

Antiviral activity of Ellagic Acid against envelope proteins from Dengue Virus through *Insilico* Docking

Giridharan Bupesh*

Ramalingam Senthil Raja

Krishnan Saravanamurali

Vijayan Senthil Kumar

Natrajan Saran

Mohan Kumar

Subramanian Vennila

Kaleefathulah Sheriff

Krishnasamy Kaveri*

Palani Gunasekaran*

King Institute of Preventive Medicine & Research, Guindy, Chennai-600 032.

Corresponding Authors:

Giridharan Bupesh

Email: bupeshgiri@gmail.com

Research Scientist

Palani Gunasekaran & Krishnasamy

Kaveri

Email: gunzking@gmail.com

Director & Deputy Director

Abstract:

Arbo viral infection such as dengue, chikungunya, japanese encephalitis, west nile viruses and other flaviviruses have transmembrane envelope proteins. These proteins (glycoproteins) form spike-like projections responsible for virus attachment to target cells and acid-activated membrane fusion. Further it targets numerous serologic reactions and tests including neutralization and hemagglutination inhibition. These viruses showed wide range of antigenic cross reactions and caused by seven antigenic complexes from 30 species, huge subtypes and varieties. This protein is the chief site for most neutralizing epitopes, highly conserved with cross-reactive epitopes. In the present study, the ellagic acid (4,4',5,5',6,6'-Hexahydroxydiphenic acid 2,6,2',6'-dilactone) was evaluated for the antiviral activity through *Insilico* docking against drug target envelope proteins from dengue viruses. Ellagic acid showed good docking score with all the four glycoproteins from dengue 1-4 viruses. Among the glycoprotein receptors the glycoprotein-1 and 4 demonstrates the highest docking score with energy minimization. This highlights that the ellagic acid have potent antiviral activity against the dengue viruses.

Keywords: Ellagic acid, Glycoprotein, Flaviviruses, Dengue virus, *Insilico* docking

Introduction

Dengue is an acute illness caused by dengue virus (DENV) which is transmitted to humans by *Aedes* mosquitoes with hemorrhagic fever. DENV infection results in a spectrum of disease ranging from a mild febrile illness or dengue fever to a severe disease or dengue hemorrhagic fever/dengue shock syndrome. It causes most illness and death in the arboviral family. It has been estimated that 50-100 million dengue infections occur each year in tropical urban areas around the world with 20,000-25,000 deaths (1). The virus possesses an icosahedral nucleocapsid

core surrounded by a host-derived lipid membrane (envelope), in which the envelope E protein and membrane (M) protein are embedded. In this in-silico study, we designed an inhibitor which showed inhibitory activity towards Dengue virus and this study will be a best illustrated model for designing new leads for other flavi virus such as Japanese encephalitis virus, Murray Valley encephalitis virus, Usutu Virus and West Nile Virus. (DENV, JEV, MVEV, USUV, and WNV) envelope proteins. The binding interactions between this inhibitor and NS3 protein were studied by docking methods using Auto dock vina

software. The present study targets the potent ligand that could inhibit polyprotein processing of Flavi virus such as Dengue viruses. Further to understand the interactions between the glycoprotein receptors from Dengue prototype viruses and the lead binding via computational docking methods.

Materials and Methods:

Dengue Receptors:

The Structure of Dengue Receptor proteins were mined from National center for Biotechnology Information. Four different receptors of dengue were screened for the *Insilico* analysis. The Dengue virus-1 glycoprotein structure PDB ID: 1TG8, Dengue 2 Virus Envelope Protein Crystal structure PDB ID: 1OAN, Dengue virus type 3 envelope glycoprotein structure PDB ID: 1UZG, dengue virus type 4 envelope glycoprotein structure PDB ID: 2JSF, were chosen for the *Insilico* antiviral study.

3-Dimension structure of Ellagic acid:

Three dimensional structure of Ellagic acid (4,4',5,5',6,6'-Hexahydroxydiphenic acid 2,6,2',6'-dilactone) was mined from the pubchem compound library. The structure displays the 3-Dimensional entity of Ellagic acid. The file was imported as structure data file and it was converted into protein data file using molecular file convertor.

