Journal of Computational Intelligence in Bioinformatics
ISSN 0973-385X Volume 4, Number 2 (2011) pp. 209-220

http://www.ripublication.com/jcib.htm

Insilco Homology Modeling & Drug Designing of Crystal Structure of the Von Willebrand Factor a Domain of Human Capillary Morphogenesis Protein Involved in Anthrax

[1]Tappa Mohammad Munawar and [2]P. Mamatha

[1]Department of Biotechnology, [2]Department of Chemistry,
J.N.T.U.A. College of Engineering, Pulivendula-516390 Andhra Pradesh, India.
E-mail: E-mail: munna686@gmail.com, mkandukuri@gmail.com

Abstract

Computational data provides new opportunities for finding optimal targets among previously unexplored cellular functions based on an understanding of their related biological processes in bacterial pathogens and hosts. Anthrax is an acute infectious disease caused by the spore-forming bacterium Bacillus anthracis. Anthrax most commonly occurs in wild and domestic lower vertebrates. Symptoms of disease vary depending on how the disease was contracted, Cutaneous, Inhalation, Intestinal, signs include nausea, loss of appetite, vomiting, fever are followed by abdominal pain, vomiting of blood, and severe diarrhea. This project study is based on molecular docking for Crystal Structure of the von willebrand Factor a domain of human capillary Morphogenesis protein involved in Anthrax. Finally, I have analyzed the quality factor for the protein by energy minimization and problematic aminoacids are rectified. The present study involves the drug docking analysis for the existing drugs for Anthrax. Form the results obtained the synthesized drug molecule and their binding to the target protein show its maximum efficiency. Finally, Fosphenytoin has the lowest distance 1.34 Angstroms and was found to be the best drug for Anthrax disease.

Keywords: Bacillus anthracis, Homology modeling, Molecular docking, Anthrax.

Introduction

Computational data and experimental data of genomes and microorganisms and their

host facilitate novel opportunities for finding best drug targets among previously unexplored cellular functions. The bioinformatics approach is assumption that the potential target must play a prominent role in the pathogen's survival and constitute a critical component in its metabolic pathway. At the same time, this target should not have any well-conserved homolog in the host. This would preclude possibilities of unacceptable cross-reactivity that might prove detrimental to the host. Drug targets identification is essentially substractive because we use a substraction template while comparing the genome under consideration. This project study is based on molecular docking for Crystal Structure of the von willebrand Factor a domain of human capillary Morphogenesis protein involved in Anthrax. Analyzing the quality factor for the protein by energy minimization and problematic aminoacids are rectified. The present study involves the drug docking analysis for the existing drugs for Anthrax.

Anthrax is one of the oldest recorded diseases of grazing animals such as sheep and cattle and is believed to be the Sixth Plague mentioned in the Book of Exodus in the Bible. Anthrax ("siberian ulcer") is now fairly rare in humans, although it still regularly occurs in ruminants, such as cattle, sheep, goats, camels, wild buffalo, and antelopes, in hind-gut fermenters such as zebras and rhinos, and in other wildlife such as elephants and lions in certain endemic areas of the world [1].

Anthrax is an acute infectious disease caused by the spore-forming bacterium Bacillus anthracis. Bacillus anthracis bacteria spores are soil-borne, Gram-positive, spore forming rod, 1 - 1.2µm in width x 3 - 5µm in length. The bacterium can be cultivated in ordinary nutrient medium under aerobic or anaerobic conditions [2] Anthrax most commonly occurs in wild and domestic lower vertebrates. Symptoms of disease vary depending on how the disease was contracted, Cutaneous, Inhalation, Intestinal, signs include nausea, loss of appetite, vomiting, fever are followed by abdominal pain, vomiting of blood, and severe diarrhea. Symptoms may also include lesions and soreness in the throat. Anthrax is diagnosed by culture and isolation of the causative bacterium, B.anthracis by detecting the bacterial DNA or antigens or by measuring specific antibodies in the blood of persons with suspected cases [3]. The bacteria can be cultured from the blood, skin lesions, fluid from the lungs or respiratory secretions, spinal fluid, or other affected tissues prior to the start of antibiotic treatment. Detection of the DNA or antigens of the bacteria, and detection of antibodies in the blood of suspected cases, are important tools for diagnosis because positive culture is unlikely after antibiotic treatment has been started [4].

