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Full Length Research Paper

Structural modeling of natural citrus products as potential cross-strain inhibitors of Dengue virus

Naveeda Riaz[1*#], Ayma Aftab[1#], Fouzia Malik[1], Ahsan Sheikh[2], Waseem Akram[3] and Saima Kalsoom[1]

[1]Department of Bioinformatics and Biotechnology, International Islamic University, Sector H-10, Islamabad, Pakistan.
[2]Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan.
[3]Department of Agriantamology, University of Agriculture, Faisalabad, Pakistan.

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There are four serotypes of Dengue virus and there are existing drugs used against specific serotype. There is no drug that is effective against all strains of this virus. In this research, bioinformatics tools were used to predict the affinity of natural ligands for the glycoprotein E of Dengue virus by considering the conserved domains. Molecular docking studies were carried out by using Autodock 3.0. Computational analysis which showed that two ligands have the potential to inhibit the site in glycoprotein E and control of all strains is now possible by these ligands.

Key words: Bioinformatics, multivariate drug designing, Dengue virus, *in silico* drug for dengue, glycoprotein E, conserved domain.

INTRODUCTION

The use of computers and computational methods saturate all aspects of drug discovery today and forms the core of structure-based drug design (Kapetanovic, 2008). *In silico* methods can help in identifying drug targets through bioinformatics tools. They can also be used to evaluate target structures for possible binding/ active sites, to generate candidate molecules and check for their drug-like properties. In addition, such methods can be used to dock these molecules with the target, rank them according to their binding affinities and further optimize the molecules to improve binding characteristics.

Dengue virus is a member of Flaviviridae family. Four strains of Dengue strains were designated as DEN-1, DEN-2, DEN-3 and DEN-4, and infection by any one serotype leaves individuals susceptible to infection by the remaining three serotypes. This disease cannot be transmitted from man to man.

*Corresponding author. E-mail: naveeda.riaz@iiu.edu.pk. Tel: 92 51 9258016.

#These Authors contributed equally to this work.

Dengue viruses have 60 to 80% homology to each other. The basic difference is on structural basis that is also associated with the pathogenecity. Phylogenetic relationship of Dengue strains show that DEN-1 and DEN-3 are closely related (Qil and Chil, 2008). During Dengue virus intracellular development, the viral precursor membrane (prM) matures in the last step of virion assembly, most probably to avoid catastrophic activation of the viral fusion peptide induced by the acidic pH of the trans-Golgi network. Envelop E protein contains class 2 fusion peptide sequences that are responsible for invasion of attachment to host cell (Modis et al., 2004).

In the acid conditions during endocytosis in endosome, the E proteins undergo a dramatic structural change from dimmer into trimmer. These trimmers lead to formation of curvature that is thought to promote fusion (Eliana et al., 2008).

MATERIALS AND METHODS

Protein sequences of E-protein of all four strains were obtained from the Viral Zone (http://www.expasy.ch/viralzone/) and were submitted to Clustal W, available at www.ebi.ac.uk/clustalw/-21k. Identified highly conserved regions in all strains within prefusion

Table 1. Conserved sequences in 4 serotypes of dengue of envelop protein

Conserved sequence	Position
MRC	1 - 3
G	5
NRDFVEG	8 - 14
SG	16,17
VLEHG	24 - 28
CVTTMA	30 - 35
KPTLDFEL	38 - 45
CIE	60 - 62
N	67
TT	69,70
RCPTQGE	73 - 79
VDRGWGNGCGLFGKG	97 - 111
C	185
PR	187,188
G	190
DFNEM	192 - 198
L	198
W	206
VH	208,209
QWF	211 - 213
DLPLPW	215 - 220
VLGSQEGAMH	252 - 261
ALTGATE	263 - 269
ETQHGT	314 - 319
EPPFG	370 - 374

protein E, were then confirmed by T-Coffee 7.44. Some of the conserved amino acids are postulated to form a contour that can be used as the potential drug target and are mentioned in Table 1.

Identification of domains

Dengue virus protein E contains 3 domains that mainly consist of beta sheets. Domain 2 contains a loop at its tip with a hydrophobic sequence that is conserved among all flaviviruses. This loop ranges from 97 to 111 amino acids. The image of these amino acids from the Python Molecule Viewer (PMV 1.5.2) is given in Figure 1.

