Molecular docking of potential curcuminoids inhibitors of the NS1 protein of dengue virus

Aurineide Ribeiro Lima[1], Jacilene Silva[1], Lucas Lima Bezerra[1],Márcia Machado Marinho[2], Emmanuel Silva Marinho[1]

[1] State University Ceará- From Brazil, [2] Federal University of Ceará- Brazil

Abstract—Dengue fever (DF) and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) are considered the most medically important and widespread mosquito-borne viral diseases.Dengueis an arbovirus that has a large occurrence in humans around the world. It is an infectious and debilitating disease caused by the dengue virus (DENV), by an arbovirus belonging to the family Flaviviridae and to the genus Flavivirus. The genome of this virus (DENV) encodes a long polyprotein, which is cleaved by viral and cellular proteases, giving rise to three structural proteins and seven non-structural proteins, among which is an NS1 protein, which is a highly protein Conserved, does not act in the stage of viral replication, being also linked to virulence and morphogenesis of the virus. Thus, knowing that NS1 is a protein that plays an important role without DENV virus, acting on the replication of its viral genome, it was sought to use it as a target molecule in molecular docking tests, to identify binding molecules that They can interact with this protein and may be potentially active against this virus. As the binding molecules are introduced two curcuminoids, Curcumin and Monodemethylcurcumin. By means of the donation between this target molecule and these binding points, it could be observed that for both couplings it has ten attractive twists between these molecules. (NS1), a bond between the H6 of the curcuminoid binder with a phenylalanine (PHE) 565 E chain of the protein, obtained the lowest value for a distance (3.5 Å). As for monodemethylcurcumin with the protein, the shortest distance obtained (3.8 Å) was observed at the binding of H15 of the ligand. Monodemethylcurcumin with the amino acid Glycine (GLY) 408 of the D chain of the protein. These ligands, therefore, are shown to be promising for Docking tests with a dengue virus (NS1) protein.

Keywords: Dengue virus. Docking Molecular. Protein NS1. Curcumin.Monodemethylcurcumin.

1 INTRODUCTION

Dengue is an arbovirus, an infectious and debilitating disease caused by dengue virus (DENV), an arbovirus belonging to the Flaviviridae family and to the genus Flavivirus, presenting four antigenically distinct serotypes (DENV-1, DENV-2, DENV-3 and DENV-4). The same is transmitted to humans through arthropods, specifically by the bite of mosquitoes of the genus Aedes, such as Aedes albopictus and Aedes aegypti, the latter being the main vector, being responsible in most cases Dengue fever (DF) and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) are considered the most medically important and widespread mosquito-borne viral diseases. [1].

This is the arboviruses that has the highest occurrence in humans worldwide [2], being the cause of large urban epidemics, which makes it a major public health problem in many developing countries, including Brazil [3]. Currently, about 3.5 billion people live in countries with a tropical and subtropical climate and are susceptible to dengue infection [4], as their vectors are present in the most populous tropical and subtropical areas of the world [1]. Research has revealed that Brazil is the country that points to the highest incidence of dengue cases in the Americas, compared to other countries. Whereas, in the last decade, more than 4 million cases occurred in the American continent, of which about 80% belonged to Brazil [5]. On an annual basis, the incidence of 390 million cases of this disease is estimated [4].

Dengue has become one of the main cases or targets of study in the world, due to its great impact and being a major public health problem in the world, 6], since this is a disease, the virus of which has a great diversity and genetic variability, as DENV has a progressive size and density of the host / vector population, which facilitates rate growth Of transmission, and consequently, viral population and amount of replication [7].

DENV belong to the family Flaviviridae, genus Flavivirus, and occur as four antigenically related but distinct serotypes designated DEN-1, 2, 3 and 4, As for the, it consists of a small spherical virus, which has a diameter of approximately 50 nanometers (nm) [3] [8] [9]. Its genome consists of a single-stranded RNA of positive polarity [23], which behaves as a messenger RNA (mRNA) [3], it has an average of 11 kilobases (Kb) and an approximate molecular weight of 3.3x106 Daltons Da) [10]. It is surrounded by untranslated regions (UTRs) 5 'and 3', and the secondary structures of these regions, 5'UTR and 3'UTR, are linked and related in viral

- *Emmanuel S. Marinho has a PhD degree in Biochemistry from the Federal University of Ceará. master in Biochemistry at the Federal University of Ceará.Brazil. currently adjunct professor at the State University of Ceará (UECE)- Brasil.email: emmanuel.marinho@uece.br*
- *Marcia M. Marinho has a master's degree in biotechnology by the Federal University of Ceará. PhD student Doctoral degree in Famacêuticas Sciences, Federal University of Ceará. Brazil. email: marinho.marcia@gmail.com.*
- *Aurineide Ribeiro Lima degree course student in chemistry by the State University of Ceará (UECE), Brazil.*
- *Jacilene Silva degree course student in chemistry by the State University of Ceará (UECE), Brazil.*
- *Lucas Lima Bezerra degree course student in chemistry by the State University of Ceará (UECE), Brazil.*

replication, translation and in the packaging of the viral genome [11] [12].

