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An *in silico* Drug Repurposing Study to Inhibit the Spike Protein of SARS-CoV2

Soha Abdollahpour[1], Azam Rahbar[2], Reza Mohammadhassan[3*], Yasamin Talebi[4] and Yegane Bahrami Hesari[5]

[1]Amino Techno Gene Private Virtual Lab (NGO), Post Code: 1495845983, Tehran, Iran (soha.abdollahpour@gmail.com).
[2]Amino Techno Gene Private Virtual Lab (NGO), Post Code: 1495845983, Tehran, Iran (azam.rahbar@gmail.com).
[3]Amino Techno Gene Private Virtual Lab (NGO), Post Code: 1495845983, Tehran, Iran (*rezarmhreza22@gmail.com, ORCID: https://orcid.org/0000-0002-0509-4187).
[4]Amino Techno Gene Private Virtual Lab (NGO), Post Code: 1495845983, Tehran, Iran (yasmintalebi81@gmail.com).
[5]Amino Techno Gene Private Virtual Lab (NGO), Post Code: 1495845983, Tehran, Iran (yeganebahrami71@gmail.com).

ABSTRACT

SARS-CoV2 has caused the recent mortal pandemic known as COVID-19. The drug repurposing approach can be employed to find the potential drugs capable of binding SARS-CoV2 structural and nonstructural proteins. The present study aimed to repurpose some common FAD-approved antiviral and non-antiviral drugs computationally for SARS-CoV2 treatment. In the *in silico* study, 89 FDA-approved drugs and Remdesivir, as the control, were analyzed by molecular docking to inhibit the SARS-CoV2 spike (S) protein as the key player in virus-cell binding. First, the Uniport website was used to find receptor and ganglioside binding domains (RBD and GBD, respectively) of the S protein as the target. The structure of the target was downloaded from RCSB, and 'the ligands' structures were downloaded from PubChem. All structures were refined using SPDV and PyRx software. AutoDock Vina was employed for the docking process. The result showed that 8 drugs, including Ledipasvir, Montelukast, Domperidone, Aprepitant, Folic acid, Losartan, Ticagrelor, and Rivaroxaban, can bind S protein and then inhibit the protein function. In addition, Ledipasvir, Montelukast, and Domperidone can bind GBD of the S protein with higher binding energy (-8.2, -8, -7.9 kcal/mol, respectively). On the other hand, higher RBD binding energy was calculated for Ticagrelor (-6.9 kcal/mol), Folic acid, Montelukast, and Domperidone (-6.5 kcal/mol). Generally, the ligands could inhibit GBD more than RBD. According to the binding energy to S protein and low side effects of the studied medications, Ledipasvir and Losartan can be introduced as the most effective candidates for repurposed drugs. Also, Gly496 and Asn137 are the most engaged amino acids in the ligand-receptor interaction from RBD and GBD, respectively.

Keywords: Drug Repurposing, Molecular Docking, COVID-19, SARS-CoV2, Spike Protein.

1. INTRODUCTION

A novel coronavirus species was observed in people who deal in the Huanan Seafood Wholesale Market in Wuhan, China, in December 2019. The International Committee on Taxonomy of Viruses (ICTV) has termed the novel virus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), and the World Health Organization (WHO) has

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declared the viral infection caused by SARS-CoV2 as coronavirus disease 2019 (COVID-19) on 22 February 2020 (Abosheasha et al., 2022). Many SARS-CoV2-infected patients were observed in 202 countries during the first three-month COVID-19 outbreak; 170,000 cases and 1800 deaths were reported during the first 50-day outbreak. On 11 March 2020, WHO announced the COVID-19 outbreak as a pandemic due to the fast worldwide prevalence of the disease (Shi et al., 2020).

It has been demonstrated that SARS-CoV2 can be transmitted among individuals via respiratory aerosols (droplets) produced by coughing and sneezing. The symptoms of COVID-19 include fever or chills, cough, shortness of breath or difficulty breathing, fatigue, headache, sore throat, nausea or vomiting, and diarrhea (Barrios et al., 2018). The viral infection sometimes leads to pneumonia, severe acute respiratory syndrome, kidney failure, and even death (Bayoumy et al., 2020).

There is a sub-classification for coronavirus including α, β, γ, and δ. SARS-CoV2 has been classified as β-coronavirus, causing two other fatal viral epidemics during the last two decades (Shi et al., 2020). The genome of SARS-CoV2 is a single positive-sense RNA (+ssRNA) with a 5' cap and a 3' poly-A tail. The genome is directly translated into the host cell. The genome size is about 30 kbs and includes 14 open-reading frames (Mohammadian et al., 2021). The genome encodes structural and nonstructural proteins. The nonstructural proteins play critical roles in the virus replication and the final virus aggregation process. The structural proteins include Spike (S), Membrane (M), Envelope (E), Nucleocapsid (N), and other sub-proteins (Prajapat et al., 2020). The S protein of the SARS-CoV2, like other coronaviruses, can bind angiotensin-converting enzyme 2 (ACE2) on the external surface of the lung cells. Then, the virus enters the cells (Chmielinska et al., 2020). Therefore, S protein recombination could probably increase the transmission rate of SARS-CoV2 (Abosheasha et al., 2022).

