SCIREA Journal of Chemistry

http://www.scirea.org/journal/Chemistry

May 15, 2023

Volume 8, Issue 2, April 2023

https://doi.org/10.54647/chemistry150313

Analysis of molecular interactions between flavones and dengue DENV E – 3 protein by *In silico* approach

Cecilia Espíndola[1]

[1]Department of Physical Chemistry, University of Seville, C / Profesor García González 1, 41012 Seville, Spain.

Abstracts

The DENV dengue virus belongs to the Flaviviridae family, a group of four serotypes that circulate freely in the different endemic regions causing dengue disease. This disease is transmitted by the female *Aedes aegypti* and *Aedes albopictus* mosquitoes. Due to the variety of serotypes and genotypes existing in the same zone, it has been difficult to develop a vaccine due to the complexity of the immune response against dengue disease. Flavones such as *7,8-dihydroxyflavone*-tropoflavin, *5,6,7-trihydroxyflavone*-baicalein, and *3',4',5,6-tetrahydroxyflavone*-luteolin show antiviral activity. The different types of H-Bonds, π-π stacking, and π-cation molecular interactions that occur in the tropoflavin, baicalein, and luteolin, and DENV E-3 protein complexes has been analyzed, and different $K_{binding}$ has been identified. Similarly, the linkages between the different flavone and DENV E-3 protein domains for the application of the flavones tropoflavin, baicalein, and luteolin as anti-DENV E-3 agents has been analyzed. Results presented in this study could be useful for compound design and antiviral studies.

Keywords: Antiviral pharmacology, Flavones, drug interactions, non-covalent interaction, DENV E-3 protein, Docking molecular, protein interaction.

Introduction

Dengue disease is transmitted by the female *Aedes aegypti* and *Aedes albopictus* mosquitoes. Dengue is a viral infection caused by the DENV virus which is transmitted by the bite of this mosquito and causes severe clinical conditions such as fever and circulates in the bloodstream leading to hemorrhage, hypovolemic shock, and even death. It is distributed in tropical and subtropical countries in all regions of the world (1). With epidemiology corresponding to 284 - 528 million infections annually (2).

The dengue virus DENV belongs to the Flaviviridae family, a group of four serotypes DENV 1, DENV 2, DENV 3, and DENV 4, which circulate freely in the different endemic areas causing dengue disease. DENV serotype 3 was first reported in 1953 in Asia (Philippines and Thailand), in 1963 in the Americas (Puerto Rico), and in 1984-1985 in Africa (Mozambique) (3).

The different serotypes share approximately 65% similarity in amino acid sequences. In turn, each serotype includes several genotypes. DENV E serotype 3 includes four genotypes: Genotype I include strains from Indonesia, Malaysia, Philippines, and South Pacific Islands; genotype II includes strains from Thailand, Vietnam, and Bangladesh; genotype III with strains from Sri Lanka, India, Africa, Samoa and Thailand and genotype IV includes strains from Puerto Rico, Latin America, Central America, and Tahiti (4).

DENV has a monocatenary linear RNA encoding structural proteins: Capsid C, pre-membrane and membrane (prM/M), and envelope-E, and seven non-structural proteins such as NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. The E protein is composed of domains I, II, and III of which domain III is responsible for binding to the receptor. The E protein is the only protein that allows virus particles to bind to host cell receptors and is responsible for virulence, tropism, and host range. It has been shown that the E protein can agglutinate red blood cells (2). Some molecules such as ICAM non-integrins, 2CD209, Rab 5, GRP 78, and mannose receptors present on the cell surface can interact with the DENV E protein (5).

The E protein of dengue virus DENV E can adopt either dimer or trimer conformations. The dimer is found in the mature virion while the trimer is found in the immature state of the

virion in the endosome of the host cell. Two conserved histidine residues in glycoprotein E can act as pH sensors, residues H209 and H7 (2,5). The dengue virus E protein DENV E-3 contains two conserved N-glycosylation sites at residues N67 and N153, which are associated with cell attachment and virus entry (6).

Numerous investigations have studied the activity of flavonoids mainly as antioxidants (7, 8, 9, 10) in coronary heart disease, anti-inflammatory, vascular activity, anticancer, antimicrobial, and neuroprotective, among others (11). In addition to the above pharmacological activities, flavones such as **1** *7,8-dihydroxyflavone*, **2** *5,6,7-trihydroxyflavone*, and **3** *3',4',5,6-tetrahydroxyflavone* (figure 1) present antiviral activity (12, 13, 14).

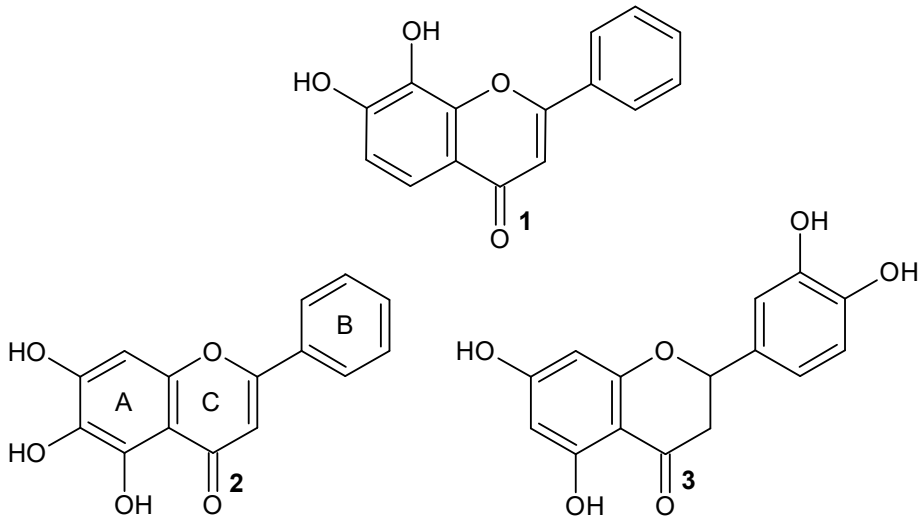


Figure 1. 1. ***7,8-dihydroxyflavone,*** **2.** ***5,6,7-trihydroxyflavone.*** **3.** ***3',4',5,6-tetrahydroxyflavone.***

It has been demonstrated that tropoflavin has anti-enterovirus EV71 activity at a concentration of 50 Mm. It inhibits 40% of viral IRES-internal ribosome entry site (IRES) activity by interfering with virus replication (15,16).

In turn, Baicalein has antiviral effects against the replication of the Chikungunya virus (CHIKV), transmitted by a mosquito that causes incapacitating arthritis in infected individuals. It is considered that the compound exerts its antiviral action in the first hours of treatment and in the early stages of infection, decreasing the production of CHIKV virus proteins at concentrations of 100 μ/ml (17).

Zandi et al., 2012 (13) evaluated the properties of the flavone Baicalein as an anti-dengue type 2 agent. They found that Baicalein has antiviral activity at all stages of DENV 2 virus replication in both the adsorbed and intracellular replication states of the virus. Similarly, Baicalein exhibits strong direct intivirucidal action. They concluded that this potent antiviral

activity of Baicalein against DENV 2 virus may be due to its ability of Baicalein to bind and/or inactivate structural and nonstructural proteins of DENV 2 virus.

Peng et al., 2017 (14), evaluated the antiviral effect of 3',4',5,7-tetrahydoxyflavone-luteolin on various serotypes of DENV E. They observed that luteolin can obstruct the late stages of the DENV E viral cycle in infected cells by inhibiting the host furin convertase proprotein.

Flavonoids are compounds that have the general structure of a 15-carbon skeleton, consisting of two phenyl rings and a heterocyclic ring. In these compounds, the hydrogen atoms have electropositive charge properties, while the charge properties of the oxygen atoms of the heterocyclic ring and the keto and hydroxyl groups are electronegative. Both hydrogen and oxygen atoms can participate in the formation of hydrogen bonds (17).

H-bond interactions are the most important interactions in biological recognition processes (18). Molecular interactions between hydroxyl and carbonyl groups, ester and ether (19), NH and carbonyl groups (20), H-bond interaction and aromatic rings (21) have been studied, and H-bond energies have been predicted (22).

The molecular docking studies provide the identification of a ligand, its binding site to the protein and its most energetically favorable binding mode. With Autodock the binding free energy, ligand efficiency, inhibition constant (μM) is obtained. The binding energy is the sum of intermolecular energy, total internal energy (desolvation energy, van der Waals and electrostatic energy), torsional energy and unbound energy. In the best binding mode, the binding energy (lowest), the inhibition constant (lowest) and the highest number of H-bond interactions with the active site of the protein are considered.

For the free energy function, an approximation is implemented using the thermodynamic cycle of Wesson and Eisenberg. The free energy function is expressed as

$$\Delta G = \Delta G_{vdW} \sum_{i,j} \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} \right) + \Delta G_{hbond} \sum_{i,j} E(t) \left(\frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}} + E_{hbond} \right) + \Delta G_{elec} \sum_{i,j} \frac{q_i q_j}{\varepsilon(r_{ij}) r_{ij}} + \Delta G_{tor} N_{tor} + \Delta G_{solv} \sum_{i_c j} S_i V_j e^{(-r_{ij}^2/2\sigma^2)}$$

where ΔG is the free energy to be minimized, ΔG_{vdw} is the energy of the typical molecular mechanical terms for dispersion and friction; ΔG_{hbond} is the energy contributed by the hydrogen bonds; ΔG_{elec} is the electrostatic energy, ΔG_{tor} models the constraint of the internal rotations of the overall conformation, and ΔG_{sol} represents the models that explain the hydrophobic effect of the already docked structure (23).

In turn, AutoDock Vina automatically predicts the interaction between ligands and biomacromolecules. It improves the precision of conformational predictions, calculates energy maps and clusters the results. It utilizes a variant of X-score (24) and a scoring function that considers inter- and intramolecular contributions.