Materials and Methods

Materials

Software's used are BLASTp, GENO3D, SPDBV.PROCHECK, CASTp, SAVS, HEX 4.5 [5].Databases used SWISSPROT [6], PBIL [5], and DRUG BANK [7].

Homology modeling and model evaluation methodology

The sequences related to disease anthrax have been retrieved from the swissprot database. Then the corresponding FASTA sequence was submitted to BLASTp server then from the list of templates. BLAST search results showed the structure of

template of Bacillus anthracis (GFP E1) (Fig 1) and target protein of Homo sapiens (Fig 2) was the most suitable homology among BLAST comparison hits. The overall sequence identity between structure of template sequence and target protein was 58%. The resultant of BLAST search identified structure template (PDB ID 1SHU) and the expected value is 4.1e-14 [8].This desired template has been selected and then submitted to launch geno3D, and then modeled protein has been retrieved. Then by using SPDBV the template and target sequences was made best fit then structures was viewed using Ramachandran plot (Fig 3&4).The result of predicted model of Ramachandranplot in procheck (Fig 5) illustrates 97.3% residues in allowed region [9]. Optimize the structure energy minimization and optimized structure(Fig 6) was submitted to SAVS server for further statistical analysis and the good quality model would be expected to have 92.39 %[10].The optimized model was submitted to CASTp server to predict active sites for optimized model. Then from the result the filename with.poc extension was selected. Then the sequence in filename.poc file was copied to notepad by using SPDBV active sites were added to the optimized model and the whole complex was modeled.

There is urgent need to develop new classes of antibacterial drugs to tackle effective drug targets in bacterial pathogens which unable to grow in *invitro conditions* [11]. The drug targets were retrieved from the database Drug Bank related to corresponding disease by using its name. The appropriate drug targets used for the docking found with the following names are Captopril, Conjugated estrogens, Methacyclin, Clavulanate, Fosphenytoil, Atenolol, Trandolapril, Norfloxacin. To dock the model and corresponding drug targets the software tool HEX 4.5 was used. The ligand and receptor molecules were loaded in to the tool then by the graphics option dot surfaces were selected. Then from the option controls matching and docking options were selected to perform the docking with default parameters [12].

The result was saved and viewed in SPDBV to analyze the docking between corresponding receptor and ligand. The docked complex was loaded into SPDBV then unknown and remaining residues were modeled with different colors by using the option build hydrogen bonds were computed. The display option was used to view the model in 3D view then the distance of hydrogen bonds between ligand and receptor complex was observed. As the distance between the target molecule and the drug molecule is less than 3Angstroms, it shows high efficiency. The specificity of the drug molecule varies according to the binding sites. The obtained mean values of all bond lengths and bond angles are satisfied with smaller molecular experimental data proposed by Kabsch et al [13]. Form the result of the docking the distance between the target and the drug molecule are less than 3 Angstrom units The appropriate drug targets used for the docking found with the following names are Captopril (Fig 8), Conjugated estrogens (Fig 9), Methacyclin (Fig 10), Clavulanate (Fig 12), Fosphenytoil (Fig 12), Atenolol (Fig 13), Trandolapril (Fig 14), Norfloxacin (Fig 15) shows bond lengths less than 3Angstroms, and their bond lengths are tabulated. Finally, by the tabular column, Fosphenytoin has the lowest distance 1.34 Angstroms and was found to be the best drug for Anthrax disease.

Results

Target Protein Result

Protein sequences in FASTA format

>SP|Q9H6X2|ANTR1_HUMAN ANTHRAX TOXIN RECEPTOR 1 OS=HOMO SAPIENS GN=ANTXR1 PE=1 SV=2

MATAERRALGIGFQWLSLATLVLICAGQGGRREDGGPACYGGFDLYFILD
KSGSVLHHWNEIYYFVEQLAHKFISPQLRMSFIVFSTRGTTLMKLTEDREQIR
QGLEELKVLPGGDTYMHEGFERASEQIYYENRQGYRTASVIIALTDGELHEDL
FFYSEREANRSRDLGAIVYCVGVKDFNETQLARIADSKDHVFPVNDGFQALQ
GIIHSILKKSCIEILAAEPSTICAGESFQVVVRGNGFRHARNVDRVLCSFKINDS
VTLNEKPFSVEDTYLLCPAPILKEVGMKAALQVSMNDGLSFISSSVIITTTHCS
DGSILAIALLILFLLLALALLWWFWPLCCTVIIKEVPPPPAEESEEEDDDGLPKK
KWPTVDASYYGGRGVGGIKRMEVRWGEKGSTEEGAKLEKAKNARVKMPEQ
EYEFPEPRNLNNNMRRPSSPRKWYSPIKGKLDALWVLLRKGYDRVSVMRPQ
PGDTGRCINFTRVKNNQPAKYPLNNAYHTSSPPPAPIYTPPPPAPHCPPPPPSAP
TPPIPSPPSTLPPPPQAPPPNRAPPPSRPPPRPSV

