

***In silico* Study to Identification of Potential SARS-CoV-2 Main Protease Inhibitors: Virtual Drug Screening and Molecular Docking with AutoDock Vina and Molegro Virtual Docker**

Mohammad Amin Manavi

Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Received 17 July 2021

Accepted 7 December 2021

Abstract

Coronavirus disease 2019 (COVID-19) has emerged in Wuhan, China, and because of fast transmission, it has led to its extensive prevalence in almost all countries, which has made it a global crisis. Drug repurposing is considered a fast way to discover new applications of the current drugs. This study aims to recognize a possible small molecule as a primary protease inhibitor versus the main protease protein of SARS-CoV-2 by computational programs. Virtual screening procedures like using Molegro Virtual Docker, AutoDock Tool, and AutoDock Vina, were done for more than 1600 FDA-approved medicines downloaded from the ZINC database, were employed to characterize new implied molecule inhibitors for the recently published crystal structure of the main protease protein of SARS-CoV-2. Virtual screening results indicated, many drugs including ARBs, cephalosporins, some kinase inhibitors, HMG CoA reductase, and leukotriene receptor antagonist, may inhibit the main protease of SARS-COV-2. Velpatasvir, Molnupiravir, and Ivermectin were selected by virtual screening methods for further studies to find an efficient ligand for the treatment of COVID-19. Due to some other beneficial features, including anti-infectious, anti-inflammatory properties, and ADME profile, they could be a promising drug nominee for repurposing to the treatment of COVID-19. Velpatasvir was selected by some virtual screening methods for further studies to find a suitable ligand for the treatment of COVID-19. Furthermore, more studies need to approve this data and finally clinical trial needs to be done to examine the efficacy of Velpatasvir for the treatment of covid-19 as an anti-viral agent.

Keywords: Velpatasvir, Virtual screening, COVID-19, Molecular Docking, Repurpose, Main Protease Inhibitor

Introduction

The appearance of the new coronavirus (new CoV-19) has influenced human health and also human lifestyle on a global scale. Discovery of the novel targeted drug(s) is needed quickly and has taken the main step in combating the coronavirus disease-19 (COVID-19) pandemic. The SARS-CoV-2 caused an efficacious supplementary strange global public health warning, with comparably high mortality and high transmission speed. The SARS-CoV-2 main protease is necessary for viral replication and could be an important drug target (Soga et al., 2021). It's more than 1 year since WHO announced that COVID-19 has become a pandemic, and to date, there are more than 260,000,000 Confirmed cases, more than 5,230,000 Confirmed deaths and, 223 Countries, areas, or territories are involved in this crisis (Akl et al., 2013).

The SARS-CoV-2 main protease is one of the most critical proteins for transcription and

replication of the virus, which cleaves the polyproteins into smaller fragments. Drug repurposing is considered as a way to discover new applications of the current drugs in handling several diseases. The main protease of a virus, like the new coronavirus, plays an essential role in reproduction and expansion (Osipiuk et al., 2021). *In silico* drug repurposing is an alternative efficacious approach to neutralize COVID-19 (Choudhury et al., 2021). This procedure may speed up the process of determining the therapeutic compounds for the newly emerged sicknesses (Polamreddy and Gattu, 2019).

Under the current emergency situation, it operated virtual screening tools to search for drugs and natural products that have been deposited in the Drug data Bank to accelerate drug discovery. This research was performed to estimate and determine whether FDA-approved medicines might be considered as COVID-19 main protease inhibitors. The aim of this study was to explore whether FDA-approved drugs could help manage COVID-19 by

* Corresponding author's e-mail address:

ma-manavi@student.tums.ac.ir

directly affecting the virus particle, as, a rapid and relatively highly accurate method for screening a large number of ligands is *in silico* methods such as molecular docking.

Materials and Methods

To identify the suitable ligand with the desirable interaction with the main protease of SARS-CoV-2, all FDA-approved drugs, containing 1615 medicines, were obtained from the ZINC database. Drugs were screened through molecular docking simulations over the main protease binding site of SARS-CoV-2. The crystal structure of the main protease of SARS-CoV-2 which has been published recently, was downloaded as a PDB file from a website related to the protein data bank (<http://www.rcsb.org>) with PDB ID: 6wtl (Sharun et al., 2020).

