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Research Article

Computational Screening of Repurposed Drugs Targeting Sars-Cov-2 Main Protease By Molecular Docking

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Abstract

Background: COVID-19 (Coronavirus disease 2019) is caused by the severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2), which poses significant global health and economic crisis that urges effective treatment.

Methods: A total of 11 molecules (baricitinib, danoprevir, dexamethasone, hydroxychloroquine, ivermectin, lopinavir, methylprednisolone, remdesivir, ritonavir and saridegib, ascorbic acid, and cepharanthine) were selected for molecular docking studies using AutoDock VINA to study their antiviral activities via targeting SARS-CoV's main protease (Mpro), a cysteine protease that mediates the maturation cleavage of polyproteins during virus replication.

Results: Three drugs showed stronger binding affinity toward Mpro than N3 (active Mpro inhibitor as control): danoprevir (–7.7 kcal/mol), remdesivir (–8.1 kcal/mol), and saridegib (–7.8 kcal/mol). Two primary conventional hydrogen bonds were identified in the danoprevir-Mpro complex at GlyA:143 and GlnA:189, whereas the residue GluA:166 formed a carbon–hydrogen bond. Seven main conventional hydrogen bonds were identified in the remdesivir at AsnA:142, SerA:144, CysA:145, HisA:163, GluA:166, and GlnA:189, whereas two carbon–hydrogen bonds were formed by the residues HisA:41 and MetA:165. Cepharanthine showed a better binding affinity toward Mpro (–7.9 kcal/mol) than ascorbic acid (–5.4 kcal/mol). Four carbon–hydrogen bonds were formed in the cepharanthine-Mpro complex at HisA:164, ProA;168, GlnA;189, and ThrA:190.

Conclusion: The findings of this study propose that these drugs are potentially inhibiting the SAR-CoV-2 virus by targeting the Mpro protein.

Keywords: repurposed drug, COVID-19, Mpro, docking

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1. Introduction

The outbreak of novel coronavirus disease (COVID-19) back in December 2019 confronts a global health crisis due to its fast transmission and mutation nature, and the lack of definitive treatment. To date, over 328 million cases have been reported with more than 5.55 million deaths [1]. This disease is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the symptoms extend from mild to severe illness, including acute respiratory distress syndrome and severe pneumonia that requires intensive care admission [2]. The vaccination program has been driven and accelerated globally in the past one year to control this devastating COVID-19 pandemic, approximately 60% of the world population received at least one dose of the vaccine [3]. A full vaccination has been reported to reduce disease transmission, hospitalization, and mortality [4]. However, this is complicated with the abilities of SAR-CoV-2 to mutate and diminish the effectiveness of vaccines, particularly the recent emergence of the fast-spreading Omicron variant [5]. Hence, an effective and potent treatment is still needed in combating this disease.

Drug repurposing is a promising area in drug discovery to identify novel therapeutic indications for previously studied drugs. It offers a great opportunity for drug discovery by reducing the drug development timeline and cost, as well as overcoming the high attrition rate from the de novo drug discovery process [6]. Repurposed drugs like remdesivir, baricitinib, hydroxychloroquine, dexamethasone, danoprevir, ritonavir, and lopinavir were selected for treating COVID-19 due to their therapeutic effect against human coronaviruses, such as SARS-CoV-1 and the Middle East Respiratory Syndrome coronavirus (MERS-CoV) [7]. Some large-scale randomized clinical trials have been completed and others are underway to further evaluate the effectiveness of these repurposed drugs in managing COVID-19 patients. However, controversial findings were reported in terms of the effectiveness of these drugs.

Computer-aided drug discovery and development is a recent approach used to enhance the efficiency and productivity in the drug discovery and development pipeline. Through the computational structure-based virtual screening using molecular docking strategies, the interactions between the tested compound and binding site can be predicted and its binding affinity can be calculated from the software [8]. Leveraging chemical and biological information between tested compounds and binding sites is crucial in identifying and optimizing new potential drugs.

