DOCKING MOLECULAR STUDIES BETWEEN THE BIXIN AND NORBIXIN CAROTENOIDS AND THE DENGUE FEVER VIRUS (NS1)

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Abstract— Dengue fever is a disease that affects about 100 countries, where it is spent 12 billion a year for the treatment and control of the mosquito that transmits this disease. This virus is part of the family Flaviviridae and the genus Flavivirus, which presents a total of four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4), so a person can acquire dengue up to 4 times during life. The proposed ligands were bixin and norbixin, where these molecules are found in the annatto (Bixa orellana L.) seed, which has antipyretic, cardiotonic, antidiarrheal, antimalarial, antifebrin, hypoglycemic, antimicrobial and anticarcinogenic activity. The present study had the objective of characterizing the possible interaction between bixin and norbixin with the dengue virus, aiming at its possible inactivation. The study was carried out with the NS1 protein of dengue virus (for DENV-1) and bixin and norbixin, where molecular docking was performed to verify if there was interaction between the molecules and their receptor site, and whether this interaction was likely and stable. Ten attractive twists were observed for each binder, where in the case of bixin, we had twist # 1.4 with the bonding distance in the value of 2.584Å, and norbixin, we had twist # 1.6 with the binding distance in the value of 1,701Å °. Being the present work a milestone for future studies of biological activity, aiming the inactivation of Dengue virus type I.

Keywords: Bixin. Dengue Fever. DENV-I. Molecular Docking. Norbixin.

1 INTRODUCTION

Dengue is a disease of global character that is transmitted through the bite of the Aedes aegypti mosquito, although there is another species, Aedes albopictus, which has similar proliferative capacity and morphology and is also responsible for some outbreaks of the disease in mainland countries Asian. This disease is seasonal, that is, occurring with greater probability in hot and high humidity periods, because these conditions help mosquito proliferation. The dengue virus is part of the Flaviviridae family and the Flavivirus genus, which presents four serotype series (DENV-1, DENV-2, DENV-3 and DENV-4) [1], where there may be coexistence of serotypes in the same region, increasing the chances of complications such as hemorrhagic fever. The main clinical forms of dengue fever are classical dengue fever (CD), dengue fever with complications (DCC), dengue hemorrhagic fever (DHF) and the most serious of all, which is dengue shock syndrome (DCD) can acquire dengue up to four times throughout his life [1]. After the infected mosquito bite in an individual, the average incubation period is 4 to 7 days, where the patient may or may not exhibit symptoms, depending on the age, immune status, virus strain. Then there is the onset of a sudden fever and constitutional symptoms that last from 5 to 6 days [2].

Dengue virus replicates within cells of the phagocytic mononuclear system (macrophages, monocytes and B cells). In addition, infection of mast cells, dendritic cells and endothelial cells is known to occur. The virus can infect leukocytes from the peripheral blood, liver, spleen, lymph nodes, bone marrow, thymus, heart, kidneys, stomach, lungs and possibly the brain, suggesting passage through the blood-brain barrier [2]. After the fever stage, the individual may recover or progress further in dengue, leading to dengue hemorrhagic fever or dengue shock syndrome, the severity of dengue infection is related to

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peak plasma viremia and circulating levels of dengue. non-structural NS1 protein. Dengue becomes even more serious when the individual is infected again, for a different serotype, it is not known for certain the reason for this fact, however, it has been suggested that the residual antibodies produced during the first infection are unable to neutralize the new infection by another serotype, and new infection under the influence of amplifying antibodies results in severe infection and disease. This phenomenon is called antibody-dependent immune amplification [2].

