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Research Article
In silico Antivirus Repurposing and its Modification to Organoselenium Compounds as SARS-CoV-2 Spike Inhibitors

Manaman Huang, Mutiara Saragih and Usman Sumo Friend Tambunan

Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Indonesia, Depok, West Java 16424, Indonesia

Abstract

Background and Objective: The COVID-19, which has been circulating since late 2019, is caused by SARS-CoV-2. Because of its high infectivity, this virus has spread widely throughout the world. Spike glycoprotein is one of the proteins found in SARS-CoV-2. Spike glycoproteins directly affect infection by forming ACE-2 receptors on host cells. Inhibiting glycoprotein spikes could be one method of treating COVID-19. In this study, the antivirus marketed as a database will be repurposed into an antiviral SARS-CoV-2 and the selected compounds will be modified to become organoselenium compounds. **Materials and Methods:** The research was carried out using *in silico* methods, such as rigid docking and flexible docking. To obtain information about the interaction between spike glycoprotein and ligands, MOE 2014.09 was used to perform the molecular docking simulation. **Results:** The analysis of binding energy values was used to select the ten best ligands from the first stage of the molecular docking simulation, which was then modified according to the previous QSAR study to produce 96 new molecules. The second stage of molecular docking simulation was performed with modified molecules. The best-modified ligand was chosen by analyzing the ADME-Tox property, RMSD value and binding energy value. **Conclusion:** The best three unmodified ligands, Ombitasvir, Elbasvir and Ledipasvir, have a binding energy value of -15.8065, -15.3842 and -15.1255 kcal mol^{-1}, respectively and the best three modified ligands ModL1, ModL2 and ModL3 has a binding value of -15.6716, -13.9489 and -13.2951 kcal mol^{-1}, respectively with an RMSD value of 1.7109 Å, 2.3179 Å and 1.7836 Å.

Key words: Spike glycoprotein, *in silico*, antivirus, repurposed, modification, QSAR, binding energy

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Corresponding Author: Usman Sumo Friend Tambunan, Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Indonesia, Depok, West Java 16424, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The SARS-CoV-2 is the cause of the COVID-19 disease, which has been sweeping the globe since the end of 2019. At the end of 2019, an unknown pneumonia case emerged in Wuhan, Hubei Province, China. The clinical characteristics of this disease are very similar to pneumonia in general[1]. According to data, the first patient infected with COVID-19 came from Hubei Province in China. The first cases of pneumonia detected in Wuhan were reported to the WHO (World Health Organization) in late 2019 and the COVID-19 outbreak was declared a pandemic in March, 2020. Coronaviruses exhibit significant serologic and strand variation, with the S gene (the gene encoding spike glycoprotein) showing the most significant diversity[2]. Changes in the spike glycoprotein structure caused by S gene mutations can change the virus's character. The virus's high infectivity level is one reason for the high number of COVID-19 cases. This virus's infectivity is linked to the spike glycoprotein in the virus's structure[3]. Reports state that the bat SARS-CoV was recombined with an unidentified -CoV to create the spike glycoprotein SARS-CoV-2[4]. Fluorescent research demonstrated that SARS-CoV-2 enters cells through binding to and activating the ACE-2 (Angiotensin Converting Enzyme 2) receptor[5].

Selenocysteine is the 21st amino acid discovered to date[6]. Selenocysteine is not one of the standard 20 amino acids. The UGA codon is used to express selenocysteine. The UGA codon, however, is a stop codon, as previously stated. In addition to serving as a stop codon, UGA can now be translated into selenocysteine[7]. The antibody-drug conjugates (ADC) method has been used to conjugate selenium to drug molecules for cancer treatment. The ADC is considered the most promising method for future use and many biotechnology companies are currently researching it[8].

Drug repurposing is a strategy for discovering new applications for marketed drugs that are not covered by the original medical indication[9]. Drug repurposing can be done in an emergency, such as the recent pandemic. Drug repurposing is done to shorten the time it takes to find a cure for a disease. Some drug repurposing has been successful, such as rituximab, which was repurposed from a cancer drug to a rheumatoid arthritis drug[10].

This experiment was carried out to investigate the interactions that spike glycoprotein has with antiviral molecules and modified molecules, as well as to analyze the ADME-Tox properties of all the best molecules chosen based on specific parameters, to identify the best ligands that can be proposed as new drugs to treat COVID-19.

