Bixinoids potentially active against dengue virus: a molecular docking study

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

[1] State University Ceará(UECE)- From Brazil, [2] Federal University of Ceará(UFC)- Brazil

Abstract—Dengue fever is a disease caused by arboviruses, belonging to the family Flaviviridae, genus Flavivirus, presents four serotypes (DENV 1-4), being transmitted by the mosquito Aedes aegypti (female). The Flavivirus genome contains a single open reading frame, which encodes a single polyprotein and is then cleaved to generate three structural proteins that make up the viral particle: C (capsid), E and prM and seven non-structural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. Protein E, approximately 60 kDa , is the largest structural protein in the virion, accounting for the fundamental biological properties of the virus. Its structure consists of three distinct domains: domain I or central domain, domain II and domain III, where domain III of protein E (ED3) is involved in receptor binding and is the highest target of neutralizing antibodies. The bixin and norbixin binders are major carotenoids found in *Bixa orellana L*. (urucum) belonging to the Bixaceae family, one of which is liposoluble and the other is water soluble, respectively. These substances are responsible for the reddish coloration of annatto, a plant native to South America. In this work, the interaction between the DENV-4 protein and the Bixin and Norbixin ligands, using computational simulation, aiming at molecular Between bixinoids ligands and dengue virus protein serotype four (DENV-4). The present study was developed in four stages: (1) Obtaining the structures of Bixin and Norbixin ligands in the chemspider repository (http://www.chemspider.com/); (2) Preparation of bixinoid ligands using Arguslab software; (3) Obtaining the protein from DENV-4 in the protein data bank repository and preparing it for docking; (4) Molecular protein-binding docking using the Chimera software. After completion of the molecular coupling between bixin-protein, the following values were found; RMSD l.b: 3,601 Å and RMSD u.b: 9,113 Å, Score of -4.3 and 17 active twists. As well as the values found for the molecular docking between the norbixin ligand and the DENV-4 protein were; RMSD l.b: 2,699 Å and RMSD u.b: 3,609 Å, and Score of -5.2, it being possible to identify that both ligand-protein complexes reached 17 active twists in the Chimera software. Using the Arguslab and Chimera software, it was possible to determine the spatial orientation of the bixinoids ligands to the active site of the DENV-4 protein, where the obtained distances of 2,4 Å between the protein and the Bixin ligand and 2,3 Å between The protein and the Norbixin ligand, indicate stability of the binding-protein compound, which reflects in great possibilities of the dockings realized to be used in the production of new drugs against dengue fever.

Keywords: DENV-4.Protein E.Bixin. Norbixin. semi-empirical. Docking Molecular.

1 INTRODUCTION

Dengue fever is a disease caused by arboviruses, belonging to the family Flaviviridae, genus Flavivirus, presenting four serotypes (DENV 1-4), being transmitted by the mosquito Aedes aegypti (female). In Brazil, the transmission has been occurring continuously since 1986, registering until December 2016 (1,487,673) cases of dengue [1], [2]. The Flavivirus genome contains a single open reading frame, which encodes a single polyprotein and is then cleaved to generate three structural proteins that make up the viral particle: C (capsid), E (envelope) and prM (precursor of the M membrane) and seven non-structural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 [3].

Protein E, approximately 60 kDa (kilodalton), is the largest structural protein in the virion, accounting for the fundamental biological properties of the virus. In addition to being the main viral antigenic component, protein E acts as a viral hemagglutinin, has fusogenic capacity with host cell membranes, induces protective immune response and interacts with receptors on the surface of target cells [4], [5]. Responsible for the phenotypic, antigenic, tropism and by virus entry into the cell [6], [7]. Its structure consists of three distinct domains: domain I or central domain, domain II and domain III involved in the in vivo and in vitro production of neutralizing antibodies against DENV [8]. The domain III of protein E (ED3) is involved in receptor binding and is the highest target of neutralizing antibodies, so it is suggested that this domain contains residues responsible for tropism and virulence in flaviviruses [9], [10]. The same has been studied as a candidate for the development of subunit vaccines against various Flaviviruses.