Structurally, the SAR-CoV-2 comprises four (4) structural proteins: spike proteins (S), membrane protein (M), nucleocapsid (N), and envelope proteins (E). N protein forms the

capsid outside the genome (i.e., a single-stranded positive-sense RNA) and is further packed by an envelope that associates with M, S, and E proteins [9]. These proteins (specifically proteases and spike proteins) are the targeted protein for molecular docking to understand the protein–ligand interactions at the active binding site in this virus [10]. Main protease (Mpro), also known as 3-chymotrypsin-like protease (3CLpro), is one of the vital targets that is potentially targeted by an antiviral agent to suppress viral replication. The Mpro plays an important role in posttranslational modifications of replicase polyproteins that further catalyzes the processing of the viral protein [11]. Hence, this study aims to investigate the potential inhibitory action of several selected repurposed drugs targeting Mpro through molecular docking. In addition, this study also highlights the possible inhibitory action of potential natural compounds (ascorbic acid and cepharanthine) against Mpro.

2. Methods

2.1. Protein retrieval and preparation

SARS-CoV-2 Mpro was used as the main target for this study. The crystal structure of the COVID-19 Mpro in complex with an inhibitor N3 (PDB ID: 6LU7, chain A, 2.16 Å) [12] was downloaded from the Protein Data Bank (www.rcsb.org) and chosen as the model of Mpro. The ligand N3 (N-[(5-methylisoxazol-3yl)carbonyl]alanyl-l-valyl-n~1~-((1R,2Z)-4-(benzyloxy)-4-oxo-1-{[(3R)-2-xopyrrolidin-3-yl]methyl}but-2-enyl)-l-leucinamide) was used as a control. The retrieved crystal structure was prepared using BIOVIA Discovery Studio Visualizer (BIOVIA, Dassault Systèmes, BIOVIA Discovery Studio Visualizer, Version 20.1.0.192, San Diego: Dassault Systèmes, 2021) to remove water and N3 ligand molecules. The binding site residues of the N3 ligand were identified and their binding pocket is shown as a red balloon in Mpro (Figure **1**), whereby their key residues are presented in Table 1. The prepared .pdb file was then saved and reviewed for its Ramachandran plot to evaluate its readiness for docking analysis using the RAMPAGE server (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) [13].

Table 1: Grid selection and targeted key residue.

PDB ID	Key residues	Center
6LU7	Thr:24, Thr:25, Thr:26, Leu:27, His:41, Thr:45, Ser:46, Met:49, Phe:139, Leu:140, Asn:141, Gly:142, Ser:143, Cyc:144, His:163, Met:165, Glu:166, His:172	X: -10.7292 Y: 12.4176 Z: 68.8161

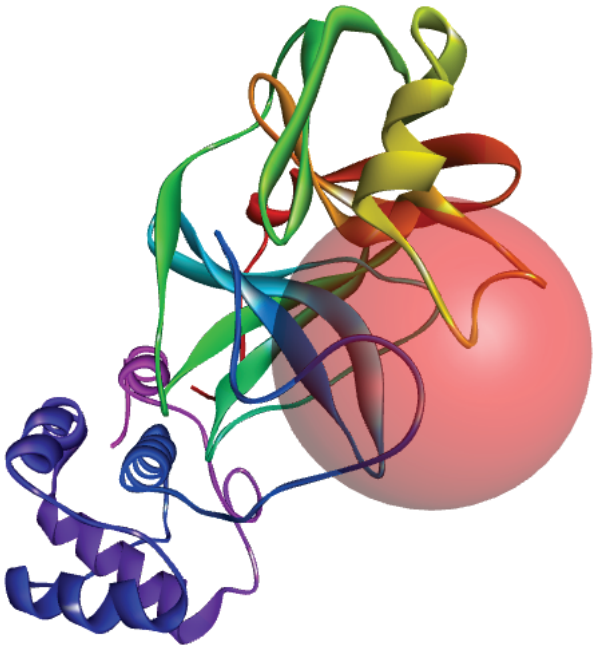

Figure 1: Binding pocket (red color balloon) of SARS-CoV-2's main protease.

