



## Original Article

SARS-CoV-2 and Angiotensin-Converting Enzyme-2 Receptor Interaction Blocker –an *In-Silico* Approach

Zoha Khan<sup>1</sup>, Muhammad Suleman<sup>2</sup>, Hamna Tariq<sup>3</sup>, Mehvish Mumtaz<sup>3\*</sup>, Hafiz Muhammad Husnain Azam<sup>4</sup>, Nazim Hussain<sup>5</sup>, Hadia Sarfraz<sup>3</sup>, Zulqarnain Baqar<sup>6\*</sup>, Haris Munir<sup>3</sup> and Moeen Zulfiqar<sup>3</sup>

<sup>1</sup>School of Biochemistry and Biotechnology (SBB), University of Punjab, Quaid-e-Azam Campus, Lahore, Pakistan

<sup>2</sup>School of Biological Sciences (SBS), University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan

<sup>3</sup>Department of Molecular Biology, University of Okara, Renala Khurd, Pakistan

<sup>4</sup>National Institute for Biotechnology and Genetic Engineering, NIBGE, Faisalabad, Pakistan

<sup>5</sup>Centre for Applied Molecular Biology (CAMB), University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan

<sup>6</sup>Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Kowloon, Hong Kong SAR, China

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**\*Corresponding Author:**

Mehvish Mumtaz

Department of Molecular Biology, University of Okara, Renala Khurd, Pakistan  
[mehvishgcu@gmail.com](mailto:mehvishgcu@gmail.com)

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## ABSTRACT

The global COVID-19 pandemic, instigated by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has led to substantial morbidity and mortality on a worldwide scale. While COVID-19 vaccines offer hope, the emergence of mutated viral strains necessitates the development of FDA-approved drugs to address future outbreaks. **Objective:** To examine prospective antiviral medications through an analysis of the interaction between the spike protein of SARS-CoV-2 and Angiotensin-converting enzyme-2 (ACE-2) receptors, which play a pivotal role in facilitating viral entry into host cells. **Methods:** Molecular docking was employed to assess the binding affinities of various protease inhibitors with ACE-2 receptors. Natural proteases, including Furin and Transmembrane serine protease 2 (TMPRSS2), cleave viral spike proteins into S1 and S2 subunits, facilitating fusion with ACE-2 receptors. We assessed the binding energies of Indinavir, Nafamostat, Fosamprenavir, Lopinavir, and Boceprevir to inhibit this interaction with a sense of optimism for their potential therapeutic applications. **Results:** Our findings suggest potential treatments for COVID-19, with Indinavir, Nafamostat, Fosamprenavir, Lopinavir, and Boceprevir displaying promising binding energies of -9.6 kcal/mol, -8.4 kcal/mol, -7.7 kcal/mol, and -7.5 kcal/mol, respectively. **Conclusions:** While promising, further clinical trials are important to potentially evaluate the efficacy and safety of these proposed drugs in combating COVID-19 and its variants.

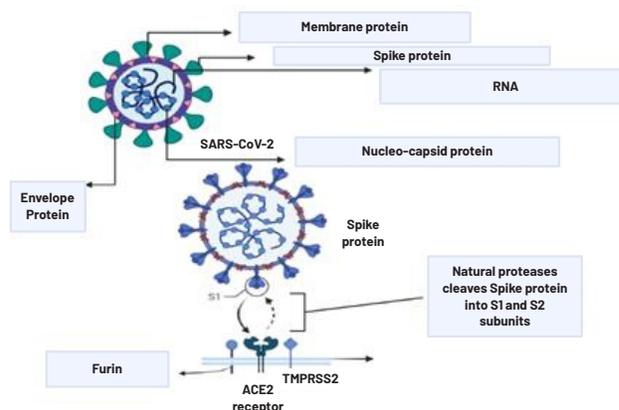
## INTRODUCTION

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) caught nearly the entire world off-guard by a pandemic outbreak that began in the last quarter of 2019. Since the world witnessed the first outbreak of this virus in Wuhan, China, it quickly went viral. The WHO then until March of 2020 officially announced that it was an epidemic [1]. After all that, the virus did its job and the death tolls keep rising during the pandemics that have suddenly

