VOLUME-7, ISSUE-11, NOVEMBER-2018 • PRINT ISSN No 2277 - 8160

Original Research Paper

Chemistry

COMPUTER AIDED DRUG DESIGNING: ANALYSIS ON MYCOBACTERIUM TUBERCULOSIS PROTEIN KINASE B (PKNB) COMPLEXED WITH IN SILICO GENERATED COMPOUND 'DOHARENE'

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ABSTRACT Mycobacterium tuberculosis is the pathogen responsible for tuberculosis (TB) resulting in 2 million deaths per year. PknB is a protein kinase involved in control of cell growth and viability of the bacterial infection. Here we have analyzed homologues of various compounds by molecular docking with native PknB. Binding energy of ligand with the active site of the protein and hydrogen bonds were analyzed. The protein was first energetically stabilized to maximum extent and optimized. Then using chemsketch and swiss pdb viewer, a final set of eight compounds was selected to undergo docking at the active site in order to select the best ligand. The complex was prepared in silico. The structure of the complex with the selected compound, which was named 'Doharene' reveals that it formed two hydrogen bonds with the active site. Further analysis of the drug molecule was conducted.
Using ADMET servers, its bioavailability, toxicity, and various other parameters were analyzed. Further studies need to be undertaken to confirm likeliness as a drug

KEYWORDS : *Mycobacterium tuberculosis* PknB, computer aided drug designing; *in silico, ADME*

I. INTRODUCTION

Computer Aided Drug Development (CADD) in pharmaceutical chemistry is one of the emerging trends in new frontiers of science. Traditionally, blind screening approach was used. Earlier, *in vitro* and *in vivo* screens were employed which required 10-20 years in synthesis and testing, which was also quite expensive and time consuming. In recent times, we employ Rational Drug Designing that takes place with knowledge of biological target. Identification and structure determination of molecular target critical to disease is important in this case.

- **What Is CADD?**

The goal in drug design is to predict if a given molecule can bind to a target. Molecular mechanics (dynamics) is most often used to determine the intermolecular interaction between the small molecule and its biological target.

- **Ligand Based Drug Design**

Ligand-based drug design (or indirect drug design) depends on data available about molecules that bind to the biological target of interest.

- ***In silico* Generation**

Chemical structures are generated using Chemsketch (Molecular editing tool). This is a tool for creating and modifying chemical structures. Molecular editors can generate these chemical structures in 2-dimentional or 3-dimentional format. They are further converted to protein data bank format (.pdb) using Open Babel.

- **Protein Optimisation and Energy Minimisation**

Protein optimization removes previously present residues.
Energy minimization repairs distorted geometries and bring back protein to lowest energy state. (SPDBV Swiss- Pdb Viewer was used for this purpose.)

- **Grid Parameter and Dock Parameter**

Grid Parameter defines 3-dimensional space and labels atoms to use.

Dock parameter specifies the algorithm to use for docking, the number of runs and energy evaluations to use. (Autodock 4.0 was used for the creation of these files namely, '.gpf' & '.dpf'.)

- **Molecular Docking**

This predicts the preferred orientation of one molecule to another when bound to the protein in order form a stable complex.

The focus is to computationally simulate the molecular recognition process.

The aim is to achieve an energetically favorable conformation for both the protein and the ligand. The relative orientation between the two should be such that binding energy of overall system is least.

- Running the Docking Algorithm (Lamarckian Genetic Algorithm)

Cygwin was used to provide Linux environment on windows in which autodock4 and autogrid4 algorithms are run.

From these results we tabulate ligands based on minimum binding energy and Lipinski's rule.

- Lipinski's Rule and Binding Energy

Lipinski's rule of five is a rule of thumb used to evaluate drug likeness. It evaluates if a chemical compound with certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans.

The association of drugs with their macromolecular targets in the cell depends on the formation of energetically favorable bonding interactions between the two partners. The equation that relates the free energy of binding (ΔG) to Keq is shown below:

$$\Delta G = -RT \ln K_{eq}$$

- What Types of Weak Bonds?

Ionic interactions, Hydrogen bonds (H-bonds), van der Waals force and hydrophobic effects.

In order to achieve strong binding, the drug must "fit cozily" into this binding pocket and must place itself in a suitable way such that functional groups remain in close proximity to functional groups near the active site.

- Hydrogen Bonding Analysis:

H-bonds keep molecule tightly bound to proteins. Groups of hydrophobic protein atoms enclose the hydrophobic ligand atoms. Hydrophobically enclosed H-bonds also strengthen the H-bond. The ligand needs to associate as well as dissociate from the active site. Hence analysis is of utmost importance.

- ADME and Tox (ADMET)

Absorption, Distribution, Metabolism, Excretion and Toxicity or ADMET tells how the drug is likely to interact inside our body.

Traditionally animals were used for pre-human testing. However, these are expensive, time-consuming and ethically undesirable.

