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IN SILICO DRUG DESIGNING STUDIES ON DENGUE VIRUS NS2A TRANS-MEMBRANE DOMAIN

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ABSTRACT

The key proteins involved in causing dengue are the seven proteins, both structural and non-structural, which are considered as the major therapeutic targets for development of drugs against dengue. Recent studies have reported positively for NS2A trans-membrane domain 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 NS2A trans-membrane domain through Insilico methods. By our virtual screening and docking result, we found that the Compound D has the highest binding affinity with the NS2A trans-membrane domain 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.

KEYWORDS: NS2A trans-membrane domain, Binding interaction, molecular docking, dengue.

INTRODUCTION

Pitaya is one of the rare known tropical fruits which has high economic potential [1]. It belongs to cactaceae family.[2] The unique feature of this plant is that it can tolerate extreme conditions. The most common species of pitaya *Hylocereus undatus*,red pitaya with white; *Hylocereus polyrhzius*, red pitaya with red pulp and *Selenicereus megalanthus*, yellow pitaya with white pulp.[3] Pitaya is 3mm in diameter with delicate and juicy pulp which contains numerous black edible seeds. This fruit is considered highly nutritious. It has large amounts of sugar, minerals and antioxidants.[1] The presence of betacyanin, a pigment which give red color to the fruit is found to have antioxidant properties.[4] Previous studies on peel and pulp of pitaya was found to contain similar amounts of phenols.[5] The anti-cancerous properties on pitaya was reported upon conducting studies on the cancer cell line such as PC3 (Human Prostate cancer cell line), BCAP-37 (Human Breast cancer cell line) and MGC803-(Human gastric cell line).[6] Pitaya, especially the *Hylocereus undatus* variety was found to have wound healing property.[7] It is reported that, pitaya seed oil contain mild laxative effect.[8] The recent studies on pitaya seed was found to possibly contain compounds which could have anti-microbial, anti-cancerous, anti-inflammatory and many more effects. Some of the compounds reported were tetradecanoic acid, phytol, octadecanoic acid, n-hexadecanoic acid, phytol, 9, 17-octadecadienal, 9, 12-octadecadienoic acid.[9]

In the recent time, dengue fever has been a major outbreak, especially in India. Dengue maybe caused by genetically similar and antigenically different serotype namely, DENV1, DENV2, DENV3 and DENV4.[10] Recent study has reported the presence of the fifth serotype, the DENV5.[11] They have seven essential proteins present in the viral structure.[12] The protein used for this study was the trans-membrane domain of the NS2A of dengue virus type 2. NS2A is a non structural protein and it is a component of viral replication complex which is functionally active in the assembly of the virion and also it acts as an antagonist to the host immune response.[13]

Bioinformatics is a multidisciplinary branch of science. It uses many fields such as biology, computer science, mathematics and statistics. In the recent times, this branch of science has been emerging important for much more studies. Bioinformatics is a science in which huge biological data can be analyzed used computational techniques. It has been widely used in the cancer research and also for many other diseases.[14] PDB is a bioinformatic tool which has a wide range of structures of proteins, ligands and other macromolecules stored in them.[15] Docking analysis gives the information on the

binding affinity of the ligands with the proteins.[16] The binding affinities are given in terms of binding energy expressed in kcal/mol. Based on the binding energies, the best compound or ligand which fits the protein can be determined. Lower the binding energy, higher is the binding affinity and vice-versa.[17]

MATERIALS AND METHODOLOGIES

Preparation of NS2A trans-membrane domain

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. NS2A trans – membrane domain 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 Chem Sketch.[17] 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.[17] 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.

