In-silico Study of the Developed Hydroxychloroquine-based ACE2 Inhibitor Molecules Against COVID-19: Molecular Modeling and Docking

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Abstract-In the present study, we will verify the action of hydroxychloroquine-based derivatives on ACE2 which is considered to be the main portal of entry of the SARS-CoV-2 virus and constitutes an exciting target given its relative genetic stability compared to viral proteins. Thus, 81 molecules derived from hydroxychloroquine by substitutions at 4 different positions were generated in-silico and then studied for their affinity for ACE2 by molecular docking. Only 4 molecules were retained because of their affinity and bioavailability demonstrated by molecular dynamics and molecular docking calculations using COSMOTHERM and Material Studio software.

Keywords—hydroxychloroquine; molecular modeling; Covid-19; ACE2; affinity

I. INTRODUCTION

Covid-19 is caused by SARS-CoV-2 [1] which is one of the emerging respiratory viruses, including MERS (Middle East Respiratory Syndrome) and SARS-CoV (Severe Acute Respiratory Syndrome), which all belong to the same family of coronaviridae [2]. The most common symptoms include fever, dry cough, muscle fatigue, headache, and diarrhea in some cases. Discovered in Wuhan in China in December 2019, this disease quickly spread throughout the world [3]. A few months after the start of the pandemic, more than 2,500,000 people have already died, and more than 100 million have been infected. To date, there is no effective cure against Covid-19 [4] and it is necessary to wait several months before the use of vaccines reaches a satisfactory level. During this time, the treatment of the disease relies on drugs to relieve the severe symptoms of the disease. To this end, more than 200 drugs have already been the subject of clinical trials, including hydroxychloroquine [5].

By targeting the SARS-CoV2 virus, it has been shown that chloroquine and several of its derivatives can bind to several viral proteins [6, 7]. Besides, chloroquine and its derivatives can also bind to ACE2 [8, 9]. The choice of this target is based on two main facts: the first is that ACE2 is the main entry point for the virus and the second is because of its genetic stability
Chloroquine and hydroxychloroquine have been the subjects of several studies to explain their effect on the SARS-CoV2 virus proteins [12, 13] such as NSP3, main protease, RNA dependent polymerase, spike glycoprotein, ADP-ribose-1 monophosphatase, and NSP9 replicase protein [6]. These interactions, determined in silico, mean that chloroquine and its derivatives are potential active molecules against SARS-CoV2 [14, 15]. It appears-in many studies- that hydroxychloroquine (version 17.1) interacts with ACE2 (PDB code: 6M18) [8].

We performed the present work to study, in silico, the effect of a series of hydroxychloroquine-derived molecules on ACE2. Thus, 81 molecules were obtained by substitution of hydroxychloroquine on 4 different positions. The study aims to estimate the affinity of the selected molecules for ACE2, the prediction of their bioactivity, molecular docking, and their electronic properties by COSMO-ROS using COSMOTHERM software (version 15.0). The second part of this work is focused on the selection of the preferred molecules according to their physicochemical properties calculated by molecular modeling and molecular docking method using Materials Studio software (version 17.1).

II. MATERIALS AND METHODS

The chemical properties of hydroxychloroquine were calculated using SwissADME (Table I). Four positions, 4, 11, 12, and 13, were chosen to undergo modifications by substituting the original chemical groups with others (Table II).

TABLE I. HYDROXYCHLOROQUINE PROPERTIES CALCULATED BY SWISSADME

| Properties            | Values                   |
|-----------------------|--------------------------|
| Molecular weight      | 335.9g/mol               |
| XLogP                 | 3.6                      |
| Hydrogen acceptors    | 4                        |
| Hydrogen donors       | 2                        |
| Rotative bonds        | 9                        |
| Exact mass            | 335.17644g/mol           |
| Mono-isotopic mass    | 335.17644g/mol           |
| TPSA (Topological polar surface area) | 48.4Å² |
| Heavy atoms           | 23                       |

TABLE II. CHEMICAL GROUPS SELECTED TO CREATE NEW MOLECULES.

| Position | Original | First modification | Second modification |
|----------|----------|--------------------|---------------------|
| 4 - R1   | H        | CH₂                | F                   |
| 11 - R2  | CH₃      | CH₂OH              | CHO (aldehyde)      |
| 12 - R3  | CH₂      | CH₂-NH₂            | CH-CH₂              |
| 13 - R4  | CH₂      | -O-                | CH₂OH               |