The Bixa orellana species has antipyretic, cardiotonic, antidiarrheal, antimalarial [4,5], hypoglycemic [6], antimicrobial [7,8] and anticarcinogenic activity [9]. Bixin is a apocarotenoid a group derived from the carotenoids, the apocarotenoids, comes from the oxidative cleavage of the carotenes with a chain of 25 carbons which constitutes, on average, 2.5% of the dried seeds of Bixa Orellana, containing a carboxylic acid and a methyl ester at the ends, occurring naturally in the cis form [4]. This molecule is present in a higher concentration in the aryl of the *urucum* seed, where this is the main substance responsible for the dyeing characteristics of the dyes obtained from that seed. In its crude seed, its concentration can reach up to 5.0%, however some seeds have contents lower than 2.0%, its commercial value of the seed is based on the percentage of bixin. Latin America is the world's largest producer of annatto (Bixa orellana L.), with an annual production of approximately 17,000 tons, of which 12,000 originate in Brazil [4]. In the concentrations predicted by the regulatory agencies, the consumption of these carotenoids is safe and in addition to the applications as a dye, can be used by the industry as natural antioxidants, providing an alternative that can replace or minimize the use of synthetic additives in meat products. Norbixin is also found in annatto seed and, due to the structural differences between this molecule and bixin, gives bixin liposoluble properties due to the presence of the methyl ester in the molecule, while norbixin presents greater water solubility due to the presence of the carboxyl group. With the removal of the methyl ester group from bixin, norbixin which is a dicarboxylic acid is produced [10].

According to estimates by the World Health Organization (WHO): more than 100 countries now face dengue, most of them in tropical areas and there are already records of outbreaks in non-endemic countries, 390 million people are infected with the virus dengue fever per year and about 90 million of these cases are severe, while the rest is mild or asymptomatic, 2.5% of people in severe cases die every year, about 6 billion are spent per year for the treatment of dengue fever and another 6 billion for mosquito control. During the last major epidemic from 2001 to 2003, there were about 1,564,112 cases of dengue in Brazil, where 4,123 were hemorrhagic, causing 217 deaths. If we consider that these notifications represent only about 15% of the total notified, it is possible that the number of cases was around 10 million [11].

From the molecular docking, different spatial conformations of the ligand are obtained, allowing the analyst to identify which of these is the most probable in the target ligand interaction. From each spatial conformation, free energies of binding (between binder and target) are obtained, where the lowest energy is considered the most probable to justify the conformation of the interaction [11]. The interactions between the drug and proteinaceous target occur through intermolecular forces of the dipole type induced and permanent dipole. Among the dipole permanent dipole forces the most common in these systems are the hydrogen bonds, the dipole dipole induced may be forces of Van Der Waals or London [12]. From these computational resources, the cost for the study is much lower when compared to laboratory costs to synthesize and pharmacologically produce various substances. The present work aimed to characterize the possible interaction between bixin and norbixin with NS1 protein of the dengue virus, aiming at its possible inactivation.

2 METHODOLOGY

In this work, we used the software UCSF Chimera [13], free access software based on the Windows operating system. The first step was to obtain the structure and its properties of the dengue virus protein (for DENV-1 and its access code in PDB is 4OIG), through the Protein Data Bank repository (http: // www. rcsb.org/pdb/home/home.do). The second step was to

use the bixin and norbixin molecules, where their structures were obtained from the Pubchem repository (whose code is 6376436 and 5281249, respectively) and its access site is: https: // www.ncbi.nlm.nih.gov / pccompound. The methodological tool used for modeling in this work was molecular docking, where the equations used for molecular anchorage calculations were developed by researchers in the last century and implemented in computational packages used today [14]. The docking was done through USFChimera® software [13], which is available for download at: https://www.cgl.ucsf.edu/chimera/download.html. This software can provide density maps and estimates of the free energy binding between the protein and the ligand, where different spatial conformations of the ligand are obtained, and it is possible for the analyst to identify which of these conformations is most likely in the target binding interaction. Then, a comparison was made with the results obtained by SILVA and collaborators [15], where the same ligands were used, however, the protein was distinct in relation to this study.

3 RESULTS AND DISCUSSIONS

Molecular docking is a dynamic computational simulation method because it provides several spatial conformations between the protein and the binder, where for each spatial conformation is obtained binding free energies, so the smaller one with less energy is more likely to justify the conformation of the interaction. Then from the obtained results, it is possible for the analyst to verify which spatial conformation is more feasible for protein-binder binding.

