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IN SILICO DRUG DESIGNING STUDIES ON DENGUE VIRUS NS2B/NS3 PROTEASE

[1]Sushmitha.H.S and [2]Balasubramanian Sathyamurthy

[1]Department of Biochemistry, Ramaiah College of Arts, Science and Commerce, Bangalore

[2]Professor, Department of Biochemistry, Ramaiah College of Arts, Science and Commerce, Bangalore

Abstract:

The seven key proteins are involved in causing dengue, which are considered as major therapeutic targets for dengue drug development. Recent studies have reported positively for dengue virus NS2B/NS3 protease in dysregulation of causing dengue process in humans. Dragon fruit seed phytochemicals are reported to have antioxidant and antiviral properties. In the present study we studied binding efficiency of 11 compounds that are present in the dragon fruit seeds with NS2B/NS3 protease through Insilico methods. By our virtual screening and docking result, we found that the Compound J and Compound K have highest binding affinity with the NS2B/NS3 protease and also we predicted the binding site amino acid residues and the nature of hydrogen bonding. However more invivo experimental validation of our results with animal models will enlighten the development of more potent drugs from these compounds for treatment of dengue.

Key words: *NS2B/NS3 protease, Binding interaction, molecular docking, dengue*

Corresponding author:

Dr. Balasubramanian Sathyamurthy

Professor,
Department of Biochemistry,
Ramaiah College of Arts, Science and Commerce,
Bangalore – 560054

QR code

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INTRODUCTION:

Pitaya also called as "Dragon Fruit" in English belongs to the Cactacea family. The generic name of Dragon fruit is *Hylocereus* [1]. The three varities of Dragon fruit include *Hylocereus undatus, Hylocereus polyrhizus* and *Hylocereus megalanthus. Hylocereus undatus* and *Hylocereus polyrhizus* differ by the colour of their pulp and both have red peel. *Hylocereus megalanthus* has White pulp with yellow peel. It is a hybrid of *Hylocereus costaricensis* and *Selenicereus inermis.* Small, black seeds that are edible are present interspersed in the pulp of the fruit [2]. The stems of the dragon fruit plant bear 4-7 fruits which are 4-10 centimeters long. The colour of the fruit varies from grayish-brown to blue-green. The fruits are 3-8 centimeters thick. Dragon fruit is now transported by Europeans to Central America, but it is believed to be a native of Mexico [3]. Dragon fruit is now cultivated in many of the Southeast Asian countries like Taiwan, Sri Lanka, India, Bangladesh [4]. The peel and the pulp extracts of the fruits were found to be useful in the treatment of various infectious diseases [5]. These extracts were also found to have antioxidant and anti-microbial properties [6]. Betacyanin, a nitrogen-containing compound was found important to give the red colour to the fruit and also it is reported to have high antioxidant activity [7]. Polyunsaturated fatty acids (PUFAs) such as linolenic acid and linoleic acid were found to be present in the seeds of Dragon fruit [8]. A recent study on the dragon fruit seeds reported the most probable compounds present in them such as 9,12-octadecanoic acid, tetradecanoic acid, phytol, octadecanoic acid, 12-chloroethyl linoleate, 9,12,15-octadecatrienoic acid, 8-hexadecyne present in them [4].

Dengue is a haemorrhagic fever [9] which can be caused by the four serotypes of dengue virus DENV-1, DENV-2, DENV-3 and DENV-4 [10]. These viruses contain ten proteins out of which three are structural proteins and seven are non-structural proteins [11]. NS2B-NS3 protease is a crucial enzyme for the viral replication [12]. This protein is heterodimeric protein of NS2B and NS3 protein The N-teminal of the NS3 protein forms associates with the NS2B cofactor which is important for the viral replication. NS2B-NS3 protease has an important role in the viral life cycle [13]. The protein used for this study was the NS2B-NS3 protease from the Dengue Virus Type-2 [14].

