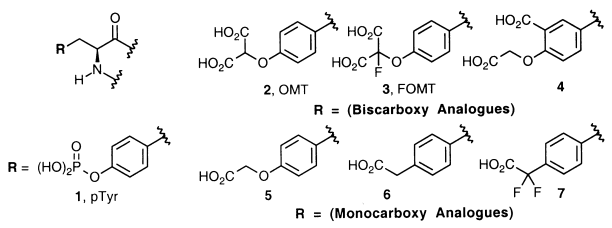

Figure 1.

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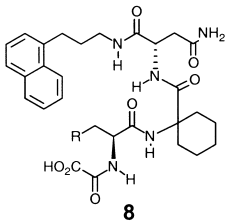
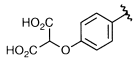
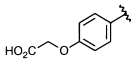
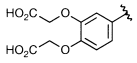
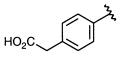
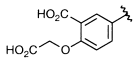
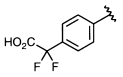
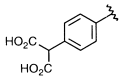
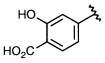
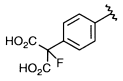
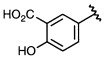
Table 1. Effect of phenylphosphate mimetics on Grb2 SH2 domain affinity[a]



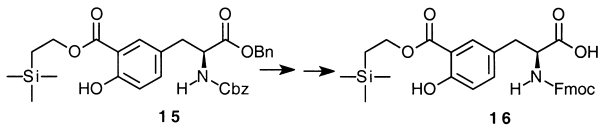
8

No.	R	IC_{50} (μM)	No.	R	IC_{50} (μM)
8a		1.1[b]	**8f**		15[d]
8b		>>100[c]	**8g**		0.6[d]
8c		1.3[c]	**8h**		2[d]
8d		0.07[b]	**8i**		≈50[e]
8e		0.17[b]	**8j**		>>100[e]

[a]IC_{50} values were determined using Grb2 SH2 domain fusion protein in either plasmon resonance or ELISA assays. Except for compounds **8i** and **8j**, values have been previously reported as indicated.
[b]Determined by ELISA as reported in ref 26.
[c]Determined by plasmon resonance as reported in ref 6.
[d]Determined by plasmon resonance as reported in ref 8.
[e]Determined by ELISA techniques according to procedures reported in ref 26.

Preparation of Inhibitors

Synthesis and Grb2 SH2 domain binding affinities of **8a–h** have been reported as referenced in Table 1. Preparation of isomeric salicyl-containing analogues **8i** and **8j** was according to general procedures previously described, using N^{α}-F_{moc}-protected amino acids **14** and **16**, respectively.[6] Synthesis of **14** relied on the coupling of benzylic bromide **11** (prepared from **10**) with commercially available Williams lactone **12**[10,11] to give **13**, which was hydrogenolytically deprotected to the free amino acid and reprotected in its N^{α}-Fmoc form **14** (Scheme 1).[8] Preparation of **16** utilized the previously

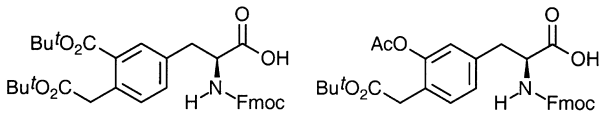


15 **16**

Scheme 2.

reported **15**,[7] which was hydrogenolytically deprotected then re-derivatized to its N^{α}-Fmoc form (Scheme 2).[12]

