PostDoc Journal
Vol. 1, No. 12, December 2013

Journal of Postdoctoral Research
www. Postdocjournal.com

In-silico drug design approach to discover novel anti-coagulants

Kashi Reddy Methuku, PhD
Department of Chemistry and Biochemistry, University of Wisconsin, Milwaukee, WI
Email: methuku@uwm.edu

Keywords: Virtual Screening, Coagulation, Thrombin, Drug Design.

In-Silico drug design tools are extensively used to improve the drug discovery program by reducing the cost associated with initial screening, exploring larger chemical space, and eliminating the non drug-like molecules at very initial stages of discovery.[1-3] With advancement in the computational field and development of various computational chemistry software suites, *in-silico* approach emerges with greater predictive ability in identifying the initial lead candidate.[4-5] Molecular docking and virtual screening are more commonly used these days to identify and design novel inhibitors with a rational design approach. Availability of crystal structures of various proteins via open sources has made structure-based drug design approach increasingly common. *In-silico* approach has been successfully applied to the discovery of inhibitors for enzymes such as proteases and kinases targeting numerous diseases. Here, we specifically discuss two cases of virtual screening approach to discover direct and allosteric inhibitors of thrombin.[6-9] Thrombin is a key enzyme involved in regulation of the coagulation cascade. Quick search on the NCBI database reveals more than 900 structures of wild or mutant-type thrombin. A thorough understanding of Thrombin structure will facilitate designing an inhibitor targeting the active site and exosites of thrombin.[10-16] Several successful attempts have been made to design such inhibitors using ligand or structure-based drug design approach using various *in-silico* tools. Not only the active site of thrombin, but also the allosteric sites were potential targets for an efficient inhibitor design. However, rationally designing allosteric inhibitors is still difficult due to the lack of understanding of allosteric connections and shallow nature of its exosites.[8-9]

Recent work by Sidhu *et al*[13] *shows that* allosteric inhibitors of thrombin were discovered using ligand-based virtual screening approach. In their study, pharmacophore model was generated from a library of allosteric inhibitors of thrombin targeting the exosite II of thrombin. The important features required for mediating allosteric inhibition of thrombin were identified. The generated pharmacophore was used to perform virtual screening on Zinc Database containing a collection of approximately one million compounds. The hit molecules identified from virtual screening were modified to resemble the actual structures. Two-step docking filter was applied using GOLD docking software to select the hits that fit best in binding site. In the first filter, docking studies were performed to identify hit molecules that bind in a defined binding region with high binding score. In the second-step, molecules selected from the first filter were docked in triplicate. The binding modes generated from all three independent runs were compared. The molecules with similar binding pose in three independent runs were selected based on R^2 value. The ten molecules that were chosen from the library showed good binding score and consistent binding poses. Three molecules were purchased and sulfated using microwave-based sulfation protocol. All three molecules showed good thrombin inhibition potency and were found to be allosteric in nature. Also, the plasma clotting studies showed good anti-coagulation activity. The present work is a novel *in-silico* approach to identify allosteric inhibitors of thrombin.

In another study by Loganathan *et al*,[17] direct inhibitors of thrombin targeting the active site were discovered. To generate a hypothesis model, 40 structurally diverse inhibitors of thrombin were selected from the literature. These were divided into training and test sets to generate a pharmacophore model and to validate it, respectively. The best pharmacophore model contains two aromatic rings, two hydrogen bond donors and one hydrogen bond acceptor, which were considered as an essential feature for designing inhibitors of thrombin. The generated model was validated using Fisher's Randomization method. Virtual screening was performed using the best predictive model on the vast NCI and Maybridge databases to identify potential lead candidates, which will be synthesized and tested further. A total of 48,227 hits were obtained during initial screening. Secondary filters such as Lipinski's Rule and ADME were performed to eliminate non-drug like molecules leading to a total of 591 molecules that were selected to proceed for molecular docking studies. Molecular docking studies were performed using LigandFit docking program to identify the binding mode of molecules in the active site of thrombin and to predict the binding affinity. Various scoring functions, molecular interactions and binding modes were obtained and compared with that of Melagatran. Based on these parameters, four molecules were selected which are shown to perform better than the control group. However, some substitutions on these molecules were unfavorable for interaction with binding sites. Further optimization was done by changing the substitutions and a library of 40 molecules was generated. Out of the 40 substitutions, 18 molecules showed good score value as compared to control group. These molecules were found to have more polar and hydrophobic interaction with residues in the active site of thrombin. The novelty of these molecules was confirmed using Scifinder Scholar and Pubchem structure search tools.