It should be noted that only these 4 positions leave the receptor site relatively stable. These substitutions made it possible to generate 81 new molecules, which were subjected to the study of their affinity for ACE2 by examining two essential parameters: Ligand Efficiency (LE) and Lipophilic Ligand Efficiency (LLE). Molinspiration Chemoinformatics software carried out the prediction of the bioactivity of the selected molecules. This step allows classifying the molecules according to their capacity to bind to the protein. The study on the protein ACE2-ligand complexes was investigated using SeeSAR software. In the last step, we studied the electronic properties of these molecules by COSMO-ROS software.

III. RESULTS AND DISCUSSION

A. Construction of a Series of Inhibitor Molecules from Hydroxychloroquine

After having done the modifications mentioned above, we managed to formulate 81 different molecules from Hydroxychloroquine with the help of the SeeSAR [16]. The main objective was to obtain a candidate molecule closer to Hydroxychloroquine but increase the receptor ACE2 (Table III).
mathematical equation is given by:

\[ \text{LE} = \frac{(1.37 \times \text{plC50})}{\text{HA}} \]  

\( (1) \)

pIC50 corresponds to the Ligand concentration occupying 50% of the receptor, and HA represents the number of atoms other than hydrogen (heavy atoms).

**B. Affinity Study**

Parameters that explain in detail the affinity of each molecule for the receptor site include:

**LE:** Refers to each atom's bond energy in a molecule toward the receptor. In other words, it refers to the ratio between \( \Delta G \) (Gibbs energy) and the number of atoms other than hydrogen in a molecule. If the LE value is greater than 0.3, the molecule in question will have a greater probability of fixing itself to the receptor [17]. The corresponding mathematical equation is given by:

\[ \text{LE} = \frac{(1.37 \times \text{plC50})}{\text{HA}} \]  

\( (2) \)

Every molecule was docked with the receptor ACE2 model to show the possibility of forming a stable complex. From 81 molecules, it was found that only 19 were proved to interact with the specified target favorably. A drug's capacity to interact with a receptor is directly linked to its affinity for the receptor [20]. The estimated affinity for the 19 candidate molecules was calculated, and the results are shown in Table IV. The results show that only 8 molecules are having a good affinity when compared with molecule_01 (Hydroxychloroquine).

**C. Bioactivity Prediction**

The selected 8 molecules were examined in Molinspiration Chemoinformatics software to know the preferred binding protein of each molecule [21]. The results of the bioactivity prediction are summarized in Table V. The symbol "XXXX" means that the molecule can interact with 4 types of protein, one of them being an enzyme inhibitor, and symbol "0" means that the molecule can interact with 5 types of protein, one of them being an enzyme inhibitor, and symbol "0" means that there is no particular interaction with any protein. Only 4 molecules can have the same activity as Hydroxychloroquine.

**D. Study on the Protein-Ligand Complexes**

Following the analysis of the candidate molecules by Molinspiration, bioactivity results and the molecules' properties proved that only 4 of the 19 molecules had the desired
properties (had the estimated affinity closer to that of Hydroxychloroquine). These are Molecule_07, Molecule_09, Molecule_18, and Molecule_73. Figure 1 shows the candidate molecules in interaction with the receptor ACE2 and energy values in kJ/mol calculated from desolvation and interaction energies. SeeSAR was used to study these complexes. Each sphere represents atoms in the molecule, and each atom interacts differently with the atoms of the receptor (Figure 1).

![Fig. 1. Interaction of the 4 potential candidate molecules with the protein ACE2.](image)

Each sphere represents an atom, and the greater the sphere's size, the greater the interaction with the receptor site. When the sphere is colored green, this signifies that the contribution of this atom is favorable. On the contrary, the sphere colored in red characterizes the unfavorable atom's interaction contribution. If the sphere is not colored, the value of $\Delta G$ is null or close to zero. Therefore, the contribution of the corresponding atom is negligible. Blue color represents polar regions, while yellow color represents hydrophobic regions [22]. The following points describe the interpretation of the results obtained in Figure 1.

- **Molecule 01**: The Hydroxychloroquine molecule shows a low affinity with its receptor site. Most of its atoms colored with green have a low $\Delta G$ values, and it has an unfavorable contribution formed by the N17 (2.7kJ/mol) and C2 (2.3kJ/mol).