The scoring function for Vina is defined as:

$$C = \sum_{i<j} f_{t_i t_j}(r_{ij})$$

where the summation is performed over all pairs of atoms that can move with each other, excluding atoms separated by up to three consecutive covalent bonds, t_i is the type of index atom i, r_{ij} is the interatomic distance and the interaction function is defined as $ft_it_j(r_{ij}) \equiv ht_it_j(dij)$ where $d_{ij} = r_{ij} - Rt_i - Rt_j$. Rt_j is the van der Waals radius for the atom of type t and is expressed in Å.

Some studies on molecular docking were carried out carried to evaluate the interaction of flavonoids and predict their binding site on the surface of the DENV E protein. Were utilized groups of various flavonoids to determine the binding sites with the DENV E protein. Molecular docking with DENV 2, DENV 3, TBEV, and JEV protein and sequence alignment studies of DENV E 2-My proteins were performed. The flavonoids utilized were baicalein, baicalin, fisetin, flavone, glabranin, hyperoside, ladanein and quercetin. It was determined that the E protein of DENV E 2-My has 82% similarity to the DENV E-3 protein (25).

We have carried out studies of the molecular interactions between flavones such as tropoflavin, baicalein, and luteolin and the DENV E -3 protein as antiviral agents to identify the binding sites and modes of binding of these flavones to the DENV E-3 protein via a computational approach. Results presented in this study could be useful for compound design and antiviral studies.

Materials and Methods

DENV E-3 protein was obtained from Protein Data Bank (PDB) crystal structure code 1UZG (6). The ligands utilized were **1** *7,8-dihydroxyflavone*-Tropoflavin PubChem ID 1880; **2** *5,6,7-trihydroxyflavone*-Baicalein PubChem ID 5281605 and **3** *3',4',5,7- tetrahydroxyflavone*-Luteolin PubChem ID 5280445.

Molecular Docking

Molecular docking studies were carried out utilizing the Autodock -ADT tools to prepare input files for both the DENV E - 3 protein and the ligands **1**,**2** and **3** (phase I in figure 2). Molecular docking was carried out with AutoDock 4.0 (26) and AutoDock Vina 4.0 (27). To prepare the ligands **1**, **2**, and **3**, the partial atomic charges were added according to the Gaisteger-Marsili model, which is a semi-empirical model, and non-polar hydrogen atoms were fused. All torsions were allowed to rotate during the docking process. Autogrid 4.0 software was utilized to generate the energy maps. Initially, blind docking was performed on the whole protein structure to find the possible binding sites of the ligands **1**,**2,** and **3** with the DENV E-3 protein. A second molecular docking was then performed according to the analysis of the binding energies of the protein-ligand complex (phase II). Analysis of the interactions of each tropoflavin, baicalein, and luteolin—DENV E-3 complex was supported on the binding energy score of the H-bond, π-cation, and π-π stacking interactions and the orientation of the compound docked to the binding site (phase III) (Figure 2).

To prepare the DENV E-3 protein, water molecules, additional ligands, and metals were removed; the protonation states, the orientation of the oxygens on the OH-containing residues, and the histidine tautomers were established. Information on the partial atomic charges of the individual atoms was added since some of the most important interactions in the ligand-protein interaction are dipole-dipole interactions and electrostatic interactions. It was determined that all atoms of the DENV E-3 protein are bonded, it contains 4698 non-polar hydrogens, i.e., bonded to carbons, and fusion of the non-polar hydrogens and carbon atoms was realized as the interactions occurring are van der Waals interactions and London dispersion. The blind docking between tropoflavin, baicalein, and luteolin with the DENVE-3 protein was carried for both autodock and vina interactions. The grid box for protein E was size X =88, Y = 74, Z =145, and grid center x = 11.939, y = -2.392, z = 1.398. DENV E-3 protein grid box was carefully constructed to ensure that the entire protein was included inside the box. The grid small with the lowest energy result for each flavone was realized with a grid size X = 58, Y= 52, Z= 60, and grid center x = -5.394, y = 3.561, z = 12.501 (5).

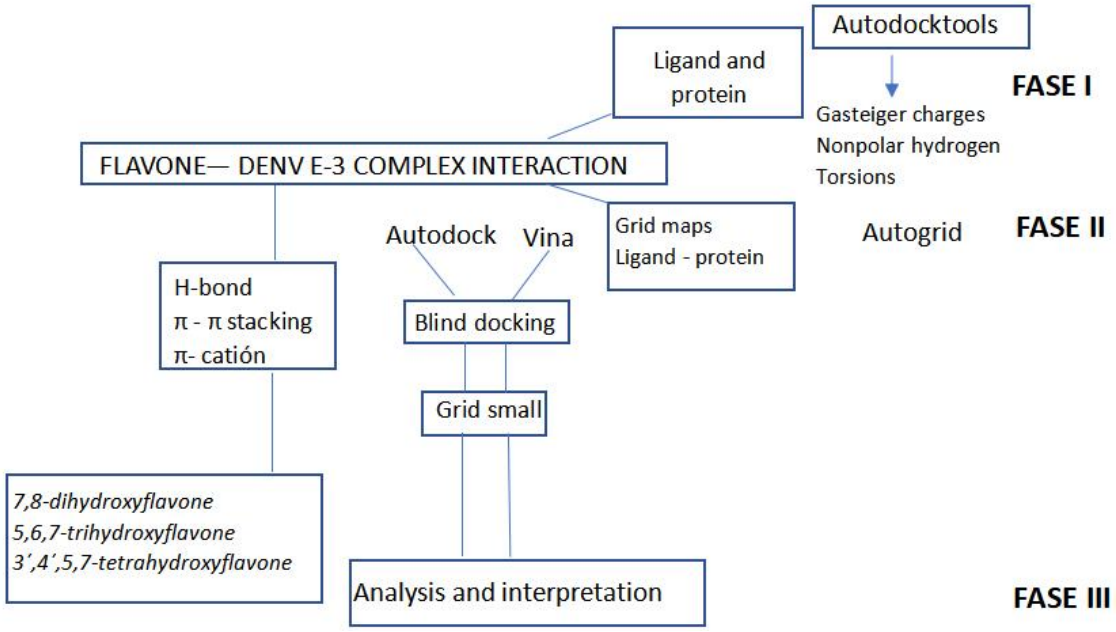


Figure 2. Scheme for molecular docking phases in Flavone - DENVE -3 complex interaction.

Results and Discussion

DENV E-3 protein crystal structure 1UZG code is a dimer with a molecular weight of 88.72 kDa. Each monomer consists of three domains. Domain I, consist of an eight-stranded roll, domain II consists of 12 β-strands and two α-helices and domain III has 10 β-strands (Figure 3) as described by Modis *et al*., 2005 (6).

In the H-bond interaction between N353—tropoflavin, the amino group of the residue donates H to the *O*-pyran of the C-ring of tropoflavin with a distance of 2.37 Å; the 7-OH group of the A-ring donates H to the keto group of A35 with a distance of 1.96 Å, while the amino group of L349 donates H to the *4-oxo* group of the C-ring of tropoflavin with a distance of 2.38 Å.

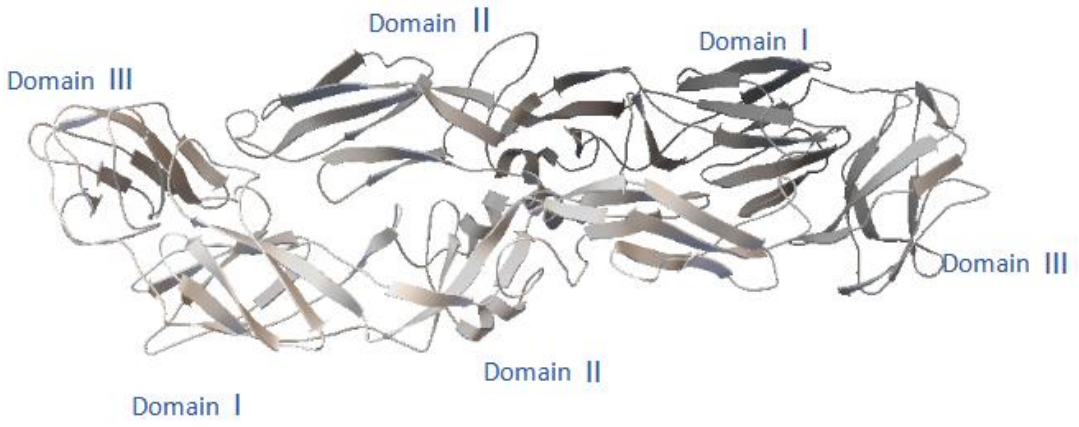


Figure 3. DENV E-3 protein structure.

A π-cation interaction occurs between the ε-amino group (-CH_2-NH_3^+) of K36 and the B-ring of tropoflavin with an ΔG=-7.5 kcal/mol and distance of 5.68 Å (Table 1). At ΔG=-7.1 kcal/mol value the π - π stacking and H-bond interactions between W229—baicalein and the H-bond interaction between Q120—baicalein occurred. The π - π stacking interaction occurred between the pyrrole ring of W229 and the B-ring of baicalein with 5.29 Å. Similarly, W229 presented an H-bond interaction with the *O*-pyran of the C-ring of baicalein with 1.75 Å in which it is the NH of the pyrrole ring that donates the H. In turn, Q120 presents H-bond interactions with the *4-oxo* group of the C ring of baicalein with 2.56 Å and with the 5-OH group of the A ring with 1.98 Å, being the NH_2 of the residue that donates the H to baicalein.

