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The efficacy of Paxlovid against COVID-19 is the result of the tight molecular docking between M\(\text{pro}\) and antiviral drugs (nirmatrelvir and ritonavir)

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ABSTRACT

Purpose: Currently, a number of medications for coronavirus disease 2019 (COVID-19) treatment are tested in clinical trials; however, credible clinical studies are becoming increasingly difficult to come by. Paxlovid is a ritonavir-boosted nirmatrelvir drug that the U.S. Food and Drug Administration (FDA) authorized for the treatment of COVID-19. This study aimed to demonstrate the interaction of nirmatrelvir and ritonavir on the active site of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) main protease (M\(\text{pro}\)).

Materials and methods: To locate the optimal docking between M\(\text{pro}\) and antiviral drugs, and to conduct dynamic simulations between atoms in the fusion areas, various bioinformatics and mathematical equations were applied.

Results: According to the docking data, nirmatrelvir has a stronger interaction with M\(\text{pro}\) than ritonavir, which has more multiple bonds. Molecular docking of antiviral drugs such as Paxlovid has a significant impact on the treatment of COVID-19 virus.

Conclusions: According to this study, Paxlovid may work on new strains, including Omicron, because the M\(\text{pro}\) mutation P132H in the Omicron variant has no direct effect on the protein.

1. Introduction

Coronavirus disease 2019 (COVID-19) drew worldwide attention, touching every corner of the globe and altering the world's social and economic conditions. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel strain of corona viral diseases. It caused COVID-19, which affected many countries in a very short period of time and negatively impacted economies and human lives. There is no specific treatment for COVID-19 developed yet except a number of vaccines. Therefore, exploration of various novel corona viral targets is urgent to combat the disease virulence [1,2]. Even though the virus mostly causes a mild respiratory infection, a considerable number of people develop acute illness and die as a result of it. Furthermore, there are many asymptomatic illnesses that might transfer the virus to others. Patients who have underlying diseases are at a higher risk of developing a severe condition [3,4].

Several antiviral drugs are now being tested in clinical trials, but credible clinical studies are reportedly getting more difficult to conduct as the public's appetite for readily available therapies develops. Remdesivir is an antiviral that is now being evaluated in clinical trials to treat COVID-19. It is hypothesized to reduce RNA synthesis by targeting RNA-dependent RNA-polymerase (RdRP) [5,6].

For many viruses, the protease enzyme is important for viral protein maturation because it cleaves proproteins after they are translated into the cytoplasm of the host cell. Protease is a common target for viral replication. Protease is present in case of human immunodeficiency virus (HIV) and hepatitis C virus (HCV). Therefore, protease inhibitors could be repurposed against COVID-19. Structure-based screening of existing protease inhibitors had been carried out utilizing docking simulation as standard barometer within very limited time under the critical lock down situations [7,8]. As a result, proteases are frequently used as therapeutic candidates. The suppression of viral protease can prevent mature viral particles from forming [9]. The main ORF1ab gene encodes the main coronavirus protease nsp5 (M\(\text{pro}\), 3CL\(\text{pro}\)), which cleaves two overlapping proteins into 16 non-structural proteins that are critical for viral replication and maturation. During viral replication, M\(\text{pro}\), a cysteine protease, is involved in the maturation cleavage of polyproteins. It is a key player in the entry of the virus into the host cell. The M\(\text{pro}\) is a three-domain homodimer containing two protomers [10,11].

Many antiviral medicines targeting proteases have been created to
SARS-CoV-2 [14,15]. It is a cytochrome P450 (CYP) 3A inhibitor that increases nirmatrelvir exposure whereas ritonavir is an antiretroviral protease inhibitor and a potent variety of other constraints [16]. The ability to establish the crystal structure of viral proteases in conjunction with potential inhibitors is crucial because it allows for inhibitor customization depending on the structural dynamics of the active site in the target enzymes (monomer or dimer) [17,18].

In this study, nirmatrelvir and ritonavir, two current HIV-protease combo inhibitors, were repurposed to target SARS-CoV-2 major protease (M\textsuperscript{pro}) and investigate their likely method of inhibition.

