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Mechanistic Characterization and Designing Possible Molecular Ligand Interactions with RdRp from CHIKV

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ABSTRACT
To date, no suitable vaccine or specific antiviral drug is available to treat Chikungunya viral (CHIKV) fever. Hence, it is essential to identify drug candidates that could potentially impede CHIKV infection. The present study focused with the development of Designing Possible Docking and Molecular Ligand Interactions with RdRp from CHIK-V protein based on the crystal structure. When, Rifapentine was interact with RdRp viral protein which were clearly showed the significantly excellent glide score of -5.690530 (Kcal/mol) as well as poor glide score of 2.874727 (Kcal/mol). The docking results showed that among the four ligand molecules Efavirenz have the lowest binding values among the other ligands because it has residue contact with total of 13 residues. Two of them were Glut-31, Glut-46, which are catalytic site residues. It is expected that this ligand could prevented the catalytic process. Rimantadine peptide has hydrogen bond interaction with five other residues and them binded with GLU-28, ASP-38 and ILE-45. Based on docking result visualization, it is known that Rifapentine and Rifampin peptide ligand was bound with RdRp enzyme inside the cavity also viral RNA entry when it covets to begin initiation and elongation process. From this study clearly revealed, the ligands such as Rifapentine, Rifampin and Rimantadine may inhibit the RNA dependent RNA polymerase protein activity in chikungunya virus. Furthermore, the backbone structural scaffolds of these four lead compounds could serve as building blocks when designing drug-like molecules for the treatment of Chikungunya viral fever.

Keywords: Chikungunya, Ligands, Docking, Efavirenz, Rifapentine, Rifampin, Rimantadine.

INTRODUCTION

Chikungunya virus (CHIKV), a member of the Alphavirus genus belongs to the family Togoviridae and it is primarily transmitted to humans by two main vectors, *A. aegypti* and *A. albopictus.* [1] The scarcity of scientific knowledge on various epidemiological aspects intimidated the outburst of epidemic and the unavailability of suitable vaccineand/or specific antiviral agent added fuel to the fire. [2] Hence, there is an immediate need to initiate research on this newly re-emerging evolutionary potent CHIKV infection. Due to the heavy monsoon and floods, the modern epidemic of chikungunya is found in south Indian states like Kerala, Tamil Nadu, Andhra Pradesh and Karnataka. [3] More difficulties are currently augmented to distinguish CHIKV infections from a spectrum of other viral infections as its symptoms are very much similar to other viral symptoms including nausea, vomiting, myalgia, rash and arthralgia and in some instance, the observation of painful puffy feet and ankles experiencing the chronic polyarthralgia, a discernible symptom of rheumatoid arthritis. The rapid developments in science have brought many changes in human life. [4] As one example, advances in biological sciences and bioinformatics have brought a better understanding of the organism functions in cellular and molecular scale. As a result of this progress, most research in the pharmaceutical industry has started to identify suitable targets in the organism and to design drugs, which interact with the target [5] This type of drug designing is known as target oriented drug or rational drug design. In a rational drug design, drug design process begins with knowing the structure of the target protein and then form a database that contains a collection of compounds that are expected to interact with the target protein. [6] Docking techniques is designed to find the most suitable conformation of ligand and its receptor. [7-8] Molecular dynamics simulation is a computation approach in which

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atoms and molecules allowed to interact with each other during a certain time period so that system behaviour can be observed. [9] Fast and inexpensive docking protocols can be combined with accurate but more costly MD techniques to predict more reliable protein ligand complexes. The strength of this combination lies in their complementary strengths and weaknesses. [10] Previously [11] identified Protein 3D structure is mandatory to predict the function and drug binding studies. Again 3Dstructure of Chikungunya virus is not discovered in Protein Data Bank which is a public repository of Protein 3D Structure. Homology Modeling or Comparative modelling [12-13] is the prediction of 3D structure with the help of Homologous or highly similar structure. [14-15] Chikungunya is an alphavirus, which carried by the mosquito of Aedes aegypti and spreaded through stagnated water. The main symptoms include severe temperature, body pain, pain in all the major joints in legs and hands with swelling, due to arthritis affecting multiple joints. The mode of action of Chikungunya virus, by which it causes the disease remain to be investigated in detail and its mechanism of action has not yet been fully characterized accept the fact that it causes major histopathological changes in the skeletal muscle tissue, severe inflammation and necrosis of skeletal muscle.

