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Investigation of Nirmatrelvir with Different Crystal Structures Effective on SARS-CoV-2 by *In Silico* Approaches

Burçin TÜRKMENOĞLU[1]

ABSTRACT: A pandemic has been declared in the world with the Covid-19 disease caused by the SARS-CoV-2 virus. Scientists on this disease, which is of antiviral origin, have been seeking treatment against SARS-CoV-2 with experimental and computational methods since December 2019. Nirmatrelvir (PF-07321332; NMV), the antiviral component of PAXLOVID™, has been introduced as an inhibitor of the main protease (M^{Pro}) of this disease, which is a threat to human health, SARS-CoV-2. By analyzing the binding interactions between the target and the ligand as *in silico* with the molecular docking method of Computer Aided Drug Design (CADD), parameters such as amino acids in the binding site, docking score values, binding energy values can be determined. In this study, to six different binding parameters (Docking score, XP GScore, Glide evdw, Glide energy, Glide emodel, MM-GBSA ΔG_{Bind}) of Nirmatelvir, an orally taken drug, on the effective crystal structures (7O46, 7QBB, 7NEO, 7B77, 7B2U, 7B2J, 7NBT and 7TVX) of M^{Pro} in SARS-CoV-2, were investigated with Schrödinger 2021-2 (Schrödinger, LLC New York, ABD) software. It is presented in this study that different crystal structures have different interactions.

Keywords: SARS-CoV-2, M^{Pro}, Nirmatrelvir, molecular docking

[1] Burçin TÜRKMENOĞLU (**Orcid ID:** 0000-0002-5770-0847), Erzincan Binali Yıldırım University, Faculty of Pharmacy, Department of Analytical Chemistry, Erzincan, Türkiye

*** Corresponding Author:** Burçin TÜRKMENOĞLU, e-mail: burcinnturkmenoglu@gmail.com

INTRODUCTION

COVID-19 has entered our lives as a disease produced by the SARS-CoV-2 virus, which is responsible for the current pandemic in the world (Catalan et al., 2022). Over the past two decades, strains of coronavirus have caused serious infections and deaths in humans. In December 2019, the third new strain of severe acute respiratory syndrome coronavirus, SARS-CoV-2, is known to have emerged in a seafood market in Wuhan, China (Asrani et al., 2020; Asrani et al., 2022). COVID-19, caused by the severe acute respiratory syndrome SARS-CoV-2, led to more than 478 million infections and more than 6,134 million deaths by 25 March 2022 (https://www.worldometers.info/coronavirus/). These data on the disease have shown that Covid-19 will continue to be in our lives for a long time.

The main protease (M^{Pro}) enzyme is one of the most important viral protease enzymes responsible for the proteolysis, viral replication, and infection process in the SARS-CoV-2 replication process (Allam et al., 2021; Boozari and Hosseinzadeh, 2021). Recombinant neutralizing monoclonal antibodies are used in the clinical treatment of COVID-19 (Hoffmann et al., 2022; Ullrich, Ekanayake, Otting, and Nitsche, 2022). The orally active drug Nirmatrelvir (PF-07321332, Paxlovid™ as a combination drug with ritonavir as a booster) was first approved for emergency use in the United Kingdom and United States in late 2021 (Ullrich et al., 2022). Nirmatrelvir shown in Figure 1 is an orally available peptidomimetic targeting M^{Pro} that uses a nitrile warhead to covalently bind the catalytic cysteine residue at the active site of the protease (Painter et al., 2021).

To provide a therapeutic option for the treatment of SARS-CoV-2 and its variants, Nirmatrelvir, the antiviral component of PAXLOVIDTM, a recently authorized oral outpatient treatment for conditional or emergency use treatment of COVID-19, was developed to inhibit SARS-CoV-2 replication (Rai et al., 2022). Nirmatrelvir (PF-07321332) is a specific inhibitor of the coronavirus M^{Pro} with potent antiviral activity against many human coronaviruses, including SARS-CoV-2, SARS-CoV and MERS (Owen et al., 2021; Rai et al., 2022).