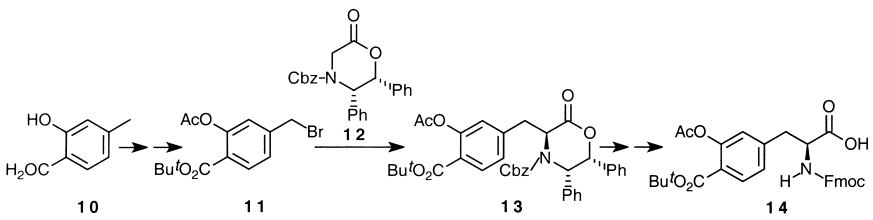
PTP-directed hexapeptides **9b–e** and **9i** were previously prepared using F_{moc}-based solid-phase techniques as reference in Table 1. Remaining hexapeptides were synthesized in similar fashion using N^{α}-F_{moc}-protected *p*Tyr mimetics having *tert*-butyl protection of side-chain carboxyl functionality. For **9f**,[13] **9g**,[13] **9j**,[14] and **9k**,[14] preparation of these residues has been described, while peptides **9n** and **9o** utilized analogues **14** and **16**, respectively (described above in Schemes 1 and 2). Peptides **9h** and **9l** were prepared using residues **17** and **18**, respectively, which were obtained by procedures similar to those described in Scheme 1.[12,15]



17 **18**

Results and Discussion

Recognition and binding of substrate by PTPs involves important interactions both internal and external to the catalytic cleft.[16] In principal, one structure-based approach to competitive PTP inhibitor design is the utilization of high affinity peptide sequences as display platforms for non-hydrolyzable *p*Tyr-mimicking residues. While maintaining peptide binding interactions outside the catalytic cleft, such display vehicles could potentially highlight structural motifs which bind within the catalytic cleft that could then be further incorporated into smaller non-peptide structures.[2] This approach has already proved useful in the identification of the 'difluorophosphonomethyl aryl' motif as a basis for small molecule PTP inhibitor design,[17] based on the finding that replacement of X = *p*Tyr in the EGFr-derived sequence, 'D-A-D-E-X-L'[18] with X = difluorophosphonomethyl phenylalanine (F_2Pmp),[19] turns a good substrate into a high-affinity inhibitor.[20] In theory, since the *p*Tyr structure presents critical recognition elements for binding of ligands to both SH2 domains and PTPs, *p*Tyr mimetics that show good affinity



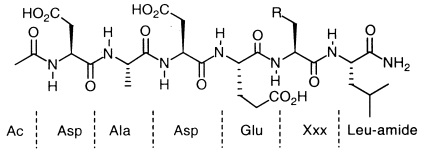
10 **11** **12** **13** **14**

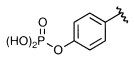
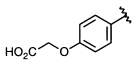
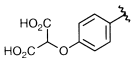
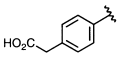
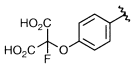
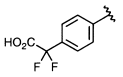
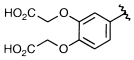
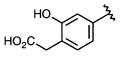
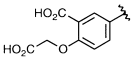
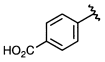
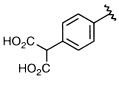
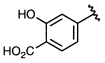
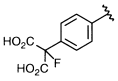
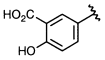
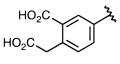
Scheme 1.

towards SH2 domains, could also potentially provide leads for PTP-binding motifs. In order to examine this hypothesis, several newly reported high affinity SH2 domain-binding *p*Tyr mimetics were examined in a PTP context. Since recognition and binding of *p*Tyr residues by Grb2 SH2 domains,[21] is highly homologous to other families of SH2 domains,[22] it served as a model system for investigating SH2 domain–*p*Tyr interactions for purposes of potential PTP lead identification.

As summarized in Table 1,[23] employing β-bend mimicking tripeptide **8**,[6] which is based on a potent Grb2 SH2 domain binding motif disclosed by Novartis,[24] the affinities of several series of carboxy-based *p*Tyr mimetics have recently been reported. Among these are analogues **8a**–**8e**, which utilize biscarboxy phenylphosphate replacements that show inhibitory potencies ranging from IC_{50} > 100 μM for **8b**, to $IC_{50} = 0.07$ μM for **8d**, depending on the arrangement of the carboxyl groups. A series of monocarboxy analogues **8f**–**8j** has also been examined, and in general these have been shown to exhibit less affinity than the dicarboxy-based compounds. The overall reduced potency of monocarboxy analogues **8f**–**8j**, which range from IC_{50} >100 μM for **8j**, to $IC_{50} = 0.6$ μM for **8g**, is consistent with the bidentate mode of native *p*Tyr binding, in which two positively charged Arg residues form ionic bonds to the doubly charged tyrosyl 4′-*O*-phosphate group.[21] It could be expected that a minimum of two anionic groups on the phosphate mimicking group would be required to take full advantage of the range of binding interactions displayed by the parent phenyl phosphate moiety. The moderate potency of monocarboxy analogue **8g** reflects the 4′-carboxymethyl group's ability to replace with good fidelity, one of the parent phosphate anionic oxygens.[25] Molecular modelling[8,25] further supports binding data which also indicates that the 4′-carboxymethylphenyl unit closely approximates the interaction of a single anionic phosphate oxygen with one arginine residue. Accordingly the high affinity of analogue **8d** (which is the most potent dicarboxylic-based inhibitor yet reported)[26] can be