emerged all over the world. Moreover, the continual evolution of the virus and emergence of new strains through mutation undermine the high-levels of attention the virus demands in the context of public health and safety. Scientists' consistent efforts in research and development are keys in fighting COVID-19, through both vaccine developments as well as finding proper and defined treatment approaches [2, 3]. A study event is

literally based on investigation of molecular interactions between the host cell and a pathogen system. The virus comes to the host cells, just like the rest of the spike proteins on SARS-CoV-2 take the main focus in vaccine development [4]. Accordingly, these spike proteins, which are three dimensional in nature, facilitate the binding to and entry of the virus into the target cells by attaching to definite host cell receptors. Among the sensors the Angiotensin-converting enzyme 2 (ACE-2) receptor has been discovered as the most suitable one which is responsible for the viral entrance to begin with. The lungs, heart, kidney and the intestinal tract being only few examples of the tissues and organs of which ACE-2 receptors are highly expressed [5]. The virus life cycle of course is not complete without the interaction of the spike protein with the cellular receptor ACE-2 which was the initiator of the internal processes that lead to virus replication. The spike protein helps the virus link with ACE-2 and as a result, the cell membrane is fused with the virus, which then penetrates inside the cell. The toxicity of the virus may be further emphasized by these fine-tuned molecular interactions that also serve as potential points of intervention for the therapeutic process [6]. The advancements in the treatment options focusing on breaking the spike protein-ACE-2 resulting bridge has largely been made possible through the acquired understanding of the processes behind the connection. This task can be achieved by way of employing protease inhibitors to interrupt the cleavage of spike protein and consequently suppress its contact with the ACE-2 receptors. These inhibitors act through specific processing proteases like Furin and Transmembrane serine protease 2 (TMPRSS2) and thus, they can be considered as potential antiviral drugs that can prevent the virus entering and reproducing inside the human [7]. However, ligand-target protein interaction prediction is also an important method to be used in the drug development at this stage since it can predict the binding affinity between small molecules, or ligands, and target proteins [8]. Using molecular docking we assess the affinity and specificity of possible drug candidates by imitating how protease chain inhibitors bind to the spike protein-ACE-2 association. Peptidase inhibitors are purposely evaluated, such as Indinavir, Nafamostat, and Fosamprenavir. They may also include Lopinavir and Boceprevir. These compounds could eventually restrict the inhibition of COVID-19 and thus minimize its severity. Meanwhile, the development of COVID-19 vaccinations brings a new chapter in its attack containment, with a possibility of reduced transmission and stable herd immunity. Variations of the virus, apart from their emergence, can overpower the established vaccine's efficacy in due course of time, and with it a timely

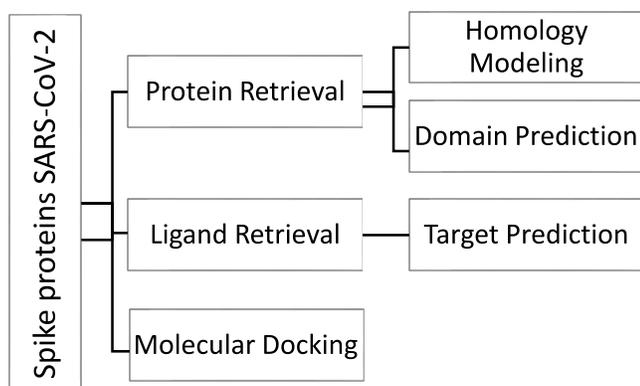
amendment of the vaccination schedule is equally important [9]. The interaction between the ACE-2 receptor and the spike protein significantly influences the pathogenesis of SARS-CoV-2 and may indicate a line of treatment. Through the application of different approaches such as the vaccine development, molecular docking and proteolytic restraint, scientists work out the most effective methods to fight the COVID-19, hence lessen the effect of the condition on the world [10]. The S1 and S2 proteins from SARS-CoV-2 are the main components for its activation to ACE2 receptors. The S protein is divided into these two subcomponents by the proteases, such as Furin and/or TMPRSS2, found naturally in human bodies. S2 favors the merging of membranes while S1 primarily belongs to ACE-2 [11]. The interaction of the SARS-CoV-2 spikes proteins and the ACE-2 receptors were investigated via single molecule force spectroscopy. Their evidence illustrated that the ACE-2 receptors and the S1 subunit interact differently through a specific binding pathway, and both of them being the strongest ones with their intrinsic value (around 120 nM) and kinetic and thermodynamics having about the same features [12]. SARS-CoV-2 S protein is held back by the electrostatic forces to the ACE-2 primarily.