The molecules which showed H-bonds in our analysis with the active site are run on online ADMET servers.

For Molecular Properties, molsoft.com whereas for ADMET studies ilab.acdlabs.com was used. ACD labs was used for physical properties prediction.

I. MYCOBACTERIUM TUBERCULOSIS PROTEIN KINASE B (PKNB) (2FUM)

Tuberculosis is caused by a devastating human pathogen *Mycobacterium tuberculosis*. "Monitoring the environment with serine/threonine protein kinases is critical for growth and survival of *Mycobacterium tuberculosis*."

Mycobacterium tuberculosis protein kinase B also plays a key role in multiple cellular processes such as glucose metabolism, apoptosis, cell proliferation, transcription and cell migration. Hence it is a popular drug target due to involvement in crucial cellular processes. Exposure to *Mycobacterium tuberculosis* (Mtb) can result in a latent form of tuberculosis (TB) infection. This can further reactivate and Grow into AN active disease.

"PknB is predicted to consist of 626 amino acids with a transmembrane segment dividing the protein into an N-terminal intracellular domain and a C-terminal extracellular domain. The N-terminal domain of PknB includes a kinase domain and juxtamembrane linker of 52 residues."

Fig. 1. Catalytic domain of protein kinase PknB from *Mycobacterium tuberculosis* (optimized).

III. MATERIALS AND METHODS

A. Dataset

In this study, molecular docking was carried out on catalytic domain of protein kinase PknB from *Mycobacterium tuberculosis* in complex with mitoxantrone (PDB ID: 2FUM). The ligand mitoxantrone was removed from the PDB file. The molecule was prepared for docking analysis by energy minimization and further adding kollman charges to the protein.

B.Analysis

Eight ligands were selected for further binding energy analysis. After docking the ligands at the active site of *Mycobacterium tuberculosis* PknB, it was found that one of the molecules is of special interest. While it had an appreciable minimum binding energy with the protein kinase, it also at the same time formed two hydrogen bonding with the active site VAL95.A . Hence the name 'Doharene' comes from the fact of two bonds which is of special interest. It also has no stereoisomers.

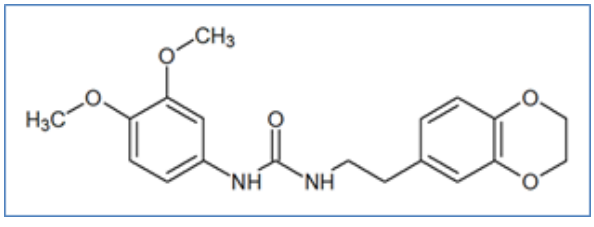


Fig. 2. Doharene

C. Molecular Docking

Molecular Docking was done using Autodock Tools 4.0 for Doharene to determine binding energy and inhibition constant. AutoGrid program with searching function and AutoDock with scoring function was used to determine docked complex. The best confirmation was selected on the basis of minimum binding energy of the run.

TABLE. 1. Results of Binding Energy

Compound	Binding Energy on PknB (kcal/mol)
Doharene	-8.59

D. Hydrogen bonding Analysis

Using University of California, San Francisco (UCSF) Chimera, the hydrogen bonding analysis was undertaken between atoms of ligand and protein. Two hydrogen bonds formed ensures the orientation of this ligand with the binding partner and also helps it act as a kinase inhibitor. Most importantly it helps high affinity towards the active site.

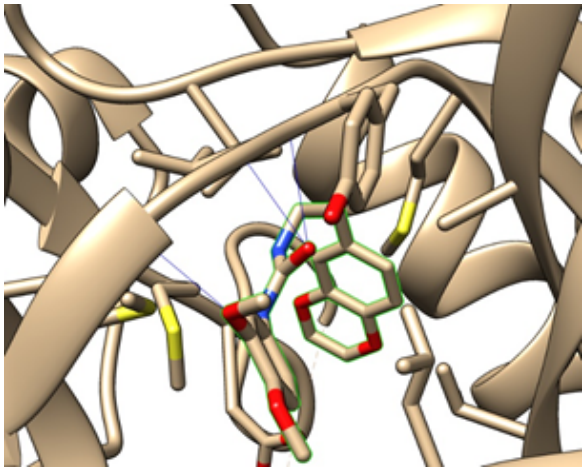


Fig. 3. Docked complex with Doharene. Hydrogen bond visualized using blue lines.

TABLE. 2. Molecular Docking Results with Doharene

Hydrogen Bonds	Ligand Interactions	Predicted Inhibition Constant (nM)	
3	O…VAL95.A O VAL95.A N…O GLY 97.A N…O	506.81	

E. ADME and Tox Studies

Further Doharene was analyzed using online servers for test of Absorption, Distribution, Metabolism, Excretion, Toxicity (ADME and Tox).

