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Modeling and synthesis of 3-(2,6-disubstituted 5-pyrimidyl) propionic acids as inhibitors of Low Molecular Weight Protein Tyrosine Phosphatase (LMW-PTP)

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Modeling and Synthesis of 3-(2,6-disubstituted 5-pyrimidyl)propionic acids as Inhibitors of Low Molecular Weight Protein Tyrosine Phosphatase (LMW-PTP)

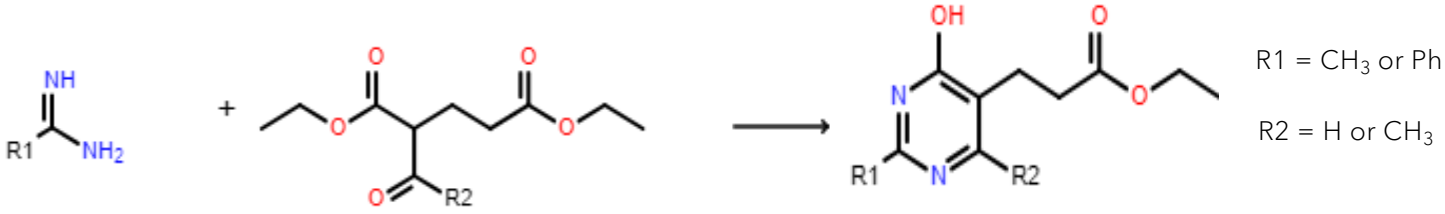
Kennedy Bueno, Madisen Carter, Edward McIntee*

Abstract

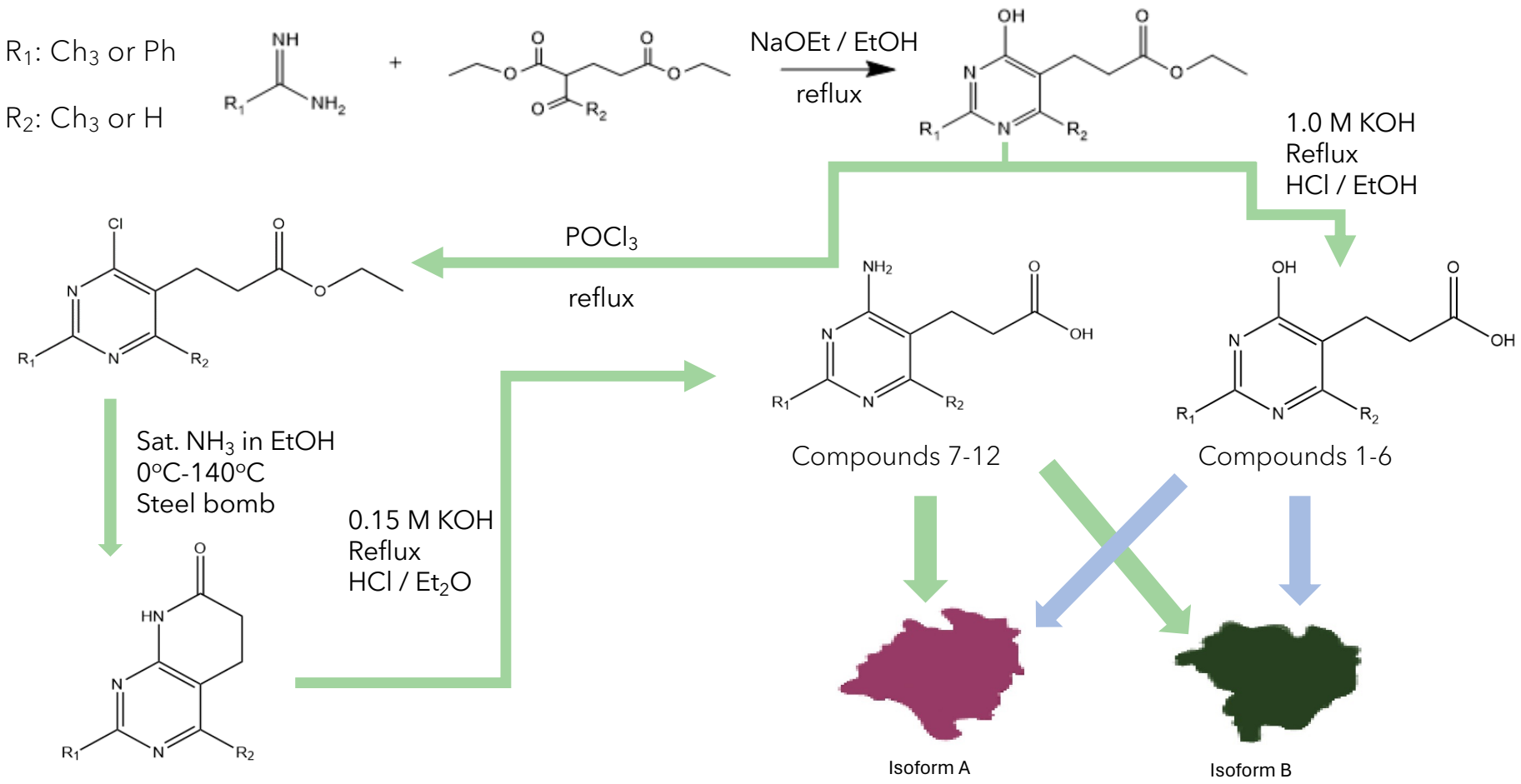
Low molecular weight protein tyrosine phosphatase (LMW-PTP) is an enzyme and a known signal pathway for growth factors and cellular transformation in eukaryotic cells. Our research aims to synthesize 12 new potential inhibitors of LMW-PTP and analyze their inhibitory activity and binding affinity. The inhibition of both isoforms of LMW-PTP has been the primary focus of research due to their potential role in breast, colon, and other cancers as well as type II diabetes. One known inhibitor, pyridoxal 5'- phosphate (PLP), is essential for various enzymatic reactions within the body, such as the synthesis of neurotransmitters, and is therefore impractical for inhibitory needs. Our current research involves performing computer modeling and the experimental synthesis of 3-(2,6-disubstituted 5-pyrimidyl) propionic acids as potential inhibitors of LMW-PTP. Amidine derivatives are condensed with molecules containing an aldehyde or ketone. These molecules undergo intramolecular cyclization and aromatization to form compounds A-F. The resulting 6 aromatic compounds are subjected to one reaction pathway where the ethyl ester is cleaved. The aromatic compounds can also be subjected to a second reaction pathway where the chloro esters are cyclized and alkaline hydrolysis of the lactams yields the potential inhibitors. All compounds were analyzed using IR, ^{13}CNMR, ^{1}HNMR, and COSY spectra. All compounds were docked into isoform A and isoform B of LMW-PTP to predict a binding affinity. The potential inhibitors were designed in the Spartan program in VMware Horizon Client and docked using SwissDock.

