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Docking studies of doxycycline on pathogenic Leptospira Species with common pharmacopore

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ABSTRACT

Every disease can be cured if its roots are traced; means there is cure for every disease. Leptospirosis is a hidden and emerging public health problem as it causes fever, acute renal failure and jaundice. The major outer membrane lipoprotein "LipL32" is expressed during infection which is highly conserved among pathogenic leptospira. It is absent in non-pathogenic avirulent leptospira. Insilico comparison of amino acid sequences of LipL32 showed 97.8% average sequence identity among the pathogenic species. In this study some 3D model were built for LipL32 protein using molecular modelling. Protein hydrophobicity plots were utilized to locate the antigenic sites. Docking studies of retrieved structures and modeled LipL32 proteins with doxycycline revealed that doxycycline can inhibit LipL32 proteins of *Leptospira santarosai*, *Leptospira weilii* and *Leptospira kirschner* as they have bound docking with doxycycline. They share a common pharmacopore within a genus and a common drug may be utilized to combat all the related species.

Introduction

Mankind has been exposed to numerous pathogens; Leptospirosis is probably the world's most widespread zoonosis (16) caused by a gram-negative bacteria *Leptospira interrogans serovar lai str*. The disease was first recognized as an occupational disease of sewer workers in 1883. In 1886, Weil described the clinical manifestations in humans who had severe jaundice, fever, and hemorrhage with renal involvement. The first human case of leptospirosis was described in 1886 as a severe icteric illness and was referred to as Weil's disease. However, most human cases of leptospirosis are nonicteric and are not lives threatening (2).

In India leptospirosis was reported in Andaman and Nicobar Islands in 1920's. Later during 1988-1997, 527 cases were reported of which 104 were serious. It is evident that leptospirosis is spread all over India. Fever, acute renal failure and jaundice are the symptoms in humans. Leptospirosis was also reported in cattle. As there is no proper system of notification (to public health authorities), leptospirosis is grossly under-reported. Therefore, it appears to be a hidden and rising health problem.

Antigenic protein:

LipL32 is the major leptospiral antigenic protein present in the outer membrane which is a lipoprotein expressed during infection and is immunodominant antigen. It is recognized during the humoral immune response in humans(1,4,7). It is the most abundant lipoprotein found in the leptospiral total protein profile, and is having a calculated molecular mass of 26.7 kDa after the cleavage of its signal peptide, but an observed electrophoretic mobility of approximately 32 kDa (8,9). LipL32 is highly conserved among pathogenic leptospires but has no orthologs in the saprophytic *Leptospira biflexa*. It has been shown to enhance hemolysis mediated by sphingomyelinase SphH, and for this reason, the protein was also identified as hemolysis-associated protein "Hap-1" which is expressed at high levels both during cultivation and during natural infection (13).

Novel aspects of LipL32 were defined in the immunogenic portions of the molecule, which indicate that both the C terminus and the intermediate portion of LipL32 are recognized by human sera, with the C terminus being detected earlier in the course of infection. LipL32 could contribute to tissue invasion and colonization by interacting with extracellular matrix (ECM). LipL32 has been evaluated as an antigen for immunodiagnosis and as a vaccine antigen, showing protection against *Leptospira interrogans* challenge in animals immunized with recombinant adenovirus, DNA vaccine, or recombinant *Mycobacterium bovis* BCG (17).

Doxycycline:

The advent of cutting-edge technologies has opened the ways to know more about the disease, by producing broad spectrum antibiotics which are mild with few side effects. Doxycycline is one such drug. Doxycycline has a high degree of lipid solubility and a low affinity for calcium binding. It is highly stable in normal human

serum. The efficacy of azithromycin was not inferior to that of doxycycline for the treatment of both leptospirosis (15). Doxycycline is derived from tetracycline is considered for the study. "Vibramycin is the trade name of doxycycline". Doxycycline is a synthetic tetracycline derivative with similar antimicrobial activity. Animal studies suggest that it may cause less tooth staining than other tetracyclines. Doxycycline is an effective drug listed as treatment for 15 conditions; alternative treatment for 0 conditions; preventive treatment for 2 conditions; research treatment for 1 condition. It can have toxic effects on development of bone in the fetus and not recommended to pregnant (14).

