RESEARCH ARTICLE

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Investigation of Various Antibiotics in the Structure of Sars Cov-2 Mpro by Molecular Docking and Molecular Dynamics Simulation Method

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ABSTRACT: Since the development of new antiviral agents to treat Sars-Cov-2 infection takes a long time, the reusability of existing drugs made more sense to investigate. The SARS-CoV-2 Mpro construct is the most likely biological target for antiviral drugs. Our aim in this study is to target and investigate various antibiotics to N3 inhibitor, which is sars cov-2 Mpro inhibitor. The protein structure of Sars-Cov2 Mpro (PDB ID:6LU7) was retrieved from the Protein Data Bank. Antibiotic compounds were obtained from PubChem. Molecular docking work was done using AutoDock Vina program. Molecular dynamics simulation study was done in Desmond Maestro software. According to the results of molecular insertion, GPER cell modulation in immunity, inflammation and consultation, compared with the antibiotic compounds N3 inhibitor, amoxicillin has better binding affinity while sulcaktam has lower binding affinity. Other antibiotic compounds showed close binding affinity with the N3 inhibitor. According to the molecular dynamics simulation results, we found that the antibiotic compounds complexes we placed on Sars-Cov2 Mpro had good conformational stability. When we look at the MM/GBSA result, amoxicillin has the best binding energy compared to the N3 inhibitor. Sulbactam has low binding energy and has shown close results with other N3 inhibitory antibiotic compounds. As a result, our results support that amoxicillin, which shows better binding affinity than N3 inhibitor, and clavulanic acid, ampicillin, amikacin sulfate, azithromycin, and cefuroxime sodium compounds, which show close binding affinity, will be a usable inhibitor in Sars-Cov-2 Mpro target.

Keywords: Sars-Cov-2 Mpro, Antibiotics Coumpounds, Molecular Docking, Molecular Dynamics Simulation.

INTRODUCTION

Antiviral treatments are needed for the COVID-19 outbreak, which has emerged as severe acute respiratory syndrome coronavirus-2 (Sars-Cov-2). Many researchers are working around the world to develop antiviral compounds and new antiviral agents already available for SARS-CoV-2 (Zhu et al., 2020). The cycle of SARS-CoV-2 to date provides information on possible targets for drug development studies (Ghosh et al., 2020).

When we look at the inhibition mechanism of Sars-Cov-2 protein, there are important targets. These are Sars-Cov-2 Mpro, the papain-like protease PLpro, cysteine proteases that process polyproteins translated from viral RNA in the replication of SARS-CoV-2 virus. Inhibiting the activity of these enzyme constructs will block the viral life cycle. In addition, SARS-CoV-2 Mpro has a distinctive feature from human proteases. This property is its ability to break down peptides after a glutamine residue (Muramatsu et al., 2016).

In the Sars Cov-2 protein structure, the most well-characterized inhibitor structure so far is Mpro. This is because Mpro inhibitors act by a covalent mechanism. For identification, they share a medium-sized peptidomimetic scaffold with a glutamine at the P1 position and a branched lipophilic group at the P2 positions (Dai et al., 2020; Jin et al., 2020; Zhang et al., 2020) (Figure 1).

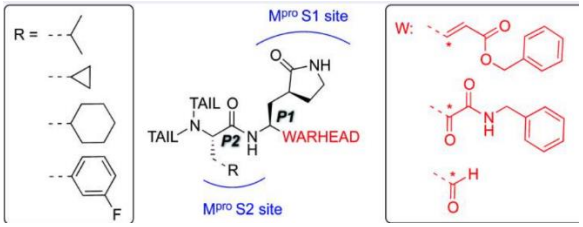

Figure 1. *Structure of Mpro, the covalent inhibitor known so far from Sars CoV-2 protein structures. R: P2 residue, P2 residue is defined as the lipophilic group. The tail is variable in shape and size. It contains Mpro subunits (S3 and S4), which cannot be identified in Figure 1.*

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Activated carbonyl derivatives, including alpha-ketoamides and aldehydes, from Michael acceptors(MAs) of SARS-CoV-2 inhibitors have been illustrative for warheads used so far. A few drugs containing the MA class are examples for covalent modification of cysteine residues (Latorre et al., 2016; Jackson et al., 2017; Gehringer et al., 2019; Voice et al., 2019). MAs are widely used against cysteine proteases because they are effective in enzyme inhibition (Bauer, 2015). Find as a promising inhibitor for Sars Cov-2 N3 inhibitor is a covalent inhibitör (Figure 2).

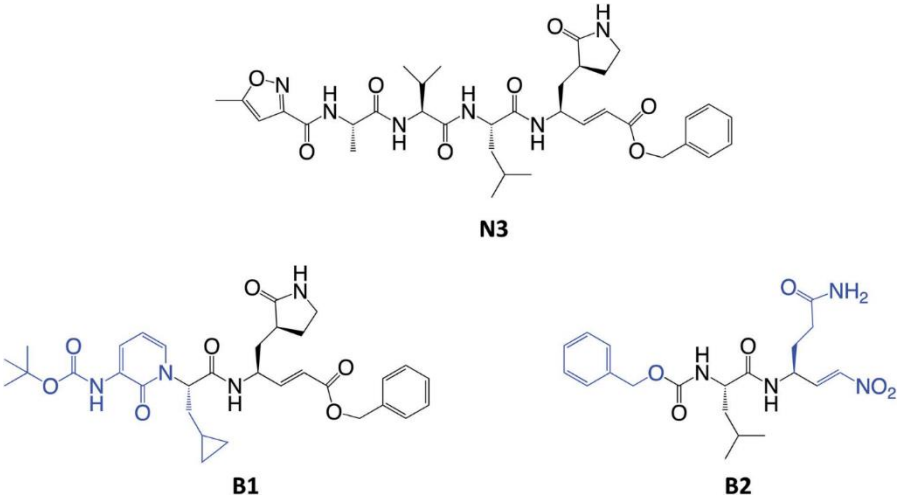


Figure 2*. Structure of N3 inhibitor of Sars-Cov-2 Mpro and structures of Michael acceptors (B1 and B2)*

The binding targets of Sars-Cov-2 Mpro as a result of its complex structure with the N3 inhibitor are shown in Figure 3.