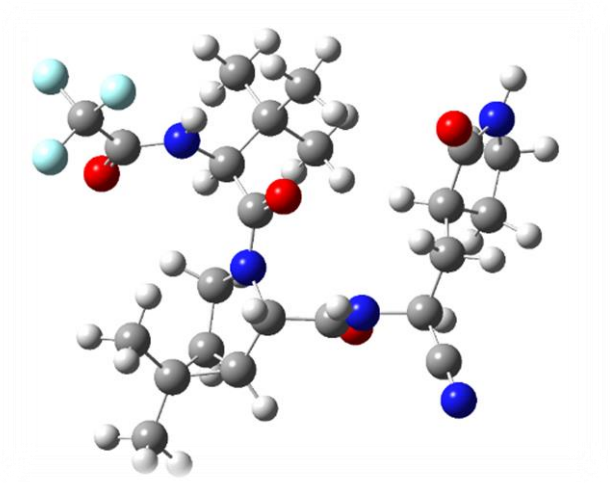

Figure 1. 3D representation of Nirmatrelvir in Gaussian 09 (Frisch and Clemente, 2009)

In studies conducted in recent years, researchers receive support from computer-aided drug design (CADD) methods to support experimental data or before starting experimental studies. In this way, the interaction between the reference drug and the target can be better understood and new lead compounds can be designed. With molecular docking, which is one of the leading CADD methods, binding sites between ligand-target, binding parameters (Glide score, binding energy, Glide emodel, Glide energy, etc. values) can be determined. In this study, the activities of SARS-CoV-2 main

protease enzyme was interacted with Nirmatrelvir drug *in silico* and its interaction on different crystal structures were discussed.

MATERIALS AND METHODS

Computational Methods

It is one of the Structure-Based Drug Design (SBDD) methods, which is a sub-branch of the molecular docking CADD method. The interaction of ligands in the target determined by this method is analyzed by *in silico* approach. In addition to estimating the strength and affinity of M^{Pro} ligand by molecular docking method, it makes it possible to determine the ligands and binding site residues in the crystal structure of the target enzyme and their interactions. In this study, to understand these interactions, all calculations were performed with the 2021-2 version of the Schrödinger 2021-2 (Schrödinger, LLC New York, ABD) software (Schrödinger Release 2021-2: Glide) graphical user wizard. In the method part of this study, the molecular docking protocol was applied as in previous studies (Anil et al., 2022; Çöl et al., 2022; Dadou et al., 2022; Kuzu et al., 2022).

Preparation of ligands

Nirmatrelvir was optimized using the LigPrep wizard utility of Schrödinger 2021-2 (Schrödinger, LLC New York, ABD) software (Anil et al., 2022; Çöl et al., 2022; Dadou et al., 2022) Schrödinger Release 2021-2: LigPrep) . With this method, a net negative change of substituents was produced in each case using possible tautomeric states Epic at pH 7.0 ± 2.0 (Anil et al., 2022; Çöl et al., 2022, Kuzu et al., 2022).

Determination, retriaval and preparation of M^{Pro} crystal structure from PDB

Crystal structures related to M^{Pro} were obtained by examining the protein database (https://www.rcsb.org/). Crystal structures that can be effective in SARS-CoV-2 have been calculated for more than ten crystal structures by molecular docking *in silico*. However, the best results shown in Table 1. The crystal structures 7O46 (Luttens et al., 2022), 7QBB (Luttens et al., 2022), 7NEO (Luttens et al., 2022), 7B77 (Luttens et al., 2022), 7B2U (Luttens et al., 2022), 7B2J (Luttens et al., 2022), 7NBT (Luttens et al., 2022) and 7TVX were interacted with Nirmatrelvir, respectively. Crystal structures were prepared with the "Protein Preparation Wizard" interface of Schrödinger 2021-2 (Schrödinger, LLC New York, ABD) software (Anil et al., 2022; Kuzu et al., 2022; Schrödinger Release 2021-2: Protein Preparation Wizard; Epik).

Molecular docking steps in previous articles were used for protein preparation and MM-GBSA procedures (Anil et al., 2022; Çöl et al., 2022; Dadou et al., 2022; Kuzu et al., 2022). In this process, Schrödinger 2021-2 (Schrödinger, LLC New York, USA) software (Schrödinger Release 2021-2: Glide) is created with the Glide module and grid boxes are defined as 20x20x20 Å grid box-centered. Molecular docking calculations were completed with the Schrödinger Glide program and the best poses were determined depending on the parameters (docking score, XP GScore, Glide evdw, Glide energy, Glide emodel, MM-GBSA ΔG_{Bind}). Prime MM/GBSA analysis of Schrödinger 2021-2 software (Schrödinger Release 2021-2: Prime) is used to estimate ligand binding energies, including the OPLS-2005 force field, VSGB solvent model (Li et al., 2011).

