In silico molecular docking analysis for repurposing approved antiviral drugs against SARS-CoV-2 main protease

Ibrahim Khater [a], Aaya Nassar [a,b,*]

[a] Biophysics Department, Faculty of Science, Cairo University, Giza, Egypt
[b] Department of Clinical Research and Leadership, School of Medicine and Health Sciences, George Washington University, Washington DC, USA

A R T I C L E   I N F O

A B S T R A C T

Developing a safe and effective antiviral treatment takes a decade, however, when it comes to the coronavirus disease (COVID-19), time is a sensitive matter to slow the spread of the pandemic. Screening approved antiviral drugs against COVID-19 would speed the process of finding therapeutic treatment. The current study examines commercially approved drugs to repurpose them against COVID-19 virus main protease using structure-based in-silico screening. The main protease of the coronavirus is essential in the viral replication and is involved in polyprotein cleavage and immune regulation, making it an effective target when developing the treatment. A Number of approved antiviral drugs were tested against COVID-19 virus using molecular docking analysis by calculating the free natural affinity of the binding ligand to the active site pocket and the catalytic residues without forcing the docking of the ligand to active site. COVID-19 virus protease solved structure (PDB ID: 6LU7) is targeted by repurposed drugs. The molecular docking analysis results have shown that the binding of Remdesivir and Mycophenolic acid acyl glucuronide with the protein drug target has optimal binding features supporting that Remdesivir and Mycophenolic acid acyl glucuronide can be used as potential anti-viral treatment against COVID-19 disease.

1. Background

For over a year, the world has been battling the rise in the number of the confirmed cases of the highly infectious coronavirus disease (COVID-19). The viral infection has been spreading widely affecting hundreds of thousands across the entire world creating a global health fear putting many lives at risk. COVID-19 infect humans causing highly prevalent disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 has been monitored since December 2019 and the infection has been spreading aggressively affecting people worldwide. The World Health Organization (WHO) declared the disease associated with the new coronavirus first strain as COVID-19 and the etiology of this new infection is attributed to a novel virus belonging to the coronavirus family [1–3]. As of April 05, 2021, the disease statistics showed approximately 131, 965, 495 confirmed cases and 2,867,083 announced death reported in 220 countries and territories worldwide [4].

Several viral epidemics related to coronaviruses were reported in earlier years including the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) between 2002 and 2003 and the H1N1 influenza in 2009 while the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) was first identified in Saudi Arabia in 2012 [1] where a significant pathogen affecting the human respiratory system is the human coronavirus (HCoVs). Previous outbreaks of coronaviruses included both 229E and NL63 strains which belong to the Alpha coronaviruses, and OC43, HKU1, SARS, and MERS strains which belong to Beta coronaviruses [3]. SARS and MERS were shown to be the most spread strains of coronaviruses [5].

COVID-19 is contagious and SARS-CoV-2 virus belongs to Betacoronaviruses genus and has shown to be related to SARS and MERS [6]. The genomic sequence analysis of SARS-CoV-2 showed 88% identity with SARS [7,8]. Symptoms of COVID-19 infection appear to start approximately 5 days after contracting the virus [9]. The time from the initial COVID-19 disease symptoms to reported death cases may range from 6 to 41 days with a median of 14 days depending on the patient's age, the status of the patient's immune system, and comorbidity of underlying health conditions [8]. The analysis of the disease etiology showed similarities in the symptoms between SARS-CoV-2 and Betacoronaviruses family [10]. The reported most common symptoms of COVID-19 are chills, fever, dry and productive cough, fatigue, headache,

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* Corresponding author. Biophysics Department, Faculty of Science, Cairo University, Giza, Egypt.
E-mail addresses: ikhater@sci.cu.edu.eg (I. Khater), aaya_nassar@cu.edu.eg (A. Nassar).

Table 1

Docking scores (Kcal/mol) calculated by AutoDock Vina against SARS-CoV-2 M^{pro}. The docking was repeated 10 times for each ligand and the probabilities to bind to active-site pocket of protein were calculated. Glecaprevir, Elbasvir, and Ledipasvir showed the highest docking scores. Remdesivir and Mycophenolic acid acyl glucuronide showed the highest probabilities.