- *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 Pharmaceuticals Sciences and Pharmaceutical. Federal University of Ceará. Chemistry graduateby the State University of Ceará.Brazil. email: marinho.marcia@gmail.com*
- *Jacilene Silva degree course student in chemistry by the State University of Ceará (UECE), Brazil*
- *Aurineide Ribeiro Lima 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.*

Bixin and norbixin binders are major carotenoids found in *Bixa orellana L.* (Urucum) belonging to the

Bixaceae family; One of which is liposoluble and the other is water soluble, respectively. These substances are responsible for the reddish color of urucum [11]; A plant native to South America, has as its main product the intense red color seed, which has a rich pericarp in bixin dye that belongs to the group of carotenoids [12]. The medicinal properties of urucum are still poorly studied; however, bixin may contribute to the protection of cells and tissues against free radicals [13].

This study aimed to study the molecular docking between bixinoids ligands (bixin and norbixin) and dengue virus serotype four (DENV-4). Molecular docking consists of a molecular modeling method that aims to find the most likely orientation and conformation of a binder anchored at a given target, allowing the prediction of the spatial structure of the receptor-ligand complex and its respective free energy formation [14], [15]. The study of the binding-protein interaction is of great importance, since its understanding allows the discovery of drugs with high potency, greater selectivity and fewer adverse effects [16]. Thus, the aim of this work is to analyze the interactions between the DENV-4 protein and the Bixin and Norbixin ligands, using computational simulation.

2 METHODOLOGY

2.1. Computer Resources

For the accomplishment of this work, all the software of simulation used, are software with license free of charge for academic purposes.

All simulations were performed on a personal computer, with Windows 7 Ultimate 64 Bit Operating System, with x64 processor with Intel® Core ™ i3-5005U CPU @ 2.0 GHz, 4 GB of RAM .

2.2. Obtaining the molecular structures of the ligands Bixin and Norbixin

From the ChemSpider repository (http://www.chemspider.com/), the Bixin and Norbixin ligand structures were obtained under identification codes ID 4444638 and ID 4444661.

2. 3.Preparation of Ligands for simulation routines

After obtaining the bixinoid ligand structures, the structural optimization and electronic characterization of ligands were performed using the Arguslab® software [18]; (HF-SCF) open shell (UHF-Unrestricted Hartree-Fock), configured for 200 interactions (using the Austin Model 1 (QM-AM1) (NDDO) method). 1000 cycles), with a convergence value of 10-10 kcal mol-1 using STO-6G base function sets, with the purpose of minimizing energy, aiming at the identification of the electronic and structural properties of the molecule [19].

2. 4.Obtaining and preparation of DENV-4 protein

The crystalline structure of the DENV-4 ED3 mutant protein with L387I PDB code (5B1C) was obtained from the protein databank repository, which is a database for 129,367 biological macromolecular structures [17]. Then the preparation of the protein for the molecular docking using the Chimera® software [20] was carried out, in which the residues (H2O and SO4) that could influence the satisfactory coupling result were removed, later the structure was saved in Mol2 format.

2. 5.Molecular docking simulations

The molecular dockings simulations were performed in the Chimera® software, and the inputs were prepared to execute the simulation routines under the conditions of flexible bixinoids and rigid protein (PROTEIN E- DENV4).

3 RESULTS AND DISCUSSIONS

Arguslab® and Chimera® software are important tools used in scientific development, they help and guarantee practicality and speed in molecular modeling and coupling processes [21]. Molecular modeling produces information essential for the development and discovery of new drugs, making it

possible to obtain specific properties of a molecule that can interact with the receptor [22].

The bixin ligand molecule (Figure 1) has molecular formula C25H30O4, mass of 394,503 Da and mass of the monoisotope of 394.214417 Da.Structurally it can be classified as a mixed-chain organic compound, because it has two functional groups, carboxyl and methoxyl, branched as it presents fourth methyl, polyunsaturated substituent groups being classified as belonging to the norcarotenoid family.