The utility of mathematics, computer and statistics to analyse the biological data is Bioinformatics which is an interdisciplinary branch of science [15]. Protein Data Bank (PDB) is a bioinformatic tool which stores the structures of proteins, ligands and macromolecules [16]. Docking analysis can be conducted to analyse the fitness and interaction between the protein and the ligand in the form of energy. This interaction can be used as a pharmaceutical basis for drug production [17].

MATERIALS AND METHODOLOGIES:

Preparation of macromolecule NS2B/NS3 protease:

The protein data bank (PDB) was used to obtain the three-dimensional structure of the macromolecule. PDB contains large number of proteins which are experimentally determined and stored in this site. The structures are downloaded and saved either in mm CIF or pdb format. NS2B/NS3 protease of dengue virus was used for this study. The 3D structure of this protein was downloaded from PDB and saved in pdb format. The downloaded protein was viewed in Py-Mol viewer.

Preparation of ligands:

Ligands selected were from the previous studies on this fruit seeds. 11 ligands were used for the study. Ligands were constructed using ChemSketch [18]. The constructed ligands were optimized to add the hydrogen bonds and the obtained structures were saved in mol for docking analysis.

Docking study:

Docking studies were conducting using iGEMDOCK software. iGEMDOCK (Generic Evolutionary Method for molecular DOCKing) is a graphical-automatic drug design system for docking, screening and post-analysis [18]. The protein and the ligands were loaded and the out path was set. Standard docking parameters were used for docking (population size=200, generations=70 and no.of solutions=2). The docking process was initiated. After the docking process, the best docking pose for the individual ligands can be obtained. The best binding pose, the binding affinity and the total binding energy values were saved in the output folder. The saved files were visualized in Py-Mol viewer.

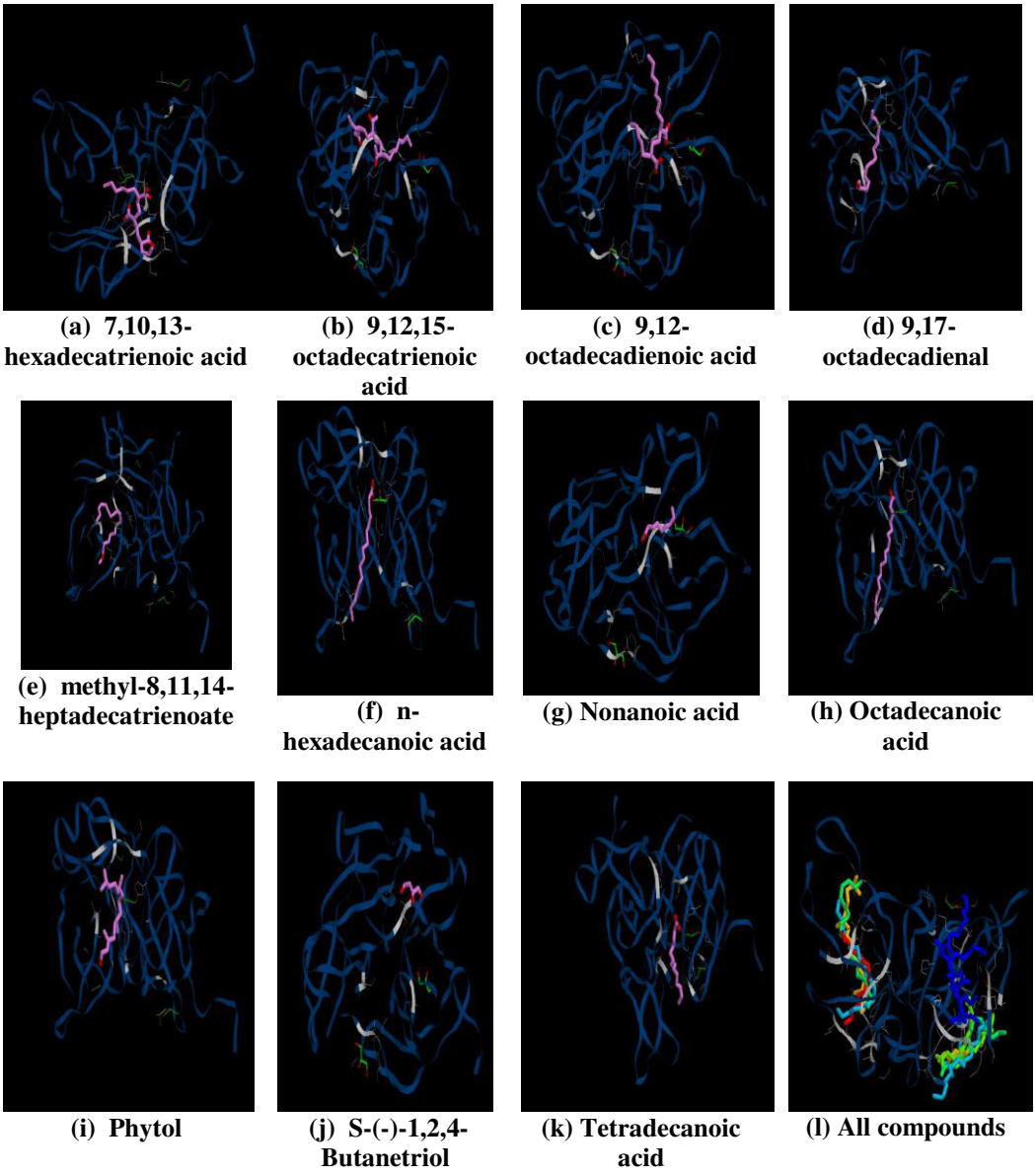
RESULTS AND DISCUSSION:

Table – 1: The fitness and the interaction profile of the NS2B/NS3 protease with the ligands

Ligands	Compounds	Total Binding Energy	Vander Waal's Force	E(pharma)	V-S Lys 75	V-S Leu 76	V-S Trp 83	V-M Ile 165	H-Bond Energy	Electrostatic Force	AverCon Pair
				Z-score=>	1.97	1.90	2.79	2.62			
				W(pharma) =>	0.71	0.68	1.00	0.94			
A	7,10,13-hexadecatrienoic acid	-107.64	-82.13	-107.6	0	0	0	0	-24.05	-1.45	24.12
B	9,12,15-octadecatrienoic acid	-114.93	-100.08	-90.4	0	0	0	0	-14.85	0	23.04
C	9,12-octadecadienoic acid	-90.44	-68.38	-114.9	0	0	0	0	-17.46	-4.60	20.75
D	9,17-octadecadienal	-84.92	-84.92	-84.5	-2.4	-4.5	-5.9	-7.3	0	0	25.14
E	methyl-8,11,14-heptadecatrienoate	-81.84	-78.34	-80.6	-7.4	-5.2	-5.6	-8.1	-3.5	0	24.35
F	n-hexadecanoic acid	-75.71	-70.33	-100	-5.8	-3.6	-5.4	-4.1	-5.38	0	24.5
G	Nonanoic acid	-69.57	-47.87	-84.9	0	0	0	0	-20.58	-1.12	28.18
H	Octadecanoic acid	-92.12	-88.62	-92.9	-5.2	-5.1	-6.6	-6.9	-3.5	0	28.2
I	Phytol	-82.80	-82.80	-68.9	-4.5	-6.2	-6	-9.1	0	0	25.91
J	S-(-)-1,2,4-Butanetriol	-57.92	-32.50	-81.8	0	0	0	0	-25.43	0	30
K	Tetradecanoic acid	-85.49	-78.49	-96.4	-7.4	-4.9	-6.7	-7	-7	0	29.44