Fusion loop of sE protein

Three hydrophobic residues in the fusion loop conserved among all the flavivirus–Trp 101, Leu 107 and Phe 108 (Figure 2a and b). This fusion loop maintains the same structure in both the dimeric and trimeric form. So, this loop is considered as drug target in this study because fruitful results of drug binding can be helpful in controlling this fatal disease caused by any of the strain of Dengue just by a single drug. As Trp 101, Leu 107 and Phe 108 are highly conserved within all strains, they ensure the validity of selection of 97 to 111 amino acids as target.

Calculation of hydrophobicity

Hydrophobicity was calculated by ProtScale available at www.expasy.ch/tools/protscale.html.

ProtScale

Using the scale Hphob/Kyte and Doolittle, the individual values for the 20 amino acids are:

Ala: 1.800; Arg: -4.500; Asn: -3.500; Asp: -3.500; Cys: 2.500; Gln: 3.500; Glu: -3.500; Gly: -0.400; His: -3.200; Ile: 4.500; Leu: 3.800; Lys: -3.900; Met: 1.900; Phe: 2.800; Pro: -1.600; Ser: -0.800; Thr: -0.700; Trp: -0.900; Tyr: -1.300; Val: 4.200, -3.500, -3.500 and -0.490.

Weights for window positions 1... 9, using linear weight variation model:

1	2	3	4	5	6	7	8	9
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	Edge			center			edge	

MIN: -2.711
MAX: 2.422

Figure 3 shows areas that are more hydrophobic, predicted by these calculations. According to PMV 1.5.2, there are 6296 atoms, 844 residues and 4 chains in E- protein. Two conserved N-linked glycosylation sites are Asn-67 and Asn-153. Asn-153 is conserved in most flavivirus, while site of Asn-67 is unique in type-3 virus. These two sites are involved in cellular attachment and viral entry (Mathers et al., 2007; Khan et al., 2008).

Ligand selection

Potential ligands were chosen from natural compounds that have been extracted from *Citrus limonia* by Doctor Waseem Akram, Department of Agriantamology, University of Agriculture, Faislabad. Two of such candidate molecules are nomilin and limonin.

Drug characteristics of nomilin and limonin

Table 2 shows the properties of both nomilin and limonin. The chemical structures for the test ligands “limonin” and “nomilin” were taken from the PubChem (http://pubchem.ncbi.nlm.nih.gov/). Atoms in nomilin and limonin are shown in figures taken from The Dundee PRODRG2 Server (http://davapc1.bioch.dundee.ac.uk/prodrg/).

Pocket identification

Amino acids 97 through 111 are conserved in all strains and are considered as the fusion peptide and the pocket.

Autodock 3

Autodock 3 was used to provide modeling flexible ligand binding to the rigid receptor. Minimum energy for nomilin -7.27 kcal/mol with 40 clusters and the root mean square deviation (RMSD) = 0.00.

Final intermolecular energy = -6.92 kcal/mol
Final internal energy of ligand = -0.35 kcal/mol

