# In silico study of the drug oseltamivir and its interactions with influenza hemagglutinins 5C0r and 5C0s

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**Abstract**—Influenza is globally recognized for its capacity to generate epidemic and pandemic waves with annotations of the main virological, clinical and epidemiological characteristics, including its mortality and lethality and the context of its occurrence in a pandemic. However, the drugs currently produced, loses their efficacy to each viral mutation, becoming a problem in various economic and social sectors. Based on this context, this research aimed at the chemical-quantum evaluation of an anti-influenza molecule using molecular modeling. In the methodology of this work, the semi-empirical AM1 method was used, performing structure optimization, calculations of molecular properties, electrostatic potential map, HOMO-LUMO frontier orbitals and Mulliken population analysis. Mulliken's analysis showed a variation of load differences, obtaining the minimum and maximum values in the oxygen atoms (ranging from -0.2262 to 0.2712), nitrogen (ranging from -0.2147 to -0.0036) and carbon (ranging from -0.5077 to 0.4555). In electronic terms, the HOMO-LUMO density maps showed a more concentrated density in the atoms O2, C14, C15, C18, C19, C21 and C22 contributing significantly to the formation of the same. The MEP electrostatic potential map indicated that the O1, O2, O3, O4, N6 and C12 atoms presented higher numbers of negativity of electrostatic potentials due to electronegative oxygen properties. The ligand structure was obtained by molecular modeling. The docking results were performed using the UCSF Chimera® software version1. 8.1. The analysis of the docking results showed that the molecule interacted with the protein specifically at the hydrophobic site, varying its energy from -4.8 Kcal.mol-1 to -7.3 Kcal.mol-1, which it was possible to visualize the interactions of the hydrophobic and hydrophilic regions of the protein. With this it is understood that, the results obtained are of great relevance, because approach through the computational simulation highlighted virtual screening can be a fundamental step for the development process of new drugs. Thus, it can be concluded that the study of this molecule could serve as a basis for future research on structural improvement in the construction of inhibition analogues for future influenza.

Keywords: Docking. Molecular modeling. Semi empirical. Theoretical chemistry.

## 1 INTRODUCTION

influenza is considered the infection that caused most illnesses and deaths to date. It is an acute respiratory system disease, caused by the Influenza virus, having high transmission capacity and global distribution [1]. Influenza virus infections cause seasonal epidemics and occasional pandemics, when new viruses are introduced into humans [2]. One such pandemic occurred in 2009 when an outbreak of an influenza A (H1N1) influenza virus (H1N1) began in Mexico and was rapidly spread throughout the world [3]. Despite efforts to vaccinate, WHO estimates that the influenza virus results in 1 billion infections, 3-5 million cases of serious illness and 300,000-500,000 deaths per year [4].

The etiologic agent of influenza is Myxovirus influenzae, also called influenza virus [5]. Influenza virus has a nucleus of 70 nm is a spherical particle having an internal diameter of approximately 110 nm. The surface is covered by proteins of approximately 10 nm in length with functions essential to the virus: a lectin, a hemagglutinin (HA) responsible for the entry of the virus into our cells where it will multiply; and neuraminidase (NA) allows the release of the new viruses that will conquer new cells [6].

Until today in the midst of modern medicine there is little research that shows the effectiveness of new drugs in combating the influenza virus. There are few products developed to combat this problem, products that in addition to being expensive, sometimes are difficult to access and can cause quite unpleasant side effects to the human organism [7].

Influenza virus is always changing, the difficulty of producing drugs that have effectiveness is rather scarce, so the principle of difficulty of this research will

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be to produce a compound that can demonstrate an antiviral effect in different modifications of the virus.

Although vaccination is the first-choice tool for influenza prevention, specific antivirals are important agents in the treatment of this disease. Vaccine development has only been partially effective in controlling influenza because of the high mutational capacity that the virus presents. Currently available in Europe and the United States are four influenza-specific antiviral drugs: classic drugs, amantadine and rimantadine, and second-generation antivirals, zanamivir and oseltamivir [8].