Table 2. Effect of phenylphosphate mimetics on PTP binding affinity[a]



No.	R	IC_{50} (μM)	No.	R	IC_{50} (μM)
9a		$K_m = 3.2$[b]	**9i**		1200[g]
9b		10[c]	**9j**		2500
9c		1[d]	**9k**		650
9d		800[e]	**9l**		13,000
9e		19[f]	**9m**		4400
9f		1500	**9n**		5300
9g		430	**9o**		4600
9h		3700			

[a]Inhibition constants were determined as indicated in ref 18. Values for compounds **9a**–**9e** and **9i** have been previously reported.
[b]Ref 18.
[c]Ref 2.
[d]Ref 3.
[e]K_i previously reported as 199 μM; ref 7.
[f]K_i previously reported as 3.6 μM; ref 7.
[g]K_i previously reported as 480 μM; ref 7.

rationalized by the fact that its 4′-malonylphenyl structure can be viewed as a 4′(-carboxymethyl)phenyl group having a second carboxyl attached at the α-carbon.

Of note however are significant differences in the manner in which SH2 domains and PTPs interact with *p*Tyr residues.[16,22,27] While SH2 domains typically utilize two arginine residues to effect bidentate chelation of the $PO_3^{(-2)}$ group, PTPs employ a single arginine.[28] Because of this, in principle mono-charged *p*Tyr mimetics such as **5**, **6**, or **7** could potentially exhibit higher affinity in comparison to doubly charged mimetics such as **2**, **3**, or **4** in a PTP systems, relative to what is observed in SH2 domain binding systems. In light of these considerations, a series of 'D-A-D-E-Xxx-L'-based peptides having Xxx = *p*Tyr mimetics roughly paralleling those shown in Table 1, was examined against PTP1B (Table 2).[29] Peptides **9b**–**9e** containing dicarboxy-based *p*Tyr mimetics and **9i**, having a monocarboxy-based *p*Tyr mimetic have previously been reported (as indicated in Table 2). Of particular interest among the remaining newly reported peptides, is the poor affinity of 4-malonyl-based mimetic **9f**, which exhibited the highest Grb2 SH2 domain affinity of all analogues (Table 1). Here the dramatic loss of PTP potency incurred by removal of the ether oxygen (compare **9b** and **9f**) is in direct contrast to that observed in the SH2 domain system, where this resulted in significant binding enhancement (compare **8a** and **8d**; Table 1).[26] Equally interesting is that introduction of fluorine enhanced PTP affinity for dicarboxylic (**9b** to **9c** and **9f** to **9g**) as well as monocarboxylic (**9j** to **9k**) inhibitors, while the reverse was observed in SH2 domain binding potency (**8d** to **8e** and **8g** to **8h**).