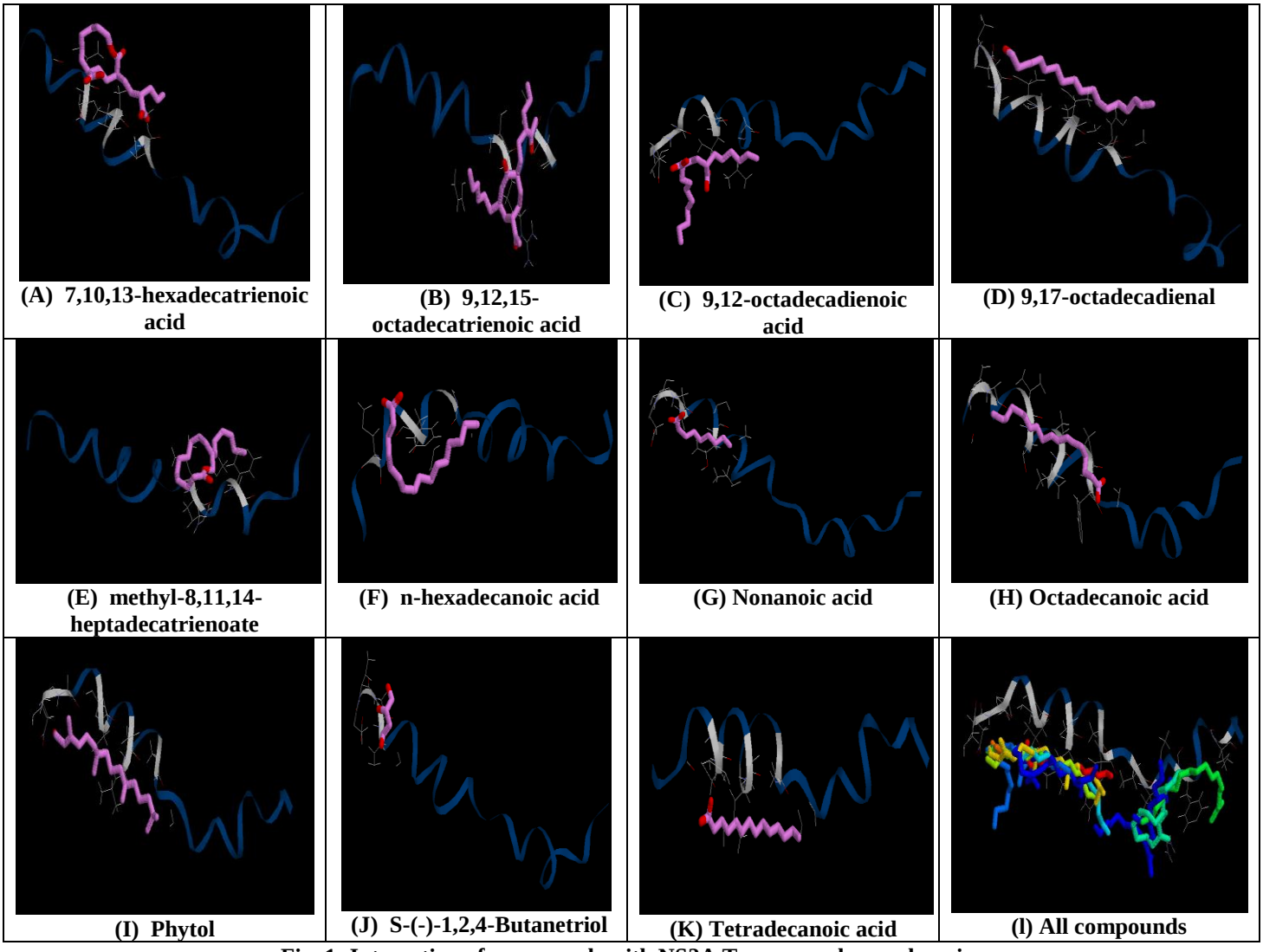
RESULTS AND DISCUSSION

Table 1: The fitness and the interaction profile for NS2A Trans-membrane domain with the ligands.

Ligands	Compounds	Total Binding Energy (kcal/mol)	Vander Waal's Force (kcal/mol)	E (pharma)	V-S Val 6	V-S Thr 7	V-S Leu 11	V-S Val 17	H-Bond Energy (kcal/mol)	Electrostatic Force (kcal/mol)	Aver Con Pair
				Z-score=>	2.17	2.94	1.74	1.70			
				W(pharma)=>	0.74	1.00	0.59	0.58			
A	7,10,13-hexadecatrienoic acid	-70.34	-68.38	-80.7	-8.3	-8.1	-6.2	-5.6	-1.97	0	17.32
B	9,12,15-octadecatrienoic acid	-78.88	-68.96	-52.6	-9.7	-4.4	-6.8	-5.1	-9.91	0	16.85
C	9,12-octadecadienoic acid	-76.99	-65.71	-90.4	0	0	0	-20.1	-13.19	1.91	19.04
D	9,17-octadecadienal	-89.51	-77.93	-75.1	-11.2	-5.6	-7.3	-1.6	-11.58	0	22.76
E	methyl-8,11,14-heptadecatrienoate	-67.98	-64.48	-68.5	-9.5	-12	-6.9	0	-3.5	0	19.85
F	n-hexadecanoic acid	-64.53	-64.53	-72.2	-16.5	-6.6	-8.6	-2.4	0	0	21.11
G	Nonanoic acid	-63.85	-52.89	-85.5	0	0	0	-12.7	-13	2.04	29.46
H	Octadecanoic acid	-78.41	-78.41	-73.4	-8.1	-6.9	-8.4	-6.5	0	0	24
I	Phytol	-80.20	-71.36	-87.7	0	0	0	-6.3	-8.85	0	22.38
J	S-(-)-1,2,4-Butanetriol	-46.76	-33.00	-92.3	-7.5	-9.4	-3.7	0	-13.76	0	28.57
K	Tetradecanoic acid	-64.84	-61.34	-91	-1.6	-4.5	0	0	-3.5	0	2275