Docking: In docking, we attempt to predict inter-molecular interactions between two or more molecules. Docking is widely used to suggest the binding modes of protein inhibitors. Most docking algorithms are able to generate a large number of possible structures, and so they also require a means to score each structure to identify those of most interest. The 'docking problem' is concerned with the generation and evaluation of plausible structures of inter-molecular complexes which include ACE which is Atomic Contact Energy(3), Area which is approximate interface area of the complex and GSC Score which is Geometric Shape Complementarity Score. The docking problem involves many Degrees Of Freedom (DOFs). There are six degrees of translational and rotational freedom which include 3 rotational angles and 3 translational parameters for one molecule relative to the other molecule.

Materials and Methods

NCBI:

National Center of Biotechnology Information (http://www.ncbi.nlm.nih.gov/) is a menu driven program which is an integrated, text-based search and retrieval system called Entrez. NCBI for the major databases, including Pub Med, Nucleotide and Protein Sequences, Protein Structures, Complete Genomes, Taxonomy, and others. Amino acid sequences of LipL32 protein of pathogenic leptospires are retrieved from NCBI.

PDB:

The Protein Data Bank (www.rcsb.org/pdb/) is the single worldwide archive of structural data of biological macromolecules. It is an archive for crystal structures of biological macromolecules. PDB provides a variety of tools and resources for studying the structures of biological macromolecules and their relationships to sequence, function, and disease. Elucidated LipL32protein structure 2ZZ8 (19) were retrieved from PDB using the "PDB ID or keyword" from the database.

Maestro 9.0:

Prime structure prediction panel in maestro 9.0 from Schrodinger has a built-in database for Structure Prediction, Refinement and MM-GBSA (molecular mechanics, the generalized Born model and solvent accessibility method). The retrieved sequences whose 3D models are not available in PDB are modeled using prime.

Protein Hydrophobicity Plots: Hydrophobicity plots (http://www.vivo.colostate.edu/molkit/hydropathy) are designed to identify the antigenic sites in protein, which are mostly hydrophobic in nature. This type of analysis has the goal of predicting membrane – spanning segments (highly hydrophobic) or regions that are likely exposed on the surface of proteins (hydrophilic domains) and therefore potentially antigenic The authors suggest that, using a window size of 6, the region of maximal hydrophilicity is likely to be an antigenic site (11,12).

DrugBank

he DrugBank (http://www.drugbank.ca/) database is a unique bioinformatics and cheminformatics resource that combines detailed drug data with comprehensive drug target information. Each DrugCard entry contains more than 150 data fields with half of the information being devoted to drug/chemical data and the other half devoted to drug target or protein data. Through data mining helped in identifying the available drugs for leptospirosis and selecting a mild activity with fewer side effects. Doxycycline is one of such kind antibiotic used to treat leptospirosis which is a broad spectrum drug in terms of efficacy and side effects (5,10).

PatchDock:

PatchDock(http://bioinfo3d.cs.tau.ac.il/PatchDock/) is an algorithm for molecular docking. The input is two molecules of any type: proteins, DNA, peptides, drugs. The output is a list of potential complexes sorted by shape complementarity criteria. PatchDock algorithm is inspired by object recognition and image segmentation techniques used in Computer Vision. Docking can be compared to assembling of a puzzle (6,18). The LipL32 proteins of 6 pathogenic leptospires were docked with doxycycline drug

Biodesigner:

Biodesigner is a general-purpose molecular modeling and visualization software tool for homology building of proteins or DNA. The docked molecules are visualized using Biodesigner to analyze whether the drug is interacted within the same pharmacopore for all the proteins.

