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Venom Peptides of *Crotalus atrox* Against SARS-Cov-2 Spike Protein and Human ACE2 Receptor by Molecular Docking Analysis

Suleyman ILHAN *1

Abstract

Venoms are composed of about 100 to 500 pharmacologically active compounds. Less than 0.01% of these compounds have been identified and a significant majority of them act on unknown receptors. Here, the potential Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) activities of selected *Crotalus atrox* venom peptides (CVPs) including Atrolysin D (AD), vascular apoptosis-inducing protein-1 (VAIP-1), Catrocollastatin (CC), and Calcium-Free Phospholipase A2 (CFP) were investigated via molecular docking analysis. CVPs were docked against human angiotensin-converting enzyme-2 (ACE-2) and 3-chymotrypsin-like protease (3CLpro) viral spike protein. All CVPs had low binding energies to both 3CLpro and ACE2, suggesting that they interacted strongly with the active sites of enzymes, compared to the reference drugs lopinavir and ritonavir. The binding energy of 3CLpro was -139.517 kcal/mol, -96.239 kcal/mol, -121.590 kcal/mol, -259.424 kcal/mol with AD, VAIP-1, CC, and CFP, respectively. CFP showed a very strong binding activity with 3CLpro, suggesting that it could be a very effective compound in inhibiting the SARS-CoV-2 virus. The binding energy of ACE2 was -101.165 kcal/mol, -73.064 kcal/mol, -106.918 kcal/mol, -82.830 kcal/mol with AD, VAIP-1, CC, and CFP, respectively. AD made a much stronger bond with ACE2 than reference drugs, showing that it could be used as a virus-protective component in humans. The results suggest a potential drug candidate for the development of therapeutics against Coronavirus disease 2019 (COVID-19). *In vitro* and *in vivo* experiments are needed to confirm these compounds' potential preventive and therapeutic effects.

Keywords: Crotalus atrox venom, COVID-19, SARS-CoV-2, 3CLpro, ACE-2.

1. INTRODUCTION

Many people have died as a result of the breakout and quick spread of the coronavirus disease 2019 (COVID-19) epidemic brought on by the SARS-CoV-2 coronavirus. New mutations have emerged, extending to the disease's complexity. According to estimates from the World Health Organization, the SARS-CoV-2 epidemic killed more than 6 million people and infected more than 700 million individuals across many different nations. As a result, the fight to stop this epidemic has spread globally. The COVID-19 epidemic continues to represent a serious threat to humanity despite several

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vaccinations and pharmacological treatment attempts because of the weakened immune system.

Angiotensin-converting enzyme, also known as ACE, produced on the surface of host cells is bound by spike protein (glycoprotein S) by the SARS-CoV-2 virus, which preferentially targets lung cells [1]. Angiotensin-converting enzyme I (ACE-I) and angiotensin-converting enzyme II (ACE-II) are both enzymes that are involved in the production of angiotensin. ACE-II found in the human body is involved in controlling blood pressure by converting angiotensin I to angiotensin II. It has been determined that SARS-CoV-2 in humans uses ACE2 as its cellular entrance receptor [2].

The viral particle that gets inside the cell is not encoded, and when the -coronavirus genome is transcribed, it often results in an 800 kDa polypeptide. Pp1a and pp1ab in ORF1a and ORF1b are prepared for polyprotein synthesis [3]. These polyproteins are proteolytically broken down into a variety of proteins by the enzymes papain-like protease (PLpro) and 3-chymotrypsin-like protease (3CLpro). 3CLpro can cleave the polypeptide at various sites into 16 different nonstructural polypeptides to produce various proteins involved in viral genome replication and transcription [4]. 3′ terminus of the gene, which exhibits extreme polymorphism, contains 3CLpro, which is crucial for the replication of viral particles. It is also thought to be a key target for halting the spread of the illness by obstructing the viral polyprotein's active cleavage sites. This information on SARS-CoV-2 has led to the acceptance of ACE2 and 3CLpro as prospective targets for the creation of antiviral medications [4].