Table 2: The cluster table for NS2A Trans-membrane domain and the ligands.

Ligands	Compound	H – Bond	Amino Acid Position	H – Bond Energy (kcal/mol)
A	7,10,13-hexadecatrienoic acid	-	-	-
B	9,12,15-octadecatrienoic acid	H-S	Arg (18)	-9.9
C	9,12-octadecadienoic acid	H-M	Gly (3)	-6.2
		H-S	Asp (1)	-2.5
D	9,17-octadecadienal	H-M	Gly (3)/ Arg (28)	-3.5
		H-S	Asp (1)	-2.5
E	methyl-8,11,14-heptadecatrienoate	H-S	Arg (18)	-3.5
F	n-hexadecanoic acid	-	-	-
G	Nonanoic acid	H-M	Gly (3)	-7
		H-S	Asp (1)	-2.5
H	Octadecanoic acid	-	-	-
I	Phytol	H-M	Gly (3)	-3.5
		H-S	Asp (1)	-2.5
J	S-(-)-1,2,4-Butanetriol	H-M	Gly (3)	-7
		H-S	Asp (1)	-4
K	Tetradecanoic acid	H-M	Thr (7)	-3.5



(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 NS2A Trans-membrane domain.

From the Table – 1, the 3D structure coordinates of NS2A trans-membrane domain is optimized and 11 compounds from dragon fruits seeds are identified. Their total binding energy was calculated using iGEMDOCK. Evaluation of binding conformation of 11 compounds with NS2A trans-membrane domain 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 NS2A trans-membrane domain is analyzed and the one having lowest ligand binding energy with NS2A trans-membrane domain 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 D was found to have lower ligand binding energy value than other analogs. Compound "D" has least binding energy score with target protein (binding energy value = - 89.51 kcal/mol). We further analyzed the docked pose for finding the binding mode of compound "D" to NS2A trans-membrane domain to validate the reasonable binding conformations.

Docking of compound – D into NS2A trans-membrane domain

From Table – 2 and Figure – 1, the docking simulation of compound - D is performed for NS2A trans-membrane domain. From the docking study, we observed that compound – D has best binding affinity with the target protein. Interaction analysis of binding mode of compound –D in the target protein reveals that it forms three strong hydrogen bonds, one with the side chains of Asp 1 having the bond energy of -2.5kcal/mol and the other two with the backbone of Gly 3 and Arg 28 having the bond energy of -3.5kcal/mol. A close-up view of binding mode of compound – D with NS2A trans-membrane domain is shown in Fig.2.

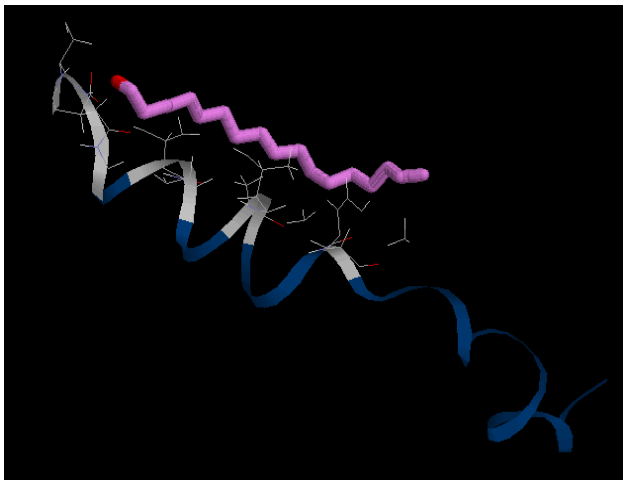


Fig. 2: A close-up view of binding mode of compound – D with NS2A trans-membrane domain.