Molecular property and bioactivity prediction by molinspiration:

The molecular properties were predicted by web based software molinspiration. It is used to predict parameter such as drug likeness, MiLogP and TPSA. MiLogP, is calculated by the methodology developed by molinspiration as a sum of fragment based contributions and correction factors. MiLog P parameter is predicts permeability across the cell membrane. Total polar surface area predicts

the hydrogen bonding potential of compound. The total volume of the compounds was calculated using molinspiration. Number of rotatable bonds predicts the molecular flexibility. This interprets the absorption index and bioavailability of drugs. The molinspiration works through knowledge based networks which can be predicted the molecular properties and structure feature with respect to profiled known drugs by virtual screening in lead optimization.

Computational Methods:

Docking calculations were carried out using Docking server. The docking parameters were set default before the docking (10,11). In the ligand atoms the gasteiger partial, Kollman united charges were added, the rotatable bonds has to be selected and defined. Further the Essential hydrogen atoms, Non polar hydrogen bonds were merged and salvation parameters were implemented for molecular docking using auto Dock tools (9).

Docking calculations were carried out on ELLAGIC ACID with Glycoprotein receptor model. Affinity (grid) maps of xx A grid points and 0.375.A spacing were generated using the Autogrid program (9).

Results:

Resistance of antiviral drugs to flavi virus infection and other multidrug resistance viral infection may fall in the post antibiotic epoch. Screening of novel potent plant pure compounds were less in side effects and have other synergetic health ailments such as antioxidant and healing properties. In the present study the plant pure compound ellagic acid was evaluated for the antidengue viral activity using *Insilico* docking. The lead compound Ellagic acid was chosen as the ligand for the dengue receptor. The Ellagic acid was evaluated for the QSAR studies (Quantitative

structure activity relationship and drug like lines score) using drug likeliness molecular property predictor. It was presented in the figure 1 A & B. The structure of the ellagic acid was submitted in the molinspiration tools using Jmol chemical structure drawer. The Molecular property was found as Molecular formula was found as C₁₄H₈O₈, Molecular weight was 304.02, Number of Hydrogen bond 4 and polar surface area was found as 108.90A². The Molecular volume is appeared as 288.06A³. The stereo centers were obtained as 2. The drug like lines score was found as -0.45. The Bio activity is very essential for recognizing the drug by the *in vivo* drug recognizing and signaling receptors shown in figure 1 B.

The bioactivity parameters such as G-Protein coupled receptor (GPCR), Ion channel modulator, Nuclear receptor and Protease inhibitor is predicted as -0.29, -0.27, 0.11 and -0.18. Where inhibitor modulation for ion channel was predicted as -0.01 shown in the figure 1 C. The docking score profiles were presented in the table 1 and the docking cycles and other parameters were used according to the Murriss good self method.

The docking of ellagic acid with glycoprotein-1, 2, 3 and 4 from prototype dengue viruses were showed in figure 3. The active site of glycoprotein-1 of dengue -1 residues involved in the docking was predicted as C7-Threonine, C8-Aspergine, C13-isoleucine shown in figure 3a. The figure 3b represents the active site residues glycoprotein in dengue virus-2 were predicted as C2-histidine, C2so-Threonine.

The figure 3C displays the activity residues of dock-3 glycoprotein residues in the dengue virus-3 were predicted as Glutamic acid, Histidine and Isoleucine and the dengue-4 Glycoprotein

receptor were predicted as C12-Isoleucine, Threonine, and Histidine in figure 3d. The Docking score and energy parameters were tabulated in the table 1. The score shows 90% of receipting activity in glycoprotein dengue prototype virus -1 Ellagic acid, 80% docking with glycoprotein dengue prototype virus -1 Ellagic acid was found, as 70% of docking activity was found with glycoprotein dengue prototype virus -2 with Ellagic acid and 50% of docking activity was found with glycoprotein dengue prototype virus -3 with Ellagic acid.