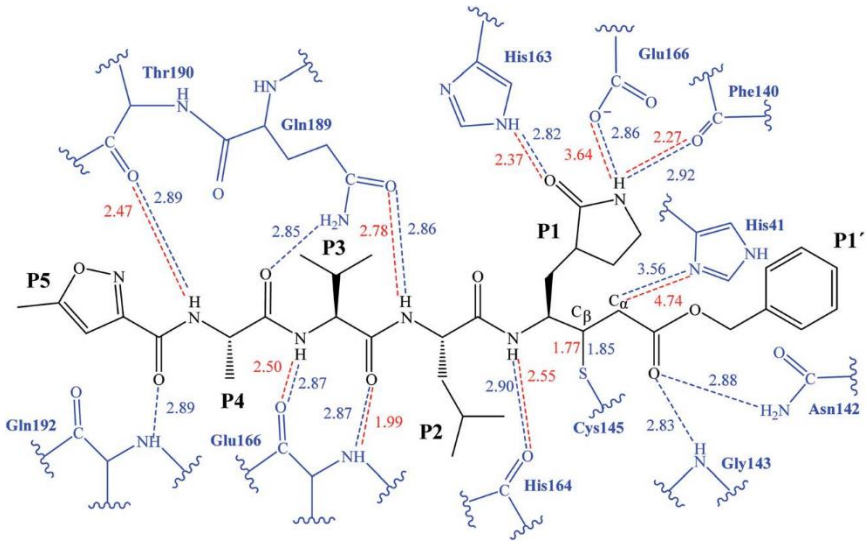


Figure 3. *Complex structure of Sars-Cov-2 Mpro with N3 inhibitor*

We think that Sars Cov 2 Mpro will block the catalytic function of the virus with ligands characterized by target amino acids Gln192, Thr190, Gln189, Glu166, His163, Cys145, Phe140, Asn142 and His41 in its active site. For this reason, we thought that the molecular docking targeting of the compounds in the antibiotic structure should remove the binding affinity and binding sites. Our aim in this study is to target and investigate various antibiotics to N3 inhibitor, which is Sars cov-2 Mpro inhibitor.

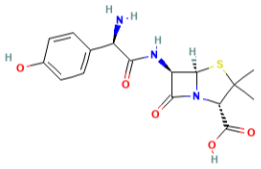
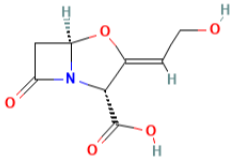
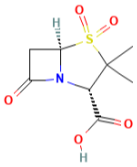
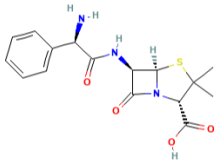
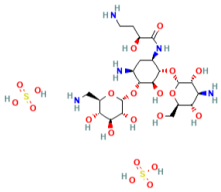
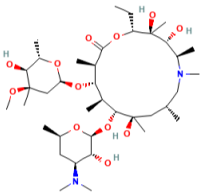
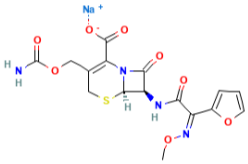
MATERIALS AND METHOD

Molecular Docking Method

Ligand Preparation

The amoxicillin, clavulanic acid, sulbactam, ampicillin, amikacin sulfate, azithromycin, and cefuroxime sodium used in this study were retrieved from the PubChem database (Table 1). (https://pubchem.ncbi.nlm.nih. gov). Converted from Open Babel GUI program to PDB format.

Table 1. *T-test chart for Microplastic awareness levels of men and women*

	Ligand Name	Ligand 2D Structre
1	Amoxicillin	
2	Clavulanic acid	
3	Sulbactam	
4	Ampicillin	
5	Amikacin sulfate	
6	Azithromycin	
7	Cefuroxime sodium	

Protein Preparation

3D structure of the Sars-Cov-2 main protease (PDB ID: 6LU7) was retrieved from the Protein Data Bank (PDB) website. (http://www.rcsb.org/pdb/.). The resolution of the PDB ID: 6LU7 protein is 2.16 Å and this value is good for the protein structure.

Ligands and water molecules in the 6LU7 protein structure were removed from the receptor. In the next step, polar hydrogen and a charge (colman charge) were added together with the receptor in the protein structure. All preparatory processes were carried out using AutoDock 4 software (Morris et al., 2009).

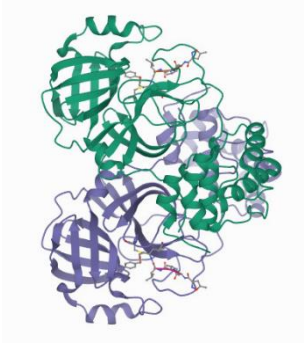


Figure 4. *The crystal structure of COVID-19 main protease in complex with an inhibitor N3*

Validation Method

N3 inhibitor from Sars cov-2 inhibitor protein (belonging to PDB ID:6LU7) was extracted with AutoDock 4 (Jin et al., 2020). Docking was performed without adding N3 inhibitor, which is Sars cov-2 Mpro ligand. Also, the mean square of difference (RMSD) value in PyMOL is checked to see the validation of our study. If the RMSD value is less than 2.0 Å, it indicates that the method is valid. This can manage to reduce the cost (Bell and Zhang, 2019).

Molecular Docking Simulation

It was carried out by applying all parameters valid for molecular docking simulation. 3D structure antibiotics compounds coupled to Sars-Cov-2. The active site site coordinates of the MPro receptor are x=-9.732, y=11.403 and z=68.925. Grid-box dimensions are 64 Å, 60 Å and 60 Å, respectively. 100 replicates were made for each active compound to ensure the correctness of the binding energy and amino acid interactions. Molecular docking operation was performed with AutoDock 4 (Laskowski, 1995).

Molecular Dynamics Simulation Method

The stability of the inserted antibiotics-Sars cov-2 Mpro (PDB ID:6LU7) complexes was investigated by performing a 20 ns molecular dynamics simulation study (Laskowski, 1995). The complex in the solver system with the OPLS3 force field was used by the Desmond program of Schreodinger 2019-4. The molecular system was resolved with crystallographic water (TIP3P) molecules under orthorhombic periodic boundary conditions for the 10Â buffer region. Overlapping water molecules were removed and Na+ was added as an ion to neutralize the system (Jorgensen et al., 1983). An assembly of Nose-Hoover thermostat and barostat (NPT) was applied to maintain constant temperature (300K) and pressure (1 bar) of the systems, respectively (Martyna et al., 1992). A hybrid energy minimization algorithm with the steepest descent with 1000 steps followed by conjugate gradient algorithms was used. Another algorithm, the limited memory Broyden-FletcherGoldfarb-Shanno (LBFGS) algorithm with a convergence threshold gradient of 1kcal/mol/Å was also used for energy minimization. A Smooth Particle Mesh Ewald method was used to calculate long-range electrostatic interactions with a cutting radius of 9Å for short-range van der Waals and Coulomb interactions. Multi-time step RESPA integration (reference system emitter algorithms) was used for near and far coupled interactions, coupled with 2, 2, and 6 fs, respectively, in the dynamic study. Data was collected for every 100ps and the resulting trajectory was analyzed with the Maestro graphical interface.