MATERIALS AND METHODS

The study was carried out in January to May, 2022 at the Bioinformatics Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Indonesia, Indonesia.

Preparation for docking simulation: The spike glycoproteins' and antiviral ligands' 3D structures were obtained from the RSCB, PDB and PubChem, respectively. The MOE 2014.09 was used to prepare the spike glycoprotein 3D structure (PDB ID: 6VYB), the preparation includes deleting the water and other molecules, minimizing the energy and fixing the charge and hydrogen atoms on the proteins. The 3D structures of antiviral ligands were obtained as SDF files from PubChem and continued to be prepared using MOE 2014.09, including protonation, partial charge optimization and energy minimization. The prepared structures of protein and ligands were saved as PDB files.

First stage of a molecular docking simulation: The MOE 2014.09 was used for all molecular docking simulations. The first simulation examined the interaction between the spike glycoprotein and antiviral ligands. The MOE 2014.09 retrieved the protein's binding site from Site Finder. Only the rigid receptor mode was used to dot the docking simulation at this stage.

Ligand modification: The top ten antiviral ligands from the previous docking simulation were chosen to be modified. Based on the results of the QSAR study, selenocysteine was attached to a specific functional group of antiviral ligands.

Second stage of a molecular docking simulation: The rigid receptor and flexible docking were conducted in this simulation. The simulation analyzed the interaction between the spike glycoprotein and the modified best ligand. The chosen binding site used in this stage is the same as the previous simulation.

Pharmacological test: SwissADME (http://www.swissadme.ch/) was used to determine the best modified antiviral ligands by analyzing the ADME-Tox properties[11]. The SMILES used to determine the ADME-Tox properties were retrieved from ChemBioDraw Ultra 14.0.

RESULTS AND DISCUSSION

Optimisation of protein and ligands structure: The protein structure (PDB ID: 6VYB) was determined using electron microscopy at 3.20 Å[12]. The MOE 2014.09 was used to prepare the structure. The AMBER10:EHT forcefield was used to prepare the structure and the solvation was set to gas phase to represent simulation without any solvent (H_2O). Furthermore, the hydrogen and partial charge were adjusted and calculated using the 'fix charge' and 'fix hydrogen' protocols. The molecular simulation requires every atom to have a calculated charge to quantify the potential electrostatic interactions[13]. Following the completion of the protein preparation, the binding site was determined using the 'SiteFinder' menu. On sequence 712-1144, the selected binding site contains approximately 65 amino acids per chain. The S2 unit was chosen because it is more conserved than the S1 unit[14], additionally, the selected sequences contain the fusion peptide sequence, allowing the virus and host cell fusion[15].

The antiviral ligands retrieved from PubChem were prepared using MOE 2014.09. The preparations of the ligands are divided into three stages: 'Wash,' 'partial charge' and 'energy minimizes' available on the 'compute' menu. The ligand is protonated after the 'wash' step, which means protonation in strong bases and deprotonation in strong acids. It is hoped that the ligand will have properties suitable for the experimental conditions by passing this stage. The MMFF94x force field parameter is used to calculate the partial charge of the atoms on the molecule in the 'partial charge' step. The MMFF94x force field is recommended for use in 'partial charge' and 'energy minimize' processes due to its high accuracy for geometry optimization[16]. 'Energy minimize' was conducted to obtain the most stable (lowest energy) pose, with an RMSD gradient of 0.001. The optimized 3D structure of spike glycoprotein is shown in Fig. 1.