Table – 2: The cluster table for NS2B/NS3 protease and the ligands

Ligands	Compound	E (pharma)	H - Bond	Amino Acid Position	H – Bond Energy
A	7,10,13-hexadecatrienoic acid	-107.6	H-M	Ser (75)/ Glu (90)/ Asn (105)	-3.5
			H-S	Arg (107)	-5.7
B	9,12,15-octadecatrienoic acid	-108.6	H-M	Asp (58) /Ile (76)	-3.5
			H-S	Arg (55)	-5.2
C	9,12-octadecadienoic acid	-120	H-M	Arg (55)	-3.2
			H-S	His (60)	-8.3
D	9,17-octadecadienal	-87.4	-	-	-
E	methyl-8,11,14-heptadecatrienoate	-72.3	H-S	Asn (152)	-3.5
F	n-hexadecanoic acid	-113	H-S	Trp (83)	-5.4
G	Nonanoic acid	-111.4	H-M	Asp (58)	-3.5
			H-S	Arg (60)	-13.2
H	Octadecanoic acid	-103	H-S	Trp (83)	-3.5
I	Phytol	-72.3	-	-	-
J	S-(-)-1,2,4-Butanetriol	-115.6	H-M	Asp (58)	-6.8
			H-S	Arg (55)	-7
K	Tetradecanoic acid	-114	-	Trp (83)	-7



(a) 7,10,13-hexadecatrienoic acid (b) 9,12,15-octadecatrienoic acid (c) 9,12-octadecadienoic acid (d) 9,17-octadecadienal

(e) methyl-8,11,14-heptadecatrienoate (f) n-hexadecanoic acid (g) Nonanoic acid (h) Octadecanoic acid

(i) Phytol (j) S-(-)-1,2,4-Butanetriol (k) Tetradecanoic acid (l) All compounds

Fig. 1: Interaction of compounds with NS2B/NS3 protease

From the Table – 1, the 3D structure coordinates of NS2B/NS3 protease is optimized and 11 compounds from dragon fruits seeds are identified. Their total binding energy were calculated using iGEMDOCK. Evaluation of binding conformation of 11 compounds with NS2B/NS3 protease protein is performed using iGEMDOCK. From docking study, we listed binding affinity of 11 compounds based on ligand binding energy (Table.1). The binding pose for each ligand molecule into the NS2B/NS3 protease is analyzed and the one having lowest ligand binding energy with NS2B/NS3 protease among the different poses are generated. The lower energy scores represent better target protein-ligand binding affinity compared to higher energy score. Among the 11 analogs, compound J and K are found to have lower ligand binding energy value than other analogs. Compound "J" has least binding energy score with NS2B/NS3 protease (binding energy value = -115.6 kcal/mol) and compound "K" has ligand binding energy value of -114 kcal/mol. We further analyzed the docked pose for finding the binding mode of compound "J" and compound "K" in to NS2B/NS3 protease protein to validate the reasonable binding conformations.

Docking of compound – J into NS2B/NS3 protease:

From Table – 2 and Figure – 1, the docking simulation of compound - J is performed for NS2B/NS3 protease. From the docking study, we observed that compound – J has best binding affinity with the target protein. Interaction analysis of binding mode of compound –J in NS2B/NS3 protease reveals that it forms two strong hydrogen bonds, one with branched chain residue Asp 58 having -6.8 kcal/mol as its bond energy and another hydrogen bond is at the branched residue of Arg 55 with – 7.0 kcal/mol as bond energy. A close-up

view of binding mode of compound – J with NS2B/NS3 protease is shown in Fig.2.