It was performed to calculate the total energy of the binding between the Prime MM/GBSA and the ligand receptor. The binding parameters of Nirmatrelvir in different crystal structures were determined as a result of molecular docking studies.

RESULTS AND DISCUSSION

Due to this positive development, *in silico* approaches were made using the Schrödinger 2021-2 (Schrödinger, LLC New York, ABD) software to determine the binding site of Nirmatrelvir in different crystal structures and to calculate the binding parameters. According to the results in the Schrödinger 2021-2 software with different crystal structures, it is aimed to lead the next studies. Crystal structures from the protein database were individually interacted with Nirmatrelvir after performing molecular docking procedures. As a result of this interaction, resolution, docking score, XP Gscore, Glide evdw, Glide energy, Glide emodel and MM-GBSA ΔG_{Bind} energy values of 7O46 (Luttens et al., 2022), 7QBB (Luttens et al., 2022), 7NEO (Luttens et al., 2022), 7B77 (Luttens et al., 2022), 7B2U (Luttens et al., 2022), 7B2J (Luttens et al., 2022), 7NBT (Luttens et al., 2022) and 7TVX crystal structures and Nirmatrelvir were determined and presented in Table 1.

According to Table 1, when the results obtained after interacting with the crystal structure of Nirmatelvir and PDB ID: 7B77 (Resolution= 1.60 Å) via *in silico* molecular docking method were examined, the docking score value is -8.015 kcal mol^{-1}, the Glide evdw value is -41.742 kcal mol^{-1}, Glide energy value was determined as -52.847 kcal mol^{-1}, Glide emodel value as -77.247 kcal mol^{-1} and ΔG_{Bind} energy value as -94.40 kcal mol^{-1}. In this study, it can be said that the best determined result according to Table 1 for both binding energy and docking score values is according to PDB ID: 7B77.

Table 1. Resolutions of different crystal structures interacting with nirmatrelvir and results of six different binding parameters

PDB ID	Resolution (Å)	Docking score (kcal mol^{-1})	XP GScore (kcal mol^{-1})	Glide evdw (kcal mol^{-1})	Glide energy (kcal mol^{-1})	Glide emodel (kcal mol^{-1})	MM-GBSA ΔG_{Bind} (kcal mol^{-1})
7O46	2.23	-6.554	-6.554	-41.850	-50.407	-74.529	-81.98
7QBB	2.00	-6.669	-6.669	-37.127	-46.625	-68.685	-92.02
7NEO	1.64	-5.169	-5.169	-42.831	-51.138	-67.750	-55.89
7B77	1.60	-8.015	-8.015	-41.742	-52.847	-77.247	-94.40
7B2U	1.55	-5.994	-5.994	-38.789	-51.922	-75.591	-76.11
7B2J	1.55	-7.157	-7.157	-42.152	-53.602	-78.331	-90.86
7NBT	1.63	-5.942	-5.942	-36.010	48.041	-64.183	-68.88
7TVX	2.09	-5.065	-5.065	-37.917	-46.900	-62.644	-48.43

According to Table 1, when analyzed according to the binding energy values of Nirmatrelvir ligand formed as a result of the interaction in crystal structures, 7O46's -81.98 kcal mol^{-1}, 7QBB's -92.02 kcal mol^{-1}, 7NEO's -55.89 kcal mol^{-1}, 7B2U's -76.11 kcal mol^{-1}, 7B2J was -76.11 kcal mol^{-1}, 7NBT was -68.88 kcal mol^{-1}, and 7TVX was -48.43 kcal mol^{-1}. After the 7B77 crystal structure, it can be interpreted according to Table 1 that the best binding energy values are 7QBB, 7B2J and 7O46 structures, respectively.

Of course, *in silico* approaches, it can be interpreted theoretically whether it has a good interaction not only according to the binding parameter values, but also according to the amino acid residues in the binding site.