Drug Tested	Drug class	ΔG (Kcal/mol)	Probability of binding to active site-pocket
Glecaprevir	Hepatitis C antiviral	−10.85 ± 0.31	10%
Grazoprevir	Hepatitis C antiviral	−8.00 ± 0.71	0%
Simeprevir	Hepatitis C antiviral	−8.23 ± 0.29	0%
Voxilaprevir	Hepatitis C antiviral	−9.24 ± 0.052	0%
Elbasvir	Hepatitis C antiviral	−10.38 ± 0.042	0%
Beclabuvir	Hepatitis C antiviral	−8.56 ± 0.29	0%
Ledipasvir	Hepatitis C antiviral	−10.93 ± 0.22	0%
Paritaprevir	Hepatitis C antiviral	−13.2 ± 0.05	0%
Daclatasvir	Hepatitis C antiviral	−9.99 ± 0.12	0%
Remdesivir	Broad-spectrum antiviral	−8.71 ± 0.52	70%
Avigan	Influenza antiviral	−7.74 ± 0.37	30%
Tamiflu	Influenza antiviral	−5.72 ± 0.27	60%
Plaquenil	Malaria medication	−5.59 ± 0.19	30%
Mycophenolic acid	Immunosuppressant medication	−6.62 ± 0.23	30%
Mycophenolic acid acyl glucuronide	Phenolic derivative	−7.48 ± 0.34	80%
GRL-0667	SARS antiviral	−7.59 ± 0.45	40%

hemoptysis, nausea, diarrhea, dyspnea, olfactory loss of taste or smell and lymphopenia [8,10,11], and [12].

Featuring the largest known RNA virus genomes, coronaviruses are positive-sense enveloped single-stranded RNA viruses, ranging from 26 to 32 kb and containing 14 open reading frames (ORFs) in SARS-CoV-2 [52]. The major reading frame ORF 1 ab encodes for two overlapping polyproteins (pp1a, pp1ab), which are cleaved into 16 non-structural proteins (nsp 1-16) by the main protease M^{pro}, also known as 3-chymotrypsin-like protease (3CLpro) [13,14], and the papain-like protease PL^{pro} [15–17], and [18]. The M^{pro} cleaves the polyprotein at least for 11 conserved sites involving Leu-Gln, (Ser, Ala, Gly) sequences, and is required for proteolytic maturation and for regulating the immune defense and polyproteins cleavage of the functional protein RNA polymerase, endoribonuclease and exoribonuclease, which highlights the importance of targeting M^{pro} enzyme to inhibit SARS-CoV-2 [13,19].

Developing a safe, fast, and effective antiviral therapeutic treatment takes more than a decade, however, when it comes to COVID-19, time is a sensitive matter and to speed the process of finding the effective treatment, efforts were directed into screening previously approved antiviral drugs that may work against COVID-19. Drug repurposing is an effective strategy for COVID-19 treatment by identifying known therapeutic agents of the approved drugs and testing their efficiency inhibiting the virus, therefore, finding the desired treatment in less time and with less cost compared to developing new drugs [20,21].

A new wave of antiviral drugs related approximately 61 direct-acting antiviral agents (DAA) in clinical trials [22,23] and The United States Food and Drug Administration (FDA) approved DAA medications in 2014. Since then, DAA has revolutionized the landscape for hepatitis C virus (HCV) treatment. Antiviral therapy with DAA combinations

provides high rates of virological response [24], and many of them have been screened and tested using molecular docking analysis since the emergent of COVID-19 [25–27].

Extensive research studies suggested suitable drug targets against SARS-CoV-2 that includes the main protease (M^{pro}) [13,26,27] and [28]. The main protease M^{pro} plays a key role in processing polyproteins which are the viral RNA transcription products [29]. The inhibition of M^{pro} would block the viral replication, and since there are no known homologs of M^{pro} in humans with identical cleavage specificity, so such inhibition is safe and is unlikely to develop adverse toxic effect.

