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SAR of non-hydrolysable analogs of pyridoxal 5′-phosphate against low molecular weight protein tyrosine phosphatase isoforms

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ABSTRACT

Kinases and phosphatases are key enzymes in cell signal transduction pathways. Imbalances in these enzymes have been linked to numerous disease states ranging from cancer to diabetes to autoimmune disorders. The two isoforms (IFA and IFB) of Low Molecular Weight Protein Tyrosine Phosphatase (LMW-PTP) appear to play a role in these diseases. Pyridoxal 5′-phosphate (PLP) has been shown to act as a potent but, impractical micromolar inhibitor for both isoforms. In this study, a series of non-hydrolysable phosphonate analogs of PLP were designed, synthesized and tested against the two isoforms of LMW-PTP. Assay results demonstrated that the best inhibitor for both isoforms was compound **5** with a K_{is} of 1.84 μM (IFA) and 15.6 μM (IFB). The most selective inhibitor was compound **16**, with a selectivity of roughly 370-fold for IFA over IFB.

Post translational modifications by kinases and phosphatases are an integral part of signal transduction pathways. Protein tyrosine kinases (PTKs) have been a target for drug discovery for a number of years. More recently, protein tyrosine phosphatases (PTPs) have emerged as a druggable target for such diseases as cancer, diabetes and autoimmune disorders.[1,2] There are over 100 enzymes in the PTP family that can be divided into four subgroups: classical pTyr specific, dual specificity, Cdc25, and low molecular weight phosphatases (LMW-PTP). LMW-PTP has received attention lately as a potential target for a variety of disorders ranging from cancer, to metabolic disorders, and even antibacterial therapies.[1,2]

LMW-PTP is active in the regulation of many important cell proliferation pathways including JAK-STAT, PDGF, and EPHA2. LMW-PTP is an 18 kDa acid phosphatase that has been isolated from a wide variety of eukaryotic and prokaryotic organisms. The mechanism(s) by which these pathways are controlled by LMW-PTP is not fully understood at this time. There are two known catalytically active isoforms of LWM-PTP; isoform A (also known as IF1 or the "fast" isoform) and isoform B (also known as IF2 or the "slow" isoform). Dysregulation of both isoforms of LMW-PTP has been observed in human breast, colon, prostrate, and cervical cancers as well as type II diabetes and insulin resistance.[3,4] The role that each isoform plays in these disease states is still under much debate but, the ability to modulate the activity of each has become a goal of several labs.[3,5,6] If a selective inhibitor for either isoform could be found, the role that each isoform plays in these disease pathways could then be better delineated.

Several studies have looked at screening libraries of compounds both virtually and *in vitro* to find selective, tight binding inhibitors for either isoform.[7,8,9] Both isoforms are structurally almost identical, which makes designing selective inhibitors difficult. Both isozymes have a conserved $12C(X)_5R18$ active site sequence and identical amino acid sequences except for residues 40–73 which are proximal to the phosphate binding-loop (P-loop) in the tertiary structure of the proteins.[10] These screening studies have provided new structural leads for inhibitors. One inhibitor, however, that has been known for some time is pyridoxal 5′-phosphate (PLP).[11]

PLP is a potent but, impractical inhibitor because of its physiological role in the body. PLP, the active form of vitamin B6, is a coenzyme in a variety of enzymatic reactions including transaminations, deaminations, decarboxylations, racemizations, and aldol cleavage reactions, just to name a few. It is involved in essential pathways such as the synthesis of neurotransmitters, amino acid metabolism, glycogen and lipid metabolism, and heme biosynthesis.[12,13,14] PLP has also been shown to be a poor substrate for both LMW-PTP isoform A (IFA) and B (IFB) and actually functions as a potent competitive inhibitor for both (K_i = 13 μM and K_i = 7.6 μM, respectfully).[11] In most other protein-PLP complexes that have been studied, covalent interactions occur between a lysine side chain and the aldehyde on PLP to form Schiff base intermediates. With LMW-PTP, there are no such covalent interactions observed with PLP. Zhou and Van Etten characterized the interactions

