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Discovery and SAR of Novel, Potent and Selective Protein Tyrosine Phosphatase 1B Inhibitors

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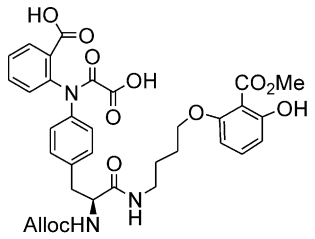
Abstract—A salicylate second site binder was linked to three classes of phosphotyrosine mimetics to produce potent protein tyrosine phosphatase 1B (PTP1B) inhibitors which exhibit significant selectivity against other phosphatases including the most homologous member, TCPTP.

Diabetes is becoming a prevalent disease in both US and other industrialized countries, among which type 2 diabetes is the most common. A distinguishing feature of type 2 diabetes is that the patients are insulin-resistant. The phosphorylation state of the insulin receptor (IR) is controlled by a balance between the relative activities of the insulin receptor kinase and cellular protein tyrosine phoshatases (PTPs). In type 2 diabetes, insulin receptor autophosphorylation and tyrosine kinase activity are impaired in skeletal muscle, fat and liver. PTP1B has been demonstrated to dephosphorylate IR in intact cells and to act as a negative regulator of insulin signaling.[1,2] The levels and activity of PTP1B have been found to be elevated in muscle and adipose tissue in insulin resistant states in both man and rodents. Recently, two independent labs have reported that PTP1B KO mice have increased insulin sensitivity and decreased weight gain on a high fat diet.[3] A potent and selective PTP1B inhibitor would be predicted to increase insulin sensitivity by blocking the negative effect of PTP1B-mediated dephosphorylation on substrates including IR and IR substrates (IRS) and therefore useful as therapeutics for type 2 diabetes.[4]

Since there are ~40 PTPs in the body, it is important that the inhibitors are selective for PTP1B over other PTPs in order to minimize potential side effects.[5] Phosphatases LAR,[6] CD45,[7] SHP-2,[8] cdc25c[9] and T-cell PTP (TCPTP)[10] share 51, 48, 52, 44 and 80% homology to PTP1B (homology in the catalytic domains), which presents a challenging task of achieving selectivity, especially TCPTP. Since the catalytic site of TCPTP and PTP1B are essentially identical, it was necessary for the inhibitor to interact with regions outside the catalytic site in order to be selective. A non-catalytic phosphotyrosine binding site (site 2) identified by Zhang and colleagues[11] seems to be ideal since the site is (1) close to the catalytic site; and (2) less homologous between the PTP1B and TCPTP when the amino acid sequences are compared. Based on these considerations, a salicylate-based ligand



1 Ki = 120 nM (against PTP1B)

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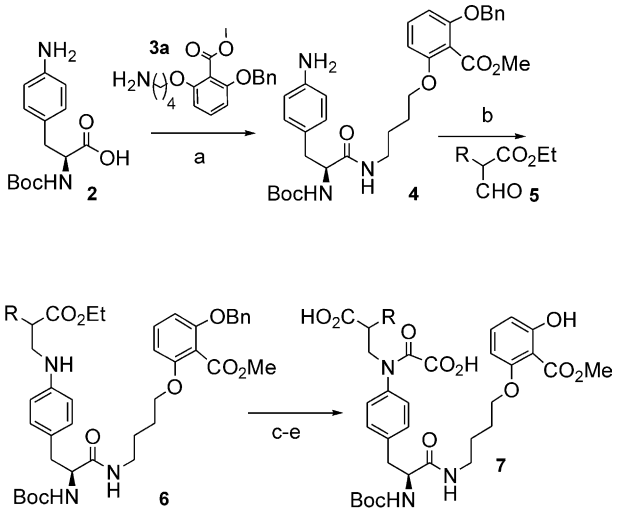
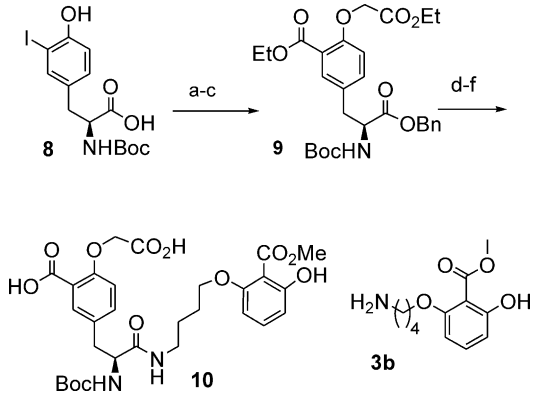
for site 2 was identified and then successfully linked to our benzoyl oxamic acid pharmacophore as exemplified by compound **1**.[12] Here we wish to report the SAR of our potent and selective PTP1B inhibitors with the second-site binder incorporated into other phosphotyrosine mimetics.

The preparation of oxamyl propionic acid is outlined in Scheme 1. Coupling of acid **2** and amine **3a** (synthesized by Mitsunobu coupling of methyl 2,6-dihydroxybenzoate and *N*-Boc-4-amino-1-butanol followed by benzyl protection of the phenol group) gave amide **4**, which underwent reductive amination with aldehyde **5** to yield amine **6**. Treatment of amine **6** with ethyl oxalyl chloride, hydrogenolysis of the benzyl group and basic hydrolysis of the ester groups afforded diacid **7**. The methyl ester group is very stable to the basic conditions due to steric hindrance.