High-throughput approved antiviral drugs screening and repurposing have suggested some potential compounds against SARS-CoV-2. Research studies suggested Oolonghomobisflavan-A as a potential bioactive molecule to act as an inhibitor for the M^{pro} of SARS-CoV-2 [30], as well, suggested DSPD-2, DSPD-6, and DSPD-5 as potential inhibitors for SARS-CoV-2 M^{pro} [31]. Moreover, other studies found hydroxychloroquine (Ki = 0.36 μM) and chloroquine (Ki = 0.56 μM) to potently inhibit SARS-CoV-2 M^{pro} [53]. Whereas Coelho et al. studied several known SARS-CoV M^{pro} inhibitors and the study findings suggested them as potential inhibitors against SARS-CoV-2 M^{pro} including the organo-mercuric compounds thimerosal and phenylmercuric acetate. Additionally, Benzophenone derivatives and Evans blue, a sulfonic acid-containing dye, were also identified as potential inhibitors for main protease M^{pro} [32]. The in silico molecular docking analysis of Deshpande et al. suggested Ritonavir, Lopinavir and Remdesivir as potential treatment for COVID-19 disease [25]. Eriodictyol had emerged as well as a new repurposing drug that can be used in COVID-19 treatment.

The current work examines few commercially available antiviral drugs to repurpose them against SARS-CoV-2 using the structure-based in-silico screening seeking potential inhibitors of SARS-CoV-2 using molecular docking analysis [25,33]. The study investigates a number of recently researched drugs for their binding interactions and to evaluate their potential use in COVID-19 therapeutic treatment. Remdesivir, or GS-5734, is an adenosine triphosphate analog first described in 2016 as a potential treatment for Ebola [34] and has been suggested as a potent treatment for COVID-19 [35]. Remdesivir is a prodrug that metabolizes into its active form Remdesivir nucleoside triphosphate. The purpose of this study is to test potential inhibitors of SARS-CoV-2 using molecular docking analysis and introducing a new parameter called the probability of docking inhibitors to the active site. The study investigates a number of recently researched drugs for their binding interactions and to evaluate their potential use for COVID-19 treatment. The findings obtained from the in-silico approaches could easily be tested in in-vitro or in-vivo conditions as safe treatment.

2. Methods

2.1. SARS-CoV-2 main protease M^{pro}

Among the best characterized drug targets for coronaviruses is the main protease (M^{pro}) [13], which is an essential enzyme for processing polyproteins that are translated from the RNA [29]. The crystal structure of SARS-CoV-2 main protease form was selected [36] and the solved structure is retrieved from the protein data bank (PDB ID: 6LU7) where heteroatoms and water molecules were removed for the molecular docking analysis purpose. The active site residues of M^{pro} are HIS 41 and CYS 145 [37].

2.2. DAAs optimization and molecular docking

Molecular docking is one of the common structure-based methods that calculates binding affinities via noncovalent protein ligand interactions such as electrostatic, Van der Waals, coulomb, hydrogen bonds and predicts ligand conformations in the binding site of the macromolecule. Predicted binding poses are ranked as docking scores. Autodock Vina calculates Gibbs free energy of binding (ΔG) as the
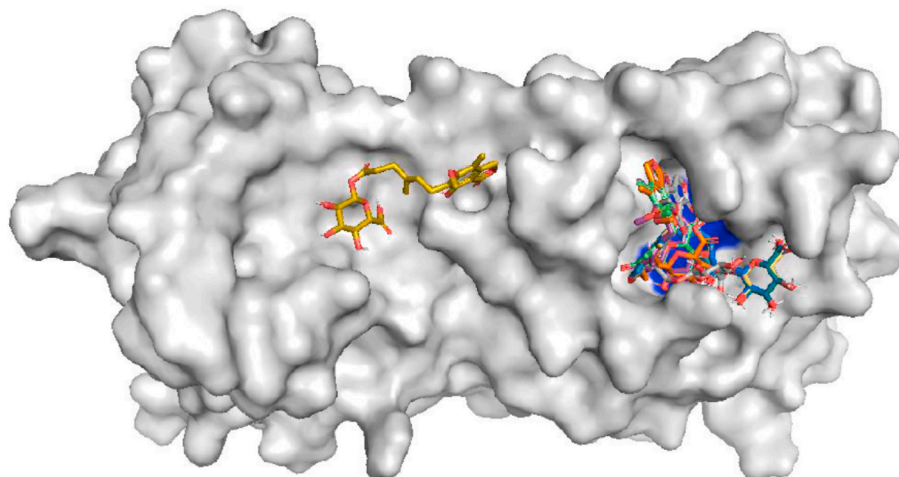
Remdesivir
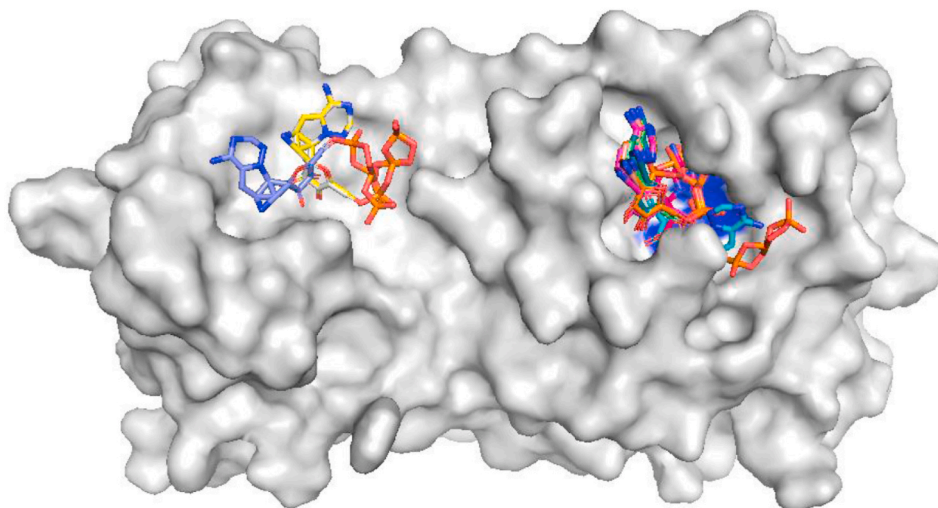
Mycophenolic acid acyl glucuronide