There are many studies ongoing to search for new vaccines/drugs for COVID-19. Researchers from around the world are working together to accelerate the development of potential treatments and vaccines for the virus. Many organizations, including the WHO, and the National Institutes of Health are dedicated to researching new treatments and vaccines for the virus. Natural compounds (herbs, spices, and medicines made from animals) are the richest source of reference for the search for anti-viral molecules [5–8]. Plants are investigated because of their high antioxidant and immune-enhancing effects, and animal venoms are also investigated because they contain active compounds with different mechanisms of action. Among the natural compounds, snake venoms are a source of potentially helpful therapeutic chemicals because of their biological activities [9, 10]. They might contain substances useful in the design or development of pharmaceuticals, leading to the identification of novel proteins and protein families. *Crotalus* sp. belongs to the family of pit vipers. They are venomous and are typically found in parts of North, Central, and South America. They have a distinctive rattle at the end of their tail that they use as a warning signal when they feel threatened. *C. atrox*, also known as the western diamondback rattlesnake, is a venomous species of rattlesnake. The venom of *C. atrox* consists of several proteins including phospholipases A2, C-type lectins, metalloproteinases, hyaluronidase, and bradykinin-potentiating peptides. These proteins have various effects on prey such as paralysis, pain, and hemorrhagic activity. Since the venom proteins of *C. atrox* have a wide variety of protein cocktails, it is conceivable whether there is an interaction between the spike protein of COVID-19, which may be relevant in evaluating potential therapeutic treatments.

A computer method called molecular docking is used to estimate the interactions between two molecules, usually small molecules like pharmaceuticals. The two molecules must fit into a binding site, and the fit is then evaluated by evaluating the energies of their interactions [11]. It is a research method that fuses physical and chemical principles with complex computational algorithms to provide a useful tool for analyzing the source and mechanism of potential new compounds [12].

Therefore, to comprehend the underpinnings of COVID-19,
computational research to shed light on snake venom protein interactions would be useful.

In this study, the interactions of *C. atrox* venom peptides (CVPs) Atrolysin D (AD), vascular apoptosis-inducing protein-1 (VAIP-1), Catrocollastatin (CC), and Calcium-Free Phospholipase A2 (CFP) with 3CLpro and ACE2 receptors were investigated, and potential anti-viral molecules targeting SARS-CoV-2 were explored.

2. MATERIALS AND METHODS

2.1. Receptor Preparation

The SARS-CoV-2 main protease 3CLpro (PDB ID: 6LU7) and ACE2 (PDB ID: 1R42) were chosen as receptors. The protein databank's PDB format was used to download the three-dimensional (3D) structures of the 3CLpro and ACE2 proteins (https://www.rcsb.org/). The load distribution, hydrogenation, and water removal processes all made use of the PyMOL software. Then, using MG Tools by AutoDock Vina program, hydrogen atoms were added to the acceptor molecule [13]. For future investigations, the structure was saved in PDB format.

2.2. Ligand Preparation

Identified snake venom proteins' three-dimensional structure were retrieved in PDB format from PubChem: Atrolysin D (PDB ID: 1ATL), vascular apoptosis-inducing protein-1 (PDB ID: 2ERO), Catrocollastatin (PDB ID: 2DW2), Calcium-Free Phospholipase A2 (PDB ID: 1PP2). Autodock Vina 4.2.5.1 software was used for water removal, hydrogenation, and adjusting the load distribution.

2.3. Molecular Docking

Computational Docking uses statistical and machine-learning methods to predict the interaction between molecules. It can be used to visualize and study protein-ligand interactions, design novel drugs, and predict drug effectiveness. By using Autodock Vina, high throughput molecular docking was performed. The grid center for 3CLpro was set as X= 21.41, Y=3.62 and Z=21.94 with dimensions of the grid box 60 A˚× 60 A˚× 60 A˚. The grid center for ACE2 was set as X=19.81, Y=-5.57 and Z=14.73 with the grid box 60 A˚× 60 A˚× 60 A˚. After calibration and optimization, the same grid box size and other parameters were applied to the docking experiments of all four proteins, and the entire setup was performed to generate different docked conformations. To see how molecules' secondary structures resembled, PyMOL was utilized.