Blast Output

A simple BLASTp searching method was carried out against Protein data bank [8].The resultant of BLAST search identified structure template (PDB ID 1SHU) with 58% identity and the expected value is 4.1e-14.

Structure of the Template

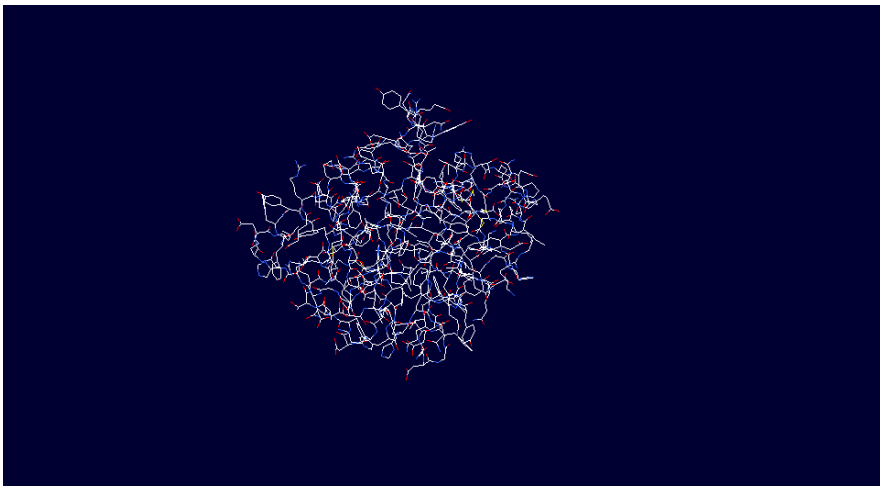


Figure 1: Structure of template.

Structure of Target

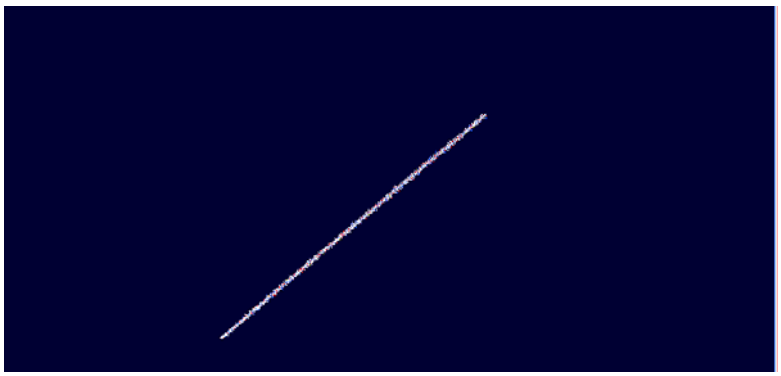


Figure 2: Structure of template.

Structure of Target and Template

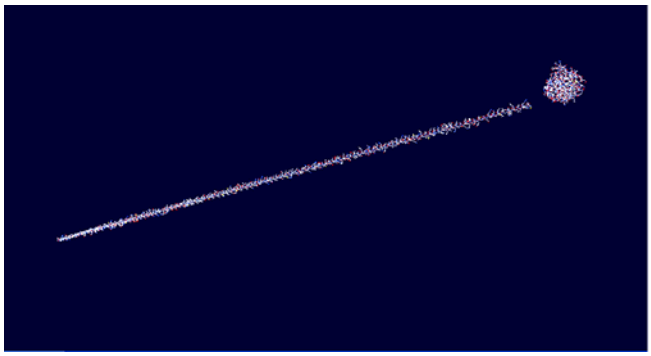


Figure 3: Structure of target and template.

