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Research Article

A COMPUTATIONAL DRUG DESIGN AND ADME STUDY TO DESIGN SMALL MOLECULE INHIBITORS AGAINST CHIKUNGUNIYA VIRUS NSP3 PROTEASE

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ABSTRACT

The purpose of our project was to computationally design small molecule inhibitors against Chikunguniya Virus (nsp3) using combined De-novo, pharmacophore, molecular docking and ADME study to cure Chikunguniya Viral fever. Successful development of drugs against Chikunguniya might provide valuable insight for design and development of new antiviral drugs. We collected the target protein structure from Protein Data Bank (RCSB PDB). We used CastP to identify the drug binding pockets in the target protein. The pocket information was used for de-novo ligand design using the e-LEA3D web server. These ligands were used to generate a pharmacophore using LigandScout for the selected pocket. The designed pharmacophore was implied to the web server Pharmit for virtual screening of small moleculues from Pubchem database and the screened small molecules were docked into the target pocket of the proteins using the software Autodock Vina. Best 5 docked molecules were identified with binding affinities of 8.6, 8.5, 8.5, 8.4 and 8.3 kcal mol-1 respectively. Finally, we analyzed the ADME properties of the best five ligands using the webserver SwissADME. All the five small molecules were proven to be the ideal candidates for further drug development.

KEY WORDS: ADME, de-novo drug design, Docking, Prion, PDB, Pharmacophore.

INTRODUCTION

Chikungunya is a mosquito (arthropod)-borne viral disease, caused by the Chikungunya virus (family: Togaviridae, genus: Alphavirus). It was first isolated in 1952 in the Makonde Plateau of the southern province along the border between Mozambique and Tanganyika (current part of Tanzania). After emerging in Africa, a number of outbreaks have been found in India, Bangladesh and Philippines. [1] The Chikungunya virus is transmitted to humans by the bite of an infected female mosquito of *Aedes* (Ae) genus. There are 3 distinct lineages of Chikungunya strains those have been demonstrated by phylogenetic analyses: West Africa (*Ae. furcifer*, *Ae. luteocephalus*, and *Ae. taylori*), Asia (*Ae. aegypti* and *Ae. albopictus*), and East/South/Central Africa (ESCA) (*Ae. furcifer* and *Ae. cordellieri*). Till now *Aedes aegypti* and *Aedes albopioctus* are widely recognized vectors for the transmission of the CHK virus. The two main Chikungunya urban mosquito vectors (*Ae. aegypti*, and *Ae. albopictus*) have been found with enhanced ability to spread to new regions of permissive environmental conditions for viral transmission. The transmission cycle needs the infection of female mosquitoes through a viraemic bloodmeal taken from a susceptible vertebrate host and the transmission is occurred to another vertebrate host by the bite of that particular mosquito following a suitable extrinsic incubation period. After an incubation period, most patients suffer from polyarthralgia and myalgia that harms their quality of life significantly. [2] The Chikungunya is single-stranded, positive-sense RNA genome virus with a 60-70 nm capsid and a phospholipid envelope. RNA viruses are