This genome also has the open reading frame (ORF), encoding a long polyprotein, which is cleaved by viral and cellular proteases, generating three structural proteins: C (capsid), prM / M (Pre-membrane / membrane) and E (envelope); And seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5)[8] [13] [14].

Because proteins are the most abundant and important organic compounds in cells, they are found in all cells, playing a key role in structure and cellular functions. These functions performed by proteins are directly related to the native spatial structure, and the forces involved in their envelopment may be in the sequence of amino acids that make up their chain and in their interactions with the medium [15].

Thus, among the Dengue virus proteins, the NS1 non-structural glycoprotein is the first non-structural protein with 353 to 354 amino acids, being highly conserved [16], acting in the early phase of viral replication, being involved in the Viral morphogenesis, and associated with virulence [12].

This protein acts in the initial phase of viral infection, and can occur in three forms: inside the plasma reticulum of infected cells (resident), where it co-localizes with the viral replication complex; On the surface associated with the cell membrane (anchored) and in the extracellular (secreted) environment, free in the serum of an infected individual [8] [17]. Since this latter form (secreted form) plays an important role in the pathogenesis of this arboviruses [18]; And it was also considered useful for the diagnosis of DENV infections in serum samples from individuals [3].

Therefore, with the existence of distinct pathogenic serotypes, and with the proteins produced by them, researchers face great challenges to carry out research and development to prepare vaccines against this virus [2].

Therefore, in the same way that there are great challenges in the scientific world, when it comes to seeking cure or new drugs and / or treatments for certain diseases (such as dengue, for example), new Techniques or tools that can help in the search for and attainment of these objectives. As for example, molecular modeling techniques, such as Molecular Docking, used in Computational and Medicinal Chemistry. The modeling has a great contribution to the development of bioligands, since by means of this technique, a detailed characterization of the structure, the intermolecular interactions, and even of the chemical reactions between the bioligands and the biomacromolecule [19].

In this way, the Molecular Docking is a technique can be associated as a game of "key" and "lock", where we try to find the relatively correct orientation of the "key" that will open the "lock", that is, the most favorable conformation of a linker with a protein, respectively. In the search for this favorable orientation, the ligand and the protein adjust their conformations, to find the one with the lowest possible global energy, also called "induced adjustment" [20].

The docking, therefore, aims to find the affinity as well as the orientation and the most probable and stable conformation of a binder, anchored in the active site of a receptor target [21] [22]. Since, during the molecular combination process, where both molecules, enzyme and inhibitor, undergo conformational changes, occurring simultaneously to the interactions between them [23], it is possible to later identify which compounds have bound to the given receptor, and to classify Which of these compounds presented greater affinity in this active site [24] [25].

In this way, since molecular modeling has computational and theoretical tools and methods that aim to understand, predict and describe the behavior of real systems; As the protein docking study aims to test the affinity of a linker to a given substrate or binding site; And how these techniques have proved to be efficient for conducting research [15]. We attempted to perform some Molecular Docking tests using the NS1 protein of Dengue Virus (DENV1) as the target molecule, since it plays an important role in the replication of the viral genome, and activity in the modulation of cellular signaling pathways [26].

As ligands, Curcuminoids, the main active compounds of the curcuma plant (Curcuma longa Linn), were used. These were used, since they are highly pleiotropic molecules, which interact with their various molecular targets, being able to directly bind and modulate their activity or indirectly regulate their functions [27]. Allowing them to demonstrate a great therapeutic potential, since these are responsible for a wide variety of activities, such as: anti-inflammatory, antiviral, antibacterial, antioxidant, antifungal, anticarcinogenic, among other therapeutic functions [28]. What makes them stand out and as an important object of study in the scientific community [29].

The Docking tests were performed through a program that provides visualization of molecular structures in an interactive way, the UCSF Chimera® Software [15] [30]. The aim of this research was to use two curcuminoids (Curcumin and Monodemethylcurcumin) as ligand molecules to identify if these binding molecules can interact with the virus protein (DENV), that is, they could be Potentially active

against this virus. Finding, identifying and predicting compounds from a database capable of interacting with the molecular target binding site, ordering these molecules according to their affinity for the receptor site, to identify promising ligands with potential pharmacological activity.