Fitting of Target with Template

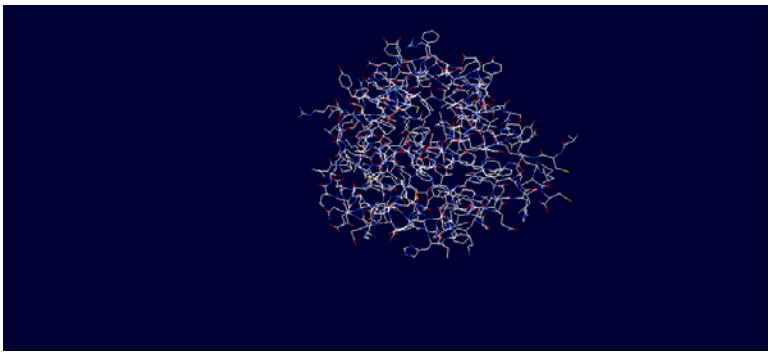


Figure 4: Fitting of target and template.

Ramachandran Plot Analysis of Amino Acids of Template Protein

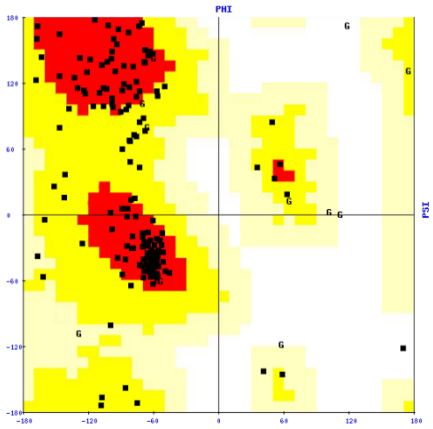


Figure 5: The Ramachandran plot of Template protein. The dark grey area represents most allowed regions, whereas the medium gray areas represent allowed regions.

SAVS Result

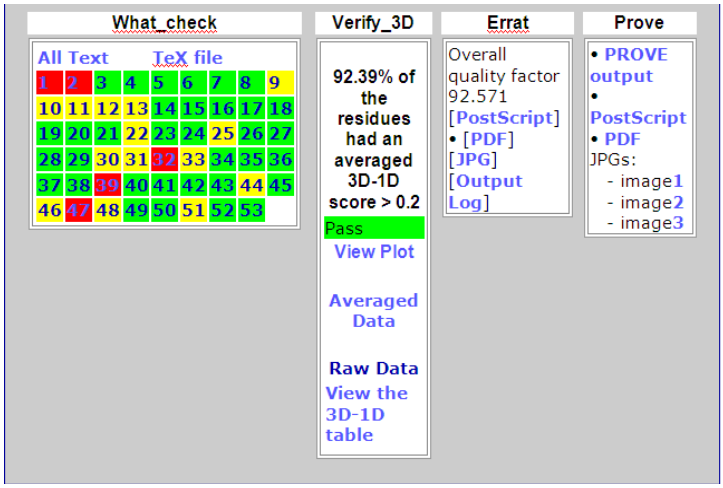
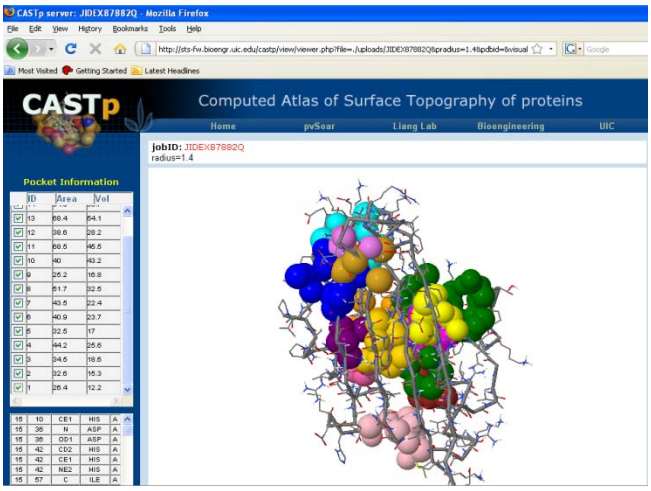


Figure 6: Savs Report.

