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Development of Pharmacological Target using Oxacyl Reductase Protein in Tuberculosis.

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ABSTRACT

Tuberculosis (TB) usually attacks the lungs (as pulmonary TB) but can also affect the central nervous system, the lymphatic system, the circulatory system, the genitourinary system, the gastrointestinal system, bones, joints, and even the skin. Other mycobacteria such as *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium canetti*, and *Mycobacterium microti* also cause tuberculosis, but these species are less common. Infection with Mycobacterium transmission occurs cough, sneeze, speak, split or drops. In TB without prior knowledge of Mycobacterium life cycle and its respective pathway we can not make the drug. The paper reports on study of Mycobacterium life cycle, binding site prediction & De novo design against Oxacyle reductase (MabA) protein for Tuberculosis.

Keywords : Oxacyl reductase, tuberculosis, swissport, drug target, pharmacophore

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INTRODUCTION

Drug design is the approach of finding drugs by design, based on their biological targets. Typically a drug target is a key molecule involved in a particular metabolic or signalling pathway that is specific to a disease condition or pathology, or to the infectivity or survival of a microbial pathogen. Bioinformatics nowadays entails the creation and advancement of databases, algorithms, computational and statistical techniques, and theory to solve formal and practical problems arising from the management and analysis of biological data. Over the past few decades rapid developments in genomic and other molecular research technologies and developments in information technologies have combined to produce a tremendous amount of information related to molecular biology [1-7]. Common activities in bioinformatics include mapping and analyzing DNA and protein sequences, aligning different DNA and protein sequences to compare them and creating and viewing 3-D models of protein structures. The primary goal of bioinformatics is to increase our understanding of biological processes. What sets it apart from other approaches, however, is its focus on developing and applying computationally intensive techniques (e.g., data mining, and machine learning algorithms) to achieve this goal. Major research efforts in the field include sequence alignment, gene finding, genome assembly, protein structure alignment, protein structure prediction, prediction of gene expression and protein-protein interactions, and the modeling of evolution [5,6,10-14].

Ab initio- or *de novo*- protein modelling methods seek to build three-dimensional protein models "from scratch", i.e., based on physical principles rather than (directly) on previously solved structures. There are many possible procedures that either attempt to mimic protein folding or apply some stochastic method to search possible solutions. These procedures tend to require vast computational resources, and have thus only been carried out for tiny proteins. To predict protein structure *de novo* for larger proteins will require better algorithms and larger computational resources like those afforded by either powerful supercomputers (such as Blue Gene or MDGRAPE-3) or distributed computing. Although these computational barriers are vast, the potential benefits of structural genomics (by predicted or experimental methods) make *ab initio* structure prediction an active research field [6,7,10].

Some approaches attempt to stop the functioning of the pathway in the diseased state by causing a key molecule to stop functioning. Drugs may be designed that bind to the active region and inhibit this key molecule. However these drugs would also have to be designed in such a way as not to affect any other important molecules that may be similar in appearance to the key molecules. Sequence homologies are often used to identify such risks. Other approaches may be to enhance the normal pathway by promoting specific molecules in the normal pathways that may have been affected in the diseased state [8-12].

The structure of the drug molecule that can specifically interact with the biomolecules can be modeled using computational tools. These tools can allow a drug molecule to be constructed within the biomolecule using knowledge of its structure and the nature of its active site. Construction of the drug molecule can be made inside out or outside in depending on whether the core or the R-groups are chosen first. However many of these approaches are plagued by the practical problems of chemical synthesis. Newer approaches have also suggested the use of drug molecules that are large and proteinaceous in nature rather than as small molecules. There have also been suggestions to make these using mRNA. Gene silencing may also have therapeutical applications [10-14].

Denovo drug designing is an interactive process in which the three dimensional structure of the receptor is used to design newer molecule. It involves structural determination of the lead target complexes and design to lead modification using molecular modelling tools. It can also be used to design a new chemical classes of the compound that present similar substituent's to the target using a template which is chemically distinct from previously characterized leads. Denovo drug design provides an *in silico* toolkit for the design of novel small molecular structure to set of specified structural constraints. It involves manipulation of input and modification of structural constraint.