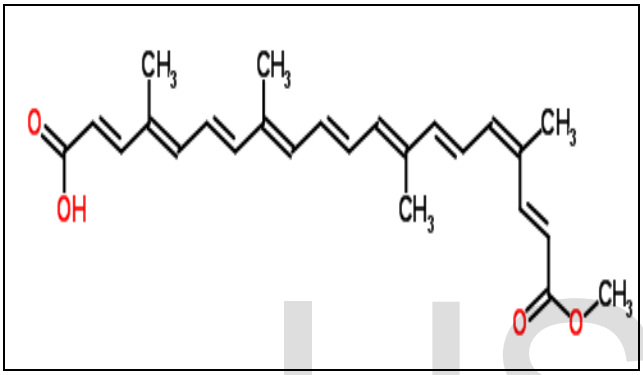

Fig.1. Molecular structure of the bixin ligand obtained from the ChemSpider repository.

However, the norbixin binder (Figure 2) has a molecular formula C24H28O4, a mass of 380,477 Da and a mass of the monoisotope of 380.198761 Da.Structurally it can be classified as an organic compound belonging to the carboxylic acid function because it has two carboxyls at the ends,indicating a higher acidity character than bixin, branched because it presents fourth methyl, polyunsaturated substituent groups.

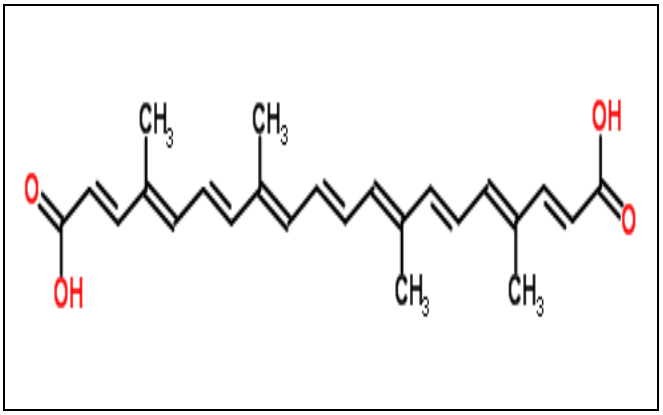

Fig.2. Molecular structure of the Norbixin ligand obtained from the ChemSpider repository

Initially the bixin and norbixin ligand structures were submitted to the semi-empirical calculations of the Arguslab® program, where it was possible to characterize and optimize the bixinoids (Figure 3).

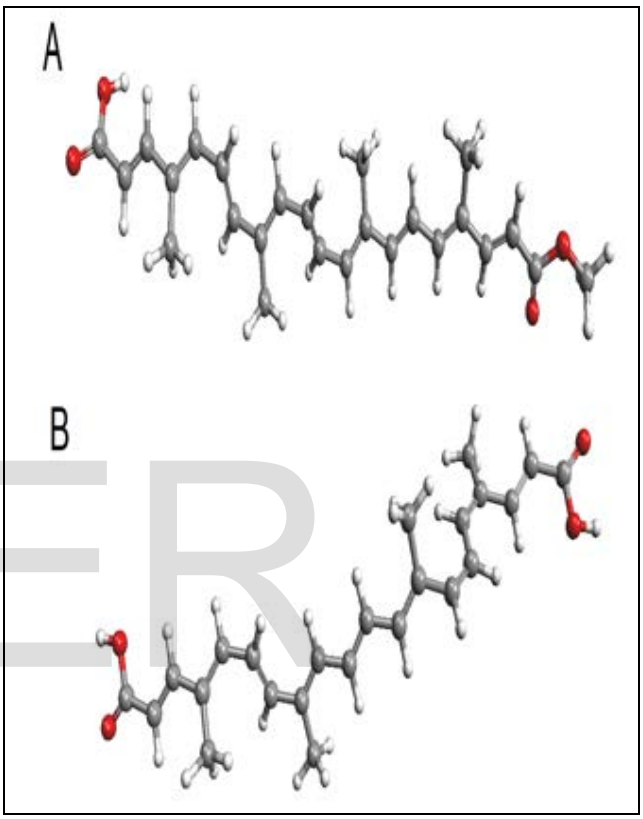

Fig.3. Molecular structures of the ligands Bixina [A] and Norbixina [B], optimized by Arguslab software.