CASTp Result
Active Site Prediction



Docking Results
Captopril

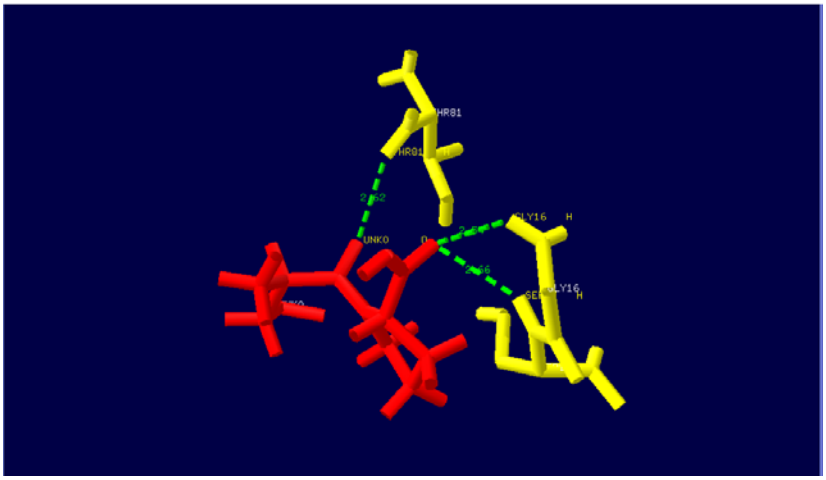


Figure 8: Docking of Captopril drug with modeled Protein.

S.NO	AMINO ACID	BOND LENGTH
1	THR 81	2.62 **Aº**
2	GLY 16	2.54 **Aº**
3	SER 17	2.66 **Aº**

Conjugated Estrogens

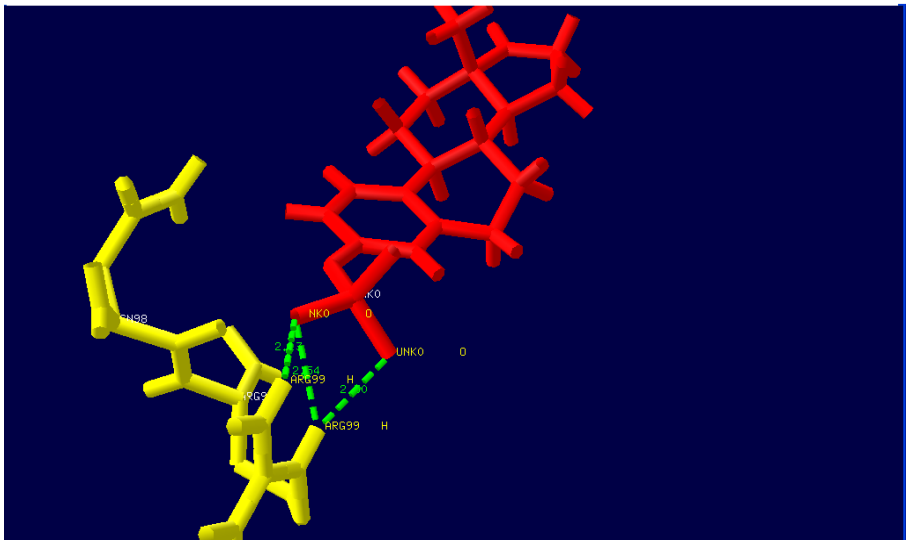


Figure 9: Docking of conjugated estrogens drug with modeled Protein.

S.NO	AMINO ACID	BOND LENGTH
1.	ARG 99	2.50Aº
2.	ARG 99	2.13Aº
3.	ARG 99	2.54Aº

Methacycline

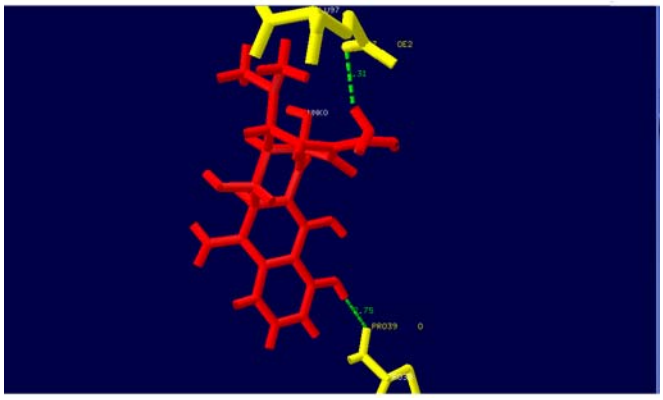


Figure 10: Docking of methacycline drug with modeled Protein.