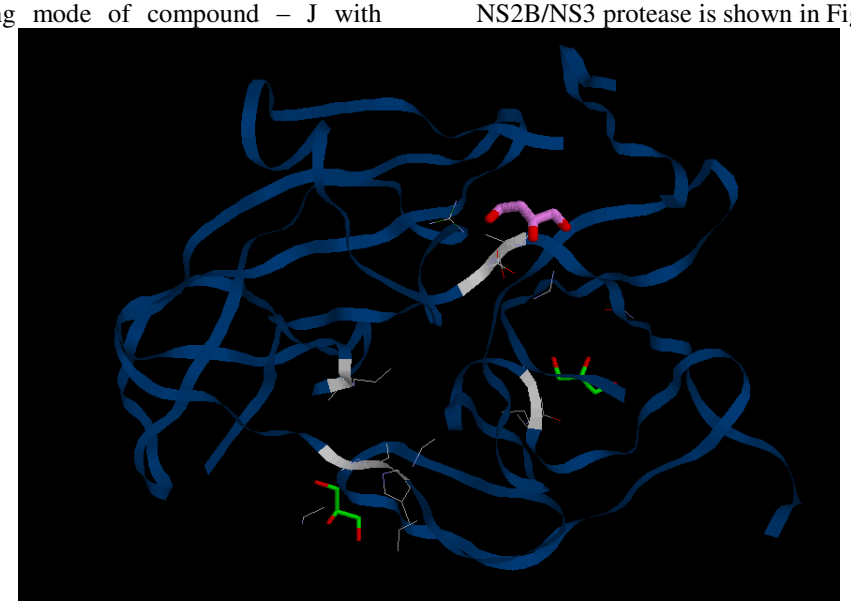


Fig. 2: A close-up view of binding mode of compound – J with NS2B/NS3 protease

Docking of compound - K into NS2B/NS3 protease:

From Table – 2 and Figure – 1, the docking studies of 11 compounds are performed for the target protein. In our results on the binding conformation modes of compounds with NS2B/NS3 protease, compound - K shows higher affinity with the NS2B/NS3 protease. In examining the binding interaction and position of the compound K with NS2B/NS3 protease ligand binding site predicted by your docking procedure, it is found to have one strong hydrogen bond which is at Trp 83 with the bond energy -7.0 kcal/mol. A close-up view of binding mode of compound – K with NS2B/NS3 protease is shown in Fig.3.

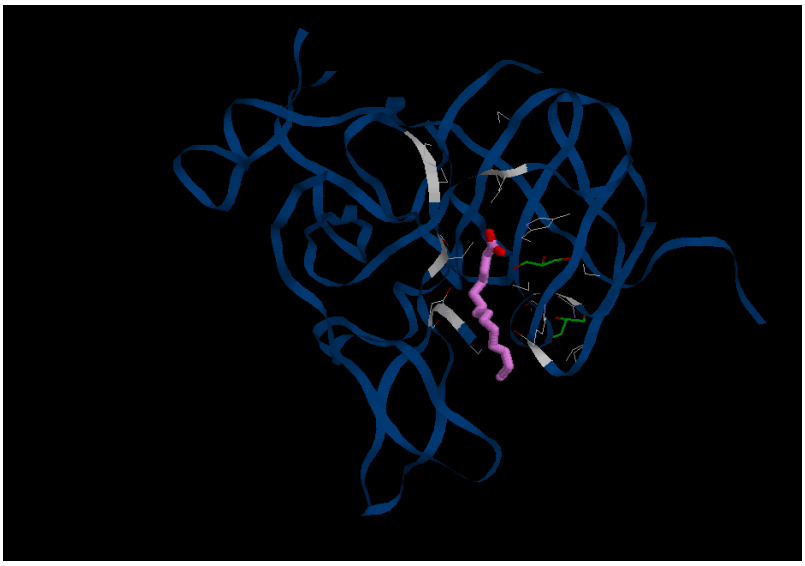


Fig. 3: A close-up view of binding mode of compound – K with NS2B/NS3 protease

CONCLUSION:

Our molecular docking studies explored the possible binding modes of 11 compounds that are present in dragon fruit seed with NS2B/NS3 protease. It revealed that all the 11 compounds show minimum affinity with NS2B/NS3 protease. Especially the compound J (S-(-)-1,2,4-Butanetriol) and compound K (Tetradecanoic acid) shows best result when compared with other compounds. On comparing the binding energy and the binding site residues, we found that all compounds differ either in their binding modes or with the binding site residues for hydrogen bond formation. The conclusion drawn from our virtual screening and docking result was that the Compound J and Compound K have highest binding affinity with the NS2B/NS3 protease. Though, there are many reports on the in vitro analysis of these compounds

and its antioxidant properties, but there are no in silico studies that predict the binding and active regions especially with NS2B/NS3 protease. Our study is probably the first such attempt to predict the binding site, However validation of our results through *invivo* and *invitro* experiments and also with animal models will enlighten hope for the future development of more potent drugs for the treating Dengue.

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