Table 1. Flavone—DENV E-3 interactions

Flavone	Flavone—DENV E-3	Interaction	ΔG (kcal/mol)
Tropoflavin	N353, L349, A35	H-bond	-7.5
	K36	π - cation	
Baicalein	W229, Q120	H-bond	-7.1
	W229	π- π stacking	
Luteolin	R348, F335, K38	H-bond	-8.0

H-bond interactions

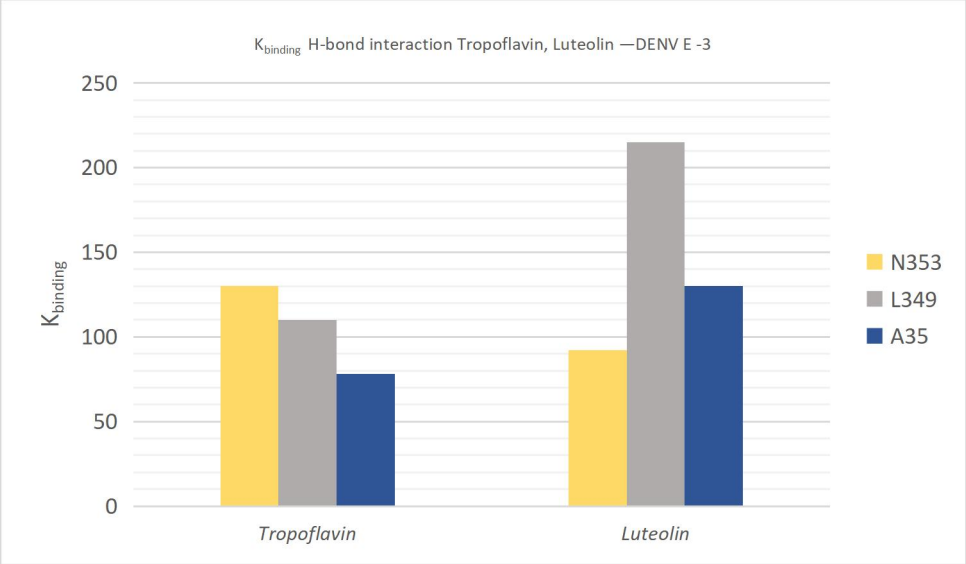
Both strength and weakness of an H-bond are influenced by the presence of neighboring acceptor and donor groups. Generally, H-bonds are not isolated interactions. There are few cases where the phenyl rings from the ligand accept H-bond bonds from the NH groups of protein amides and it is unlikely that these interactions are the main cause of affinity energy gains (16).

For the interaction between luteolin—DENV E-3, at ΔG=-8.0 kcal/mol, H-bond interactions between R348 and the *O*-pyran of the C-ring of luteolin and between R348 and 3'-OH of the B-ring were presented (Table 1). In the first interaction, it is the NH_2 of the guanidinium ion from R348 that donates the H to the *O*-pyran at 2.49 Å and in the second interaction, it is the protonated guanidinium ion (-C-$(NH_2)_2^+$) that donates the H to the 3'-OH of the B-ring of luteolin at a distance of 2.27 Å. H-bond also occurred between F335 and 4'-OH of the B-ring of luteolin which donates H to the keto group of F335 at a distance of 2.27 Å and between K38 and 5-OH which donates H to the keto group of K38 at a distance of 2.12 Å.

The $K_{binding}$ was obtained from the equation

$\Delta = - \quad (\quad b)$

for the H-bond interactions. The $K_{binding}$ value for the H-bond between residue N353 and tropoflavin was approximately 130 while the $K_{binding}$ obtained for the H-bond interaction between residue L349 and 7,8-DHF was approximately 120 (Graphic 1). It was observed that residues N353, L349, and A35 exhibit H-bond interaction with both tropoflavin and luteolin.



Graphic 1. $K_{binding}$ for H-bond interactions between N353, L349, A35—tropoflavin, and luteolin.

Table 2 evidence the binding modes of both flavones with N353, L349, and A35 having H-bond interaction and the corresponding binding energies.

Table 2. H-bond interaction Tropoflavin, Luteolin — DENV E-3.

Residue	Flavone	H-Bond donor/acceptor	ΔG (kcal/mol)
N353	Tropoflavin	7,8-DHF:O—N353:NH_2	-7.5
		7,8-DHF:O—N353:NH_2	-6.8
		7,8-DHF:8-OH—N353:NH_2	-6.7
	Luteolin	Luteolin:O—N353:NH_2	-7.2
		Luteolin:4′-OH—N353:NH_2	-6.4
L349	Tropoflavin	7,8-DHF:4=O—L349:NH_2	-7.5
		7,8-DHF:4=O—L349:NH_2	-7.0
		7,8-DHF:4=O—L349:NH_2	-6.7
		7,8-DHF:4=O—L349:NH_2	-6.4
	Luteolin	Luteolin:7-OH—L349:O=	-7.7

		Luteolin:7-OH—L349:O=	-7.0
A35	Tropoflavin	7,8-DHF:8-OH—A35:3=O	-6.7
	Luteolin	Luteolin:5-OH—A35:3=O	-7.0

Analyzing the $K_{binding}$ obtained for the H-bond interactions between N353, L349, and A35 that occur with both tropoflavin and luteolin, it was observed that tropoflavin has the highest $K_{binding}$ 130 in the interaction with N353, while for luteolin the highest $K_{binding}$ occurred with L349. In the same way, the $K_{binding}$ for A35 is higher in luteolin than in tropoflavin as depicted in graphic 1.

For N353 the NH_2 —*O*-pyran interaction has the same binding energy value = -7.2 kcal/mol for the interactions with tropoflavin and with luteolin. However, for R348 this same NH_2 —*O*- pyran interaction has a binding energy = -7.63 kcal/mol for coupling with luteolin and -6.6 kcal/mol for coupling with tropoflavin (Figure 3).

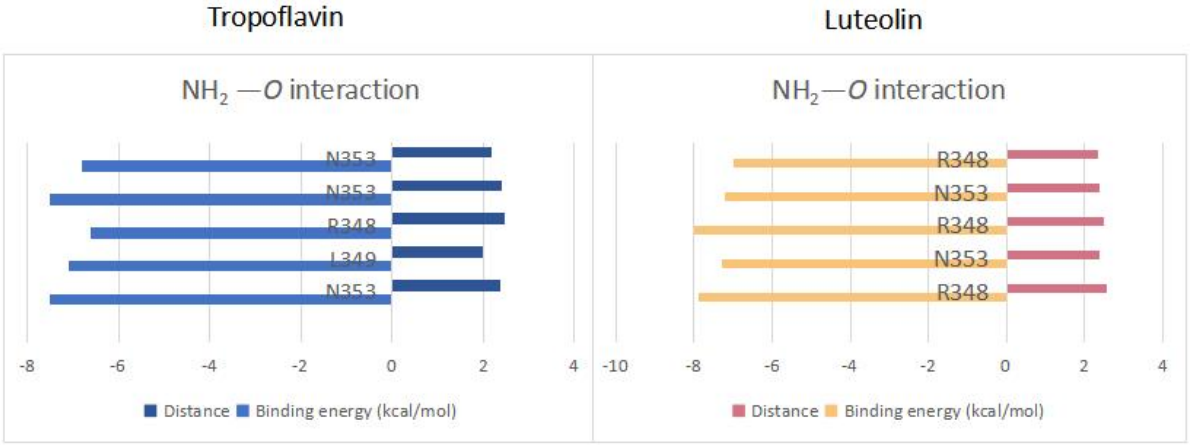


Figure 3. NH_2 —*O* bond binding energies for Tropoflavin, and Luteolin—DENV E-3 interaction.

The domain III of the DENV E -3 protein (Figure 3) is responsible for the host cell receptor binding site. The presence of the N residue in this domain is directly or indirectly related to receptor binding (6). So, focusing on the study of DENV E protein to find inhibitors of virus entry is one of the suitable strategies to inhibit the virus (25). Our results agree with the results obtained by other authors.

In addition to the H-bond interactions presented in Table 2, some of which are found in domain III of the DENV E-3 protein, and corresponding to the lowest energy values, flavones **1**,**2** and **3** also exhibit interactions with N353 and R348 also found in domain III of the DENV E-3 protein at different ΔG values (Tables 3 and 5).

Table 3. H-bond flavones—Asn353 interaction

Residue	Flavone	H-Bond donor/acceptor	Ki μM	ΔG (kcal/mol)
N353	Tropoflavin	Tropoflavin:4=O—N353:NH_2	148.26	-5.22
	Baicalein	Baicalein:O—N353:NH_2	319.09	-4.77
	Luteolin	Luteolin:O—N353:NH_2	255.65	-4.9

Both Baicalein and Luteolin exhibit the same binding mode with N353 with $K_{binding}$ = 3.7 and Ki 319.09 and 255.65 μM respectively. In this interaction, it is the NH_2 group of N353 that donates the H to the *O*-pyran of the C-ring of both Baicalein and luteolin (Table 3 and Figure 4B and C). While with a higher $K_{binding}$ 6.2 it is the NH_2 group of N353 that donates the H to the *4-oxo* group of the C-ring of Tropoflavin with a Ki 148.26 μM (Table 3 and Figure 4A).

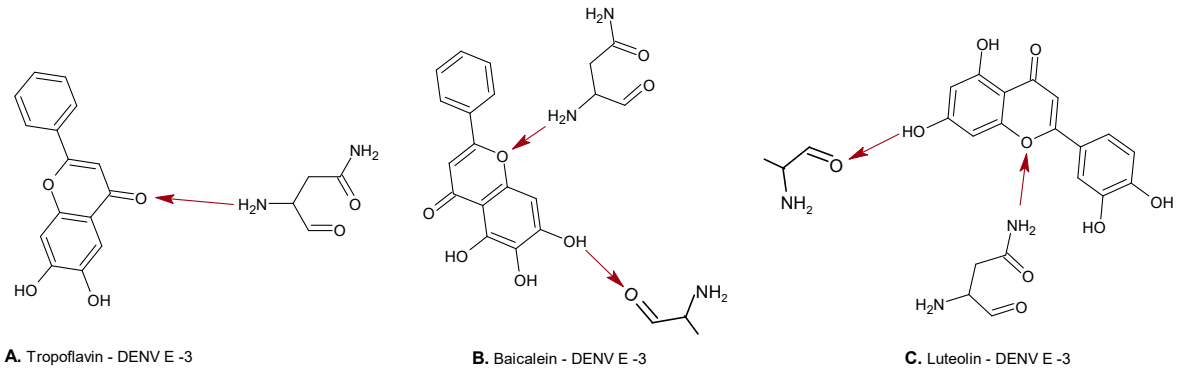


Figure 4. Flavone — DENV E-3 H-bond interaction

The H-bond N353 interaction with *O*-pyran of the C-ring of baicalein has a distance 2.30 Å and bond angle 90.57, while the same interaction with luteolin has 2.48 Å and bond angle of 133.81. The interaction of this residue with the *4-oxo* group of the C-ring of tropoflavin has 2.44 Å and an angle of 95.11.