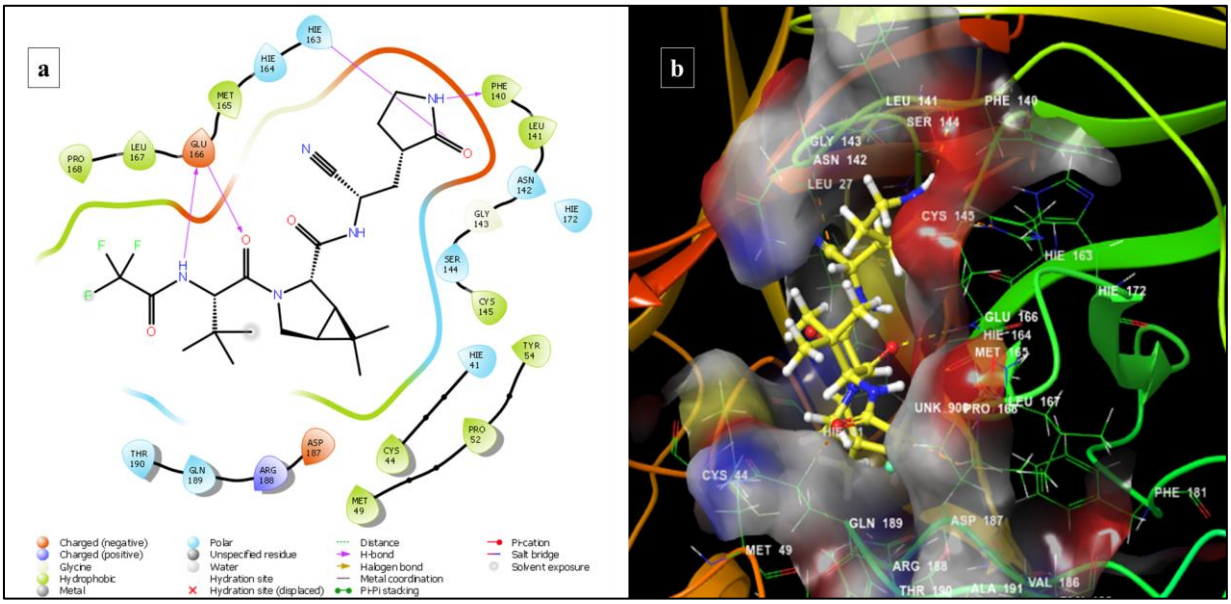

Figure 2. (a) 2D interaction diagram of Nirmatrelvir ligand with 7B77. (b) Molecular docked structure of Nirmatrelvir ligand to7B77 crystal structure

In Figure 2 represented (a) 2D interaction diagram of Nirmatrelvir and PDB ID:7B77 crystal structure after interaction with MM-GBSA analysis in molecular docking, (b) 3D representation of the binding site between Nirmatrelvir-7B77 complex. Figure 2 shows the amino acids in the binding site of the 7B77 crystal structure, which has the best binding energy, in interaction with Nirmatrelvir. It has been determined that hydrogen bonding takes place with Phe140, Hie163, Glu166, and amino acids such as Hie164, Met165, Cys145 are docked in the active binding site.

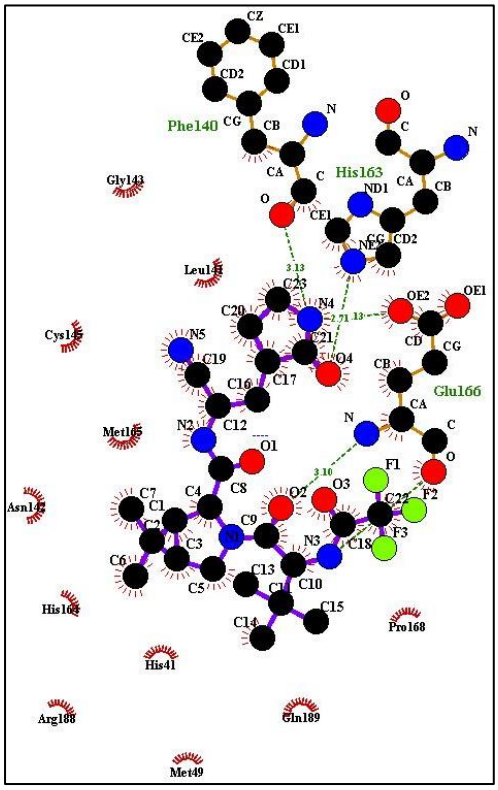

Figure 3. 2D interaction diagram of Nirmatrelvir and PDB ID: 7B77generate in Ligplot+ software (Laskowski and Swindells, 2011)

After the model with the best binding energy was determined with the Schrödinger 2021-2 software (Schrödinger Release 2021-2: Glide; Schrödinger Release 2021-2: Prime), the investigation of the interaction of the 7B77 target structure with the Nirmatrelvir ligand was also performed using the LigPlot+ software (Laskowski and Swindells, 2011). Figure 3 shows the 2D interaction diagram of the 7B77-Nirmatrelvir docked construct obtained with LigPlot+ software. In Figure 3, bond lengths at the bond between the 7B77 crystal structure and Nirmatrelvir interacting *in silico* were determined. Nirmatrelvir's distance bonds with Phe140 amino acid 3.00, His163 amino acid 2.7, Glu166 amino acid 3.10 distance bonds shown in LigPlot+'s 2D diagram.