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of PLP with LMW-PTP IFB via ^{1}H NMR. It forms several key intermolecular interactions that lead to the tight binding of PLP and LMW-PTP IFB. [11] The pyridine nitrogen of PLP is protonated to give the pyridinium form of the ring which interacts with the deprotonated form of Asp-129 via ion-ion interactions. The phosphate group on PLP interacts with the P-loop through a series of hydrogen bonds. The hydroxyl and aldehyde groups on PLP may interact with residues close to the active site such as Asn-50 and Trp-49. Zhou and Van Etten proposed that these two groups act to increase the basicity of the pyridine nitrogen which favors the protonated form.

In the current study, several non-hydrolysable analogs of PLP were synthesized to probe which groups on PLP are essential to its binding and to determine if these requirements differ between the two isoforms of LMW-PTP. Results from these studies should be helpful in designing new selective isoform inhibitors.

Phosphonate derivatives of PLP were synthesized as previously described by Knobloch *et al.*[15] Synthetic schemes and experimentals are available in Supporting Information.

In addition, molecular modeling studies were performed using Auto Dock Vina (version 1.1.2). Protein Databank files for the crystal structures of human LWM-PTP isoform A (5PNT) and isoform B (1XWW) were used in docking studies with all 17 compounds plus PLP. Potential ligand-protein interactions were explored using Discovery Studio Visualizer (version 20.1.0.19295). Docking scores and 2-D ligand-protein interaction diagrams are available in Supporting Information.

Looking at the structural requirements for binding to IFA as presented in Tables 1 and 2, the pyridine nitrogen appears to be critical to binding. Comparing compounds **2** and **4** to compounds **3** and **5** there is an increase in selectivity from 5- to 70-fold, respectively, when the pyridine nitrogen is introduced as opposed to just a benzene ring. Modeling studies did not suggest any additional intermolecular interaction between the pyridine nitrogen and the protein. Modest results are seen when looking at compounds **10** and **11** (2-fold increase in binding preference). Interestingly, however, when compounds **12** and **13** are compared there is an approximate 3-fold bias for the benzene ring over the pyridine ring. This might suggest that the alkene phosphonates might bind differently in the active site or that the entropic gain from the restricted rotation assists in binding or that the pyridine nitrogen is not as critical for binding of the phosphonates as was suggested for PLP or potentially some combination of all of these factors.[11] Docking studies also suggest that there would be these slight preferences. Please see Supporting Information for docking scores.

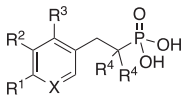
Introduction of a methyl group at R^1 position increases the selectivity from 4-fold (comparing compounds **2** and **4**) to 56-fold (comparing compounds **3** and **5**). According to modeling studies, this is probably due to hydrophobic interactions of the methyl group with tyrosines 131 and 132. However, when examining the alkene phosphonates, there is only a minimum increase in binding preference when a methyl group is introduced at the R^1 position. Again, this is suggestive of a potential alternative binding within the active site for these alkene phosphonates. Modeling studies suggest that the alkene phosphonates without a methyl group in the R^1 position bind within the active site but also possess an unfavorable donor-donor interaction between the phosphonate group and Gly14 and Leu13. The entropic decrease by having an alkene substituent might be offset by unfavorable ligand protein interactions and may lead to these mixed results.

As seen from the data in Tables 1 and 2, introduction of a hydrogen bonding moiety such as a hydroxyl group in position R^2, has a strong effect on binding. For the conformationally unrestrained phosphonates an increase in binding bias for the hydroxyl group ranged from 36-fold (comparing compounds **4** and **7**) to 42-fold (comparing compounds **2** and **6**). Modeling studies suggest that the hydroxyl group is potentially interacting with either tyrosine 132 (compound **6**) or glutamic acid 50 (compound **7**). The hydroxyl group in the restricted rotation alkene containing phosphonates gave mixed results. There was still a preference for the hydroxyl group when comparing compounds **10** and **14** (30-fold preference) but, very little when comparing compounds **12** and **15** (1.3-fold difference).