**Figure 1:** An Interaction Between SARS-COV-2 and ACE-2 Receptor

## METHODS

Computer-aided drug design tools were used to check the interaction of target protein (ACE-2 receptors) with ligands (protease inhibitors) after their retrieval from the protein data bank (PDB) and Pub-chem, as shown in a flowchart in figure 2.



**Figure 2:** Flow Chart of Steps for CADD of COVID-19

### Protein Retrieval

The Protein Data Bank is a database for 3D structural data of proteins. These structures can be easily downloaded in PDB format and used wherever required. For the current study, the protein was retrieved from the Protein data bank with PDB id 6M0J, a crystal structure of spike proteins of the SARS-CoV-2 domain bound with ACE2 as it gains entry into the cell through ACE2 receptors.

### Homology Modeling

An atomic resolution model of the target protein is made from an amino acid sequence and an experimental 3D structure of a related homologous protein through SWISS MODEL [13].

### Domain Prediction

InterProScan was used for domain prediction of protein. Inter-Pro Scan provides the functional analysis of proteins by their classification into families and prediction of domains and important sites. Ligand Retrieval Ligand was retrieved from PubChem. It is an open database of chemical molecules and their activities against biological assays, managed by the National Center for Biotechnology Information (NCBI). It contains small and larger molecules such as nucleotides, carbohydrates, lipids and chemically modified macromolecules. Camostat was used for SARS and MERS, but it caused some diseases. So, in the current study, we use different protease inhibitors belonging to different drug families.

### Toxicity Prediction

Toxicity prediction of the drug was conducted through ADMET analysis to assess potential adverse effects during its action. This analysis evaluates the drug's absorption, distribution, metabolism, and excretion rates, as well as establishes quantitative structure-toxicity relationships.

### Target Prediction

Target prediction was made to find phenotypical side effects and cross-reactivity caused by small molecules. SWISS target prediction was used to estimate a small molecule's most probable molecular targets so it gets easier to predict which molecule would mostly act on it.

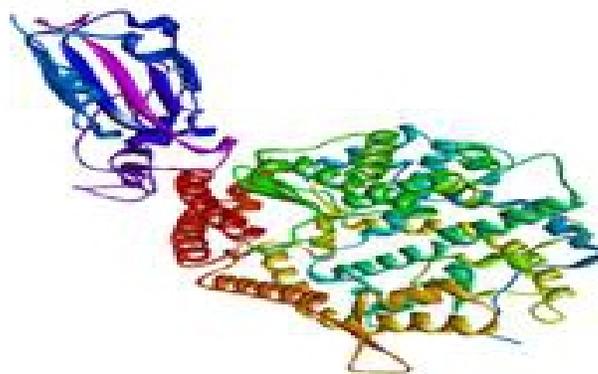
### Molecular Docking

In order to identify the essential amino acid interactions between the protein and ligands, molecular docking is employed. The interactions of the ligands were characterized by scoring functions to predict the binding affinity with the receptor. Molecular docking was utilized to examine the interactions between drugs and SARS-CoV-2 spike proteins which are protease inhibitors. These protease inhibitors act as ligands in molecular docking. The binding energy values depict the best candidate for COVID-19. Before docking, the ligands and water molecules were eliminated from the protein utilizing Discovery Studio Visualizer. Protein-ligand interactions were assessed through docking employing AutoDock Vina. Polar hydrogens were incorporated into the protein, and a grid box was positioned at coordinates: Center\_X = -26.873, Center\_Y = 18.465, and Center\_Z = -14.035, with dimensions Size\_X, Y, Z = 26. Ligand torsions were set to 6, and both protein and ligand files were saved in PDBQT format. Docking was executed, and outcomes were visualized and presented using Discovery Studio Visualizer. Binding poses were evaluated based on binding energy in kcal/mol.