Background

- Low Molecular Weight Protein Tyrosine Phosphatase (LWM – PTP) is a known enzyme present in signal transduction pathways and important in cell growth and regulation.
- It been found to play a role in several types of cancers when the enzyme is abnormal (i.e. colon and breast cancer)
- Pyridoxal 5′-Phosphate (PLP) is a known inhibitor of LMW-PTP but is impractical due to its various roles in the body.
- Attempting the modeling and synthesis of 12 different potential inhibitors for LWM –PTP (isoforms A and B) to find a binding affinity comparable to the known inhibitor.
- Using a condensation and cyclization reaction below to make 6 starting compounds which will be further refined to inhibitors through other reactions.



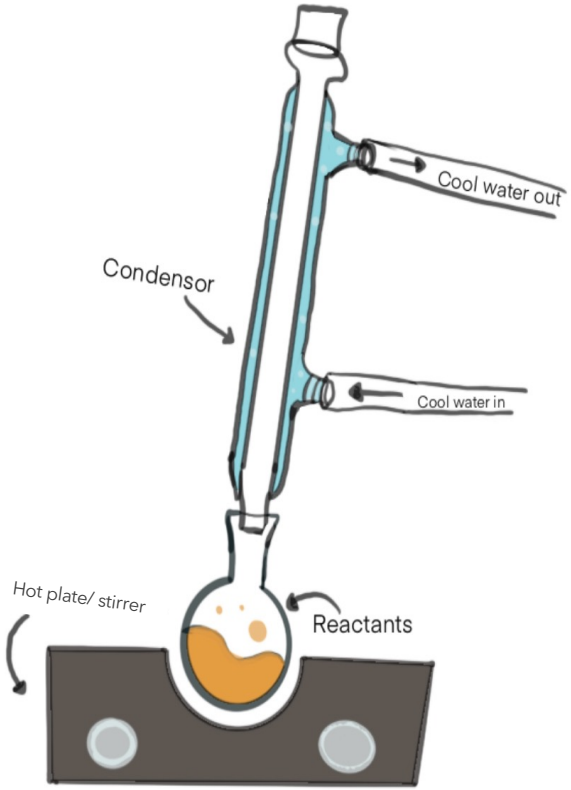
The two reaction pathways to synthesize the potential inhibitors of LMW-PTP