Hence the objective of the present work was to construct the 3D structure of Chikungunya virus. Since the adversity of X-Ray Crystallography and other in vitro methods for predicting the 3Dimensional structure, we used Homology modelling for the prediction of 3D structure of Chikungunya virus. As a limiting parameter of homology modelling, the template undertaken for backbone alignment should have identical amino acids with 30 or >30% when sequentially aligned with query protein. [16-17]

MATERIALS AND METHODS

RdRp dengue virus enzyme crystal structure

Searching of RdRp Enzyme structure in PDB format was performed at Research Collaboratory for Structural Bioinformatics (RCSB) site (http://www.rcsb.org/pdb/). After the 3D structure was obtained, the analysis to determine the binding site was conducted. The binding site determination was performed using molecular modelling software.

Preparation of peptide ligands

Peptide ligands were drawn in 3D by using ACD Labs program. The peptide was modelled as cyclic peptide where cysteine residue was added at its end to form a disulfide bridge and it was composed of negatively charged amino acid residue, aspartic acid and glutamic acid. [18]

RdRp enzyme preparation

Water molecule, chlorine ion and tryethylene glycol was eliminated by using Pymol program. The force field CHARMM22_PROT optimization was conducted, with steepest descent and conjugate gradient methods, by using VegaZZ program.

Docking of peptide ligand and enzyme

The docking parameter was prepared by using AutoDock Tools. In the enzyme molecule, the polar hydrogen atom was added. In the ligand, the Gasteiger charge was added and every bond was rotated. The docking calculation was conducted in AutoDock 4.0 program, by using Lamarckian Genetic Algorithm (LGA). The utilized parameters are population sizes 150, energy evaluations 2, 5.106 and 50 times runs. The Grid box was prepared with 0, 375 Å grid spacing and RMSD value of each cluster must not higher than 1.

Analysis of docking result toward peptide-RdRp enzyme complex

The docking analysis was conducted by examining the conformation which has the lowest energy value from the most populated cluster. Then, the binding and Ki (inhibition constant) values between peptide-enzyme was examined. This procedure was performed to describe the interaction, analyze the hydrogen bonding between peptide and enzyme and determination of which enzyme residue that had certain contact with peptide ligand.

RdRp enzyme 3D structure: Chikungunya virus (CHIKV) RdRp enzyme structure with ID 2J7U was downloaded from PDB database. [19]

The parameter preparation of RdRp (CHIKV)

Enzyme: The preparation was conducted in accordance with the parameters from the first batch which were elimination of water molecule, chlorine ion and polyethylene glycol. These were performed to separate the enzyme from other irrelevant ions, which could obstruct the catalytic process. Protonation was conducted to change the macromolecule ionization state with Protonate 3D option. The partial charges addition, hydrogen atom and gas phase solvation were utilized based upon the minimization energy of force field MMFF94x calculation. This enzyme optimization process was performed by using MOE 2008.10 software.

Peptide ligand preparation as inhibitor

The ligand optimization was done by using MOE database viewer (dv). Every ligand was 'washed' in order to repair its 3D structure and charged by using MMFF94 force field calculation. The molecular energy structure minimization was done until the RMS gradient reached 0,001 kkal mol^{-1} Å. Other parameters were left at default value.

Peptide ligand docking with RdRp enzyme

The docking simulation was performed by using MOE-dock program. The ligand applicant database was arranged to interact with the chosen enzyme residues. They were Arg-737, Arg-729 and Ser-710. During this process, the enzyme was made rigid and the ligand was left free to rotate. The utilized placement method was triangle matcher, which is useful for generating ligand energy calculation for each 2, 5 106 iteration pose. The result of this last selection step was only displaying the most suitable molecule based on one retain. The docking result analysis was based on G binding (S) values. The result is a ligand which would be suitable as drugs and would be analyzed further.

