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Insilco homology modeling &Drug designing of Crystal Structure of the Fusion Glycoprotein E1 from Semliki Forest virus protein involved in Chikungunya

Tappa Mohammad Munawar[1] and P. Mamatha*

[1]Department of Biotechnology, JNTUA College of Engineering, Pulivendula, 516390 Andhra Pradesh, India.
**Department of Chemistry, JNTUA College of Engineering Pulivendula, 516390, Andhra Pradesh, India.*
**Corresponding Author Email: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. Chikungunya is an arboviral disease transmitted by aedes mosquitoes. Chikungunya virus is a member of the genus Alpha virus and the family Togaviridae. The disease typically consists of an acute illness characterized by fever, rash, and incapacitating arthralgia. The word chikungunya, used for both the virus and the disease, means "to walk bent over" in some east African languages, and refers to the effect of the joint pains that characterize this dengue-like infection. This project study is based on molecular docking of Crystal Structure of the Fusion Glycoprotein E1 from Semliki Forest virus protein involved in Chikungunya. 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. Form the results obtained the synthesized drug molecule and their binding to the target protein show its maximum efficiency. Finally, Lumiracoxib has the lowest distance 1.26 Angstroms and was found to be the best drug for Chikun Gunya disease.

Keywords: Aedes mosquitoes, Alpha virus, Homology modeling, Molecular docking, Chikungunya

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 subtractive because we use a subtraction template while comparing the genome under consideration. This project study is based on molecular docking for Crystal Structure of the Fusion Glycoprotein E1 from Semliki Forest virus protein involved in Chikungunya. 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 Chikungunya.

Chikungunya is an arboviral disease transmitted by aedes mosquitoes. The virus was first isolated in 1953 in Tanzania. Chikungunya virus is a member of the genus Alpha virus and the family Togaviridae. The disease typically consists of an acute illness characterized by fever, rash, and incapacitating arthralgia. The word chikungunya, used for both the virus and the disease, means "to walk bent over" in some east African languages, and refers to the effect of the joint pains that characterize this dengue-like infection. Chikungunya is a specifically tropical disease, but it is geographically restricted and outbreaks are relatively uncommon. It is only occasionally observed in travelers and military personnel. More than 2, 66, 000 people have been infected during the ongoing outbreak in Reunion, in which Aedes albopictus is the presumed vector. In the ongoing Indian outbreak, in which Aedes aegypti is the presumed vector, 1, 40, 000 cases of chikungunya were reported during 2006. The reasons for the re-emergence of chikungunya on the Indian subcontinent, and for its unprecedented incidence rate in the Indian Ocean region, are unclear [1].

Chikungunya virus is a member of the genus Alpha virus and the family Togaviridae. The virus is transmitted by infected humans to Aedes aegypti and Aedes albopictus. Virus infects during its life cycle arthropod and vertebrate hosts Aedes aegypti (the yellow fever mosquito), a household container breeder and aggressive daytime biter which is attracted to humans, is the primary vector of CHIKV to humans. Aedes albopictus (the Asian tiger mosquito) may also play a role in human transmission is Asia, and various forest-dwelling mosquito species in Africa have been found to be infected with the virus [2]. Viral host belongs to the Domain Eucarya, Kingdom Animalia, Phylum Arthropoda and Chordata, Subphylum Hexapoda, Class Insecta,Subclass Pterygota,Order Diptera[3]. The incubation period (time from infection to illness) can be 2-12 days, but is usually 3-7 days. The main symptoms include severe temperature, body pain and pain in all the major joints with swelling, due to arthritis affecting multiple joints. In some cases, skin rashes, severe headache and conjunctival infection can also be found. Usually the body temperature or Chikungunya fever will be completely normal within 2 to 3 days, but the joint pain and swellings will last longer, usually 7 days to even 2 to 3 months.