Methods

Reaction Methods:

- Equimolar amounts of starting material and sodium ethoxide were combined in ethanol.
- The combined chemicals were refluxed and stirred for 24 hours at 85°C using a reflux condenser.
- The solution was extracted using a rotary evaporator, dichloromethane and water, a separatory funnel, and a Hirsch funnel.
- Spectroscopy of the final product was collected and analyzed using IR, ^{13}CNMR, ^{1}HNMR, and COSY.

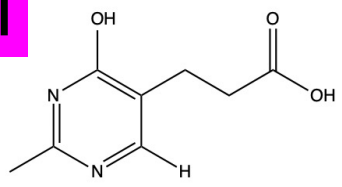


Docking Methods:

- SwissDock was used to combine the potential inhibitors to each isoform.
- Chimera was used to analyze the binding affinity values of the resulting docked compounds.
- Chimera data was imported to PyMOL and then into LigPlot+ where specific IMFs were shown and analyzed.

Potential Synthesized Inhibitors: Compounds 1-12

1



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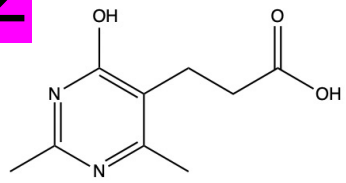

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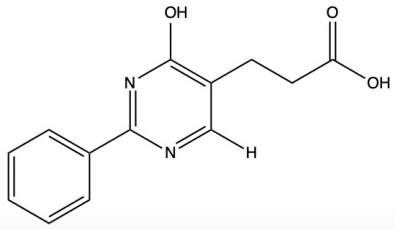

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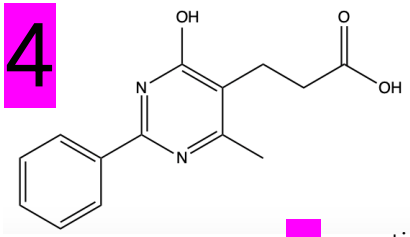
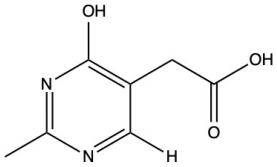


reaction pathway 1

reaction pathway 2

5



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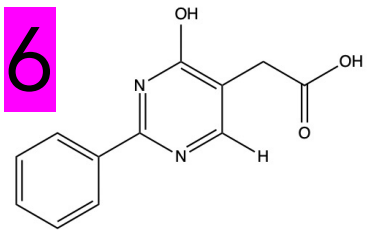

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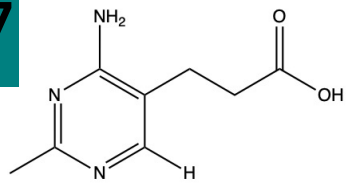

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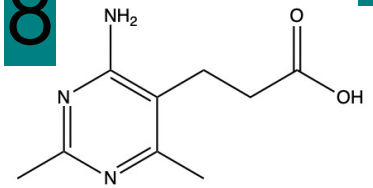

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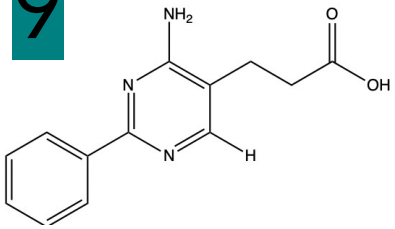

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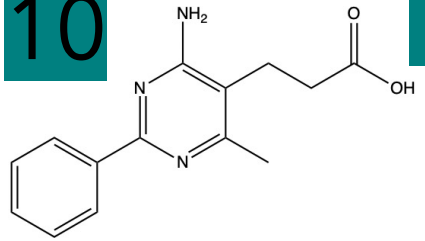