2.2. Ligand retrieval and preparation

A total of 12 ligands were retrieved in SDF formats from the PubChem database (NCBI) (http://pubchem.ncbi.nlm.nih.gov) (Table 2). Optimization was performed in each downloaded ligand for their molecular geometry, torsional barriers, and intermolecular-interaction geometry by using MMFF94 partial forcefield in AutoDock VINA in PyRx (https://pyrx.sourceforge.io/home). Before the molecular docking study, all ligands were saved in AutoDock-specific coordinate file .pdbqt format where 'pdbqt' stands for protein data bank (pdb) format file along with charges (q) and AutoDock atom types (t).

2.3. Docking visualization, validation, and analysis

All the prepared proteins and ligands were submitted for docking analysis in the built-in AutoDock VINA in the PyRx program (https://pyrx.sourceforge.io/home) [14]. Site-specific molecular docking was conducted according to the grid selection parameter (Table 1**)**. The binding result table and the best model for each protein–ligand interaction were saved. The Discovery Studio 2021 (BIOVIA) was used for conducting docking visualization to construct 3D and 2D binding conformation diagrams [15]. Docking visualization and analysis were accomplished in Microsoft Windows PC with Intel® Core^{TM} i5-8265U CPU @1.60 GHz, 8 GB RAM.

Table 2: Ligands to target Mpro (6LU7).

No.	Ligand name	Pubchem CID
List of repurposed drug		
1	Baricitinib	44205240
2	Danoprevir	9460579
3	Dexamethasone	5743
4	Hydroxychloroquine	3652
5	Ivermectin	6321424
6	Lopinavir	92727
7	Methylprednisolone	6741
8	Remdesivir	121304016
9	Ritonavir	392622
10	Saridegib	25027363
List of potential natural compounds		
11	Ascorbic acid	54670067
12	Cepharanthine	10206

3. Results and Discussion

We evaluated a total of 12 ligands (mixtures of repurposed drugs and natural bioactive compounds), where most of them are currently in clinical trials for the COVID-19 disease. The antiviral mechanisms of these promising drugs are still poorly understood. The viral Mpro, (also called 3CLpro) controlling the coronavirus replication activities has become an attractive target for therapy. Therefore, in this study, the potential inhibitory action of several ligands targeting Mpro was investigated. Here, N3 was used in the docking studies as a control. The binding affinity of N3 was reported at –7.1 kcal/mol [16], while other reports showed the binding energy at –7.85 kcal/mol [17]. Further, the average binding affinity of N3 at –7.48 kcal/mol was calculated and used for subsequent comparative analysis.

The binding affinity of each ligand (repurposed drugs and natural compounds) toward the protein target is shown in Table 3. Among the 10 tested drugs, only three drugs indicated stronger binding affinity toward Mpro than N3 (control): danoprevir (–7.7 kcal/mol), remdesivir (–8.1 kcal/mol), and saridegib (–7.8 kcal/mol).

Figure **2**B shows the 2D and 3D molecular interaction of danoprevir targeting Mpro. There were two main conventional hydrogen bonds identified in this danoprevir–Mpro complex at GlyA:143 and GlnA:189, whereby the residue GluA:166 formed a carbon–hydrogen bond. The formation of hydrogen bonds is crucial as it gives a stabilization effect in ligand–protein interaction [18]. Besides hydrogen bonds, other key residues

interact through hydrophobic bonds (alkyl [ProA:168] and π-sigma [AlaA:191]) and electrostatic bonds (van der Waals). The presence of hydrophobic bonds could be attributed to the interaction between hydrophobic amino acids with a polar solvent [19].

Table 3: The binding affinity energy of ligands and their interactions targeting SARS-CoV-2 main protease (Mpro).