Conclusion

In these studies, pharmacophore model for identifying the inhibitors of thrombin targeting the active site and exosites of thrombin was generated and validated using various *in-silico* drug discovery tools. The best model was used to identify the potential lead molecules by virtually screening of large databases of chemical structures. These potential molecules were further filtered using Lipinski's Rule, ADME and molecular docking to select the drug-like molecules and those that bind to the active site similar to known inhibitors of thrombin.

References:

1) Sousa S. F.; Cerqueira N. M.; Fernandes P. A.; Ramos M. J.; Virtual screening in drug design and development. *Comb. Chem. High Throughput Screen.* **2010**, *13*, 442-453.
2) Klebe G.; Virtual ligand screening: strategies, perspectives and limitations. *Drug Discov. Today* **2006**, *11*, 580-594.
3) Ripphausen P.; Nisius B.; Peltason L.; Bajorath J.; Quo V.; virtual screening? A comprehensive survey of prospective applications. *J. Med. Chem.* **2010**, *53*, 8461-8467.
4) Liao C.; Sitzmann M.; Pugliese A.; Nicklaus M. C.; Software and resources for computational medicinal chemistry. *Future Med. Chem.* **2011**, *3*, 1057-1085.
5) Brooijmans N.; Kuntz I. D.; Molecular recognition and docking algorithms. *Annu. Rev. Biophys. Biomol. Struct.* **2003**, *32*, 335-373.
6) Warren G. L.; Andrews C. W.; Capelli A. M. et al. A critical assessment of docking programs and scoring functions. *J. Med. Chem.* **2006**, *49*, 5912-5931.
7) Fauman E. B.; Rai B. K.; Huang E. S.; Structure-based druggability assessment – identifying suitable targets for small molecule

therapeutics. *Curr. Opin. Chem. Biol.* **2011**, *15*, 463-468.
8) Peach M. L.; Nicklaus M. C.; Combining docking with pharmacophore filtering for improved virtual screening. *J. Cheminform.* **2009**, *1*, 6.
9) Waszkowycz B.; Towards improving compound selection in structure-based virtual screening. *Drug Discov. Today* **2008**, *13*, 219-226.
10) Verghese, J.; Liang, A.; Sidhu, P. S.; Hindle, M.; Zhou, Q.; Desai, U. R.; First steps in the direction of synthetic, allosteric, direct inhibitors of thrombin and factor Xa. *Bioorg. Med. Chem. Lett.* **2009,** *19*, 4126-4129.
11) Sidhu, P. S.; Liang, A.; Mehta, A. Y.; Abdel Aziz, M. H.; Zhou, Q.; Desai, U. R.; Rational design of potent, small, synthetic allosteric inhibitors of thrombin. *J. Med. Chem.* **2011,** *54*, 5522-5531.
12) Abdel Aziz, M. H.; Sidhu, P. S.; Liang, A.; Kim. J. Y.; Zhou, Q.; Farrell, D. H.; Desai, U. R.; Designing allosteric regulators of thrombin. Monosulfated Benzofuran dimers selectively interact with Arg173 of exosite 2 to induce inhibition. *J. Med. Chem.* **2011**, *55,* 6888–6897.
13) Sidhu, P. S.; Mosier, P. S.; Desai, U. R.; On Scaffold Hopping: Challenges in the Discovery of Sulfated Small Molecules as Mimetics of Glycosaminoglycans. *Bioorg. Med. Chem. Lett.* **2013,** *23*, 355–359.
14) Sidhu, P. S.; Hamdy, M. H.; Sarkar, A.; Mehta, A. Y.; Zhou, Q.; Desai, U. R.; Designing Allosteric Regulators of Thrombin. Exosite 2 Features Multiple Sub-Sites That Can Be Targeted By Sulfated Small Molecules for Inducing Inhibition. *J. Med. Chem.* **2013**, *56*, 5059-5070.

15) Sidhu P. S.; Designing allosteric inhibitors of thrombin. PhD Dissertation, Virginia Commonwealth University, VA, December **2011.**

16) Sidhu P. S.; Allosteric inhibitors of thrombin. *Journal of Postdoctoral Research*, **2013**, *1*, 46-47.
17) Chandrasekaran L.; Sugunadevi S.; Keun W. L.; Senthamaraikannan k.; Chandrasekaran M.; Pharmacophore Design, Virtual Screening, Molecular Docking and Optimization Approaches to Discover Potent Thrombin Inhibitors. *Comb. Chem. High Throughput Screening*, **2013**, *16*, 702-720.