Results

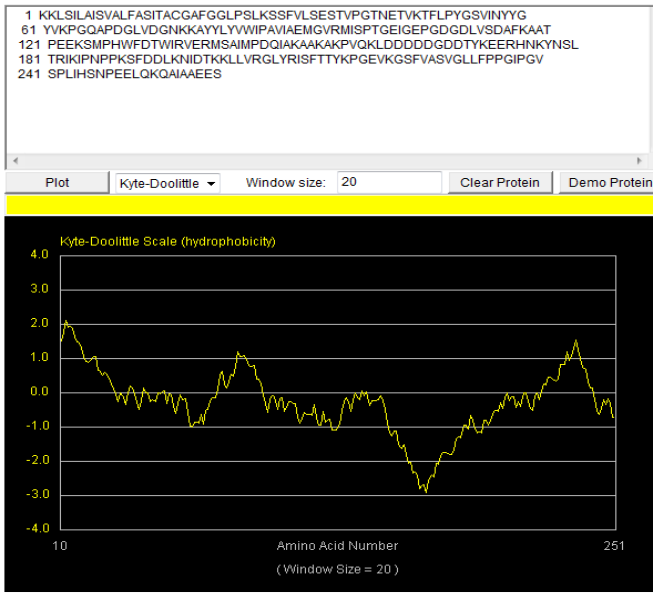

Figure: 1 showing hydrophobicity Plot of *Leptospira santarosai*

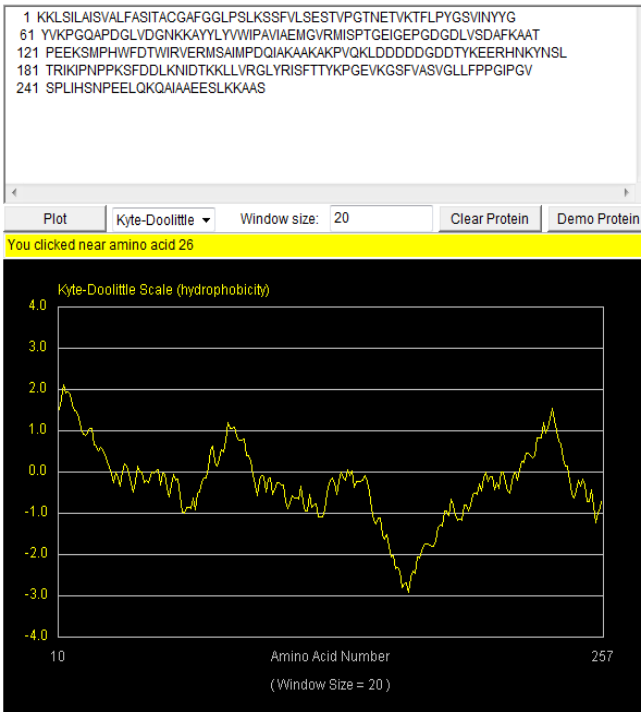

Figure: 2 showing hydrophobicity Plot of *Leptospira weilii*

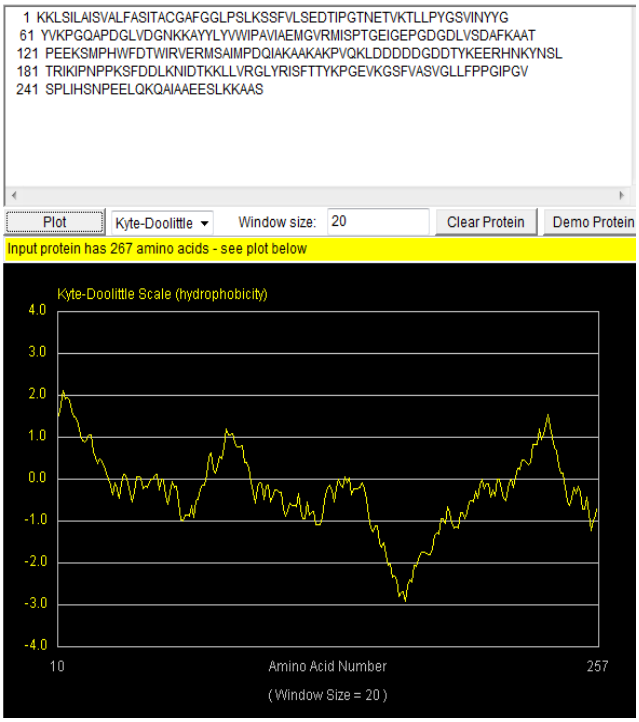

Figure: 4 showing hydrophobicity Plot of *Leptospira noguchii*

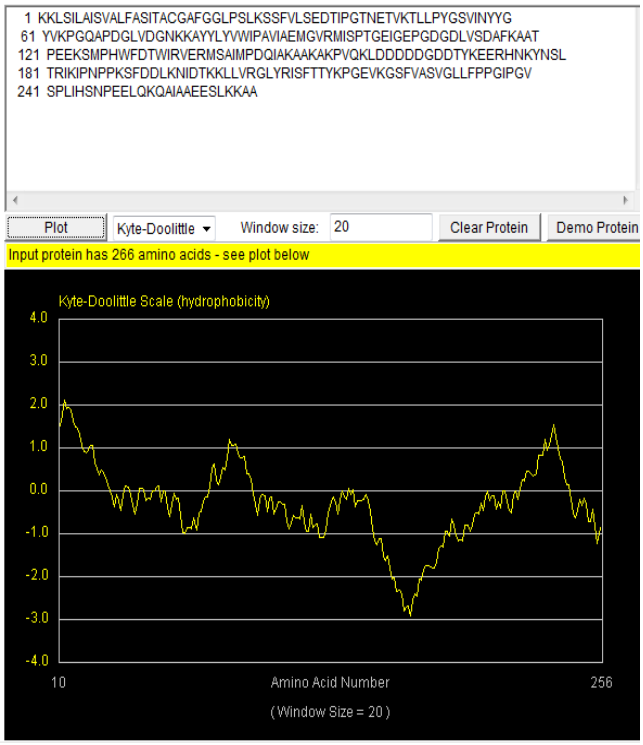

Figure: 3 showing hydrophobicity Plot of *Leptospira kirschneri*

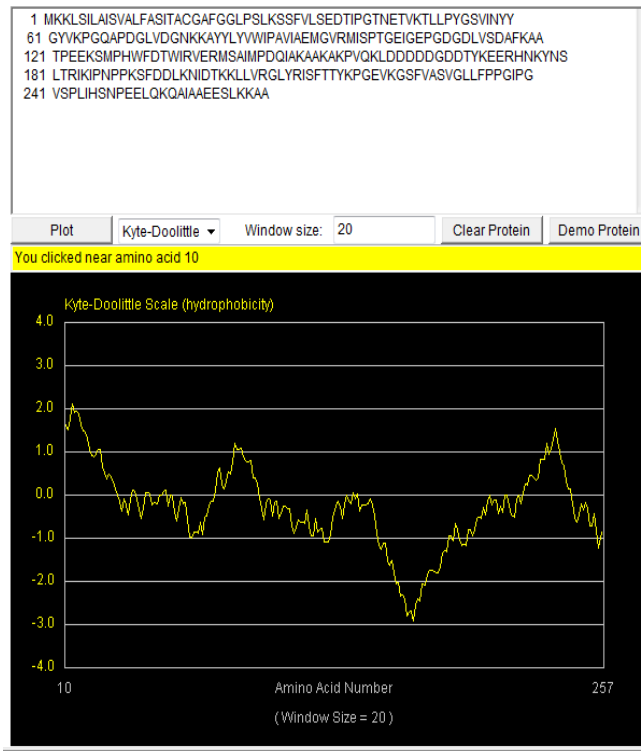

Figure: 5 showing hydrophobicity Plot of *Leptospira interrogans*

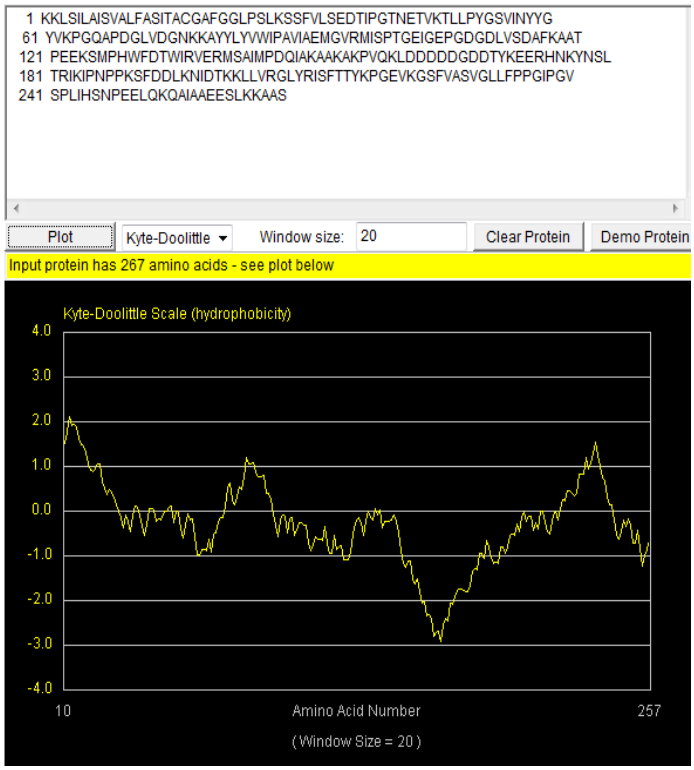

Figure: 6 showing hydrophobicity Plot of *Leptospira borgpetersenii*

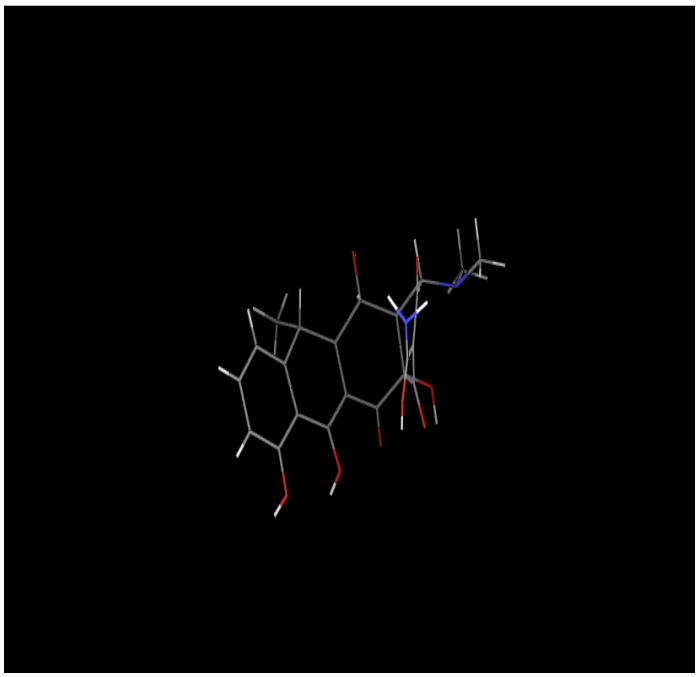

Figure: 7 showing doxycycline drug molecule in Biodesigner