Abbreviations: RMSD/ub: root mean square deviation (upper bound), RMSD/lb: root mean square deviation (lower bound).

### 2. Methods

#### 2.1. Preparation of the structure and dynamic investigations

The initial structure of SARS-CoV-2 M\textsuperscript{pro} was retrieved from the Protein Data Bank (PDB) under the ID 6Y2E [19]. To compare with wild type, Omicron (BA.1.1.7) was acquired from Global Initiative on Sharing All Influenza Data (GISAID). The Omicron variant has only one mutation in the nsp5. Proline is replaced with histidine in residue 132. Using PyMOL, P132H was loaded onto the wild strain. The 2D structures of nirmatrelvir and ritonavir were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov) as SDF files (Fig. 1). The 2D structures were converted to 3D structures and optimized using PyMol software (https://pymol.org). The BIOVIA (San Diego, CA, USA) software [20] was used to remove the heteroatoms (HETATM) and water molecules from the M\textsuperscript{pro} and add the polar hydrogen. The AutoDock Vina [21] in PyRx 0.9.x (https://pyrx.sourceforge.io) was used to perform molecular docking of the proteases and inhibitors. The InterEvDock2 [22] and SeamDock [23] servers were used to accomplish the double docking of nirmatrelvir + ritonavir with M\textsuperscript{pro}. The most relevant technique to judge the validity of the interaction procedure is to compare how well the minimize energy postures - predicted by the object scoring function - match up. The outcomes of the docking were validated by redocking a number of times. The optimal stance was determined by employing the Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) methods force field [24].

#### 2.2. Estimate docking simulation

The protein-ligand interaction complex structure was assessed using molecular docking simulation. GROMACS 2018 [25] and VMD [26] software were used for all of the trajectory simulations and analysis. Initially, 2000 steepest-descent energy was used to minimize energy. The reaction complex was dissolved with about 18,000 TIP3P water molecules to analyze the docking modes and this happened after energy conservation. To achieve mathematical equilibration, 0.1 ns constraints of heavy protein and ligand atoms were used. The restrictions were released, and the molecular simulation was run for 100 ns. The Root Mean Square Deviation (RMSD) from the starting structure was calculated to explore the dynamic stability of poses and to confirm the sampling process’s reasoning.

FUpred (https://zhanggroup.org/FUpred) was used to determine domain boundaries based on map contact. It has a much better ability to predict domain boundaries than threading-based approaches and computational modeling is a new field of study that helps to improve the success of drug development efforts. It is influenced by a variety of factors such as protein-ligand geometry, chemical interactions, and a variety of other constraints [16]. The ability to establish the crystal structure of viral proteases in conjunction with potential inhibitors is crucial because it allows for inhibitor customization depending on the structural dynamics of the active site in the target enzymes (monomer or dimer) [17,18].

Fig. 1. The chemical structure and formula of nirmatrelvir and ritonavir.

| M\textsuperscript{pro} – Ligand interaction | Binding Affinity | RMSD/ub | RMSD/lb |
|-------------------------------------------|------------------|---------|---------|
| 6Y2E- Nirmatrelvir Model 1                | −8.1             | 0       | 0       |
| 6Y2E- Nirmatrelvir Model 2                | −7.3             | 30.262  | 26.506  |
| 6Y2E- Nirmatrelvir Model 3                | −7.2             | 31.754  | 29.604  |
| 6Y2E- Nirmatrelvir Model 4                | −7.1             | 3.177   | 2.232   |
| 6Y2E- Nirmatrelvir Model 5                | −7.1             | 32.018  | 28.37   |
| 6Y2E- Nirmatrelvir Model 6                | −7.1             | 18.233  | 15.128  |
| 6Y2E- Nirmatrelvir Model 7                | −7               | 31.688  | 29.968  |
| 6Y2E- Nirmatrelvir Model 8                | −7               | 33.158  | 29.419  |
| 6Y2E- Nirmatrelvir Model 9                | −6.8             | 18.434  | 15.526  |
| 6Y2E- Ritonavir Model 1                  | −6.9             | 0       | 0       |
| 6Y2E- Ritonavir Model 2                  | −6.4             | 23.226  | 19.276  |
| 6Y2E- Ritonavir Model 3                  | −6.4             | 25.013  | 19.654  |
| 6Y2E- Ritonavir Model 4                  | −6.4             | 2.084   | 1.434   |
| 6Y2E- Ritonavir Model 5                  | −6.3             | 6.531   | 2.747   |
| 6Y2E- Ritonavir Model 6                  | −6.3             | 22.59   | 18.955  |
| 6Y2E- Ritonavir Model 7                  | −6.3             | 33.699  | 26.307  |
| 6Y2E- Ritonavir Model 8                  | −6.3             | 15.723  | 9.352   |
| 6Y2E- Ritonavir Model 9                  | −6.3             | 33.158  | 29.419  |