genetically very diverse and the high mutation rates improves their fitness and pathogenicity for survival. The genome of CHIKV is about 11.7 kb in length and it is encoded with 2 open reading frames (ORF) and flanked by 50 and 30 un-translated regions. Genomic RNA translates 50 ORF by a cap-dependent mechanism that yields the formation of 5 structural (envelope proteins E1-E3 forming trimeric spikes on the virion's surface, capsid and 6K/TF) and 4 non-structural proteins (nsP1–4), where glycoprotein, GP E1 is responsible for fusion within endosomes of target cells and the release of nucleocapsid, GP E2 interacts with cellular receptors to get the entry into the cell and the small glycoprotein E3 mediates pH-protection while virus biogenesis is in process and prevents E1 from premature fusion. The capsid protein of the virus acts as a serine protease for self-cleavage that is helps in the interaction with viral spike proteins while virion forms and serves a major function in nucleocapsid formation. The 6K/TF protein is necessary for formation and budding of new virions. Two-thirds of the RNA of the CHIK virus encodes non-structural polyprotein precursors nsP1-4 with RNA helicase, nucleoside triphosphatase and RNA dependent 50 triphosphatase enzymatic activity. [2] The major 3 CHIKV genotypes were isolated in Asian, a West African and an East/Central/Southern African subtype (ECSA). The Sequencing ECSA and Asian genotype strains reveals that 96.8% of amino acids is similar between these strains. The biggest genetic diversity was found in ns-proteins, the 6K and the E3 epitope. Moreover, the length of genome differs between genotypes and is longer in West African (11,843 to 11,881 nucleotides) and Asian (11,777 to 11,999 nucleotides) strains than in the ECSA lineage (11,557 to 11,789 nucleotides). Indian ocean lineage genomic sequence analysis discovers a new viral variant characterized by the substitution of Alanine instead of Valine within the E1 protein that is the major envelope surface protein. These novel mutations in the envelope glycoproteins cause adaptive evolution of the virus to local vector abundance and allow CHIKV to use *Aedes albopictus* and *Aedes aegypti* as major vectors and resultantly increases its distribution beyond tropical areas to the Western world. Analysis with E1 protein suggested that the new mutation provided better vector specificity and improved fitness of CHIKV. [3] The symptoms of chikungynya are analogous to other viral symptoms including nausea, vomiting, myalgia, rash and arthralgia and in some cases, painful puffy feet and ankles experiencing the chronic polyarthralgia are observed, among these polyarthralgia is a obvious symptom of rheumatoid arthritis. Besides these, severe temperature, body pain, pain in all the major joints in legs and hands, [4] as well as Neurological manifestations have also been detailed during the most recent epidemics in India, where disorders such as encephalitis, peripheral neuropathy, myelopathy, myeloneuropathy, and myopathy have been observed.[2] Eye infection (chikungunya neuroretinitis) has also been reported where patients suffered from a sudden, painless decrement of vision in both eyes. [5], [6] There is currently no exclusive unique antiviral treatment for chikungunya. During the recent prevalence that occurred in the Indian Ocean nations, only symptomatic treatments were available, which were upon NSAIDs, nonsalicylate analgesics and plenty of fluids. There is a belief that mild exercise could decrease the joint stiffness, but heavy exercise may excerbate the rheumatic pain. During chronic CHIKV infection, corticosteroids may be used to help decrease the inflammation.[7] The status of drug discovery for the CHIKV is still in the very early stages with no drugs currently in clinical trials. Targeting the CHIKV nsp2 protease activity within the C-domain would have an inhibitory effect on the viral replication. By blocking the intracellular furin-mediated cleavage of viral envelope glycoproteins: the E2E3 or p62 precursor infection by alphaviruses can be inhibited in vitro Chloroquine and quinine, Among the other potential lead ribavirin, 6-Azauridine, Trigocherrin A, Trigowiin A, Prostratin, Harringtonine, and 12-O-Tetradecanoylphorbol, 13-Acetate are worth mentioning. Meanwhile, arbidol which acts by the inhibition of inosine monophosphate dehydrogenase (IMPDH), an enzyme evolved in the de novo biosynthesis of guanine nucleotide. Besides these purine based inhibitors, polyinosinic acid, gene silencers, envelope protein antagonists are also potential candidates for lead compounds . [8]

The assembly of stress granules is inhibited with the recruitment of G3BPinto cytoplasmic foci by nsP3. Both for nsP3-G3BP interaction and viral RNA replication the conserved nsP3 SH3 binding motif is required. G3BP is an enzyme for human cells and a member of the heterogenous nuclear RNA-binding protein, which plays a major role during infection as well as in the assembly of stress granules. In response to cellular stress these stress granules, which are membranous cytoplasmic focal structures (foci) aggregates. This very action leads to impaired translation of most mRNAs. These stress granules are thought to have antiviral activity that is inhibited by CHIKV replication by the nsP3 SH3 domain-binding motif and if this nsP3 can be impede somehow, consequently the stress granules may work against the infection by CHIKV. Probably this will lead to a potential molecule to stop CHIKV replication in human body. By Considering all these points into our consideration we have taken the nsp3 macro domain ass our target protein. [8]

MATERIALS AND METHODS

To find the prospective drug against Prion protein, 3D structure of the CHKV nsp3 is needed. For this purpose, the structure of the protein is obtained Protein Data Bank (*RCSB PDB*).