The binding energies between Sars-Cov-2 Mpro and antibiotic complexes were calculated by the MM/GBSA method. The mean binding free energy (ΔG binding) with standard deviation was calculated using the thermal_mmgbsa.py script. MM/GBSA binding energies were calculated using 10 ns squares for the trajectory of the entire system.

RESULTS

Validation Results

Revalidation with N3 inhibitor was performed to reveal the strength of binding affinity. The result of our validation is shown in Figure 5. The ligand had an RMSD value of 1.61 Å and the binding energy was -6.9 kcal/mol. This molecular docking is valid due to the RMSD value 2.0 Å below the method used.

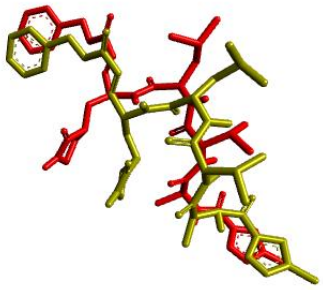


Figure 5*. Status of N3 inhibitor with Sars-Cov-2 MPro inhibitor before reconfirmation (red), status of N3 inhibitor after insertion (yellow)*

Molecular Docking Results

Binding affinities were determined by the number of key binding interactions and the insertion score, with specific reference to hydrogen bonds. The best binding conformations of the antibiotic compounds targeted to the Sars-Cov-2 Main protease structure were selected (Table 2). According to the result of antibiotic compounds targeted to the active site of Sars-CoV-2 main protease, amoxicillin showed the best binding affinity when compared with N3 inhibitor. Other antibiotic compounds revealed that it binds with lower affinity than the N3 inhibitor. N3 inhibitor has a binding affinity of -6.9 kcal/mol, while amoxicillin is -7.8 kcal/mol. The binding affinities of clavunic acid, sulbactam, ampicillin, amikacin sulfate, azotromycin and cefuroxime sodium compounds are -6.0, -5.3, -6.6, -6.0, -6.6 and -6.4 kcal/mol, respectively. Basic attachment models are also presented (Table 2).

Table 2. *Binding affinities and insertion sites of various antibiotics in the Sars CoV-2 main protease structure (PDB ID: 6LU7)*

Ligand Name	**PDB ID**	**Hydrogen Bonds**	**Hydrofobics Interactions**	**Docking Score(kcal/mol)**	
				Ligand	**N3 İnhibitor**
Amoxicillin	**6LU7**	**LEU141 GLY143 SER144 CYS145 ARG188**	**MET165**	**-7.8**	**-6.9**
Clavulanic acid		**LEU141 CYS145**		**-6.0**	
Sulbactam		**LEU141 GLY143 CYS145 GLU166**		**-5.3**	
Ampicillin		**THR26 PHE140 GLY143 SER144 CYS145 GLU166**	**GLU166**	**-6.6**	
Amikacin sulfate		**THR26 LEU141 CYS145**	**THR25 ASN142 MET165**	**-6.0**	
Azithromycin		**SER144**	**PRO168**	**-6.6**	
Cefuroxime sodium		**THR24 THR25 GLY143 GLU166 LEU141**		**-6.4**	

The best binding patterns were revealed according to the molecular docking results. It was observed that amoxicillin, which showed the best binding affinity for Sars Cov-2 main protease (PDB ID: 6LU7), made hydrogen bonding with LEU141, GLY143, SER144, CYS145 and ARG188 and hydrophobic interaction with MET165 at the binding site. It was observed that clavunic acid made hydrogen bonds with LEU141 and CYS145 at the binding site. It was observed that sulbactam made hydrogen bonds with LEU141, GLY143, CYS145 and GLU166 at the binding site. It was observed that ampicillin made hydrogen bonding with THR26, PHE140, GLY143, SER144, CYS145, GLU166 and hydrophobic interaction with GLU166 at the binding site. It was observed that amikacin sulfate formed hydrogen bonds with THR26, LEU141 and CYS145 at the binding site and hydrophobic interaction with THR25, ASN142, MET165. It was observed that azothromycin made hydrogen bonding with SER144 and hydrophobic interaction with PRO168 at the binding site. It was observed that cerfuroxime sodium made hydrogen bonds with THR24, THR25, LEU141, GLYS143 and GLU166 at the binding site (Figure 6-12).

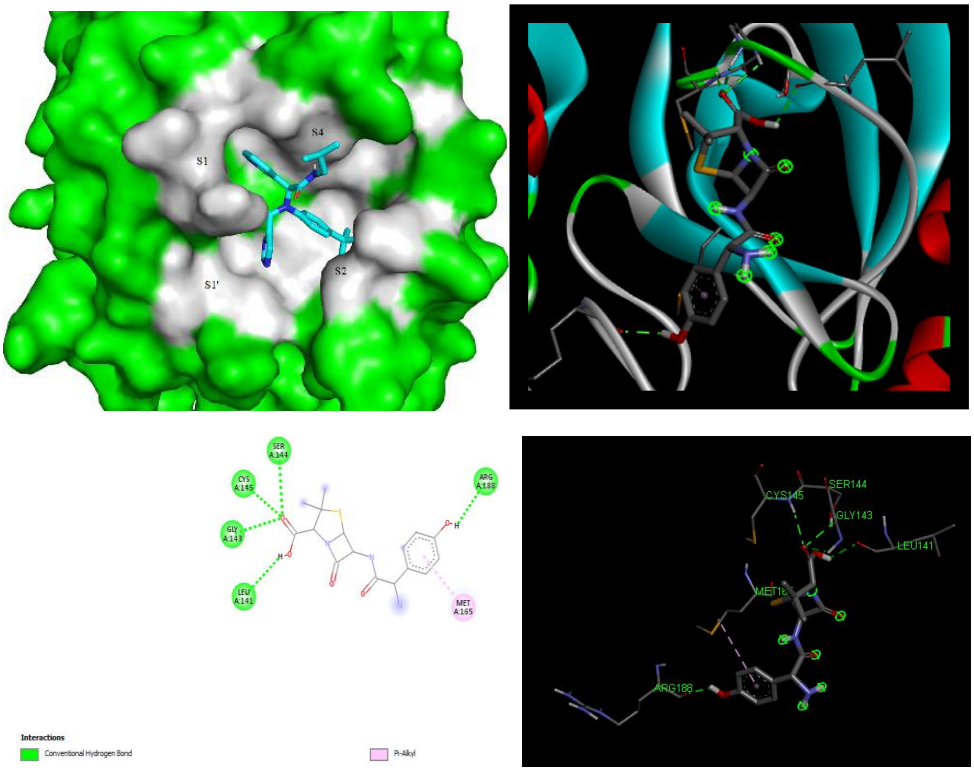


Figure 6. *Insertion of amoxicillin into the active site of Sars Cov-2 Mpro (PDB ID: 6LU7), 2D and 3D interaction model*