3. RESULTS AND DISCUSSION

A computational technique called *in silico* docking is used to anticipate the binding affinity of a small molecule drug to a target protein. *In silico* docking can be used to find possible small molecule inhibitors for antiviral drug development that can attach to viral proteins and interfere with their function, reducing viral reproduction and infection. Snake venoms are produced in venom glands and contains several proteins, enzymes, and peptides with various biological activity. While the majority of a snake's venom is utilized for defense and predation, some of the venom's components have been discovered to have medicinal qualities and are used as medications for a variety of medical disorders. Some examples of drugs developed from snake venom can be listed as anti-venoms, blood pressure medications, pain medications, anti-cancer agents and neurological medications [9]. Overall, drugs obtained from snake venoms have great potential for treating a variety of medical conditions. Here, to explore a possible therapeutic target for COVID-19 disease, 3CLpro and human ACE-2 receptors were docked with CVPs using *in silico* methods. Since hydrogen bonds (H-bonds) and steric interactions (such as Van der Waals

interactions) are vital for ligand-target protein interactions and binding affinity, we investigated the binding affinity of the venom between the SARS-CoV-2 target protein 3CLpro and human ACE2. PubChem CID, molecular formula and 2D protein structures of the venom were obtained from the PubChem library database. The binding energies, H-bond interaction scores and amino acid interactions with 3CLpro and ACE2 ligands are presented in Table 1.

All CVPs had low binding energies to 3CLpro, suggesting that they interacted strongly with the enzyme's active sites. The binding energies of these CVPs ranged from -96.239 to -259.424 kcal/mol for 3CLpro which were comparable with the binding energies of reference drugs lopinavir (-126,713 kcal/mol) and ritonavir (-108,731 kcal/mol). The binding energy of 3CLpro was -139.517 kcal/mol, -96.239 kcal/mol, -121.590 kcal/mol, -259.424 kcal/mol with AD, VAIP-1, CC, and CFP, respectively (Table 1). The most prominent binding energy values were calculated as -139.517 kcal/mol for AD and -259.424 kcal/mol for CFP, which were lower than the reference drugs lopinavir and ritonavir, indicating a strong binding affinity. CFP is a human neutrophil-calcium modulating protein isolated from *C. atrox* venom [14]. Although its biological activities have not been studied much, it has many interesting properties such as heat stability, activity on non-aqueos and lipid molecules. CFP showed a very strong binding activity with 3CLpro, suggesting that it can also be very effective in virus. CFP also formed hydrogen interaction with Cys29, and steric interactions with Asp49 and Phe5 (Figure 1A-B). AD is a hemorrhagic metalloproteinase isolated from *C. atrox* venom. It is a reprolysin subfamily of zinc metalloproteinases and is an effective inhibitor of platelet aggregation [15]. AD had high binding affinity with 3CLpro and formed hydrogen interactions with Arg167, Gly169, Glu143 and Pro168. It also formed steric interactions with Val138, Leu170, His142, Leu108, Arg167 and Glu143 (Figure 1C-D). CC is another peptide isolated from *C. atrox* venom, is an inhibitor of collagen-induced platelet aggregation prothrombin activator [16]. It had a -121.590 kcal/mol binding energy which was lower than reference drug Ritonavir. It formed hydrogen interactions with Leu447, Ala479, Cys 481 and steric interactions with Glu480 (Figure 2A-B). VAIP-1 is an apoptosis-inducing peptide that target vascular endothelial cells and has the lowest binding energy with 3CLpro [17]. It formed hydrogen interactions with His18 and Arg13, and steric interactions with Gln16 and Arg13 (Figure 2C-D). All CVPs also displayed low values of binding energy to ACE2. These CVPs had binding energies that varied from -73.064 to -106.918 kcal/mol (Table 1). All tested CVPs had binding energies with ACE2 higher or similar to the reference drugs Lopinavir (-80.524 kcal/mol) and Ritonavir (-73.550 kcal/mol).

Table 1 Interaction of the SARS-CoV2 Main Protease 3CLpro with *C. atrox* venom proteins.