- **Molecule 07**: atoms N17 and N3 are colored in red, with $\Delta G$ values of 9.3 and 1.6 kJ/mol, respectively. Therefore, the interaction with the receptor site is unfavorable. However, most of the atoms have negative $\Delta G$ values C19 (-5.7kJ/mol), O46 (-5.2kJ/mol), C18 (-3.7kJ/mol), C41 (-3.1kJ/mol), C10 (-2.9kJ/mol), and Cl6 (-1.9kJ/mol), signifying that their interaction with the receptor site is favorable.

- **Molecule 09**: C21 is the only atom colored in red ($\Delta G = 6.4kJ/mol$), corresponding to unfavorable interaction. Atoms C47 (-3.7kJ/mol), C2 (-3.6kJ/mol), C19 (-
from these results, it can be concluded that Molecule 9 will have the best interaction with the receptor site.

E. Prediction of Electronic Properties by COSMO-RS

Conductor-like Screening Model for Real Solvents (COSMO-RS) is a quantum chemistry method based on thermodynamics, which helps to determine chemical potentials for solutions [23]. This method can predict sigma charge densities as well as chemical potentials for each species in the solution. The calculation is done in two main steps: Firstly, geometrical optimization on the molecule was done with the module Dmol3 [24] of the software BIOVIA Material Studio 2017 [25]. Secondly, the obtained cosmo-files were used to calculate sigma profiles and sigma potentials with the COSMOTHERM software [26]. The sigma profile is divided into 3 distinct regions:

- HBD Region: Hydrogen bond donor region: the sigma values are less than -0.01e\(\text{Å}^{-2}\). The negative sigma values mean positive polarities [27].
- Non-Polar Region: \(\sigma\) values are given in the interval -0.01e\(\text{Å}^{-2}\) to +0.01e\(\text{Å}^{-2}\) [28].
- HBA Region: Hydrogen bond acceptor region: the \(\sigma\) values are greater than 0.01e\(\text{Å}^{-2}\). Positive sigma values represent negative polarities [29].

Figure 2 shows that the highest picks for all the selected molecules are found in the non-polar region. They are showing the tremendous non-polar character of the molecule surfaces. That said, the 5 molecules have net peaks in the HBD and HBA regions, making hydrogen bonds as acceptors and donors with the ACE2. The selected molecules meet the criteria that a candidate-molecule must possess to interact with the protein target: HBA, HBD, and hydrophobic sites [30].

Fig. 2. Electronic charge densities of the candidate molecules.

Molecule 01 (Hydroxychloroquine) possesses a small HBD and HBA region. Molecule 07 shows a small HBD region and also some HBA regions. In Molecule 09, there are 3 regions capable of accepting hydrogen atoms and a small hydrogen donor region. Molecule 18 possesses a large enough hydrogen donor region as well as a hydrogen bond acceptor region. A tiny region in Molecule 73, almost negligible, can donate a hydrogen atom (colored in blue) while a more significant part,
colored in red, represents the hydrogen acceptor region; The most prominent picks in the range -0.01eÅ²<σ<-0.01eÅ² are due to the non-polar chemical groups such as CH3, CH2, CH, which are more abundant in the molecules. Negatively charged atoms such as O constitute hydrogen acceptors (region HBA) [31]. The positively charged atoms or atoms with lone pairs of electrons are responsible for the picks in the region HBD (NH, NH2, and NH3). Figure 3 proves the results obtained from the sigma profiles and the energies of desolvation and interaction. All candidate molecules have strong affinities HBD and HBA well balanced. This demonstrates that they have sufficient solubility in water and blood after administration [28].

Fig. 3. Sigma potentials of the four candidate molecules.

IV. CONCLUSION

Taking hydroxychloroquine as the primary molecule, we built 81 new derivative molecules, of which only 4 molecules had improved affinity for ACE2. The modifications of the hydroxychloroquine structure at critical positions enhance properties such as affinity for the receptor site, solubility, and permeability and allow reconsidering the hydroxychloroquine derivative molecules for therapeutic use as a ligand for ACE2. Nevertheless, in-vitro and in-vivo studies will help confirm the results obtained in this study.

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Zaher et al.: In-silico Study of the Developed Hydroxychloroquine-based ACE2 Inhibitor Molecules

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