The recognition that the enzymes hemagglutinin and neuraminidase are of fundamental importance for the success of the infection opens the possibility of interfering in the activity of one or both enzymes of the life cycle of influenza virus, which can slow the process of cellular invasion, allowing the immune system enough time to be effective against the virus. However, hemagglutinin has a very common mutative capacity, making it difficult to attack prolonged drugs. And it was precisely by knowing the life cycle of the influenza virus and understanding how it is possible to intervene in this cycle that today there are two medicines for the treatment of influenza: Relenza® [zanamivir] and Tamiflu® [oseltamivir], Roche and GlaxoSmithKline, respectively [9].

Oseltamivir has already been responsible for the inhibition of influenza virus Hemagglutinin, NS1 and NS2 proteins in some previous years. In general, bioinformatics using the molecular docking method would be very useful for the anchoring of the oseltamivir ligand and its theoretical analogues in the structure of the HA proteins of influenza 2015 and 2016, in order to verify a possible molecular interaction, and could contribute to studies future vaccines. Since these viruses can return at any time, as was the case this year [10].

Molecular modeling consists of a set of tools for the construction, editing and visualization, analysis and storage of complex molecular systems. These tools can be applied in strategies of direct and indirect modeling of new drugs [11].

The AM1 (Austin Model 1) method is a semi-empirical method developed on the MNDO (Modified Nehlect of Diatomic Overlap) method, which has become quite popular. In addition, the AM1 method predicts formation enthalpies more accurately and rapidly [12].

The discovery and improvement of new drugs has its foundation in the molecular planning of new structures capable of presenting desired pharmacological effects, with safety, adequate bioavailability for its therapeutic use and posology convenience [13]. The aim of the present work was to use molecular modeling (AM1 method) to analyze the chemical-quantum properties of the anti-influenza molecule oseltamivir and molecular anchorage in two H1N1 influenza viruses of 2015 and 2016.

## 2 METHODOLOGY

The structure of Oseltamivir was obtained from the PUBCHEM molecule repository (https://pubchem.ncbi.nlm.nih.gov/) in output format "mol2". In the Arguslab® software [14], the structure of the molecule was optimized for three-dimensional. Then, the molecule was sent in mol format to the ACD / ChemSketch® [15] for analysis of structure properties.

Then the software Arguslab® (http://www.arguslab.com) was used, in which the software was defined to optimize the structure by the semi-empirical calculations (Austin model 1 - QM-AM1) following the approach of Hartree -Fock for the maximum of 300 interactions [16]. Using the output files, we obtained the electrostatic potential map (MEP) and the frontier orbitals (HOMO-occupied largest molecular orbital), (LUMO - unoccupied minor molecular orbital).

Next, the molecular anchorage simulation was performed, using the Microsoft Windows Seven® Ultimate 62-bit Operating System with Intel® Celeron® processor, through the UCSF Chimera® [17] version 1 computational package. 8.1 (https://www.cgl.ucsf.edu) of free access.

In the molecular coupling, two hemagglutinin proteins from the influenza A virus of 2015 and 2016, originating from New Caledonia of the 3rd and 4th mutation, were used [18]. The proteins were obtained from the Protein Data Bank [5] with 5C0R and 5C0S codes encoded by X-ray diffraction with resolution of 1.70Å and 2.20Å respectively. In the Chimera software, the molecular docking was performed with values of X = 12, Y = 22 and Z = 6 of the position of the ligand in the

receptor. For the analysis of the molecular coupling, the methodology described by GROSDIDIER et al. [20] was used, evaluating the total energy, estimated Gibbs free energy variation (ΔG), docking positions, molecular forms, energy, RMSD (Root Mean Square Deviation) and molecules to the fit of proteins.