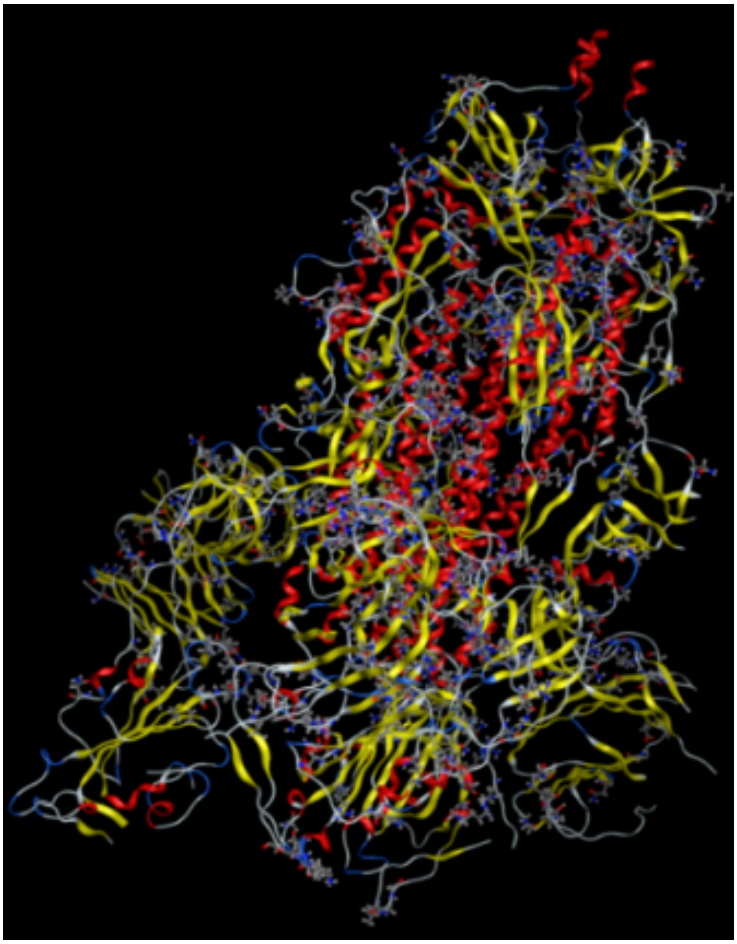

Fig. 1: 3D Structure of the spike glycoprotein after optimization

First stage of molecular docking simulation: Molecular docking was performed on a pre-prepared spike glycoprotein with 66 available ligands (1 as a control). Remdesivir is a control ligand that has been approved by the FDA (Food and Drug Administration) for use as a drug for the treatment of COVID-19 since late October 2020. The first stage of molecular docking simulation used ligands from antiviral compounds, including the ligand used as a standard, remdesivir. The 'rigid docking' process is carried out at this point. The 'rigid docking' principle employs the lock and key principle, in which a spike glycoprotein acts as a lock with minimal atomic movements and an antiviral agent acts as a key that can move freely in the selected pocket. In this study, the 'rigid docking' retain process was applied to 30:1 and 100:1. Retain is a ligand-binding interaction position[17]. In this stage, the best ten ligands were determined using only the $G_{binding}$ value. The standard ligand, Remdesivir, has a binding energy of -8.9597 kcal mol^{-1}. The top 10 ligands based on the binding energy value ranged from -15.8065 to -12.5821 kcal mol^{-1}, which can be seen in Table 1. Ombitasvir has the lowest binding energy compared to the other ligands docked.

Ligand modification: The ligand modification was conducted using the previous QSAR study: Atazanavir[18], cobicistat[19], daclatasvir[20], elbasvir[21], fosamprenavir[22], ledipasvir[23], ombitasvir[24], ritonavir[25], simeprevir[26] and telaprevir[27]. Modification is accomplished by attaching selenocysteine to specific functional groups in each drug molecule. The modification produced 96 new molecules. All new molecules will be prepared using the same protocol as the previous preparation, then continued to the second stage of a molecular docking simulation.

Second stage of molecular docking simulation: At this stage, molecular docking simulation was conducted in 2 steps, rigid receptor followed by flexible docking. The flexible docking was conducted to obtain a higher accuracy value of RMSD[28]. Flexible docking is a process that allows the two molecules to move because not all of the bonds in the two molecules are rigid. The retain used to perform the rigid docking in this stage is the same as the first molecular docking stage, but the flexible docking was conducted using 100:1 retain. The ten best ligands obtained were determined by analyzing the $G_{binding}$ and RMSD values, named ModL1 to ModL10. The ModL1 possesses the lowest binding energy with the value of -15.6716 kcal mol^{-1} with RMSD 1.7109 Å. The binding energy value ranged from -15,6716 to -12.0182 kcal mol^{-1} and the RMSD ranged from 1,7109 Å to 2.3796 Å, as seen in Table 2.