Protein Preparation

The protein preparation facility performs the final stages of the preparation of proteins for use in Glide. A typical PDB structure file consists only of heavy atoms. Therefore, hydrogen does have to be added prior to use in Glide calculations, which use an all-atom force field. The charge state of protein residues is also important to the results generated by Glide. Before running a protein preparation job, one must perform some preliminary preparation tasks that are not automated. The protein preparation facility consists of two components, preparation and refinement. After ensuring chemical correctness, the preparation component adds hydrogen and neutralizes side chains that are not close to the binding cavity and do not participate in salt bridges. The refinement component performs a restrained impact minimization of the co-crystallized complex, which reorients

side-chain hydroxyl groups and alleviates potential steric clashes. The protein preparation panel is used to set up jobs that perform these tasks.

Ligand Preparation

The structure Efavirenz, Rifaximin, Rifampin, Rifapentine, was taken for the docking studies. The crystallographically solved structure is taken in the form of PDB format and it was converted into Maestro format using Amber force field.

Docking phase: RdRp Chikungunya virus structure needs to be optimized before docking process. This step was conducted in MOE 2008.10. The optimization was performed by changing the structure into its ionization state by protonate3D option, adding partial charge and minimizing the energy until RMS gradient 0.05 reached. Meanwhile, ligands were also optimized by using MOE database viewer. Ligands were prepared with wash option to get the most favourable structure; next optimization was done by choosing MMFF94x force field to control molecular surface potential. The two ligands were arranged to interact with the selected enzyme residues, which were SER-48, ASP-17 and GLU-61. These three residues are important residues of Chikungunya virus RdRp. By choosing gas solvation state, the enzyme was made to be rigid and the ligand was free to rotate to gain the most suitable position.

Docking analysis: Result of docking simulation was saved in MOE database. This database was then analyzed to study the docking process. Analysis was carried out by comparing the binding energy between ligand and protein from the two ligands.

Molecular dynamics analysis: Analysis of MD result was performed by reviewing molecular dynamics database viewer. Ligands were marked by their residue contact with RdRp CHIK-V and their total potential energy during simulation.

Statistical Analysis

The data of Glide energy and docking score were arranged in the tabulated form from the figure. These data's were analysed with a one way of ANOVA Test using pp Version-4 Window. Result with $P<0.05$ were considered as statistically significant.

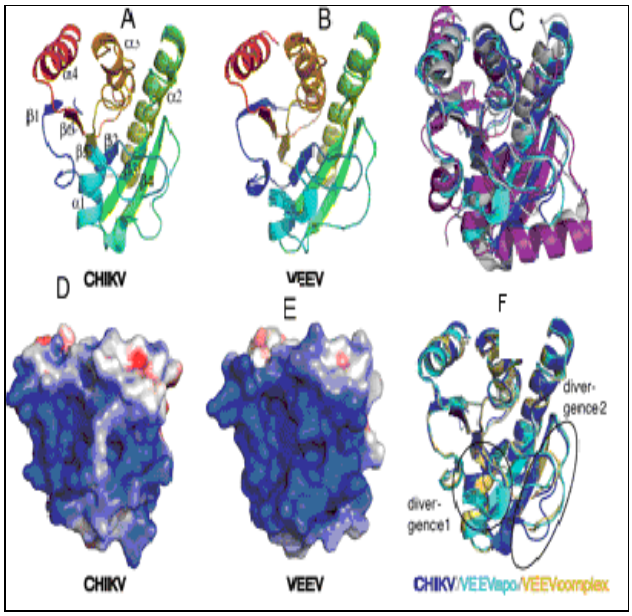

Fig: 1. RdRp dengue virus enzyme crystal structure (s)

RESULT

Peptide ligand preparation: The peptide was designed to have negative charge or acids, which involve aspartic acid (D) and glutamic acid (E). This is because negatively charged amino acid would help designed peptide to have strong interaction with the important residues in RdRp enzyme. These residues were Ser- 18, His-34 and Ala-42 which were more positively charged. The chosen three amino acids on peptide ligands was based on principle that the amount of the amino acids in the peptide chain should be kept limited, in order to make the structure agile enough to pass through the paracelluar way. The peptide ligand modelling was conducted by protonating the amino group and deprotonating the carboxyl group on it. The side chain of carboxyl group on aspartic acid residue and glutamic acid was deprotonated as well.