The structures designed in three-dimensional form are not necessarily in their more stable conformation, thus occurring some distortions in the molecule, presenting an unfavorable formation of lengths and angles of connection, in which the molecules are optimized following the process of energy minimization, used to correct these distortions [21]. However, the realization of the chemical-quantum calculations through the ArgusLab® software, allowed the optimization of the structural geometry of the molecule oseltamivir (figure 1) by the semi-empirical method (Austin Model 1 - QM-AM1), determining an energy conformation and the heat of formation (ΔHf) -201.7812 kcal mol-1, calculated by the atomization energies, using the atomization enthalpy of the atoms [16] is stable by the method of interactions SFC -12484.546 kcal mol-1.

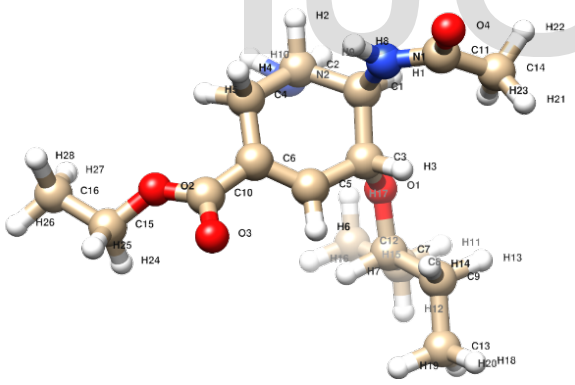


**Fig.1.** Optimized structure of the oseltamivir molecule (front).

The Mulliken population analysis is a method used to determine the partial atomic charges, whose results are strongly dependent on the set of bases used. It is a partition scheme, based on the use of density and coating matrices, to distribute the electrons of a molecular entity somehow fractional among its various parts (atoms, bonds, orbitals) [22]. However, the comparison of population analysis for a number of molecules is useful for a quantitative description of intramolecular interactions, chemical reactivity and structural regularities [23]. A very nucleophilic end in Oxygen atoms (ranging from -0.2262 to 0.2712), Nitrogen (ranging from -0.2147 to -0.0036) and Carbon (ranging from -0.5077 to 0.4555) can be observed by analyzing the oseltamivir molecule (Table 1), these being the preferred sites for reactions.

**Table 1:** Mulliken charges from oseltamivir molecule.

| Charge of Mulliken | | |
|---|---|---|
| 1 | C | 0.2233 |
| 2 | C | -0.1347 |
| 3 | C | -0.0169 |
| 4 | C | -0.1875 |
| 5 | C | -0.5077 |
| 6 | O | 0.2712 |
| 7 | O | -0.2581 |
| 8 | O | -0.0471 |
| 9 | C | 0.0413 |
| 10 | N | -0.0978 |
| 11 | N | -0.0036 |
| 12 | N | -0.0075 |
| 13 | N | -0.2147 |
| 14 | C | -0.1871 |
| 15 | C | 0.3893 |
| 16 | O | -0.0853 |
| 17 | C | 0.4555 |
| 18 | O | -0.2764 |
| 19 | C | -0.2455 |
| 20 | C | 0.3806 |
| 21 | O | -0.0707 |
| 22 | O | -0.1116 |

In the valence band, the occupied electronic level of higher energy is called (HOMO) and in the conduction band the low power unoccupied electronic level is called (LUMO - Lowest Unoccupied Molecular Orbital or Orbital Unoccupied Minor Energy). These orbitals have an essential function in delimiting the reactivity of chemical compounds as well as in the identification of excitation of the molecules. The HOMO energy is directly related to the Ionization Potential, which characterizes the susceptibility of the molecule to the attack of electrophiles. The energy of LUMO is related to electron affinity and characterizes the susceptibility to

attack by nucleophiles [24]. Using the data generated in the structural optimization of the drug oseltamivir, it was possible to plot the HOMO frontier orbitals (fig 2a) and LUMO (fig 2b), where it is possible that both forms are formed by the contribution of atoms O2, C14, C15 , C18, C19, C21 and C22, and which have an asymmetric distribution between the phases.