F. Accordance with Lipinski's Rule

Our analysis of the compound found that the drug molecule was in accordance with Lipinski's Rule

TABLE. 3. Results of Studies with Doharene [9]

S. No.	Characteristic	Value
1	Molecular Weight	358.15
2	No. of H-Bond Acceptor	5
3	No. of H-Bond Donor	2
4	MolLogP	3.25

Lipinski's Rule

- *Molecular Weight < 500*
- *HBA < 10*
- *HBD < 5*
- *MolLogP < 5*

IV. CONCLUSION

CADD is effective and efficient due to the following reasons:

- Reduction in cost
- Predictive power for unknown compounds
- Large number of molecules in lesser time
- Easy visualization and mechanism analysis
- Intricate details are revealed
- Tools to predict ADMET Response

This whole approach enables us to study inhibition based on simple docking analysis. The analysis enables us to determine binding affinity of the proposed drug, Doharene with the native PknB. It had a binding affinity in range of -8.59 kcal/mol. Molecular Docking was used to prepare the complex with the said drug and showed two hydrogen bonds and a low value of inhibition constant.

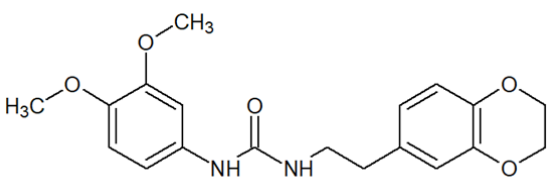
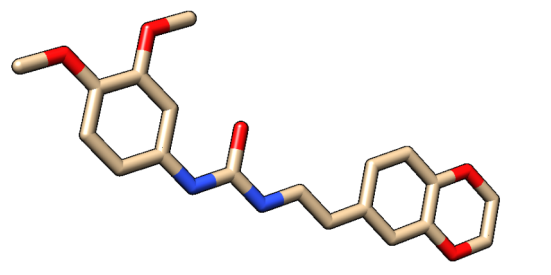
The active VAL 95.A of *Mycobacterium tuberculosis* was also involved in these interactions which to some extent confirms it ability for kinase inhibition.

Molecular modelling reveals intricate details, atomic scale binding properties that are difficult to imagine in other ways.

There has been extensive research on tuberculosis which to some extent till date contained the spread of the disease. However, it is still a major concern for the world. New drugs are necessary as it gets resistant to old ones. Protein modelling is one of the most important and reliable techniques available for determining promising drugs.

This entire approach enables us to completely stay out of the labs for the initial time and not waste resources on drugs that are ultimately destined to fail. Our study concluded that 'Doharene' may be suitable to inhibit PknB and needs further studies.

TABLE. 4. Predicted Physical and Chemical Properties

'Doharene'



IUPAC NOMENCLATURE 1-[2-(2,3-Dihydro-1,4-benzodioxin-6-yl)ethyl]-3-(3,4-dimethoxyphenyl)urea	
Chemical data (Predicted data online server)	
Molecular Formula	C19H22N2O5
Molecular Weight	358.15
Number of stereocenters	0
Hydrogen Bond Acceptors	5
Hydrogen Bond Donors	2
MolLogP (Octanol Water Partition coefficient)	3.25
Legal status	
Legal status	Not Accepted

Physical Properties (Predicted data online server)	
Density	1.255 ± 0.06 g/cm3
Boiling Point	504.4±50.0 °C at 760 mmHg
Vapour Pressure	0.0±1.3 mmHg at 25°C
Enthalpy of Vaporization	77.4±3.0 kJ/mol
Index of Refraction	1.598
Freely Rotating Bonds	6

FURTHER SCOPE

Further tests could be undertaken to determine:

- Bioavailability: The fraction of an administered dose that is finally able to reach the target unaffected.
- Absorption: It is the movement of a drug from the site of administration to bloodstream.
- LD50: Measure of the lethal dose of a toxin, radiation, or pathogen. Dose required to kill half the population after a specified test duration.

Ames test (Carcinogen Test): To test whether a given chemical can cause mutations in the DNA of the test organism.
The molecule designed 'Doharene' requires further research to determine its effectiveness as a drug.

ACKNOWLEDGMENT

I would like to thank Dr. Rakhi Thareja, Department of Chemistry, St. Stephen's College, University of Delhi for encouraging me to learn CADD.

I would also like to show my gratitude to Dr. Shabnam Johry, Head of the Department, St. Stephen's College, University of Delhi, Dr. Jyotirmoy Maity and Dr. Priyanka Thakral, Department of Chemistry, St. Stephen's College, University of Delhi for their reviews and comments on an earlier version of the manuscript although any current errors are my own and are not to tarnish the reputation of the above mentioned.

I would also like to mention Bhavnesh Jangid of Ist Year Chemistry Hons and Roshni B. Joseph of IInd Year Chemistry Hons, St. Stephen's College for their interest and support for the topic and helping me out with computer applications and understanding of biological terms.

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