2 METHODOLOGY

Computational resources

Molecular Docking was done using free access software based on the Windows 7 Ultimate 64-bit Operating System with Intel® Core ™ i3-5005U CPU @ 2.0 GHz processor, 4GB of RAM and 10TB of HD. If using the UCSF Chimera® software (https://www.cgl.ucsf.edu/chimera/), this is a Computational Chemistry program that allows interactive visualization and analysis of molecular structures, as well as Density, anchorage results, trajectories and conformational sets.

Computational strategies

We also used three repositories: the repository of protein structures, the RSCB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do), through which the structure of the non-structural protein (NS1) of Dengue Virus, which is registered with the code PDB (4OIG), being possible to obtain other information about it; The ChemIdplus (https://chem.nlm.nih.gov/chemidplus/), where through it, the structures of the Curcumin ligands were obtained, and six curcuminoids were found. However, now only two of them were subjected to Docking tests, with the others being tested for later tests. The curcuminoids used in the tests were: Curcumin (code 458-37-7; MW: 368,383 -100% similar) and Monodemethylcurcumin (code 149732-51-4; MW: 354.3562 -90.5123% similar); And the ChemSpider repository (http://www.chemspider.com/), where the respective structures mentioned above were downloaded in the mol format. After obtaining the protein (NS1) and its curcuminoid binders, both were submitted to the Molecular Docking simulation, through the software UCSF Chimera® [30] AutoDock Vina, where at the beginning, the protein preparation was carried out to perform this simulation, being removed from this molecule all residues of H2O and (SO4)-2.

During the docking, the search for the scoring function was performed, this function is used to identify and select the possible and better conformations formed between ligand-protein, being used during the same, the algorithm of conformational search, being that this algorithm Predicts and analyzes the values of Free Binding Energy (ΔG), which will locate the best coupling of the ligand in the active site of the receptor, During the same, is also obtained. After docking, the coupling region between this receptor and its respective ligands was obtained.

3 RESULTS AND DISCUSSIONS

Molecular docking, also known as molecular anchoring, is a technique that provides the researcher with various spatial conformations, that is, estimates of the free energy binding between a protein and the ligand, even before they are synthesized [31]. From each spatial conformation, the free energies of binding (between the binder and its target) are provided, and the lowest energy is considered the most likely to justify the conformation of the interaction [21], thus helping to "Targeting" the non-target compounds, those having a good interaction with the active site of the receptor [32].

Thus, the non-structural glycoprotein (NS1) of the Dengue Virus (Fig. 1) was initially obtained through the RSCB Protein Data Bank repository, which is registered with the PDB code (4OIG). Is a resolution equal to 2.7 Å.

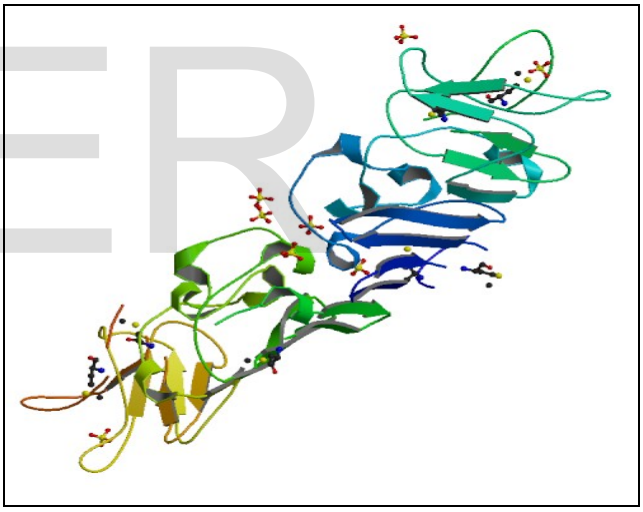

Fig.1. Protein Structure NS1 (4OIG) Source: RSCB Protein Data Bank, 2017.

Then, the molecular structures of the curcuminoid ligands were obtained. The first binder used was Curcumin (Fig.2), formally known as: (1E, 6E) -1,7-Bis (4-hydroxy-3-methoxyphenyl) -1,6-heptadiene-3,5-dione, according to IUPAC, whose molecular formula is C21H20O6; Percent composition: C 68.47%, H 5.47%, O 26.06%, and mass 368.380 Da.