Docking of Ellagic acid with DENV prototypes glycoprotein receptor falls within the range of observed values for the experimentally determined structures of similar lengths. The validity of the predicted model of Glycoprotein protein was also verified. On the whole, the best confirmation was achieved through free energy binding (ΔG bind kcal/mol) for the designed ligand. The Binding energy of four DENV glycoprotein with ellagic acid were obtained as -5.9 kcal/mol, -6.0 kcal/mol, -6.7 kcal/mol, -6.6 kcal/mol and -6.6 kcal/mol for respectively. The negative and low value of ΔG bind indicates strong favorable bonds between Glycoprotein- E receptor and the novel ligand Ellagic acid. This indicates that the ligand was in its most favorable conformations. Hydrogen bonds formed in the catalytic site residues on receptor-ligand complex confirms the potent binding and perfect docking on the ligand. Thus the study portrays the best docking and good drug likeliness profiles were achieved by ellagic acid. Therefore the drug ellagic acid binds with DENV receptors and influence the three dimensional entity of receptor for significant inhibition in viral host replication.

Discussion:

Glycoprotein – E protein receptor is one of the key pathways to the flavi viral entries. Flavi virus targets this Glycoprotein-E through receptor and mediates interactions within the virus and the components of the host cell membrane involve virus adsorption. The E protein interacts with the cell membrane during fusion (2) thus it evades and establishes the infection in host. In the present study the Glycoprotein E Receptors of Dengue prototypes from 1-4 were selected as the drug targets. Compounds that interact with sites on the E protein that binds to structures on the cellular membrane affect the adsorption and fusion process that interferes with virus penetration into the cellular cytoplasm and consequently its subsequent replication (3,4). There is no antiviral drug for treatment for any of the Flavivirus and an effective vaccine for human use is not yet available to prevent dengue. Patients of dengue viral infections needed hardly the potent antiviral agents as drugs to prevent the dengue shock syndrome. Compounds obtained from traditional medicinal plants and herbs species have been reported to have antiviral activity and a wide variety of active phytochemicals have been identified (5). Further other bioactivities were also screened through *insilico* analysis, the bioactivities tools calculates the drug solubility, absorption, digestive capacity and metabolic rate at receptor level (6, 9). Molinspiration tools were widely used to predict the molecular properties based on the combinatorial chemistry by virtual screening strategies (7,8).The Molsoft druglikeness score of ellagic acid was predicted as -0.45 and other bioactivity parameters indicates the ellagic acid was a potent lead for the infectious diseases.

The phytal compound ellagic acid have good drug likeliness score than the other polyphenol compounds. Therefore the Ellagic acid was docked with Glycoprotein E Receptors of Dengue-1,2,3 and 4 through the Docking server. Among the 4 prototypes glycoprotein E receptor glycoprotein 1,2 and 4 demonstrates well docking activity , this evidences that the ellagic acid had good receiving activity which posses potent antiviral activity against dengue virus.

Conclusion:

Insilico drug discovery serve a significant role in the development of novel drugs for pharmaceutical and clinical industry. It reduces the duration for finding novel lead solution to many infectious diseases. New drugs will be essential for many drug resistant viral infections in near future. Therefore the present study contemplates towards the same to find a novel drug to dengue infections. Ellagic acid was evaluated for the antidengue activity by *Insilico* docking through glycoprotein E receptors in dengue 1-4 prototype viruses. All the four envelope proteins were well docked with Ellagic acid and reveal the good molecular and bioactivity. Among the four glycoproteins 1uzg and 2jsf receptors demonstrates significant docking profiles and scores in the study. Finally this study concludes that the ellagic acid was a promising drug which has a significant druglikeness for the therapeutic of dengue infection.

Acknowledgements:

The authors would like to express their deep gratitude to ICMR, (Under Grade-1 Viral Diagnostic Laboratory Network (VDL-1) Government of India for funding and King Institute

of Preventive Medicine and Research, Guindy, Tamilnadu, India for providing the adequate

laboratory facilities in the successful completion of this research work.