| M\textsuperscript{pro} – Ligand interaction | Binding Affinity | RMSD/ub | RMSD/lb |
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| 6Y2E- Nirmatrelvir Model 1                | −8.1             | 0       | 0       |
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| 6Y2E- Nirmatrelvir Model 3                | −7.2             | 31.754  | 29.604  |
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| 6Y2E- Nirmatrelvir Model 5                | −7.1             | 32.018  | 28.37   |
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| 6Y2E- Nirmatrelvir Model 8                | −7               | 33.158  | 29.419  |
| 6Y2E- Nirmatrelvir Model 9                | −6.8             | 18.434  | 15.526  |
| 6Y2E- Ritonavir Model 1                  | −6.9             | 0       | 0       |
| 6Y2E- Ritonavir Model 2                  | −6.4             | 23.226  | 19.276  |
| 6Y2E- Ritonavir Model 3                  | −6.4             | 25.013  | 19.654  |
| 6Y2E- Ritonavir Model 4                  | −6.4             | 2.084   | 1.434   |
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| 6Y2E- Ritonavir Model 8                  | −6.3             | 15.723  | 9.352   |
| 6Y2E- Ritonavir Model 9                  | −6.3             | 33.158  | 29.419  |
machine learning-based methods, according to benchmark studies. The FUscore with a continuous domain was recorded in the FU-curve and FU-map topologies after analysis.

### 2.3. Calculating stereochemical parameter and Z score-RMS

To calculate the stereochemical parameter, Z score, Root Mean
Square (RMS), several servers, mathematical equations, and computational programs, were employed. Programs were employed to confirm and achieve the intensity of the double docking of Paxlovid's two antiviral drugs with the M\text{PRO}. The intensity of the double docking got different peaks according to the software type.

### 2.4. Ethical issues

The ethical approval was not required for this study.

### 3. Results

#### 3.1. Molecular docking

The docking procedure and the interaction between the protein and the antiviral drugs were carried out. As indicated in Table 1, the AutoDock Vina program determined the optimal degrees of docking for the two ligands; at a given energy, there are 9 alternative models of interaction. When the degrees of RMSD lower bound (RMSD/lb) and RMSD upper bound (RMSD/ub) equaled zero (0), the optimal model for docking was the first model for both nirmatrelvir and ritonavir. The M\text{PRO} active site interaction region was similar to those of the two antiviral drugs, with minor differences. The Gln-110 and Thr-111 residues of M\text{PRO} interact with nirmatrelvir in two conventional hydrogen bonds, whereas the Pro-108 and Gly-109 residues interact with ritonavir. Nonetheless, ritonavir interacts with the two preceding residues (Gln-110 and Thr-111) through two carbon-hydrogen interactions.

Fig. 2 shows that the two antiviral medicines have additional convergent residuals.

According to our results, the position of the P132H mutation of the Omicron variant does not have an effect on the interaction region of the two antiviral drugs. The distance between proline 132 (PRO\text{132}) or histidine 132 (HIS\text{132}) and the carbon atom 6 of nirmatrelvir was 16.6 Å, while for ritonavir the distance was 10.1 Å (Fig. 3).

The superposition of the coordinates of each complex in a trajectory (308 structures) onto the starting structure allowed us to observe the progression of the RMSD. The average structure of compounds was reduced by the superposition of energy coordinates. According to our study of 118 structures with a resolution of at least 2.0 Å and an R-factor of no more than 20%, a fine quality model should have more than 90% in the most popular positions. The Ramachandran plot revealed that the preferred zones contain approximately 244 residues (92.1%). The entire Ramachandran plot analysis in Fig. 4 indicates the number of favored, liberally allowed regions, and glycine and proline residues (Table 2).