The docking result analysis

The docking process was conducted 50 times for each peptide ligand. The objective is to form 50 different conformations when peptide ligand binds to the enzyme. The Auto Dock program will classify the same conformation in one cluster. If the cluster has the most population, then it could be inferred that the cluster conformation was more favourable for ligand binding with its binding site. Low binding values signify that the peptide ligand was in the most stable conformation when bound with enzyme (The binding chance of 80-90%). The interaction profile generated over the docking experiments was sorted in the following order: Hydrogen bond (D-H---A), Hydrogen bond distance (Å), Docking score (Kcal/mol) and Glide energy (Kcal/mol).

When the most populated cluster was in the first cluster rank, the ligand-enzyme conformation is the most stable. Based on existing data, more than half of the ligand fulfilled the most stable ligand conformation when they bound with the enzyme. This study shows the six various favourable ligands, which oust from the criteria, because they had uncertain conformation. The ligands were fulfilled with their criteria's such as Efavirenz, Rifapentine, Rifampin and Rifaximin based upon the kinds of active site with amino acid residues. After the ligands selection, the next process was to evaluate docking free energy value. If the rotatable binding value was smaller, the Gtorsional would be decreasing as well. When the rotatable bonds amount decreased by one point, Gtorsional would also decreased with constant value ~0.17 kkal mol^{-1}. The Gintramolecular values were affected by bond length, bond angle and dihedral angle of the ligand molecules. Based on data above, there is tendency that if the Gintramolecular is increased (near positive value), the rotatable bond amount will be smaller. Then, we find a residue on enzyme which has ligand contact, by using Chimera program. Based on residual contact evaluation, it was perceived that those five ligands have contact with 3 important binding site residues. They were Ser-35, Ala-43 and Glut-31, Glut-46. The docking result showed that Efavirenz ligand has the lowest Gbinding value among the others. It has the most residue contact, with total of 13 residues. Two of them were Glut-31, Glut-46, which are catalytic site residues. It is expected that this ligand could prevented the catalytic process. Rimantadine peptide has hydrogen bond interaction with five other residues and them binded with GLU-28, ASP-38 and ILE-45. Besides of having hydrogen bond interaction with those residues, Rifapentine peptide ligand was forming salt bridge with COO^- group side

chain with Gly-45 The salt bridge interaction is considered important for G-intermolecular value, because its stabilization value is stabilizing the hydrogen bond. Based on docking result visualization, it is known that Rifapentine and Rifampin peptide ligand was bound with RdRp enzyme inside the cavity. It is viral RNA entry when it covets to begin initiation and elongation (NTP Tunnel). It was inferred from the docking result, that the cyclic peptide ligand with Efavirenz combination (His- Asp-Glu-Glu-Asp) could be applied as potential inhibitor to block the RdRp enzyme activity. The supporting conditions are as following: It has the lowest binding energy value among the ligands when bound with RdRp enzyme, which is -9. 04 kkal. mol^{-1}. It has Ki value of nM scale (43, 44 nM), indicates that stable peptide ligand-enzyme complex was formed. It has the most contact with other residues and includes contact with catalytic site, Asp-38 and Asp-33, also Glut-31, Ser -35, Ala--43 and His-34, which have been strong influence on RNA virus Initialization (Fig. 2a, b, c & d).

Efavirenz, the widely prescribed drug for treating Chikungunya infections was found to be the top ranked docked conformer with energy of -30.863939Kcal/mol in accordance with our previous observation on HCVns5B polymerase. Whereas other docking profiles resembling the core scaffold of rifapentine, rifampin, Rimantadine was observed to be the best docked conformations down the clustered hierarchy with an energy distributed over the range of -85.4173 and -78.049 Kcal/mol. Though, all the four ligand molecules showed the significant Glide energy such as -30.234548**, -35.620214**, -42.953510** and -21.426416** for Efavirenz, rifapentine, rifampin and Rimantadine respectively (Table1-4).

Efavirenz which shows the best glide score of -5.855736 and shows the best glide energy of -29.358769. Moreover, the hydrogen bond interaction with the following peculiar residues such as SER- 35, HIS- 34 and GLU- 41 of the target protein. Among the three residues SER-34 was almost highly bind with RdRp protein than the remaining residues (Table-1). Another ligand was Rifapentine, when this one interact with RdRp viral protein which were clearly showed the significantly excellent glide score of -5.690530 (Kcal/mol) as well as poor glide score of 2.874727 (Kcal/mol) **(**Table-2**).** Along with another category of the result shows the minimum and maximum observed glide energy was -46.231002 and -29.629230 (Kcal/mol). Moreover, it has been interact with following hydrogen bonds with the residues ASP38, ASP33, ASP-17, SER35, SER-48, GLU-28, GLU-41 ALA-42 and ILE-40 of the target protein.