In the alignment of the interaction of the three flavones with the DENV E-3 protein (Figure 5) it is observed that baicalein and luteolin have the same binding mode, while tropoflavin has a different binding mode.

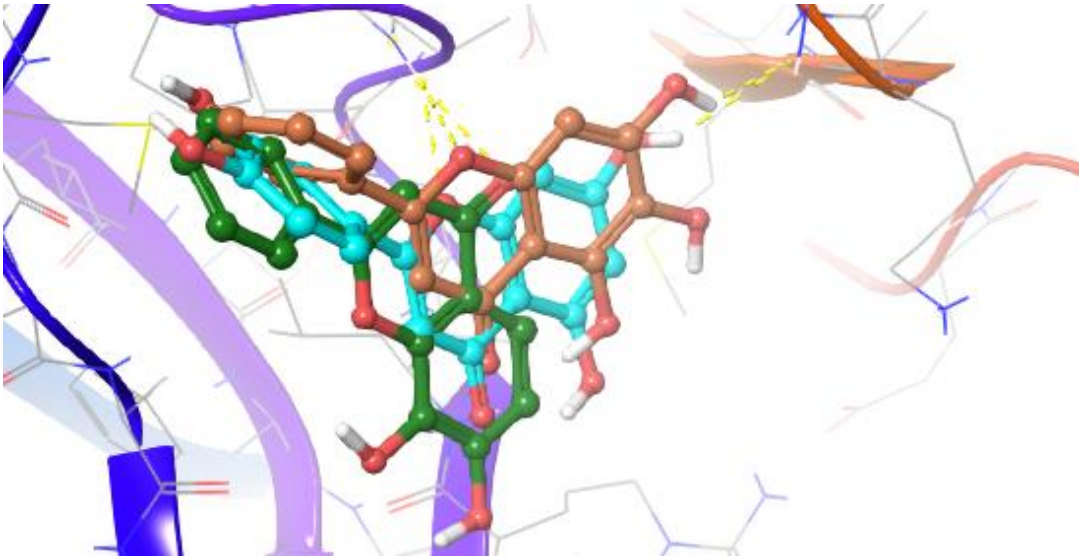


Figure 5. 3D-alignment of Tropoflavin-green ΔG=-5.22 kcal/mol, Baicalein-brown ΔG=-5.02 kcal/mol and Luteolin-blue ΔG=-4.9 kcal/mol, with N353 and A35 interaction.

With a ΔG =-5.02 kcal/mol baicalein presented H-bond interaction with A35 in which the 7-OH group of baicalein donates H to the keto group of A35 (Figure 4B, Table 4, and Figure 5).

Tabla 4. H-bond flavone ⎯Ala35 interactions

Residue	Flavone	H-Bond donor/acceptor	Distance (Å)	ΔG (kcal/mol)
A35	Baicalein	Baicalein:7-OH—A35:O=	2.09	-5.02
	Luteolin	Luteolin:7-OH⎯A35:O=	2.76	-4.9

The same binding mode is presented by A35 with the 7-OH group of luteolin but at ΔG=-4.9 kcal/mol, with distance different (Figure 4C, Table 4, and Figure 5). It is important to mention that A35⎯baicalein interaction was realized with Ki of 209.67 μM while with luteolin Ki was 255.65 μM.

Respect the flavone-R348 interaction which is found in domain III of the DENV E-3 protein (Table 1), R348 also interacts with these flavones at different lower energy values (Table 5). In this is NH_2 of the guanidinium ion from R348 that donates the H to the 8-OH of tropoflavin with a ΔG=-7.0 kcal/mol and distance 2.42 Å, while in the interaction with baicalein and luteolin this same group donates the H to the *O*-pyran of the C-ring with distances of 2.49 and 2.57 Å, respectively (Figure 6).

Table 5. H-bond flavone—R348 interaction

Residue	Flavone	H-Bond donor/acceptor	ΔG (kcal/mol)
R348	Tropoflavin	Tropoflavin:8-OH—R348:NH_2	-7.0
	Baicalein	Baicalein:O—R348:NH_2	-7.3
	Luteolin	Luteolin:O—R348:NH_2	-7.9
		Luteolin:O—R248:NH_2^+	

With ΔG = -7.0 kcal/mol R438 interacts with 8-OH of tropoflavin via an H-bond at 2.42 Å and bond angle of 109.35 (Table 5 and Figure 6). The bond angles of NH_2 interaction from R348 with *O*-pyran from ring C of both baicalein and luteolin are 103.00 and 126.19 respectively. For its part, the 3'-OH group of luteolin receives the H from the protonated NH_2^+ of R348 guanidinium ion with 2.18 Å, a bond angle of 98.870 and an ΔG=-7.9 (Table 5 and Figure 6).

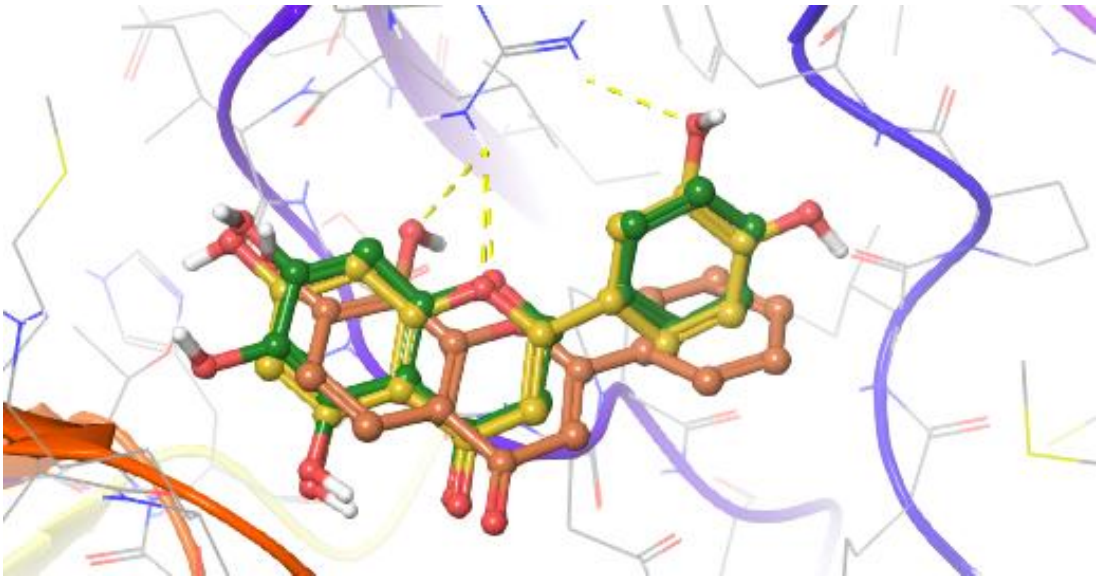


Figure 6. 3D-alignment of tropoflavin-brown ΔG=-7.0 kcal/mol, baicalein-green ΔG=-7.3 kcal/mol and luteolin-yellow ΔG=-7.9 kcal/mol with R348 interaction.

In the above alignment where the flavone-R348 interaction is present, it is observed that tropoflavin, baicalein, and luteolin have the same binding mode, and were found with the lowest energy values.

Previous studies on flavonoid interactions with DENV E-3 protein indicate that the binding site of some flavonoids is on residues of domain I of chain B (residues 4-9, 151-154) and

domain II of chain A (residues 98-103, 244-247), which includes the conserved region of the fusion peptide. Similarly, it has been found that the region where the fusion peptide is formed (residues 98-108) is a highly conserved region among flaviviruses. Although the DENV E-3 protein has a high similarity to the DENV E-2 protein, the flavonoids that interact with the DENV E-3 protein present very different results from those of the other E proteins evaluated. Baicalein, presented interaction in a different chain than the other flavonoids evaluated for interaction with DENV E-3. The explanation for this different response resides in the different orientations presented by domains I and II of the crystallized DENV E-3 protein (1UZG.pdb) (25).

In our study carried out with vina on the molecular interactions of the baicalein—DENV E-3 complex, at a value of ΔG=-6.4 kcal/mol a π-cation interaction between the baicalein A-ring and the alkylammonium ion (-CH2-$NH3^+$) of KA:244 occurred at 3.72 Å. With this same ΔG value, an H-bond interaction with GB:28 is presented where it is the NH2 group of GlyB:28 that donates the H to the 7-OH group of baicalein at 2.37 Å. Similarly, an H-bond interaction is presented with EB:44 at 1.82 Å where it is the 6-OH that donates the H to the keto group of EB:44 at the same ΔG value. The interaction of baicalein with the KA:244 residue is found in domain II of the A chain of the DENV E-3 protein coinciding with the baicalein binding sites with the DENV E-2 protein reported (25).

With autodock, at an ΔG = -3.3 kcal/mol an H-bond interaction with RA99 and 2.45 Å was presented coinciding with the flavonoid binding site reported in domain II of chain A, a region in which the fusion peptide is included. In this interaction, it is the NH_2 group of RA:99 that donates the H to the *4-oxo* group of baicalein (Table 6).