## RESULTS

### Homology Modeling and Stability Validation

Protein with PDB id 6M0J was retrieved from Protein data bank. Figure 1 shows the 2D and 3D structures of protein. Homology modeling was done to build accurate structural models of proteins. A Ramachandran plot was generated to visualize energetically favorable regions for the backbone dihedral angles Phi against Psi of amino acid residues within the protein structure. The Ramachandran plot revealed that 96.80% of residues resided within the favored region. Additionally, the MolProbity score, a key indicator of protein quality statistics, was determined to be 2.62, representing the central MolProbity score (Figure 3)



**Figure 3:** 3D Structure of a Protein by Discovery Studio

### Domain Prediction of Protein

The residues 1-598 resemble the Peptidase-M2 family by the IntePro family. Residues from 10-581 show

resemblance with M2 ACE by CDD; there is an active side residue T at position 329, active site residue D at position 350 in Conserved Protein domain CDD, H residue at position 356, D residue at position 364, E residue at 384, IL residue at 385. Residues from 208-235, 242-263, 351-367, 379-399, 421-446, 456-483, 484-512 resemble the Peptidyl-dipeptidase A (M2) metalloprotease family. Residues from 1-598 resemble the Angiotensin-Converting Enzyme by PANTHER, and from 3-588 show a resemblance with PEPTIDASE M2 by Pfam. It resembles Metallo-proteases ("zincins"), catalytic domain Superfamily from 4-589 and with ANGIOTENSIN-CONVERTING ENZYME 2 by PANTHER from 1-598.

### Ligand Retrieval

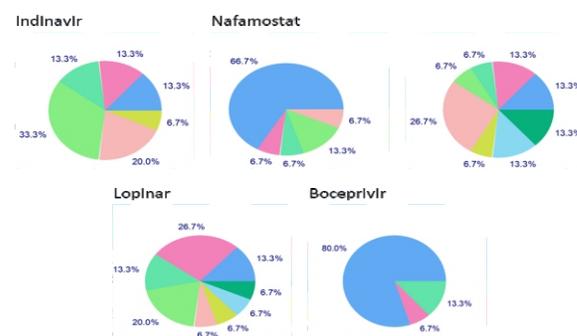
Indinavir, Nafamostat, Fosamprenavir, Lopinavir and Boceprevir were retrieved from PubChem. The PubChem ID of these drugs with their 2D structures is given in Table 2. These are all protease inhibitors belonging to different families.

**Table 1:** Retrieved Ligand Information from PubChem

Sr. No	Drugs	PubChem CID	2D structure	Target (protein)
1	Indinavir	5362440		6M0J
2	Nafamostat	4413		
3	Fosamprenavir	131536		
4	Lopinavir	92727		
5	Boceprevir	10324367		

### Target prediction

Swiss Target Prediction by (<https://www.swisstargetprediction.ch>) was logged on, and Canonical Smiles of Nafamostat were inserted for target prediction. The pie-chart for Nafamostat predicts 66.7% Proteases, the pie-chart for Indinavir 13.3 % for proteases, that for Fosamprenavir and Lopinavir 26.7% for proteases and that for Boceprevir 80.0% (Figure 4).



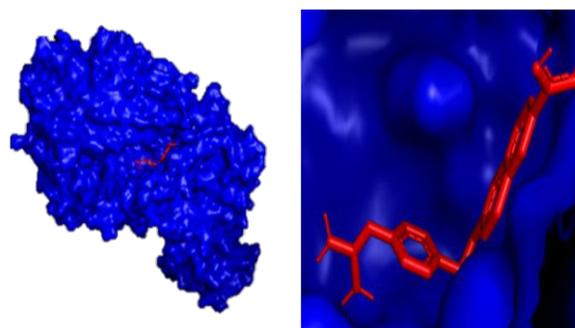
**Figure 4:** Pie-charts for Target Prediction of Drugs