The clinical manifestations of chikungunya fever resemble those of dengue fever. Laboratory diagnosis is critical to establish the cause of diagnosis and initiate specific public health response.Three main laboratory tests are used for diagnosing Chikungunya fevers are Virus isolation, serological tests, Molecular technique of Polymerase Chain Reaction (PCR)[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 Chickungunya 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 Alpha virus (FGP 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 63%.The resultant of BLAST search identified structure template (PDB 1I9W A) and the expected value is 2e-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 Ramachandran plot 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 88.78%[10].The optimized model was submitted to CASTp server to predict active sites for optimized model. Then from the result the filenamewith.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 cefepime, chlorempheniсol, enoxacin, loracarbef, Loteprednol Etabouate, lumiracoxib, oseltamivir, spermine. 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 cefepime (Fig 8), chloremphenicol (Fig 9), enoxacin (Fig 10), loracarbef (Fig 12), Loteprednol Etabouate (Fig 12), lumiracoxib (Fig 13), oseltamivir (Fig 14), spermine (Fig 15) shows bond lengths less than 3Angstroms, and their bond lengths are tabulated. Finally, by the tabular column, Lumiracoxib 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:

>tr|C8YZ73|C8YZ73_CHIKV Structural polyprotein OS=Chikungunya virus PE=4 SV=1

MEFIPTQTFYNRRYQPRPWTPRPTIQVIRPRPRPQRQAGQLAQLISAVNKLTM
RAVPQQKPRRNRKNKKQKQKQQAPQNNTNQKKQPPKKKPAQKKKKPGRRE
RMCMKIENDCIFEVKHEGKVTGYACLVGDKVMKPAHVKGTIDNADLAKLAF
KRSSKYDLECAQIPVHMKSDASKFTHEKLEGYYNWHHGAVQYSGGRFTIPTG
AGKPGDSGRPIFDNKGRVVAIVLGGANEGARTALSVVTWNKDIVTKITPEGA
EEWSLAIPVMCLLANTTFPCSQPPCTPCCYEKEPEETLRMLEDNVMRPGYYQ
LLQASLTCSPHRQRRSTKDNFNVYKATRPYLAHCPDCGEGHSCHSPVALERIR
NEATDGTLKIQVSLQIGIKTDDSHDWTKLRYMDNHMPADAERAGLFVRTSAP
CTITGTMGHFILARCPKGETLTVGFTDSRKISHSCTHPFHHDPPVIGREKFHSRP
QHGKELPCSTYVQSTAATTEEIEVHMPPDTPDRTLMSQQSGNVKITVNGQTV
RYKCNCGGSNEGLTTTDKVINNCKVDQCHAAVTNHKKWQYNSPLVPRNAE
LGDRKGKIHIPFPLANVTCRVPKARNPTVTYGKNQVIMLLYPDHPTLLSYRN
MGEEPNYQEEWVMHKKEVVLTVPTEGLEVTWGNNEPYKYWPQLSTNGTAH
GHPHEIILYYYELYPTMTVVVVSVATFILLSMVGMAAGMCMCARRRCITPYE
LTPGATVPFLLSLICCIRTAKAATYQEAAIYLWNEQQPLFWLQALIPLAALIVL
CNCLRLLPCCCKTLAFLAVMSVGAHTVSAYEHVTVIPNTVGVPYKTLVNRPG
YSPMVLEMELLSVTLEPTLSLDYITCEYKTVIPSPYVKCCGTAECKDKNLPDY
SCKVFTGVYPFMWGGAYCFCDAENTQLSEAHVEKSESCKTEFASAYRAHTA
SASAKLRVLYQGNNITVTAYANGDHAVTVKDAKFIVGPMSSAWTPFDNKIV
VYKGDVYNMDYPPFGAGRPGQFGDIQSRTPESKDVYANTQLVLQRPAVGTV
HVPYSQAPSGFKYWLKERGASLQHTAPFGCQIATNPVRAVNCAVGNMPISIDI
PEAAFTRVVDAPSLTDMSCEVPACTHSSDFGGVAIIKYAASKKGKCAVHSMT
NAVTIREAEIEVEGNSQLQISFSTALASAEFRVQVCSTQVHCAAECHPPKDHIV
NYPASHTTLGVQDISATAMSWVQKITGGVGLVVAVAALILIVVLCVSFSRH.

BLAST OUTPUT

A simple BLASTp searching method was carried out against Protein data bank[8].The resultant of BLAST search identified structure template (PDB 1I9W A) with 63% identity and the expected value is 2e-157.