Protein name	Ligand	Docking Score (Binding Energy, Kcal/mol)	H Bond	Amino acid Residue
Atrolysin D	3CLPro	-139.517	-8.573	Val138, Leu170, Arg167, Ile165, Gly169, His142, Thr139, Glu143, Leu108, Pro168
	ACE2	-101.165	-9.278	Gly109, Glu143, Thr139, Cys164, Tyr176, Val138, Ile165, Arg167, Gly169, Leu170, Pro168
Vascular Apoptosis-Inducing protein-1	3CLPro	-96.239	-6.138	Glu480, Cys481, Ala479, Leu447, Glu445
	ACE2	-73.064	-9.247	Asp582, Leu587, Met585
Catrocollastatin	3CLPro	-121.590	-8.440	Asp416, Glu407, Gly442, Asn425
	ACE2	-106.918	-6.147	Gln424, Asn422, Cys417, Glu407, Asp416
Calcium-Free Phospholipase A2	3CLPro	-259.424	-5.00	Cys29(R), Asp49(R), His48(R), Phe5(R)
	ACE2	-82.830	-5.140	Leu19, Glu6, Cys29, His48
Lopinavir	3CLPro	-126,713	-7.414	Cys95, Thr96
	ACE2	-80.524	-11.160	Asn98, Thr96, Cys95, Gly94, Ile3, Pro1
Ritonavir	3CLPro	-108,731	-3.045	Asn98, Ile3, Thr96, Gln2
	ACE2	-73.550	-15.621	Asn98, Ile3, Thr96, Pro1

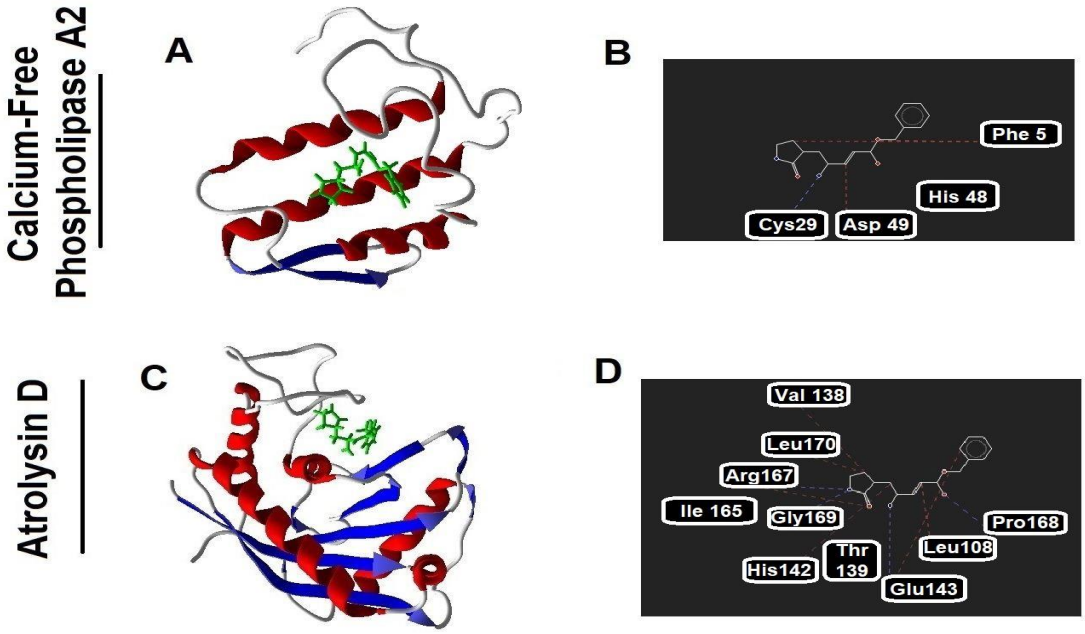

Figure 1 (A) Molecular docking of SARS CoV-2 main protease (3CLpro) and Calcium-free phospholipase A2 (B) interactions with key residues. (C) Molecular docking of SARS CoV-2 main protease (3CLpro) and Atrolysin D and (D) interactions with key residues (Red dashes show steric interactions and blue dashes show hydrogen bonds)