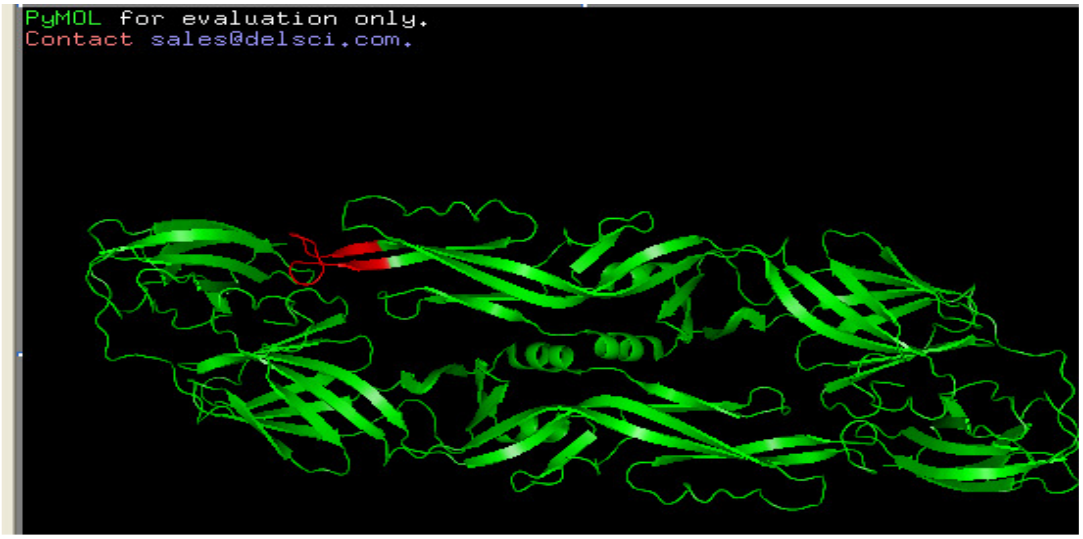
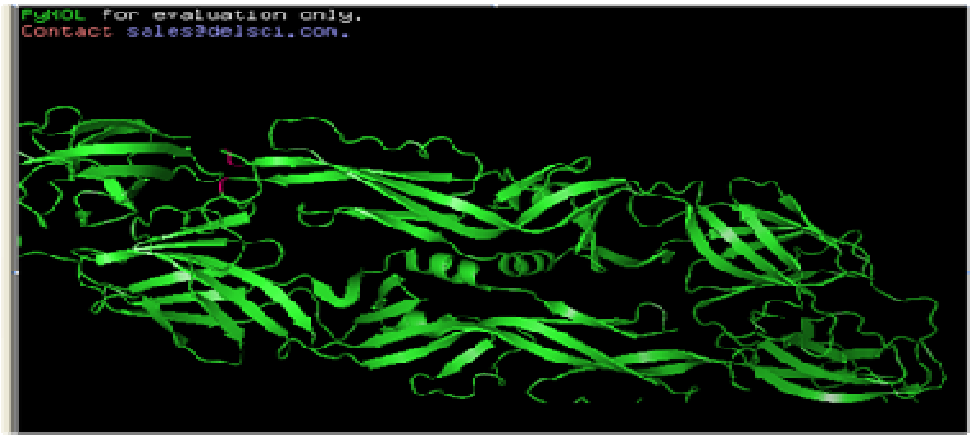
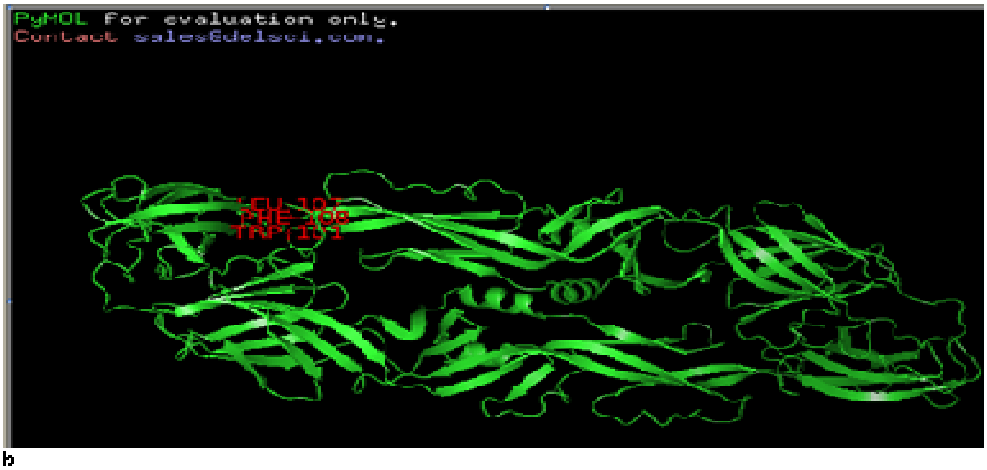


Figure 1. Conserved sequence of amino acid 97 to 111 (high lighted with red color).



a



b

Figure 2. (a) Hydrophobic areas in 1OAN.pdb (Trp 101, Leu 107 and Phe 108). (b) Hydrophobic areas in 1OAN.pdb (Representation of area where Trp 101, Leu 107 and Phe 108 are present).