The NS1 protein of the dengue virus is a conserved glycoprotein, where it is membrane bound and secreted with replication and immune evasion functions. This secreted protein is a hexameric lipo-protein that is in the form of a barrel that may or may not bind to the plasma membrane of cells. For the docking, this non-structural glycoprotein was used (Figure 1) through the Protein Data Bank [16] repository, it is registered in the PDB with the code 4OIG and has a resolution of 2.69 Å.

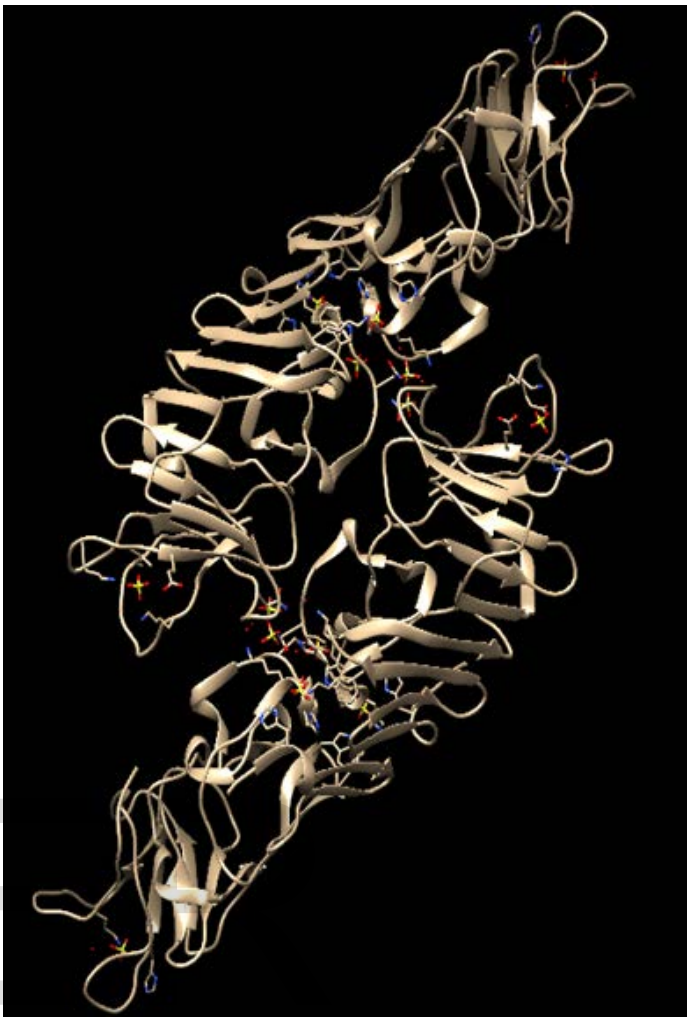

Fig.1. Structure of the NS1 protein (40IG). Source: RSCB Protein Data Bank, 2017.

The second step to perform molecular docking was to obtain the bixin binder, i.e., 2E, 4E, 6E, 8E, 10E, 12E, 14E, 16Z, 18E) -20-methoxy-4,8,13,17- tetramethyl-20-oxoicosa-2,4,6,8,10,12,14,16,18-nonaenoic acid, (Fig.2), where this compound has the following molecular formula C25H30O4, which may exist in cis and trans form. Bixin has liposoluble properties, due to its methyl ester in its structure and is cited as a carotenoid with a higher concentration in the colorless liposoluble solutions of *urucum*.