When submitted to hydrolysis in alkaline media, bixin loses one molecule of methanol, producing norbixin [23]. In that it is possible to notice that bixin has the methyl ester in its structure and norbixin has the carboxyl. Therefore, the difference between bixinoid ligands is easily identified by the simple analysis of their structures.The pharmacological effect of a molecule is due to its interaction with receptors, which are in most cases, macromolecules present in the cell membrane [24]. Proteins are an important group of drug receptors that normally serve as receptors for ligands [25].In this perspective, Figure 4 demonstrates the molecular docking between the bixin ligand and the DENV-4 protein run in the Chimera® software.

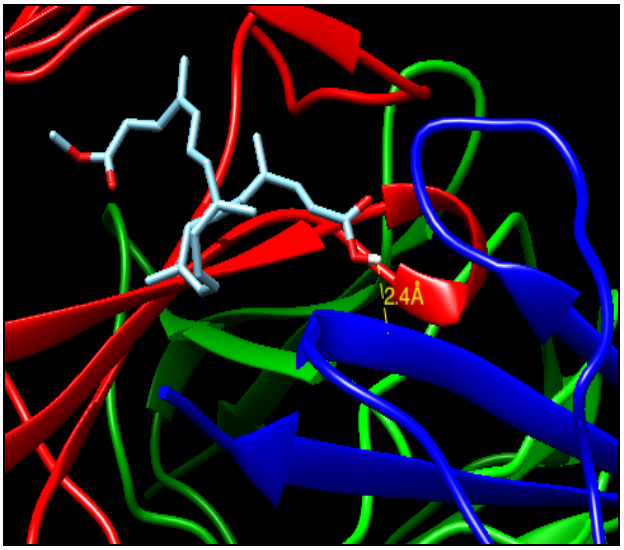

Fig.4. Molecular docking between Bixin Binder and DENV-4 Envelope E Protein using Chimera® software.

After termination of the molecular coupling, the deviation between the bixin binder and the 5B1C protein was analyzed, where RMSD values (mean root mean square deviation) between the structures were calculated. The following values are found; RMSD l.b: 3,601 Å and RMSD u.b: 9,113 Å, Score of -4.3 and 17 active twists. It was also possible to observe that the H30 atom of the linker is closest to the amino acid ASP77 (Aspartic Acid), located in the B chain (represented by the blue color) of the DENV-4 protein at 2.4 Å of distance.

The active site is associated with a specific region of the macromolecule and can be considered isolated from the remainder of this [26], in Figure 5 we can identify the active site of 5B1C protein coupled to bixin ligand,Indicating the potential of this ligand in interacting with the active site of the proteins.

The molecular docking methodology proposes the assembly of the experiment through four situations: rigid ligand and rigid protein, rigid ligand and flexible protein, flexible ligand and rigid protein, and flexible ligand and flexible protein. While more options for flexibility, more functions, or variables are included for calculations, the more computationally the experiment is [27]. Therefore, performing the molecular docking between the Norbixin ligand and the protein (Figure 6) was developed using the flexible linker and the rigid protein.