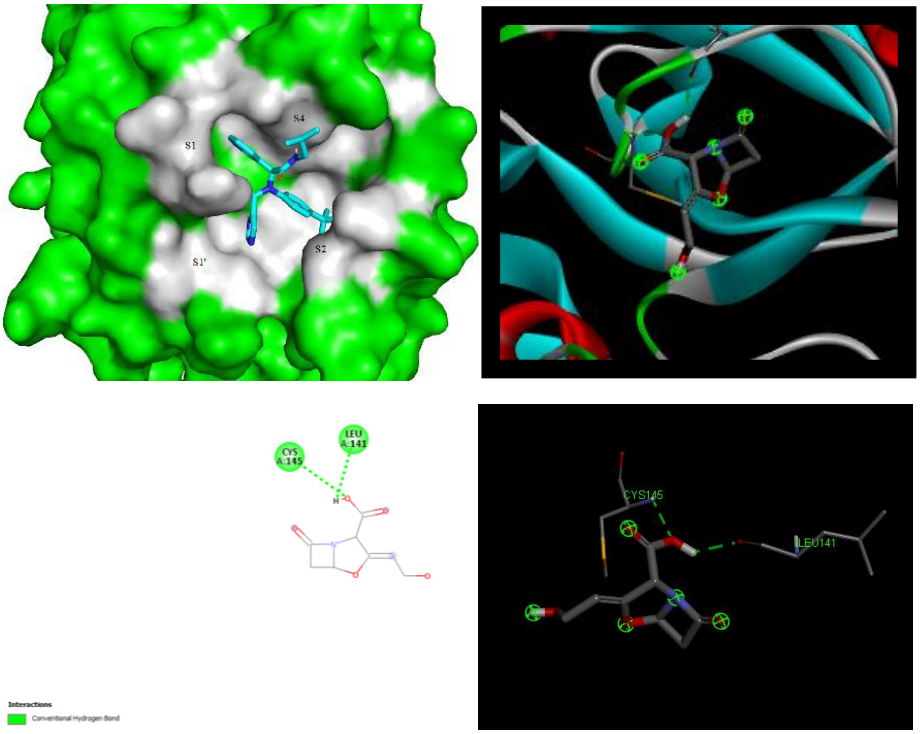


Figure 7. *Placement of clavunic acid Sars Cov-2 Mpro in the active site (PDB ID: 6LU7), 2D and 3D interaction model*

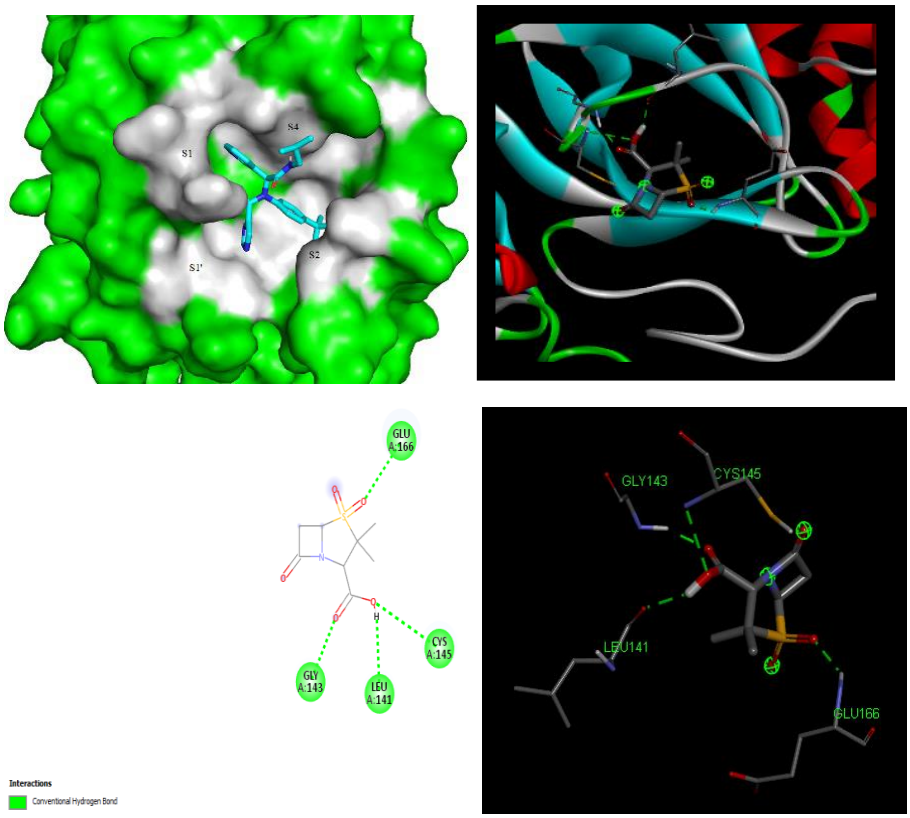


Figure 8. *Placement of Sulbactam Sars Cov-2 Mpro in active site (PDB ID: 6LU7), 2D and 3D interaction model*