Disease information-tuberculosis

Tuberculosis (abbreviated as TB for *tubercle bacillus* or Tuberculosis) is a common and often deadly infectious disease caused by mycobacteria, mainly *Mycobacterium tuberculosis* (1-5). Tuberculosis usually attacks the lungs (as pulmonary TB) but can also affect the central nervous system, the lymphatic system, the

circulatory system, the genitourinary system, the gastrointestinal system, bones, joints, and even the skin. Other mycobacteria such as *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium canetti*, and *Mycobacterium microti* also cause tuberculosis, but these species are less common [6-11].

The classic symptoms of tuberculosis are a chronic cough with blood-tinged sputum, fever, night sweats, and weight loss. Infection of other organs causes a wide range of symptoms. The diagnosis relies on radiology (commonly chest X-rays), a tuberculin skin test, blood tests, as well as microscopic examination and microbiological culture of bodily fluids [8]. Tuberculosis treatment is difficult and requires long courses of multiple antibiotics. Contacts are also screened and treated if necessary. Antibiotic resistance is a growing problem in (extensively) multi-drug-resistant tuberculosis. Prevention relies on screening programs and vaccination, usually with Bacillus Calmette-Guérin (BCG vaccine) [11-14].

MATERIALS AND METHODOLOGY

NCBI database used

Established in 1988 as a national resource for molecular biology information, NCBI creates public databases, conducts research in computational biology, develops software tools for analyzing genome data, and disseminates biomedical information - all for the better understanding of molecular processes affecting human health and disease.

- PDB
- The Protein Data Bank (PDB) is a repository for the 3-D structural data of large biological molecules, such as proteins and nucleic acids. (See also crystallographic database). The data, typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world, are released into the public domain, and can be accessed at no charge on the internet. The PDB is overseen by an organization called the Worldwide Protein Data Bank, wwPDB.

The PDB is a key resource in areas of structural biology, such as structural genomics. Most major scientific journals, and some funding agencies, such as the NIH in the USA, now require scientists to submit their structure data to the PDB. If the contents of the PDB are thought of as primary data, then there are hundreds of derived (i.e., secondary) databases that categorize the data differently. For example, both SCOP and CATH categorize structures according to type of structure and assumed evolutionary relations; GO categorize structures based on genes.

Software Used

Discovery studio 2.1

Discovery Studio 2.1, the most advanced computational drug discovery environment available, features significant new science and usability enhancements. Access a novel QM/MM method for refining docked poses and optimizing specialized interaction motifs such as cation-pi interactions. Learn about enhanced capabilities to easily customize DS workflows with an unprecedented level and quality. Join Accelrys scientists for this exciting seven-part webinar series as they present the latest scientific advancements and usability upgrades available to help excel your drug discovery research.

METHODOLOGY

- Selection of Disease
  Selection of Disease is main step of the De Novo drug design.In this project Tuberculosis has been taken as the disease.
- Selection of Protein
  Selection of protein is the second next step of the De Novo drug design.In this project MabA(3-oxoacyl reductase) has been taken as a protein.This protein is taken from PIR.
- Retrieval of Protein structure
  The protein structure has retrieved from the PDB.
- Binding Site Analysis

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Binding site analysis is necessary for Ligand Binding.So,in this target 1st site is taken.because it is largest binding site.

- Docking Analysis

  In the Docking Analysis De Novo Receptor Protocol is used.

  De Novo Receptor :-

  De Novo Receptor protocol is used to generate fragments from the binding sites.

- QSAR

  QSAR is a protocol which is used to check the molecular properties of the target molecule.

- Pharmacophore

  Pharmacophore is a protocol which is used to check the flexibility of drug or target molecule inside the body.

  - Diverse Conformation Generation
  - Common feature pharmacophore
  - Interaction Generation

ADMET is a protocol which is used to check solubility level & drug likeness of the molecule & it is also used check Hapatotoxicity & blood brain barrier level.

RESULT AND DISCUSSION

- UNIPROT:

  Identifying the target protein information from the swissprot

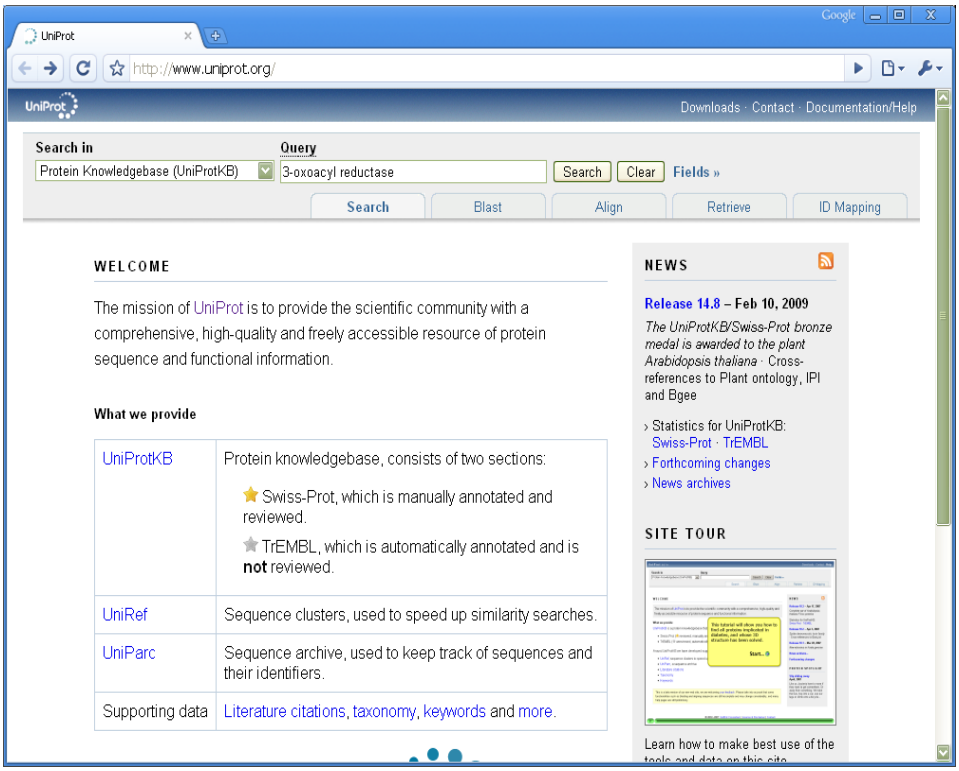

Figure 1: The above figure shows that entry of the target molecule MabA retrieved from swissport database. The target ID of protein of P0A5Y4/FABG_MYCTU.

Retrieving the protein information:

The structure of protein MabA was retrieved from the protein data bank. The PDB ID of 3-Oxoacyl Reductase used for study was 2NTN.

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Figure 2: Retrieving protein information from PDB

By considering this as our target we can proceed with Binding site prediction so that we can generate fragments from the Binding site. The Binding site or the cavities available were calculated

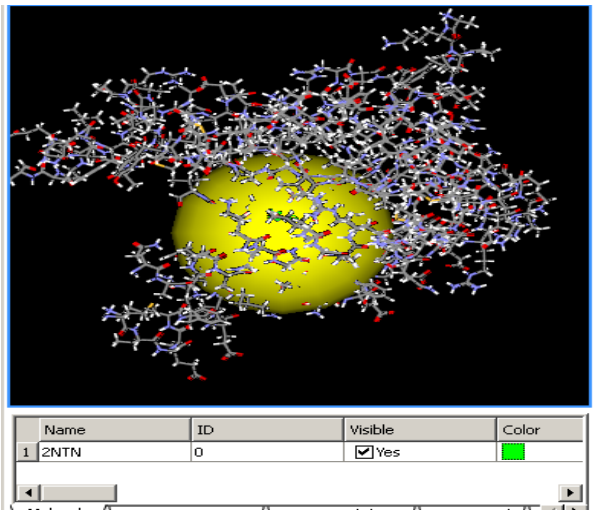

Figure 3: The picture shows one Binding site. The site1 is green color seen in the forefront of the picture is the largest Binding site, which is consider to be the best site for ligand Binding.

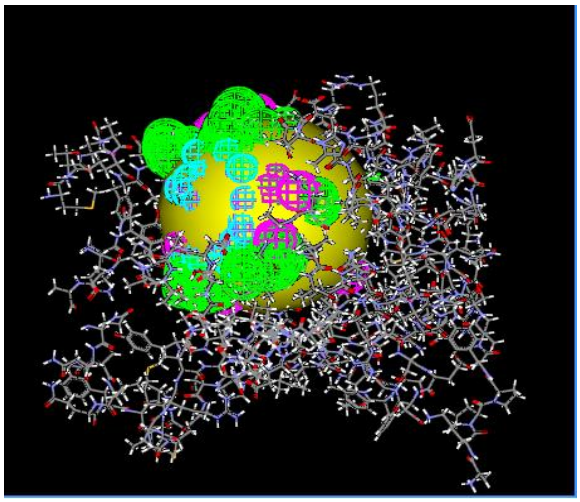

Figure 4: The above picture shows possible Pharmacophoric Features present in the binding site region.