Data collection:

Protein Data Bank (*RCSB PDB*) (https://www.rcsb.org/pdb/home/home.do) was used for the collection of the structure of prion protein. The structure of the CHKV nsp3 (PDB ID:3gpg) was downloaded from this database. [9]

Energy Minimization:

The energy minimization of the target protein was conducted using the YASARA Energy minimization web server, (http://www.yasara.org/minimizationserver.htm) which runs molecular dynamics simulations of models in the solvents specifically explicit solvent. It uses a new partly knowledge-based all atom force field which is basically derived from Amber and the parameters have been optimized to minimize the damage done to protein crystal structures. [10]

Validation:

Target protein structure was validated by using the Webserver RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) and interpreted the results with Ramachandran plot. Once the structure of the protein was validated, it then directed to the pocket identification procedure. [11]

Pocket Identification:

In this work, CastP webserver is used to determine pockets and their dimensions. [12]

Structured Based De Novo Ligand Design:

The techniques used for ligand design was structure based *de-novo* drug design. The web-server used for this purpose was e-LEA3D,(http://chemoinfo.ipmc.cnrs.fr/) which uses PLANTS (Protein-Ligand ANT System) algorithm. In the de-novo drug design, small molecules are used as a fragment and it is docked in the protein receptor site. Here, Genetic algorithm is used to generate initial population of small molecules, once searched the favorable site inside the receptor and docking, their performance is calculated. Based on the calculations, new population is generated and this is how the algorithm evolves and find out most suited generation which will be the specific small molecule for that particular receptor-binding site.[13] For the target protein following x, y and z coordinate values were used: (x,y,z=-5.343, -27.419, -12.658)

Pharmacophore Modeling and Pharmacophore Based Virtual Screening:

LigandScout software package was used for pharmacophore modeling. In this case, merged pharmacophore feature was applied in order to obtain pharmacophore from the molecules derived from de-novo design. Here, three-dimensioal Pharmacophore models are constructed which are based on a defined set of six types of chemical features and volume constrains, which are sufficiently enough

selective to identify the described binding mode and are thus a useful tool for in-silico screening of large compound databases. [14] For the Pharmacophore design, we kept the molecules in the test set having cluster ID 1 and other molecules were in the training set having different cluster ID. The ligand set were clustered according to their 3D pharmacophore characteristics and all other criteria's were set as default. Then the webserver Pharmit(http://pharmit.csb.pitt.edu/) was used for pharmacophore-based virtual screening purpose.[15] For the screening purpose, at first we studied the Physical and Chemical properties of the FDA approved Antiviral drugs. Then, we selected some specific criteria included in the Table 2 for the pharmacophore-based virtual screening. In this experiment, Molport database was set for the virtual screening operation.

Molecular Docking:

Molecular docking was conducted using the software Autodock Vina. Based on the binding free energy score, small molecules were categorized and directed to ADME testing. [16]

ADME Testing:

ADME (Absorption, Distribution, Metabolism, and Excretion) analysis is a vital factor in drug design. Some properties including intestinal absorption, solubility level, BBB Penetration levels, CYP enzyme and their substrate inhibition were analyzed for the selected compounds after docking. In the case of ADMET analysis, we considered the following properties to be present in a molecule such as BBB ratio which must be less than 0.3:1; Unlikely to inhibit CYP2D6 enzyme, Moderate or good intestinal absorption etc. SwissADME(http://www.swissadme.ch/) is a webserver that is used to compute the physiochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties. Besides, it also helps to predict druglike nature and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery.[17]

RESULTS AND DISCUSSION

In this section, all the results obtained from each step mentioned in the previous section are shown in the respective manner as presented in methodology.