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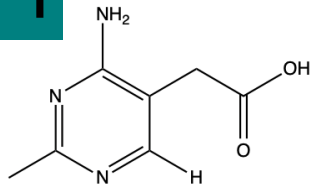

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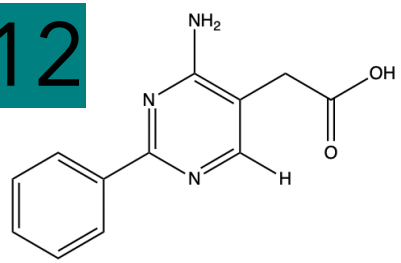


Table 1. The binding site and affinity of the 12 potential inhibitors to both isoforms A and B of LMW-PTP

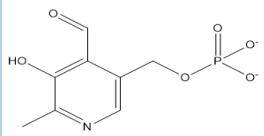
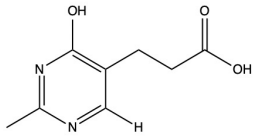
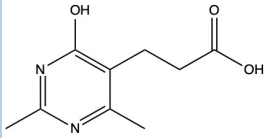
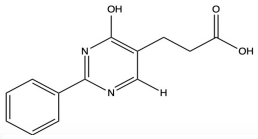
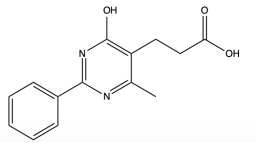
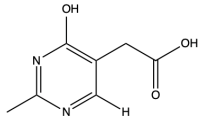
Compound	Structure	ΔG Isoform A	Binding Site Isoform A	ΔG Isoform B	Binding Site Isoform B	Ki
PLP		-10.465	active	-7.065	active	7.6 uM (pH 5.0)
1		-7.156	active	-6.356	allosteric	-
2		-7.472	active	-6.808	active	-
3		-8.099	active	-7.728	active	-
4		-7.355	active	-6.415	allosteric	-
5		-7.224	active	-6.945	active	-

Table 1. This displays the ΔG binding affinity and binding site of PLP and each potential inhibitor, compounds 1-12, to isoform A and isoform B of LMW-PTP. Swiss dock was used to identify the binding affinity and site of each compound. The highlighted cells located in Table 1 are the 3 best bound compounds out of the 12 compounds docked.

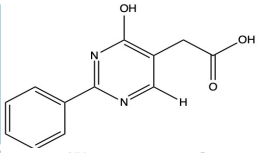
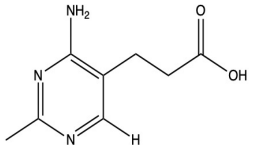
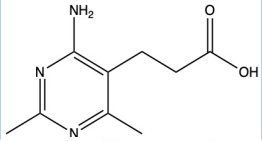
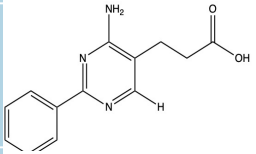
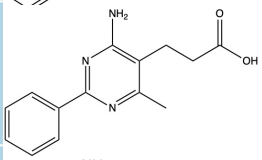
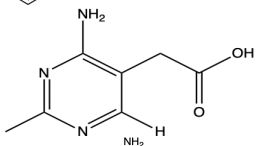
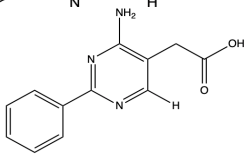
6		-7.680	active	-7.017	active	-
7		-7.362	active	-7.436	active	-
8		-6.945	active	-6.506	active	-
9		-7.971	active	-6.933	allosteric	-
10		-7.505	active	-6.443	allosteric	-
11		-6.975	active	-6.948	active	-
12		-7.728	active	-6.779	allosteric	-