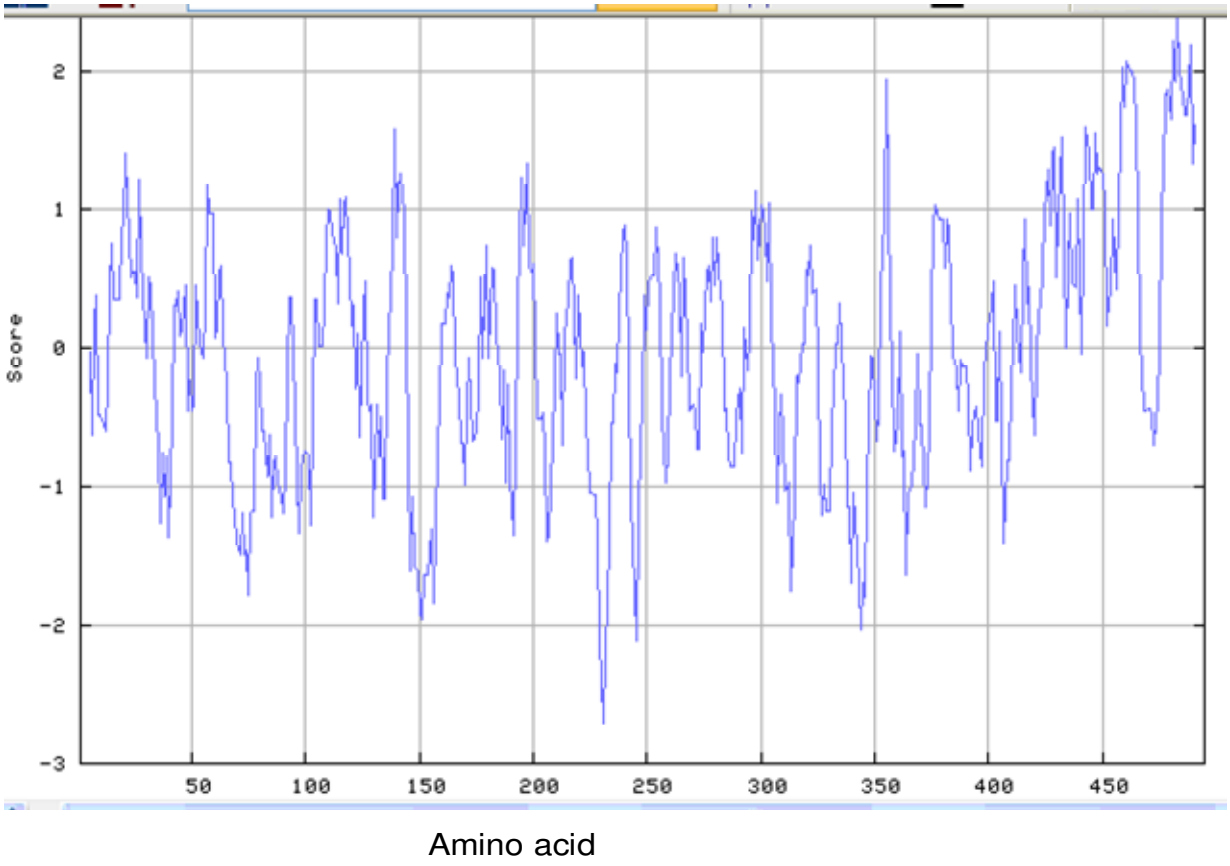


Figure 3. Hydrophobicity calculation by ProtScale (2D view).

Table 2. Physicochemical properties of limonin and nomilin.

Physical property	Limonin	Nomilin
Molecular weight	470.5116 [g/mol]	514.56416 [g/mol]
Molecular formula	$C_{26}H_{30}O_8$	$C_{28}H_{34}O_9$
XLogP3-AA	1.8	2.6
H-Bond donor	0	0
H-Bond acceptor	8	9
Rotatable bond count	1	3
Tautomer count	2	2
Exact mass	470.194068	514.220283
MonoIsotopic mass	470.194068	514.220283
Polar surface area	105	122
Heavy atom count	34	37
Formal charge	0	0
Complexity	1010	1080
Isotope atom count	0	0
Defined atom stereo center count	8	0
Undefined atom stereo center count	1	9
Defined bond stereo center count	0	0
Undefined bond stereo center count	0	0
Covalently-bonded unit count	1	1