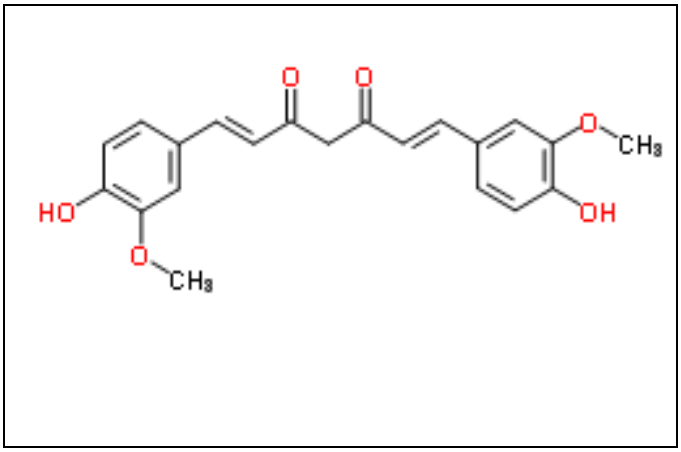

Fig.2. Molecular structure of Curcumin
Source: ChemSpider Repository, 2017

The second ligand used in the Docking tests was the curcuminoid Monodemethylcurcumin (Fig. 3), its nomenclature according to IUPAC is (1E, 6E) -1- (3,4-Dihydroxyphenyl) -7- (4-hydroxy-3-methoxyphenyl) -1,6-heptadiene-3,5-dione, its molecular formula is C20 H18 O6, and its mass is 354,353 Da.

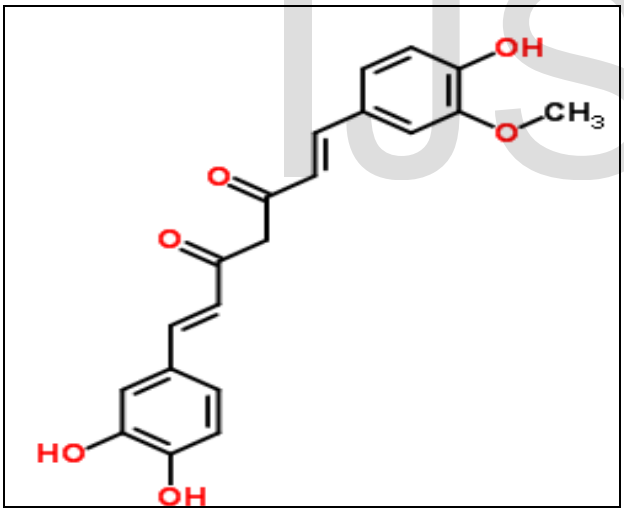

Fig.3. Molecular structure of Monodemethylcurcumin
Source: ChemSpider Repository, 2017

In short, molecular docking has two important steps: in the first it provides the complexes, formed with the protein and the binder, and in the second, it analyzes these possible complexes to identify the most favorable ones, recognizing those that present a Good steric and electrostatic complementarity between ligand-receptor, classifying the most potent compounds [33]. For this, the Docking [34] conformational search algorithm, which predicts and analyzes the Free Binding Energy (ΔG) values, is used to locate the best positioning of the ligand in the active site of the receptor [34]; And the scoring function, which is used to describe the intensity of the association or binding affinity between the molecules (ligand-receptor), identifying and selecting the best conformations formed [20].

Thus, after performing the molecular anchoring between the target molecule (NS1) and the respective binding molecules (Curcumin and Monodemethylcurcumin), it was observed that for both couplings, ten attractive twins were found between them, and the data of these twins were Available in table form (TABLE 1).

TABLE 1

Attractive NS1 protein docking with curcumin ligand

CURCUMIN			
CHIMERA MODEL	SCORE	RMSD L.B	RMSD U.B
#1.1	-5.6	0.0	0.0
#1.2	-5.6	11.888	16.641
#1.3	-5.3	12.85	16.127
#1.4	-5.2	1.103	2.576
#1.5	-5.2	54.092	55.653
#1.6	-5.1	1.063	1.966
#1.7	-5.0	53.905	56.465
#1.8	-4.9	33.825	37.1
#1.9	-4.8	53.434	55.418
#1.10	-4.8	2.81	4.167

After analyzing the interactions between the Curcumin binder and the NS1 protein, it was observed that among the molecular coupling distances between them, the torsion that obtained the shortest distance was # 1.6, which obtained a value equal to 3.5 Å (Fig. 4); In this torsion, the H6 of the curcuminoid binder is linked to the phenylalanine (PHE) 565 of the E chain of the protein (shown in red); As for the scoring function, which is the parameter used to classify the binder according to its affinity, a value equal to 5.1 was found in this torsion, and for the mean root mean square deviation (RMSD) values: RMSD lb 1.063 Å and RMSD ub 1.966 Å. In AutoDock Vina, the lower the value of the

score function, the better is the affinity between ligand-receptor, and a high affinity between a ligand-receptor usually occurs when these compounds have a low number of torsions and favorable steric interactions [35].