PDB Structure of the CHKV nsp3:

The PDB structure of the prion protein was obtained from the Protein Data Bank (*RCSB PDB).*

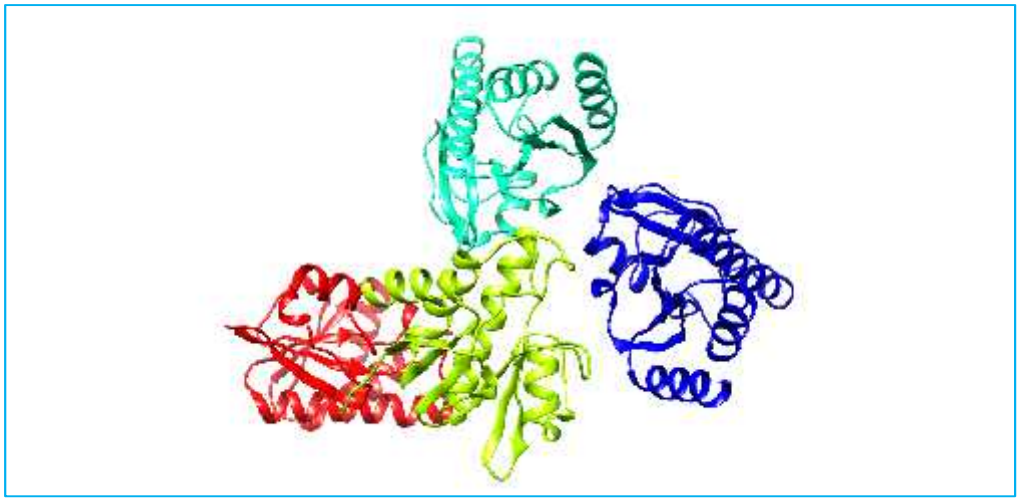

Figure 1. nsp3 Macro Domain of CHKV

Energy Minimization:

We have minimized the energy of the structure so that we can find the best nearby conformation. Another purpose of the energy minimization is to remove very high energy configurations (steric

clashes) that can result in a physical perturbation and instability of the simulation.

Validation of the Structure:

Validation of the Structure was done by using the webserver RAMPAGE and interpreting the Ramachandran plot.

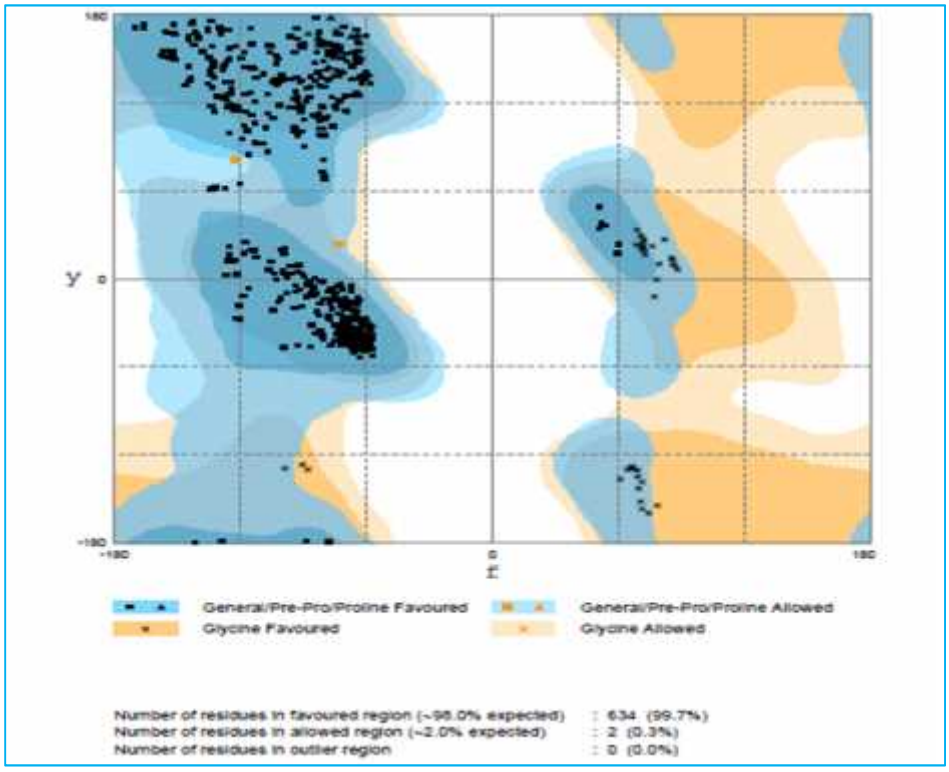

Figure 2. Ramachandran plot of the CHKV nsp3 macro domain

As stated earlier, by using the Ramachandran plot, we considered the structure as validated one. As a rule of thumb, if the favored region is more than 88% then the protein is suitable for in-silico study. As seen in the results that both of the proteins have the value of Favorable +Allowed (F+A) >89%, so it can infer that protein structure can be used for further in-silico analysis. [18]