No.	Ligand name	Binding affinity energy (kcal/mol)	Interactions with Mpro residues[#]		Total number of interactions	
			Hydrogen bond	Hydrophobic interaction	Hydrogen bond	Hydrophobic interaction
1	Baricitinib	–7.4	**Gly143**, **Thr190**	Cyc145, His163	2	2
2	Danoprevir	–7.7	**Gly143**, **Gln189, Glu166**	**Pro168**, **Ala191**	4	2
3	Dexamethasone	–7.1	**Glu166**	–	2	0
4	Hydroxychloroquine	–6.0	Leu141, Asn142	**His41**, **Met165**	2	3
5	Ivermectin	–7.9	–	–	1	0
6	Lopinavir	–7.4	Asn142, **Met165**, **Glu166**, **Gln189**	Leu27, **His41**, **Met49**, Cys145, Pro168	6	6
7	Methylprednisolone	–7.1	**Glu166**	–	1	0
8	Remdesivir	–8.1	Asn142, Ser144, Cys145, His163, **Glu166**, **Gln189**	**His41**, **Met165**	9	2
9	Ritonavir	–7.4	Asn142, His163, **His164**, **Glu166**	Leu141, Cys145, **Met165**, **Leu167**, **Pro168**	8	7
10	Saridegib	–7.8	Asn142, **Gly143**, **Gln189**	Cys145	3	1
11	Ascorbic acid	–5.4	Leu141, Asn142, **Gly143**, Ser144, Cys145	–	7	0
12	Cepharanthine	–7.9	**His164**, **Pro168**, **Gln189**, **Thr190**	**Met165**, **Pro168**	4	3

[#]Residues in bold are binding sites of N3 on Mpro.

Two types of hydrogen bonds are detected: conventional hydrogen bond and carbon–hydrogen bond. In comparison, the carbon–hydrogen bond (CH···O) is known to be weaker than the conventional hydrogen bond and this is due to CHO's average distance, which is longer than the conventional hydrogen bonds (NH···O, OH···O, OH···N, and NH···N). Several reports have stated that the carbon-hydrogen (CH···O) bond plays an essential role in the molecular recognition process [20-22].

Danoprevir is a new NS3/4A protease inhibitor with potent and broad-spectrum antiviral activity against the hepatitis C virus [23]. It was proposed that SARS-CoV-2 Mpro has a similar structure to other RNA viruses, including the hepatitis C virus [24]. From our molecular docking simulation, danoprevir demonstrated strong interactions with Mpro. This finding is congruent with the findings reported by da Costa *et al.* [25]. This strong binding affinity may explain its promising effect in a clinical trial. Treatment of danoprevir boosted with ritonavir improved the clinical condition of patients with