Third ligand was Rifampin showed the greatest glide score of -5.185533 (Kcal/mol) as well as lowest glide score -3.626609 (Kcal/mol). Despite, the highest glide energy was also noted on -44.190408. Though, a nearly seven hydrogen bond interacted with the following amino acid residues such as ASP-17, ALA-43, SER- 35, SER- 48, GLY-45, GLY-61 and HIS-34 of the target protein (Table-3). The inhibitor Ribavirin which shows the best glide score of -6.418661 (Kcal/Mol) and shows the best glide energy of -20.665363 (Kcal/Mol) and it has been almost noted six hydrogen bonds with the amino acid residues GLU-46, HIS-34, GLU-46 and ASP-38 of the target protein. ILE-40, ILE-47, LEU-37, HIS-34, ASP-38 and GLU-28 (Table-4).

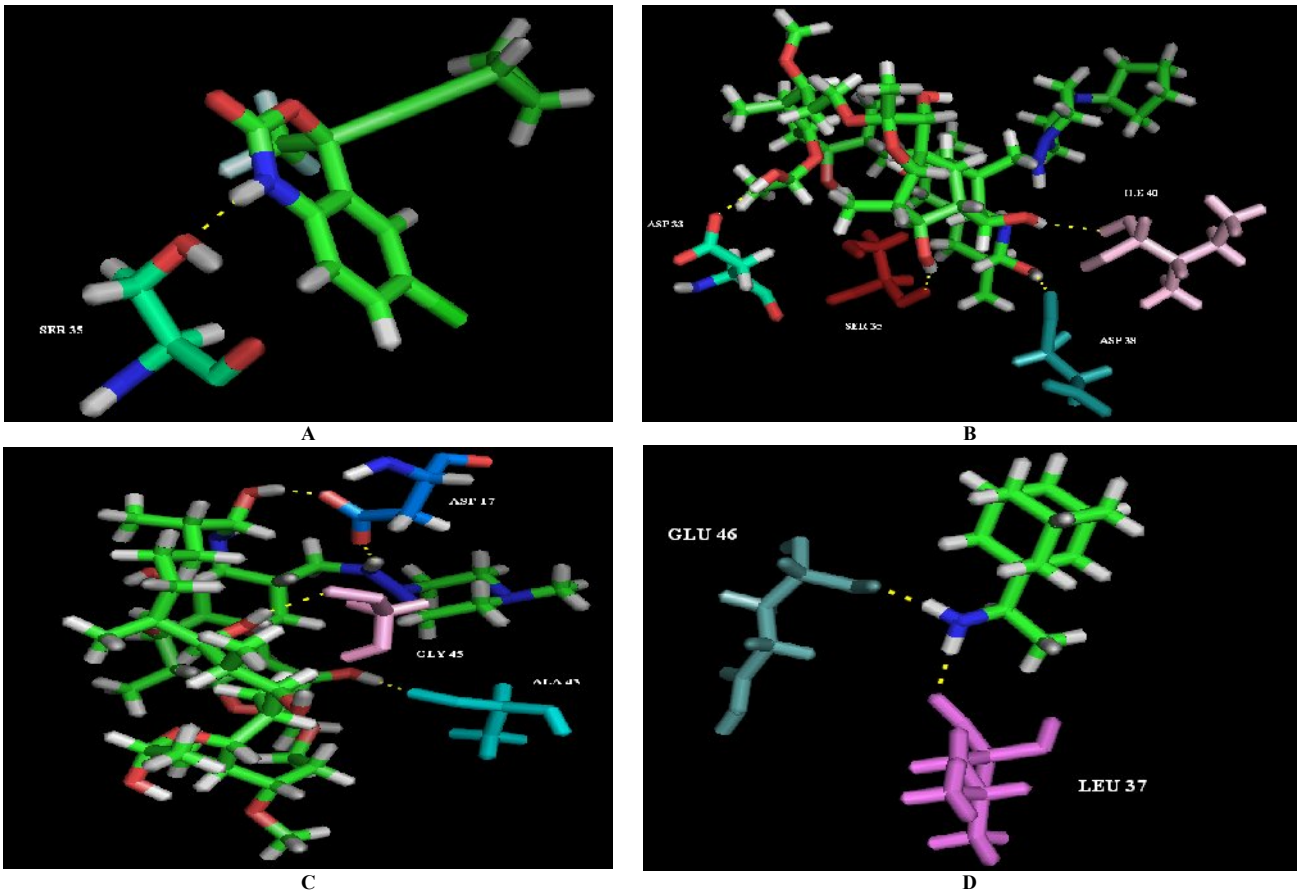

Fig. 2: Interaction between A) Efavirenz and RdRp B) Rifapentine and RdRp C} Rifampin and RdRp, D) Rimantadine and RdRp

Table 1: Induced Fit Docking Scores Hydrogen Bond Interaction of the Ligand *Efavirenz* with Rdrp

Pose	Hydrogen bond D-H-–A	Hydrogen bond distance(Å)	Docking score (Kcal/mol)	Glide energy (Kcal/mol)
1.	N-H---O (SER-35)	2.806**	-5.855736	-29.358769
2.	N-H---O (SER-35)	2.878	-5.413428**	-27.933231
3.	N-H---O (HIS-34) N-H---O (SER-35)	3.244 3.142	-5.928003	-31.691677
4.	N-H---O (HIS-34) O-H---O (SER-35)	2.957 2.904	-5.066238	-30.234548**
5.	N-H---O (GLU-41)	2.896	-5.330889	-30.863939
6.	N-H---O (HIS-34)	2.948	-3.755243	-28.037908
7.	N-H---O (HIS-34)	2.936	-3.518497**	-26.544112

**- Significant at 5% level

Table 2:Induced fit docking scores hydrogen bond interaction of the ligand Rifapentine with RdRp