First, databases such as ZINC were used to download the SDF file format of small molecules for searching the DrugBank database and followed by molecular docking. Auto Dock Tool (ADT, Ver.1.5.6) was used to prepare the input files and analyze the result (Morris et al., 2009). 3D structures of FDA-approved drugs were downloaded from the ZINC database (Sterling and Irwin, 2015) in structure-data file (SDF) format. Molegro Virtual Docker (MVD) ver. 6, was used for the first step of molecular docking. The docking was performed with these steps: 1) importing SDF file of ligands and PDB file format of the protein; 2) searching for all probable cavities on the protein surface which led to the selection of five cavities; 3) setting the binding site and grid space to 0.3Å; 4) setting the search algorithm on the energy-minimization; and 5) hydrogen bonds optimization, then run the software and saving the docking results for the following analysis. Docking scores represent calculated ligand-receptor (protein) interaction energy; hence, more negative scores indicate better binding bias (Jadhav and Karuppaiyil, 2021).

In the next step, all FDA-approved drugs that included 1615 drugs, or in other words, ligands, were screened. Every ligand was docked ten times with each cavity. After *in silico* screening of drug spaces, they were sorted based on their affinity to main protease and 18 drugs, as potential SARS-CoV-2 main protease inhibitor, were selected to be examined for further analyses. In the next step, the selected ligands by Molegro Virtual Docker, were prepared for docking with AutoDock Tool. Some investigated medications of COVID-19 such as Ivermectin, Molnupiravir and Remdesivir were also chosen to be examined as positive controls.

For protein input file preparation, all water molecules, ligands, and ions were removed from the PDB file. Then polar hydrogens were added, the Kollman-united charge was used to determine the partial atomic charge, and the prepared file was saved in PDBQT format for use in the following steps. Ligand with structure data file (SDF) format should be converted to PDB format, so Open Babel (version 2.3.1) was used to do so (Nosrati et al., 2018). Protein with PDB format was chosen as a macromolecule and was saved as a PDBQT file. Later, 90×90× 90Å (x, y, and z) grid box was centered on the protease binding pocket with 0.375 nm spacing for each dimension and a grid center at dimensions of -15.845, 30.799 and 11.939 was determined for x, y, and z, respectively.

Result and Discussion

Initially, a screening procedure was used to screen 1615 ligands. After docking, those ligands with a mol MolDock score lower than 160 were selected and chosen for the next steps. therefore 18 compounds were found and chosen for next step. AutoDock tool was run, and compounds were sort based on their binding energy.

Pharmacophore studies have been indicated that, some amino acids are more responsible for interaction between ligands and active site of main protease, such as: Tyr 237, Glu 288, Lue287, Ile 249, Arg4 and Phe 294. Trypan Blue, Venetoclax, Indocyanine green (Pourhajibagher and Bahador, 2020) had the highest scores among the chosen drugs, but others could be better choices because of the side effects and lower availability of these three drugs.

Some statins like atorvastatin have been suggested as useful drugs in COVID-19 patients mainly. In addition to the different helpful effects of atorvastatin, its high ability to inhibit the main protease enzyme made it an ideal choice for further studies. (Li et al., 2021) Many cephalosporins indicated high affinity to 6wtl protein. These medicines have an excellent anti-upper respiratory infection and could be considered as satisfactory prophylaxis and treatment for pneumonia caused by covid-19, although because of possible antibiotic resistance it could not be a good option for inhibition of virus loading, but could be used as an adjuvant therapy (Kumar et al., 2021).

