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Modification of the N-Terminus of Peptidomimetic Protein Tyrosine Phosphatase 1B (PTP1B) Inhibitors: Identification of Analogues with Cellular Activity

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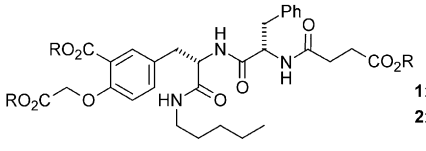
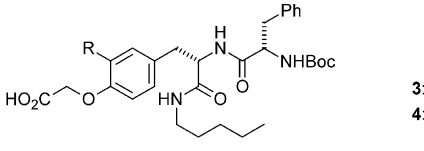
Abstract—Low molecular weight peptidomimetic compounds based on *O*-malonyl tyrosine and *O*-carboxymethyl salicylic acid are potent inhibitors of PTP1B. Modifications of the N-terminal Boc-Phe moiety were undertaken in an effort to improve physical chemical properties and to achieve cellular activity. Although Phe ultimately proved to be the optimal N-terminal amino acid, several viable replacements for the Boc group were identified, two of which afforded analogues that were effective at enhancing the insulin-stimulated uptake of 2-deoxyglucose by L6 myocytes.

In Type II diabetes mellitus, the most prevalent form of the disease, tissues develop resistance to the actions of insulin even though, in most instances, the insulin receptors in those tissues are structurally normal and are in near normal abundance.[1] One strategy to combat this insulin resistance therapeutically is to maintain insulin receptors (IR) in the active tyrosine-phosphorylated form by inhibiting enzymes that catalyze IR dephosphorylation. Based on substantial evidence that protein tyrosine phosphatase 1B (PTP1B) catalyzes IR dephosphorylation and is involved physiologically and pathologically in terminating insulin signaling, this enzyme has emerged as an attractive therapeutic target.[2] A molecular mechanism that describes the interaction of PTP1B with activated IR has been proposed[3] and a variety of small molecule inhibitors of PTP1B have been described, some of which potentiate insulin action on cells or in animal models of diabetes.[4]

We recently reported the discovery that the simple tripeptide Ac-NH-Asp-Tyr(SO_3H)-Nle-NH_2 is a highly effective inhibitor of PTP1B ($K_i = 5$ μM).[5] An analogue program was subsequently pursued with the goal of attenuating peptidic character and improving physical chemical properties, resulting in the identification of small molecular weight competitive inhibitors incorporating carboxyl groups as surrogates for the tyrosine phosphate group.[6,7] Although some of these compounds exhibited K_i values in the submicromolar range (e.g., **1**), evidence for insulin-sensitizing activity in whole cells was lacking, with the exception of a triester prodrug **2** that effected modest augmentation of insulin-stimulated 2-deoxyglucose uptake into myocytes.[5] Recognizing that multiple carboxyl groups were likely precluding cell penetration, attempts were made to jettison the *N*-terminal carboxyl, leading to the discovery that the simple *N*-Boc analogue **3** retained significant activity against PTP1B.[6] An investigation of carboxyl bioisosteres was concurrently undertaken, ultimately resulting



1: R = H (K_i = 0.22 μM)
2: R = Me



3: R = CO_2H (K_i = 2 μM)
4: R = 5-tetrazolyl

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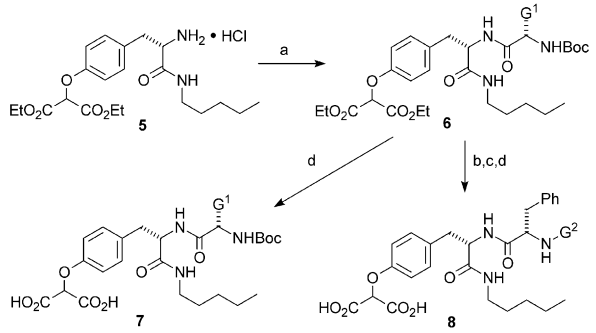
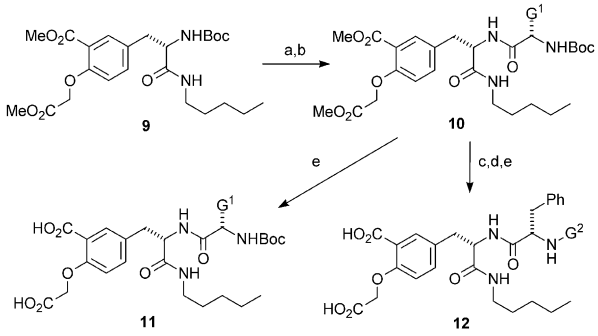
in the identification of a tetrazole-containing analogue **4** with modest cellular activity.[8]

The results of several X-ray co-crystal structures[5,6,8] indicated that the N-terminus was likely to be amenable to further manipulation. Inhibitor electron density was relatively diffuse in that area, suggesting that projection into solvent was occurring to some extent, and a guanidine moiety (Arg 47) was not being fully engaged by the Boc group.