Table 6. H-bond interactions between flavones—DENV E-3 at flavonoid binding sites.

Flavone	ΔG (Kcal/mol)	H-bond interaction with DENVE-3	Residue/flavone interaction functional groups	Distance (Å)
Tropoflavin	-7,0	G:152	C=O—8-OH	2.37
	-4.49	KA:245	NH_3^+—7-OH	2.42
Baicalein	-3.3	RA:99	NH_2—C=O	2.45
Luteolin	-5.19	KA:245	C=O—3′-OH	2.45

We also observed that tropoflavin presents H-bond interaction with KA:245 at an ΔG =-4.49 kcal/mol (Table 6), coinciding with residues of chain A of domain II reported by Ismail and Jusoh, 2017 (25). This interaction occurs between the alkylammonium ion ($-CH_2-NH_3^+$) of KA:245 and the 7-OH group of tropoflavin at 2.42 Å.

At a ΔG=-7.0 kcal/mol, tropoflavin presented an H-bond interaction with G:152. In this interaction, the 8-OH group of tropoflavin donates H to the keto group of G:152 at a distance of 2.37Å. At this same energy value, a π-π stacking interaction occurs between the C-ring of tropoflavin and the guanidinium ion of RB:99 at 5.36Å. Similarly, an H-bond interaction between the 7-OH group of tropoflavin and the keto group of RA:2 was also presented. In turn, luteolin also interacts with KA:245 at an ΔG =-5.19 kcal/mol and 2.45 Å (Table 6).

It is important to highlight that tropoflavin in interaction with G:152 presents N153 as a close contact. Dengue virus protein E has two conserved N-glycosylation sites. The N-glycosylation site at N153 is conserved in most flaviviruses, while N67 appears in only a few. Both glycans are involved in host cell attachment and virus entry (28, 29, 30, 31, 32, 6, 5, 25).

The side chains of N are electrically neutral; however, it is a very polar amino acid and is frequently found on the surface of proteins. The polar amide groups of N also form hydrogen bonds with side chains of other amino acids (33). Mondote *et al*., 2007 (34) studied the function of N-glycosylation in the different stages of DENV viral replication and found that the glycan at position N153 slightly increases viral particle production in the virus, but dramatically increases viral infectivity in mammalian host cells.

The π-cation interaction between baicalein and KA:244 has tight contact with residues: IB:276, TB:274, KB:47, QB:46, IB:140, RB:2, EB:154, EB:44, GB:28, HA:242, at an ΔG=-6.4kcal/mol.

π -π stacking interaction

Hydrophobic interactions mainly that of the aryl rings of amino acids such as W, F, and H are common in proteins and generally expose their aromatic side chain to the binding site. Thus, the shape and electronic properties of the aromatic rings give rise to high polarizability and a significant quadrupolar moment. This leads to various interaction geometries between two π-systems, mainly edge-to-face T-shape and stacking arrangement. The arrangement of the hetero aromatic rings for the stacking interaction is influenced by the alignment of the partial positive and negative charges and molecular dipoles. The edge-to-edge aliphatic-aromatic and aromatic-aromatic interactions have similar stabilization levels. However, an increase in the

interaction energy in T-shaped aliphatic-aromatic interaction has been observed by increasing the acidity of the interacting CH unit (18).

The side chains of Y, F, and mainly W interact most frequently with polarized CH groups as donors (35,36). Therefore, π -π stacking interaction also occurs in the molecular interaction of baicalein—DENV E-3 with Y96, F212, and W210 (Table 7).

For its part, luteolin also presents π -π stacking type interaction, with H27, H242, H259, and tropoflavin presenting this interaction with HB:242 (Table 7). It is important to highlight that the interactions of luteolin with histidines are found in domains I and II of the DENV E-3 protein, and in these domains, the presence of histidines detects pH changes causing a molecular rearrangement involving them (5).

The π -π stacking Edge to face interactions, are not limited to hetero aromatic rings, as they also occur with the guanidinium-carboxylate pairs of R, E, or N side chains, or with the π faces of amide bonds. These motifs require H-bond interactions within the π-plane but are polar and highly polarizable in the perpendicular direction (16). In the realized luteolin—DENV E-3 interaction, π -π stacking interaction between the guanidinium ion from R348—ring A of luteolin was presented with ΔG=-7.0 kcal/mol and at 5.48 Å. At 4.88 Å and ΔG=-6.7 kcal/mol, the same binding mode was present in the π-π stacking interaction between R57—baicalein. For its part tropoflavin presents π-π stacking interaction with R99 at 5.36 Å and an ΔG= -7.0 kcal/mol with the same binding mode as the other two flavones (Table 7).

Table 7. π —π stacking interactions flavone—DENV E-3

Flavone	ΔG (Kcal/mol)	π-π stacking interaction	Residue/flavone interaction functional groups	Distance Å	Angle
Tropoflavin	-7.0	R99	Guanidium ion—B ring	5.36	77.79
	-4.37	HB:242	Imidazole ring—B ring	4.91	78.54
Baicalein	-6.7	F212	Phenyl ring—B ring	3.85	64.49
	-6.7	R57	Guanidium ion—B ring	4.88	83.33
	-6.5	Y96	Phenol ring—B ring	3.87	7.76
	-5.46	W229	Benzyl ring—B ring	5.40	79.88
	-4.77	W210	Imidazole ring—A ring	4.16	21.63

Luteolin	-7.0	R348	Guanidium ion—A ring	5.48	86.02
	-3.24	H27	Imidazole ring —A ring	4.06	13.24
	-3.24	H242	Imidazole ring—A ring	5.20	77.65
	-5.44	H259	Imidazole ring—B ring	5.16	61.81

At ΔG=-7.14 kcal/mol, the π - π stacking interaction between W229—DENV E-3 was found. As shown in the following graph 2 and figure 7, it is important to highlight that the π - π stacking distance is much larger than the H-bond distance (Graphic 2).

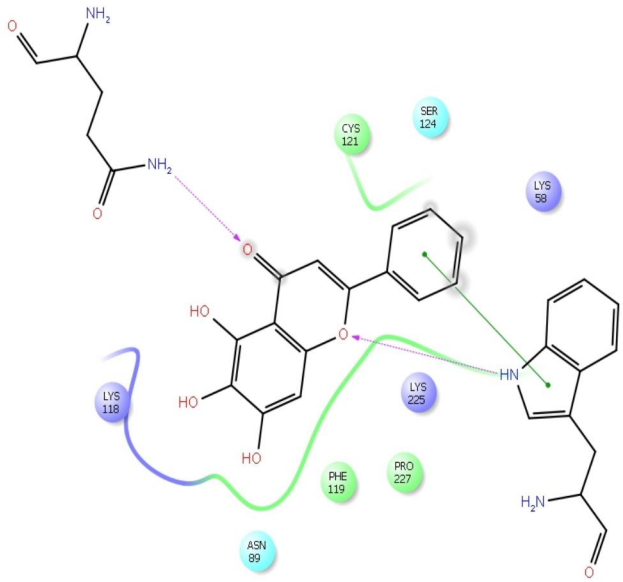
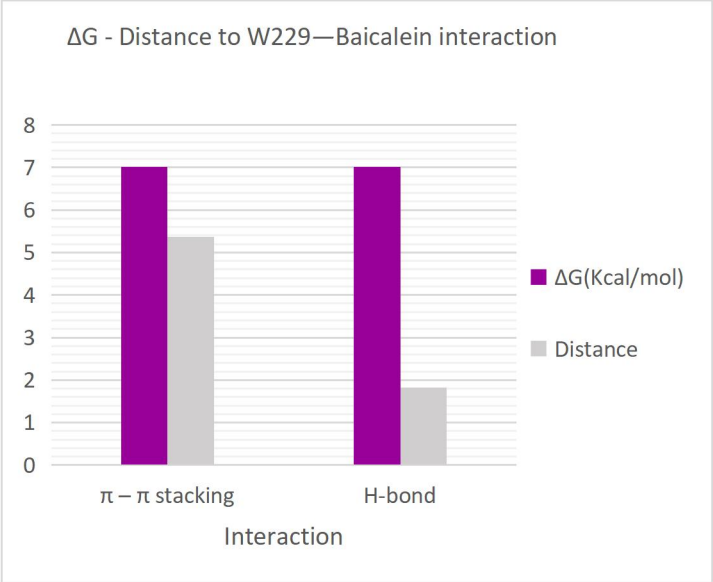


Figure 7. H-bond interaction Q120, W229, π – π stacking W229— Baicalein. ΔG=-7.14 kcal/mol



Graphic 2. π – π stacking Vs. H-bond distance

The figure above shows the π - π stacking interaction between the pyrrole ring of W229 and the B-ring of baicalein with 5.44 Å and a bond angle of 72.42. Equally, W229 presents an H-bond interaction with the *O*-pyran of the C-ring of baicalein with 1.90 Å in which it is the NH of the pyrrole ring that donates the H. For its part, Q120 presents H-bond interactions with the *4-oxo* group of the C-ring of baicalein with 2.56Å, being the NH_2 of the residue that donates the H to baicalein (figure 7).

π – cation interactions

In protein structures, π-cation interactions have been extensively studied. It has been found that such interactions are very rarely hidden (37) and that R forms such interactions more frequently than K (38). Through mutational studies, it was found that the interaction strength of W and the side chain of K, R, or H ranges from -0.8 to -0.5 kcal/mol (39). With increasing

solvent exposure, the interaction energy decreased significantly. An important example is the study of the complex structure of the periplasmic receptors of the K, R, and ornithine-binding protein-LAO, in which binding to K and R with the same affinity is present (40).