Some ARBs like losartan and Azilsartan, and Candesartan indicated a high affinity to the main protease of new coronavirus which made them a potential medicine for managing this crisis. Many studies discussed about interaction between ARBs

and COVID-19 (Bavishi et al., 2021) and there was no significant difference in hospitalization or death rate, So, these drugs could not be a good choice. Although other studies should be done to find ARB-like drugs that inhibit the main protease of new coronavirus. Velpatasvir also indicated a good affinity to main protease of new coronavirus. It is an NS5A inhibitor, which is used in combination with Sofosbuvir in the treatment of hepatitis C. It is just shown that this drug could be useful for treatment of COVID19 through *in silico* study before (Bharti and Shukla, 2021). As it has been shown in figure 1, there are 4 amino acids that have a role in interaction between Velpatasvir and the main protease, including: GLY 275, MET 276, LEU 286 and ALA 285.

Furthermore, the investigated medication of COVID-19 has been utilized in this study. Some particular medicines have been studied in various clinical trials for the treatment of COVID-19. The affinity of Remdesivir to the main protease of new coronavirus is higher than other drugs which are under investigation in clinical trials, and the FDA has recently emergency approved this drug for this illness (Elfiky, 2020)

It is recognized that Remdesivir targets SARS-CoV-2 RNA-dependent RNA polymerase (RDRP). It acts as an RNA nucleotide structure scaffold and

can be incorporated into the replicating strand, therefore terminating RNA chain stretching out interfered by the SARS-CoV-2 RDRP complex (Mei and Tan, 2021).

Ivermectin is also another option which is investigation for the treatment of the COVID-19 infection. It can be a potent inhibitor for SARS-CoV-2 to enter into the human cell and even inhibits RNA duplication of that. Ivermectin is a broad-spectrum antiparasitic agent, that has inhibitory potential on many viral infections. It has also been found that ivermectin could prevent SARS-CoV-2 replication *in vitro*. Binding Affinity in AutoDock Tool for ivermectin was Interestingly high (-15.8) but in Molegro Virtual Docker ligand and receptor indicated no affinity. (Perišić, 2020).

In one study it was revealed that Molnupiravir (EIDD-2801) could be useful for the treatment of COVID-19 infection and also, its inhibitory activity has also been demonstrated against coronaviruses including SARS, MERS and SARS-CoV-2. Moreover, it was shown that EIDD-2801 exhibits some level of efficiency in the inhibition of SARS-CoV-2 mRNA replication. (Cox et al., 2021)

In figure 2, it has been shown that Ivermectin, Molnupiravir, Remdesivir and also Velpatasvir could inhibit the main protease of SARS-COV-2.