Introduction of a hydrogen bond acceptor or hydrogen bond acceptor/donor at the R^3 position increased the inhibitory ability of the compounds whether the phosphonates had free rotation or restricted rotation. Interestingly, there is a slight preference for a hydrogen bond acceptor when the phosphonate has restricted rotation and a hydrogen bond donor when the phosphonate has free rotation about the carbon-carbon bond. Modeling studies suggest that when there is free rotation about the carbon-carbon bond the hydrogen bond donor group in the R^3 position is able to form a conventional hydrogen bond with aspartic acid 129, whereas placing a hydrogen bond acceptor in this position results in simply a diploe/dipole interaction with aspartic acid 129. In the phosphonates with restricted rotation, aspartic acid 129 is still able to interact using dipole/diploe interactions with the hydrogen bond acceptor in R^3 position but, is not able to hydrogen bond with a hydrogen bond donor group in that position as is seen with those phosphonates with free rotation.

Those inhibitors that bind well to IFA are shown below in Fig. 1. They all essentially take advantage of similar interactions with the enzyme. The charged phosphonate head interacts with arginine 18 through ion/ion interactions. The rest of the phosphonate is held in place by a network of hydrogen bonding with residues Cys12, Cys17, Gly14, Ile 16 and Asn 15. The aromatic ring on the inhibitors interacts with tyrosine 131 via pi-stacking interactions. And finally, side chains in the R^1, R^2 and R^3 positions interact with residues Tyr132, Asp129,

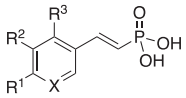
Table 1
In vitro activity of phosphonates against LMW-PTP isoforms



Compound	X	R^1	R^2	R^3	R^4	K_{is} IFA (μM)	K_{is} IFB (μM)	X Fold Selectivity for IFA
1	CH	H	H	H	F	359 ± 82	N.I.	> 28
2	CH	H	H	H	H	552 ± 278	N.I.	> 18
3	N	H	H	H	H	103 ± 36	1075 ± 293	10.4
4	CH	CH_3	H	H	H	130 ± 60	323 ± 75	2.5
5	N	CH_3	H	H	H	1.84 ± 0.47	15.6 ± 8.9	8.5
6	CH	H	OH	H	H	13.0 ± 1.7	52.2 ± 4.8	4
7	CH	CH_3	OH	H	H	3.59 ± 0.84	39.9 ± 4.6	11
8	N	CH_3	OH	CH_2OH	H	10.4 ± 2.1	552 ± 134	53
9	N	CH_3	OH	CHO	H	74.9 ± 26.1	218 ± 124	2.9

N.I. No inhibition where K_{is} > 10 mM.

Table 2
In vitro activity of alkene containing phosphonates against LMW-PTP isoforms



Compound	X	R^1	R^2	R^3	K_{is} IFA (μM)	K_{is} IFB (μM)	X Fold Selectivity for IFA
10	CH	H	H	H	482 ± 160	N.I.	> 21
11	N	H	H	H	243 ± 80	1070 ± 248	4.4
12	CH	CH_3	H	H	66.8 ± 20.2	323 ± 75	4.8
13	N	CH_3	H	H	213 ± 54	842 ± 243	4
14	CH	H	OH	H	15.7 ± 4.4	580 ± 141	37
15	CH	CH_3	OH	H	50.3 ± 24.9	42.4 ± 5.7	0.8
16	N	CH_3	OH	CH_2OH	16.3 ± 4.3	6060 ± 3910	372
17	N	CH_3	OH	CHO	5.14 ± 1.26	113 ± 24	22

N.I. No inhibition where K_{is} > 10 mM.