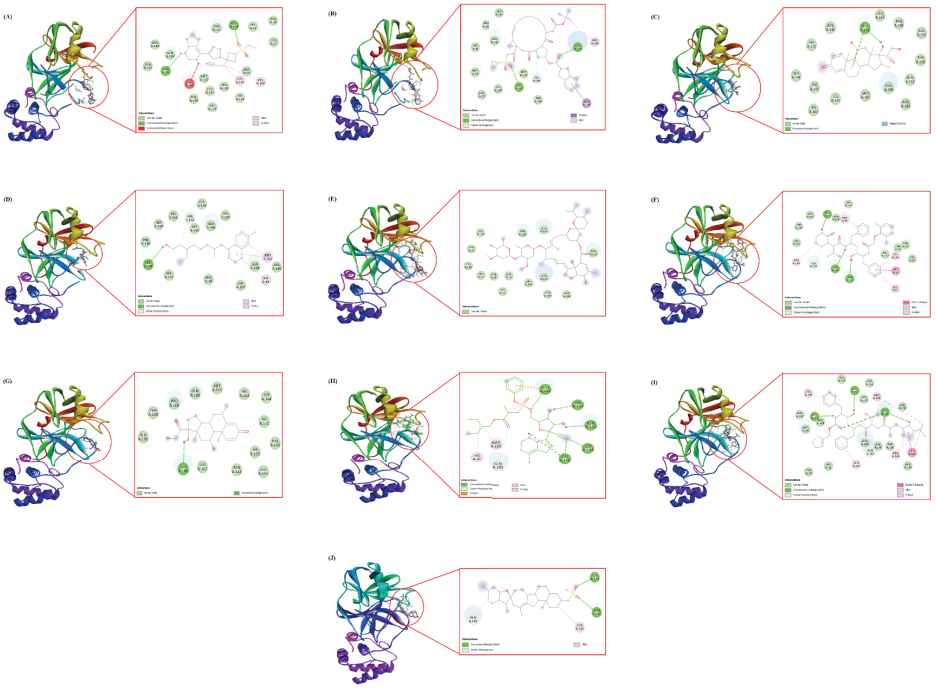

Figure 2: Molecular interaction of (A) baricitinib, (B) danoprevir, (C) dexamethasone, (D) hydroxychloroquine, (E) ivermectin, (F) lopinavir, (G) methylprednisolone, (H) remdesivir, (I) ritonavir, and (J) saridegib against SARS-CoV-2 main protease (Mpro).

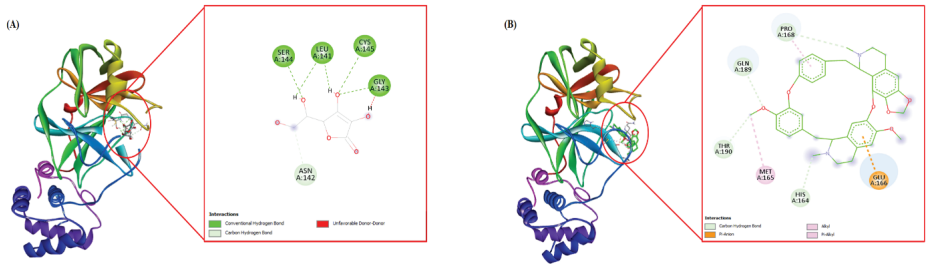

Figure 3: Molecular interaction between (A) ascorbic acid and (B) cepharanthine against SARS-CoV-2 main protease (Mpro).

moderate COVID-19 and increased the discharge rate without adverse outcomes [26]. This further confirms the potential of danoprevir in treating COVID-19 infection.

Among all the tested ligands, remdesivir showed the strongest inhibitory action against Mpro with the strongest binding affinity energy at –8.1 kcal/mol. This could be due to a high number of hydrogen bonds formed in the remdesivir–Mpro complex (Figure **2**H). There are seven primary conventional hydrogen bonds identified in the remdesivir at AsnA:142, SerA:144, CysA:145, HisA:163, GluA:166, and GlnA:189, whereas two carbon–hydrogen bonds were formed by the residues HisA:41 and MetA:165. The lower binding affinity of remdesivir is more likely associated with the double bonding of key residues, such as GluA:166 interacts in hydrogen and π-anion binding. While other

key residues interact via hydrophobic bonds through the π- alkyl (HisA:41) and alkyl (MetA:165) interactions. The π-interaction is believed to give an important impact on the ligands' binding energy to their receptors [20].

Remdesivir drug is one of the agents authorized by the Food and Drug Administration (FDA) to treat COVID-19 patients since October 2020 and remains active against various SARS-CoV-2 variants, including Alpha, Beta, Gamma, Delta, and Omicron [27]. It is a wide-spectrum antiviral agent effective against various viruses, including the Ebola virus, measles and mumps virus, and coronaviruses [28]. Our study findings are consistent with other studies, which also suggested that remdesivir strongly binds to the Mpro of SARS-CoV-2 [29, 30]. These interactions may explain its significant positive outcomes in randomized clinical trials by reducing the recovery time, mortality, and preventing the progression into serious respiratory diseases and oxygen requirement among those patients who received supplementary oxygen [31].