Structure of Template

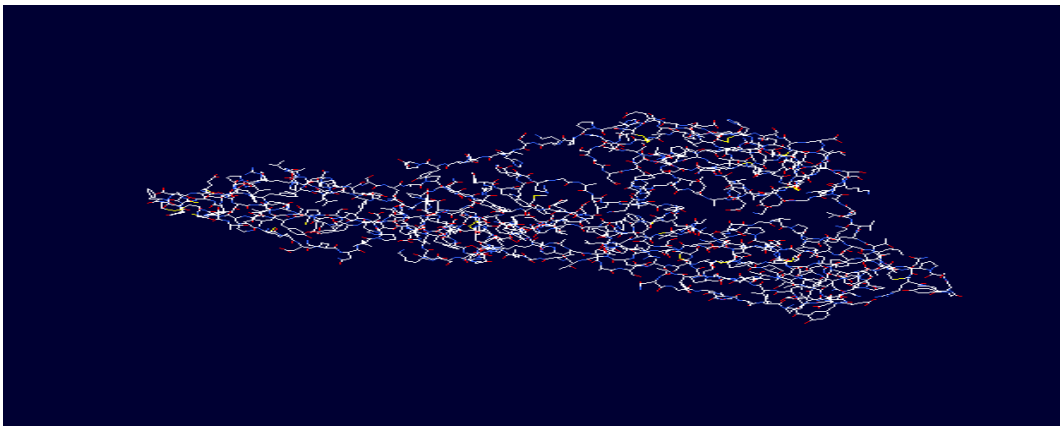

Fig.1. Structure of template.

Structure of Target

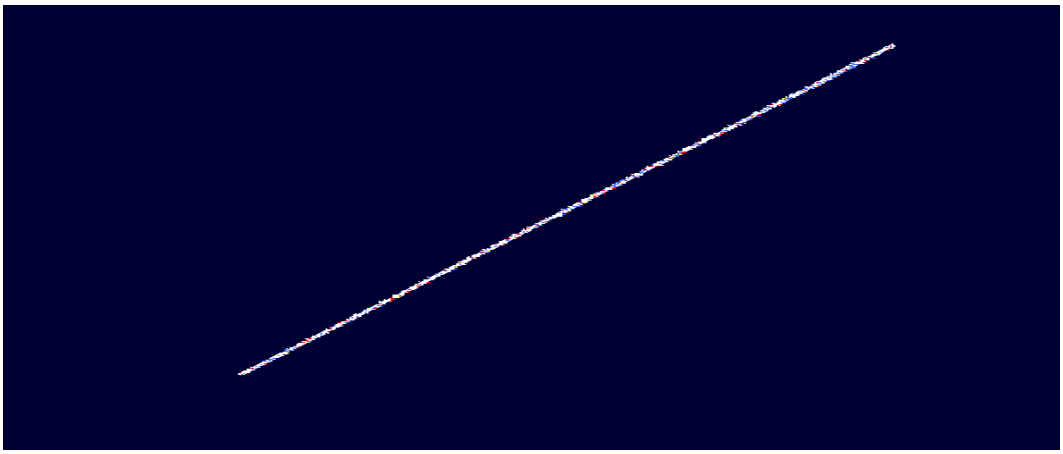

Fig. 2. Structure of target.

Structure of Target and Template

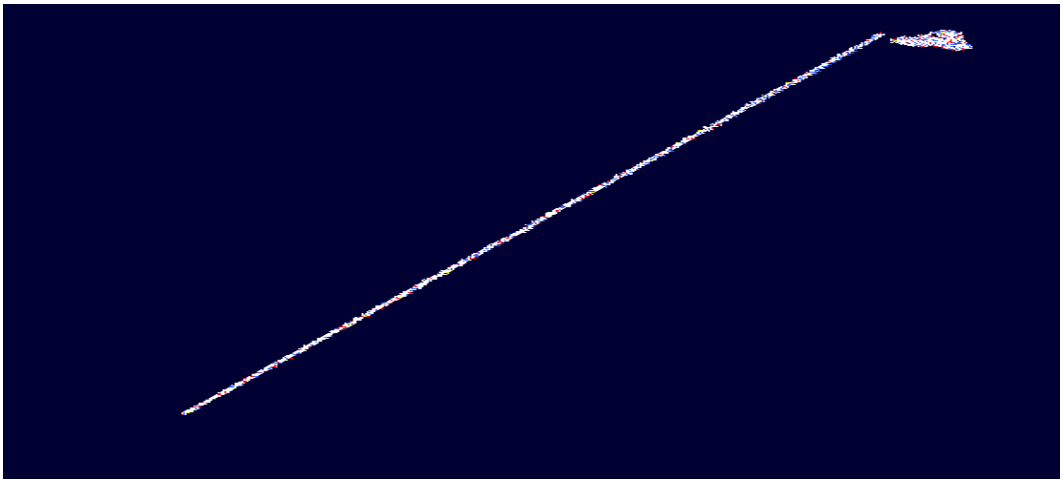

Fig.3. Structure of target and template.