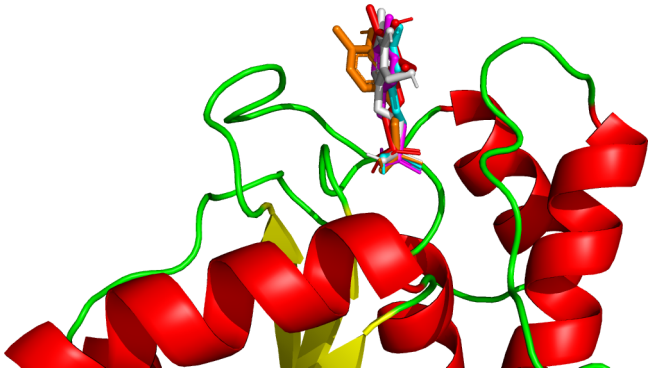


Fig. 1. Binding of compounds **5** (magenta), **6** (cyan), **7** (orange), **8** (grey) and **17** (red) to IFA.

Glu50, and Tyr49 to varying degrees.

Looking at the structural requirements for binding to IFB as presented in Tables 1 and 2, again the pyridine nitrogen plays a role in binding. Comparing compounds **2** and **4** to compounds **3** and **5** there is an increase in activity from 9- to 20-fold respectively when the pyridine nitrogen is introduced as opposed to just a benzene ring. Similarly, moderate results are seen when looking at compounds **10** and **11** (9-fold increase in binding preference). Again, modeling studies did not suggest any additional intermolecular interaction between the pyridine nitrogen and the protein. Interestingly, however, when compounds **12** and **13** are compared there is an almost 3-fold binding bias for the benzene ring over the pyridine ring.

As can be seen from the data presented in Tables 1 and 2, introduction of a methyl group at R^1 position increases the binding selectivity from 14-fold (comparing compounds **14** and **15**) to 30-fold (comparing compounds **2** and **4** or compounds **10** and **12**) to approximately 70-fold (comparing compounds **3** and **5**). Similar as with IFA, this is probably due to an additional hydrophobic interaction of the methyl group with tyrosine 131. However, there is only a minimum increase in selectivity when a methyl group is introduced at the R^1 position for compounds **11** and **13** or **6** and **7**. There is also a preference for a hydrogen bond acceptor in position R^3. In fact, when comparing compound **16** (hydrogen bond donor/acceptor) with compound **17** (hydrogen bond acceptor only) there is a 54-fold preference for the hydrogen bond acceptor group. Modeling studies did not show interactions with the protein and R_3 group from either of these inhibitors. Instead, the binding of the pyridine ring rotated between the two inhibitors changing what residues and the extent that the other substituents and the pyridine nitrogen interacted with.

Our results are in agreement with those of Zhou and Van Etten, showing that the pyridine nitrogen is important in the binding of PLP like compounds.[11] However, the role of the hydroxyl group is probably not to just increase the basicity of the pyridine nitrogen. When comparing compound **15**, which contains a benzene ring, to compounds **16** and **17**, which contain a pyridine ring, the presence of a hydroxyl group does not enhance activity when a pyridine nitrogen is present. Just the opposite was observed. Additionally, for the binding of PLP-like compounds to IFB, there is definitely a preference for a hydrogen bond acceptor in position R^3 as opposed to a hydrogen bond donor. As modeling studies suggest, this might lead to distinctly different orientations of these inhibitors within the active site. Additionally, the hydrophobic interactions at the R^1 position appear to play a more pronounced role in binding that was first proposed. In each case, the addition of a methyl group at the R^1 position enhanced the activity of the PLP-like compounds. The steric tolerance for this position was not examined but, will be considered for future studies.

Those inhibitors that bind well to IFB are shown below in Fig. 2. They all essentially occupy similar space and take advantage of similar interactions with the enzyme. The charged phosphonate head interacts with arginine 18 through ion/ion interactions. The rest of the phosphonate is held in place by a network of hydrogen bonding with residues Cys12, Cys17, and Ile 16. The aromatic ring on the inhibitors interacts with tryptophan 49 and tyrosine 131 via pi-stacking interactions. Hydroxyl groups in the R^2 position appear to hydrogen bond with tyrosine 132.