S.NO	AMINO ACID	BOND LENGTH
1.	GLU 97	2.31Aº
2.	PRO 39	2.75Aº

Clavulanate

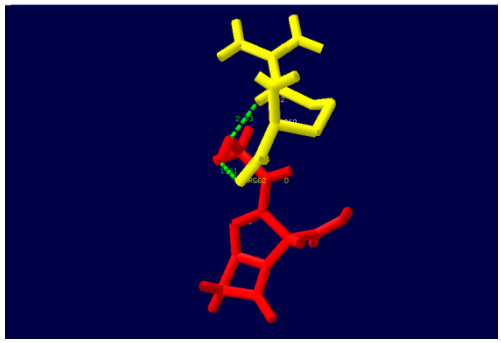


Figure 11: Docking of clavulanate drug with modeled Protein.

S.NO	AMINO ACID	BOND LENGTH
1	ARG 62	2.03 Aº
2	ARG 62	1.61 Aº

Fosphenytoin

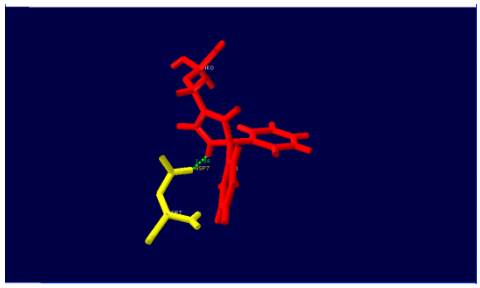


Figure 12: Docking of fosphenytoin drug with modeled Protein.

S.NO	AMINO ACID	BOND LENGTH
1	ASP 7	1.34 Aº

Trandolapril

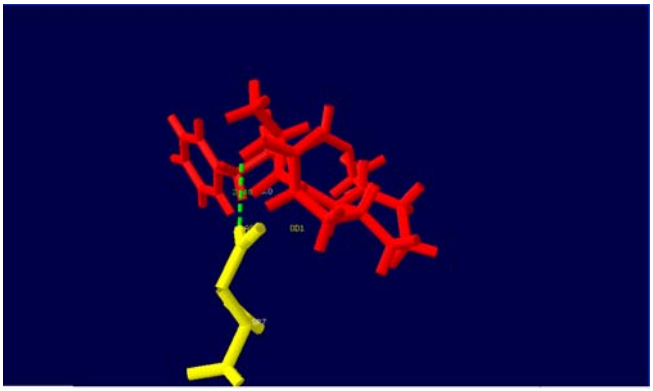


Figure 13: Docking of trandolapril drug with modeled Protein.

S.NO	AMINO ACID	BOND LENGTH
1.	ASP 7	2.40 Aº

Atenolol

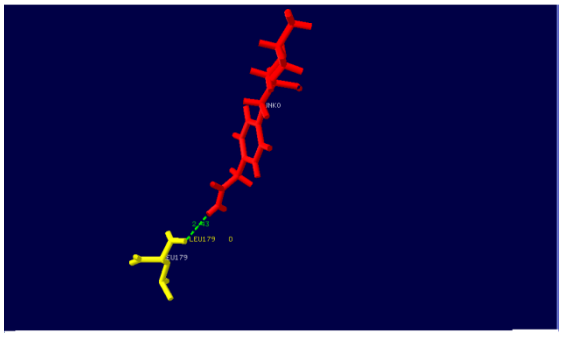


Figure 14: Docking of Atenol drug with modeled Protein.

S.NO	AMINO ACID	BOND LENGTH
1	LEU179	2.43 Aº

Norfloxacin

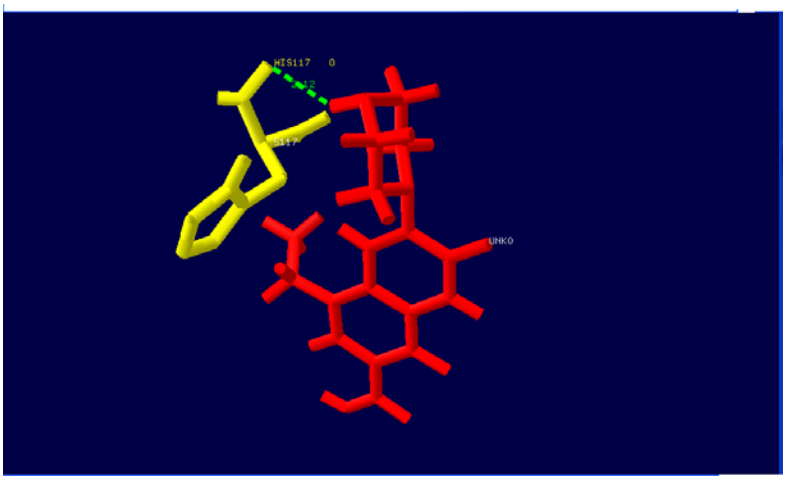


Figure 15: Docking of Norflaxin drug with modeled Protein.