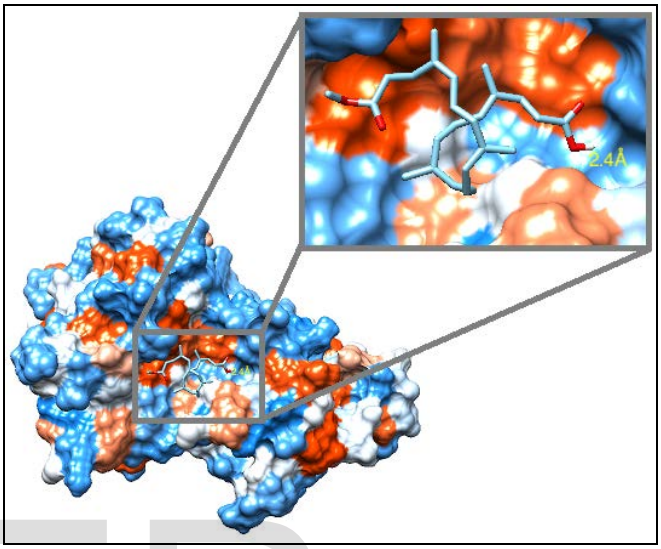

Fig.5. Active site of Dengue Virus 4 Protein 5B1C, bound to bixin; Where the hydrophobic regions are shown in orange and the hydrophilic regions in blue.

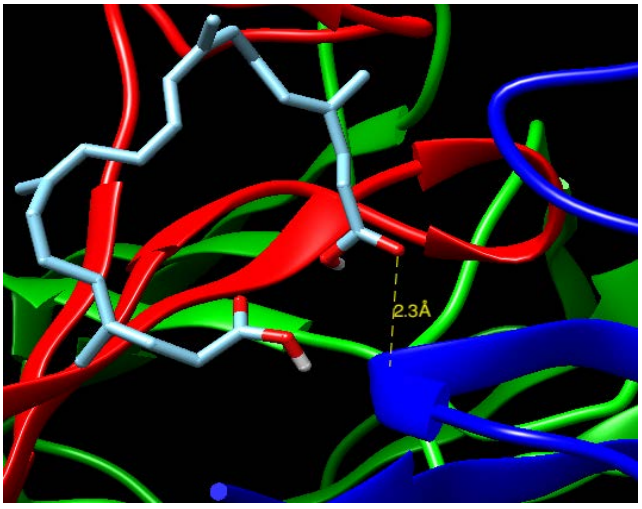

Fig.6. Molecular docking between the Norbixin binder and the DENV-4 protein, using the Chimera software.

In figure 7, it is possible to observe that norbixin binds to the protein, by means of the hydrophilic region, highlighted in blue color.

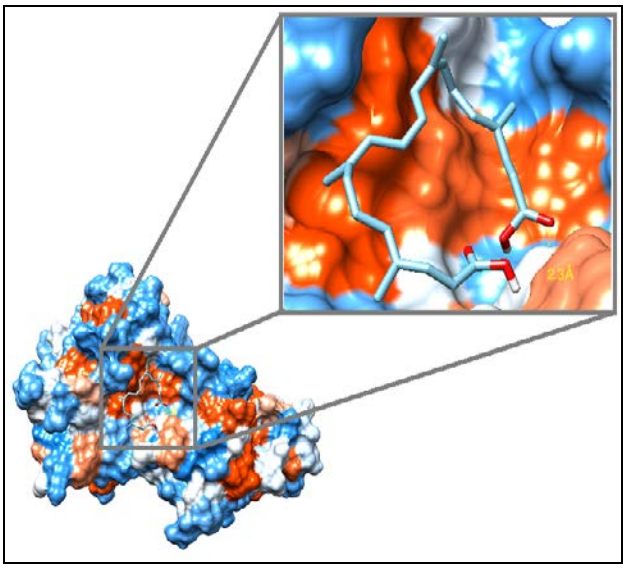

Fig.7. Molecular docking between the Norbixin binder and the DENV-4 protein, using the Chimera software,Application of the protein binding interaction

The RMSD values found for the Norbixin ligand were; RMSD l.b: 2,699 Å and RMSD u.b: 3,609 Å, and Score of -5.2, as well as the bixin binder, norbixin also achieved 17 active twists in the Chimera software. The four oxygen atom (O4) of the linker is closest to the amino acid ASP77, located in the B chain (Blue) of the protein at 2.3 Å away.