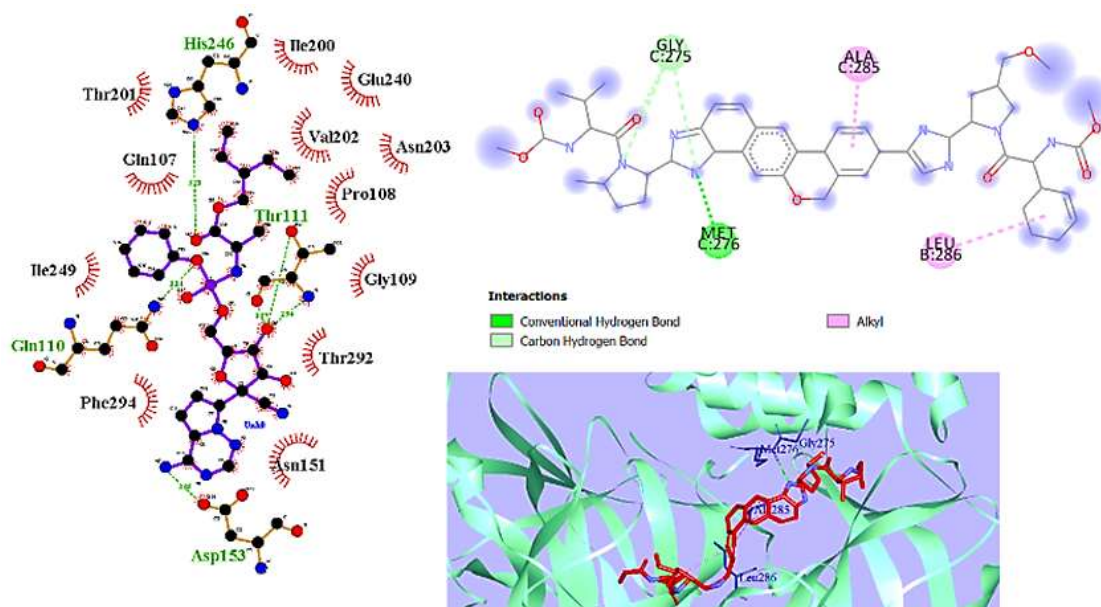


Figure 1. 2D ligand-protein interactions: integration of 6wtt with Velpatasvir designed by LigPlus+ (Left), by Discovery Studio (right-UP). 3D ligand-protein integrations of 6wtt with Velpatasvir designed by Discovery Studio (right-down).

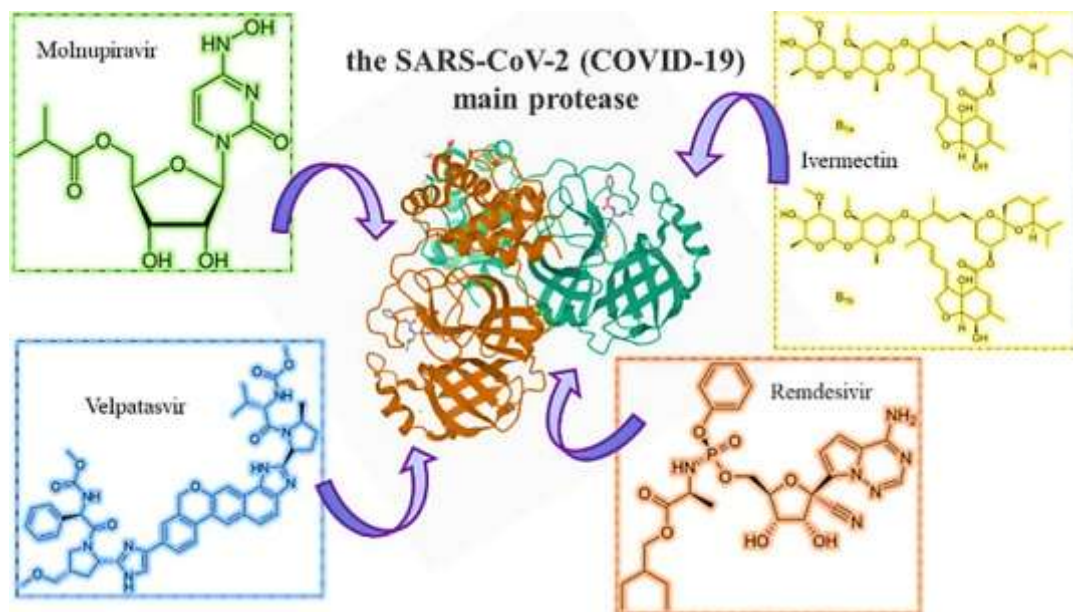


Figure 2. Ivermectin, Molnupiravir, Remdesivir and Velpatasvir as of SARS-COV-2 main protease inhibitors.

Conclusions

In this study, an *in silico* molecular docking experiment was performed on the interaction of FDA-approved drugs with the SARS-CoV-2 main protease enzyme. The virtual screening result consisted of many drugs include 3 ARBs, 5 cephalosporins, a kinase inhibitor and an HMG CoA reductase and leukotriene receptor antagonist, and some other medicines. Velpatasvir is the recommended drug in this study that could be useful for treatment of COVID-19 infection. Moreover, it could be mentioned that the limitation of this study is related to the reliability of *in silico* studies. In this regard, further experimental investigations are needed to confirm the potential of these compounds as treatment for COVID-19 infection.

