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In Silico Inhibition Potential of Artemisinin Derivatives Against SARS-CoV-2 Main Protease

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Abstract: The outbreak of COVID-19 caused by the SARS-CoV-2 virus has recently affected millions worldwide. The natural compounds obtained from medicinal plants have been proven to be the source of many treatments throughout history. Efforts to combat Sars-CoV-2 generally focused on repositioning drugs or finding treatments with natural compounds and have been rapidly ongoing. Main protease (Mpro) is a vital protein of SARS-CoV-2 and an important target of drug research. The present study evaluated seven artemisinin derivatives: artemisinin, artemether, arteether, artesunate, dihydroartemisinic acid, dihydroartemisinin and artemisinic acid. For this purpose, the molecular docking study was carried out to investigate the potency of artemisinin derivatives against the SARS-CoV-2 Mpro. As a result, artesunate, dihydroartemisinic acid and dihydroartemisinin had promising results in Mpro inhibition with the binding energies between -8.42 and -9.35 kcal/mol.

Keywords: Artemisinin, Artesunate, COVID-19, Main protease

Artemisinin Türevi Bileşiklerin Sars-CoV-2 Ana Proteaz Proteinine Karşı In Silico İnhibisyon Potansiyeli

Öz: SARS-CoV-2 virüsünün neden olduğu COVID-19 salgını son zamanlarda dünya çapında milyonlarca insanı etkiledi. Şifalı bitkilerden elde edilen doğal bileşiklerin, tarih boyunca birçok tedavinin kaynağı olduğu kanıtlanmıştır. Sars-CoV-2 ile mücadele çabaları genellikle ilaçları yeniden konumlandırmaya veya doğal bileşiklerle tedaviler bulmaya odaklandı ve hızla devam ediyor. Ana proteaz (Mpro), SARS-CoV-2'nin hayati bir proteinidir ve ilaç araştırmalarının önemli bir hedefidir. Bu çalışmada, artemisinin, artemether, arteether, artesunate, dihydroartemisinic acid, dihydroartemisinin ve artemisinic Acid olmak üzere 7 artemisinin türevinin değerlendirilmesi amaçlanmıştır. Bu amaçla, artemisinin türevlerinin SARS-CoV-2 Mpro'ya karşı potansiyelini araştırmak için moleküler modelleme çalışması yapılmıştır. Sonuç olarak, artesunate, dihydroartemisinic acid ve dihydroartemisinin, -8.42 ile -9.35 kcal/mol arasında bağlanma enerjisi ile Mpro inhibisyonu açısından umut verici sonuçlar ortaya çıkarmıştır.

Anahtar Kelimeler: Artemisinin, Artesunate, COVID-19, Ana proteaz

1. Introduction

The outbreak of coronavirus caused by a novel RNA virus emerged at the end of 2019. Although symptomatic treatments are applied, there is no specific treatment yet. Many countries have aimed to reduce mortality by applying phytotherapy throughout the treatment course in COVID-19 patients. Currently, only supportive therapies are available for the COVID-19 pandemic and community restrictions to reduce transmission. Pharmacologically, initial treatment with lopinavir/ritonavir and chloroquine/hydroxychloroquine has been tried [1]. As it affects many

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people worldwide in different ways, the potential of natural molecules for treatment or protection against COVID-19 has been intensively studied from the moment the pandemic was declared [2]. Primary drug targets for SARS CoV-2 include the vital proteins of the virus, 3-chymotrypsin-like protease (3CLpro) or known as Mpro, papain-like protease (PLpro), RNA-dependent RNA polymerase, and spike (S) protein [3,4]. These structural proteins have some similarities with other known viruses such as hepatitis B, hepatitis C, Ebola, MERS-CoV and human immunodeficiency virus (HIV), paving the way to retarget anti-viral drugs approved by the FDA [5,6]. Multiple sequence alignment analysis revealed that 3CLpro was conserved, with 100% identity among all SARS-CoV-2 genomes. The fact that the SARS-CoV and SARS-CoV-2 3CLpro sequence similarity rates are 100% in the active site and 96% in the whole structure indicates that the mutation rate is not very high in this protein [7]. The 3CLpro of SARS-CoV-2 has a Cys-His catalytic dyad (CYS145 and HIS41), consistent with that of SARS (CYS145 and HIS41), Transmissible Gastroenteritis Coronavirus 3CLpro (CYS144 and HIS41) and Human coronavirus 3CLpro (CYS144 and HIS41) [8]. Therefore, inhibitors targeting the 3CLpro protein of SARS-CoV-2 may offer mutation-independent treatment and also inhibit the other type of coronavirus.