Pocket Detection:

Pockets were identified by using CastP and following results were obtained. The Blue colored regions marked with the red circle selected as target site for drug design showing in the following figure.

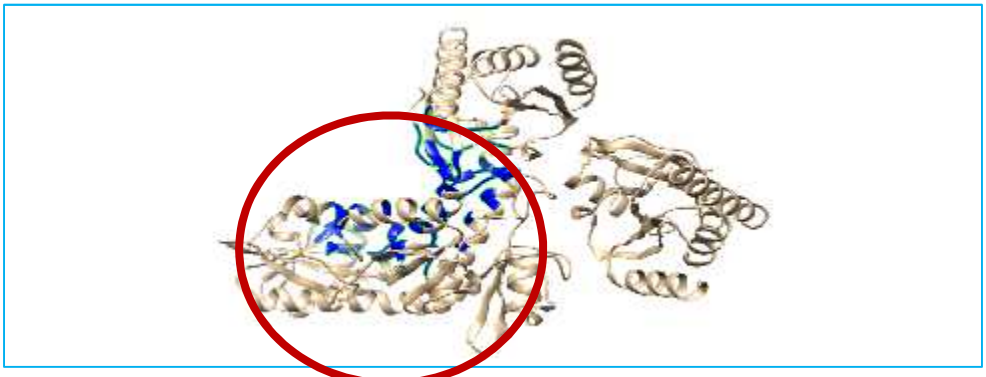

Figure 3. Pocket of the target protein marked with blue color

By using the CastP webserver, we obtained 76 pockets for the target protein. But, we selected the largest pocket having the highest volume and area because as experimentally proven, the largest pocket is the ideal site for a ligand binding. [19]

Structure Based *De-Novo* Drug Design:

For this study, we used e-LEA3D, an online web server for structure based *De-Novo* drug design. The purpose of selecting *de-novo* drug design strategy is to design molecules possesses best binding affinities. [20] For selected binding pocket, e-LEA3D delivered 11 molecules showed in the Table 1.

Table 1. Canonical Formula of the small molecules obtained from *De-Novo* Drug Design for the target protein

Serial No	Canonical Formula of the Molecules
1	CCCCCCCCCC(=O)O[C@H]1CC[C@H]2[C@@H]3CCC4=CC(=O)CC[C@@H]4[C@H]3CC[C@]12C
2	CCCCCCCCCC(=O)O[C@H]1CC[C@H]2[C@@H]3CCC4=CC(=O)CC[C@@H]4[C@H]3CC[C@]12/C=C/C[C@@](C)(CCCC)O
3	CCCCCCCCCC(=O)O[C@H]1CC[C@H]2[C@@H]3CCC4=CC(=O)CC[C@@H]4[C@H]3CC[C@]12/C=C/C[C@@](C)(CCCC)O
4	CCCCCCCCCC(=O)Oc1cccc2C(=O)N([C@H](CS(=O)(=O)C)c3ccc(c4nc5ccccc5[nH]4)c(CCCCCCC(=O)OC)c3)C(=O)c12
5	CCCCCCCCCC(=O)Oc1cccc2C(=O)N([C@H](CS(=O)(=O)C)c3ccc(c4nc5ccccc5[nH]4)c(CCCCCCC(=O)OC)c3)C(=O)c12
6	CCCCCCCCCC(=O)ON1C(=O)c2cccc([C@H](CS(=O)(=O)C)c3ccc(c4nc5ccccc5[nH]4)c(CCCCCCC(=O)OC)c3)c2C1=O
7	CCCCCCCCCC(=O)Oc1cccc2C(=O)N([C@H](CS(=O)(=O)C)c3ccc(c4nc5ccccc5[nH]4)c(CCCCCCC(=O)OC)c3)C(=O)c12
8	CCCCCCCCCC(=O)Oc1cccc2C(=O)N([C@H](CS(=O)(=O)C)c3ccc(c4nc5ccccc5[nH]4)c(CCCCCCC(=O)OC)c3)C(=O)c12
9	CCCCCCCCCC(=O)ON1C(=O)c2cccc([C@H](CS(=O)(=O)C)c3ccc(c(CCCCCCC(=O)OC)c3)c3cccc4C(=O)N(C(=O)c34)OC(=O)CCCCCCCCC)c2C1=O
10	CCCCCCCCCC(=O)ON1C(=O)c2cccc([C@H](CS(=O)(=O)C)c3ccc(c(CCCCCCC(=O)OC)c3)c3cccc4C(=O)N(C(=O)c34)OC(=O)CCCCCCCCC)c2C1=O
11	CCCCCCCCCC(=O)ON1C(=O)c2cccc([C@H](CS(=O)(=O)C)c3ccc(c(CCCCCCC(=O)OC)c3)c3cccc4C(=O)N(C(=O)c34)OC(=O)CCCCCCCCC)c2C1=O