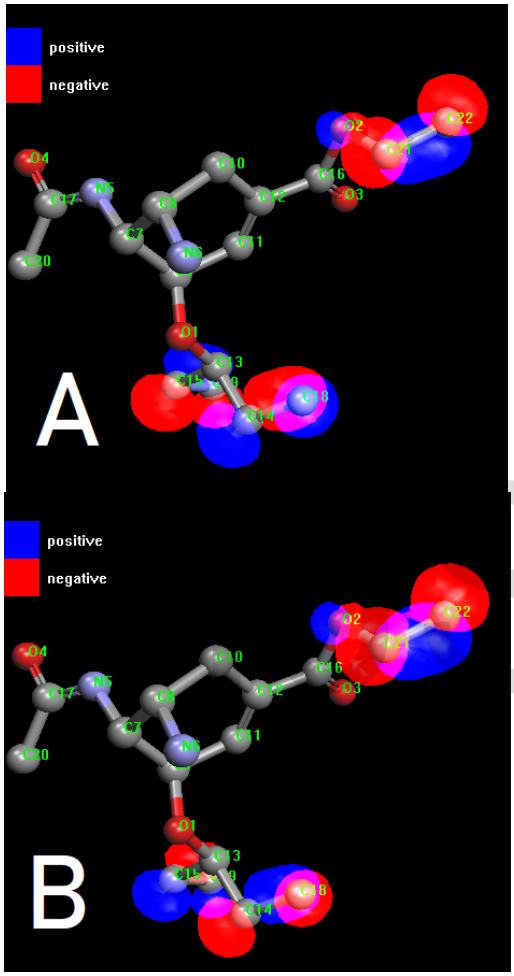


**Fig.2.** Frontier orbitals for oseltamivir: (a) HOMO Orbital; (b) LUMO Orbital.

The maps of electrostatic potential (MEP) measures the interaction of a positive charge point with nuclei and electrons of a molecule [25]. The interaction between molecules occurs between regions of opposite electrostatic potential.

Figure 3 shows the maps of electrostatic potential (in hartrees) of the drug molecule oseltamivir, in which the "white" color zones indicate positive values of the potential, that is, of electron defi ciency; while the "red" color zones indicate negative values of the electrostatic potential, that is, regions rich in electrons, emphasizing that the atoms O1, O2, O3, O4, N6 and C12 presented greater potential for electrostatic negativity, this fact is due to the high electronegativity of oxygen. With this test carried out, it is noted that the oxygen atoms will be more regioselective in docking processes than the others. With this test performed, it is noted that the oxygen atoms will be more selective in docking processes than the others.

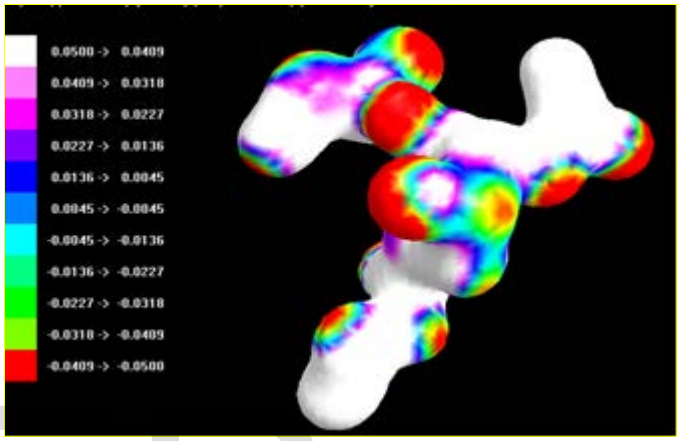


**Fig.3.** Electrostatic potential map of Oseltamivir.

According to CARNEIRO and collaborators (2017) [26], describes that Molecular Docking is a computational technique performed through calculations that shows the fit of two molecules in three-dimensional space, thus seeking a preferred way with which a small ligand accommodates itself in the active site of a biological macromolecule and to estimate its binding affinity. From the molecular anchoring methodology a (Figure 4) shows the molecular docking between the oseltamivir ligand and the hemagglutinin protein (5C0R).