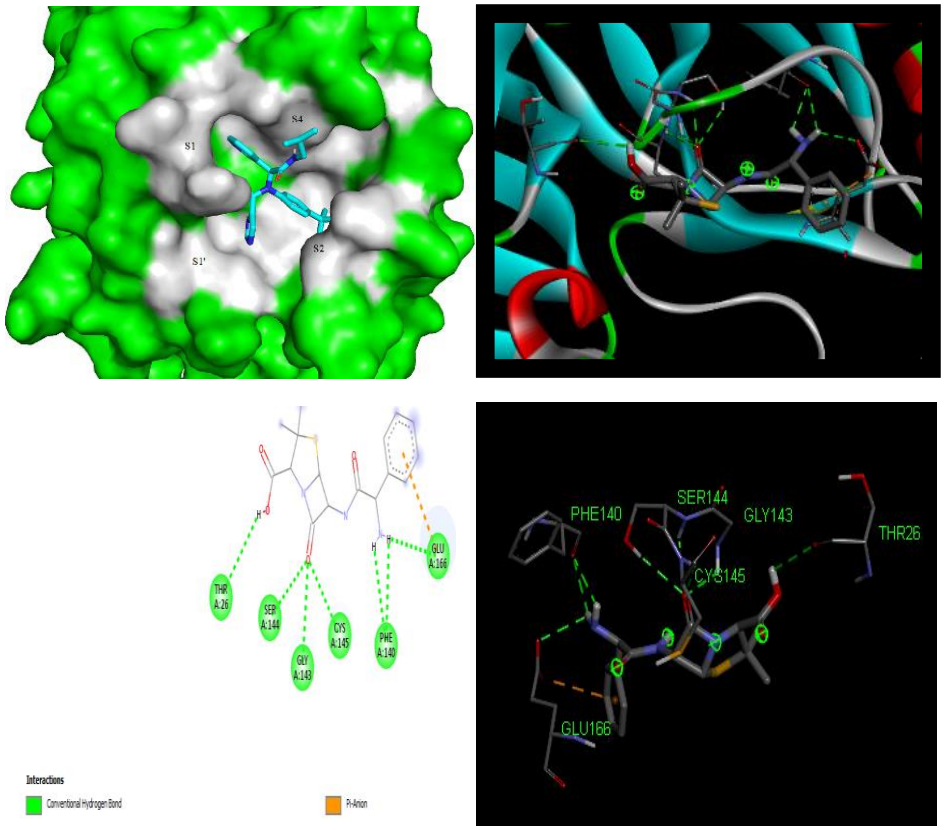


Figure 9. *Placement of Ampicillin in the active site of Sars Cov-2 Mpro (PDB ID: 6LU7), 2D and 3D interaction model*

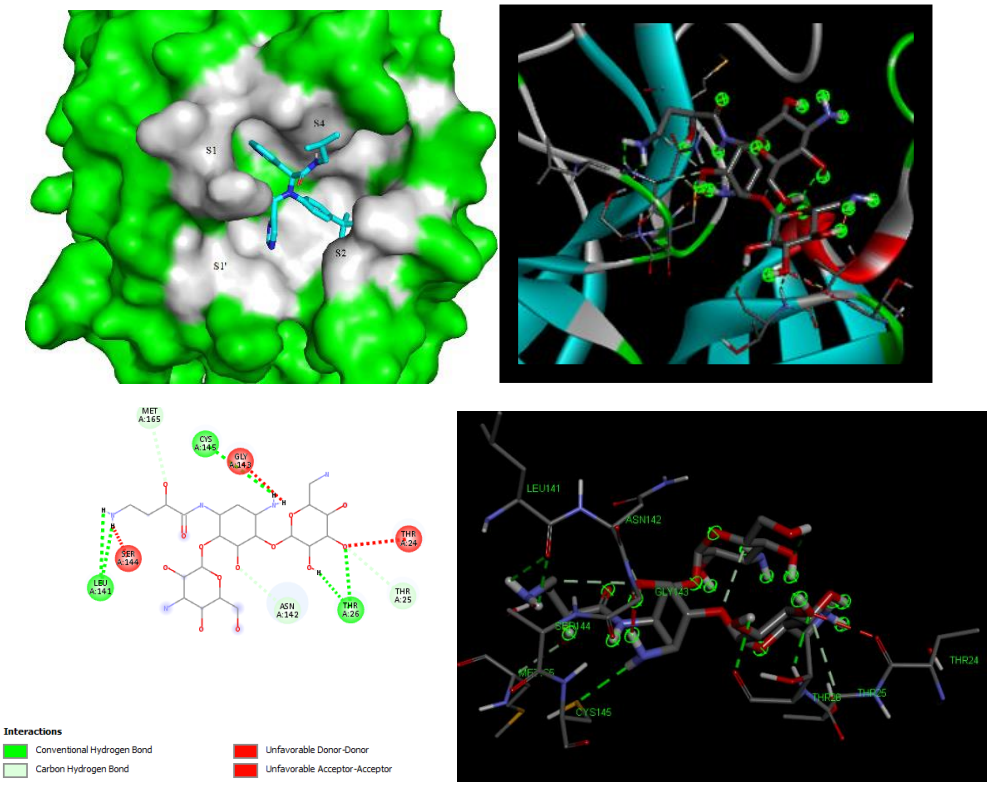


Figure 10. *Insertion of Amikacin Sulfate Sars Cov-2 Mpro in the active site (PDB ID: 6LU7), 2D and 3D interaction model*

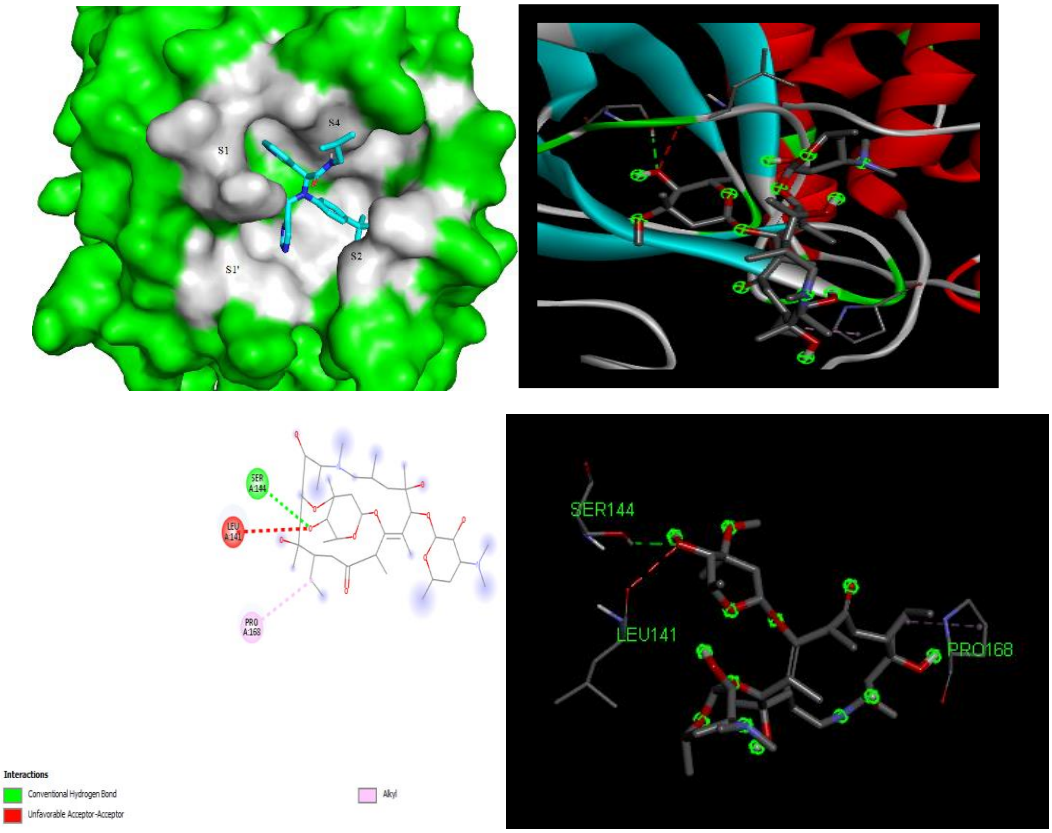


Figure 11. *Insertion of Azothromycin Sulfate Sars Cov-2 Mpro in the active site (PDB ID: 6LU7), 2D and 3D interaction model*