Of particular interest are new monocarboxy-based *p*Tyr mimetics **9j**–**9o**, which had been anticipated to potentially exhibit good binding potency. Such an expectation was supported by the recent report of a high affinity non-peptide PTP1B inhibitor in which a carboxymethyloxyphenyl group was postulated to mimic a phenylphosphate moiety.[30] Surprisingly, in spite of the fact that carboxymethyl-based *p*Tyr mimetics showed reasonable SH2 domain binding affinity (**8g** and **8h**; Table 1), neither **9j** nor **9k** bound well to PTP1B, nor was potency improved by adding *ortho*-substituted functionality (carboxy, **9h** or hydroxy, **9l**). The poor affinity of carboxydifluoromethyl analogue **9k** is consistent with recent observations that 2-(carboxydifluoromethyl) naphthalene is poor inhibitor of PTP1B relative to the corresponding difluoromethylphosphonate.[31] Finally, the less extended monocarboxy analogue **9m** as well as the isomeric salicyl-based *p*Tyr mimetics **9n** and **9o** also showed extremely poor affinity. The failure of these analogues to bind, particularly in light of recently reported high affinity of small molecule inhibitors bearing similar phenylphosphate mimicking structures,[32] raises questions regarding the limitations of peptides as *p*Tyr mimetic display platforms for the discovery of small molecule PTP inhibitors. Although such peptide-based agents allow the study of interactions within the catalytic site while maintaining peptide binding outside the *p*Tyr pocket, they do so at the cost of limiting the orientation and insertion depth of the *p*Tyr mimicking side chain. A potential consequence of such limitations is that phenyl phosphate-mimicking moieties found inactive in a peptide context, might exhibit much higher affinity when expressed in a small molecule setting that allows greater freedom for orientation within the catalytic pocket.

In conclusion, the present study has demonstrated significant differences for relative affinities of carboxy-based *p*Tyr mimetics in two biologically important systems. It has also shown that while *p*Tyr mimetic-containing peptides may serve as valuable tools in small molecule PTP inhibitor discovery, results of such studies must be interpreted with caution, particularly in extending from peptide to small molecule contexts, low inhibitory potency found for *p*Tyr mimicking structures.

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References and Notes

1. Burke, T. R., Jr.; Yao, Z.-J.; Smyth, M. S.; Ye, B. *Curr. Pharm. Des.* **1997**, *3*, 291.
2. Burke, T. R.; Zhang, Z. Y. *Biopolymers* **1998**, *47*, 225.
3. Kole, H. K.; Ye, B.; Akamatsu, M.; Yan, X.; Barford, D.; Roller, P. P.; Burke, T. R., Jr. *Biochem. Biophys. Res. Commun.* **1995**, *209*, 817.
4. Ye, B.; Akamatsu, M.; Shoelson, S. E.; Wolf, G.; Giorgetti-Peraldi, S.; Yan, X. J.; Roller, P. P.; Burke, T. R. *J. Med. Chem.* **1995**, *38*, 4270.
5. Burke, T. R.; Ye, B.; Akamatsu, M.; Ford, H.; Yan, X. J.; Kole, H. K.; Wolf, G.; Shoelson, S. E.; Roller, P. P. *J. Med. Chem.* **1996**, *39*, 1021.
6. Yao, Z. J.; King, C. R.; Cao, T.; Kelley, J.; Milne, G. W. A.; Voigt, J. H.; Burke, T. R. *J. Med. Chem.* **1999**, *42*, 25.
7. Burke, T. R.; Yao, Z. J.; Zhao, H.; Milne, G. W. A.; Wu, L.; Zhang, Z. Y.; Voigt, J. H. *Tetrahedron* **1998**, *54*, 9981.
8. Burke, T. R., Jr.; Luo, J.; Yao, Z.-J.; Gao, Y.; Milne, G. W. A.; Guo, R.; Voigt, J. H.; King, C. R.; Yang, D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 347.
9. 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.; Betageri, R.; Cardozo, M.; Hickey, E. R.; Ingraham, R.; Jakes, S.; Kabcenell, A.; Kirrane, T.; Lukas, S.; Patel, U.; Proudfoot, J.; Sharma, R.; Tong, L.; Moss, N. *J. Med. Chem.* **1999**, *42*, 1757.
10. Williams, R. M.; Im, M.-N. *J. Am. Chem. Soc.* **1991**, *113*, 9276.
11. Both (+)- and (−)-enantiomers of the Williams reagent can be purchased from Aldrich Chemical Co.
12. All compounds provided analytical data consistent with their assigned structures.
13. Gao, Y.; Burke, T. R., Jr. *Synlett* **2000**, 134.
14. Yao, Z. J.; Gao, Y.; Voigt, J. H.; Ford, H.; Burke, T. R. *Tetrahedron* **1999**, *55*, 2865.
15. An additional step of treatment with *n*-butylamine is required to remove *O*-acetyl groups.
16. Jia, Z. C.; Barford, D.; Flint, A. J.; Tonks, N. K. *Science* **1995**, *268*, 1754.