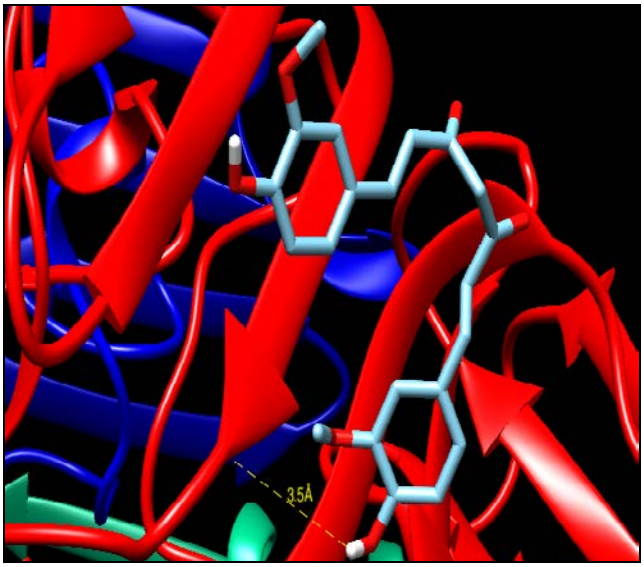

Fig. 4. Molecular coupling of NS1 Protein with Curcumin ligand

The best coupling region, also called the "induced fit", can be observed between the ligand and the active site of the target molecule (Fig. 5), that is, where the best Curcumin ligand interacted with the active site of the target molecule. Target molecule (NS1), this being the orientation and the most favorable and stable conformation between them.

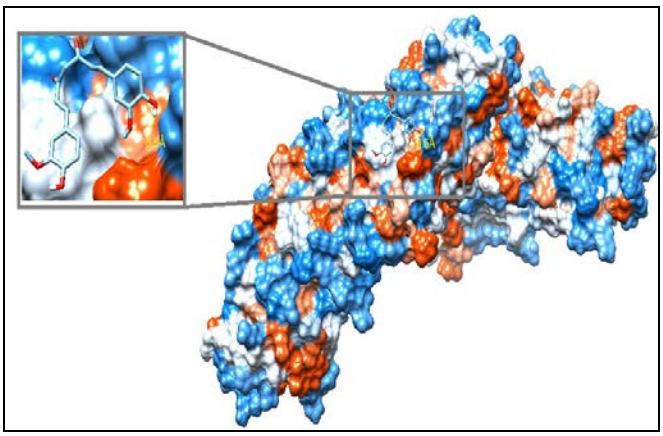

Fig. 5. Molecular coupling of the Curcumin ligand at the active site of the target molecule (NS1).

As for the molecular anchoring of NS1 with the second binder, Monodemethylcurcumin, ten attractive twists were also found, ie ten favorable interactions between both molecules (TABLE 2).

TABLE 2

Attractive NS1 protein doping twist with Monodemethylcurcumin ligand

MONODEMETHYLCURCUMIN			
CHIMERA MODEL	SCORE	RMSD L.B	RMSD U.B
#1.1	-6.3	0.0	0.0
#1.2	-6.2	40.444	43.732
#1.3	-6.0	23.982	26.743
#1.4	-5.9	53.188	57.609
#1.5	-5.9	21.37	24.109
#1.6	-5.9	3.116	6.754
#1.7	-5.8	33.435	39.004
#1.8	-5.8	41.779	45.171
#1.9	-5.7	2.155	4.741
#1.10	-5.7	21.886	24.626

When analyzed, it was observed that the coupling that obtained the shortest distance was # 1.6, whose distance was 3.8 Å (Fig.6), where it can be observed that the H15 of the binder Monodemethylcurcumin is bound to the amino acid Glycine (GLY) 408 of the D chain of the protein (represented by green color); For this torsion the values obtained for the scoring function and root mean square deviation (RMSD) were: score (-5.9), RMSD l.b 3.116 Å and RMSD u.b. 6,754 Å.

Since this coupling was the one that obtained the shortest distance, it is possible to observe this region where the best docking of the Monodemethylcurcumin occurred in the active site of the target molecule (NS1) (Fig.7).

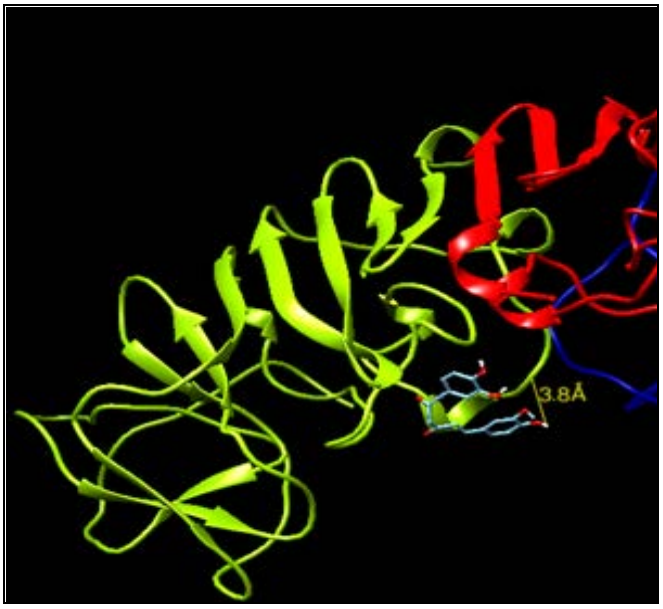

Fig. 6. Molecular coupling of NS1 Protein with Monodemethylcurcumin ligand.

