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Docking Studies for Designing Novel Inhibitors of PknB Protein Kinase for Combating Mycobacterium Tuberculosis

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Abstract—*In this paper, we report the design and molecular docking study of PknB with mitoxantrone,an ATP-Competitive inhibitor of Mycobacterium tuberculosis. Ten designed compounds and the original PknB were docked based on their interaction with mycobacterium receptor binding target in complex with mitoxantrone.*

The docking resulted in the five top-ranked compounds, namely, compounds 5, 2, 7, 3, and 4, which have a lower ΔG binding energy, better affinity and stronger hydrogen bonding interactions to the active site of PknB .

Among those five top-ranked compounds, analogue compounds 5 and 7, respectively, exhibited the strongest hydrogen bond interaction, formed high stability conformation, and demonstrated the greatest inhibitory activity on the catalytic site of PknB. ADME predictions of 5 top selected compounds were done.

The information generated from the present study should be useful in the design of more potent PknB inhibitors as anti tuberculosis agents.

Keywords*: Design, Docking,ADME , Analogue, PknB, Mycobacterium tuberculosis.*

1. INTRODUCTION

Mycobacterium tuberculosis is a pathogenic bacterial species in the family Mycobacteriaceae and the causative agent of most cases of tuberculosis. It has an unusual, waxy coating on its cell surface (primarily due to the presence of mycolic acid), which makes the cells impervious to Gram staining. The Ziehl-Neelsen stain, or acid-fast stain, is used instead. The physiology of *M. tuberculosis* is highly aerobic and requires high levels of oxygen. Primarily a pathogen of the mammalian respiratory system, it infects the lungs. Once rare in developed countries, tuberculosis infections began increasing in 1985, partly because of the emergence of HIV, the virus that causes AIDS. HIV weakens a person's immune system so it can't fight the TB germs. In the United States, because of stronger control programs, tuberculosis began to decrease again in 1993, but remains a concern. (http://www.mayoclinic.org/diseases-conditions/tuberculosis/basics/definition)

Tuberculosis is caused by bacteria that spread from person to person through microscopic droplets released into the air. This can happen when someone with the untreated, active form of tuberculosis coughs, speaks, sneezes, spits, laughs or sings. Although tuberculosis is contagious, it's not easy to catch. We're much more likely to get tuberculosis from someone we live with or work with than from a stranger. Most people with active TB who've had appropriate drug treatment for at least two weeks are no longer contagious. Many strains of tuberculosis resist the drugs most used to treat the disease. People with active tuberculosis must take several types of medications for many months to eradicate the infection and prevent development of antibiotic resistance.

The main risk factors for weakened immune system, traveling or living in any certain area, poverty and substance abuse, etc. So, I tried to find out efficient target for treatment of diabetic condition in human being. One of the way choose by researchers are by molecular docking studies. The design of new chemical entities is based on the requirement of active binding site present in the enzyme. The inhibitory action of design compound may be predicted by using different molecular docking softwares in this paper Autodock 4.2 that help out for predicting designed molecule's efficiency with respect to binding site. The target such as mainly highlighted by me is PknB in complex with mitoxantrone ,an ATP-Competitive inhibitor from mycobacterium tuberculosis in complex with mitoxantrone. PknB is an essential serine/threonine kinase of Mycobacterium tuberculosis with possible roles roles in a number of signalling pathways involved in cell division and metabolism.

2. METHODS

2.1 CHEMSKETCH

In this software we are going to draw the structure of ligand/molecule. It is a chemically intelligent drawing interface that allows you to draw almost any chemical

structure including organics, organometallics, polymers, and Markush structures.

2.2 RCSB Protein Data Bank

The PDB is the single, global archive for information about the 3D structure of biomacromolecules and their complexes, as determined by X-ray crystallography, NMR spectroscopy and cryoelectron microscopy, and includes more than a few Nobel Prize winning structure. PknB enzyme was downloaded from Protein data bank with the specific resolution and the PDB id is 2FUM.

2.3 Swiss Pdb Viewer

Swiss-PdbViewer (aka DeepView) is an application that provides a user friendly interface allowing to analyze several proteins at the same time. The proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts. Amino acid mutations, H-bonds, angles and distances between atoms are easy to obtain thanks to the intuitive graphic and menu interface.

2.4 AutoDock

AutoDock is a molecular modeling simulation software. It is especially effective for Protein-ligand docking. The molecular docking tool, AutoDock 4.2 (Scripps Research institute) software was used for ligand docking studies in to the (PknB) binding pocket. AutoDock 4.2 is one of the most accurate docking tool available for ligand-protein, protein-protein binding studies. Autodock was found to produce least number of inaccurate poses and 85% of binding models had an RMSD of 1.4 A0 or less from native co-crystallized structures.

2.5 Chimera

UCSF Chimera is a highly extensible, interactive molecular visualization and analysis system. Chimera can read molecular structures and associated data in a large number of formats, display the structures in a variety of representations, and generate highquality images and animations suitable for publication and presentation.

2.6 ADMET

The molecules which have shown H-Bond with the active site residue or any other residue of the binding pocket note down those molecules and then run these molecules on the following online ADMET servers. For molecular properties and drug likeness score we use Molsoft insilico where we can draw or import the ligand file from ChemSketch. For toxicity and bioactivity we go to OSIRIS Property Explorer.For ADMET studies we can use ACD/I Lab where we can draw or import the ligand file from ChemSketch.