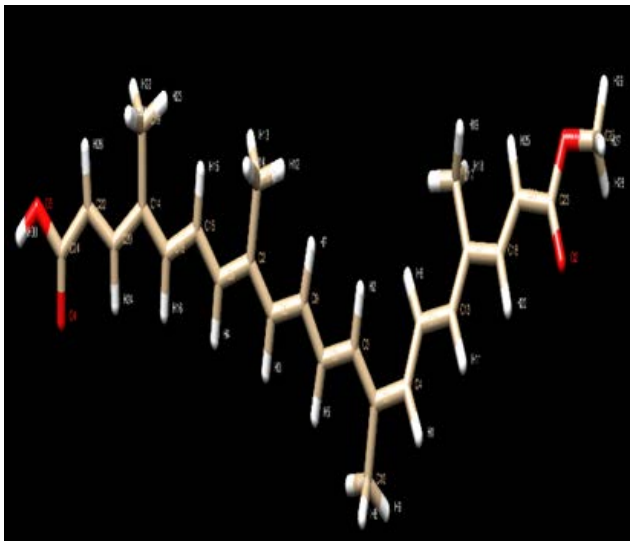

Fig.2. Molecular structure of Bixin. Source: PubChem Repository.

The other binder used in the tests with the molecular docking was norbixin(iupac name 2E, 4E, 6E, 8E, 10E, 12E, 14E, 16E, 18E) -4,8,13,17tetramethylicosa2,4,6,8, 10,12,14,16,18-nonaenedioic acid) (Fig.3), the compound has molecular formula C24H28O4 and molecular mass 380,464 mol / L. Norbixin has higher water solubility due to the presence of the carboxyl group and this compound is mentioned with carotenoid of greater concentration in the colorless solutions of urucum.

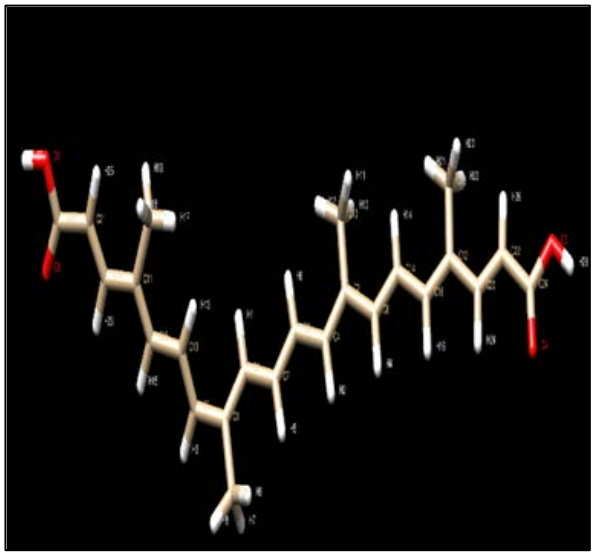

Fig.3. 3D structure of Norbixin. Source: PubChem Repository.

After the molecular docking between the target protein and the respective molecules (bixin and norbixin), ten attractive twists were obtained for the two couplings, where the data were shown in table form (TABLE 1).

TABLE 1

Attractive twists of NS1 protein doping with bixin

Chimera model	Score	RMSD L.B	RMSD U.B
#1.1	-6,2	0,0	0,0
#1.2	-5,8	54.811	55.653
#1.3	-5,8	16.797	23.336
#1.4	-5,5	55.377	55.275
#1.5	-5,2	16.856	23.497
#1.6	-5,1	43.928	48.485
#1.7	-5,0	5.842	18.191
#1.8	-5,0	35.369	44.34
#1.9	-4,9	17.772	21.369
#1.10	-4,9	45.509	48.135

After performing the analysis of the interactions between the NS1 protein and the bixin binder, the torsion # 1.4, presented the smallest distance among all the torsions in the value of 2.584 Å (Fig. 4), regarding the score function, was found in the value of -5.5 and for mean root mean square deviation (RMSD) root mean values: RMSD lb 55.377 and RMSD ub 55.275. From Figure 4, it was possible to verify the region where the molecular docking interacted best with the active site of the target molecule (NS1), where this orientation and conformation was the most favorable and stable among all other regions.