Figure 1a. Binding sites on Isoform A

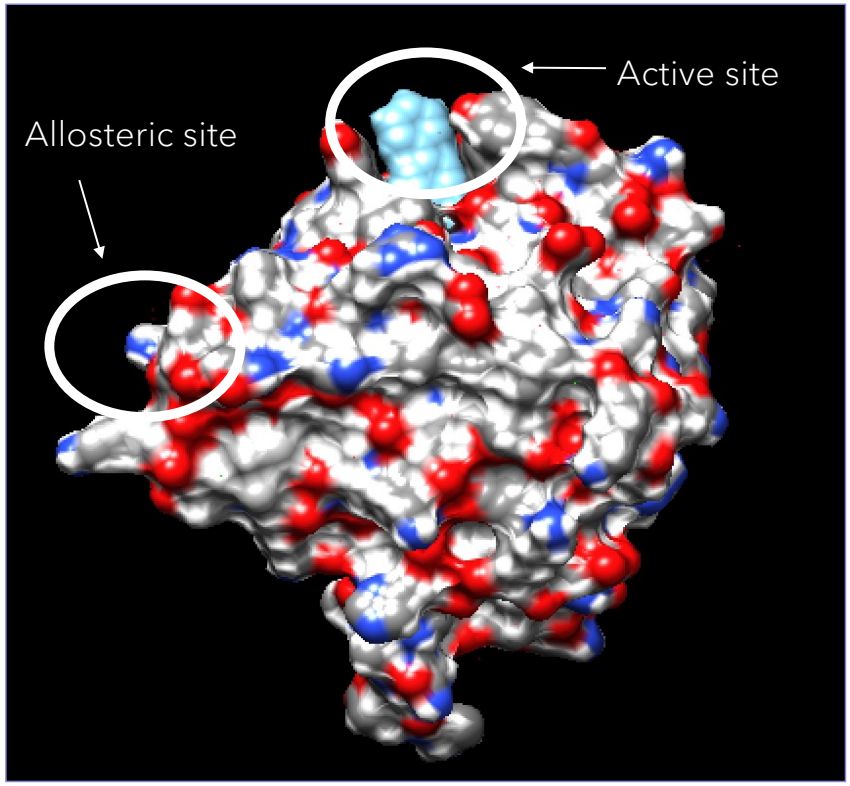


Figure 1b. Binding sites on Isoform B

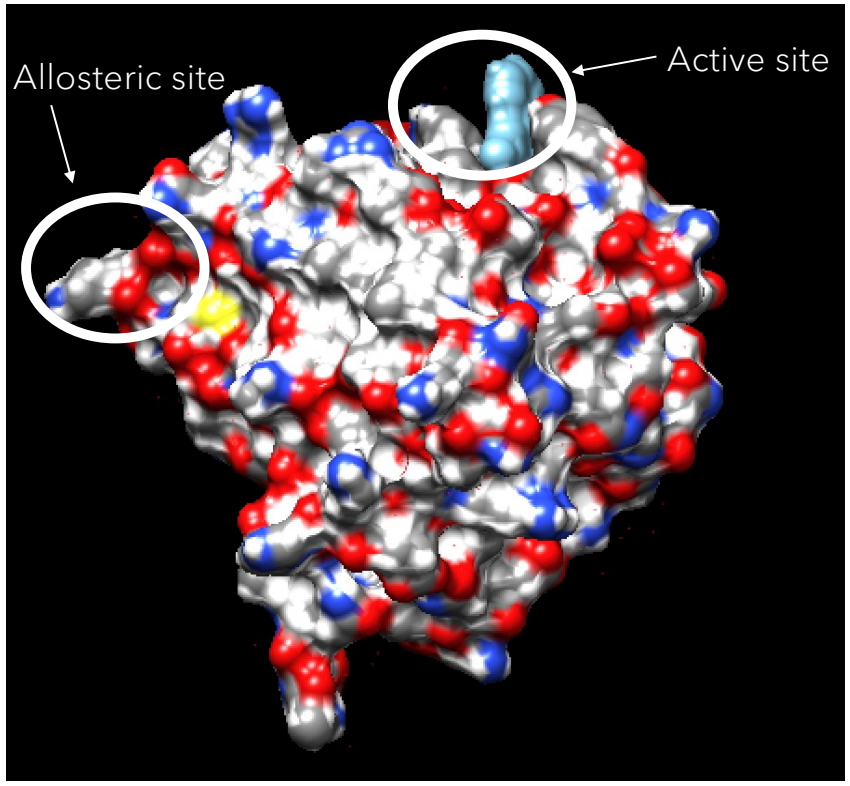
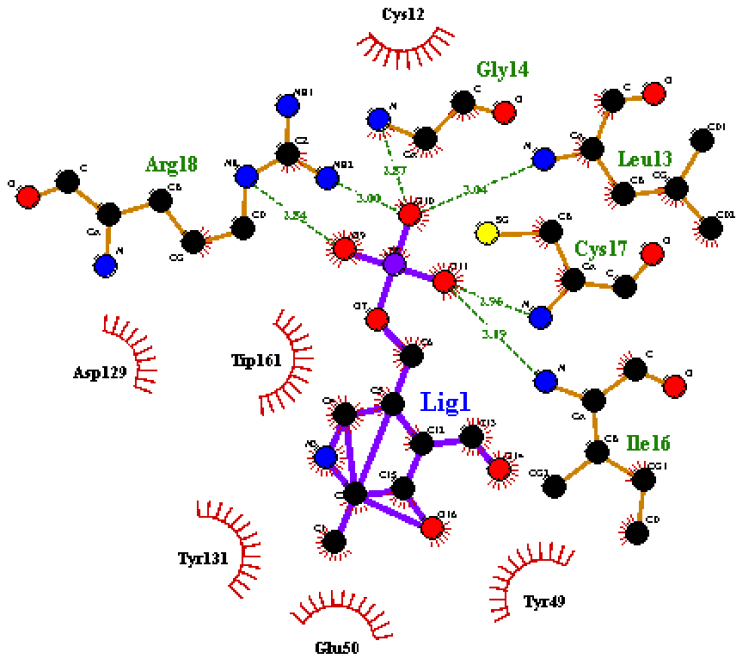