Pose	Hydrogen bond D-H---A	Hydrogen bond distance in (Å)	Docking score (Kcal/mol)	Glide energy (Kcal/mol)
1.	O-H---O (ASP-33) O-H---O (SER-35) O-H---O (ASP-38) O-H---O (ILE- 40)	2.742 2.796 2.812 3.072	-5.690530	-46.231002
2.	O-H---O (ASP-38) O-H---O (GLU-41) N-H---O (GLU-41)	2.829 2.945 2.493	-5.051135	-35.330184
3.	O-H---O (ASP-33) O-H---O (GLU-28) N-H---N (HIS-34) O-H---O (ALA-42)	2.857 2.628 3.122 2.783	-4.304221	-35.620214**
4.	O-H---O (SER-48) O-H---O (ASP-17)	2.835 2.973	-3.056129	-27.819675
5.	O-H---O (GLU-28)	2.936	-2.874727**	-29.629230

**- Significant at 5% level

Table 3: Induced Fit Docking Scores Hydrogen Bond Interaction of the Ligand Rifampin with RdRp

Pose	Hydrogen bond D-H---A	Hydrogen bond distance in (Å)	Docking score (Kcal/mol)	Glide energy (Kcal/mol)
1.	O-H---O(ALA- 43) O-H---O(GLY- 45)	2.817 2.837	-5.185533	-42.953510**
2.	N-H--O(GLY-45) O-H---O(ASP-17) O-H--O(GLY-61) O-H---O(SER-18)	2.897 2.764 2.770 2.904	-4.852445	-44.190408
3.	O-H---N(HIS-34) N-H---O(SER-35)	2.828 3.046	-4.207621	-30.590080
4.	O-H--O(ALA-43) O-H--O(GLY-45) O-H--O(ASP- 17)	2.781 2.950 2.897	-4.243471	-43.252049**
5.	O-H---O(ASP-16) O-H---O(ASP-17) O-H---O(ASP-17)	2.767 2.512 2.861	-4.229910	-40.551512
6.	O-H---O(ALA-43) N-H---O(ASP-17) O-H---O(GLY-45)	3.325 2.959 2.890	-3.995968	-36.892717
7.	O-H---O(ASP-17)	2.878 2.864	-4.090960	-42.175998**
8.	O-H---O(GLY-61) N-H---O(GLY-45)	2.889 3.063	-3.626609	-40.311410
9.	O-H---O(SER-48)	3.005	-4.798695	-41.784272