There has been significant evidence that the mixture of the protease inhibitors, lopinavir and ritonavir is partially effective against the SARS virus [9]. Lopinavir is an antiretroviral drug that acts by inhibiting the protease enzyme. It is also used in conjunction with ritonavir, as it increases the half-life of ritonavir, a cytochrome enzyme inhibitor. In many countries, the lopinavir/ritonavir mixture decreases the hospitalization duration of patients infected with the SARS CoV-2 virus [10]. Research on plant-based drugs and their acceptability has been increasing in recent years. Since ancient times, natural products and derivatives have been used in folk medicine to treat numerous diseases, including viral infections [11,12]. Phytotherapy research has suggested that it can help discover potentially beneficial compounds against coronaviruses [13]. Globally, herbs having preliminary evidence of anti-viral activity and phytotherapeutic drugs with immunostimulating properties appear to be therapeutic candidates against coronaviruses [13–17].

Organic extracts of *Artemisia Annua* are effective in the treatment of malaria [18,19]. Artemisinin, a sesquiterpene lactone with a peroxide linkage with low toxicity, is stated to be the compound responsible for the biological activity of *Artemisia Annua* extract [20]. According to the literature, the efficacy of artemisinin derivatives as potent anti-virals in the treatment of diseases caused by human herpes virus-6 (HHV-6), human immunodeficiency virus (HIV), HPV bovine viral diarrhea virus (BVDV), and human cytomegalovirus (HCMV) has been proven [21]. Artemisinin-based combination therapy (ACT) has demonstrated in vitro inhibition of SARS-CoV-2 replication after clinical use in malaria at recommended doses [22]. This anti-viral potential has led to artemisinin-derived compounds being one of the promising candidates in the fight against COVID-19. The most important artemisinin derivatives are artemether, arteether, artesunate and dihydroartemisinin, while the main precursors are artemisinic acid and dihydroartemisinic acid [20].

This study reveals how effectively act the artemisinin derivative compounds at the 3CLpro binding site and ultimately how their mode of interaction occurs. Considering the importance of the Mpro protein on the vital cycle of the SARS CoV-2 virus, the disruption of the function of this protein by plant-derived molecules, which are easier to obtain, will be an important step in combating the epidemic. The aim of this study was to examine the inhibitor potential of artemisinin-derived compounds that may be used to combat the COVID-19 infection.

2. Material and Methods

The Maestro Molecular Modeling platform of Schrödinger, LLC was used for molecular docking studies [23]. The PLIP (Protein-Ligand Interaction Profiler) web service was used to provide

atomic-level information on binding properties to identify non-covalent interactions between target macromolecules and ligands [24].

2.1. Protein Preparation

The crystal structure of Sars-CoV-2 main protease in complex with an inhibitor N3 was obtained with 1.7 Å resolution (PDB id: 7BQY) by X. Liu and co-workers recently [25]. The target protein was downloaded from the Protein Data Bank [26] and prepared using protein preparation wizard in Maestro [27]. The missing hydrogens, side chains, and residues were fulfilled using the Prime Module [28]. Since the water molecule in the active site of the target enzyme plays an important role, water molecules were removed except within 5 Å from the bound ligand. The protein structure was further optimized using the OPLS3 force field [29]. Propka was used for protonation states of amino acid residues [30]. The centroid of the bound ligand was considered as the active region.

2.2. Ligand Preparation

The 3D-Structures of the artemisinin derivatives were downloaded from the PubChem database [31], namely artemisinin, artemether, arteether, artesunate, dihydroartemisinic acid, dihydroartemisinin and artemisinic acid. Since it is an anti-viral drug currently used for treatment, lopinavir was used as a standard drug molecule to compare the binding affinity. The downloaded 3-D structures were exposed to ligand preparation procedure on the LigPrep module to produce the lowest energy 3D conformations under the neutral pH using the OPLS3 force field.