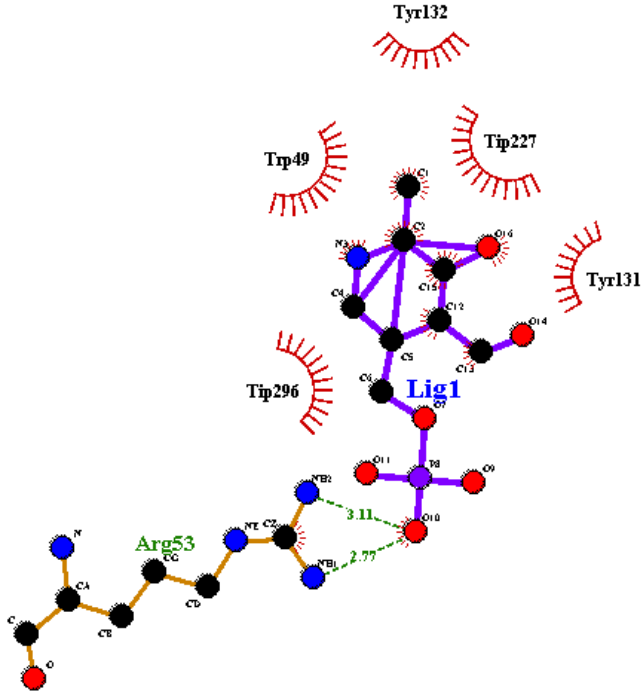
Figure 1a -1b. The location of the active site and allosteric site on Isoform A and Isoform B of LMW-PTP.

Figure 2a. LigPlot+ diagram of PLP binding to the active site of isoform A of LMW-PTP.



IsoformA_PLP

Figure 2b. LigPlot+ diagram of PLP binding to the active site of isoform B of LMW-PTP.



