Potent Inhibitors for SARS-CoV-2 Main Protease: An in-Silico Study for Drug Development

Ibrahim M. Khater [a] and Aaya M. 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, U.S.A.

[*]Corresponding author:

Aaya Nassar, Ph.D.
ORCID 0000-0002-5542-9387
Tel: +20 2 33767406
Fax: +1-888.959.4981
E-mail: aaya_nassar@cu.edu.eg

Short Running Title:

In Silico Study of SARS-CoV-2 Potent Inhibitors

Abstract

Background: The emergent of the new coronavirus (SARS-CoV-2) and consequently the viral infection that spread widely affecting hundreds of thousands across the entire world has developed a global health concern. Coronaviruses infect humans causing highly prevalent diseases.

Method: The current work is to examine commercially available drugs to repurpose them against SARS-CoV-2 by the means of structure-based in-silico screening. The present study focusses on testing the repurposing efficacy of the currently used drugs against SARS-CoV-2 main protease. The main proteases from the coronavirus are essential for the viral replication and are involved in the polyprotein cleavage and immune regulation, making them attractive and effective targets for the development of antiviral drugs. Number of approved anti-viral drugs were tested as potential SARS-CoV-2 virus inhibitors using molecular docking analysis by examining the free natural affinity of the binding ligand to the active-site pocket and catalytic residues without forcing the docking of ligand to active site. SARS-CoV-2 protease solved structure (6LU7) is targeted by repurposed drugs.

Results: 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 suggesting further experimental consideration for their treatment effectiveness.

Keywords

Coronavirus treatment, Remdesivir, Mycophenolic acid acyl glucuronide, molecular docking, anti-protease drugs.

1. INTRODUCTION

Developing a safe and effective anti-viral treatment takes more than a decade however, when it comes to coronavirus disease 2019 (COVID-19), time is a sensitive matter and to speed the process of finding the drug therapy efforts were directed into screening previously approved drugs to work against this new disease threat.

In December 2019, COVID-19 epidemic was described in Wuhan, China, and the infection has spread widely affecting hundreds of thousands of people. Since then COVID-19 has become an important public health concern across the globe. In February 2020, the World Health Organization (WHO) announced that the disease caused by the new coronavirus (CoV) was a “coronavirus disease 2019" abbreviated as COVID-19. The etiology of COVID-19 illness is attributed to a novel virus belonging to the coronavirus (CoV) family.[1] In March 2020, WHO declared the fast spreading of coronavirus outbreak and announced it as a global pandemic. [2,3] As of September 05, 2020, approximately 26,775,656 confirmed cases and 879,694 death, have been reported in 210 countries and territories worldwide.[4]

Several viral epidemics related to coronavirus were reported over the past 20 years including the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) from 2002 to 2003 and H1N1 influenza in 2009 while the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) was first identified in Saudi Arabia in 2012.[1]

Human Coronavirus (HCoVs) is one of the major pathogens that primarily targets the human respiratory system. Previous outbreaks of coronaviruses include 229E and NL63 strains which belong to Alphacoronaviruses, while OC43, HKU1, SARS, and MERS belong to Betacoronaviruses. [3] SARS and MERS are the most aggressive strains of coronaviruses.

The SARS-CoV-2 virus is contagious spreading quickly worldwide. SARS-CoV-2 is a member of Betacoronaviruses genus and is closely related to SARS and MERS.[5] The genomic sequence analysis of SARS-COV-2 showed 88% identity with SARS.[6,7] The symptoms of COVID-19 infection appears to start approximately 5 days after contracting the virus. [8] The period from COVID-19 symptoms to reported death cases ranged from 6 to 41 days with a median of 14 days depending on the age of the patient and the status of the patient's immune system and other underlying health conditions.[7] The analysis of the disease etiology showed similarities in the symptoms between COVID-19 and other Betacoronaviruses.[9] The reported most common symptoms of COVID-19 are chills, fever, dry cough, fatigue, headache, hemoptysis, diarrhea, dyspnea, olfactory loss of taste or smell and lymphopenia. [10, 9, 7, 11] In general, HCoVs are positive-sense and very long single-stranded RNA viruses (30,000 bp). HCoVs consists of two groups of proteins; four structural proteins, such as Spike (S) that characterize all coronaviruses, Nucleocapsid (N), Matrix (M), and Envelope (E), in addition to the non-structural proteins such as proteases (nsp3 and nsp5) and RNA-dependent-RNA polymerase RdRp (nsp12) and main protease (M^{pro}) and papain-like protease (PL^{pro}).[12, 13, 14, 15]