References

- Akl E. A., Jawad M., Lam W. Y., Co C. N., Obeid R. and Irani J. (2013) Motives, beliefs and attitudes towards waterpipe tobacco smoking: a systematic review. *Harm Reduction Journal* 10:12
- Bavishi C., Whelton P. K., Mancina G., Corrao G. and Messerli F. H. (2021) Renin-angiotensin-system inhibitors and all-cause mortality in patients with COVID-19: a systematic review and meta-analysis of observational studies. *J Hypertens* 39:784-794.
- Bharti R. and Shukla S. K. (2021) Molecules against Covid-19: An *in silico* approach for drug development. *Journal of Electronic Science and Technology*:100095.
- Choudhury S., Moulick D., Borah A., Saikia P. and Mazumder M. K. (2021) In search of drugs to alleviate suppression of the host's innate immune responses against SARS-CoV-2 using a molecular modeling approach. *In Silico Pharmacol* 9:26.
- Cox R. M., Wolf J. D. and Plemper R. K. (2021) Therapeutically administered ribonucleoside analogue MK-4482/EIDD-2801 blocks SARS-CoV-2 transmission in ferrets. *Nat Microbiol* 6:11-18.
- Elfiky A. A. (2020) Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RDRP): A molecular docking study. *Life Sci* 253:117592.
- Jadhav A. K. and Karuppayil S. M. (2021) Topoisomerase II as a target for repurposed antibiotics in *Candida albicans*: an *in silico* study. *In Silico Pharmacol* 9:24.
- Kumar R., Kumar V. and Lee K. W. (2021) A computational drug repurposing approach in identifying the cephalosporin antibiotic and anti-hepatitis C drug derivatives for COVID-19 treatment. *Comput Biol Med* 130:104186.

Li Y., Duche A., Sayer M. R., Roosan D., Khalafalla F. G., Ostrom R. S., Totonchy J. and Roosan M. R. (2021) SARS-CoV-2 early infection signature identified potential key infection mechanisms and drug targets. *BMC Genomics* 22:125.

Mei M. and Tan X. (2021) Current Strategies of Antiviral Drug Discovery for COVID-19. *Front Mol Biosci* 8:671263.

Morris G. M., Huey R., Lindstrom W., Sanner M. F., Belew R. K., Goodsell D. S. and Olson A. J. (2009) AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem* 30:2785-2791.

Nosrati M., Shakeran Z. and Shakeran Z. (2018) Frangulosid as a novel hepatitis B virus DNA polymerase inhibitor: a virtual screening study. *In Silico Pharmacol* 6:10.

Osipiuk J., Azizi S.A., Dvorkin S., Endres M., Jedrzejczak R., Jones K. A., Kang S., Kathayat R. S., Kim Y., Lisnyak V. G., Maki S. L., Nicolaescu V., Taylor C. A., Tesar C., Zhang Y.A., Zhou Z., Randall G., Michalska K., Snyder S. A., Dickinson B. C. and Joachimiak A. (2021) Structure of papain-like protease from SARS-CoV-2 and its complexes with non-covalent inhibitors. *Nature Communications* 12:743.

Perišić O. (2020) Recognition of Potential COVID-19 Drug Treatments through the Study of Existing Protein-Drug and Protein-Protein Structures: An Analysis of Kinetically Active Residues. *Biomolecules* 10.

Polamreddy P. and Gattu N. (2019) The drug repurposing landscape from 2012 to 2017: evolution, challenges, and possible solutions. *Drug Discov Today* 24:789-795.

Pourhajibagher M. and Bahador A. (2020) Computational Biology Analysis of COVID-19 Receptor-Binding Domains: A Target Site for Indocyanine Green Through Antimicrobial Photodynamic Therapy. *J Lasers Med Sci* 11:433-441.

Sharun K., Tiwari R. and Dhama K. (2020) Protease inhibitor GC376 for COVID-19: Lessons learned from feline infectious peritonitis. *Annals of medicine and surgery* (2012) 61:122-125.

Soga M., Evans M. J., Cox D. T. C. and Gaston K. J. (2021) Impacts of the COVID-19 pandemic on human-nature interactions: Pathways, evidence and implications. *People and Nature* 3:518-527.

Sterling T. and Irwin J. J. (2015) ZINC 15 Ligand Discovery for Everyone. *Journal of Chemical Information and Modeling* 55:2324-2337.

Open Access Statement:

This is an open access article distributed under the Creative Commons Attribution License (CC-BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.