Fitting of Target with Template

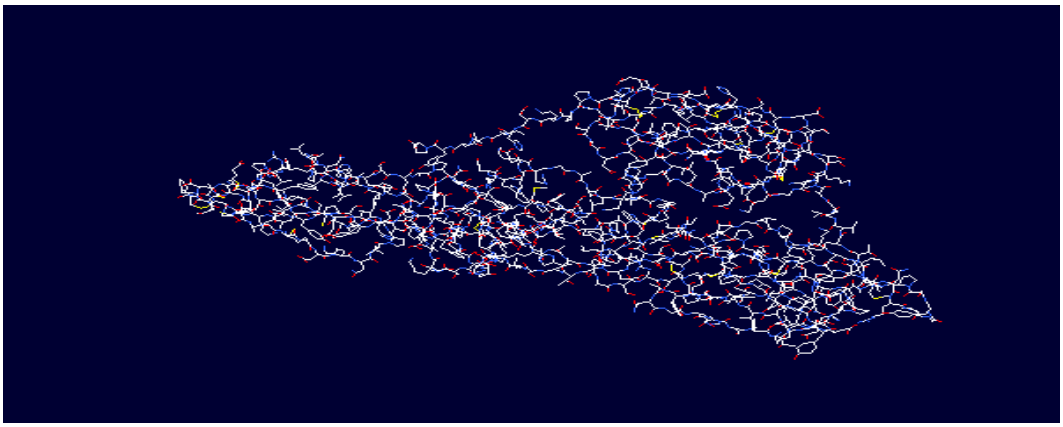

Fig. 4. Fitting of target and template.

RAMACHANDRAN PLOT ANALYSIS OF AMINO ACIDS OF TEMPLATE PROTEIN

BEFORE ENERGY MINIMIZATION AFTER ENERGY MINIMIZATION

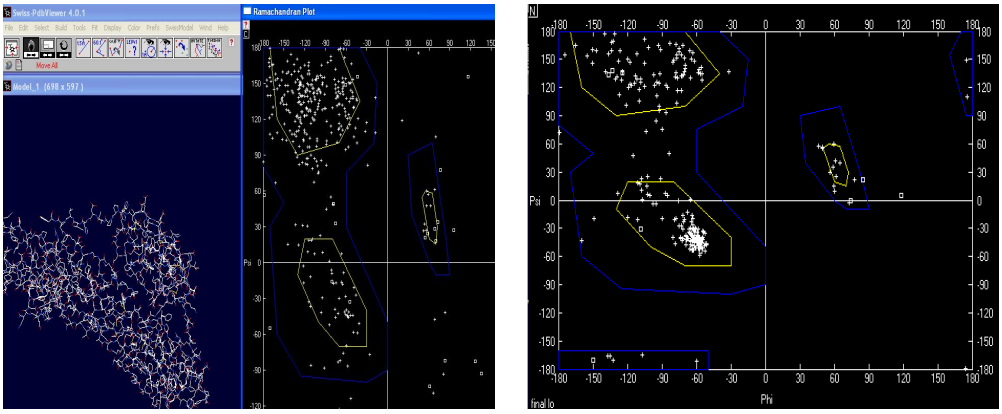

Fig. 5. The Ramachandran plot of Template protein. The dark grey area represents most allowed regions, whereas the medium gray areas represent allowed regions.

SAVS RESULT

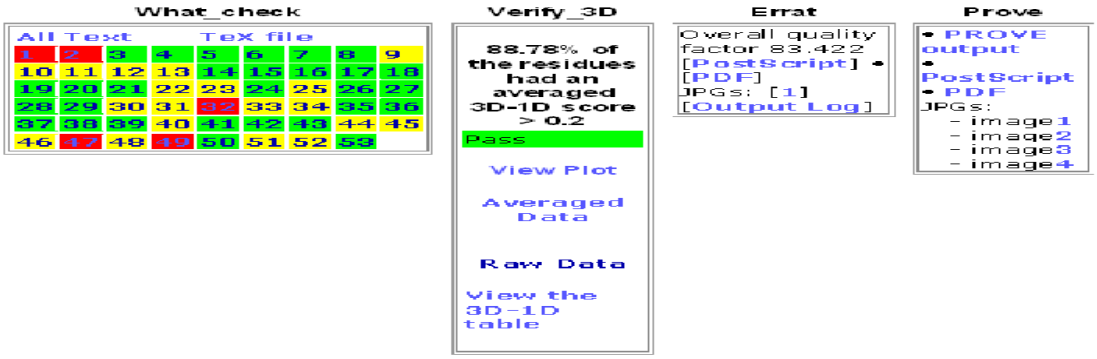

Fig.6. Savs Report.