2.3. The Protocol of Docking Study

The prepared ligands were docked into the active sites of 3CLpro of SARS-CoV-2 without any restriction with the receptor grid of radii 20 Å. Induced Fit Docking (IFD) algorithm, which has the advantage of allowing flexibility both ligand and the active site residues of the receptor, combined with the default Glide/XP method, was used during the docking simulations. A maximum number of 80 poses were generated for each ligand molecule. For residues within 5 Å of the ligand, Prime refinement was used. Glide redocking was performed for ligands within 30 kcal/mol of the best poses. The binding affinity was determined and ranked based on the docking score. Finally, the single best pose was determined for each structure.

3. Results and Discussion

To find a potential therapeutic effect for COVID-19, in silico studies were performed for common artemisinin derivatives on the binding pocket of the 3CLpro enzyme of Sars-CoV-2. Due to its vital role in the viral life cycle, 3CLpro is an attractive drug target to combat COVID-19.

Based on our results, it was found that all the studied molecules have an excellent binding affinity to the active site of the target protein and a similar interaction pattern with the standard drug molecule of lopinavir. The artesunate was located in the active region of protein more strongly than the other artemisinin derivatives with a docking score of -9.35 kcal/mol. The dihydroartemisinic acid and dihydroartemisinin with docking scores of -8,49 and -8.41 kcal/mol have the closest score to artesunate. The docking scores and interacting residues of all studied compounds were given in Table 1. The results, as shown in Table 1, indicate that all ligands have similar interactions in the active pocket of Mpro with a good binding affinity.

The artesunate could interact with Mpro making four H-bond through carbonyl groups of ligand and NH moiety of GLY143, SER144, CYS145, GLU166 polar residues with the bond distance of 2.18,

2.95, 2.16 and 1.93 Å, respectively. The carbonyl moiety of GLN189 residue is also making water bridged hydrogen bonding interactions with the oxygen atom of the artesunate with the bond distance of 1.78 Å. The hydrophobic interactions were also established between the THR25 and HIS41 residues and endoperoxide bridge moiety of the artesunate. The 2-dimensional interaction diagram of the Artesunate compound with the Mpro protein of Sars-CoV-2 and its 3-dimensional location at the electrostatic potential surface of the active site were illustrated in Figure 2-A and Figure 3, respectively.

Table 1. The docking results were obtained from the best pose of the studied ligand at the active site.

Compound	Docking Score (kcal/mol)	Hydrogen Bonds (with length in Å)	Hydrophobic Interactions (with length in Å)
Artesunate	-9,35	GLY143(2.18), SER144(2.95), CYS145(2.16), GLU166(1.93), GLN189-H_2O(1.78)	THR25(3.93), HIS41(3.68)
Dihydroartemisinic acid	-8,51	GLY143(2.01), CYS145(2.65), GLN189-H_2O(1.78)	MET165(3.64)
Dihydroartemisinin	-8,42	GLY143(1.88), SER144(3.47), CYS145(2.83), HIS 163(3.08)	GLU166(3.56)
Artemisinin	-8,19	ASN142(3.04), GLY143(2.73), SER144(2.51), CYS145(2.79)	HIS41(3.96), HIS163(4.66)
Artemisinic acid	-8,18	ASN142(1.76), GLU166(1.88)	HIS41(3.82)
Artemether	-7,74	GLY143(2.15), CYS145(3.22)	ASN142(3.60), MET165(3.95), GLN189(3.89)
Arteether	-7,87	GLY143(2.10), GLU166(3.71)	THR25(3.83), HIS41(3.97), MET165(4.00), GLU166(3.86), GLN189(3.89)
Lopinavir	-8,59	GLY143(2.74), HIS164(2.03), GLU166(2.07), GLN189(2.06), GLN189-H_2O(2.79), GLN189-H_2O(3.73)	THR25(3.16), LEU27(3.79), HIS41(3.52), ASN142(3.81), PRO168(3.52)

The Dihydroartemisinic acid interacts with Mpro making two H-bond through the COOH end group of ligand and NH and SH moieties of GLY143 and CYS145 residues with the bond distance of 2.01, 2.68 Å, respectively. The water bridged hydrogen bonding interactions have been formed with GLN189, as well. The hydrophobic interaction has been observed between the MET165 residue. The 2-dimensional interaction diagram of the dihydroartemisinic acid with the Mpro of Sars-CoV-2 was illustrated in Figure 2-B.