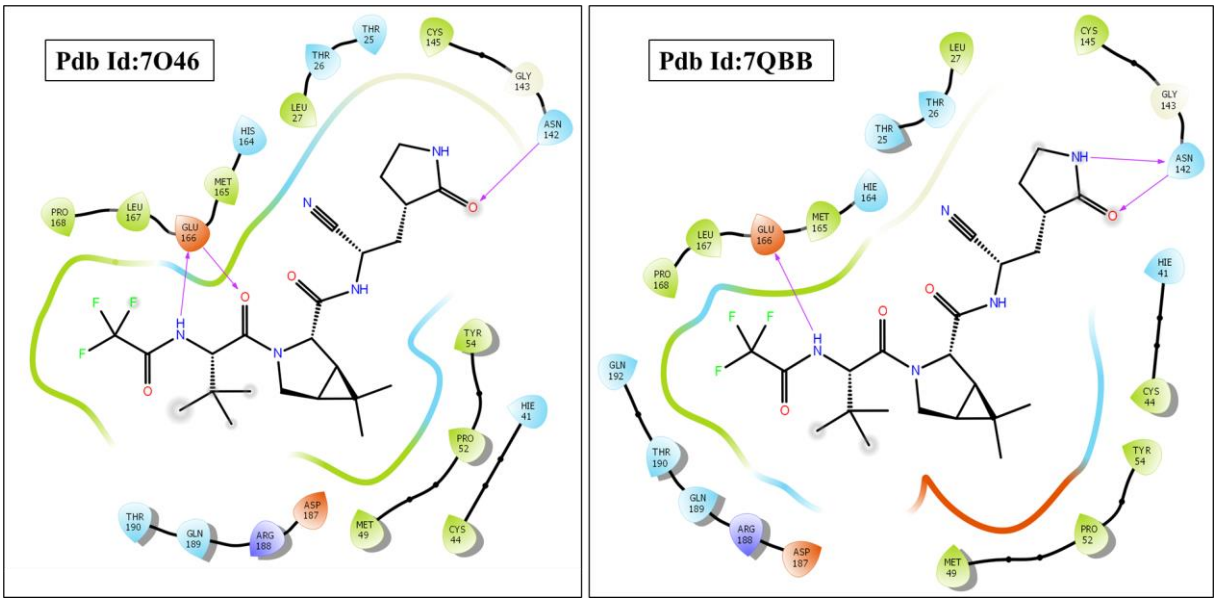

Figure 4. 2D interaction diagram of Nirmatrelvir ligand with respectively, 7O46 and 7QBB crystal structures

The crystal structures in Table 1 and the interactions of Nirmatrelvir with amino acid residues, which are the result of interactions with molecular docking, were all also examined in terms of binding site. In Figure 4, while 7O46 and Nirmatrelvir have a hydrogen bond interaction with Glu166 and a hydrogen bond interaction with Asn142, its interaction with important amino acids such as Cys145, His164, Met49 has been determined. In Figure 4, the 2D interaction diagram between 7QBB and Nirmatrelvir shows the hydrogen bond interaction with the amino acid Glu166, Asn142.