Williamson *et al*., 2009 (41), found that π-arginine cation interactions are an important factor in several heat shock protein inhibitors. This type of interaction has an important function in nucleic acid binding sites, and the adenine-arginine pair is conserved in several protein families. K is important in the interaction of proteins with biomolecules. Many studies on the assembly of nanocarriers with drugs utilize K since it forms a weak bond, which facilitates the release of the drug from the nanocarriers (42). In the flavone-DENV E-3 interaction, π-cation interactions occur mainly with K.

In the interactions between tropoflavin, baicalein, and luteolin with the DENV E-3 protein, several π-cation-type interactions were present (Table 8).

Table 8. π —cation interaction flavone—DENV E -3

Flavone	ΔG (Kcal/mol)	π-cation interaction	Residue/flavone interaction functional groups	Distance	Angle
Tropoflavin	-6.5	K308	NH_3^+—A ring	3.85	28.81
	-7.0	K36	NH_3^+—B ring	5.68	27.18
	-4.1	K200	NH_3^+—B ring	3.96	27.05
Baicalein	-5.9	K225	NH_3^+—B ring	4.08	25.76
Luteolin	-7.8	R348	NH_2^+—B ring	5.77	12.40
	-7.7	R348	NH_2^+—B ring	5.55	16.40
	-6.4	K36	NH_3^+—A ring	4.30	24.14
	-3.87	KB:244	NH_3^+—B ring	3.86	19.00
	-5.14	K202	NH_3^+—B ring	6.22	29.89
	-4.92	K200	NH_3^+—B ring	5.79	15.21
	-4.92	K202	NH_3^+—B ring	5.64	14.71
	-5.44	K200	NH_3^+—B ring	6.03	19.63
	-5.44	K202	NH_3^+—B ring	5.43	47.47

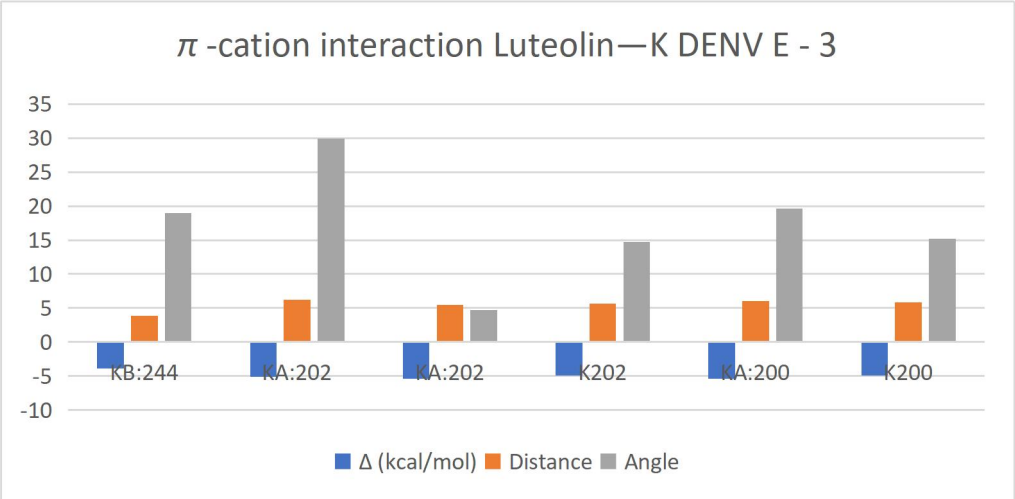
The π-cation interaction of flavones with the DENV E-3 protein occurred mainly with K residues in higher proportion and with R (Table 8). Tropoflavin presented π-cation interaction with K308, K36 and K200 with ΔG =-6.5, -7.0 and -4.1 kcal/mol respectively. The interaction with K308 is between the alkylammonium ion ($-CH_2-NH_3^+$) of the residue and the A-ring of the flavone at 3.85 Å, while the interaction with K36 and K200 is between the alkylammonium group of each residue and the B-ring of tropoflavin with distances of 5.68 and 3.96 Å respectively. Of note is the difference between the distance of the π-cation interaction with K36 concerning respect to the distance with K308 and K200.

For its part, luteolin presents π-cation interaction with residues R348, K36, KB:244, K200, and K202. The π-cation interaction with R348 occurs between the $-NH_2^+$ guanidinium ion and the B-ring of luteolin with ΔG =-7.7 and -7.8 kcal/mol and distances of 5.77 and 5.55 Å respectively. The π-cation interactions with KB:244, K200, and K202 were realized between the alkylammonium ion ($-CH_2-NH_3^+$) of the corresponding residue and the B-ring of luteolin with ΔG ranging from -3.87, -5.44 and -4.92 kcal/mol respectively.

Luteolin inhibited DENV-3 with an EC_{50} value of 5.69 mM and a selectivity index -IS of 8.07 in hepatocellular carcinoma cells, ATCC- Huh-7 cells after 48 h of incubation (14). Immature DENV particles are processed by the host enzyme furin in the trans-Golgi complex network to produce infectious mature virus particles. To evaluate the inhibitory action of luteolin, a concentration of 5-200 μM was evaluated on the catalytic activity of the human enzyme furin at a substrate concentration of 100 μM. Maximal inhibition of enzyme activity was observed at a concentration of 200 μM of luteolin. Luteolin was determined to be a non-competitive inhibitor where inhibition occurs at the binding of the enzyme-substrate complex rather than on the enzyme itself, with a Ki of 58.6 μM. They determined that luteolin acts on the furin-substrate complex to inhibit prM cleavage which prevents virus maturation in DENV-infected cells and is treated with luteolin (14).

In the interactions between luteolin and DENV E -3 in the lowest ΔG=-5.5 kcal/mol and in the following value ΔG=-5.19 kcal/mol an H-bond interaction with K 239 and K 245, and a π -cation interaction with KB:244 with ΔG=-3.87 kcal/mol and 3.86 Å was presented. Similarly, the lowest ΔG=-5.44 kcal/mol was presented in the π-cation interaction with KA:200 and KA:202 with distances of 6.03 and 5.43 Å respectively. At ΔG=-5.14 kcal/mol the π-cation interaction with KA:202 was present at 6.22 Å and the following value ΔG=-4.92 kcal/mol also π-cation interaction with KA:200 and KA:202 with distances of 5.79 and 5.64 Å was

present. It is to highlight that the largest bond angle 29.894 was presented with the largest distance of 6.22 Å in the KA:202 interaction and the B-ring of luteolin (Graphic 3).



Graphic 3. π-cation interactions between K—luteolin for ΔG different.

The interaction between the alkylammonium ions of K and aromatic rings can be considered a special kind of alkyl-aryl interaction. A positively charged nitrogen is a particularly strong electronegative substituent and, therefore, the direct interaction of an alkylated ammonium group with an aromatic ring gives rise to a strong attractive interaction. In medicinal chemistry, ammonium groups are rarely permanently charged quaternary ions, so there is at least one proton on the nitrogen atom that must be considered in the drug design process (16).

R348 also presents π-cation type interactions with the B-ring of luteolin at 5.77 Å, a bond angle 12.40 and an ΔG=-7.8 kcal/mol (Table 8 and Figure 8); with the same B-ring at 5.55 Å and angle of 16.40 with ΔG =-7.7 kcal/mol π -cation interaction is also present

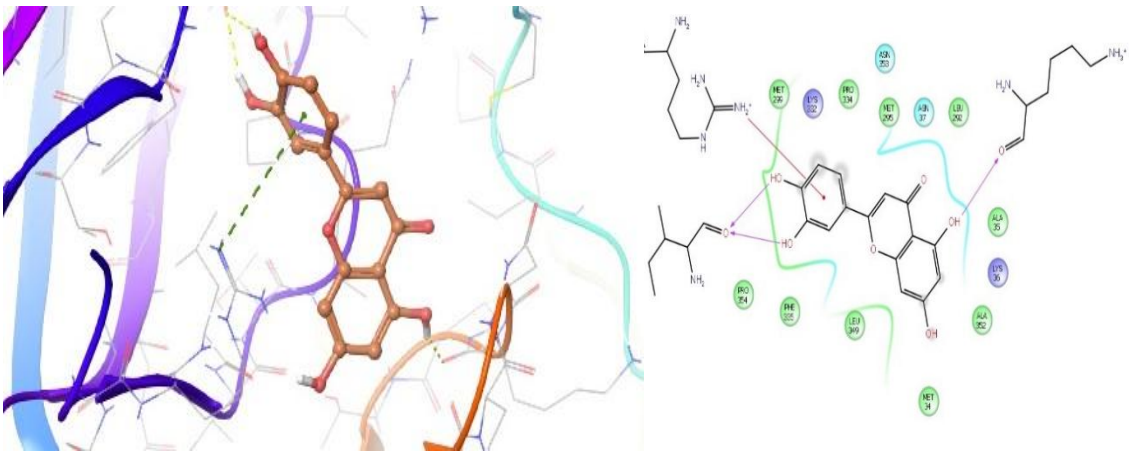
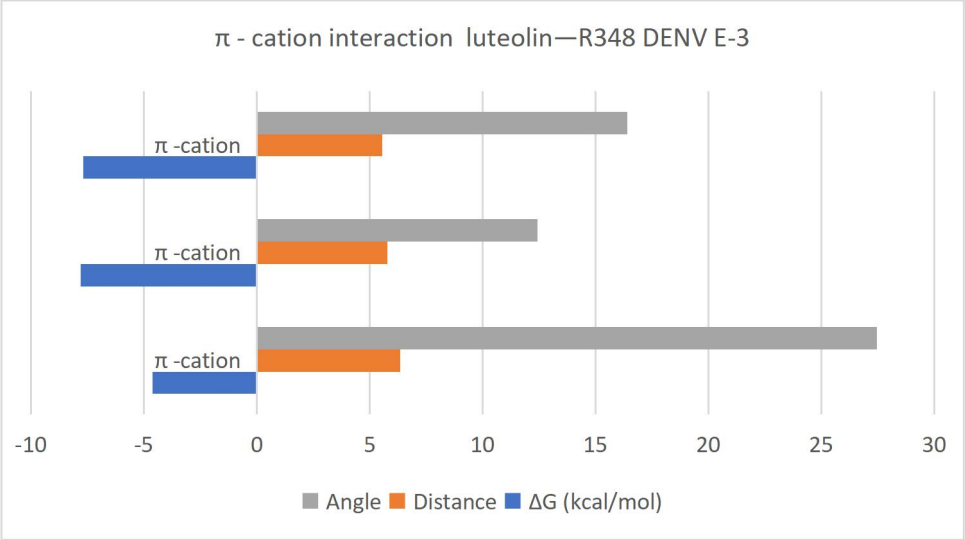


Figure 8. Luteolin—DENV E-3 interaction. π-cation with R348 and H-bond with I333 and K38. ΔG =-7.8 kcal/mol. Left 3D. Right 2D.