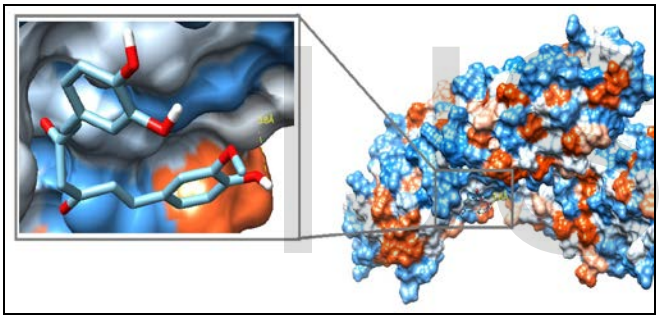

Fig. 7. Acoplamento molecular do ligante Monodemethylcurcumin no sítio ativo da molécula-alvo (NS1).

At first, by comparing both ligands, it is possible to observe that they differ in the presence of a methyl group, since the Curcumin ligand has an additional methyl group in its structure, which considerably influences the reactivity difference of both molecules. Thus, after the accomplishment of both Docking and after comparing them, it was observed that of the two couplings performed, which obtained the shortest distance (3.5 Å) of binding between the protein and the respective ligand, was what involved the Curcumin lingand.

4 CONCLUSÕES

Molecular docking consists of a computational technique that is able to predict / test the affinities and interactions between a ligand and a given receptor site, in order to identify the most probable and stable conformations in this molecular complex, that is, this technique gives the Opportunity to analyze and evaluate how molecular systems behave or can behave in the face of a medium or situation; Being that the behavior of these complexes are generated and provided by means of graphical representation.

Thus, this screening was performed with the purpose of analyzing how some interaction between dengue virus NS1 protein (DENV) with two curcuminoid ligands: Curcumin and Monodemethylcurcumin would occur or would occur. Since they were submitted to the Docking tests two curcuminoids, since these are molecules that have a great utility in popular medicine, being cited as molecules, which present antiviral activity against several types of virus. By means of the molecular docking between the protein (NS1) and both curcuminoids, it was noticeable that for each anchorage between these molecules ten attractive twists occurred, that is, ten favorable interactions. In the anchorage of NS1 with the Curcumin ligand, the interaction that obtained a lower value for the distance (3.5 Å) was the binding of the H6 of the curcuminoid linked to phenylalanine (PHE) 565 of the E chain of the protein. Already in the anchorage of NS1 with the Monodemethylcurcumin ligand, the shortest distance obtained (3.8 Å) was with the H15 binding of the Monodemethylcurcumin ligand bound to the amino acid Glycine (GLY) 408 of the D chain of the protein. Both curcuminoids were shown to be promising ligands, since their molecules interacted well with the receptor site of the protein, however, their activities are still unknown, which is why, there is a need to deepen these studies.

ACKNOWLEDGEMENTS

We thank Dr Ricardo Pires do Santos of Universidade Federal do Ceará (UFC) for his technical support. The Pró-Reitoria de Pós-Graduação e Pesquisa da Universidade Estadual do Ceará(PROPGPQ/UECE) for the support. This work was partly supported The Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico (FUNCAP), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

REFERENCES

[1] D. J. GUBLER, Epidemic Dengue/Dengue Haemorrhagic Fever: A Global Public Health Problem in the 21st Century. ASM Press, Washington, v. 21, p. 1-120. 1997. Availablein: https://pdfs.semanticscholar.org/ec9e/7ff5ce2569090ccfe3653e6b2d2124fd99be.pdf.

[2] A. M. SILVA, Caracterização molecular dos vírus Dengue circulantes em Pernambuco: Implicações epidemiológicas.

Doutorado em Saúde Pública. Fundação Oswaldo Cruz Centro de Pesquisas Ageu Magalhães. Recife, 2013.

[3] M. R. Q. LIMA, Antígeno NS1 dos Vírus Dengue: desempenho de testes disponíveis comercialmente e aplicações alternativas para o diagnóstico precoce das infecções por dengue. Instituto Oswaldo Cruz Pós-Graduação em Medicina Tropical, Rio de Janeiro, 2014.