The interaction zone may be seen in the active site because ritonavir is a nirmatrelvir booster and the residues of their fusion with M\text{PRO} are close together. P132H can be detected at an identical distance between the two antiviral drugs for the Omicron variant without influencing the protein's conformational change (Fig. 5).

During the simulation run, the interaction trend of two critical rings of each medication was observed to see whether there was a probable mechanism of action between nirmatrelvir and ritonavir on SARS-CoV-2 M\text{PRO}. These two rings created a significant contact with residues within the active site, which was surprising. These interactions pushed the active site further into the background. The residual sites for the M\text{PRO} were analyzed, and it was discovered that all of the sites are graded

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**Table 2**

Distribution of the number of double docking residues of M\text{PRO} and antiviral drugs.

| Position of Residues                      | Residue No. | % of Residues |
|------------------------------------------|-------------|---------------|
| Most favored regions [A,B,L]             | 244         | 92.1%         |
| Additional allowed regions [a,b,l,p]     | 18          | 6.8%          |
| Generous allowed regions [−a,−b,−l,−p]   | 2           | 0.8%          |
| Disallowed regions                       | 1           | 0.4%          |
| No. of non-glycine and non-proline residues | 265       | 100.0%        |
| No. of end-residues (excl. Gly & Pro)    | 4           |               |
| No. of glycine residues (shown as triangles) | 26         |               |
| No. of proline residues                  | 13          |               |
| Total number of residues                 | 308         |               |

**Fig. 4.** Ramachandran plot of the double docking between M\text{PRO} and with 2 antiviral drugs nirmatrelvir + ritonavir.

**Fig. 5.** Molecular docking of M\text{PRO} with 2 antiviral drugs (nirmatrelvir and ritonavir).
within the general site average. The arrangement of the residues by 85% at the heat map’s median axis verifies this (Fig. 6).

Fig. 7 depicts a study of ERRAT2 total double docking based on contact atoms for fusion poses and for all protein residues. Two lines are placed on the error axis at the top of the figure to illustrate the degree of certainty with which regions exceed the error value of most of the error bars, which are shown in red. Percentage of the Mpro expression for computed error value is less than the rejection level of 95%. The overall quality is produced values of less than 95%. The typical all quality factors for lower resolutions (2.5 to 3Å) are around 91%. Fig. 8 depicts the predominance of double docked residues in Mpro and nirmatrelvir + ritonavir, revealing a convergent distribution of residues with regard to the cut-off line at 0.23–0.24.

3.2. Dynamic simulation

Various criteria have been used to evaluate the stereochemical quality of the Mpro on localized atoms. The distribution of phi, psi, chi1 and chi2
torsion angles, as well as hydrogen bond energies, are examples of general parameters. There are significant relationships between these characteristics and improved resolution. The parameter distribution becomes increasingly concentrated. **Table 3** shows the main measurements of stereochemical quality that provide a direct indicator to a structure’s dependability. The side-chain parameters are illustrated in **Fig. 9**.

Volume Z-scores, which were based on atomic volume deviations from standard values calculated the quality of protein crystal structures. The volume of variance (Z-score - RMS) calculated the average magnitude of the structure’s volume abnormalities. In the present study, it was found that a smaller RMS for the Z-score is associated with a better fit for the docking model. In structures with a particular resolution or R-factor, the Z-score-RMS distribution is used. Outliers are structures beyond Z-

| Stereochemical parameter | No. of data pts | Parameter value | Comparison value | No. of band widths from mean |
|--------------------------|----------------|----------------|-----------------|-----------------------------|
| a. Chi-1 gauche minus SD | 48             | 12.8           | 13.6            | 6.5                         |
| b. Chi-1 trans SD        | 79             | 18.0           | 15.3            | 5.3                         |
| c. Chi-1 gauche plus SD  | 123            | 11.3           | 13.8            | 4.9                         |
| d. Chi-1 pooled SD       | 250            | 13.8           | 14.3            | 4.8                         |
| e. Chi-2 trans SD        | 65             | 13.9           | 17.7            | 5                            |

**Abbreviations:** SD – standard deviation.

**Fig. 9.** Stereochemical parameters of Mpro showing the side chain.
score - RMS in which constraints are defined. The Z-scores have a high correlation with the atomic B-factors. Atoms with absolute Z-scores greater than 3 exist in or near parts of the model where algorithms like PROCHECK detect anomalous stereochemistry. The Z-score analysis of Mpro is presented in Fig. 10.