Table 1: First stage docking 10 best ligands

Ligand	$\Delta G_{binding}$ (kcal mol^{-1})
Ombitasvir	-15.8065
Elbasvir	-15.3842
Ledipasvir	-15.1255
Fosamprenavir	-13.2715
Daclatasvir	-13.1087
Simeprevir	-12.8775
Cobicistat	-12.7761
Ritonavir	-12.7586
Telaprevir	-12.6429
Atazanavir	-12.5821

Table 2: Second stage docking 10 best ligands

Ligand	Origin drug	$\Delta G_{binding}$ (kcal mol^{-1})	RMSD (Å)
ModL1	Ledipasvir	-15.6716	1.7109
ModL2	Ledipasvir	-13.9489	2.3179
ModL3	Ombitasvir	-13.2951	1.7836
ModL4	Ombitasvir	-13.2155	1.1583
ModL5	Elbasvir	-12.9065	1.0589
ModL6	Elbasvir	-12.8860	2.3219
ModL7	Ombitasvir	-12.7604	2.3796
ModL8	Ombitasvir	-12.3204	2.2137
ModL9	Ombitasvir	-12.2897	1.7473
ModL10	Ombitasvir	-12.0182	2.3506

The RMSD value could indicate the stability of the complex formed, a lower RMSD indicates a higher stability complex and vice versa. The RMSD and binding energy from ModL1 showed that the ModL1 has a higher tendency to bind to the pocket than the original drug, Ledipasvir. It could be indicated that the complex formed from the ModL1 more likely has higher stability than Ledipasvir.

Ligand-protein interactions: The exploration of protein-ligand interactions is an essential portion of new drug candidates[29]. The sampling and scoring stages are the two main stages of molecular docking. A particular algorithm generates the possible binding methods during the sampling stage. The previously obtained bond mode is evaluated with a specific value function during the scoring stage. The obtained bond value is then used as a parameter to calculate bond density[30]. The best ligands show several types of interactions with the proteins. Figure 2a depicts the interaction of Ombitasvir with the protein binding site, Fig. 2b depicts the interaction of Elbasvir with the protein binding site and Fig. 2c depicts the interaction of Ledipasvir with the protein binding site. Meanwhile, Fig. 3a depicts the interaction of ombitasvir with the protein binding site, Fig. 3b depicts the interaction of Elbasvir with the protein binding site and Fig. 3c depicts the interaction of Ledipasvir with the protein binding site.