### Molecular Docking

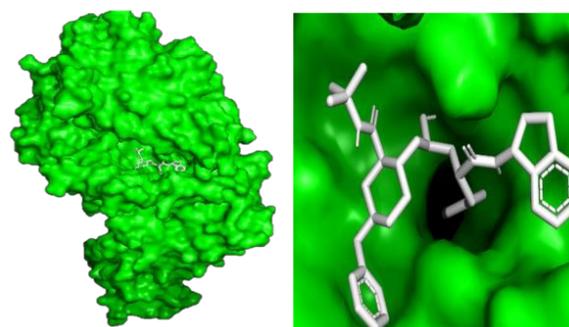
Molecular docking was conducted to assess the interactions between the protein and ligand. Polar hydrogens were added to the protein, and a grid box was positioned with the following coordinates: Center\_X = -26.873, Center\_Y = 18.465, and Center\_Z = -14.035, with dimensions Size\_X, Y, Z = 26. Ligand torsions were set to 6, and files of both the protein and ligand were saved in PDBQT format.

Subsequently, docking was performed, and the results were visualized and analyzed using Discovery Studio Visualizer. The binding poses were evaluated based on the binding energy in kcal/mol. The ligand with the lowest energy score represented the most favorable interaction with the protein (shown in Figure 5).

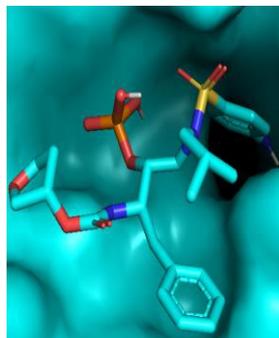
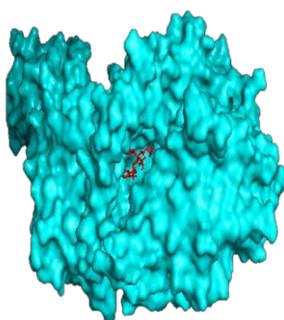
#### Nafamostat



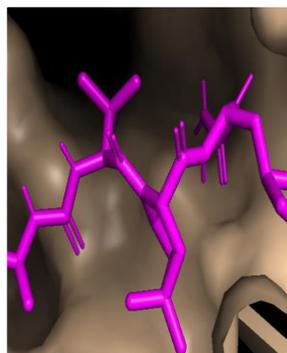
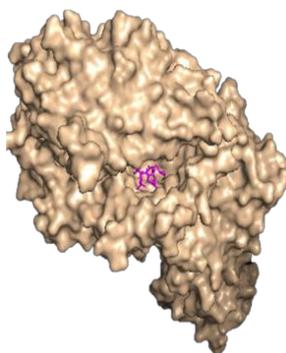
#### Indinavir



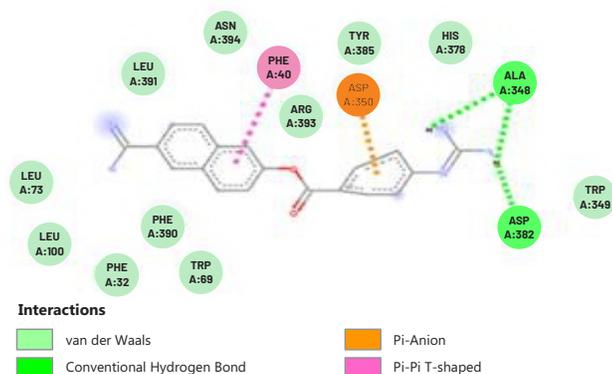
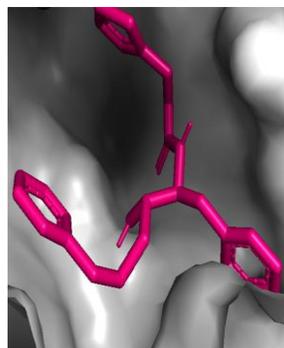
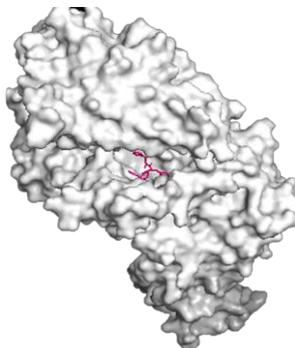
## Fosamprenavir