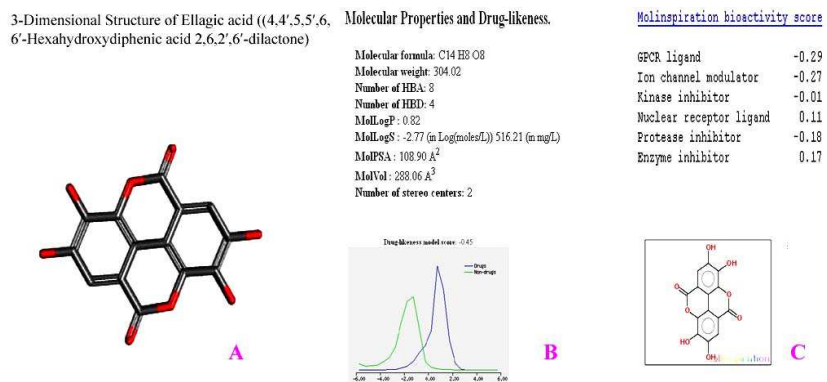


Figure 1

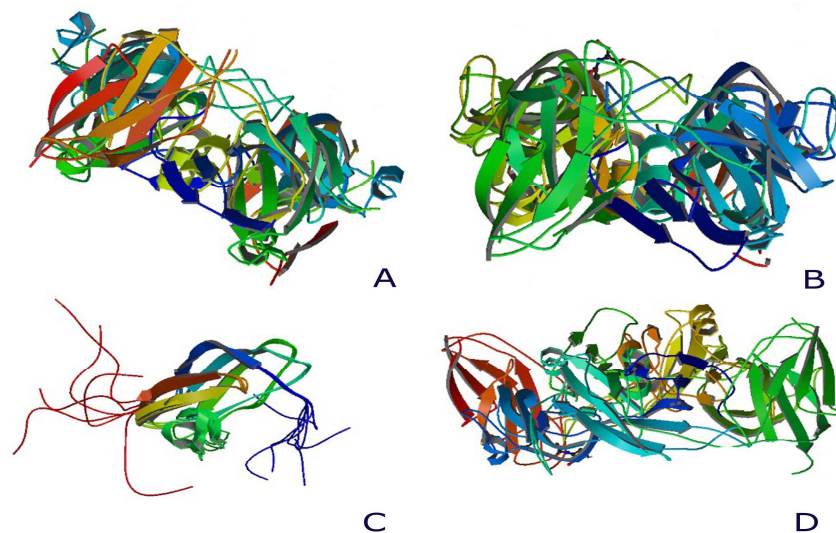


Figure 2

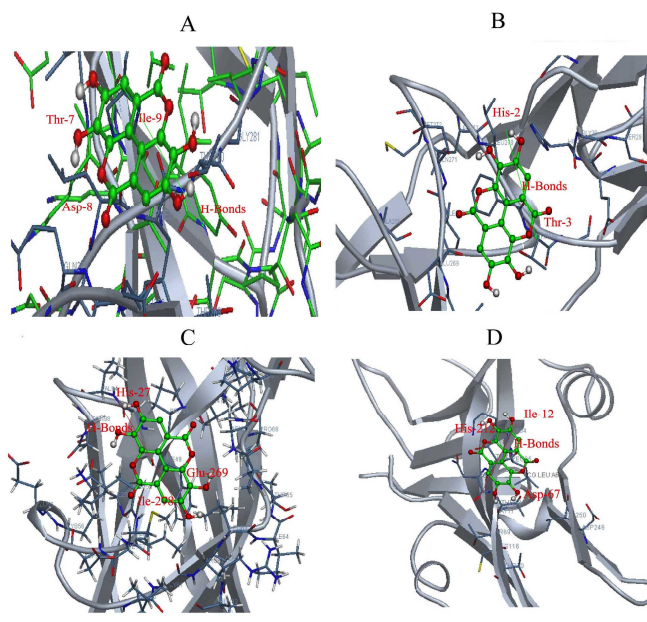


Figure 3

Table 1: *Insilico* Docking of ELLAGIC ACID to Glycoprotein

Rank	Est. Free Energy of Binding	Est. Inhibition Constant (KI)	vdW + Hbond + desolv Energy	Electro-static Energy	Total Intermolecular Energy	Frequency	Interact Surface \pm
ELLAGIC ACID to Glycoprotein 1UZG	-5.04 kcal/mol	202.92 uM	-4.46 kcal/mol	-0.16 kcal/mol	-4.62 kcal/mol	90%	448.845
ELLAGIC ACID to Glycoprotein 1TG8	-3.69 kcal/mol	1.98 mM	-3.53 kcal/mol	-0.05 kcal/mol	-3.58 kcal/mol	80%	323.37
ELLAGIC ACID to Glycoprotein 1OAN	-4.27 kcal/mol	746.94 uM	-4.13 kcal/mol	-0.07 kcal/mol	-4.21 kcal/mol	70%	338.765
ELLAGIC ACID to Glycoprotein 2JSF	-4.34 kcal/mol	657.14 uM	-3.94 kcal/mol	+0.03 kcal/mol	-3.91 kcal/mol	50%	420.909

References :

- Gubler D.J., (2006). Dengue/dengue haemorrhagic fever: history and current status. Novartis Foundation Symposium 277. In G Bock, J Goode, *New treatment strategies for dengue and other flaviviral diseases*. John Wiley, London, p: 3-16.
- Lindenbach B, Rice C, Knipe D, Howley P, (2007). Flaviviridae: In the viruses and their replication. *Fields virology*, 991-1041.
- Leysen P, De Clercq E, Neyts J (2000). Perspective for the treatment of infections with Flaviviridae. *Clin Microb Rev* , 13: 67-82.
- Patkar Ch, Kuhn R, (2006). Development of novel antivirals against flaviviruses. Novartis Foundation symposium 277. In G Bock, J Goode, *New treatment strategies for dengue and other flaviviral diseases*, John Wiley, London, 41-52.