IsoformB PLP

Isoform A Best Binding Potential Inhibitors

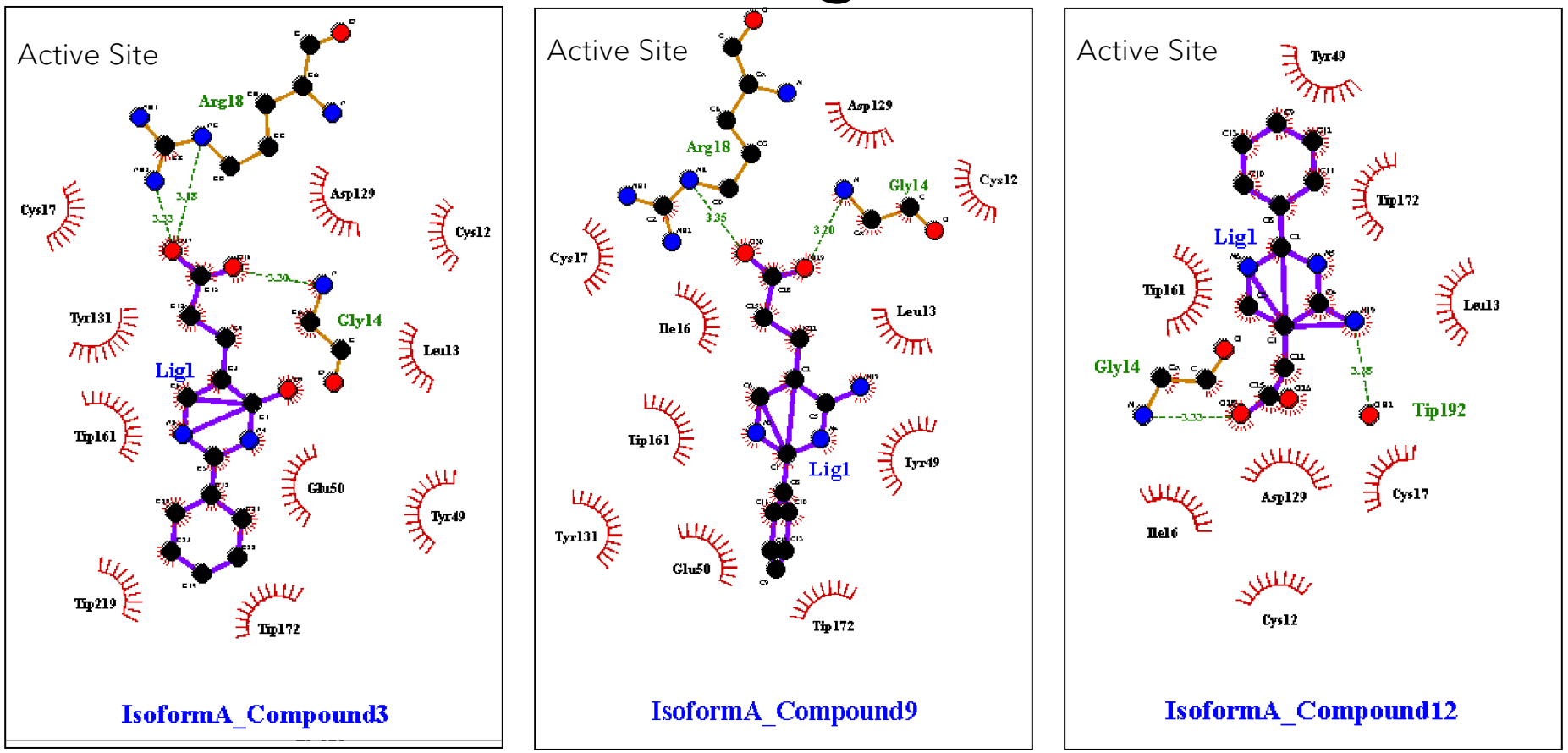


Figure 3a,3b,3c. LigPlot+ diagram of the best binding compounds to the active site of isoform A of LMW-PTP.