Pharmacophore Design and Screening:

LigandScout provided a pharmacophore model based on the supplied 11 molecules which contained basically two types of pharmacophoric features e.g. 4 hydrogen bond acceptors (HBA) and 1 hydrophobic interaction.

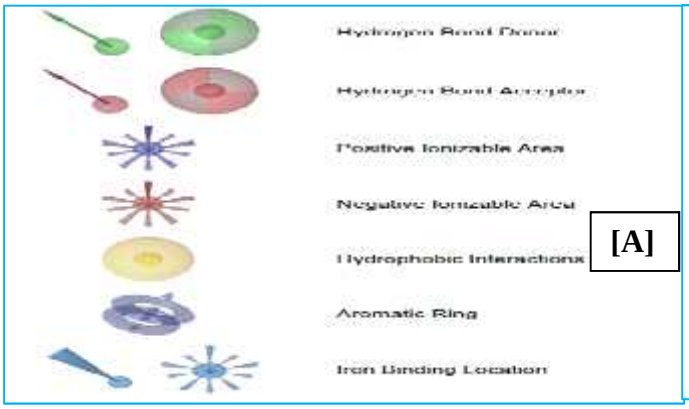

Figure 4A. Key to the pharmacophore features Figure 4B. Designed pharmacophore based on the supplied molecules.

As we had no biological active ligand for our target, so we have used cluster ID to define test and training set. With respect to pharmacophore, we considered two features while selecting. First one was the number of molecule, the more the molecules the better would be the pharmacophore. Secondly which is provided by the software is the score, the higher (0-1) the score the better the result. In our study the overall pharmacophore score was obtained 0.6668, which is favorable to conduct pharmacophore based virtual screening. The Designed Pharmacophores were screened with the web server Pharmit by using the Criteria's set according to the properties of Neurological drugs. For the target protein following criteria was set.

Table 2. Criteria which was set for the target protein

Molecular Weight	Rotatable Bonds	LogP	Polar Surface Area	Hydrogen Bond Donor	Hudrogen Bond Acceptor
180-330	3-7	1-2.5	140	4-8	1-4

We have done the screening procedure to find new ligands on the basis of the pharmacophore. By using the Pharmit Database we got overall 632 potential hits for the given pharmacophore.

Docking:

When the screened molecules were docked with the crystal structure of the target protein's binding site, it produced 10 conformations for each ligand. The screened molecules were docked with the target protein using the software Autodock Vina and the following results were obtained:

Table 3. Best 5 molecules for the target protein

Molecule No	Molport ID	Binding Affinity (kcal mol^{-1})
1	019-888-587	-8.6
2	044-179-937	-8.5
3	019-681-384	-8.5
4	000-469-280	-8.4
5	020-115-029	-8.3