17. Kole, H. K.; Smyth, M. S.; Russ, P. L.; Terrence, R.; Burke, J. *Biochemical J*. **1995**, *311*, 1025.
18. Zhang, Z. Y.; Maclean, D.; Mcnamara, D. J.; Sawyer, T. K.; Dixon, J. E. *Biochemistry* **1994**, *33*, 2285.
19. Burke, Jr., T. R.; Smyth, M.; Nomizu, M.; Otaka, A.; Roller, P. P. *J. Org. Chem*. **1993**, *58*, 1336.
20. Burke, T. R.; Kole, H. K.; Roller, P. P. *Biochem. Biophys. Res. Commun*. **1994**, *204*, 129.
21. Rahuel, J.; Gay, B.; Erdmann, D.; Strauss, A.; Garcia-Echeverria, C.; Furet, P.; Caravatti, G.; Fretz, H.; Schoepfer, J.; Grutter, M. G. *Nature Struct. Biol*. **1996**, *3*, 586.
22. Zhou, Y. Y.; Abagyan, R. *Fold. Des*. **1998**, *3*, 513.
23. Conditions for plasmon resonance and ELISA assays have been optimized, and both provide the same IC_{50} value (1 μM) for a common pSHC peptide standard. Likewise, repetitive measurement of compound **8d** in both assays ($n>10$), provided nearly identical IC_{50} values (0.071 and 0.067 μM, respectively), indicating that results from both methods are comparable.
24. Furet, P.; Gay, B.; Caravatti, G.; Garcia-Echeverria, C.; Rahuel, J.; Schoepfer, J.; Fretz, H. *J. Med. Chem*. **1998**, *41*, 3442.
25. Tong, L.; Warren, T. C.; Lukas, S.; Schembri-King, J.; Betageri, R.; Proudfoot, J. R.; Jakes, S. *J. Biol. Chem*. **1998**, *273*, 20238.
26. Gao, Y.; Luo, J.; Yao, Z.-J.; Guo, R.; Zou, H.; Kelley, J.; Voigt, J. H.; Yang, D.; Burke, Jr. T. R. *J. Med. Chem*. in press.
27. Burke, Jr., T. R.; Gao, Y.; Yao, Z.-J. In *Biomedical Chemistry: Applying Chemical Principles to the Understanding and Treatment of Disease*; Torrence, P. R., Ed.; John Wiley & Sons: New York, 2000; pp 189–210.
28. Hoff, R. H.; Wu, L.; Zhou, B.; Zhang, Z.-Y.; Hengge, A. C. *J. Am. Chem. Soc*. **1999**, *121*, 9514.
29. The IC_{50} values reported in Table 2 were measured at 2 mM pNPP concentration (close to the K_m), while the values reported in footnotes e, f, and g are K_i values. Although IC_{50} values are substrate concentration dependent and may not equal the K_i, at fixed substrate concentrations, they provide a measure of relative affinity.
30. Wrobel, J.; Sredy, J.; Moxham, C.; Dietrich, A.; Li, Z. N.; Sawicki, D. R.; Seestaller, L.; Wu, L.; Katz, A.; Sullivan, D.; Tio, C.; Zhang, Z. Y. *J. Med. Chem*. **1999**, *42*, 3199.
31. Kotoris, C. C.; Chen, M. J.; Taylor, S. D. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 3275.
32. Sarmiento, M.; Wu, L.; Keng, Y.-F.; Song, L.; Luo, Z.; Huang, Z.; Wu, G.-Z.; Yuan, A. K.; Zhang, Z.-Y. *J. Med. Chem*. **2000**, *43*, 146.