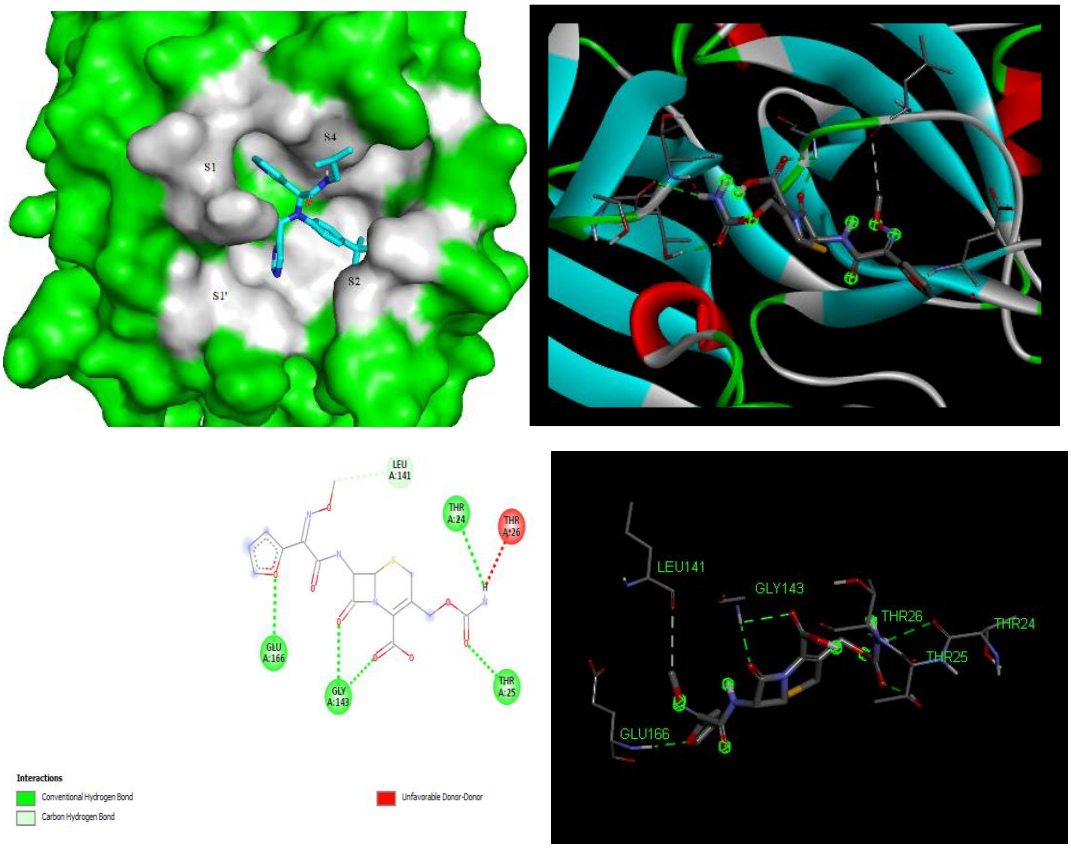


Figure 12. *Placement of Cefuroxime Sodium Sulfate Sars Cov-2 Mpro in the active site (PDB ID: 6LU7), 2D and 3D interaction model*

Molecular Dynamics Results

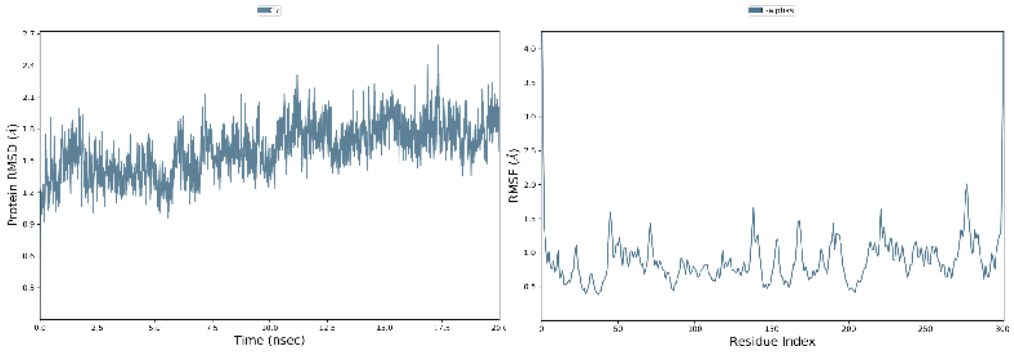


Figure 13. *RMSD and RMSF analysis of the MD simulation trajectory of the 6LU7 protein structure*

It was observed that the RMSD value of amoxicillin in the SARS-CoV-2 Mpro (PDB ID: 6LU7) complex did not change significantly during the dynamic simulation and the fluctuations were stable. Looking at the RMSD graph, we saw that Sars cov-2 Mpro and amoxicillin ligand showed a similar trend and the RMSD value continued slightly above 2 Å (Figure 14).

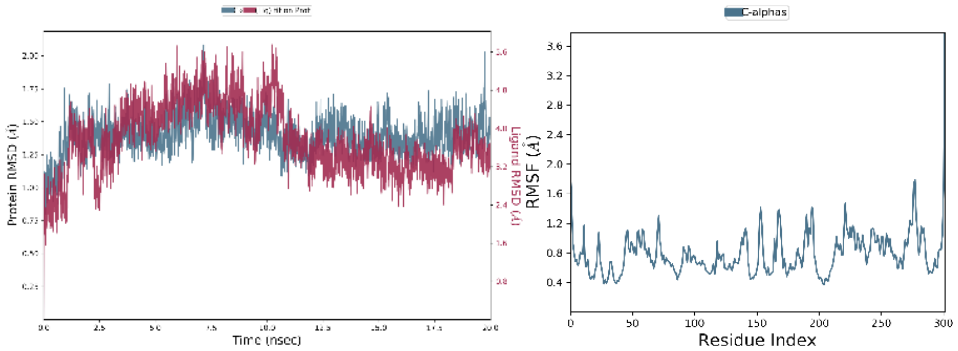


Figure 14. *RMSD and RMSF analysis of the MD simulation trajectory of the 6LU7 protein-Amoxicillin complex structure*

The complex of clavunic-acid with the Sars-Cov-2 main protease structure (PDB ID: 6LU7) fluctuated very little and peaked at 12.52 ns (2.5 Å), and clavunic acid remained stable. Finally, it made a sharp jump with RMSD values above 8 Å, down to 8.02 ns, and finally stabilized (Figure 15).

