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Identification of bioflavonoid as fusion inhibitor of dengue virus using molecular docking approach

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ABSTRACT

Dengue virus with four distinct serotypes belongs to *Flavivirus*, poses a significant threat to human health and becomes an emerging global problem. Membrane fusion is a central molecular event during viral entry into host cell. To prevent viral infection it is necessary to interrupt the virus replication at an early stage of attachment. Dengue Virus (DENV) envelope protein experiences conformational changes and it causes the virus to fuse with host cell. Hinge region movement of domain I and II in envelope protein facilitates the fusion process. Small molecules that bind in this pocket may have the ability to interrupt the conformational changes that trigger fusion process. We chose different flavonoids (baicalein, fisetin, hesperetin, naringenin/ naringin, quercetin and rutin) that possess anti dengue activity. Molecular docking analysis was done to examine the inhibitory effect of flavonoids against envelope protein of DENV-2. Results manifest quercetin (flavonoid found in *Carica papaya*, apple and even in lemon) as the only *flavone* that can interrupt the fusion process of virus by inhibiting the hinge region movement and by blocking the conformational rearrangement in envelope protein. These novel findings using computational approach are worthwhile and will be a bridge to check the efficacy of compounds using appropriate animal model under *In vivo* studies. This information can be used by new techniques and provides a way to control dengue virus infection.

1. Introduction

A major public health concern worldwide in recent years is a most prevalent mosquito-borne viral pathogen dengue virus (DENV). Presently around the world dengue is endemic in 112 countries [1,2]. Mostly in tropical and subtropical areas, each year 50–100 million individuals are infected with DENV resulting in nearly 500,000 severe life-threatening illnesses and 25,000 deaths, [3,4]. Four distinct serotypes (DENV1-4) of dengue virus are major contributors in circulation of virus in population. The virus is transmitted to humans via the bite of the *Aedes* mosquito, [5].

DENV belongs to the genus *Flavivirus of* family *Flaviviridae* containing single-stranded RNA (positive-sense) genome [6]. A polyprotein precursor is translated by ORF as NH2-C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5-COOH, [7,8]. In a mature virion, C prM and E are structural proteins while other seven are non-structural proteins, [9]. Viral nucleocapsid (RNA genome encapsulated by multiple copies of C protein) is surrounded by a host-cell-derived lipid bilayer (glycoprotein), comprises 180 anchored copies of M and E proteins arranged as 90 homodimers at the surface of mature virion, [10–13]. E (envelope) protein (54.5 kDa, 493–495 amino acid) is a major component of the virion surface plays an important role in binding to the host receptor, assists virus fusion (class II membrane fusion peptides), and induces immunogenic response in host cell, [14]. Three domains folded largely based on β sheets the N-terminus domain one (DI); (central domain), domain two (DII) called as the fusion (or dimerization) domain and domain three (DIII) immunoglobulin (IgG) like domain are reported in E protein, [15].

At neutral pH, E protein of DENV exists as dimeric structure and at the tip of Domain II between domain I and domain III the highly conserved fusion peptide buried in a hydrophobic pocket thus provides a shield from interaction with cellular membranes, [16,17]. Within the acidic environment a drop in pH due to protonation of highly conserved histidine residues triggers major rearrangement (homodimers dissociation to form intermediate monomers) in the E protein, [18–20]. As a result, As a result, the C-terminal fusion protein of E protein exposes and primes the E protein for contact with the host endosomal membrane. During this fusion process, a flexible region between domain I and II

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"hinge region", acts like a spring to bring the fusion peptide in close proximity (insertion) with the host membrane, [17,21]. Upon the transformation of the E monomers into homotrimers the fusion process ends, the anchor of the E protein then pulled back with the fusion peptide-inserted endosome membrane to complete the fusion, [22,23].

Experimental results have indicated that in the absence of the detergent *n*-octyl-β-D-glucoside (β-OG) binding in E protein, a shift in the kl β-hairpin (residues 268–280) occurs that opens the hydrophobic interface allowing the shift of hinge region to promote fusion. kl β-hairpin plays an important and basic structural role in formation of fusion component trimers by initiating the conformational changes at low pH [15]. In the presence of a detergent (any small molecule, while β-OG in this case), kl loop forms a hydrogen bond and a salt bridge with its dimer partner resulting in the closeness of the holes in hydrophobic channel at the interface between domains I and II. In contrast, absence of a detergent shifts the kl loop in such a way that the hydrophobic interface (pocket) opens up. This shift allows domain II to hinge away from its dimer partner projecting the fusion peptide at the tip toward the membrane of the target cell. These conformational changes in the presence and absence of a detergent suggest that the small molecules that bind in the ligand-binding pocket in opened kl hairpin conformation may inhibit the infection in low pH by premature triggering and preventing fusion, [21,24,25].