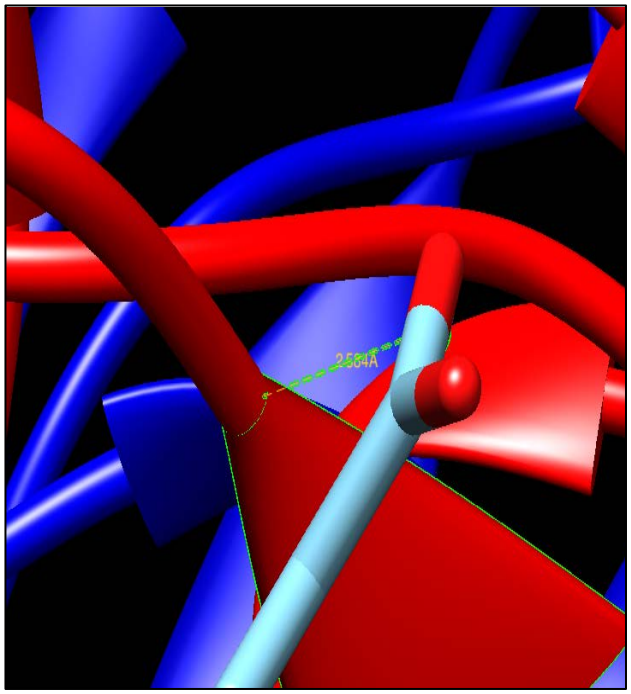

Fig.4. Molecular docking of NS1 protein with Bixin ligand.

As for the molecular anchorage between the NS1 protein and the second ligand, norbixin, ten attractive twists were found, that is, ten interactions where orientation and conformation are more favorable and stable (TABLE 2)

TABLE 2

Attractive NS1 protein docking twists with binder Norbixin

CHIMERA MODEL	SCORE	RMSD L.B	RMSD U.B
#1.1	-5.8	0.0	0.0
#1.2	-5.7	28.478	36.438
#1.3	-5.4	55.482	59.664
#1.4	-5.4	42.712	45.509
#1.5	-5.3	25.483	33.788
#1.6	-5.3	35.142	37.89
#1.7	-5.2	56.438	59.794
#1.8	-5.0	56.215	58.784
#1.9	-4.9	56.335	59.256
#1.10	-4.8	29.575	33.922

After analysis of the interactions between the NS1 protein and the Norbixin ligand, it was observed that among all the molecular coupling distances between them, the torsion # 1.6 was the one that presented the shortest distance in the value of 1.701 Å. For this torsion, the obtained values of score were -5.3 and for the mean square deviation values of Root Mean Square Deviation (RMSD), were: RMSD l.b 35.142 and RMSD u.b 37.89.

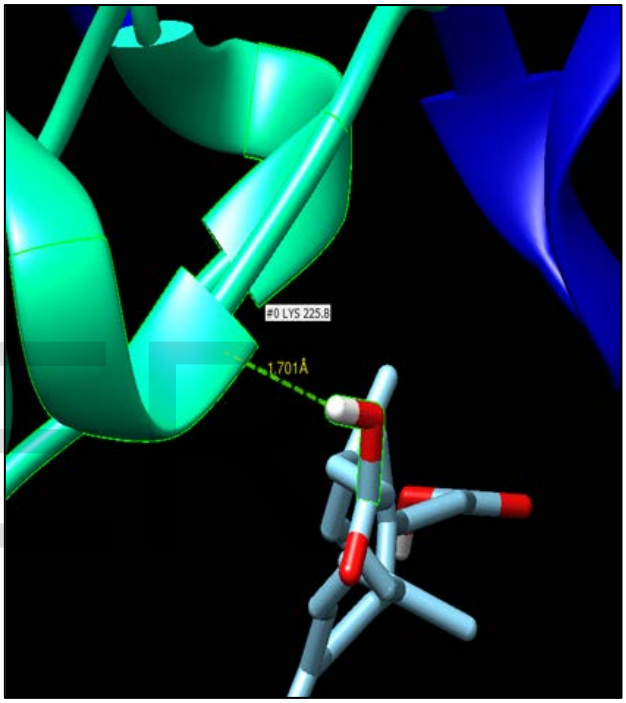

Fig.5. Molecular docking of NS1 protein with the binder Norbixin.