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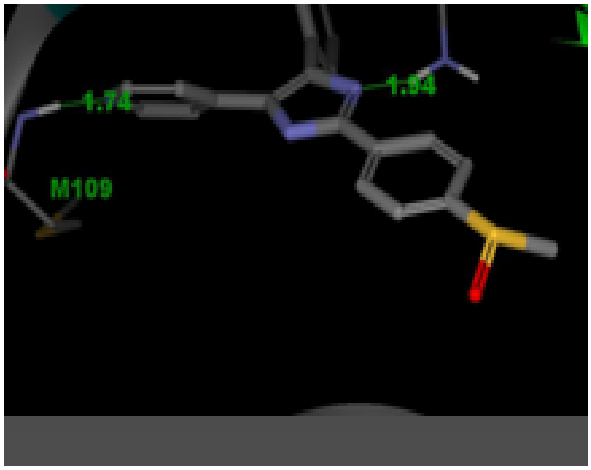

Figure 5: The above figure showed three H-bond Interaction with Gln 63 and ser 67.

The interaction of lead molecule with the receptor protein was analyzed based on the H-bond formation and dock score. Maximum two different poses of lead molecules were analyzed for their interaction with the receptor. The pose that gives a maximum dock score was considered to be the best orientation for the interaction. The active site of protein i.e amino acid that interact with lead molecule was identified by viewing H-bond. The H-bond were also calculated.

The QSAR predicts the property of G13 molecule, which undergoes the Lipinski's rule of 5. The various properties of drug are AlogP value, molecular weight, number of number of H-bond acceptor and number of H-bond donor. The above figure 4 showed the property of fragments. The pharmacophore of the lead molecule were predicted considering the most important feature like Hydrophobic region, H-bond donors and H-bond acceptor. The pharmacophoric features were predicted by analyzing different confirmation of molecule. The figure 5 showed pharmacophoric features such as hydrophobic region and H-bond donor properties.

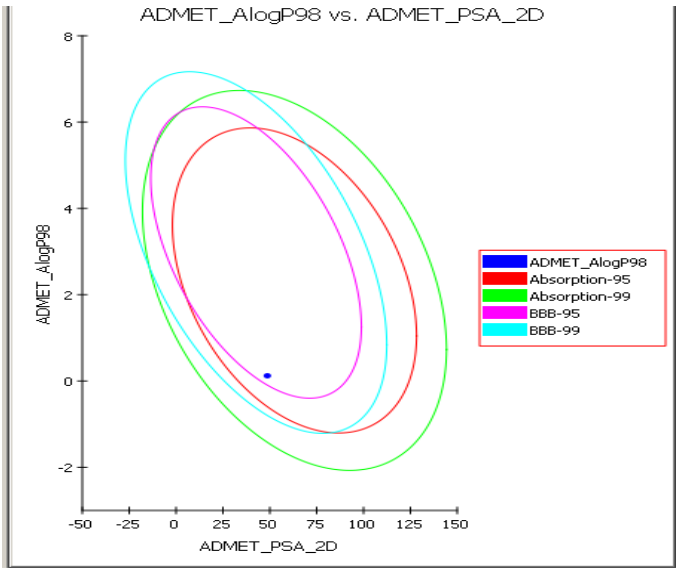

Figure 6: Admet properties have been calculated for the molecule and showed result that G13 fragment is well absorbed at the intestinal level but it has crossed the Blood Brain Barrier.

ADMET is used to check blood brain barrier, hepatotoxicity of the target molecule. In this paper MabA(3-Oxocyl reductase) of Mycobacteria was used as a target protein (Figure 6). The drug designing process was done by using the Accelrys Discovery studio 2.1. The binding site of target protein was predicted. Using De Novo concept small molecule from the binding site of the protein have been generated. The H-bond interaction between the protein and the fragment obtains and check their distance was calculated. Along with

this the fragment's ADMET, QSAR & PHARMACOPHORE properties have analysed to see whether it is a suitable for a drug or not. As these analysis showed positive result, this fragment G13 can be tried in wet laboratory so that it may be act as a lead molecule by acting on the protein MabA from *Mycobacterium Tuberculosis* which cause TB.

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