**- Significant at 5% level

Table 4: Induced Fit Docking Scores Hydrogen Bond Interaction of the Ligand Rimantadine with Rdrp

Pose	Hydrogen bond D-H---A	Hydrogen bond distance in (Å)	Docking score (Kcal/mol)	Glide energy (Kcal/mol)
1.	N-H---O(GLU46) N-H---O(LEU37)	2.681 2.731	-6.418661	-20.665363
2.	N-H---N(ILE47)	2.962	-5.535473	-17.373992
3	N-H---N(HIS34) N-H---O(HIS34)	2.917 3.019	-5.396935	-17.471786
4.	N-H---O(ILE40)	2.788	-6.200529	-21.426416**
5.	N-H---O(GLU46)	2.961	-5.436982	-16.754310
6.	N-H---O(ASP38)	2.762	-5.504866	-21.227519

**- Significant at 5% level

DISCUSSION

Protein–ligand docking aims to predict and rank the structure(s) arising from the association between a given ligand and a target protein of known 3D structure. [20] Despite the breathtaking advances in the field over the last decades and the widespread application of docking methods, several downsides still exist. In particular, protein flexibility a critical aspect for a thorough understanding of the principles that guide ligand binding in proteins is a major hurdle in current protein–ligand docking efforts that needs to be more efficiently accounted for. According to the key concepts of protein–ligand docking methods are outlined, with major

emphasis being given to the general strengths and weaknesses that presently characterized this methodology.

The sequence for RdRp is taken from NCBI with the accession id (GU013528.2) and search against PDB using PSI BLAST which ends up with RNA dependent RNA polymerase protein from Rhino virus showing high similarity and hence was taken as template. The structure was modeled using bioinformatics tool SWISS PDB VIEWER. The predicted model was cross validated using PROCHECK tool which shows the score 80.4% and four residues are in disallowed region of Ramachandran plot. Similarly this kind of results agreed by [12-15] with the 3D structure of RdRp was favoured for the good satisfactory model.

Though, another few contradictory opinion also proposed by Nayarisseri *et al.* [12] such as the reason for choosing Chikungunya virus protein was because of its function on viral attachment at the host cell surface and to alleviate the immune response at the host cell. After performing the Energy minimization of SPDBV, The final 3D Structure has given the energy of - 12063.947 KJ/Mol and RMSD Value of 0.29A^{o}. The overall G factor calculated for modelled structure came to be -2 (inside) as compared to the reference value -4. Further validation favoured by bad contacts analyzed per 100 amino acids revealed that 2.1 when compared with reference value 4.2. [21] The current study can provide valuable information of protein structure and function. It can be used for finding the target protein and ligand for the treatment of the disease and its epidemic nature as well. [21] The predicted structure was submitted in CASTP server for the identification of possible catalytic residues available in the model. The selected pocket volume is 3.2 Å and the surface area 6.1. The validated model was searched for possible ligands that may hinder the normal function of RdRp. The four ligands like Rifapentine, Rifampin, Efavirenz and Rimantadine was selected for future analysis.

The RNA dependent RNA polymerase protein was selected from Chikungunya virus and the sequence of this protein was taken from NCBI and the structure was modelled using the bioinformatics tool named Swiss model. Then the predicted model was validated and the catalytic residues were identified using CASTP. The protein modelled was optimized using molecular dynamics simulation; the junction peptides of a non structural protein complex were docked in order to investigate the possible protein–protein interactions between the ligands and RdRp. The high through put six ligands were chosen for the interaction with the protein. After minimization six compounds were again screened using XP (Xtra precision). Then all the six ligands were docked with the target protein. To allow all possible conformational degrees of Induced-fit docking algorithm provided by Schrödinger's GLIDE software is used. Based on the glide score and glide energy of the interactions reduced by ligands like Rifapentine, Rifampin and Rifaximin were better than other three compounds (Efavirenz and Rimantadine). From this study clearly revealed, the ligands such as Rifapentine, Rifampin and Rimantadine might be inhibiting the RNA dependent RNA polymerase protein activity in chikungunya virus also these compounds could be bind to the active site of RdRp protease and inhibit this enzyme.

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