[4] O.TELLE,A.VAGUET, N.K.YADAV, B.LEFEBVRE, E. DAUDÉ, R.E.PAUL, A.CABEILLAC, B.N. NAGPAL, The Spread of Dengue in an Endemic Urban Milieu-The Case of Delhi, India. Plos one, v. 11, n. 3, 2016. Availableinhttp://journals.plos.org/plosone/article?id=10.1371/journal.pone.0146539

[5] M.G.TEIXEIRA, J.R.SIQUEIRA, G.L.C.FERREIRA, L.BRICKS, G.JOINT, Epidemiological Trends of Dengue Disease in Brasil (2000-2010): A Systematic Literature Search and Analysis. PLOS Neglected Tropical Diseases. European Editorial Office, v.7, 2013. Availableinhttp://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002520.

[6] R. F. PEIXOTO, Análise dos sorotipos de vírus dengue no estado da Paraíba, no período de 2012-2015. Universidade Federal da Paraíba, João Pessoa, 2016.

[7] R.CHEN,N.VASILAKIS, Dengue - Quo tu et quo vadis? Viruses, Basel, v.3, n. 9, p. 1562-1608, 2011. Availableinhttps://www.ncbi.nlm.nih.gov/pmc/articles/PMC3187692/.

[8] B. D.LINDENBACH, H. J.THIEL, C. M. RICE, Flaviviridae: the viruses and their replication. In Knipe DM & Howley P.H. Fields Virology, Filadélfia, 4 ed., p. 1101–1152, 2007. Availablein : http://www.viala.org/img/Lindenbach2007.pdf.

[9] A. C. PINHO, Diagnóstico e caracterização molecular do vírus Dengue circulante na cidade de Salvador, Bahia, Brasil. Dissertation. Instituto de Ciências da Saúde, Universidade Federal da Bahia, Salvador, 2013.

[10] L. A. CASTRO, Avaliação da detecção da proteína NS1 no diagnóstico da infecção pelo vírus dengue-3 em comparação a outrosmétodos laboratoriais utilizados no diagnóstico da dengue. Universidade de São Paulo Faculdade de Medicina de Ribeirão Preto. Ribeirão Preto – SP. 2008.

[11] D. E.ALVAREZ, E.A.L.DE LELLA, S.FUCITO, A.V. GAMARNIK, Role of RNA structures present at the 3′UTR of dengue virus on translation, RNA synthesis, and viral replication. Virology, London, v.339, p. 200-212, 2005. Availablein: http://www.sciencedirect.com/science/article/pii/S0042682205003375.

[12] E. A.HENCHAL, J. R. PUTNAK, The dengue viruses. Clinical Microbiology Reviews, v. 3, n. 4, p. 376-396, 1990. Availablein: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC358169/pdf/cmr000049-0090.pdf.

[13] C. D. L. GUARDIA, R.LLEONART, Progress in the identification of dengue virus entry/fusion inhibitors. BioMed Research International, v. 2014, 2014. Availablein: https://www.hindawi.com/journals/bmri/2014/825039/.

[14] A. L. ROTHMAN, Dengue: defining protective versus pathologic immunity. The Journal of Clinical Investigation, Ann Arbor, v. 113, n. 7, p. 946-951, 2004. Availablein https://www.ncbi.nlm.nih.gov/pmc/articles/PMC379334/.

[15] B. C. S.NOVAES, L. P. B. SCOTT, Modelagem molecular e Docking de Proteína-Ligante. II Simpósio de Iniciação Científica da Universidade Federal do ABC, Santo André, 2009.

[16] R.BARTENSCLAGER, S.MILLER, Molecular aspects of Dengue virus replication. Future Microbiology, London, v. 3, n. 2, p. 155-165, 2008. Availablein: http://www.futuremedicine.com/doi/abs/10.2217/17460913.3.2.155?journalCode=fmb.

[17] T.J.CHAMBERS, C. S.HAHN, R.GALLER, C. M. RICE, Flavivirus Genome, Organization, Expression and Replication. Annual Reviews of Microbiology, v.40, p.649-688, 1990. Availablein:http://download.bioon.com.cn/upload/201102/26/211850b8wxf8ccuubixxxc.attach.pdf.

[18] K.CLYDE,J.L. KYLE, E.HARRIS, Recent Advances in Deciphering Viral and Host Determinants of Dengue Virus Replication and Pathogenesis. Journal of Virology, Baltimore, v. 80, n. 23, p. 11418-11431, 2006. Availableinhttps://www.ncbi.nlm.nih.gov/pmc/articles/PMC1642597/.

[19] C. M. R. SANT'ANNA, Métodos de Modelagem Molecular para Estudo e Planejamento de compostos Bioativos: Uma Introdução. Rev. Virtual de Química, v. 1, n. 1, p. 49-57, 2009.

[20] A. C FELTRIN, M. M MARTINS, H. R.APPELT, R.KERSTING, Simulação de Docking molecular de açúcares em Hexoquinase. XIV Simpósio de ensino, pesquisa e extensão. UNIFRA, 2010.