Fig. 1. The docking between Remdesivir, Mycophenolic acid acyl glucuronide and SARS-CoV-2 main protease M^{pro}. SARS-CoV-2 M^{pro} is represented as cartoon, where catalytic residues (HIS41, CYS145) are represented in blue color and the ligands are represented in licorice color. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

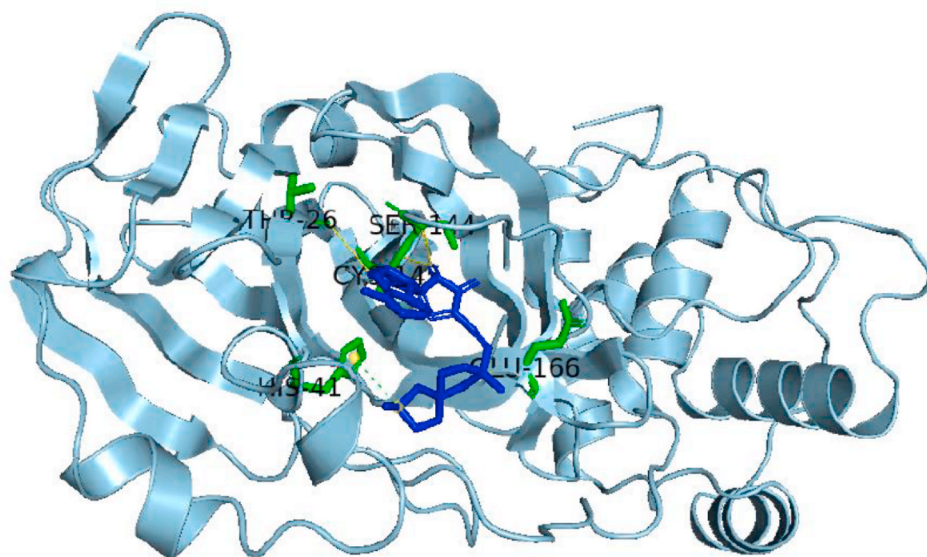docking score which can also be converted to inhibition constant (Ki) [38].

Direct-acting antiviral agents (DAAs) are newer class of drugs used to treat hepatitis C virus (HCV), they are of shorter treatment periods, less side effects, and of higher SVR blood detectable rates than older medicines. DAAs directly target the virus, making them more effective than older treatments. Coronavirus makes its own proteins to help it grow or replicate on its host. DAAs stop those proteins from working so that the virus cannot finish its life cycle and grow [39] and [54]. Known antiviral drugs like Glecaprevir, Grazoprevir, Simeprevir, Voxilaprevir, Elbasvir, Beclabuvir, Ledipasvir, Remdesivir, Avigan, Tamiflu, Plaquenil, Paritaprevir, Daclatasvir, Mycophenolic acid (MPA), Mycophenolic acid acyl glucuronide, and GRL-0667, which are used to treat other ailments, were selected as candidate ligands for docking against the viral proteins. The ligand structures were obtained from the online DrugBank which is a comprehensive database containing information on drugs and drug