The urgent need to stop the viral infection and to develop potent anti- SARS-CoV-2 agents in very short time got researchers attentions for the repurposing of known small molecules or therapeutic agents that seem to be efficient to develop a potent treatment to combat COVID-19 and identify promising inhibitors against coronavirus protease.[16, 17] COVID-19 drug repurposing means identifying other uses for approved drugs, which is considered an effective approach for COVID-19 drug discovery since it involves finding the treatment in less time and with less cost compared to the de novo drug discovery process.[18]

The coronavirus 3-chymotrypsin-like protease ($3CL^{pro}$), known as the main protease M^{pro}, is required for proteolytic maturation of the corona virus. M^{pro} has an essential role in the immune regulation and cleaving the polyproteins pp1a and pp1ab, making them potential targets for anti- SARS-CoV-2 drugs.[19] The functional proteins RNA polymerase, endoribonuclease and exoribonuclease are generated by cleavage of pp1a and pp1ab polyproteins by M^{pro}, therefore, targeting M^{pro} enzyme will inhibit the viral maturation and enhance the host innate immune response against COVID-19.[16]

Approximately 61 direct-acting antiviral agents (DAA) have been reported to contemporary drugs in clinical trials for COVID-19 treatment and many of them were under molecular docking studies to screen and identify the potent antiviral agents specifically for COVID-19.[20, 21]

The purpose of this study is to test potential inhibitors of SARS-CoV-2 using the molecular docking analysis. The study investigates number of recently researched drugs for their binding interactions and to evaluate their potential use for COVID-19 treatment.

2. METHODS

2.1. SARS-CoV-2 Main Protease M^{pro}

The full genome sequence for the newly emerged COVID-19, SARS-CoV-2, is retrieved from the National Center for Biotechnology Information (NCBI) nucleotide database (GenBank: NC_045512.2, https://www.ncbi.nlm.nih.gov/nuccore/NC_045512).

Among the best characterized drug targets for coronaviruses is the main protease (M^{pro}),[22] which is an essential enzyme for processing the polyproteins that is translated from the RNA.[23]

COVID-19 M^{pro} solved structure is retrieved from protein data bank (PDB) (PDB ID: 6LU7) available at (https://www.rcsb.org/structure/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.[24]

2.2. DAAs Optimization and Molecular Docking

Direct-acting antiviral agents (DAAs) are a newer class of drugs that have been used to treat hepatitis C virus (HCV). DAAs have shorter treatment times, fewer side effects, and higher SVR rates than older drugs; SVR means that HCV is not detectable in the blood. DAAs work by targeting the virus directly, 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.[25, 26]

The structures of the SARS-CoV-2 main protease inhibitors were downloaded from DrugBank.[27] The geometry of all inhibitors was optimized using MMFF94 force field function using Avogadro software (https://avogadro.cc).[28] The molecular docking was performed with AutoDock Vina software (http://vina.scripps.edu)[29] using the default parameters. The docking was rigid against the whole protein to examine the free natural affinity of binding ligand to the active-site pocket and the catalytic residues without forcing the docking of ligand to active site only. The molecular docking was repeated 10 times for each ligand. The evaluation of affinity of the molecular docking will depend on the score and the probability to bind to the active-site pocket of the protein.[30,31]

2.3. Analysis of Interactions between DAAs and SARS-CoV-2 Protease

Most DAAs are taken in combination with other DAAs to improve how well they work. Ribavirin and peginterferon are sometimes added to a DAA to improve how it attacks the virus. NS3/4A protease inhibitors target the NS3/4A protease enzyme, which is needed for the virus to grow and thrive. NS5B is an enzyme that is essential for the virus to replicate. NS5B inhibitors block this enzyme and stop the virus from replicating. These drugs are generally well tolerated and are less likely to develop resistance to the virus. NS5Bs tend to work equally well across many genotypes.[25, 32]

PLIP web server [33] was used to analyze the interactions formed between DAAs and SARS-CoV-2 M^{pro}.