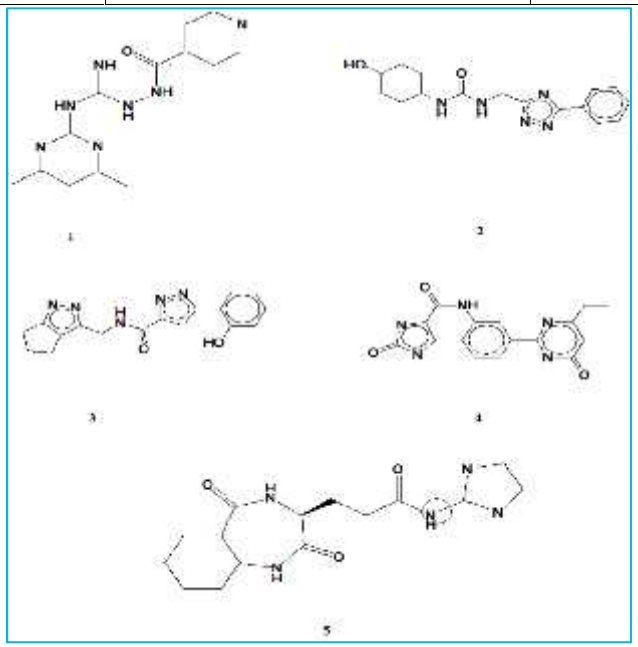

Figure 5. Best 5 Docked Molecules for the target protein

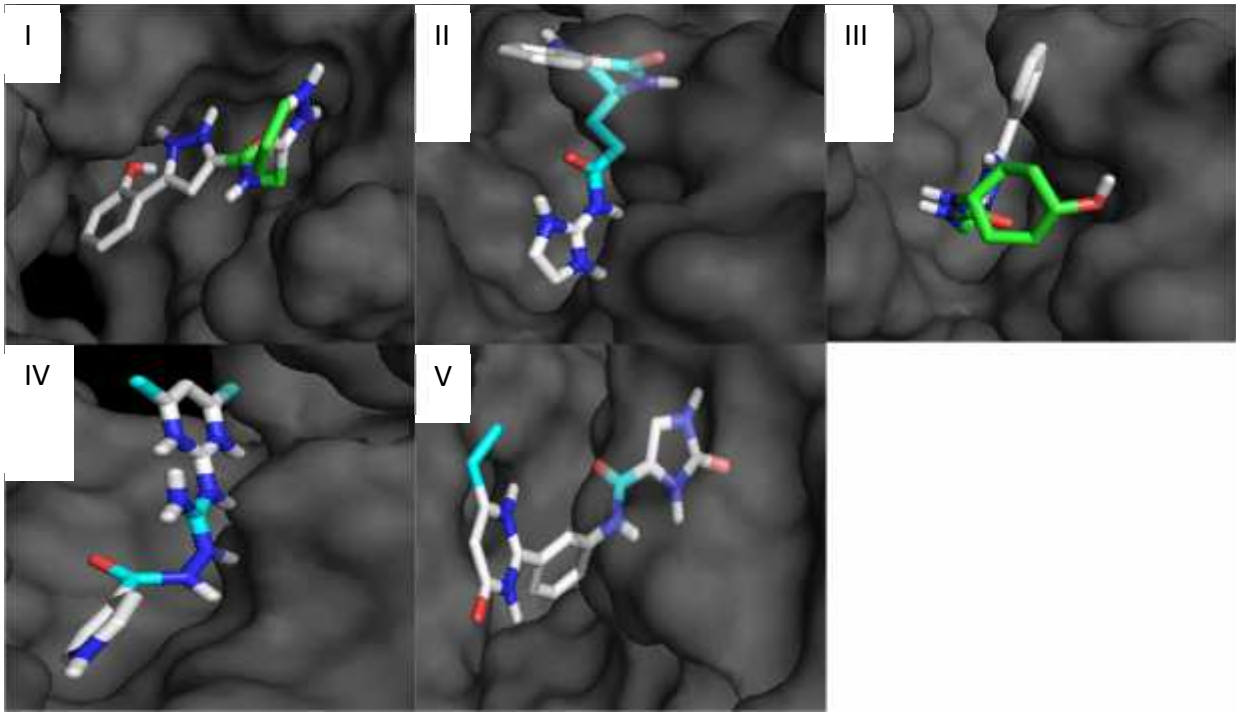

Figure 7. Docked Molecules with the target protein

The docking process resulted in a list of molecules with their binding affinities and RMSD values. After the docking process done, we selected the best 5 molecules for analyzing the binding interactions and their affinities. The binding affinities of M2 and M3 are -8.5 $kcalmol^{-1}$ and binding affinities of M4 and M5 are -7.1 and -8.3 $Kcalmol^{-1}$ respectively. In addition, the binding affinity of M1 with the target protein was -8.6 $Kcalmol^{-1}$ which is also shown in the table 3.

ADMET Testing:

By using the webserver SwissADME we predicted the ADMET profile of the protein molecules. To determine the ADMET profile of the selected molecules we have used SwissADME as it is the most widely used and accepted webserver for ADMET profile determination. The analysis revealed that all the molecules for the protein showed Physical and Chemical properties as same as neurological drugs that we considered during the screening process (Table 4). Their molecular weight was within the specified limit and the drug-likeness of those molecules were acceptable with respect to the properties of approved drugs. Besides, they followed the Lipinski's rule of five (No more than 5 hydrogen bond donors, No more than 10 hydrogen bond acceptors, a molecular mass less than 500 daltons, an octanol-water partition coefficient Log *P* not greater than 5) which means all these molecules have the characteristics that is needed to be present in a drug. The results of ADMET analysis are shown in the following tables. The aim of this study is to propose small molecule inhibitors targeting CHKV nsp3 macro domain using computer-aided techniques. The *in-silico* method of drug design is found to be very effective technique for the initial phase of drug design which can not only find the possible pockets inside the protein but also find a possible drug for that pocket. In this study, we applied de-novo drug design methodology combined with pharmacophore