[21] D. B.KITCHEN, H.DECORNEZ, J. R.FURR, J.BAJORATH, , Docking and scoring in virtual screening for drug discovery: methods and applications. Nature Reviews, v. 3, p. 935-949, nov. 2004. Available em < http://www.nature.com/nrd/journal/v3/n11/full/nrd1549.html.

[22] T. V.PYRKOV, I. V.OZEROV, E. D.BALITSKAYA, R. G. EFREMOV, Molecular docking: The role of noncovalent interactions in the formation of protein-nucleotide and protein-peptide complexes. Russian Journal of Bioorganic Chemistry, v. 36, 4, p. 446-455, 2010.

[23] B. M. REBOREDO, Modelagem molecular aplicada ao estudo de ligantes candidatos a fármacos com atividades Antileishmaniais. Fundação Universidade Federal de Mato Grosso do Sul, Campo Grande, 2012.

[24] S. A.ALENCAR, Utilização de ferramentas computacionais para o estudo do impacto funcional e estrutural de NSSNPS em genes codificadores de proteínas. Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Belo Horizonte, 2010.

[25-] L. S. SILVA, Estudos computacionais de Esfingomielinases D: Docking, Dinâmica Molecular e Métodos Híbridos QM/MM. Universidade Estadual Paulista Júlio de Mesquita Filho. São José do Rio Preto, 2015.

[26] A.C.A.CARNEIRO, A.L.A. REIS, P.F. SILVEIRA,E.M. RIBEIRA, C.S. FERREIRA, B.M. SILVA. Análise do envolvimento da proteína NS1 de dengue virus na modulação da atividade transcricional no promotor de il-6 em células hepáticas humanas. Blucher Biochemistry Proceedings, v.1, p.68-69. 2015. Availableinhttp://pdf.blucher.com.br.s3-sa-east 1.amazonaws.com/biochemistryproceedings/v-jaibqi/0007.pdf.

[27] H. ZHOU, C. S. BEEVERS, S. HUANG, Targets of curcumin. Curr Drug Targets, v. 12, n. 3, p. 332–347, 2012. Available em https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3025067/.

[28] L.L.LEON, C. A. C. ARAÚJO, Biological activities of Curcuma longa L. Mem Inst Oswaldo Cruz, Rio de Janeiro, v. 96, n. 5, p. 723-728. 2001. Availableinhttp://www.scielo.br/scielo.php?script=sci_arttext&pid=S00740276200100050026.

[29] L.COLLINO, Curcumina: de Especiaria à Nutracêutico. Trabalho de Conclusão de Curso. Universidade Estadual Paulista Júlio de Mesquita Filho, Faculdade de Ciências Farmacêuticas, Araraquara, 2014.

técnicas de QSAR-2D (HQSAR) e Docking molecular. Universidade Federal do Rio de Janeiro, Centro de Ciências da saúde Faculdade de Farmácia, Rio de Janeiro, 2012.

[33] S. D. SOUZA, Estudo de inibidores de colinesterases aplicando

[34] I. A.GUEDES, C. S.MAGALHÃES, L. E. DARDENNE, Receptor-ligand molecular docking. Biophysical Reviews, v. 6, n. 1, p. 75-87, 2014. Availablein: https://www.researchgate.net/publication/259399172_Receptor-ligand_molecular_docking

[35] U. R. BARBOSA, Seleção de compostos naturais candidatos à inibição da enzima isocitrato liase do Paracoccidioides spp.: uma abordagem por triagem virtual e dinâmica molecular. Universidade Federal de Goiás, Goiás, 2016.

[30] E.F PETTERSEN.; T.D.GODDARD, C.C.HUANG, G.S. COUCH, D.M.GREENBLATT, E.C MENG, T.E. J. FERRIN , UCSF Chimera—a visualization system for exploratory research and analysis Comput Chem. V. 25, n. 13, p. 1605-12, oct, 2004.

[31] R. L. SOUZA, **Aplicação da técnica de ancoragem molecular na otimização do fármaco hipoglicemiante metformina** 2015. Trabalho de Conclusão de Curso (Bacharelado em Farmácia). Centro Universitário Luterano de Palmas, Palmas, Tocantins, 2015.

[32] R. P.RODRIGUES,S. P. MANTOANI, J. R.DE ALMEIDA, F. R.PINSETTA, E. P.SEMIGHINI, V. B DA SILVA, C. H. P DA SILVA, Estratégias de Triagem Virtual no Planejamento de Fármacos, Revista Virtual de Química, v. 4, n. 6, p.739-736, dez., 2012. Available in:http://rvq.sbq.org.br/imagebank/pdf/v4n6a10.pdf.