CASTp RESULT
ACTIVE SITE PREDICTION:

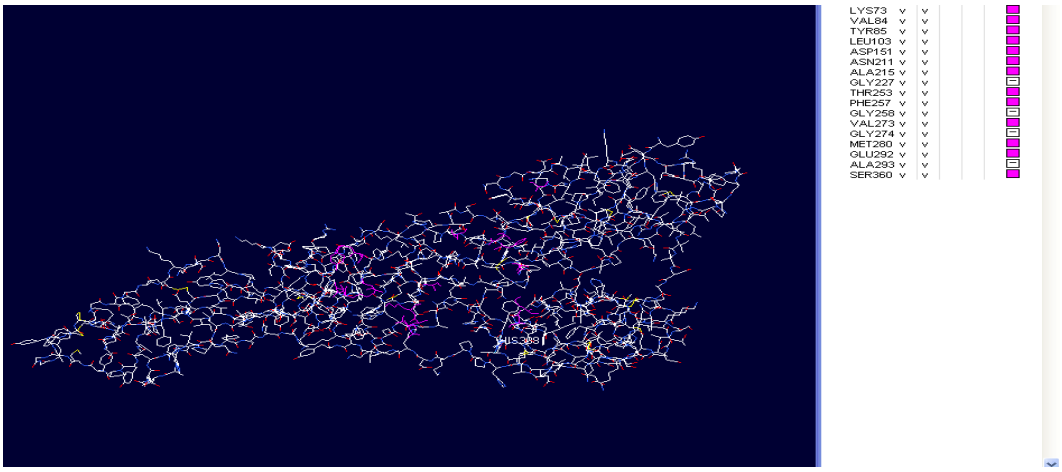

Fig 7: CASTp prediction of active sites of Modelled Protein

DOCKING RESULTS:
CEFEPIME

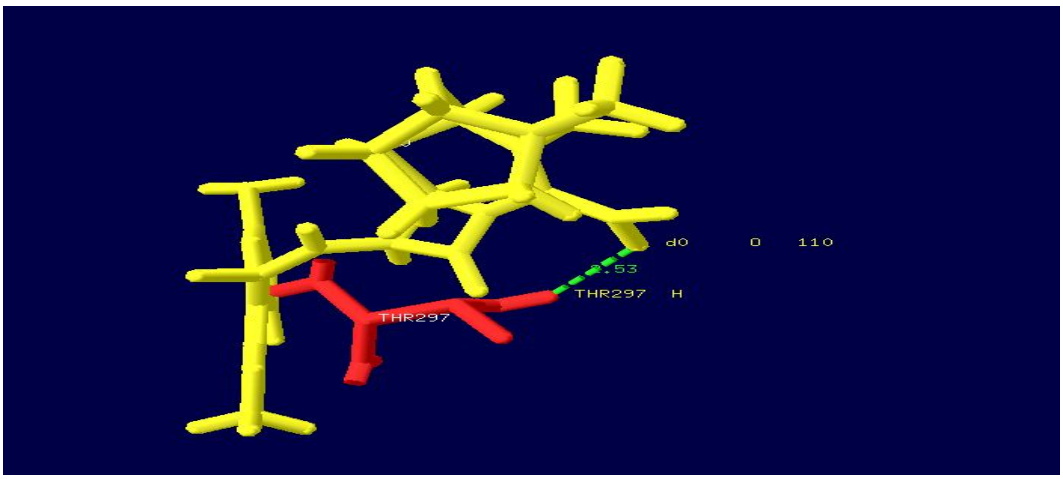

Fig. 8. Docking of Cefepime drug with modeled Protein

TABLE.1. Bond length of aminoacid of modeled Protein with Cefepime drug

S.NO	AMINO ACID	BOND LENGTH
1	THR 297	2.53Aº

CHLOREMPHENICOL

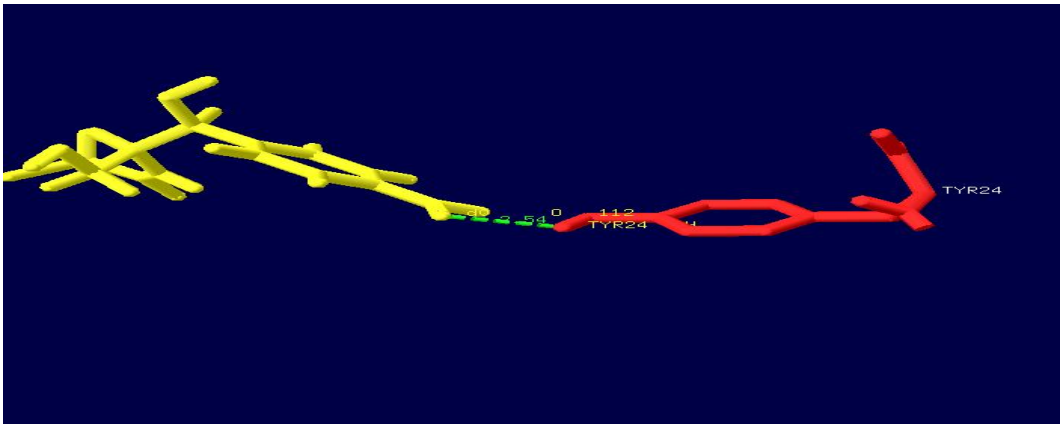

Fig.9. Docking of chloremphenicol drug with modeled Protein.