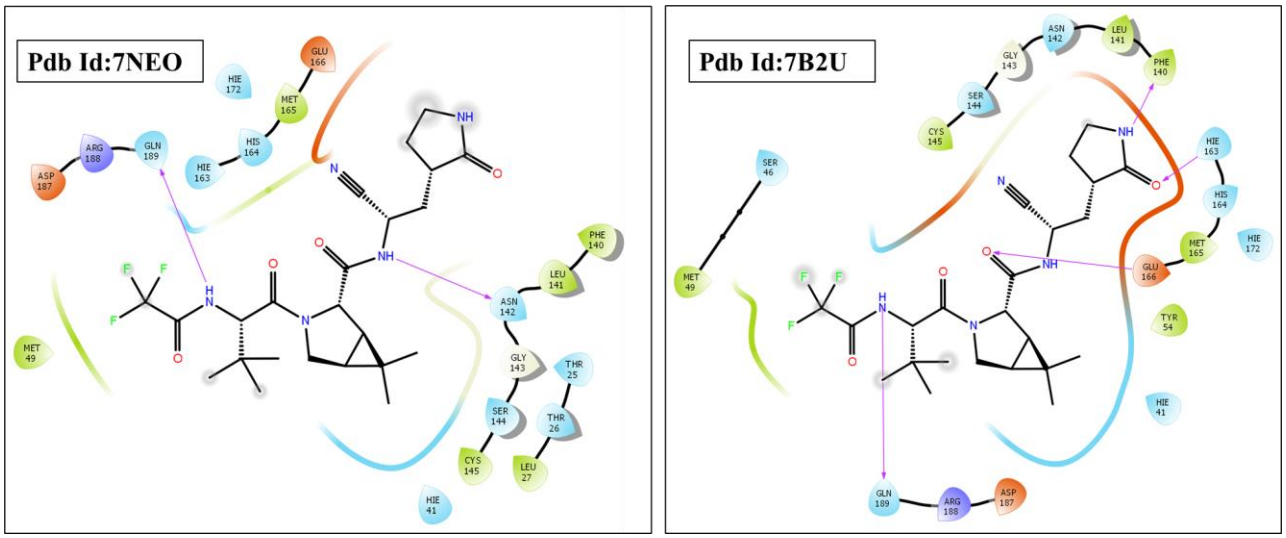

Figure 5. 2D interaction diagram of Nirmatrelvir ligand with respectively, 7NEO and 7B2U crystal structures

Figure 5 shown a 2D representation of the interaction of the 7NEO and 7B2U crystal structures in Table 1. As a result of the interaction between 7NEO and Nirmatrelvir, there is a hydrogen bond interaction between the amino acids Gln189 and Asn 142, and the ligand is docked in the region of important amino acids such as Glu166, Met165, Ser144, Cys145. When the interaction between 7B2U and Nirmatrelvir is examined, it can be interpreted that it interacts with different amino acids, makes hydrogen bond interactions with the amino acid residues Glu166, Hie163, Gln189, Phe140, and was docked in the binding site where the amino acids Cys145, Ser144, Gly143, Asn142 were docked.