Employing chemistry developed in our earlier work, the N-termini of two different templates (*O*-malonyl tyrosine and *O*-carboxymethyl salicylic acid) were probed via modification of the amino acid residue or the terminal *N*-substituent (Schemes 1 and 2). Amines **5** and **9**, prepared as previously described,[6] were neutralized or deprotected, respectively, under standard conditions prior to EDC-mediated coupling with various Boc-protected amino acids. The resulting amides **6** and **10** could be directly saponified to acids **7** and **11**, respectively. Alternatively, **6** and **10** could be *N*-deprotected as above, and the resulting free amines subsequently acylated or sulfonylated under standard conditions, affording analogues **8** and **12**. Most of the reagents used to functionalize the free amines were commercially available. Exceptions included the serine and homoserine derivatives used to prepare **37**, **39** and **40**,[9] the heterocyclic acids used to prepare **42**, **44** and **45**,[10] and the pyrrolidone phenylalanine derivative used to prepare **52**.[11] As described in our earlier work, all new



Scheme 1. Reagents and conditions: (a) L-Boc-NHCH(G^1)CO_2H, EDC, TEA; (b) HCl/HOAc; (c) G^2Cl, TEA or G^2OH, EDC, TEA; (d) LiOH, THF/H_2O.



Scheme 2. Reagents and conditions: (a) TFA/CH_2Cl_2; (b) L-Boc-NHCH(G^1)CO_2H, EDC, TEA; (c) HCl/HOAc; (d) G^2Cl, TEA or G^2OH, EDC, TEA; (e) LiOH, THF/H_2O.

compounds were assayed for their ability to inhibit a *C*-terminal truncated, soluble form of recombinant human PTP1B.[12] Specificity was determined by concurrent assays against two structurally dissimilar phosphatases, LAR and SHP-2. As a measure of the potential to enhance insulin sensitivity in cells, all active compounds were further evaluated for their ability to augment insulin-stimulated uptake of 2-deoxyglucose (2-DOG) into L6 myocytes.[12]

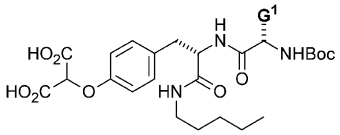
Table 1 summarizes a survey of amino acid replacements for Phe (**13**) within the malonate template **7**. Diverse residues were evaluated, in addition to residues judged to have the potential to interact with the guanidine moiety of Arg 47. Removing the aromatic ring of the phenylalanine (**14**) attenuated activity somewhat, suggesting that the aromatic ring is making some lipophilic interaction with Arg 47. Analogues replacing the aromatic ring with aliphatic residues (e.g., **21**, **24**, **25**) resulted in similar reductions in activity, as did those that moved the aromatic ring farther from the amide chain (**15**, **22**, **27**). Replacing the phenyl ring with indole (**28**) or phenol (**26**) was more promising, but neither analogue had significantly improved activity vs the lead **9**, suggesting that additional pi-stacking or electrostatic interactions with Arg 47 were not being achieved.

A preliminary investigation of N-termini was also undertaken within the malonate template (**8**, Table 2). Alterations included homologation (**32**), conversion of the Boc carbamate to a urea (**29**), replacement of the carbonyl with sulfonyl (**30**, **33**) and replacement of the *t*-butyl with aminoalkyl (**31**) or alkoxyalkyl (**34**). Only the alkoxyalkyl group offered some improvement, although again the magnitude does not suggest the establishment of a new binding interaction. Interestingly, some of these alterations appeared to improve the specificity for inhibition of PTP1B relative to LAR.

A more limited set of amino acid changes was examined in the *O*-carboxymethyl salicylic acid template (**11**, Table 3). Here, removal of the phenyl group (**36**) was even more detrimental than in the malonate template, and replacement with hydrogen bond donor or acceptor groups was not productive. Replacement of the phenyl with pyridine (**41**) similarly reduced activity. Only tyrosine (**35**) was a viable surrogate for phenylalanine, mirroring the result observed in Table 1 for the malonate template.