design and pharmacophore based virtual screening to collect potential hits. The Pharmacophore models consisted of spatial arrangement of chemical features namely hydrogen bond acceptors (HBA), hydrophobic interactions, different excluded volumes as well as shape constraints. The selected molecules were refined using several drug-like filters so that we can screen the drug-like compounds for the molecular docking study. After that, binding conformations of the best five docked compounds were analyzed to see the interactions with the target protein. Finally, five compounds for the target protein with structural diversity were selected for ADME

(Absorption, Distribution, Metabolism, and Excretion) testing. In the ADMET analysis, all the molecules showed drug-like properties specifically as antiviral drugs for our concern which can suggest us that the proposed molecules may work as further drug development process against the CHKV. This methodology which we have used in this study can be helpful for design and development of many more potential anti-prion drugs and more sophisticated techniques can be experimented to increase the accuracy of the results generated.

Table 4: ADMET Properties of the Top 5 Best Molecules for the target protein

Here, MW=Molecular Weight, RB=Rotatable Bond, HBA=Hydrogen Bond Acceptors, HBD=Hydrogen Bond Donors, TPSA=Total Polar Surface Area, GIA=GI Absorption, BBBP=Blood Brain Barrier Permeation, PgPS=P-Glycoprotein Substrate, LV= Lipinski Violation, BS= Bioavailability Score, LLV= Lead likeness Violations, SA= Synthetic Accessibility

Molecule no	MW	RB	HBA	HBD	TPSA	XLOGP3	GI A	BBBP	PgPS	CYP 1A2 Inhibition	CYP 2C19 Inhibition	CYP2C9 Inhibition	CYP2D6 Inhibition	CYP 3A4 Inhibition	LV	BS	LLV	SA
1	325.32	5	4	4	123.5	0.01	High	No	No	No	No	No	No	No	0	0.55	0	2.7
2	285.3	6	5	4	115.68	0.53	High	No	Yes	No	No	No	No	No	0	0.55	0	2.92
3	315.37	6	4	4	102.93	1.28	High	No	Yes	No	No	No	No	No	0	0.55	0	3.63
4	323.35	5	4	4	106.69	1.64	High	No	Yes	Yes	No	No	No	No	0	0.55	0	2.95
5	313.31	5	4	4	115.98	-0.15	High	No	Yes	No	No	No	No	No	0	0.55	0	2.89

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