On the other hand, I333 presents H-bond interactions with 3'-OH and 4'-OH of the B-ring of luteolin who donate the H to the keto group of I333 with distances of 2.08 and 2.07 Å respectively with ΔG =-7.8 kcal/mol (Figure 8), the same interaction occurs with 3'-OH at 1.93 Å and ΔG =-7.7 kcal/mol. Similarly, I333 presents an H-bond interaction with 4'-OH at 2.14 Å and ΔG =-7.2 kcal/mol. In the above interactions the OH groups of luteolin donate the H to the keto group of I333.

A π -cation interaction is also observed between R348 and the A-ring of luteolin at 6.34 Å and an angle of 27.46 with an ΔG =-4.61 kcal/mol (Graphic 4).



Graphic 4. π -catión interaction R348—luteolin.

The flavone baicalein present π-cation interaction with K225. This interaction occurs between the alkylammonium ion (-CH_2-NH_3^+) of K225 and the B-ring of baicalein with an ΔG =-5.9 kcal/mol and 4.08 Å (Figure 9).

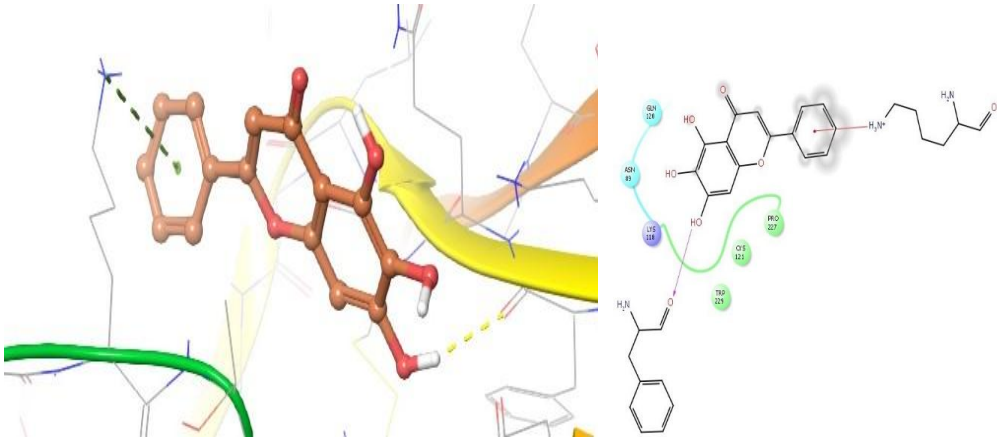
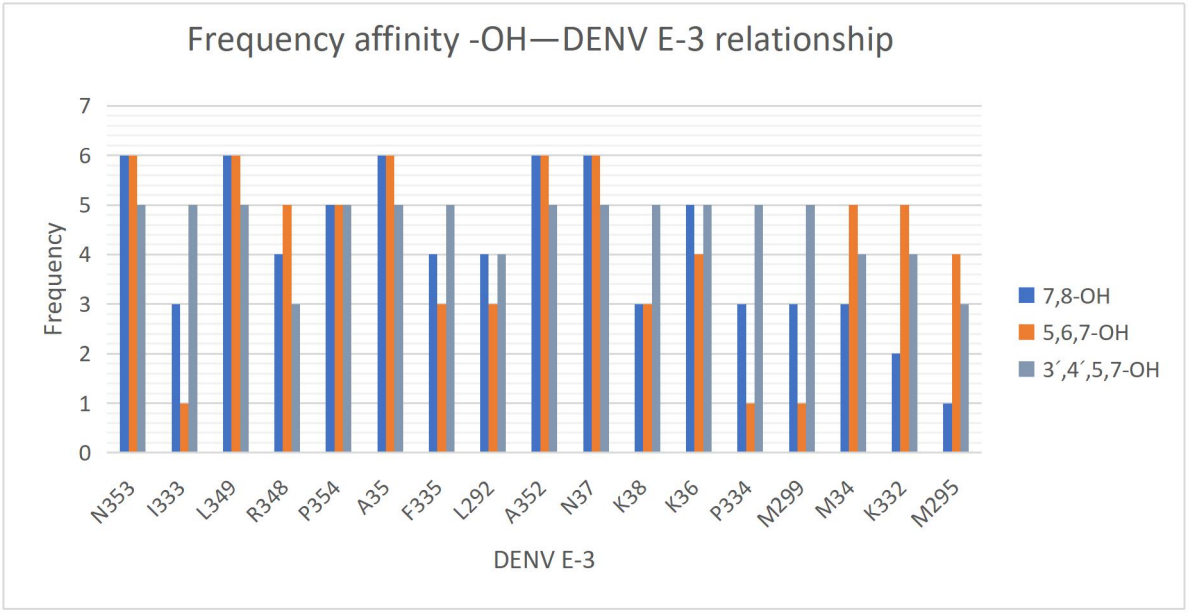


Figure 9. π-cation interaction K225—baicalein. ΔG =-5.9 kcal/mol. Left 3D. Right 2D.

Affinity -OH—DENV E-3 relationship

Analyzing the results of molecular interactions between flavones **1**, 2, and **3**, the graphic of the affinity frequencies of the residues for each flavone-DENV E-3 complex was realized.

In graphic 5, it can be evidenced that *7,8-dihydroxyflavone* presents a higher affinity frequency for N353, L349, A352, N37, and A35 present in domains III and I with ranges ΔG =-7.5 - 6.4 kcal/mol. The flavone *5,6,7-trihydroxyflavone* has a higher affinity frequency for N353, L349, A352, N37, and A35 present in domains III and I, with ranges ΔG =-7.2 - 6.6 kcal/mol. In turn, *3′,4′,5,7-tetrahydroxyflavone* present equal affinity frequency for N353, L349, P354, F335, A352, P334, M299, A35, N37, K38 and K36, residues present in domains III and I with ranges ΔG = -8.0 - 7.0 kcal/mol.



Graphic 5. Affinity frequency between flavone—DENV E-3 complexes.

The three flavones evaluated *7,8-dihydroxyflavone*, *5,6,7-trihydroxyflavone*, and *3′,4′,5,7-tetrahydroxyflavone* present equal affinity frequencies for P354 present in domain III, with ΔG values of -6.8 kcal/mol, -6.7 kcal/mol and -7.5kcal/mol respectively.

By residue K38 both **1** and **2** show equal affinity frequency with the same ΔG =-6.7 kcal/mol. However, by K36 it is **1** and **3** which present the same affinity frequency with ΔG =-6.8 kcal/mol and -7.5 kcal/mol respectively.

It is remarkable that with ΔG =-6.8 kcal/mol, *7,8-dihydroxyflavone* has an equal affinity frequency for P354, and K36. Similarly, *3′,4′,5,7-tetrahydroxyflavone* with ΔG= -7.5 kcal/mol has the same affinity frequency for both P354, and K36. Both *7,8-dihydroxyflavone*

and *3′,4′,5,7-tetrahydroxyflavone* have the same frequency of affinity for N353, L349, P353, A352, A35, and N37, residues present in domains III and I. With respect to residue I333 present in domain III, it is *3′,4′,5,7-tetrahydroxyflavone* that presents a higher affinity frequency concerning to the other flavones, with an ΔG = -7.5 kcal/mol.

Conclusions

Non-covalent molecular interactions between the flavones tropoflavin, baicalein and luteolin as antiviral agents and the DENV E-3 protein were analyzed. Tropoflavin, baicalein, and luteolin presented H-bond interaction with N353, R348 in domain III of the DENV E-3 protein. The highest $K_{binding}$ value was between N353—tropoflavin and for R348 the highest $K_{binding}$ value was between R348—luteolin. The highest number of π - π stacking interactions with the DENV E-3 protein was presented by baicalein with F, R, Y and mainly with W, followed by luteolin with R and mainly with H. The highest number of π-cation interactions was presented by luteolin with K. The flavones tropoflavin, baicalein and, luteolin present interaction with domains II and I of the DENV E-3 protein at the DENV E-3 protein binding sites reported for flavonoids. Both tropoflavin and baicalein have equal affinity frequency for residues N353, L349, A352, A35, and N37 present in domains III and I of the DENV E-3 protein.

REFERENCES

[1] Tolle M. Mosquito-borne diseases. Curr Probl Pediatr Adolesc. Health Care. 2009; 39:97-140.

[2] Harapan, H.; Michie, A.; Sasmono, R. T.; Imrie, A. Dengue: A Minireview. Viruses, 2020, 12, 829.

[3] Messina, J.P.; Brady, O.J.; Scott, T.W.; Zou, C.; Pigott, D.M.; Duda, K.A.; Bhatt, S.; Katzelnick, L.; Howes, R.E.; Battle, K.E.; Simmons, C. P.; Hay S. I. Global spread of dengue virus types: Mapping the 70-year history. Trends Microbiol. 2014, 22, 138–146.

[4] Weaver, S.C.; Vasilakis, N. Molecular evolution of dengue viruses: Contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease. Infect. Genet. Evol. 2009, 9, 523–540.