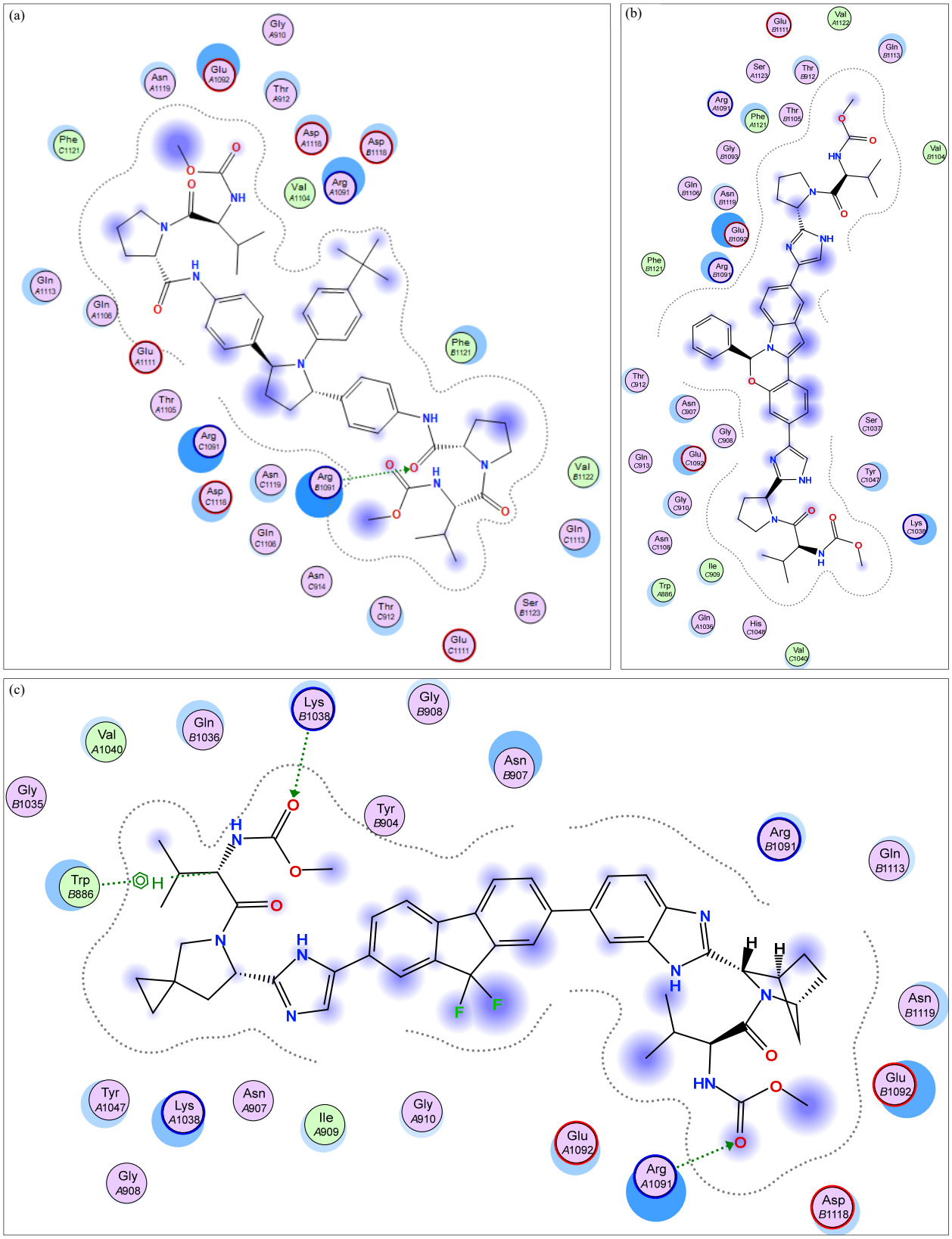

Fig. 2(a-c): 2D visualization of best 3 antiviral agents interactions with protein, (a) Ombitasvir, (b) Elbasvir and (c) Ledipasvir