Table 4 summarizes an extensive investigation of the N-terminal substituents of the *O*-carboxymethyl salicylic template **12**. Compounds **42** – **45** represent attempts to replace the N-terminal carboxyl group of **1** with less acidic heterocyclic bioisosteres,[10] a modification that could be expected to enhance cell permeability. All proved to inhibit PTP1B more effectively than the Boc derivative **3**, with mercaptotetrazole **43** being the most potent ($K_i = 0.7$ μM), but cellular activity remained elusive. A highly lipophilic stearyl derivative **46** was prepared to try to achieve passive diffusion into cells, an approach that has proven effective for a peptidic PTP1B inhibitor,[13] but intrinsic activity was lost.

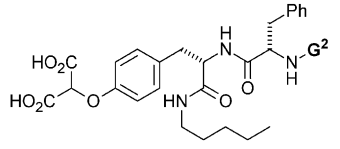
Table 1. Replacement of Phe in *O*-malonyl tyrosines (**7**)



Compd	G^1	% PTP1B inh @ 100, 10, 1 μM (K_i)	% LAR inh[a]	% SHP-2 inh[a]	% ctrl 2-DOG uptake[a,b]
13	Bn	91, 48, 7 (9 μM)	27	10	88
14	Me	83, 36, 7 (14 μM)	0	3	88
15	CH_2SBn	83, 16, 3	0	0	89
16	CH_2-2-napth	90, 29, 3	4	3	62
17	CH_2-1-napth	86, 33, 3	34	8	84
18	$(CH_2)_2SMe$	82, 25, 5	0	4	74
19	$(CH_2)_2SOMe$	67, 21, 7	0	6	70
20	$CH_2C_6F_5$	80, 25, 5	8	0	100
21	CH_2i-Pr	76, 24, 4	3	5	84
22	CH_2OBn	71, 19, 3	3	0	79
23	CH_2CONH_2	85, 40, 9	0	8	110
24	*i*Pr	72, 18, 3	0	0	
25	CH_2c-C_6H_{11}	88, 18, 1	4	0	93
26	CH_2p-HO-Ph	90, 50, 10	0	5	96
27	CH_2CH_2Ph	77, 17, 2	7	0	104
28	CH_2-3-indolyl	89, 44, 10	18	9	92

[a]Assayed at 100 μM.
[b]Uptake of 2-deoxyglucose into L6 myocytes in the presence of half-maximal concentration of insulin, expressed as a percent of the control.

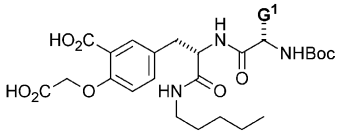
Table 2. Replacement of Boc in *O*-malonyl tyrosine inhibitors (**8**)



Compd	G^2	% PTP1B inh @ 100, 10, 1 μM (K_i)	% LAR inh[a]	% SHP-2 inh[a]	% ctrl 2-DOG uptake[a,b]
13	Boc	91, 48, 7 (9 μM)	27	10	88
29	CONH*t*-Bu	86, 40, 9	21	6	93
30	SO_2Me	86, 40, 9	0	8	93
31	$CO(CH_2)_2NEt_2$	92, 54, 12	0	14	89
32	$CO(CH_2)_2NHBoc$	93, 62, 14	2	8	87
33	SO_2CH_2Ph	62, 16, 4	6	10	98
34	$CO(CH_2)_2OMe$	94, 65, 19	6	11	104

[a]Assayed at 100 μM.
[b]Uptake of 2-deoxyglucose into L6 myocytes in the presence of half-maximal concentration of insulin, expressed as a percent of the control.

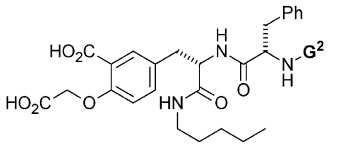
Table 3. Replacement of Phe in *O*-carboxymethyl salicylates (**11**)



Compd	G^1	% PTP1B inh @ 10, 1 μM (K_i)	% LAR inh[a]	% SHP-2 inh[a]	% ctrl 2-DOG uptake[a,b]
3	Bn	78, 30 (2.0 μM)	0	2	108
35	CH_2p-HO-Ph	81, 32	0	5	115
36	Me	43, 8	4	5	76
37	CH_2OMOM	51, 16	2		70
38	CH_2OH	41, 11	1	4	84
39	CH_2CH_2OMOM	42, 6	0	6	113
40	CH_2CH_2OH	45, 11	0	8	74
41	$CH_2$2-pyr	50, 10	2	1	106

[a]Assayed at 100 μM.
[b]Uptake of 2-deoxyglucose into L6 myocytes in the presence of half-maximal concentration of insulin, expressed as a percent of the control.