After analyzing the results obtained, it was possible to notice that the bixin and norbixin ligands were coupled in the same chain and amino acid of the DENV-4 protein, in blue chain (B) and amino acid ASP77 (Aspartic Acid), both produced 17 kinks Active in the Chimera software, differing only in the atoms bound to the protein and in the distance between binder-receptor, in which the h30 atom of bixin was 2.4 Å away from ASP77, and norbixin O4 was at 2.3 Å Away from the amino acid.

3 CONCLUSION

Using semi-empirical quantum molecular modeling and molecular docking simulations, it was possible to determine the spatial orientation of the bixinoid ligands to the active site of the DENV-4 protein, where the obtained distances of 2,4 Å between the protein And the Bixin ligand and 2.3 Å between the protein and the Norbixin ligand, indicate stability of the ligand-protein compound, which reflects the potential of these ligands in binding to the active site of that protein, this being an initial step for the development of Future drugs.

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] Ministério da Saúde. http://portalsaude.saude.gov.br/index.php/oministerio/principal/secretarias/ svs/ dengue. Acesso em: 06 de março de 2017.

[2] Ministério da Saúde. http://portalsaude.saude.gov.br/index.php/cidadao/principal/agencia saude/27153-tocantins-recebera-r-1-7-milhao-de-recurso-adicional-para-combate-ao-aedes. Acesso em: 23 de março de 2017.

[3]D.B. LINDENBACK, C.M. RICE,Flaviviridae: The viruses and their replication. In: Knipe DM, Howley PM & Fields BN: Fields Virology. 4 ed. Philadelphia: Lippincott Williams and Wilkins. New York. p. 991-1042, 2001.

[4] Y. CHEN, T. MAGUIRE, R. E. HILEMAN, J. R. FROMM, J. D. ESKO, R. J. LINHARDT, R. M. MARKS, Dengue virus infectivity depends on envelope protein binding to target cell heparan sulfate. Nat. Med. 1997. 3(8):866–871.

[5] Y. MODIS, S. OGATA, D. CLEMENTS, S. C. HARRISON, Structure of the dengue virus envelope protein after membrane fusion. Nature. 2004; 427(6972):313-9.

[6] D. W. BEASLEY, J. G. AASKOV, Epitopes on the dengue 1 virus envelope protein recognized by neutralizing IgM monoclonal antibodies. Virology, v. 279, n. 2, p. 447-58, Jan 20 2001.

[7] K.C. LEITMEYER, D.W. VAUGHN, D.M. WATTS, R. C. SALAS, C. RAMOS, R. RICO-HESSE, Dengue virus structural differences that correlate with pathogenesis. J Virol 1999 Jun;73(6):4738-47.

[8] W.M. WAHALA, A.A. KRAUS, L.B. HAYMORE, M.A. ACCAVITTI-LOPER, A.M. SILVA, Dengue virus neutralization by human immune sera: role of envelope protein domain III reactive antibody. Virology, v. 392, p. 103-113, 2009.

[9] S. S. WHITEHEAD, J. E. BLANEY, A. P. DURBIN, B. R. MURPHY, Prospects for a dengue virus vaccine. Nat Rev Microbiol. 2007. 5: 518-528.

[10]Y. MODIS, S. OGATA, D. CLEMENTS, S. C. HARRISON, Variable surface epitopes in the crystal structure of dengue virus type 3 envelope glycoprotein. J Virol, v. 79, n. 2, p. 1223-31, Jan 2005.

[11] L. R. P. LIMA, T. T. OLIVEIRA, T. J. NAGEM, A. S. PINTO, P. C. STRINGHETA, A. L. A. TINOCO, J. F. SILVA, Bixina, Norbixina e Quercetina e seus efeitos no metabolismo lipídico de coelhos. Braz. J. vet. Res. anim. Sci. São Paulo, v. 38, n. 4, p. 196-200, 2001.

[12] F. M. F. CORLETT, Qualidade fisiológica de sementes de urucum (bixa orellana L.) armazenadas em diferentes ambientes e embalagens. 2004.94p. Tese (Doutorado em Ciência e Tecnologia de Sementes.Faculdade de Agronomia Eliseu Maciel. Universiadade Federal de Pelotas.Porto Alegre, 2004.