4. Discussion

Mpro of SARS-CoV-2 has sparked a lot of interest in therapeutic research to combat the ongoing COVID-19 epidemic [27]. The effectiveness of novel and existing antiviral drugs in binding to active site of Mpro has been investigated. According to the molecular docking research, HCV NS3/4A protease inhibitors (danoprevir, sovaprevir, glecaprevir and grazoprevir) bind to SARS-CoV-2 Mpro effectively [28,29]. In another study, the FDA-approved antiviral drugs, i.e. tipranavir, lopinavir-ritonavir and raltegravir, demonstrated robust, stable, and flexible binding to active site of Mpro [30,31]. Several studies concentrate on the in silico design of effective medications targeting SARS-CoV-2 Mpro. The therapeutic application of treatments is uncertain, owing to the potential limits of passing clinical trials.

The interaction of nirmatrelvir with Mpro is greater than that of ritonavir, which has several more multiple bonds, according to the docking data. Although ritonavir is a booster, it has certain unfavorable interactions. The interaction of the two antiviral drugs on the active site of the Mpro is crucial, and this demonstrates that this relationship has a significant impact on the decision to use Paxlovid as a COVID-19 therapy [32].

The double docked complex of Mpro and nirmatrelvir + ritonavir was more stable than the single docked complex of each antiviral medication, since the inhibitors were exposed to bulk water significantly more in the double docked state. These findings suggested that molecular simulation on a long time scale using the all-atom model should be more trustworthy. The RMSD value of the crystal structure and anticipated conformation has been found to be extensively used as a measure of whether or not the software acquired the correct docking position.

By tracking the percentage occurrence of expected hydrogen bonds during the simulation, the stability of the hydrogen bond network predicted by the fusion interaction approach was studied. Molecular
dynamics trajectory studies of representative antiviral drugs reveal the presence of multiple hydrogen bonds with moderate to high frequencies between Mpro and nirmatrelvir + ritonavir.

According to our results, since the Mpro mutation P132H in the Omicron variant has no direct influence on the protein, Paxlovid may work on new strains, including Omicron.

Vaccines and antibody-based treatments are expected to be identified before small molecules. On the other hand, vaccines may not be effective, and antibodies may have immunopathological implications. As a result, it is critical to search for potential medications that target SARS-CoV-2 Mpro. An alternative solution, in case of need, may be to use existing authorized broad-spectrum drugs with appropriate modifications in design and potency. Despite enormous efforts in the search for effective inhibitors of SARS-CoV-2 Mpro, longitudinal investigations on the treatment safety and efficacy of potential medications are either limited, continuing, or have not yet been disseminated.

Although nirmatrelvir has been licensed for clinical use as an oral antiviral drug, there are still questions about its efficacy and possible hazards [35]. It is important to conduct extensive clinical studies of the new Paxlovid to support our conclusion.

5. Conclusions

Antiviral medicines, both new and old, have been studied for their ability to bind to the active site of Mpro. This study examined the intensity of molecular binding between Mpro and nirmatrelvir + ritonavir independently and found that the docking was greater when the two antiviral drugs were administered together. According to the findings, the degree of coalescence of the two antiviral drugs has a higher affinity and greater stability with the Mpro with less energy than if the coupling occurred between each drug and the Mpro separately. According to our results, Paxlovid may work on new strains, including Omicron, since the Mpro mutation P132H in the Omicron variant has no direct influence on the protein.

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The author contribution

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Data Collection: Ali Adel Dawood.
Statistical Analysis: Ali Adel Dawood.
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Declaration of competing interest

The author declares no conflict of interests.

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