Table 4. Replacement of Boc in *O*-carboxymethyl salicylates (**12**)



Compd	G^2	% PTP1B inh @ 10, 1 μM (K_i)	% LAR inh[a]	% SHP-2 inh[a]	% ctrl 2-DOG uptake[a,b]
3	Boc	78, 30 (2.0 μM)	0	2	108
42	$COCH_2S$(1,2,4-triazol-3-yl)	82, 36	0	6	80
43	$COCH_2$(5-HS-1H-tetrazol-1-yl)	91, 57 (0.7 μM)	0	10	89
44	$COCH_2S$(1,2,3-triazol-5-yl)	88, 49	0	5	86
45	$COCH_2SO$(1,2,4-triazol-3-yl)	85, 40	0	17	92
46	$CO(CH_2)_{16}CH_3$	3, 1	0	54	
47	COMe	77, 28	0	13	95
48	$CO(CH_2)_2OMe$	83, 37	0	2	89
49	$CO(CH_2)_3OH$	78, 23	0	27	108
50	$CO(CH_2)_2NHBoc$	75, 19	0	19	88
51	$CO(CH_2)_2NHSO_2Me$	73, 15	10	25	81
52	NHG^2 = 2-pyrrolidon-1-yl	71, 24	0	5	95
53	CO_2Ph	85, 43 (0.87 μM)	0	11	103
54	$COCH_2$-3-indolyl	88, 47	0	38	73
55	$COCH_2Ph$	87, 45	0	18	73
56	$CO(CH_2)_2Ph$	74, 22 (1.4 μM)	0	12	136
57	$CO(CH_2)_3Ph$	70, 19	0	17	73
58	$COCH_2$-3-HO-Ph	86, 43	6	32	108
59	$COCH_2$-4-HO-Ph	88, 46	7	34	96
60	$COCH_2$-4-Me-Ph	85, 39	6	20	77
61	$COCH_2$-4-CF_3-Ph	85, 39 (0.59 μM)	0	28	**130**
62	$COCH_2$-4-MeO-Ph	87, 46 (0.67 μM)	5	25	**141**

[a]Assayed at 100 μM.
[b]Uptake of 2-deoxyglucose into L6 myocytes in the presence of half-maximal concentration of insulin, expressed as a percent of the control.

The activity of simple *N*-acetyl derivative **47** was essentially identical to the lead **3**, suggesting that the *tert*-butyl group is not participating in binding to PTP1B to any significant extent, a finding that is consistent with the structural data obtained for **3** in our previous work.[6] Installation of a 2-methoxypropanoyl group on the terminal nitrogen (**48**) did not afford the same magnitude in potency boost as was observed in the malonate template (**34**). Inclusion of more polar groups or homologating the Boc group (**49**–**51**) were similarly ineffective at improving potency or realizing cellular activity. A pyrrolidone analogue (**52**) was prepared to reduce molecular weight and to investigate the effect of removing the N-terminal hydrogen. No significant effect on activity was observed, consistent with earlier structural data that indicated an absence of hydrogen bonding between the N-terminal NH of **3** and PTP1B.

Replacement of the *tert*-butyl moiety of **3** with phenyl (**53**) provided the first neutral N-terminal *O*-carboxymethyl salicylate analogue possessing activity significantly better than the lead. Nevertheless, cellular activity with this compound still was not observed. Compounds **54** and **55**, bearing arylacetyl groups in lieu of Boc, were similarly active. Lengthening the alkyl chain by one (**56**) or two (**57**) carbons attenuated enzyme activity, suggesting that a one carbon tether is optimal for binding. Interestingly, although the phenyl propanoyl analogue **56** possessed less intrinsic activity than the phenylacetyl derivative **55**, some evidence for augmentation of insulin-stimulated 2-DOG uptake into L6 myocytes was observed (136% control, single experiment). Variously substituted aryl rings were then examined (**58**–**62**), including weakly acidic phenols with the potential to interact electrostatically with Arg 47. Although all of these analogues ultimately proved to be essentially equipotent to **55** as inhibitors of PTP1B, two analogues (**61** and **62**) are noteworthy because, despite poor adsorptive coefficients measured in Caco-2 cells (<2 nm/s), each reproducibly stimulated insulin-dependent glucose transport by L6 myocytes (Table 4). The 41% increase observed with **62** is almost half the increase in glucose transport expected when the insulin concentration is increased from 10 nM (at which the inhibitors are tested) to a saturating insulin concentration (300 nM). Although some erosion in specificity versus SHP-2 is observed upon replacement of the Boc group with arylacetyl groups, the specificity remains greater than 100-fold.