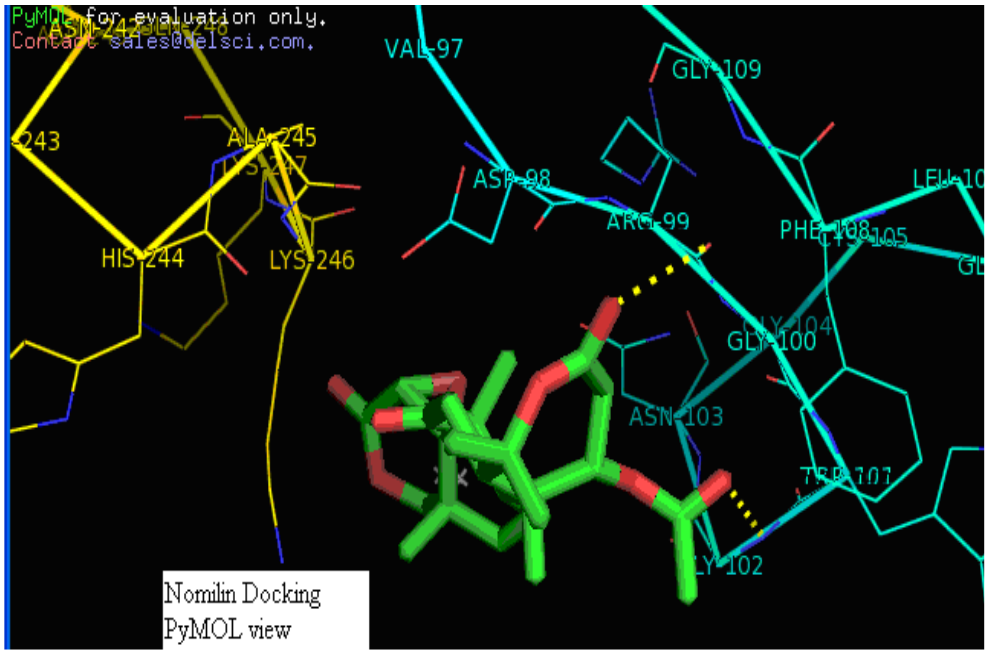


Figure 4. Hydrogen bonding shown between nomilin and 1OAN.pdb target site.

Analysis

Nomilin showed docking with amino acids ARG 99 and GLY 102 (Figure 4). Hydrogen bond formed: OAH atom of nomilin and Arg-99; OAI atom of nomilin and Gly-102.

The best and lowest docking energy of limonin that is equal to -6.83 kcal/mol and having number of clusters = 6 was obtained. PyMOL shows interaction of Limonin with Gly-109 (Figure 5). In ADT 3.05, the hydrogen bond formed is between OAD atom of limonin and Arg-99 (Figure 6).

Calculations of absorption, distribution, metabolism and excretion (ADME) property of ligands

Potential drug ADME properties of limonin and nomilin were calculated by web based tool cLogP (http://www.organic-chemistry.org/prog/). ADME property of limonin shows:

No risk of mutagenicity, score	1.0
No risk of tumorigenicity, score	1.0
No risk of irritating effects, score	1.0
No risk of reproductive effects, score	1.0

These results are the combined results of the cLogP, solubility, drug likeliness and drug score.

ADME property of nomilin shows:

No risk of mutagenicity, score	1.0
No risk of tumorigenicity, score	1.0
No risk of irritating effects, score	1.0
No risk of reproductive effects, score	1.0

These results are the combined results of the cLogP, solubility, drug likeliness, and drug score. These ADME calculations indicate that these molecules will become effective drugs without any toxicity.

DISCUSSION

According to World Health Organization, 50 million human victims for Dengue virus have been reported. So, it has become a challenge to develop some treatment for this disease because it has proven difficult to control mosquito borne diseases. To develop a tetravalent potent drug or vaccine, it is always an indispensable demand to treat this disease that is caused by 4 serotypes with different level of severity. Scientists succeeded in developing tetravalent vaccines but because of meddling of different attenuated Dengue virus, they need to be improved (Edelman et al., 2003). Identification of inhibitors of antiflavivirus opened horizons for the development of antidengue drug development. The target of these drugs can be any of the viral stage to inhibit its spread in the body but again the drug should be tetravalent. Therefore, nucleic acid based therapy was developed to inhibit replication of virus in the cell but it was able to control DEN-2 strain only (Adleman et al., 2002). Similarly, viral entry inhibitor compounds have been discovered, some of which are specific to stop only DEN-3 and to a lesser degree DEN-1 and DEN-4 (Ono et al., 2006). In this study, efforts have been made to develop a tetravalent drug. To accomplish the goal, envelop protein is selected as a target for drug because it is the first one that enters the cells by binding and hence begins the surge of infection. Moreover, the target site that is selected consists of amino acid sequence that is conserved in all the strains. So, selection of such a target