targets [40]. The geometry of all inhibitors was optimized using MMFF94 force field function using Avogadro software (https://avogadro.cc) [41]. The molecular docking was performed with Auto-Dock Vina software (http://vina.scripps.edu) [38]. using the default parameters. The docking was rigid against the whole protein to examine the free natural affinity of the binding ligand to the active site pocket and the catalytic residues without forcing the docking of the ligand to the active site only. The molecular docking was repeated 10 times for each ligand. The evaluation of the affinity of the molecular docking will depend on the score and the probability to bind to the active-site pocket of the protein [42,43].
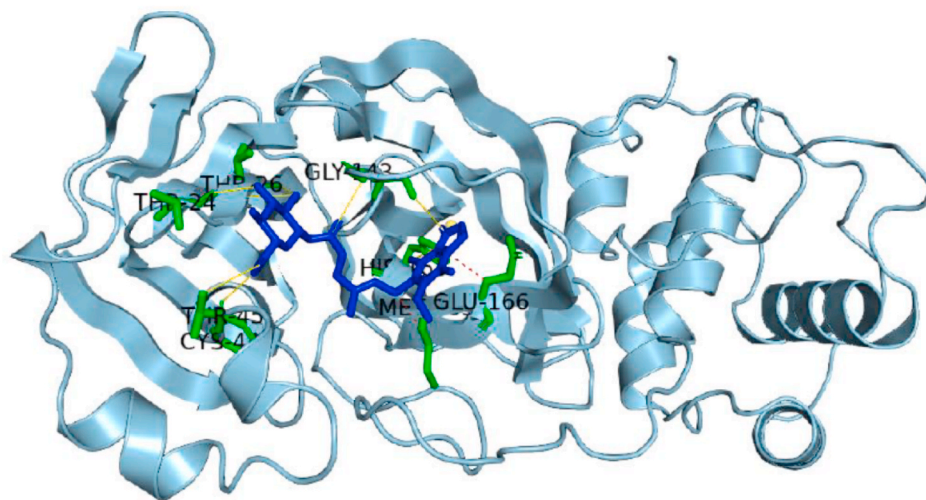
2.3. Interactions between ligand and protease

PLIP web server (Salentin, Schreiber, Haupt, Adasme, & Schroeder, 2015) was used to analyze the interactions formed between DAAs and

Remdesivir

Mycophenolic acid acyl glucuronide

Fig. 2. The formed interactions between the DAA drugs and SARS-CoV-2 M^{pro} protease after the molecular docking. The drugs, Remdesivir and Mycophenolic acid acyl glucuronide, represented in blue color, docked to SARS-CoV-2 M^{pro} (6LU7), represented in green color. The H-bonds are shown in solid yellow lines where the hydrophobic interactions are shown in red dashed lines and salt bridges are shown in green dashed lines. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

SARS-CoV-2 Mpro. The PLIP web service allows for comprehensive detection and visualization of protein–ligand interaction patterns from 3D structures and the results for each binding site are provided as 3D interaction diagrams for manual inspection.

2.4. Molecular dynamics simulation

The molecular dynamic simulation of the protein-ligand complexes was performed using the GROMACS simulation and CHARMM36 force field [44,45]. To obtain the molecular topology file of the ligand compatible with the CHARMM36 force field, we used the CGenFF web service, where the protein-ligand complex was solvated and the complete system was neutralized with the addition of Cl$^-$ ions by replacing the water molecules. After completing these steps, the energy minimization of the system was performed, which was followed by equilibration of the system using two consecutive NVT (100 ps) and NPT (100 ps)

runs. Lastly, the complex was introduced to a 1000 ps molecular dynamics simulation with a time-stage of 2 fs for each simulation. The root mean square deviation (RMSD) of the peptide (atom backbone) and the radius of gyration (Rg) are then plotted as a function of time.