In conclusion, the N-termini of peptidomimetic PTP1B inhibitors **3** and **13** were modified by alteration of the amino acid residue or the N-terminal substituent in an attempt to modify the physical properties without diminishing binding affinity. Although many changes were tolerated with retention of enzyme-inhibitory activity, only the replacement of the Boc group with arylalkanoyl groups yielded compounds with superior activity and measurable cellular activity.

References and Notes

1. Kruszynska, Y. T.; Olefsky, J. M. *J. Invest. Med.* **1996**, *44*, 413.
2. Ukkola, O.; Santaniemi, M. *J. Int. Med.* **2002**, *251*, 467.
3. Salmeen, A.; Andersen, J. A.; Myers, M. P.; Tonks, N. K.; Barford, D. *Molec. Cell* **2000**, *6*, 1401.
4. (a) Blaskovich, M. A.; Kim, H.-O. *Expert Opin. Ther. Pat.* **2002**, *12*, 871. (b) Johnson, T. O.; Ermolieff, J.; Jirousek, M. R. *Nat. Rev.—Drug Discov.* **2002**, *1*, 696.
5. Bleasdale, J. E.; Ogg, D.; Palazuk, B. J.; Jacob, C. S.; Swanson, M. L.; Wang, W.-Y.; Thompson, D. P.; Conradi, R. A.; Mathews, W. R.; Laborde, A. L.; Stuchly, C. W.; Heijbel, A.; Bergdahl, K.; Bannow, C. A.; Smith, C. W.; Liljebris, C.; Schostarez, H. J.; May, P. D.; Stevens, F. C.; Larsen, S. D. *Biochemistry* **2001**, *40*, 5642.
6. 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.
7. Larsen, S. D.; May, P. D.; Bleasdale, J. E.; Liljebris, C. L.; Schostarez, H. J.; Barf, T. Preparation of substituted phenylalanine derivatives as protein tyrosine phosphatase inhibitors. WO 9911606, 11 March 1999.
8. Liljebris, C.; Larsen, S. D.; Ogg, D.; Palazuk, B. J.; Bleasdale, J. E. *J. Med. Chem.* **2002**, *45*, 1785.
9. **37**: prepared from MOM-protected L-Boc-serine, which was made by saponification of the corresponding methyl ester (Hanessian, S.; Ninkovic, S. *J. Org. Chem.* **1996**, *61*, 5418). **39**: prepared from MOM-protected L-Boc-homoserine, which was made from L-Boc-homoserine methyl ester (Howarth, N. M.; Wakelin, L. P. G. *J. Org. Chem.* **1997**, *62*, 5441) by protection with MOMCl (DIEA, DCM) followed by saponification. **40**: prepared from TBDMS-protected L-Boc-homoserine (Altmann, K.-H.; Chiese, C. S.; Garcia-Echeverria, C. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1119).
10. Drysdale, M. J.; Pritchard, M. C.; Horwell, D. C. *J. Med. Chem.* **1992**, *35*, 2573.
11. **52** was prepared as in Scheme 2 from *N*-(2-oxo-1-pyrrolidinyl)-L-phenylalanine (Ocain, T. D.; Deininger, D. D. US 5,023,338) instead of L-Boc-NHCH(G^1)CO_2H.
12. Initial rates of PTP-catalyzed hydrolysis of p-nitrophenyl phosphate (pNPP) were determined as described[5] and, where appropriate, K_i values were computed using the direct linear method of Cornish-Bowden (Enzpack 3, Biosoft, Cambridge, UK). The various PTP enzymes used were purified recombinant enzyme constructs of human origin (PTP1B, SHP-2, LAR).[5] Glucose transport by L6 myocytes (myotubes) was monitored as the initial rate of uptake of 2-[^{3}H]deoxyglucose using L-[^{14}C]glucose as a marker of the extracellular space. Effects of PTP1B inhibitors on glucose transport were examined at a concentration of insulin (10 nM) shown previously to elicit an approximate 50% maximal uptake.[5] Data were normalized to cell lysate protein recovered and are expressed as (net insulin-dependent glucose transport in the presence of PTP1B inhibitor–net insulin-dependent glucose transport in the absence of PTP1B inhibitor)×100%. A value of 120% or higher was considered a significant increase.
13. Kole, H. K.; Liotta, A. S.; Kole, S.; Roth, J.; Montrose-Rafizadeh, C.; Bernier, M. *J. Biol. Chem.* **1996**, *271*, 31619.