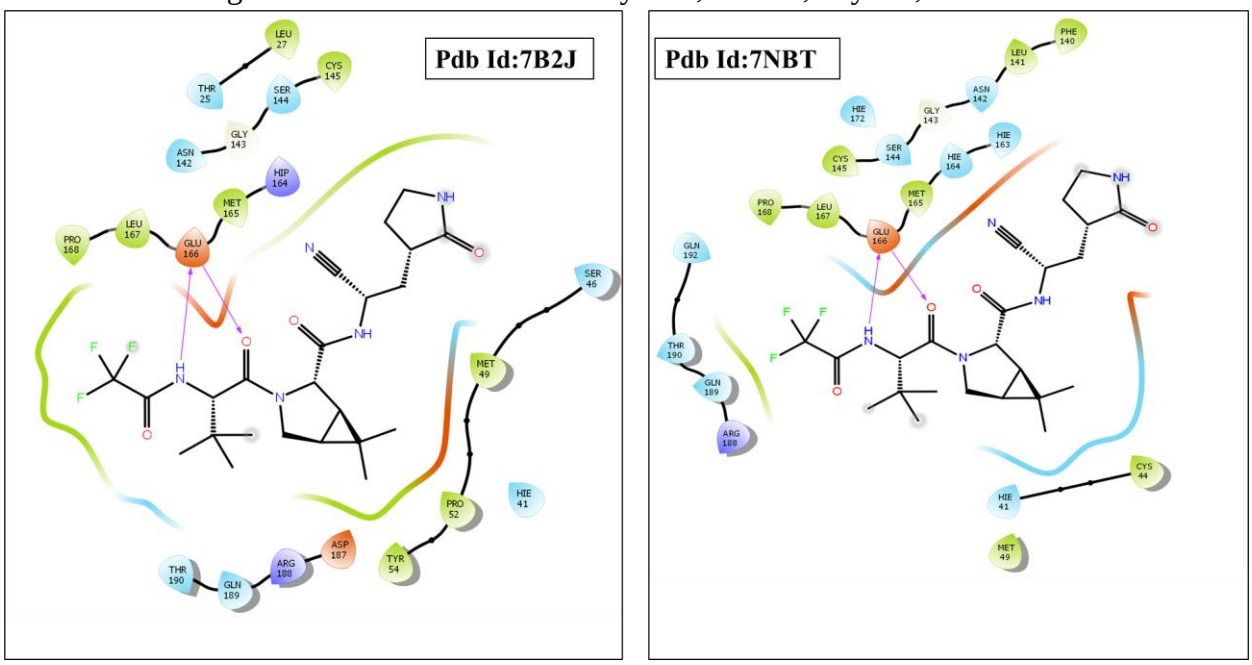

Figure 6. 2D interaction diagram of Nirmatrelvir ligand with respectively, 7B2J and 7NBTcrystal structures

In Figure 6, analysis was performed for Nirmatrelvir and 7B2J and 7NBT structures, which are also a different crystal structure. In the 2D interaction diagram between 7B2J and Nirmatrelvir, it was determined that it makes hydrogen bonds with Glu 166, and that there is an interaction in the region where the amino acids Met49, Pro52, Tyr54, Met165 are docked. Another crystal structure in Figure 6, 7NBT, has an H-bond interaction with the Glu166 amino acid of Nirmatrelvir.

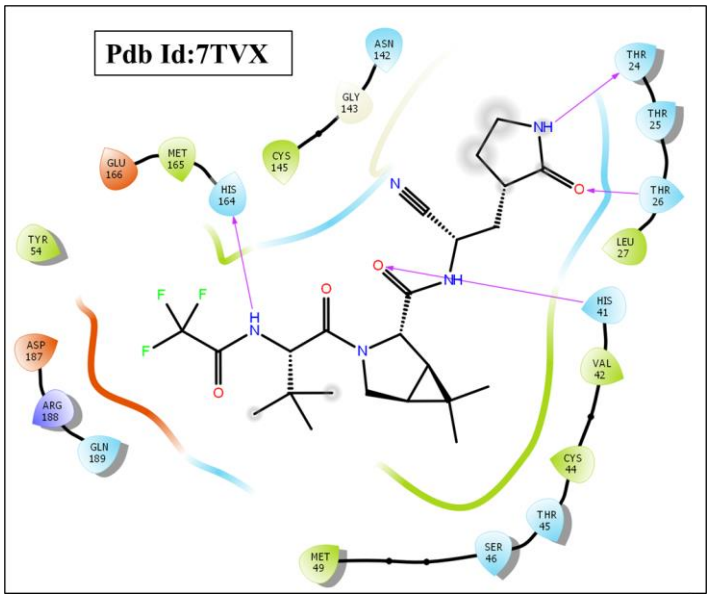

Figure 7. 2D interaction diagram of Nirmatrelvir ligand with 7TVX crystal structure

In Figure 7, the final crystal structure between 7TVX crystal structure and Nirmatrelvir were analyzed in this study. His 164, His41, Thr26, Thr24 had hydrogen bond interactions with amino acid residues.

CONCLUSION

In the study, SARS-CoV-2 of M^{Pro} different crystal structures were obtained from the protein database. Interactions with Nirmatrelvir, which has inhibitory properties on these crystal structures, were investigated using an *in silico* approach. The PDB codes of these crystal structures are 7O46, 7QBB, 7NEO, 7B77, 7B2U, 7B2J, 7NBT and 7TVX. Molecular docking studies were performed with Schrödinger 2021-2 (Schrödinger, LLC New York, USA) software. The most striking of the examined structures is PDB ID: 7B77. This is because the binding parameters between 7B77-Nirmatrelvir have the best ΔG_{Bind}, docking score and Glide emodel energy values in Table 1 and the presence of amino acids in the binding site presented in the Figure 2. In addition, it was determined that Glu166, Asn142, Hie163 and Cys145 amio acid residues, which have significant activity on M^{Pro} of SARS-CoV-2, participate in binding in all Nirmatrelvir and crystal structures. Molecular docking studies with this *in silico* approach will be pioneering for researchers working on a new drug candidate.

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Conflict of Interest

The author declares no competing interests.

Author's Contributions

B.T. contributed to all molecular docking studies, HTS analyzes, discussed the results, and wrote the manuscript.

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