A typical synthesis of (2-carboxy)phenoxyacetic acids is outlined in Scheme 2.[13] Protection of the acid group of iodide **8**, carbonylation at the iodide position, and



Scheme 1. Reagents and conditions: (a) TBTU, DMF; (b) $NaBH(OAc)_3$, $ClCH_2CH_2Cl$; (c) EtOCOCOCl, *i*$PrNEt_2$, DCM; (d) H_2, cat Pd/C; (e) NaOH, $EtOH/H_2O$.



Scheme 2. Reagents and conditions: (a) BnBr, $NaHCO_3$, Δ; (b) cat $Pd(dppf)Cl_2$, 4 atm CO, EtOH, 60 °C, 77%, two steps; (c) ethyl bromoacetate, K_2CO_3, Δ, 98%; (d) 1 atm H_2, cat Pd/C, EtOH, 100%; (e) isobutyl chloroformate, Et_3N, 0 °C, then **4**, THF, 70%; (f) NaOH, $EtOH/H_2O$.

alkylation of the phenol group with ethyl bromoacetate yielded triester **9**. Deprotection of the benzyl ester, amide coupling of the resulting acid with amine **3b**, and hydrolysis of the two ester groups yielded the final product **10**.

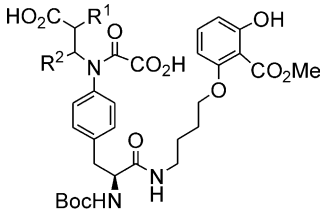
All the compounds were assayed at pH 7.5 using cloned and purified human PTP1B and *p*-nitrophenyl phosphate (pNPP) as the substrate. The inhibitory potency of the compounds was expressed in inhibitory constant (K_i) values, which was obtained as the X-intercept by linear regression of the plot of K_m/V_{max} versus inhibitor concentration. Kinetically, all the inhibitors described behave as reversible, competitive inhibitors.

The first series of analogues tested was the oxamyl propionic acids (Table 1). When there are no substituents attached to the propionic acid chain, the corresponding diacid **7a** lost potency completely. When a methyl (**7b**) or ethyl group (**7c**) was introduced to the α-position, the corresponding diacids had K_i's of 3.2 and 1.7 μM, respectively. When a phenyl group was introduced, the resulting diacid **7d** was not as potent, presumably due to the size of the phenyl group. A β-substituent is also tolerated and the corresponding diacid **7e** has a K_i of 1.8 μM.

Next we applied the same linking strategy to other known phosphotyrosine mimetics (Table 2).[14,15] To our delight, the linked diacid **10** turned out to be active with a K_i of 2.1 μM. However, when the carboxyl group was removed, the resulting monoacid **11** was not active. The protecting groups of Boc and Ac in this series make a 4.5-fold difference in potency (**12** vs **10**). When a methyl group was introduced at the α-position of the acetic acid moiety, the potency dropped 23 fold (**13** vs **10**). When a side chain of *N*-Boc-phenylalanine was introduced, the diacid **14** has a K_i of 0.16 μM. Another class of known phosphotyrosine mimetic is malonic acid.[16] When this moiety was introduced into our system, the resulting 2-oxymanolic acid **15** has a K_i of 3.2 μM.

As mentioned earlier, the selectivity of these inhibitors is of critical importance. A phosphatase panel was selected

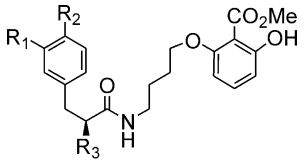
Table 1. K_i values (in μM) of oxamyl proprionic acids **7**[a]



Compd	R_1	R_2	K_i (PTP1B) (μM)[a]	K_i (TCPTP) (μM)[a]
7a	H	H	>200	>200
7b	Me	H	3.2	16.7
7c	Et	H	1.7	9.0
7d	Ph	H	15.2	97.8
7e	H	Et	1.8	9.3

[a]Values are means of two experiments.

Table 2. K_i values (in μM) of compounds **10–17**[a]



Compd	R_1	R_2	R_3	K_i (PTP1B) (μM)[a]	K_i (TCPTP) (μM)[a]	Ratio
10	CO_2H	OCH_2CO_2H	NHBoc	2.1	>30	>14.3
11	H	OCH_2CO_2H	NHBoc	220	>200	—
12	CO_2H	OCH_2CO_2H	NHAc	9.4	129.1	13.7
13	CO_2H	$OCHMeCO_2H$	NHBoc	48.6	273	5.6
14	CO_2H	$OCHMeCO_2H$	NH-(*N*-Boc-L-Phe)	0.16	1.97	12.3
15	H	$OCH(CO_2H)_2$	NHBoc	3.2	24.78	7.7
16	H	$OCH(CO_2H)CONH_2$	NHBoc	13.8	79.49	5.8
17	H	$OCH(CO_2H)COHN$	NHBoc	8.4	>200	>23.8

[a]Values are means of two experiments.