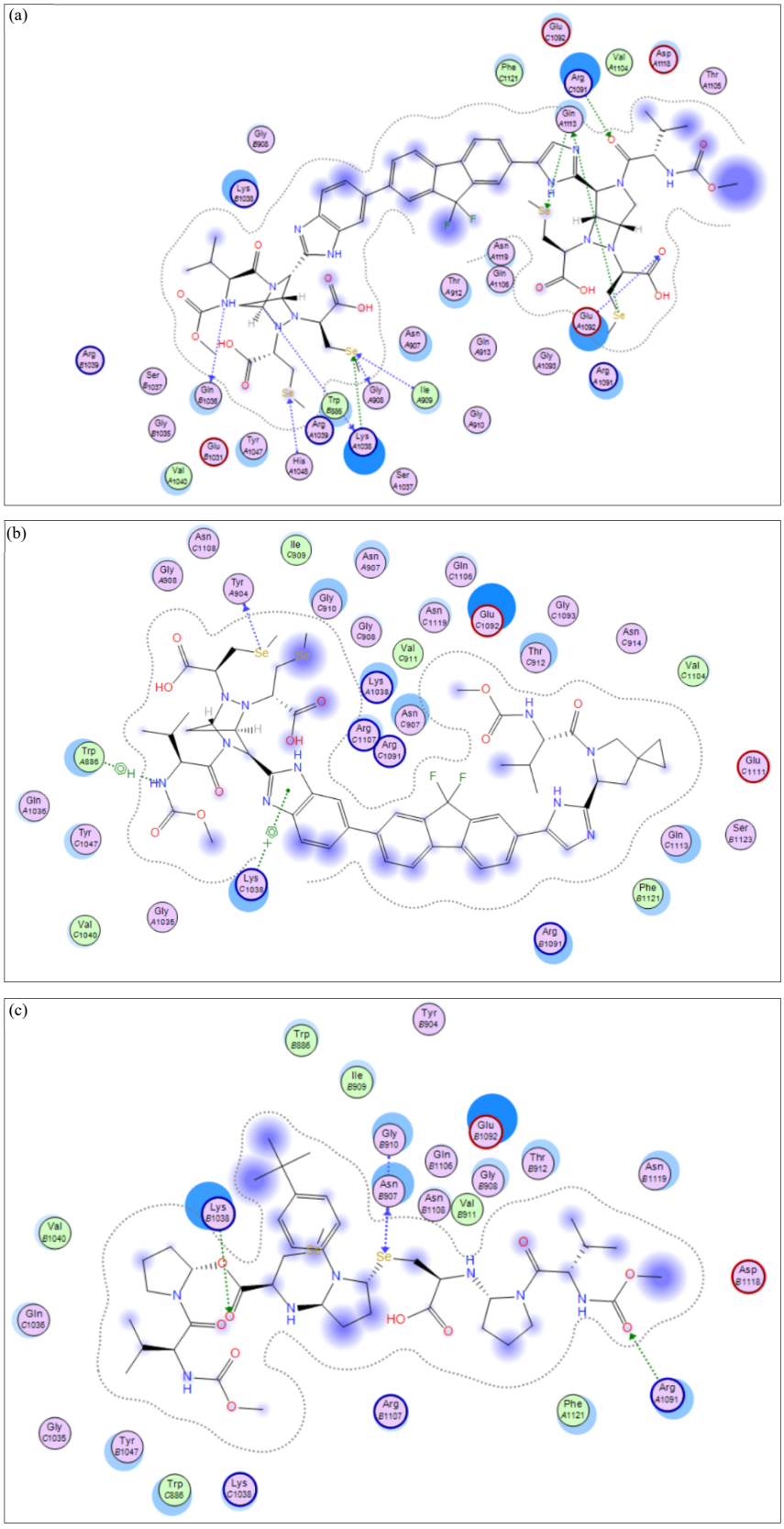

Fig. 3(a-c): 2D visualization of best 3 modified antiviral interactions with protein, (a) ModL1, (b) ModL2 and (c) ModL3

Table 3: ADME-Tox prediction of 10 best antiviral ligands (part 1)

Parameter	Ombitasvir	Elbasvir	Ledipasvir	Fosamprenavir	Daclatasvir
P-GP substrate	+	+	+	+	+
P-GP inhibitor	+	-	-	+	-
CYP450 substrate	CYP3A4	CYP2D6, CYP3A4	CYP2D6, CYP3A4	CYP3A4	CYP3A4
CYP450 inhibitor	-	CYP1A2	CYP1A2	-	CYP1A2, CYP3A4
Human intestinal absorption	73.3890	85.706	91.3810	68.1970	69.7170
Fraction unbound	0.1950	0.3790	0.3760	0.0360	0.3380
Total clearance	0.2280	0.9950	1.7660	1.4760	1.0260
hERG I inhibitor	-	-	-	-	-
hERG II inhibitor	+	+	+	-	+
Hepatotoxicity	+	-	+	+	-
Mutagenic	-	Low	High	-	-
Tumorigenic	-	-	High	-	-
Irritant	High	-	-	-	-
Reproductive effect	-	-	-	-	-
Druglikeness	-97.3320	-71.5620	-12.9070	-32.3730	-74.3050
Synthetic accessibility	7.3100	7.5200	8.6600	5.3500	6.3300

+: Present and - : Not present

Table 4: ADME-Tox prediction of 10 best antiviral ligands (part 2)

Parameter	Simeprevir	Cobicistat	Ritonavir	Telaprevir	Atazanavir
P-GP substrate	+	+	+	+	+
P-GP inhibitor	-	+	+	+	+
CYP450 substrate	CYP3A4	CYP3A4	CYP3A4	CYP3A4	CYP3A4
CYP450 inhibitor	-	CYP3A4	CYP3A4	-	CYP3A4
Human intestinal absorption	80.5900	68.9660	69.7690	55.0730	56.4210
Fraction unbound	0.2110	0.0600	0.0840	0.0580	0.0550
Total clearance	1.3240	25.6450	0.5620	2.8970	3.5400
hERG I inhibitor	-	-	-	-	-
hERG II inhibitor	-	+	+	+	+
Hepatotoxicity	+	+	+	+	+
Mutagenic	-	-	-	-	-
Tumorigenic	High	-	-	-	-
Irritant	-	-	-	-	-
Reproductive effect	-	-	-	-	-
Druglikeness	5.0690	-76.2320	-7.1470	-12.4420	-16.5420
Synthetic accessibility	7.4600	6.7400	6.4500	6.2600	6.2400

+: Present and - : Not present

Table 5: ADME-tox prediction of 10 best modified ligands (part 1)