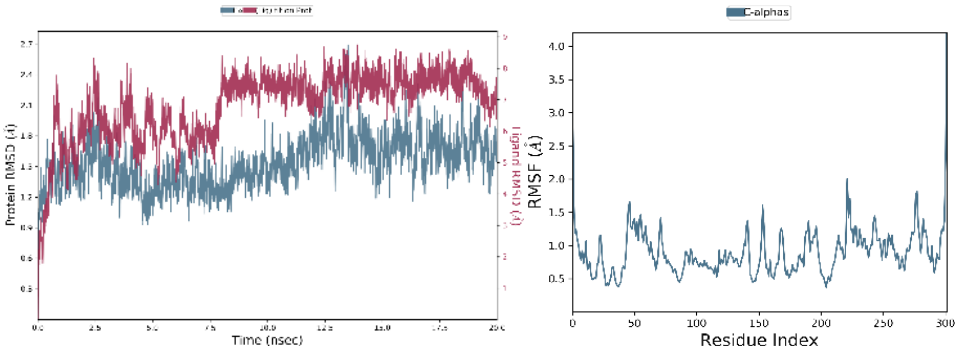


Figure 15. *RMSD and RMSF analysis of the MD simulation trajectory of the 6LU7 protein-Clavunic acid complex structure*

Looking at the RMSD graph of sulbactam and Sars-Cov-2 Mpro (PDB ID:6LU7) complex, it was seen that the RMSD value reached a maximum of 2.35 A at the end of the dynamic simulation. The RMSD value of the sulbactam ligand showed a very clear increase from 0.94 Å to 8.07 Å and remained stable after 6 ns (Figure 16).

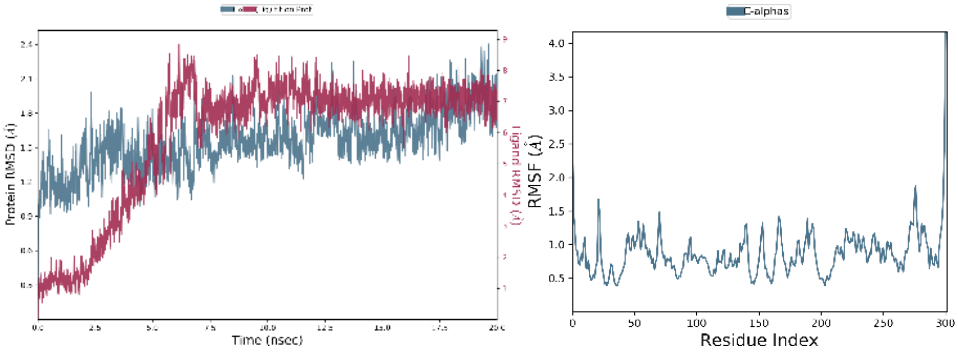


Figure 16. *RMSD and RMSF analysis of the MD simulation trajectory of the 6LU7 protein-Sulbactam complex structure*

The sars cov-2 Mpro (PDB ID:6 LU7) complex with ampicillin showed a stable ligand-protein complex over the entire simulation period at an RMSD of 0.8 Å (Figure 17).

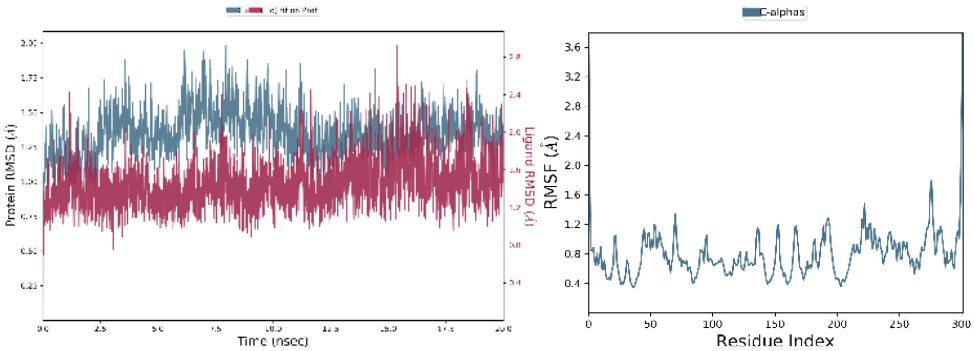


Figure 17. *RMSD and RMSF analysis of the MD simulation trajectory of the 6LU7 protein-Ampicilin complex structure*

According to the graph of amikacin sulfate and sars cov-2 Mpro, the RMSD value at 3.60 ns was 4.53 Å and fluctuations were observed after 13.47 ns(Figure 18).

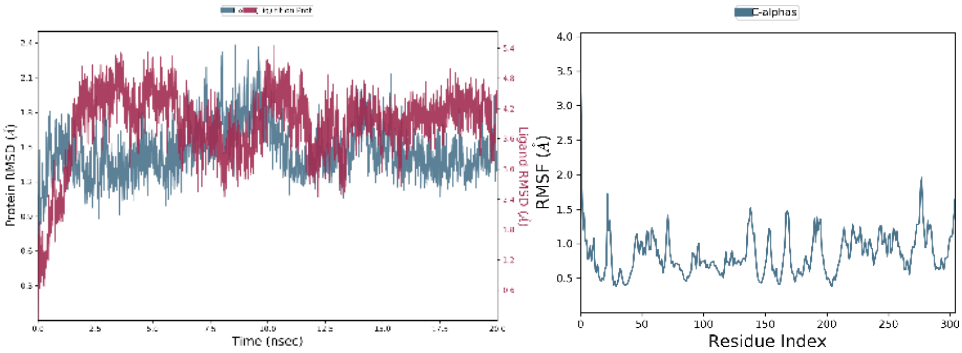


Figure 18. *RMSD and RMSF analysis of the MD simulation trajectory of the 6LU7 protein-amikacin sulfate complex structure*

On the graph of the Sars-Cov-2 Mpro (PDB ID: 6LU7) complex with azothromycin, the RMSD value at 15.82 ns was 2.22 A and was very stable, while the RMSD value below 6.05 ns showed little fluctuation at 3.1 A. A stable fluctuation was seen after 12.5 ns(Figure 19).