CONCLUSION

Our molecular docking studies explored the possible binding modes of 11 compounds that are present in dragon fruit seed with NS2A trans-membrane domain. It revealed that all the 11 compounds show minimum affinity with the target protein. Especially the compound D (9, 17-octadecadienal) shows best result when compared to 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 D, has the highest binding affinity with the NS2A trans-membrane domain. 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 the target protein. 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.

REFERENCES

1. Jeronimo MC, Orisine JVC, Maria RC. Nutritional Pharmacological and Toxicological Characteristics of Pitaya (*Hylocereus undatus*): A review of Literature. African Journal of Pharmacy and Pharmacology, 2017; 11(27): 300-304.
2. Choo JC, Koh RY, Ling APK. Medicinal Properties of Pitaya: A Review; Spatula DD, 2016; 6(2): 69-76
3. Donadio LC. Pitaya. Rev. Bras. Frutic, 2009; 31(3): 637-929.
4. Kim H, Choi HK, Moon JY, Kim YS, Mosaddik A, Cho SK. Comparative Antioxidant and Antiproliferative Activities of Red and White Pitayas and Their Correlation with Flavonoid and Polyphenol Content. J. Food Sci., 2011; 76(1): C38-45.
5. Wu LC, Hsu HW, Chen YC, Chiu CC, Lin YI, Ho JA. Antioxidant and Antiproliferative Activities of Red Pitaia Vermelha. Food Chem., 2006; 95: 319-327.
6. Luo H, Cai Y, Liu Tao, Yang S. Chemical Composition and in-vitro Evaluation of the Cytotoxic And Antioxidant Activities of Supercritical Carbon dioxide Extracts of Pitaya (Dragon Fruit) Peel .Chemistry Central Journal, 2014; 8: 1.
7. Perez GRM, Vargas SR, Ortiz HYD. Wound Healing Properties of *Hylocereus undatus* on Diabetic Rats. Phytother. Res., 2005; 19: 665-668.
8. Crane JH, Balerdi CF. Pitaya Growing in Florida Home Landscape. Extension of University of Florida (IFAS), 2005; 9.
9. Sushmitha HS, Roy CL, Gogoi D, Velagala RD, Nagarathna A, Balasubramanian S, Rajadurai M.

Phytochemical and Pharmacological Studies on *Hylocereus undatus* Seeds: An *In Vitro* Approach. World Journal of Pharmacological Research, 2018; 7(14): 986-1006.
10. Yung CF, Lee KS, Thein TL, Tan LK, Gan VC, Wong JGX, Lye DC, Ng LC, Leo YS, Dengue Serotype-Specific Differences in Clinical Manifestation, Laboratory Parameters and Risk of Severe Disease in Adults, Singapore. Am. J. Trop. Med. Hyg., 2015; 92(5): 999-1005.
11. Lt. Col Mustafa MS, Col Rastogi V, Col Jain S, Lt. Col Gupta V. Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. Medical Journal Armed Forces India, 2015; 71: 67-70.
12. Perera R and Kuhn RJ. Structural Proteomics of Dengue Virus. Curr Opin Microbiol, 2008; 11 (4): 369-377
13. Xie X, Gayen S, Kang C, Yuan Z and Shi PY. Membrane Topology and Function of Dengue Virus NS2A Protein. J. Virol., 2013; 87: 4609-4622.
14. Chowdhary M, Rani A, Parkash J, Shahnaz M, Dev D. Bioinformatics: An Overview for Cancer Research. Journal of Drug Delivery and Therapeutics, 2016; 6(4): 69-72.
15. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. Nucleic Acids Research, 2000; 28(1): 235-242
16. Ferreira LG, Ricardo N, Oliva G, Andricopulo AD. Molecular Docking and Structure-Based Drug Design Strategies. Molecules, 2015; 20: 13384-13421
17. Al-Khaboori SA, Balasubramanian S, Kumar KM. In Silico Drug Designing on Human Protein Kinases Inhibitors. Int. J. Rec. Biotech., 2015; 3(4): 10-17.