Parameter	ModL1	ModL2	ModL3	ModL4	ModL5
P-GP substrate	-	+	-	+	-
P-GP inhibitor	-	-	-	-	-
CYP450 substrate	-	CYP3A4	CYP2D6, CYP3A4	CYP2D6, CYP3A4	CYP3A4
CYP450 inhibitor	-	CYP1A2	-	-	CYP3A4
Human intestinal absorption	0	33.3130	45.0270	17.2170	65.1840
Fraction unbound	0.3810	0.3780	0.1170	0.3640	0.3780
Total clearance	2.1830	4.9320	42.9540	18.7930	17.0610
hERG I inhibitor	-	-	-	-	-
hERG II inhibitor	-	-	-	-	+
Hepatotoxicity	-	-	+	+	-
Mutagenic	High	High	High	High	-
Tumorigenic	High	High	-	Low	-
Irritant	-	-	-	-	-
Reproductive effect	-	-	-	-	-
Druglikeness	-13.2560	-13.0940	-10.9040	-14.2800	-20.3840
Synthetic accessibility	10	9.9000	7.1500	8.4300	7.8200

+: Present and - : Not present

Table 6: ADME-Tox prediction of 10 best modified ligands (part 2)

Parameter	ModL6	ModL7	ModL8	ModL9	ModL10
P-GP substrate	+	+	+	+	+
P-GP inhibitor	-	-	+	-	-
CYP450 substrate	CYP2D6, CYP3A4	CYP2D6, CYP3A4	CYP3A4	CYP2D6, CYP3A4	CYP3A4
CYP450 inhibitor	-	-	-	-	-
Human intestinal absorption	46.4730	17.1270	40.7450	1.9690	26.0770
Fraction unbound	0.3790	0.3640	0.2980	0.4140	0.3450
Total clearance	7.7980	18.7930	34.8340	2.8510	26.9150
hERG I inhibitor	-	-	-	-	-
hERG II inhibitor	-	-	+	-	-
Hepatotoxicity	-	+	+	+	+
Mutagenic	-	High	High	-	-
Tumorigenic	-	Low	Low	-	-
Irritant	-	-	-	-	-
Reproductive effect	-	-	-	-	-
Druglikeness	-16.927	-14.82	-26.411	-15.062	-14.82
Synthetic accessibility	8.02	8.43	8.46	8.23	8.39

+: Present and - : Not present

ADME-Tox prediction: When determining the best candidates, it is critical to consider the pharmacokinetic properties of the drug molecule in question, such as the ADME (Absorption, Distribution, Metabolism, Excretion) character, the toxic nature and the ligand molecule's ability to act as a drug. These characteristics will be used as parameters to assess the suitability of the relevant ligands for administering drug candidates to the body. Synthetic accessibility is a descriptor used to predict the difficulty of synthesizing a ligand. This descriptor is one of the critical descriptors to consider for the later synthesis of drug compounds. Until now, scoring from synthetic accessibility has relied on data from fragments in a large dataset, followed by scoring using a statistical approach[31]. Table 3-6 show the predicted ADME-Tox properties of unmodified and modified ligands. Table 3 shows the prediction results for the compounds ombitasvir, elbasvir, ledipasvir, fosamprenavir and daclatasvir, while Table 4 shows the prediction results for simeprevir, cobicistat, ritonavir, telaprevir and atazanavir. Meanwhile, Table 5 displays the prediction results for ModL1, ModL2, ModL3, ModL4 and ModL5 compounds, while Table 6 displays the prediction results for ModL6, ModL7, ModL8, ModL9 and ModL10 compounds.

CONCLUSION

This research determined the best ligand by analyzing parameters such as binding energy, RMSD and ADME-Tox properties. The best three antiviral agents selected from binding energy to be repurposed as the COVID-19 drugs are ombitasvir, elbasvir and ledipasvir, with binding energy -15.8065, -15.3842 and -15.1255 kcal mol^{-1}, respectively. Meanwhile, the best three modified ligands determined were ModL1 with binding energy and RMSD value -15.6716 kcal mol^{-1} and 1.7109 Å, ModL2 with binding energy and RMSD value -13.9489 kcal mol^{-1} and 2.3179 Å and ModL3 with binding energy and RMSD value -13.2951 kcal mol^{-1} and 1.7836 Å.

SIGNIFICANCE STATEMENT

This research focused on repurposing antivirals and modifying compounds to aid researchers in discovering new drugs for COVID-19 therapy. The modifications applied in the research can provide researchers with new insights that can help them reduce the toxic nature of a molecule while also increasing the molecular interaction with spike glycoprotein, which is expected to inhibit the SARS-CoV-2 virus infection and replication process.

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