3. Results

Molecular docking was performed on the solved structure PDB ID: 6LU7 of SARS-CoV-2 main protease M^{pro} where the RMSD of the peptide and the radius of gyration (Rg) was plotted as a function of time. Table 1 lists the mean values of the docking scores, ΔG (Kcal/mol), and the probability to bind to the active site. The results have shown that ledipasvir has better docking score (−10.93 kcal/mol) followed by glecaprevir (−10.85 kcal/mol) and elbasvir (−10.38 kcal/mol) however, the probabilities to bind to the active site pocket are 0%, 10%, and 0%, respectively. Therefore, these drugs did not express a beneficiary use to
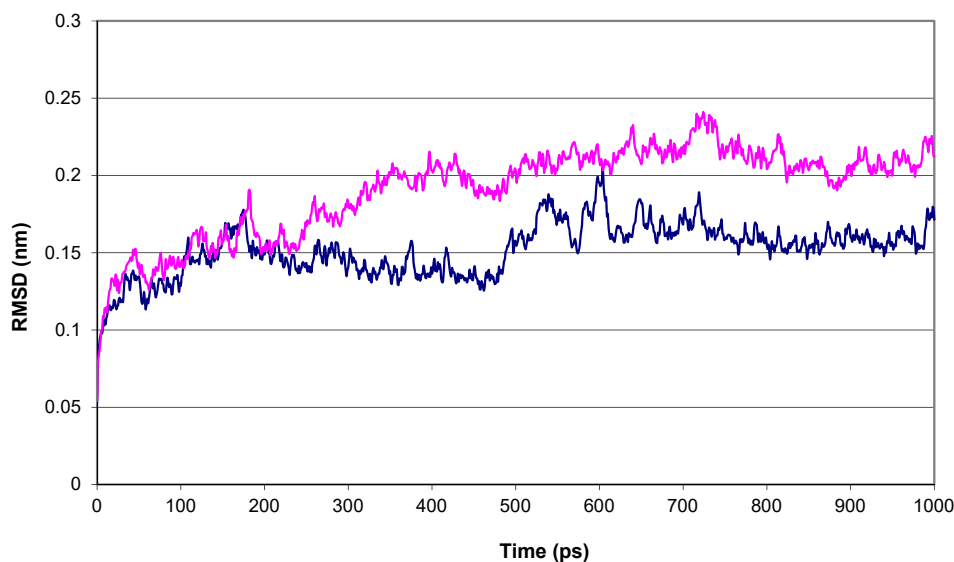
Fig. 3. Plots of Root Mean Square Deviation (RMSD) values for the backbone atoms of 6LU7 – Remdesivir complex (magenta) and 6LU7 – Mycophenolic acid acyl glucuronide complex (blue) from initial structure during 1000 ps simulation as a function of time. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
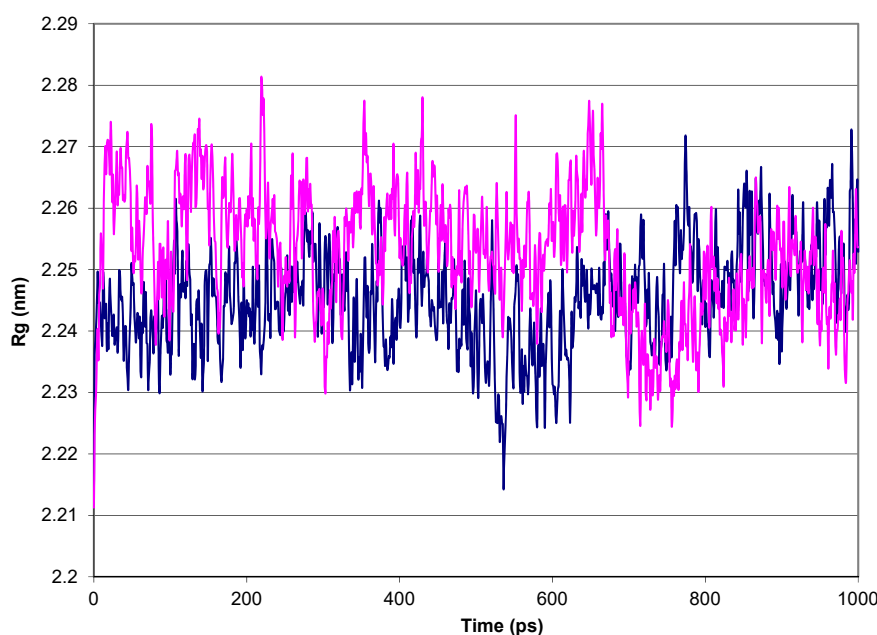