Naturally plants derived compounds are an important source for the discovery and the development of new antiviral drugs Recently, efforts have been made to find effective antiviral substance among the natural compounds because of their high accessibility and expected low side effects [26,28,29]. Numerous phytochemicals are reported as antiviral compounds against dengue viruses including flavonoids [30–33]. After literature screening, among the reported flavonoids baicalein, fisetin, hesperetin, naringenin/ naringin, quercetin and rutin were selected (Table 1) due to their potential activity against DENV at different stages of viral life cycle but none of them investigated as inhibitory ligand for E protein of dengue.

Antiviral activities have also been described for these flavonoids against many different viruses; [34–36]. Baicalein, quercetin and rutin are flavones. Antiviral activity of baicalein and quercetin is determined previously against DENV, JEV (Japanese encephalitis virus) and herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2); [35,37,38]. Quercetin also was studied for the infectivity and replication of polio-virus type 1, para-influenza virus type 3 (Pf-3), respiratory syncytial virus (RSV), porcine epidemic diarrhea virus and some types of human adenoviruses, [35,36,39–42]. Rutin has been reported as anti DENV recently, [27]. Fisetin, hesperetin and naringin are flavonones. In vitro antiviral activities of hesperetin have been reported against some RNA viruses, [43–45]. Naringenin and hesperetin showed inhibitory activity on NSV (non-specific vaginitis) infection. Antiviral activity of Fisetin and Naringin were reported against DENV as well, [27].

Therefore considering the previous studies about flavonoids and binding pocket of E protein, we are interested to find potential small molecules and lead compounds to interrupt the fusion process at very early stage of interaction with receptor. The small molecules were docked first time in our study in the opened ligand-binding pocket of E Protein of DENV2 through molecular docking approach suggesting that the bioflavonoid compounds inserted at this position have ability to interrupt further conformational changes and hence can inhibit the fusion transition.

2. Materials and methods

2.1. Target selection (model preparation)

2.1.1. Preparation of envelope (E) protein structure

The three-dimensional structure of E Protein of DENV-2 in complex with *n*-octyl-beta-D-glucoside (βOG) was retrieved from the RSC Protein Data Bank [PDB:1OKE]. By removing all crystal water molecules the E Protein structure was prepared for molecular docking processes. Small molecules (BMA, NAG, FUL and βOG) were removed. E Protein and only βOG were saved as two separate pdb files for controlled docking.

2.1.2. Pocket identification

A hydrophobic pocket occupied by a small detergent molecule βOG near the hinge region of E protein led to an interest for designing fusion inhibitors targeting this pocket of E protein (Reference [15, Figs. 1–3]). This pocket is proposed to be "fit-induced"; suggesting that molecules binding in the pocket will result in the steric hindrance to the hinge movement, hence hampering fusion process.

2.2. Ligand selection and preparation

The chemical structure of small molecules (Fig. 1) were retrieved from ZINC and PubChem databases, [46,47]. 3D Pdb structures were obtained by Chimera software, [48]. The ligand molecules were then subjected to energy minimization through PRODRG (an online tool use Gromos 96 force field for energy minimization), [49] Gasteiger charges were added to small molecules through Autodock Tools and Torsdof were set accordingly.

2.3. Molecular docking

AutoDock Vina, [50] was used to perform molecular docking due to its accuracy and its two order faster magnitude speed than AutoDock 4, [51]. AutoDock Tools was utilized to prepare the input pdbqt file for E protein [PDB: 1OKE] and to set the size and the

Table 1
Flavonoids reported as anti DENV in literature with their half maximal inhibitory concentration (IC_{50}).

Flavonoids		Food sources	Inhibitory activity (dengue serotype/cell line)	References
Group	Compound			
Flavones	Rutin	Apple and papaya	No activity (DENV-2/Vero[a])	[43]
	Quercetin	Apple, lemon and papaya	IC_{50}=35.7 μg/mL (DENV-2/ C6/36[b] and Vero monolayer cells)	[27]
	Baicalein	Plant Chinese skullcap, and Indian trumpet flower	IC_{50}=6.46 μg/mL (DENV-2/ C6/36 and Vero)	[37]
Flavonones	Fisetin	Apples, strawberries, and grapes.	IC_{50}=43.12 μg/mL (DENV-2/Vero)	[43]
	Hesperetin	Citrus fruits	No activity (DENV-2/ Vero cells)	[27]
	Naringenin/ Naringin	Grapefruit, oranges and tomato's peel	IC_{50}=52.64 μg/mL (DENV-2/Vero)	[27,43]

[a] Vero (African green monkey kidney) cell line.
[b] C6/36 mosquito cell line derived from Aedes albopictus.