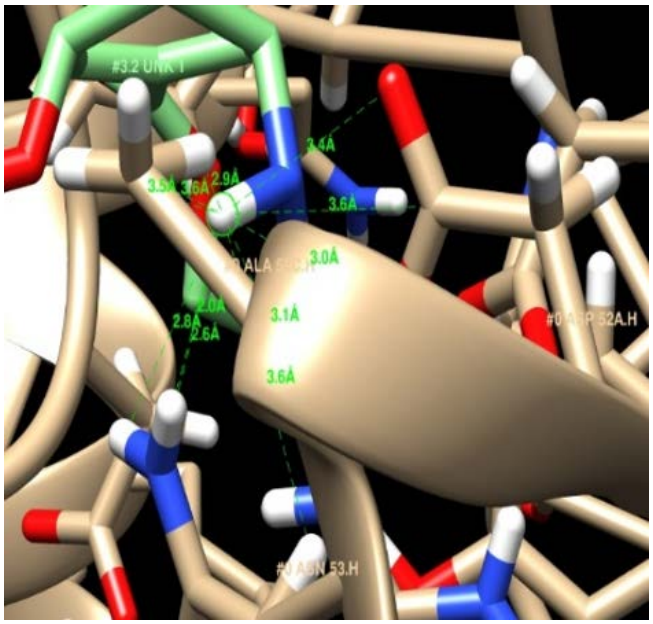


**Fig.4.** Molecular interaction between curcumin and DENV-4 visualized in the CHIMERA® Software.

After the molecular docking between oseltamivir and HA-5C0R, 321 interactions between the ligand and the protein were verified. By the screening methods [4], an interaction showed 4 bonds in the protein with values of 1.6Å, 2.0Å, 2.2Å, 2.3Å distance between C1-ALA227, O1-ALA227, O3-TYR195, O4-ASP153, respectively. The docking values of this interaction were 1.41Å (RMSD l.b.), 2.13 (RMSD u.b.) and Score -4.2, in addition to presenting 9 active torsions.

Thus, as in the blind docking (Fig. 5), all molecules interacted with the protein, however this time, they interacted specifically at the hydrophobic site, with a range of energy ranging from -4.8 Kcal mol -1 to -7.3 Kcal mol-1. These energy values characterize a favorable interaction of the compounds with the protein, in which experimental analyzes could better clarify these results [8]. Figure 5 shows the interaction with the hydrophobic (red region) and hydrophilic regions (blue region) of the protein.

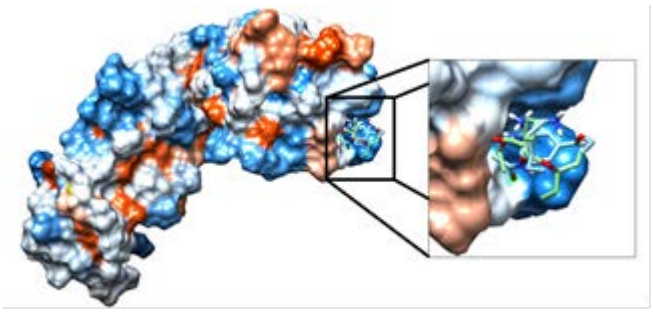

**Fig.5.** Active site between oseltamivir and Hemagglutinin-5C0R protein.

The Figure 6 shows the docking between oseltamivir and HA-5C0S, in this docking 118 interactions were shown. The selected interaction presented RMSD l.b. of 1.17Å, RMSD u.b. of 3.76Å and score of -5.2; showed extremely strong O3 bonds with GLY97, GLN90 and ASN94 with distance values respectively of 2.2Å, 2.9Å and 3.1Å. According to figure 7, the interactions entailed a binding in the hydrophobic characteristic protein (red region) while the hydrophilic region did not look like bonds.