The Hedgehog signaling pathway plays an important role in embryogenesis, tissue homeostasis, and remodeling [32]. In addition, there is emerging evidence that Hedgehog signaling is a target for some pathogens, including influenza A virus, human immunodeficiency virus, and hepatitis [32]. A recent study reported that the Sonic Hedgehog signaling pathway plays some role in patients with COVID-19-associated pneumomediastinum [33]. Therefore, saridegib is included as a tested compound in this study. Saridegib, also known as IPI-926, is a cyclopamine-derived Hedgehog pathway inhibitor that has been clinically investigated for various types of cancer [34]. Figure **2**J shows the 2D and 3D molecular interactions between Saridegib against Mpro. Like danoprevir, two main conventional hydrogen bonds were formed in the saridegib–Mpro complex at Asn A:142 and Gly A:143, and GlnA:189, whereby the residue GlnA:189 formed a carbon–hydrogen bond. While other key residues interact through hydrophobic bonds via alkyl interaction at CysA:145. To date, saridegib is not investigated preclinically or clinically as a treatment for COVID-19 patients. Interestingly, this study demonstrated that saridegib has strong inhibitory action against Mpro, which elucidates that it may have some degree of antiviral activity and further investigation is needed to confirm this finding.

Cepharanthine showed better binding affinity toward Mpro (–7.9 kcal/mol) than ascorbic acid (–5.4 kcal/mol. As shown in Figure **3**B, four carbon–hydrogen bonds were formed in the cepharanthine–Mpro complex at HisA:164, ProA;168, GlnA;189, and ThrA:190. Other key residues interact through three hydrophobic bonds (alkyl, π- alkyl, and π-π T shaped sigma) and one electrostatic bond (π- anion). The π-anion interaction is characterized as the favorable non-covalent electrostatic interaction between anions

located on top of the aromatic ring where the binding energy is controlled by the electrostatic anion-induced polarity contribution [35, 36]. Cepharanthine is an alkaloid isolated from *Stephania cepharantha Hayata*. It has been widely used in Japan since 1951 for various indications, including leukopenia, alopecia, and viper bite. It is also reported with antiviral activities against coronavirus, influenza virus, and hepatitis B and C viruses [37, 38]. Its antiviral activity against SARS-CoV-2 is postulated to be associated with its ability to hinder the SAR-CoV-2 entry phase in viral infection [39] and interactions with nonstructural proteins [40]. This study also provides an insight on interactions of cepharanthine to Mpro, which might be attributed to its potential antiviral effect against SAR-CoV-2.

Ascorbic acid (vitamin C) is a known potent antioxidant that acts by scavenging reactive oxygen species. Several studies suggested that vitamin C supplementation is effective in preventing and treating virus infection by decreasing the susceptibility to viral respiratory infections and pneumonia [41]. However, the role of ascorbic acid in treating COVID-19 patients remains unclear, and ongoing clinical trials are conducted to investigate this [41]. Based on the finding from molecular docking, ascorbic acid poorly interacts with Mpro.

The study focused on the potential of several selected repurposed drugs and some natural compounds to be used in the treatment of COVID-19 infection, based on the virtual screening of their interactions on Mpro. It provides preliminary insight into understanding the potential mechanism of these agents toward the SAR-CoV-2 virus. Further, molecular docking on other SAR-CoV-2 proteins and proteases, such as S protein, N protein, E protein, M protein, NSPs, and papain-like protease to have a better illustration in terms of interactions between the drugs with the viral proteins is recommended. This could provide valuable information in drug discovery and development.

4. Conclusion

All 12 molecules (baricitinib, danoprevir, dexamethasone, hydroxychloroquine, ivermectin, lopinavir, methylprednisolone, remdesivir, ritonavir and saridegib, ascorbic acid, and cepharanthine) showed antiviral activity against COVID-19 infection through their inhibitory action targeting Mpro. Our findings indicate the potential mechanism of these molecules by inhibiting Mpro protein in the SAR-CoV-2 virus. Nevertheless, further molecular docking on other viral proteins is warranted to understand the interactions of these molecules with other viral proteins, which is vital in discovering and developing novel treatments for the COVID-19.

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None

Ethical Considerations

Not applicable.

Competing Interests

None declared.

Availability of Data and Material

The dataset generated during this study is available from the corresponding author on reasonable request.

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