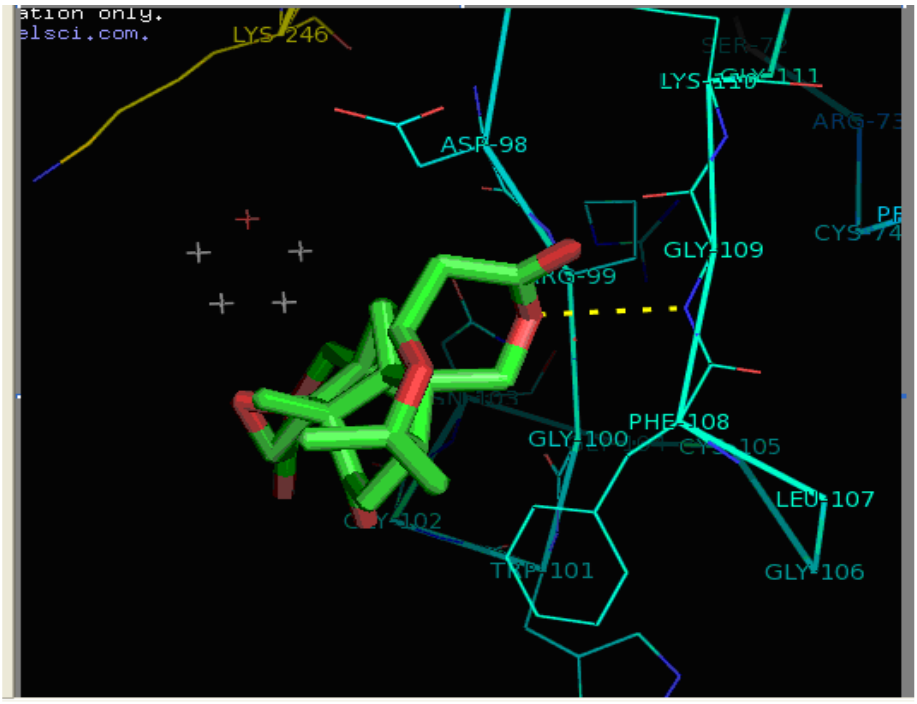


Figure 5. Hydrogen bonding shown between limonin and 1OAN.pdb target site.

Figure 6. Binding interactions of limonin and 1OAN.pdb target site.

site is important for entry into the body cells and is present in all strains of Dengue virus which is a key for developing a tetravalent drug. The ligands selected are from *C. limonia* which also showed good binding to the target site. This binding affinity shows that these ligands can inhibit the entry of virus in the cell and thus the

spread of infection as well.

Conclusion

Selection of conserved site as drug target is of core importance in focusing the problem of designing a multi-variant drug against all serotypes of dengue. Selection of conserved regions among all the serotypes has played the leading role to select such ligands that can prevent the infection from prevailing from the very beginning when the Dengue virus enters the body and the envelop protein get attached to the DC cells of the body. Natural ligands are considered because of their surety of been non-toxic to humans. Computational analysis showed that two ligands have the potential to inhibit that site in glycoprotein E that is conserved among 4 strains. When the ligand get attached to the fusion peptide of the envelop protein, it does not allow the protein to transform into tripeptide. This tripeptide binds to the body cells and is important for causing infection. So, in this way, the drug performs its function. Based on the results of Auto-dock 3 and ADME properties, it can be concluded that these two ligands can be proved as the best weapon to stop the infection of Dengue virus from spreading in humans. So, it can be hypothesized that these ligands may be found to be effective if tested in wet laboratory. Moreover, identification of various conserved sites can preferentially be used as drug targets or to induce mutations for further studies.

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