TABLE.2. Bond length of aminoacid of modeled Protein with chloremphenicol drug

S.NO	**AMINO ACID**	**BOND LENGTH**
1.	TYR 24	2.54**A°**

ENOXACIN

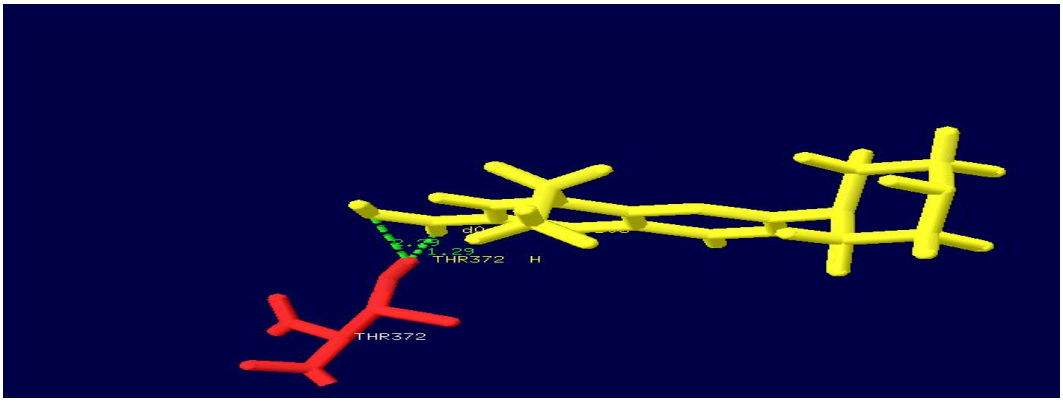

Fig.10. Docking of **Enoxacin** drug with modeled Protein.

TABLE.3. Bond length of aminoacid of modeled Protein with Enoxacin drug

S.NO	**AMINO ACID**	**BOND LENGTH**
1.	THR 372	1.29**A°**

LORACARBEF

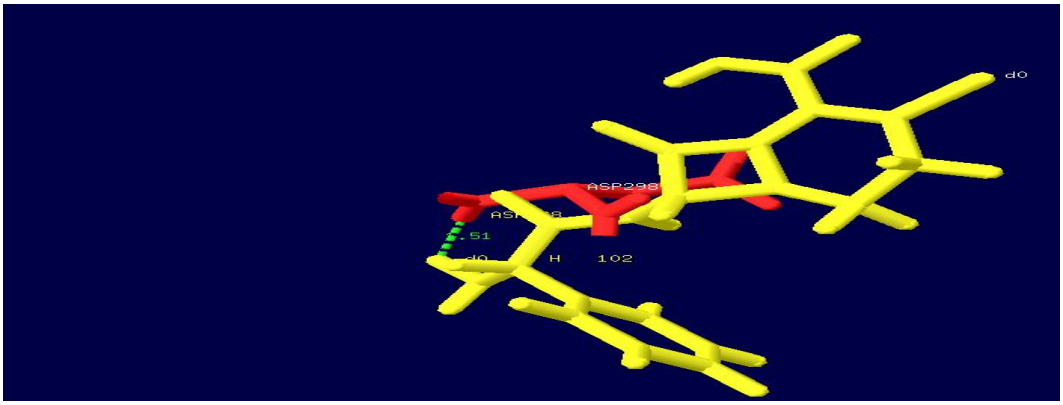

Fig.11. Docking of **loracarbef** drug with modeled Protein.