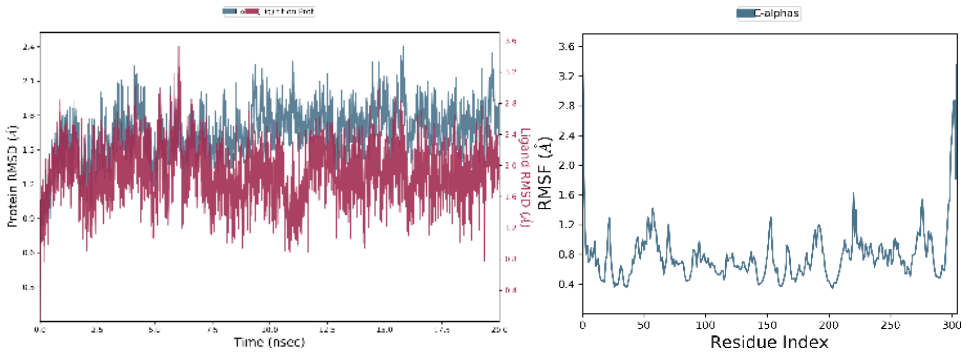


Figure 19. *RMSD and RMSF analysis of the MD simulation trajectory of the 6LU7 protein-Azothromycin complex structure*

Looking at the RMSD graph in the Sars-Cov-2 Mpro (PDB ID:6LU7) complex with cefuroxime sulfate, the fluctuation remained stable until the end of the dynamic simulation. The RMSD value, which was above 2 A at the beginning of the simulation, showed a stable fluctuation at the end of 12.58 ns(Figure 20).

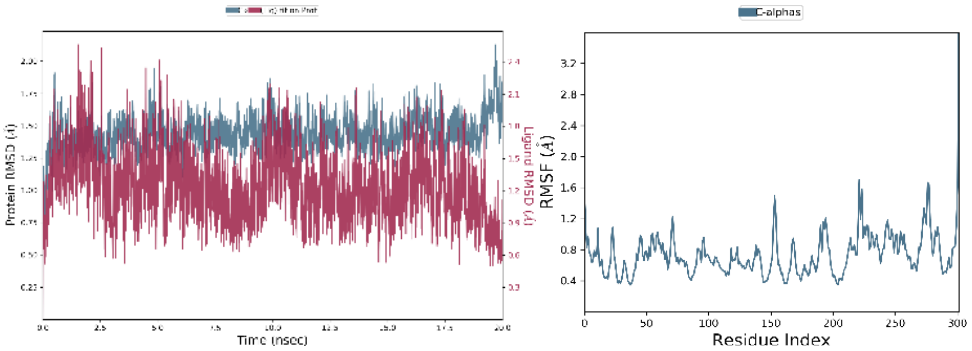


Figure 20. *RMSD and RMSF analysis of the MD simulation trajectory of the 6LU7 protein-cerfuroxime complex structure*

According to the MM/GBSA result; Amoxicillin, one of the antibiotic compounds, showed good affinity compared to the N3 inhibitor. Clavunic acid, ampicillin, amikacin sulfate, azotromycin and cefuroxime sodium compounds showed close affinity to N3 inhibitor. However, sulbactam showed low affinity compared to N3 inhibitor.

Table 3. *MM/GBSA ΔG binding scores of antibiotics compared to N3 inhibitor*

PDB ID	Ligand Name	MM/GSA ΔG bind ±SD (kcal/mol)
6LU7	N3 Inhibitor	-42.34±4.78
	Amoxicillin	-68.31±3.71
	Clavulanic acid	-36.10±2.14
	Sulbactam	-17.22±1.98
	Ampicillin	-37.88±2.33
	Amikacin sulfate	-29.91±3.45
	Azithromycin	-38.47±3.24
	Cefuroxime sodium	-35.77±3.66

DISCUSSION

According to the results of our study, when the antibiotic compounds are evaluated according to our molecular docking and molecular dynamics simulation research on Sars-cov-2 Mpro structure to the N3 inhibitor, it is seen that amoxicillin shows the best binding affinity. We determined that sulbactam has very low binding affinity. However, it showed close binding affinity with the N3 inhibitor when compared to other antibiotic compounds. Let us consider our results in more detail. The thiohemiketal

ring is formed in a reversible reaction with the nucleophilic attack of the α-keto group of Cys 145, which has catalytic activity of the antibiotic compounds (amoxicillin, clavulanic acid, sulbactam, ampicillin, amikacin sulfate) that we target as inhibitors. This is clearly reflected in the electron density and is S in all copies of amoxicillin, clavulanic acid, sulbactam, ampicillin, amikacin sulfate compounds. The oxyanion (or hydroxyl) group of this thiohemiketal is stabilized by a hydrogen bond from His41, while the amide oxygen of the amoxicillin, clavulanic acid, sulbactam, ampicillin, amikacin sulfate group is hydrogen, one of the main chain amides of Gly 143, Cys 145 and partially Ser 144. creates the bond. An advantage of α-ketoamides is that, as with other warheads such as aldehydes, they can interact with the catalytic center of target proteases via two hydrogen-bonding interactions instead of just one. The P1 γ-lactam moiety, designed as a glutamine surrogate, is deeply embedded in the S1 pocket of the protease, where the lactam nitrogen donates a tricentric (forked) hydrogen bond to the main chain oxygen. Phe 140 and Glu 166 values for the structure in the carboxylate [and the carbonyl oxygen accepts hydrogen bonding from the imidazole of His. We found that our results were also supported by the 2020 study of Zhang et al (2020).

Zhang et al. (2020) emphasized that amino acid GLU-166 interacts with NH2 terminal residues, which is a necessary switch for the catalytic activity of the substrate binding site, namely the S1 unit.

Jin et al (2020) showed that ligands, which may be the strongest inhibitors for Sars-Cov-2 Mpro target, are formed by hydrogen bonding with Glu166, Cys145 and His163.

Jin et al (2020) found that N3 inhibitor is a potent inhibitor for Sars-Cov-2. They emphasized that the N3 inhibitor will be the target for drug studies.

In the study of Braz et al. (2020), examining the potential mechanism of action of azotromycin against SARS-CoV-2 infection in silico, it was observed that the binding affinity of azotromycin in the sars cov-2 Mpro structure was high.

Sencanski et al. (2020) found that ampicillin has a binding energy of -6.8 kcal/mol in the sars cov-2 Mpro structure. A similar result was found when compared with our study.

Except for the antibiotics we discussed, no in silico studies have been conducted on the compounds in the other antibiotic group.

When compared with the N3 inhibitor, the compounds that could be potential inhibitors in the Sars Cov-2 Mpro structure found in our study are amoxicillin, ampicillin, azotromycin and cefuroxime sulfate compounds, respectively.

CONCLUSION

In summary, we think that Sars Cov 2 Mpro will block the catalytic function of the virus with ligands characterized by target amino acids Gln192, Thr190, Gln189, Glu166, His163, Cys145, Phe140, Asn142 and His41 in its active site. It is especially responsible for the hydrolysis reaction of the sulfur atom of the Cys amino acid. We predict that antibiotic compounds that react with Cys will block its catalytic function. Our results support that amoxicillin, which shows better binding affinity than N3 inhibitor, and clavulanic acid, ampicillin, amikacin sulfate, azithromycin, and cefuroxime sodium compounds, which show close binding affinity, will be a usable inhibitor in Sars-Cov-2 Mpro target.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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