Dihydroartemisinin has H-donor interaction with GLY143, SER144, CYS145 and HIS163 amino acid residues, Hydrophobic interaction with MET165. Artemisinin and the other derivatives also showed similar interaction, especially with THR25, ASN142, GLY143, SER144, CYS145, HIS41, MET165, GLU166 and GLN189 amino acid residues as well as lopinavir standard drug. The

interactions of all studied molecules and Mpro were shown in Figure 1-A to H. The interaction diagram shows many H-bonding interactions with the catalytic dyad of protein, the same as that of lopinavir. These interactions strengthen the drug potency of this compound against SARS CoV-2.

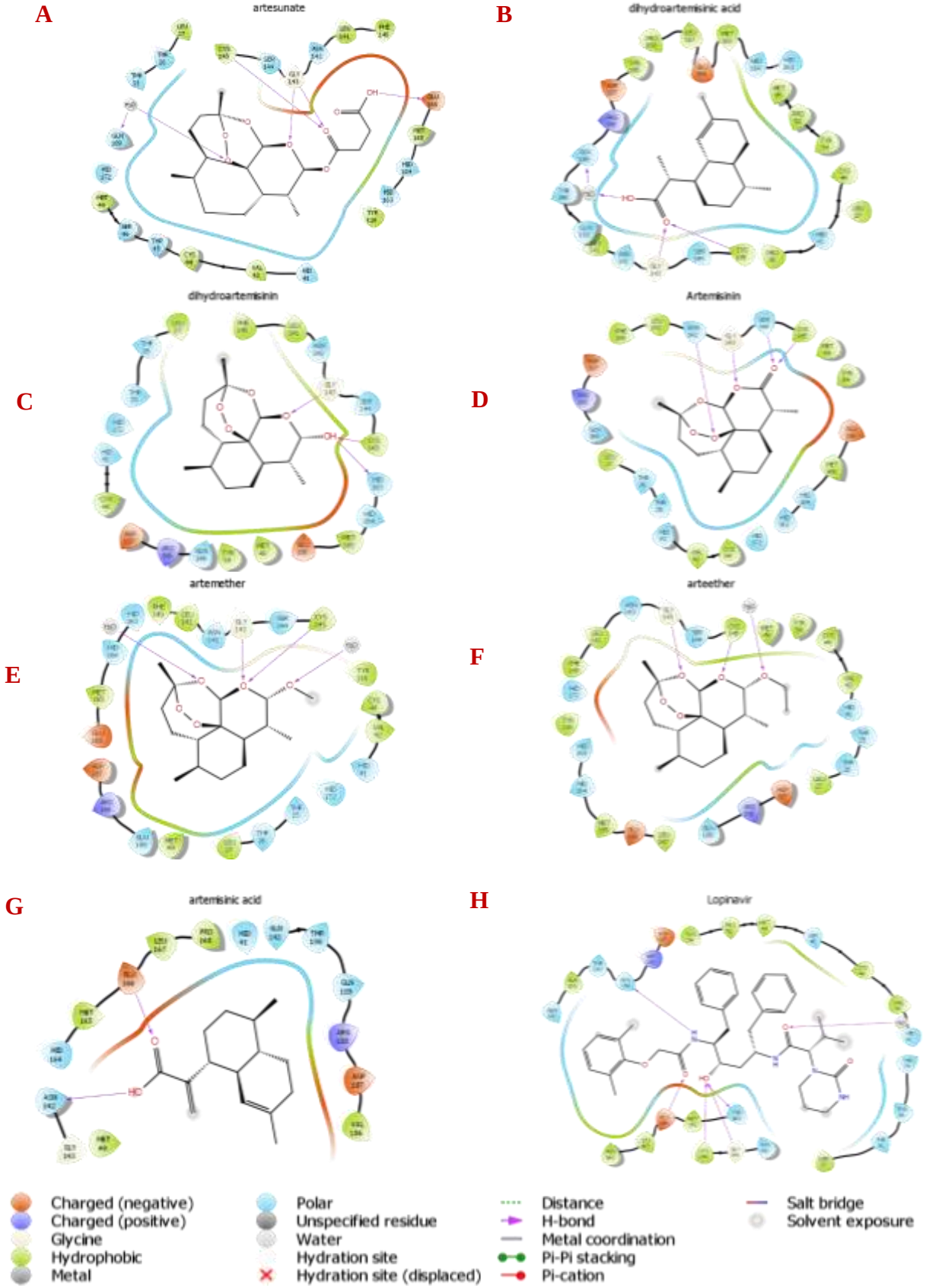

Figure 1. 2-D interactions diagrams of all the studied compounds with the Mpro of SARS-CoV-2 obtained by molecular docking study.

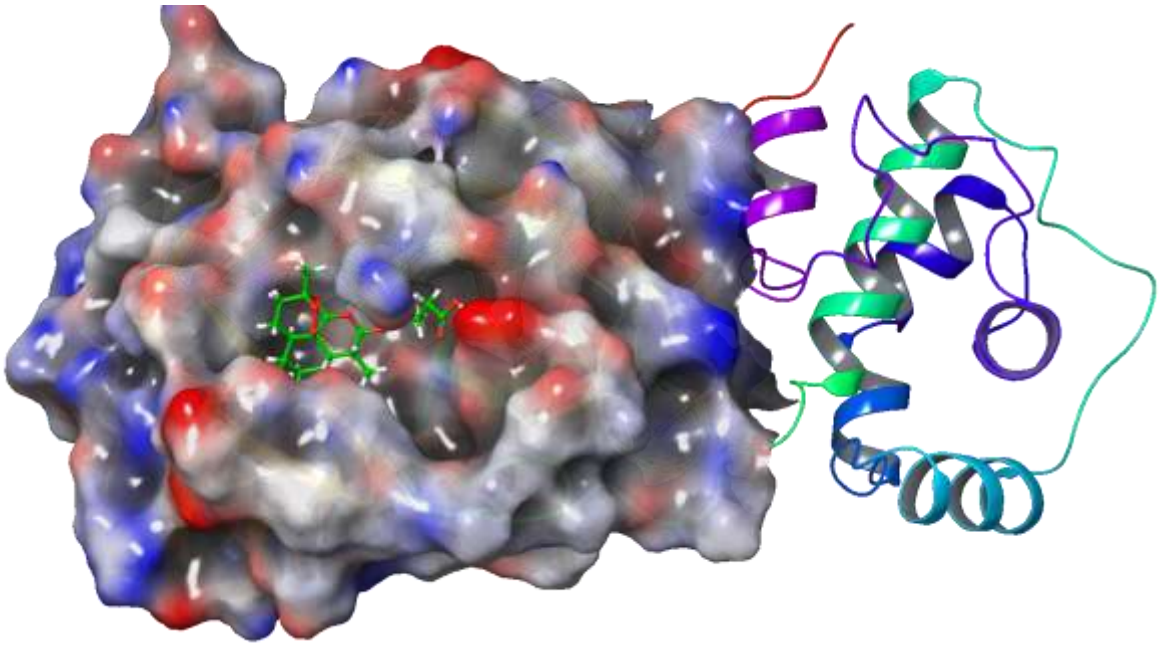

Figure 2. The docked pose of artesunate at the electrostatic potential surface of Sars-CoV-2 3CLpro

4. Conclusions

In this study, we have selected the main protease of the virus as a target to combat COVID-19. All the artemisinin derivatives are interacting via hydrogen bonding and hydrophobic interactions with the active site of 3CLpro. Based on docking scores and interaction patterns, the artesunate was found to be significantly located to the 3CLpro active site. Since the inhibitors targeting the 3CLpro of the coronavirus offer mutation-independent treatment and the doses of artemisinin-derived compounds for malaria treatment have been determined, herbal treatments containing Artesunate, Dihydroartemisinic acid and dihydroartemisinin could be useful to combat COVID-19. It should be kept in mind that this study only recommends natural compounds for the treatment of COVID-19 with the in-silico approach and should be confirmed by in vitro and in vivo studies.

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Authors' contributions

ME wrote up the article. The authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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