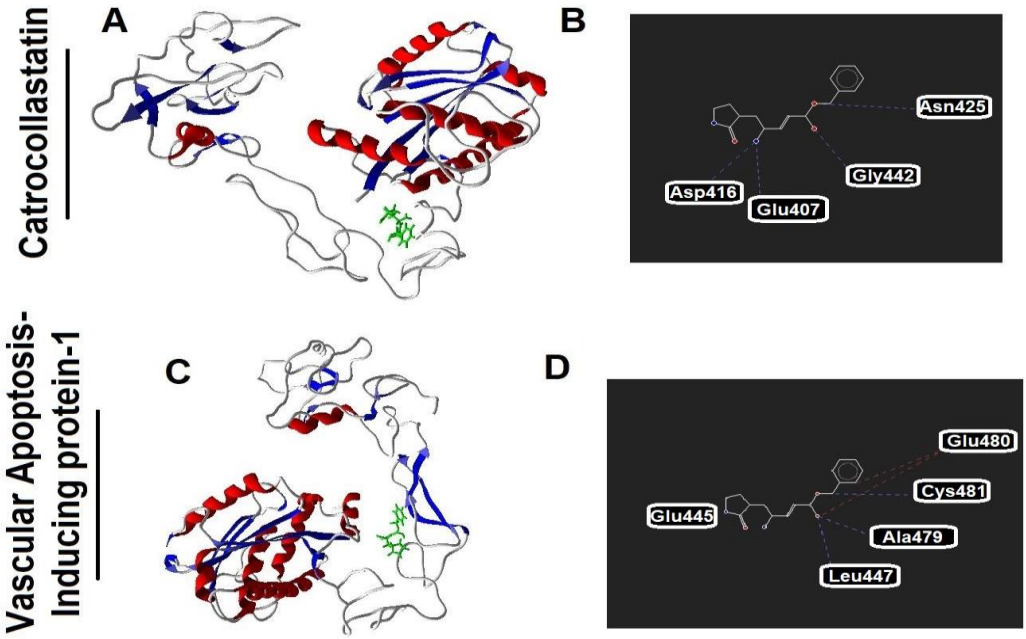

Figure 2 (A) Molecular docking of SARS CoV-2 main protease (3CLpro) and Catrocollastatin (B) interactions with key residues. (C) Molecular docking of SARS CoV-2 main protease (3CLpro) and Vascular apoptosis-inducing protein-1 (D) interactions with key residues (Red dashes show steric interactions and blue dashes show hydrogen bonds)

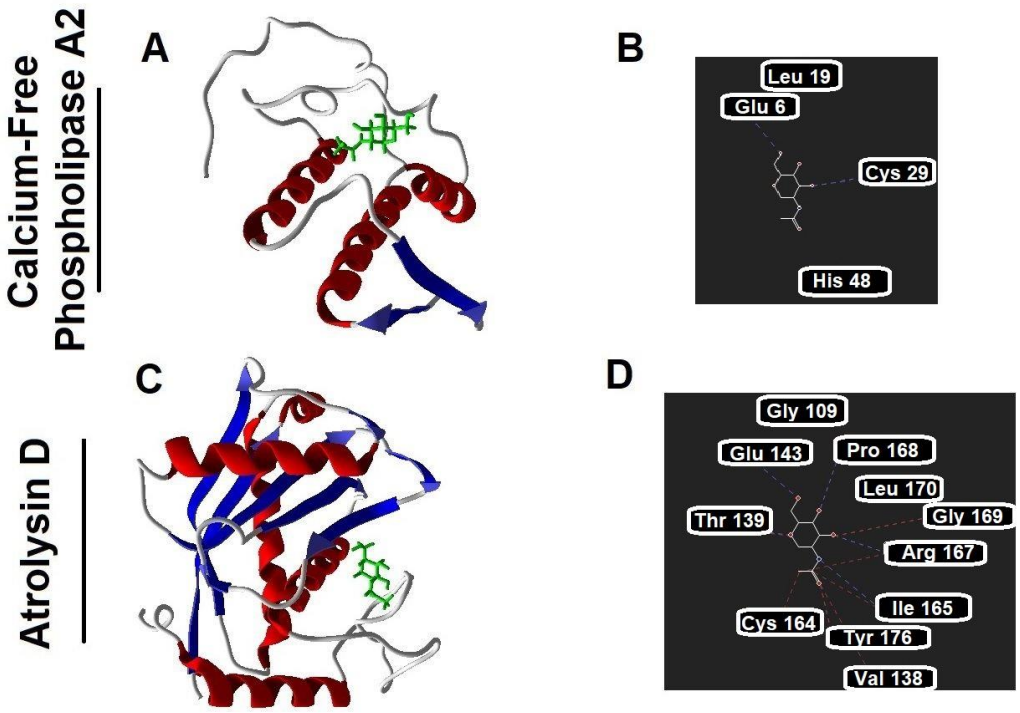

Figure 3 (A) Molecular docking of ACE2 and Calcium-free phospholipase A2 (B) interactions with key residues. (C) Molecular docking of ACE2 and Atrolysin D (D) interactions with key residues (Red dashes show steric interactions and blue dashes show hydrogen bonds)