Fig. 4. Plots of Radius of gyration (Rg) of 6LU7 – Remdesivir complex (magenta) and 6LU7 – Mycophenolic acid acyl glucuronide complex (blue) through 1000 ps of molecular dynamics simulation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

inhibit the virus main protease. On the other hand, other ligands like mycophenolic acid acyl glucuronide with docking score (−7.48 kcal/mol) showed a higher probability to bind to the active site pocket (80%) followed by Remdesivir with docking score (−8.71 kcal/mol) and 70% chance to bind to the active site pocket. The results observed from docking the selected ligands to the viral protein expressed good docking scores and high native probability of both mycophenolic acid acyl glucuronide and Remdesivir to bind to the active site pocket suggesting those drugs as potent inhibitors to the main protease of the virus active site.

4. Discussion

Extensive studies repurposed antiviral drugs against M^{pro} [9,19,46,

47]. Abd El-Mordy et al. showed that phenolic compounds like rutin, myricitrin, mearnsitrin, and quercetin 3-Ob-D-glucoside have strong interaction with SARS-CoV-2 protease with high binding energy of −8.207, −7.197, −7.586, and −7.675, respectively [48]. Most studies docked inhibitors against small box around the active site and calculated the docking score. In this study, we docked inhibitors against the whole molecule and repeated the docking 10 times and calculated the docking score then introduced a new parameter called the probability of docking inhibitors to the active site (i.e., if inhibitors docked to active site 5 times out of 10 trials the probability of binding this inhibitor to active site is 50%). Since, the binding between the inhibitor and the active site is important for inactivation of protease, this new parameter is a good measure of the effectivity of the inhibitors.

The docking between Remdesivir and mycophenolic acid acyl

glucuronide with SARS-CoV-2 main protease M^{pro} are shown in Fig. 1 where PLIP web server was used to analyze the interactions formed. Fig. 2 represents the formed interactions between the DAA drugs and SARS-CoV-2 main protease M^{pro} after the docking. Ligands are shown in blue, while protein residues are shown in green representations labeled with three-letter codes. H-bonds are shown in solid yellow lines, while the dashed red lines represent the hydrophobic interactions, and the green dashed lines represent salt bridges. The number of H-bonds for Remdesivir and mycophenolic acid acyl glucuronide are 5 and 7, respectively. One salt bridge formed with Remdesivir and mycophenolic acid acyl glucuronide reflected on the docking score. The formation of hydrogen bonds and salt bridges with the active site-pocket inhibits the function of catalytic residues and prevent them from sharing in virus replication [49].

The interactions between the legends and the protein are instantaneous through the docking process and the interaction may be unstable [50]. The molecular dynamics simulation allows providing information about the stability of the molecular interactions of the complexes. In this work, Remdesivir and mycophenolic acid acyl glucuronide expressed their highest probability to bind to the active site pocket of 6LU7. Using the RMSD, the stability of the complexes was evaluated for the backbone atoms of 6LU7 with respect to the starting structures [51]. Fig. 3 illustrates the chart of the RMSD values of 6LU7 – Remdesivir complex (in magenta) and 6LU7 – Mycophenolic acid acyl glucuronide complex (in blue), complexes were stabilized at about 600 ps indicating that Remdesivir and mycophenolic acid acyl glucuronide form stable complexes with 6LU7. Additionally, the stability of those complexes were further evaluated by plotting Rg [51]. The calculated Rg values over the simulation time scale is demonstrated in Fig. 4, where the parameter is stable for the complexes after 700 ps of simulation time.

5. Conclusion

SARS-CoV-2 main protease inhibitor is a key to reduce the spread of the COVID-19 infection and repurposing approved antiviral drugs is a fast approach in finding the safest treatment for the novel coronavirus disease. The extensive work carried out in this study is to examine the interaction of the viral protein and different antiviral drug ligands to inhibit the viral protein main protease. The main protease protein is important in the viral replication and was selected in the study because inhibiting this protein might be useful to block the initiation of the infection and the chain of replication. The results from this in silico molecular docking study enhance the use of Remdesivir and mycophenolic acid acyl glucuronide as potential candidate drugs for the treatment of COVID-19 because both drugs expressed their highest probability to bind to the active site pocket of SARS-CoV-2.

Declaration of competing interest

None.

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