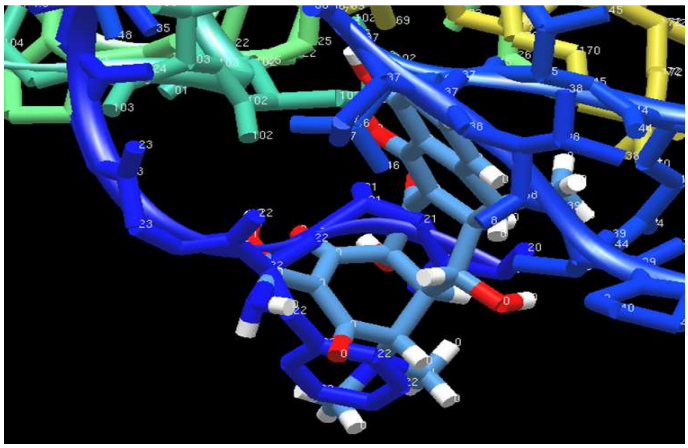

Figure: 8 showing docked view of *L.santarosai* in Biodesigner

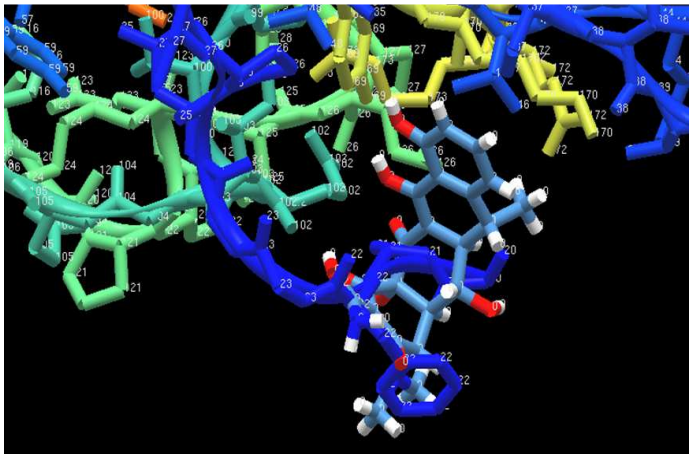

Figure: 9 showing docked view of *L. weilii* in Biodesigner

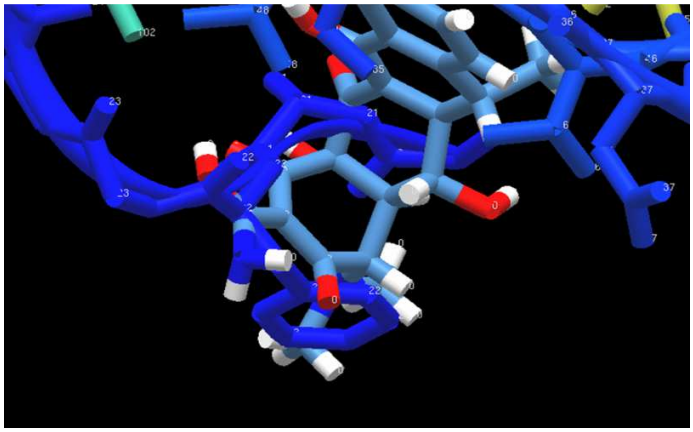

Figure: 10 showing docked view of *L. kirschneri*

Discussion:

The results revealed that the potent antigenic site for the LipL32 protein of pathogenic leptospiral species is located in-between 18th to 27th amino acid residues and 93rd to 105th amino acid residues of the LipL32 protein in protein hydrophobicity plots. the antigenic site is lying approximately in-between 12th to 42nd amino acids, 102nd to 127th amino acids and 167th to 197th amino acid residue in almost all the modeled proteins as the peaks in the Kyte-

Doolittle plots are typically >1.6 in the scale which infers the antigenic site with the window size of 20.

The docking results showed that doxycycline is exhibiting a bound docking with LipL32 proteins of *Leptospira santarosai*, *Leptospira weilii* and *Leptospira kirschneri* within the same pharmacopore site located at 21st, 22nd and 23rd amino acids which are Alanine (hydrophobic), Phenylalanine (very hydrophobic) and Glycine (amphiphilic) respectively.

The docking results in the remaining species like *Leptospira interrogans*, *Leptospira borgpetersenii and Leptospira noguchii* are varying as bound docking was not observed within the pharmacopore pocket site for the remaining species.

Conclusion:

The concept behind the work is to propose an efficient mild drug for leptospirosis, which can combat LipL32 antigenic protein within the pathogenic leptospires through insilico docking studies.

The docking results gave an understanding that doxycycline can inhibit the LipL32 antigenic proteins present in the three leptospiral species like *Leptospira santarosai*, *Leptospira weilii*, *Leptospira kirschner* which was concluded by the bound docking within the same pharmacopore as they have sequence identity. So, if few species are sharing a common sequence they may have a common pharmacopore and a common drug can be used to combat the related species.

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