2.4. Molecular Dynamics Simulation

The molecular dynamics simulation of the protein-ligand complexes was performed using the GROMACS simulation and CHARMM36 force field. [34,35] To obtain the molecular topology file of ligand compatible with the CHARMM36 force field, we used the CGenFF web service (https://cgenff.umaryland.edu), 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 1000 ps molecular dynamics simulation with a time-stage of 2 fs for each simulation. The root mean square deviation (RMSD) of peptide (atom backbone) and the radius of gyration (Rg) was plotted as a function of time.

3. RESULTS AND DISCUSSION

Molecular docking was performed on the solved structure 6LU7 of SARS-CoV-2 main protease M^{pro} where the root mean square deviation (RMSD) of peptide and the radius of gyration (Rg) was plotted as a function of time. Table 1 represents the mean values of the docking scores and the probabilities 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 show to be beneficially used as potent therapy for COVID-19. Meanwhile, mycophenolic acid acyl glucuronide with docking score (-7.48 Kcal/mol) showed 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. Consequently, these drugs have shown good docking scores and high native probabilities to bind to the active site therefore could be used as potent treatment for COVID-19.

The docking between Remdesivir, Mycophenolic acid acyl glucuronide and SARS-CoV-2 main protease M^{pro} are shown in figure 1 where PLIP web server was used to analyze the interactions formed. Figure 2 represent the formed interactions between the DAA drugs and SARS-CoV-2 M^{pro} protease after the docking. Ligands are shown in blue, while protein residues are shown in green representations labeled with three-letter code. 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 active site-pocket inhibits the function of catalytic residues and prevent them from sharing in virus replication.

Interactions between the inhibitor and the protein are instantaneous through the docking process and the interaction may be unstable. The molecular dynamics simulation allows providing the information about the stability of the molecular interactions on the complexes. In this work, Remdesivir, and Mycophenolic acid acyl glucuronide with their highest probabilities to bind to the active site of 6LU7 have been used to perform molecular dynamics simulation. Using the RMSD, the stability of the complexes was evaluated for the backbone atoms of the 6LU7 with respect to the starting structures.[36] Figure 3 shows the chart of the RMSD values of 6LU7 – Remdesivir complex (in magenta) and 6LU7 – Mycophenolic acid acyl glucuronide complex (in blue) 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.[36] The calculated Rg values over the simulation time scale are shown in figure 4; where the parameter is stable for the complexes after 700 ps of the simulation time.

4. FUTURE PERSPECTIVE AND CONCLUSION

Coronaviruses infect humans causing highly prevalent diseases. The newly emergent SARS-CoV-2 is one of the major pathogens that primarily targets the human respiratory system. COVID-19 became a pandemic and has no treatment currently including vaccine or antiviral drugs. The current work is an effort to examine commercially approved available antiviral drugs in order to repurpose those drugs against COVID-19 by the means of structure-based in-silico screening. The study focusses on testing the repurposing efficacy of the currently used and

investigated drugs against SARS-CoV-2 main protease. The main proteases from the coronavirus are essential for the viral replication and are involved in the polyprotein cleavage and immune regulation, making them attractive and effective targets for the development of antiviral drugs. Number of drugs were tested as potential SARS-CoV-2 virus inhibitors using molecular docking analysis by examining the free natural affinity of the binding ligand to the active-site pocket and catalytic residues without forcing the docking of ligand to active site. SARS-CoV-2 protease solved structure (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 suggesting their use in further experimental and clinical studies to save the life of COVID-19 patients. The Remdesivir and Mycophenolic acid acyl glucuronide docking have demonstrated that they could be potent treatment for the new strain of SARS-CoV-2.

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