[13] C. R. SILVA, L. M. ANTUNES, M. L. P. BIANCHI, Antioxidant action of bixin against cisplatin-induced chromosome aberrations and lipid peroxidation in rats. Pharmacological Research, v. 43, n. 6, p. 561-566, 2001.

[14] D. B. KITCHEN, H. DECORNEZ, J. R. FURR, J. BAJORATH, Docking and scoring in virtual screening for drug discovery: methods and applications. Nat Rev Drug Discov, v. 3, 11, p. 935-49, 2004.

[15] T. PYRKOV, I. OZEROV, E. BALITSKAYA, R. 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.

[16] I. I. BASKIN, I. G. TIKHONOVA, V. A. PALYULIN, N. S. ZEFIROV, (2003) Selectivity fields: comparative molecular field analysis (CoMFA) of the glycine/NMDA and AMPA receptors. J Med Chem. 46(19):4063-9.

[17] PROTEIN DATA BANK. Download da estrutura em formato pdb da proteína do DENV-4. Disponível em: http://www.rcsb.org/pdb/explore/explore.do?structureId=5b1c.

[18] M. A. THOMPSON, ArgusLab 4.0. 1. Planaria Software LLC, Seattle, WA, 2004. available in: http://www.arguslab.com

[19] J. SILVA, A. R. LIMA, M. M. MARINHO, R. R. CASTRO, E. S. MARINHO, Utilização do método semi-empírico (QM-PM3) para caracterização do Fármaco Ataluren: MEP,HOMO e LUMO. XXII Encontro de Iniciação à Pesquisa Universidade de Fortaleza 17 à 21 de Outubro de 2016.

[20]E. F. PETTERSEN, T. D. GODDARD, C. C. HUANG, G. S. COUCH, D. M. GREENBLATT, E. C. MENG, T. E. FERRINHO, UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. 2004 Oct;25(13):1605-12.

[21] H. L. B. BRAZ, L. C. S. CANDIDO, M. M. MARINHO, M. O. C. ROCHA, E. S. MARINHO, Estudo in silico das propriedades de interação entre Curcumina e Apolipoproteína E4 (apoE4): O novo alvo de ataque a demência da Alzheimer em condições iniciais. XXI Encontro de Iniciação à Pesquisa Universidade de Fortaleza 19 à 23 de Outubro de 2015.

[22] C. R. RODRIGUES, Processos Modernos no Desenvolvimento de Fármacos: Modelagem Molecular. Cadernos Temáticos de Química Nova na Escola, nº 3, maio de 2001. Disponível em: http://qnesc.sbq.org.br/online/cadernos/03/modelag.pdf

[23] K. C. COSTA, Estudo fitoquímico de Bixa orellana L., Bixaceae e aplicação de seu óleo em formulação cosmética. 2007. Dissertação (Mestrado em Ciências Farmacêuticas) – Setor Ciências da Saúde, Universidade Federal do Paraná, Curitiba. 2007.

[24] S. F. DIAS, C. C. TAVEIRA, Homologia de proteínas como ferramenta na construção de novos fármacos. Cenarium Pharmacêutico, Ano 4, n° 4, Maio/Nov 2011, ISSN: 1984-33801.

[25] J. S. LAZO, GOODMAN & GILMAN: As Bases Farmacológicas da Terapêutica. 11. ed. Rio de Janeiro: Editora McGraw Hill, 2006. p. 25-34.

[26] S. C. IZIDORO, Algoritmos genéticos para identificação de sítios ativos em enzimas. Tese de Doutorado da Universidade Federal de Minas Gerais. Belo Horizonte; março de 2014.

[27] K. B. LIPKOWITZ, T. R. CUNDARI, D. B. BOY, Reviews in Computational Chemistry, Nova Jersey: John Wiley & Sons Inc., v. 26, 2008.