Isoform B Best Binding Potential Inhibitors

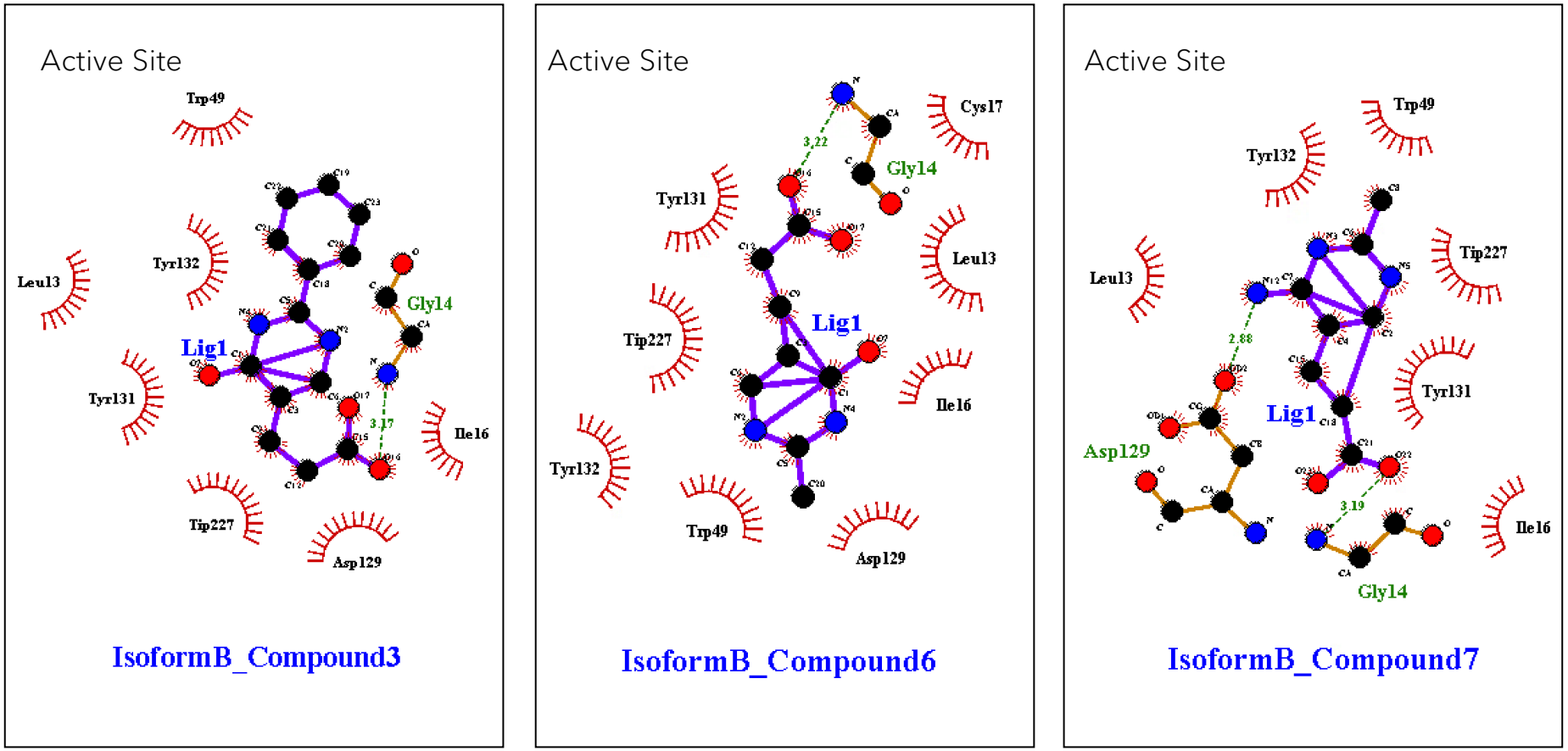


Figure 4a,4b,4c. LigPlot+ diagram of the best binding compounds to the active site of isoform B of LMW-PTP.

Conclusion and Future Work

- The delta G values of the 12 potential inhibitors did not yield values as competitive as the known inhibitor PLP for isoform A. The delta G values of compound 3 and 7 yielded values ~0.094 and ~0.052 times greater respectively, than the the delta G of PLP bound to isoform B.
- The LigPlot+ binding showed significantly fewer IMF's between the compounds and the isoforms when compared to PLP.
- There is future work to be done researching the structure of compound 3 due to its high delta G values and binding in the active site for both isoforms A and B. There is a potential for similar structures to be derived and yield more negative delta G values.
- Future work can be done to revise the other potential inhibitors that yielded similar delta G values to PLP.
- Although the condensation and cyclization reaction was successful in yielding a starting compound, it should be revised and reviewed for future research.

Acknowledgements

We would like to thank the CSB/SJU Chemistry Department for providing the necessary equipment and resources, and the CSB/SJU Undergraduate Research Program for providing funding for our research. We would also like to thank our research advisor, Professor McIntee, for the opportunity to be a part of his research, and for his guidance and mentorship throughout.

References

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2. J. Biggs.; P. Sykes.; Two Isomeric Homologues of Thiamine. *J. Med. Chem.* 1959, 1849-1855.
3. C. Seiler.; K. Richards; H. Jakubowski.; E. McIntee.; Identification of new inhibitors for low molecular weight protein tyrosine phosphatase isoform B. *Bioorganic & Medicinal Chemistry Letters.* 2013, 23, 1-4, 5912-5914.
4. M. Zhou.; R. Van Etten.; Structural Basis of the Tight Binding of Pyridoxal 5′-Phosphate to a Low Molecular Weight Protein Tyrosine Phosphatase. *Biochemistry* 1999 38 (9), 2636-2646.
5. I. Alho.; L. Costa.; M. Bicho.; C. Coelho.; Low Molecular Weight Protein Tyrosine Phosphatase Isoforms Regulate Breast Cancer Cell Migration through a RhoA Dependent Mechanism. *PLOS One* 2013, 8 (9), 1-10.