- Jassim S, Naji M, (2003). Novel antiviral agents: a medicinal plant perspective. *J Appl Microb* 95: 317-412.
- Bohm, H. J. G, Schneider H, Kubinyi R., Mannhold, and Timmerman H., (2003). *Virtual Screening For Bioactive Molecules (Methods and Principles in Medicinal Chemistry)*. Wiley-VCH. 8: 9-11
- Li R, Chen X, Gong B, Selzer P. M, Davidson E, Kurzban G, Miller R. E, Nuzum E. O, McKerrow J. H, Fletterick R. J, Gillmor S. A, Craik C. S, Kuntz I. D, Cohen F. E, and Kenyon G. L., (1996). Structure-based design of parasitic protease inhibitors, *Bioorg Med Chem*. 4: 1421-1427.
- Lyne P. D., (2002). Structure-based virtual screening: an overview. *Drug Discov Today*, 7:1047-1055.
- Jain A. N., (2004). Virtual screening in lead discovery and optimization. *Curr Opin Drug Discov Devel*, 7: 396-403.
- Goodsell D.S., Morris G.M., Olson A.J., (1996). Automated docking of flexible ligands: Applications of AutoDock. *J Mol Recognit. PubMed Abstract Publisher Full Text OpenURL*, 9:1-5.
- Bikadi Z, Demko L and Hazai E., (2007). Functional and structural characterization of a protein based on analysis of its hydrogen bonding network by hydrogen bonding plot. *Arch. Biochem. Biophys*, 461: 225-234.
- McDonald I. K., and Thornton J. M., *Satisfying Hydrogen Bonding Potential in Proteins*. *JMB*, 238: 777-793. 1994

Article History: -----

Date of Submission: 16-09-2013

Date of Acceptance: 29-09-2013

Conflict of Interest: NIL

Source of Support: NONE

SJR SCImago
Journal & Country
Rank

Powered by
SCOPUS™