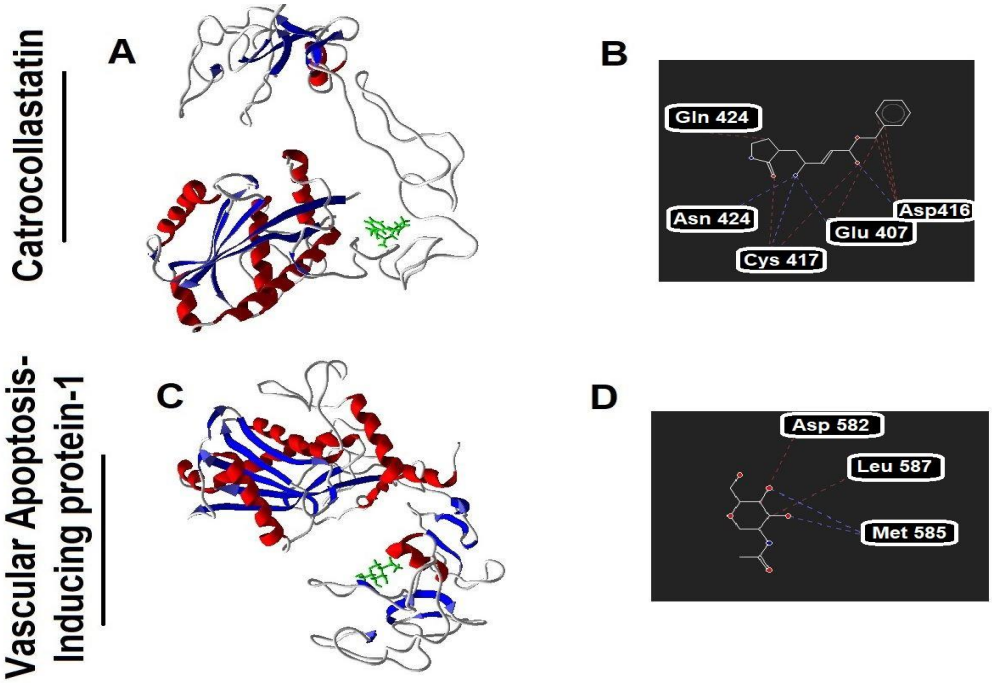

Figure 4 (A) Molecular docking of ACE2 and Catrocollastatin and (B) interactions with key residues. (C) Molecular docking of ACE2 and Vascular apoptosis-inducing protein-1 (D) interactions with key residues (Red dashes show steric interactions and blue dashes show hydrogen bonds)

The binding energy of ACE2 was -101.165 kcal/mol, -73.064 kcal/mol, -106.918 kcal/mol, -82.830 kcal/mol with Atrolysin D (AD), VAIP-1, Catrocollastatin (CC), and Calcium-Free Phospholipase A2 (CFP), respectively (Table 1). VAIP-1 with the lowest binding score almost the same as Ritonavir. It formed hydrogen bonding with Met585 and steric interactions with Leu587 and Asp582 (Figure 3A-B). AD formed hydrogen interactions with Glu143, Thr139, Pro168, Arg167, and Ile165; formed steric bonds with Thr139, Cys164, Tyr176, Val138, Ile165, and Gly169 (Figure 3C-D). The peptide with the greatest binding affinity to human ACE2 was found as CC. Compared to Lopinavir and Ritonavir, CC had a higher affinity for binding hydrogen bonds with Asn422, Cys417, Glu407, and Asp416 and steric interactions with Gln424, Cys417, Glu407, and Asp416 (Figure 4A-B). CFP had the similar results to Lopinavir and it formed hydrogen bonds with Cys29 and Glu6 (Figure 4C-D).

These *in silico* analysis results highlight the CVPs tested as potential anti-SARS-CoV-2 components. However, it is important to validate the results of *in silico* docking using *in vitro* and *in vivo* studies to ensure that the compounds are effective and safe for use as antiviral drugs.

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Authors' Contribution
The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

The Declaration of Conflict of Interest/ Common Interest
No conflict of interest or common interest has been declared by the authors.

The Declaration of Ethics Committee Approval
This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics
The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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