[5] Hassandarvish, P.; Rothan, H. A.; Rezaei, S.; Yusof, R.; Abubakara, S.; Zandi, K. In silico study on baicalein and baicalin as inhibitors of dengue virus replication. RSC Adv., 2016, 6, 31235.

[6] Modis, Y.; Ogata, S.; Clements, D.; Harrison, S.C. Variable Surface Epitopes in the Crystal Structure of Dengue Virus Type 3 Envelope Glycoprotein. JOURNAL OF VIROLOGY, Jan. 2005, p. 1223–1231

[7] Zheng, C-D.; Li, G.; Li, H-Q.; Xu, X-J.; Gao, J-M.; Zhang, A-L. DPPH-Scavenging Activities and Structure-Activity Relationships of Phenolic Compounds. Natural Product Communications. 2010, 5,1759 – 1765.

[8] Chiang, N-N.; Lin, T-H.; Teng, Y-S.; Sun, Y-C.; Chang, K-H.; Lin, C-Y.; Hsieh-Li, H. M.; Su, M-T.; Chen, C-M.; Lee-Chen, G-J. Flavones 7,8-DHF, Quercetin, and Apigenin Against Tau Toxicity via Activation of TRKB Signaling in 1K280 TauRD-DsRed SH-SY5Y Cells. Front. Aging Neurosci. 2021,13, 758895.

[9] Gao, Z.; Huang, K.; Yang, X.; Xu, H. Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of Scutellaria baicalensis. Biochimica et Biophysica Acta. 1999. 1472, 643-650.

[10] Ahmadi, S. M.; Farhoosh, R.; Sharif, A.; Rezaie, M. Structure-Antioxidant Activity Relationships of Luteolin and Catechin. Journal of Food Science. 2020, (85) 2.

[11] Veitch, N.C.; Grayer, R.J. Chalcones, Dihydrochalcones, and Aurones. In FLAVONOIDS. Chemistry, Biochemistry and Applications; Andersen O. M. and Markham K. R. Eds. CRC Press. Taylor and Francis Group. 2006; Chapter 16. Pp-1003-1071.

[12] Tsai, F-J.; Lin, C-W.; Lai, C-C.; Lan, Y-C.; Lai, C-H.; Hung, C-H.; Hsueh, K-C.; Lin, T-H.; Chang, H. C.; Wan, L.; Sheu, J. J-C.; Lin, Y-J. Kaempferol inhibits enterovirus 71 replication and internal ribosome entry site (IRES) activity through FUBP and HNRP proteins. Food Chemistry. 2011, 128, 312-322.

[13] Zandi, K.; Teoh, B-T.; Sam, S-S.; Wong, P-F.; Mustafa, M. R.; AbuBakar, S. Novel antiviral activity of baicalein against dengue virus. BMC Complementary and Alternative Medicine, 2012, 12,214.

[14] Peng, M.; Watanabe, S.; Ki-C, K. W.; He, Q.; Zhao, Y.; Zhang, Z.; Lai, X.; Luo, D.; Vasudevan, S. G.; Li, G. Luteolin restricts dengue virus replication through inhibition of the proprotein convertase furin. Antiviral Research, 2017, 143, 176-185.

[15] Wang, L.; Wang, J.; Wang, L.; Ma, S.; Liu, Y. Anti-Enterovirus 71 Agents of Natural Products. Molecules, 2015, 20, 16320-16333.

[16] Wang, M.; Tao, L.; Xu, H. Chinese herbal medicines as a source of molecules with anti-enterovirus 71 activity. Chin Med. 2016. 11:2.

[17] Zheng, Y-Z.; Zhou, Y.; Liang, Q.; Chen, D-F.; Guo, R. Theoretical studies on the hydrogen-bonding interactions between luteolin and water: a DFT approach. J Mol Model. 2016, 22: 257.

[18] Bissantz, C.; Kuhn, B.; Stahl, M. A Medicinal Chemist's Guide to Molecular Interactions. J. Med. Chem. 2010, 53, 5061–5084.

[19] Lommerse, J. P. M.; Price, S. L.; Taylor, R. Hydrogen bonding of carbonyl, ether, and ester oxygen atoms with alkanol hydroxyl groups. J. Comput. Chem. 1997, 18, 757–774.

[20] Taylor, R.; Kennard, O.; Versichel, W. Geometry of the iminocarbonyl (N-H 333 O:C) hydrogen bond. 1. Lone-pair directionality. J. Am. Chem. Soc. 1983, 105, 5761–5766.

[21] Nobeli, I.; Price, S. L.; Lommerse, J. P. M.; Taylor, R. Hydrogen bonding properties of oxygen and nitrogen acceptors in aromatic heterocycles. J. Comput. Chem. 1997, 18, 2060–2074.

[22] Nocker, M.; Handschuh, S.; Tautermann, C.; Liedl, K. R. Theoretical Prediction of Hydrogen Bond Strength for use in Molecular Modeling. J. Chem. Inf. Model. 2009, 49, 2067–2076.

[23] Morris, G. M., Goodsell, D. S., Halliday, R. S., Huey, R., Hart, W. E., Belew, R. K., y Olson, A. J. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. Journal of Computational Chemistry, 19(14): 1998. 1639–1662.

[24] Wang, R., Lai, L., y Wang, S. Further development and validation of empirical scoring functions for structure-based binding affinity prediction. Journal of computeraided molecular design, 16(1): 2002.11–26.

[25] Ismail, N. A.; Jusoh, S. A. Molecular Docking and Molecular Dynamics Simulation Studies to Predict Flavonoid Binding on the Surface of DENV2 E Protein. Interdiscip Sci Comput Life Sci. 2017, 9:499–511.

[26] Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of Computational Chemistry, 2009. 30(16), 2785–2791

[27] O. Trott, A. J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, Journal of Computational Chemistry. 2010. 31, 455-461.

[28] Guirakhoo, F.; Hunt, A. R.; Lewis, J. G.; Roehrig, J. T. Selection and partial characterization of dengue 2 virus mutants that induce fusion at elevated pH. Virology. 1993, 194:219–223

[29] Hung, S. L.; Lee, P. L.; Chen, H. W.; Chen, L. K.; Kao, C. L.; King, C. C. Analysis of the steps involved in dengue virus entry into host cells. Virology, 1999. 257:156–167.

[30] Navarro-Sanchez, E.; Altmeyer, R.; Amara, A.; Schwartz, O.; Fieschi, F.; Virelizier, J. L.; Arenzana-Seisdedos, F.; P. Despres, P. Dendritic-cell-specific ICAM3-grabbing non-integrin is essential for the productive infection of human dendritic cells by mosquito-cell-derived dengue viruses. EMBO Rep. 2003. 4, 1–6.

[31] Modis, Y.; Ogata, S.; Clements, D.; Harrison, S. C. A ligand-binding pocket in the dengue virus envelope glycoprotein. PNAS. 2003, 100, 6986–6991.

[32] Tassaneetrithep, B.; Burgess, T. H.; Granelli-Piperno, A.; Trumpfheller, C.; Finke, J.; Sun, W.; Eller, M. A.; Pattanapanyasat, K.; Sarasombath, S.; Birx, D. L.; Steinman, R. M.; Schlesinger, S.; Marovich, M. A. DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. J. Exp. Med. 2003. 197, 823–829.

[33] Horton H. R.; Moran L. A.; Scrimgeour K. G.; Perry M. D.; Rawn J. D. Principles of Biochemistry. 2006. 4th. Edition. Pearson Education, Inc., Publishing as Prentice Hall. Chapter 3.

[34] Mondotte, J. A.; Lozach, P-Y.; Amara, A.; Gamarnik, A. V. Essential Role of Dengue Virus Envelope Protein N Glycosylation at Asparagine-67 during Viral Propagation. JOURNAL OF VIROLOGY, 2007, 7136–7148.

[35] Steiner, T.; Koellner, G. Hydrogen bonds with pi-acceptors in proteins: frequencies and role in stabilizing local 3D structures. J. Mol. Biol. 2001, 305, 535–557.

[36] Imai, Y. N.; Inoue, Y.; Yamamoto, Y. Propensities of polar and aromatic amino acids in noncanonical interactions: nonbonded contacts analysis of protein-ligand complexes in crystal structures. J. Med. Chem. 2007, 50, 1189–1196

[37] Gallivan, J. P.; Dougherty, D. A. A computational study of cation-pi interactions vs salt bridges in aqueous media: implications for protein engineering. J. Am. Chem. Soc. 2000, 122, 870– 874.

[38] Gallivan, J. P.; Dougherty, D. A. Cation-pi interactions in structural biology. Proc. Natl. Acad. Sci. U.S.A. 1999, 96, 9459– 9464.

[39] Berry, B. W.; Elvekrog, M. M.; Tommos, C. Environmental modulation of protein cation-pi. interactions. J. Am. Chem. Soc. 2007, 129, 5308–5309

[40] Oh, B.-H.; Ames, G. F.-L.; Kim, S.-H. Structural basis for multiple ligand specificity of the periplasmic lysine-, arginine-, ornithine-binding protein. J. Biol. Chem. 1994, 269, 26323–26330.

[41] Williamson, D. S.; Borgognoni, J.; Clay, A.; Daniels, Z.; Dokurno, P.; Drysdale, M. J.; Foloppe, N.; Francis, G. L.; Graham, C. J.; Howes, R.; Macias, A. T.; Murray, J. B.; Parsons, R.; Shaw, T.; Surgenor, A. E.; Terry, L.; Wang, Y.; Wood, M.; Massey, A. J. Novel adenosine-derived inhibitors of 70 kDa heat shock protein, discovered through structure-based design. J. Med. Chem. 2009, 52, 1510–1513.

[42] Wang, W.; Xi, M.; Duan, X.; Wang, Y.; Kong, F. Delivery of baicalein and paclitaxel using self- assembled nanoparticles: synergistic antitumor effect in vitro and in vivo. International Journal of Nanomedicine. 2015, 10, 3737–3750.