Table 3. Selectivities of PTP1B inhibitors (K_i values are in μM)

Compd	PTP1B	CD45	cdc25c	SHP-2	LAR
7c	1.7	>30	>30	>30	>30
14	0.18	>30	>30	>30	>30
15	3.2	>30	>30	>30	>30

to include both receptor (transmembrane) and non-receptor (intracellular or non-transmembrane) members such as CD45, LAR, cdc25 and SHP-2. To our delight, the inhibitors with the second-site binder incorporated are universally selective against all these enzymes (Table 3). Furthermore, our linking strategy has produced some of the most selective inhibitors against TCPTP, the most homologous enzyme to PTP1B.[17] For instance, benzyloxyl oxyacetic acids **10** and **14** were more than 14- and 12-fold selective against TCPTP, respectively. 2-Oxymalonic acid **15** was 7.7-fold selective against TCPTP. In contrast, the corresponding inhibitors without the second-site binder are not selective against TCPTP at all (data not shown).

We have soaked a selected number of compounds into crystals of PTP1B and obtained X-ray crystal structures of these inhibitors bound to PTP1B.[18] The inhibitors were soaked into crystals at 100 mM at pH 7.1 for one to 2 h in the presence of the anti-oxidant DTT. Shown in Figure 1 is the structure of **15**/PTP1B complex.[19] First, there are several hydrogen bond interactions between the ligand and the enzyme in the catalytic site. The oxygen atoms of one carboxylic acid interact with the side-chain NH of Gln 266 (3.1 Å), both main-chain and side-chain NHs of Arg 221 (2.9 and 3.1 Å). One of the two oxygen atoms of the other carboxylic acid interacts with main-chain NH's of Ser216 (2.8 Å) and Ala217 (2.9 Å). The two nitrogen atoms in the linker form bidentate interactions with the two oxygen atoms of the side-chain carboxylic acid of Asp48 (2.8 and 2.9 Å).

Second, the salicylate second-site binder makes several interactions with the enzyme as well. The ether oxygen atom interacts with the NH_2 of Gln262 (3.0 Å), the phenolic oxygen with the side-chain NH of Arg24 (2.9 Å). the carbonyl oxygen of the salicylate forms bidentate hydrogen bonds with the two side-chain NH's of Arg254 (3.1 and 3.6 Å). Unlike our benzoyl oxamic series,[12,20] the WPD loop (Trp179 to Ser187) of the enzyme is half-closed in the **15**/PTP1B complex. Overall, diacid **15** occupies both the catalytic site and the second site of PTP1B as predicted and it is believed that the interactions at the second site contributed to the selectivity against TCPTP.

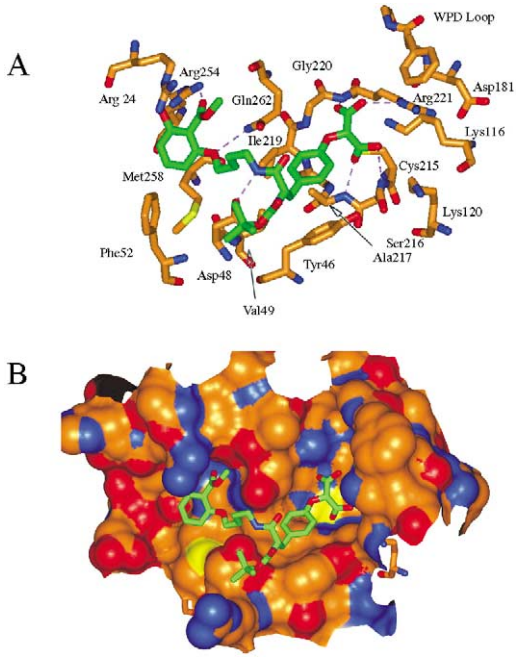


Figure 1. X-ray crystal structure of 15/PTP1B complex. Oxygen atoms are in red, nitrogen blue, carbon atoms of the ligand green. (A) Stick model with selected hydrogen bonds indicated by dashed lines; (B) enzyme with surface, showing the catalytic site (right) and second site (left).

Last, we have briefly examined the possibility of reducing the number of acid groups in the inhibitors, another major challenge in the PTP1B field. As the number of acid groups (which are ionized under physiological pH) increases, the chance for the inhibitors to penetrate the

cell membrane via passive diffusion is dramatically reduced. Our *O*-malonic acid system is chemically set up to investigate this possibility. When one of the acid groups is converted to an amide group, monoacid **16** displayed reduced potency against PTP1B with a K_i of 13.8 μM (Table 2). When a cyclopropyl is added to the primary amide, monoacid **17** maintained most of the potency of the corresponding diacid and has a K_i of 8.4 μM, and selectivity over TCPTP greater than 23.8-fold, which represent the most potent and most selective monoacid reported so far.

In summary, we have linked second-site binding salicylate to three different classes of phosphotyrosine mimetics and the resulting molecules are novel, potent and selective PTP1B inhibitors. Analogues derived from monoacid **17** are currently under investigation and inhibitors which display cellular activities will be reported shortly.

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