In general, the phosphonates were better inhibitors of LMW-PTP IFA than IFB. Additionally, the restricted rotation of the double bond

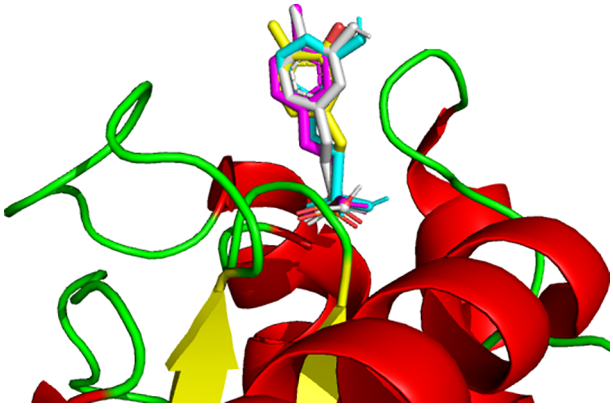


Fig. 2. Binding of compounds **5** (magenta), **6** (cyan), **7** (orange) and **15** (gray) to IFB.

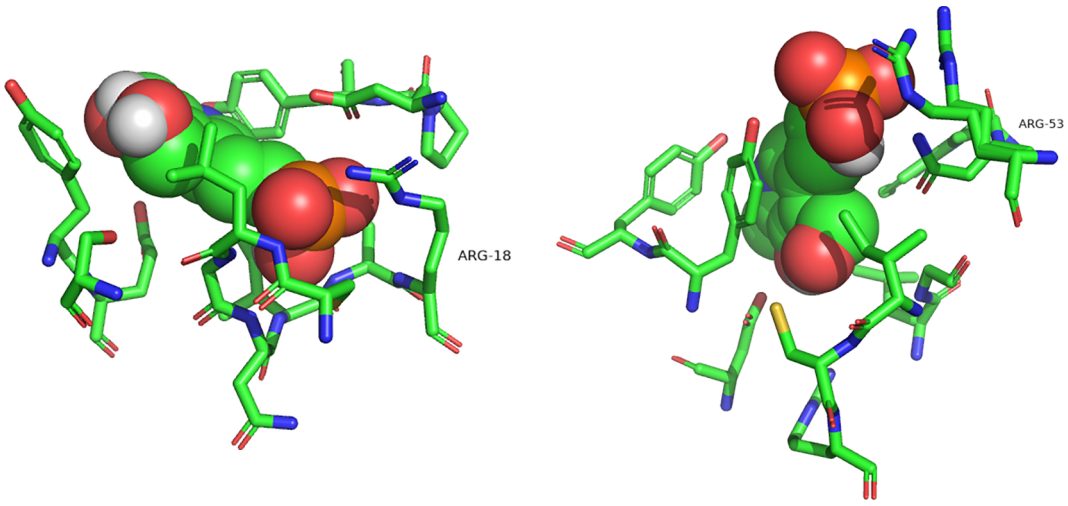


Fig. 3. Compound **16** bound to IFA (left) and compound **16** bound to IFB (right).

appeared to, in general, enhance the inhibitory effect of the phosphonates regardless of which isoform it was tested against. The best inhibitor for both IFA and IFB was compound **5** with a K_{is} of 1.84 μM and 15.6 μM, respectively. The most selective inhibitor was compound **16**, with a selectivity of roughly 370-fold for IFA (K_{is} 16.3 μM) over IFB (K_{is} 6060 μM). Modeling studies suggest that compound **16** binds differently in IFB than it does in IFA. The proposed binding of compound **16** to IFA and IFB is illustrated in Fig. 3. This indeed may be where the selectivity of compound **16** comes from. In IFA, compound **16** takes advantages of intermolecular forces within the active site as described earlier whereas, in IFB compound **16** appears to have flipped its orientation and the negatively charged phosphonate is bound to Arg53 and Asn50 on the periphery of the active site and the polar hydroxyl groups are orientated towards the active site.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127342.

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