S.NO	AMINO ACID	BOND LENGTH
1.	HIS117	2.12 Aº

Conclusion

The drug industry is one of the major players involved in the development of bioinformatics. Many pharmaceutical companies have internal units conducting bioinformatics research. The competition for finding the solution to a problem that may give the companies that crucial edge is in producing the major drug. The aim of the project is to do a comparative analysis of existing drug compounds-receptor interactions of Crystal Structure of the von willebrand Factor a domain of human capillary morphogenesis protein involved in Anthrax disease.

Molecular modeling is the method of choice when there is a close homology between the sequence of the target protein and the template. Comparative modeling provides a useful model of this protein. Swiss-pdb viewer helps to resolve the problem effectively. The quality of the crystal structure of the Von willebrand factor a domain of human capillary morphogenesis receptor was refined and was evaluated using SAVS server, the quality increased to 92. 39%. To obtain suitable already marketed drugs, a series of the sequential procedures were done. A group of drugs were identified through drug bank. From the list of compounds CAPTOPRIL, METHACYCLINE, CLAVULANATE, FOSPHENYTOIN, TRANDOLAPRIL, ATENOLOL, NORFLOXACIN for Anthrax disease was chosen based on their effectiveness. The drugs were docked with the receptor and their bond lengths were tabulated.

Based on the distance of docking region between that target and the drug molecule, the specificity, efficiency of the molecule as well as pharmaco kinetics of

the drug also can be determined. Form the result of the docking the distance between the target and the drug molecule are less than 3 Angstrom units. From the result of CASTp the active site of the molecule is predicted and the drug molecule specifically binds to these regions. As the distance between the target molecule and the drug molecule is less than 3Angstroms, it shows high efficiency. The specificity of the drug molecule varies according to the binding sites. Form the results obtained the synthesized drug molecule and their binding to the target protein show its maximum efficiency. Finally, by the tabular column, Fosphenytoin has the lowest distance 1.34 Angstroms and was found to be the best drug for Anthrax disease.

This work which was aimed to compare the drug interactions has succeeded in obtaining the goal by identifying the group of drugs. It would be worth enough to do further studies for analog preparation in pharma industries.

References

[1] ANTHRAX, the investigation of a Deadly Outbreak, Jeanne Guillemin, University of California Press, 1999, ISBN 0=520-22917-7, pg 34
[2] "Anthrax" by Jeanne Guillemin, University of California Press, 2001, ISBN 0-520-22917-7, pg. 3.
[3] Bravata DM, Holty JE, Liu H, McDonald KM, Olshen RA, Owens DK (2006), Systematic review: a century of inhalational anthrax cases from 1900 to 2005, Annals of Internal Medicine; 144(4): 270–80.
[4] Sternbach, G. (2002). "The History of Anthrax". The Journal of Emergency Medicine 24(4) 463–467.
[5] http://www.expasy.ch/sprot/
[6] http://geno3d-pbil.ibcp.fr/cgbin/geno3d_automat.pl?page=/GENO3D/geno3d_home.html),
[7] http://www.drugbank.ca/
[8] http://www.ncbi.nlm.nih.gov/blast/
[9] Ramchandran G N, Ramakrishnan C& Sasisekharan (1963), J.Mol.Biol 7, 95-99.
[10] Laskowski R.A., MacArthur M.W., Moss D.S & Thornton J.M (1993), J Appl Crystallogr 26, 283-291.
[11] McDevitt D & Rosenberg M (2001).Trends Microbiol 9, 611-617.
[12] Joesph-McCarthy D, Parris K, Huang A, Failli A et.al.Use of structural-based drug design approaches to obtain novel drugs, J Med Chem.2005 Dec 15;48(25):7960-9.
[13] Kabsch W & Christian sander (1983), Biopolymers 22, 2577-2637.