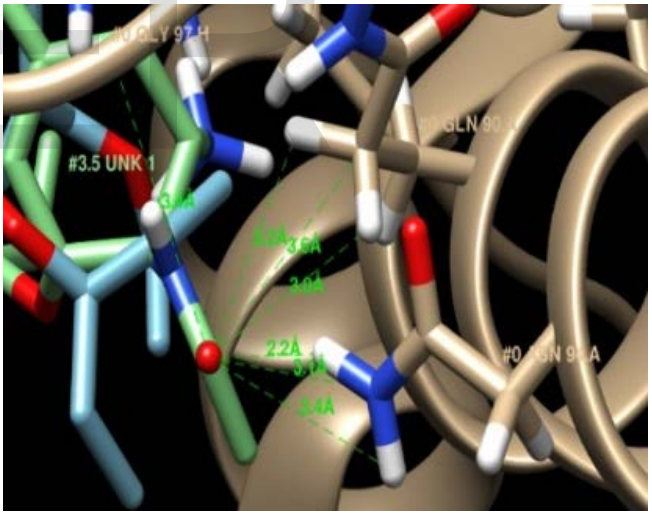


**Fig.6.** Molecular interaction between oseltamivir and HA-5C0S visualized in the CHIMERA® Software.

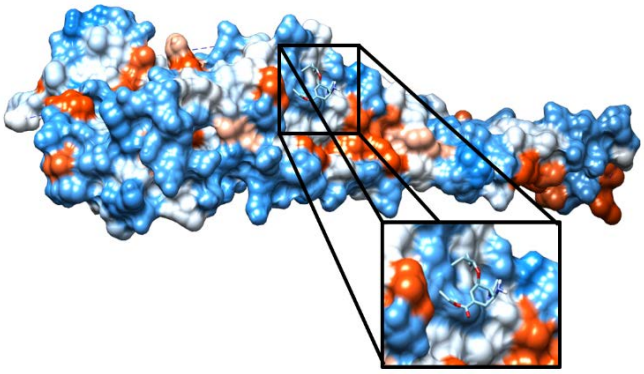

**Fig.7.** Active site between Oseltamivir and Hemagglutin-5C0S protein.

## 4 CONCLUSIONS

The present work carried out an in silico study of the molecule oseltamivir, obtaining structures that maintained the nucleophilic site, and with molecular properties without many distortions. Mulliken's analysis showed a variation of load differences, obtaining the minimum and maximum values in the oxygen atoms (ranging from -0.2262 to 0.2712), nitrogen (ranging from -0.2147 to -0.0036) and carbon (ranging from -0.5077 to 0.4555). In electronic terms, an analysis of the HOMO-LUMO orbitals revealed that the density maps showed a more concentrated density in the atoms (O2, C14, C15, C18, C19, C21 and C22), thus contributing to their formation. The electrostatic potential map MEP indicated that the atoms (O1, O2, O3, O4, N6 and C12) presented higher negativity numbers of electrostatic potentials due to electronegative oxygen properties. With this test realized, these atoms will be more selective in docking processes than the others.

Thus, studies performed by docking calculations between the oseltamivir ligand and the hemagglutinin (5COR) protein allowed to interfere with some observations common to the ligand studied. With this, we obtained the docking values of this interaction and Score of -4.2, besides presenting 9 active torsions. As discussed (Figure 7), the molecule interacted with the protein specifically at the hydrophobic site, varying its energy from -4.8 Kcal.mol-1 to -7.3 Kcal.mol-1, which showed the interactions of the hydrophobic (red region) and hydrophilic regions (blue region) of the protein.

In view of the foregoing, it is believed that the purpose proposed by this work was achieved by describing the main molecular and structural characteristics favorable to the formation of the protein-ligand complex studied in the course of this research. In view of the fact that the reactions caused may be associated with structural properties of the molecule used as a drug, which is understood that results obtained are of great relevance in the pharmacological study of this ligand.

With this we conclude that the approach through computer simulation to highlight virtual screening can be a fundamental step in the development process of new drugs.

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