TABLE.4. Bond length of aminoacid of modeled Protein with loracarbef drug

S.NO	AMINO ACID	BOND LENGTH
1	ASP 298	1.51**A°**

LOTEPREDNOL ETABOUATE

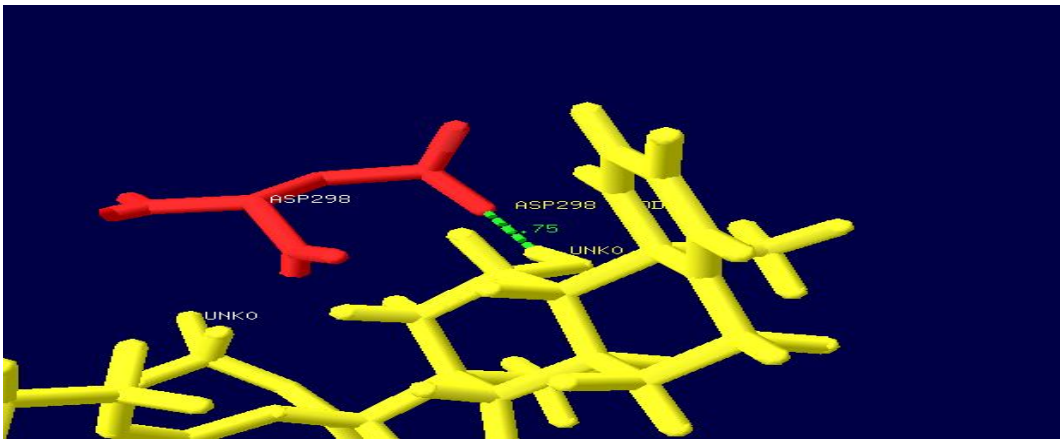

Fig. 12: Docking of Loteprednol Etabouate drug with modeled Protein.

TABLE.5. Bond length of aminoacid of modeled Protein with Loteprednol Etabouate drug

S.NO	AMINO ACID	BOND LENGTH
1	ASP 298	1.75**A°**

LUMIRACOXIB

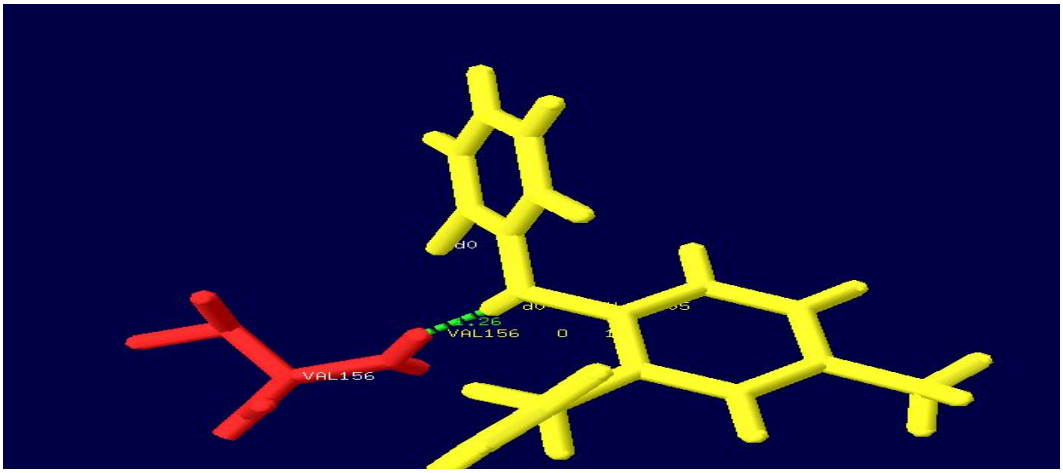

Figure 13: Docking of **lumiracoxib** drug with modeled Protein.