2.7 Protein preparation

A typical PDB structure file consists only of heavy atoms, can contain waters, cofactors, and metal ions, and can be multimeric. The structure generally has no information on bond orders, topologies, or formal atomic charges. Terminal amide groups can also be misaligned, because the X-ray structure analysis cannot usually distinguish between O and NH2. Ionization and tautomeric states are also generally unassigned. Glide calculations use an all-atom force field for accurate energy evaluation. Thus, AutoDock 4.2 requires bond orders and ionization states to be properly assigned and performs better when side chains are reoriented when necessary and steric clashes are relieved.

2.8 Ligand preparation

The LigPrep process consists of a series of steps that perform conversions, apply corrections to the structures, generate variations on the structures, eliminate unwanted structures, and optimize the structures. Many of the steps are optional and are controlled by selecting options in the LigPrep panel or by specifying command-line options. The process like convert the structure format (sd format), select the structures, add hydrogen atoms, remove unwanted molecules, neutralize charged groups, generate ionization states, generate tautomers, filter the structures, generate alternative chiralities, generate low-energy ring conformations, remove problematic structures, optimize the geometries and finally convert the output file are performed by during ligand preparation.

3. RESULTS

PTP 1B as target and design the skeleton of inhibitors:

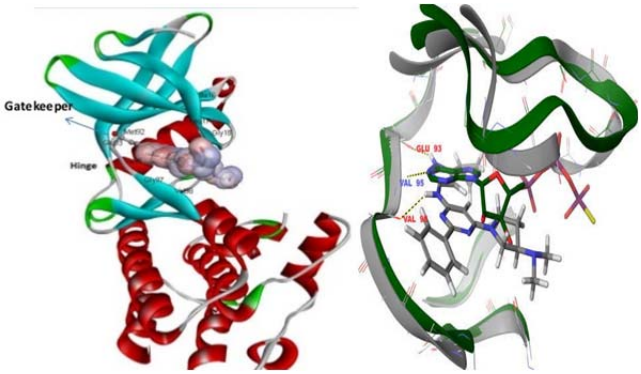

Fig. 1:a. Ribbon view of the crystal structure of protein kinase (PknB), highlighting the main regions of the protein. b. This graphic actually shows the basic regions in the pockets as well as the active site residues.(www.rcsb.org/pdb)

According to researcher studies, we found that some important properties of inhibitors. The properties like (i) For basic antihyperglycemic activity Nitrogen containing ring required; (ii) Molecule should contain phosphate like moiety that one able to bind at catalytic side of enzyme; (iii) Oxygen required for hydrophobic interaction (iv) Ring nitrogen required for selectivity at active binding site.

As requirement of PTP1B inhibitors, we design around 10 molecules of 2FUM with respect to active binding site.

Among 10 molecules, we select 5 top molecules considered for further studies.

The results of the docking studies are presented in the form of G-score, Energy, Good, Bad and Ugly Van Der Waals (vdw) interactions. The G-scores are presented as negative values, indicating that more the negative values more are the binding interactions. The docking studies were performed for the designed ligands with Pknb enzyme and the results were compared with the ligand 2FUM present within the receptor. The docked complexes of the designed compounds along with the ligand receptor poses have been shown in the **Fig.** . The designed compounds were found to display good binding affinity to the receptor.

Table 1: Docking Results of Top 5 Molecules with their RMSD values:

Molecule	Binding Energy	Run(Docking run)
5	-7.88	8
2	-7.49	10
7	-6.97	6
3	-6.57	6
4	-6.44	5

4. DISCUSSION

Computer-assisted drug design (CADD) approach has contributed to the successful discovery and design of several novel agents. Molecular Docking continues to hold great promise and agenda in the field of computer based drug design which screens small molecules by arranging and orienting them and scoring them in the binding site of a protein. Number of reports citing successful application of CADD in developing specific drugs in different therapeutic areas is expanding rapidly. There are various tools, which can be used for Computer aided drug design such as QSAR, Docking, Homology modeling, ADMET prediction etc. These all parameters are helpful to find out bioavailability and toxicity prediction in human body.

5. CONCLUSION

The major reason for failure of compounds at latter stages of drug discovery process i.e. drug like pharmacokinetic profile set up, has forced us setting filters like molecular weight, No. of H-bond donors, No. of H- bond acceptors, Polar Surface Area and number of rotatable bonds; so that only drug like compounds would be generated and resultant compounds would not have the pharmacokinetic inadequacies. But the thorough analysis of results of docking and ADMET analysis studies predicts the safer performance of our designed compounds. The most potent derivatives were subjected to molecular docking studies to get further insights of interactions of compounds with PknB. Finally 5 top compounds with good docking score will be subjected to wet lab work viz., The results of dry lab work and wet lab work will be analyzed thoroughly to find out correctness of the rational used for the design of compounds in general and optimization of pharmacophore for inhibition of PknB from mycobacterium tuberculosis in association with mitoxantrone.

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AUTHOR SUMMARY

As we know, that tuberculosis has become the deadliest disease of our nation and is spreading all over the world ,so it has become very important to curb it. My paper has outlined an important and portable process that can be used in the drug development or novel drug discovery process by various researchers and scientists to help in curbing mycobacterium tuberculosis. This paper has been designed in order to bring forward the best molecules that can be used for inhibiting tuberculosis. Mitoxantrone with PknB is believed to give the best ATP-binding inhibitors of Mycobacterium tuberculosis, and this paper has put forward five most appropriate molecules which has passed several validation, evaluation and toxicity tests and also lipinski's rule of five, so as to become the best inhibitors. This manuscript is providing an insight of bioinformatics uses in today's scientific world by explaining the uses and results of docking studies in combating such a serious and grave disease like Mycobacterium Tuberculosis.