After obtaining the molecular coupling data, a comparison was made between the results obtained by this study and that of SILVA and collaborators [15], because the ligands were the same, where only the protein was distinct, code 5B1C and was pro DENV-4 virus. 17 attractive torsions were obtained for the bixin binder, whose lowest distance value involved the hydrogen atom (H30) of the ligand that is closest to the amino acid ASP77, located in the B chain, presenting a distance value of 2.4 Å. As for norbixin, 17 attractive twists were obtained, where the smallest distance between these twists was involving the oxygen atom (O4) of the ligand that is closest to the amino acid ASP77

that is in the B chain of the protein, presenting the value of 2.3 Å from distance. As for the present study, we had ten attractive twists for the two ligands, where bixin presented 2.584 Å and norbixin of 1.701 Å. It is likely that because the binding distance of the current study is smaller with the results that were compared, the binder should have a higher probability of interaction and stability.

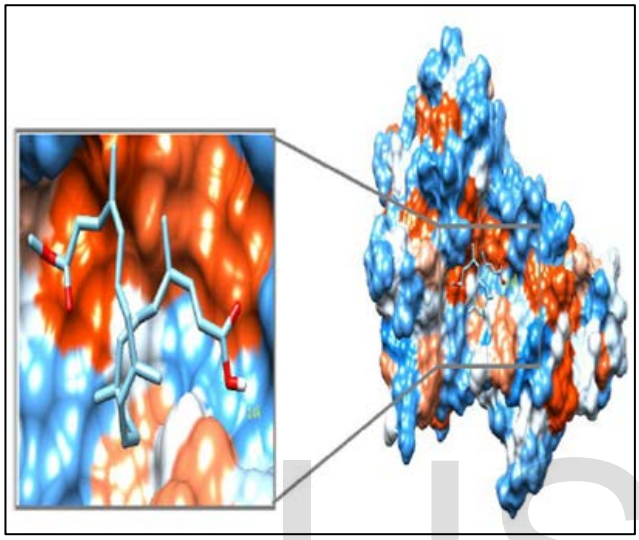

Fig 6: Active site of the 5b1C protein of dengue virus 4, bound to bixin, where the hydrophobic regions are shown in orange and the hydrophilic in blue.

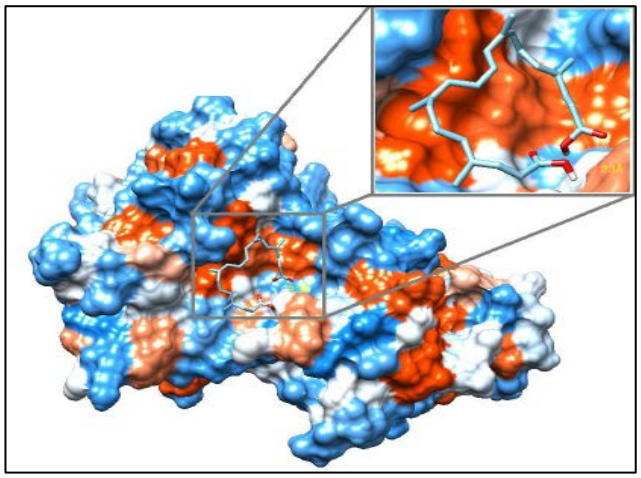

Fig.7. Active site of dengue virus protein 5B1C, linked to norbixin.

4 CONCLUSIONS

Computational chemistry has provided several benefits to society, where its computational resources can significantly reduce the laboratory costs used to synthesize and produce various substances. Molecular docking simulations allow to identify attractive binders of the ligand or different spatial conformations of the ligand, where it is possible to obtain the position of the ligand where the interaction between the molecule and its receptor site is most likely and stable. In this study, the receptor used was the NS1 protein of the dengue virus, as for the ligand, bixin and norbixin were used to perform molecular docking. After the tests, ten attractive twists were obtained for each of the ligands, where the most stable conformation for bixin was torsion # 1.4, presented the bond distance in the value of 2.584 Å , for the binder norbixin, position more stable was the twist # 1.6, where the bond distance was in the value of 1.701. The present study an initial stage for future biological assays that make possible to understand the action of bixinas on dengue virus.

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