## Boceprevir



## Lopinavir

**Table 2:** Retrieved Ligand Information from PubChem

Sr. No	Drugs	Kcal/mol	Rmsd l. b	Target (protein)
1	Indinavir	-9.6	0.000	0.000
2	Nafamostat	-8.4	0.000	0.000
3	Fosamprenavir	-7.7	0.000	0.000
4	Lopinavir	-7.5	0.000	0.000
5	Boceprevir	-7.5	0.000	0.000

## DISCUSSION

SARS-CoV-2, the pathogenic agent of COVID-19, utilizes the angiotensin-converting enzyme 2 (ACE-2) receptor as its primary means of entering cells, a process enhanced by interactions between the viral spike protein and ACE-2 receptors abundantly present in diverse human organs [14]. Augmented by proteases like Furin and transmembrane serine protease 2 (TMPRSS2), these interactions pave the way for viral attachment and subsequent internalization, culminating in viral replication and dissemination [15]. The current research supports prior discoveries that suggest the potential effectiveness of protease inhibitors in hindering the interaction between SARS-CoV-2 spike proteins and ACE-2 receptors thereby impeding viral entry into host cells [16]. The drugs examined in this study, including Indinavir, Lopinavir, Fosamprenavir, Nafamostat, and Boceprevir, exhibit strong binding affinities with ACE-2 receptors, emphasizing their potential as promising therapeutic candidates against COVID-19 [17]. Despite concerns about toxicity, this study utilizes toxicity predictions to assess their safety profile, advocating for their potential utility as therapeutic options [18]. Moreover, comparing the binding energy highlights the benefit of targeting virus entities rather than naturally occurring proteases like TMPRSS2, reducing the possibility of unintentionally interfering with vital physiological processes for host homeostasis [19]. This work offers important insights into drug-virus relationships via the use of computational tools and molecular modeling approaches, which will help with the logical design of tailored therapeutic treatments toward COVID-19 [20]. In conclusion, the findings presented herein underscore the potential of protease inhibitors as viable alternatives to conventional vaccination strategies in combating the COVID-19 pandemic. By elucidating the molecular mechanisms underlying viral entry and pathogenesis, this study contributes to the growing armamentarium of therapeutic options aimed at curbing the propagation of SARS-CoV-2 and ameliorating its detrimental effects on global health.

## CONCLUSIONS

The advent of SARS-CoV-2 has driven global attempts to identify efficient antiviral countermeasures, including the utilization of accessible medications, to treat COVID-19.

**Figure 5:** Docking Views Of Lowest Energy Ligands With Protein Are Shown, And Discovery Studio Shows A 2d View Of These Interactions

Several vaccines are available commercially to treat this deadliest disease, but some allergic reactions are seen in immunocompromised people. Moreover, mutated forms of SARS-CoV-2 have imperiled their effectiveness. The current study highlights options for combating SARS-CoV-2 pathogenesis utilizing available FDA-approved medications. The cleavage of spike protein into S1 and S2 by natural proteases of the human body, i.e., furin and TMPRSS2, respectively, facilitate entry of SARS-CoV-2 into the host cells through ACE2 receptors. By blocking the activity of these proteases, the association of the virus with the ACE 2 receptor can be halted. The interaction between FDA-approved available drugs Indinavir, Nafamostat, Fosamprenavir, Lopinavir and Boceprevir with natural proteases in the human body via autodock vina discern positive outcomes as immediate treatment for COVID-19. Further clinical trials are needed to ensure its efficacy against all reported strains of SARS-CoV-2.

### Authors Contribution

Conceptualization: ZK

Methodology: ZK, MM

Formal analysis: MS, ZK

Writing, review and editing: MS, HT, MM, HMH, NH, HS, ZB, HM, MZ

All authors have read and agreed to the published version of the manuscript.

### Conflicts of Interest

The authors declare no conflict of interest.

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