TABLE.5. Bond length of aminoacid of modeled Protein with lumiracoxib drug

S.NO	AMINO ACID	BOND LENGTH
1.	VAL 156	1.26**Aº**

OSELTAMIVIR

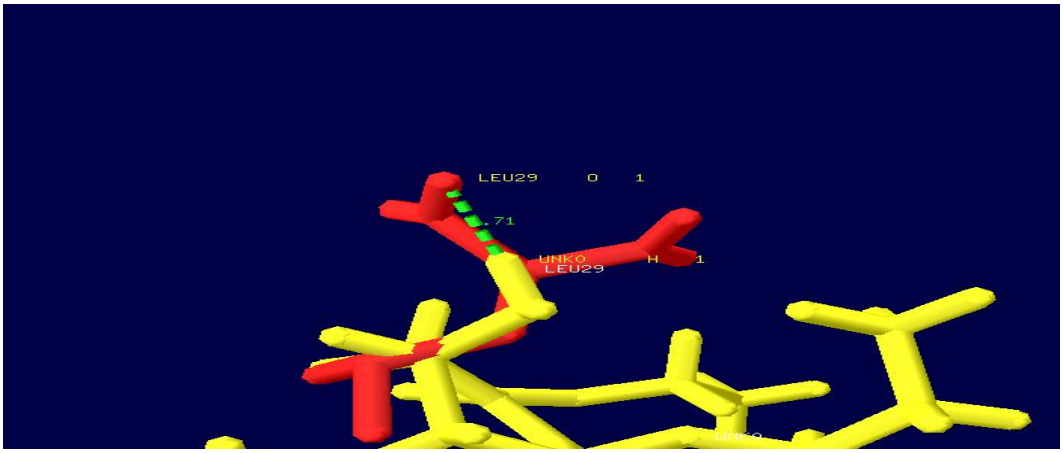

Figure 14: Docking of **Oseltamivir** drug with modeled Protein.

TABLE.6. Bond length of aminoacid of modeled Protein with Oseltamivir drug

S.NO	AMINO ACID	BOND LENGTH
1	LEU 29	1.71**Aº**

SPERMINE

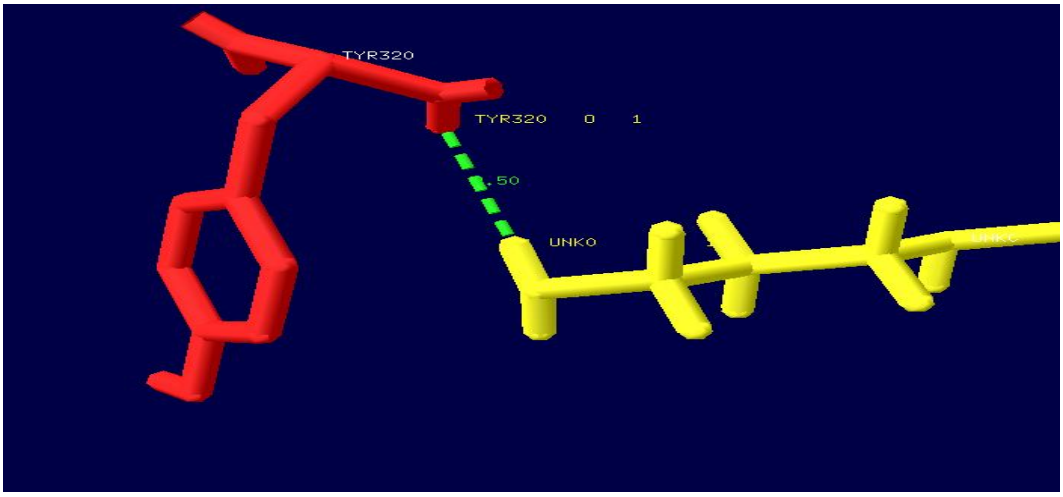

Figure 15: Docking of **Spermine** drug with modeled Protein.

TABLE.7 Bond length of aminoacid of modeled Protein with Spermine drug.

S.NO	AMINO ACID	BOND LENGTH
1.	TYR 320	2.50**A°**

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 Fusion Glycoprotein E1 from Semliki Forest virus protein involved in Chikungunya.

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 Cefepime, Chloremphenicol, enoxacin, loracarbef, Loteprednol Etabouate, lumiracoxib, Oseltamivir, Spermine. 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, Lumiracoxib has the lowest distance 1.26 Angstroms and was found to be the best drug for Chikun Gunya 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.

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