Although viral infections caused by human β-coronavirus are typically mild, two β-coronavirus epidemics, including SARS-CoV in 2002 and MERS-CoV in 2012 (Mohamadian et al., 2021), have caused more than 10,000 deaths. Mortality proportions of SARS-CoV and MERS-CoV have been estimated at 10 and 37 per cent, respectively, whilst the mortality proportion of SARS-CoV2 was about 2.9 per cent in 109 countries until March 2020. However, the critical point is the higher transmission rate of SARS-CoV2 than SARS-CoV (Mohammadhassan et al., 2016).

Recently, many biotechnological companies have developed several vaccines approved by WHO for emergency use to prevent COVID-19 (Anesi, 2021). However, it has

been reported that these vaccines cannot immunize people 100%. So, vaccinated people can be infected, but less or milder than non-vaccinated individuals. Also, this virus keeps on mutating. So, the efficiency of the vaccine needs to be rechecked for every mutation ((Shi et al., 2020).

Since, a mild infection would be probable, even after vaccination, many pharmaceutical companies are seeking an effective antiviral compound as a drug to treat COVID-19 (Kim et al., 2021). Currently, there is no reported and approved drug and treatment for COVID-19 (Huang et al., 2020). The potential treatments for COVID-19 are categorized into immune-system-targeting drugs and viral-protein-targeting drugs (Wu et al., 2020).

Computational biology can play a prominent role in novel drug discovery and development. Also, drug repurposing can be a promising approach for identifying remedial opportunities to use available drugs. In addition, it could significantly reduce the cost and time of developing novel drugs (Mohammadhassan et al., 2020). Drug repurposing is a cost-effective and fast approach to finding novel applications for the accessible drugs in the market that are analyzed by novel *in silico* tools (Shyr et al., 2020), particularly molecular docking, data mining, and machine learning (March-Vila et al., 2017). So, drug repurposing focuses on new functions of the already-approved biological properties or side properties of a medication. Pleiotropic drugs and drug discovery, particularly through the human genome, are two fundamental scientific concepts of drug repurposing (Jourdan et al., 2020). There are many repurposed medications, such as some conventional drugs, in the history of medicine. Aspirin (acetylsalicylic acid), as a good example, has been repositioned several times as an analgesic, antithrombotic, anti-inflammatory, and anticarcinogenic drug respectively (Qorri et al., 2022). Significant growth of high-processing and available computers, as the rising number and easy accessibility of identified-structure proteins and small molecules have facilitated molecular docking (Stanzione et al., 2021). The basic aim of molecular docking is the energetical and structural prediction of the molecule recognition. There are several applications of molecular docking in drug discovery and repurposing, such as finding potential ligands by *in silico* screening to fit substrates and inhibitors according to electron density (Abdelsattar et al., 2021). Since molecular docking has significantly succeeded in structure-based drug designing, the approach has been improved over time. Over 60 in-silico docking tools have been developed for academic and commercial purposes during the last two decades (Mohanty and Mohanty, 2023). Also, molecular docking has launched many

commercial medications, including Erdafitinib, Zanamivir, Imatinib, Nelfinavir, and other clinical drugs (Stanzione et al., 2021).

So, drug repurposing is used in the present study to analyze the inhibitory effects of the eighty-nine common drugs, both antiviral and non-antiviral, against the S protein of SARS-CoV2. In addition to these drugs, Remedesevir is employed as the control.

2. METHODOLOGY

2.1. Selecting and Preparing the Target Protein

S protein was selected among SARS-CoV2 proteins. As mentioned above, SARS-CoV infection depends on the fusion between the virus coat and the host cell membrane. S protein plays a crucial role in fusion. SARS-CoV2 can utilize fusion type 1 to enter the host cells. The protein is a heterotrimer; each monomer contains two subunits, S1 and S2. The receptor binding domain (RBD) of the S1 is responsible for binding the ACE2 to the host cell (Outlaw et al., 2020).

S protein (Acc. No. P0DTC2; https://www.uniport.org) was assessed to find the RBD. According to the data from the website, 13-685 and 686-1273 amino acids belong to S1 and S2, respectively. 319-541 amino acids of the S1 are the domain involved in binding to human ACE2 (hACE2), and 437-508 amino acids are the exact motif directly binding to the receptor. The amino acids of the motif interacting with hACE2 were identified via the 6ZGE structure from the RCSB database (https://www.rcsb.org/structure/6zge). It has been suggested that 111-162 amino acids of S protein can also bind to the superficial gangliosides of the host cell membrane. The amino acid region is the gangliosides binding domain (GBD). 134-137 amino acids of the GBD directly interact with gangliosides (Fantini et al., 2020; Fig 1). The most interacting amino acids of RBD and GBD, respectively, include Gly446 / Tyr449 / Gly496 / Thr500 and Gln134 / Phe135 / Cys136 / Asn137, which are used to recognize grade box.

6ZGE structure, a homotrimer, was downloaded from the RCSB database. Then, a chain was separated to use for docking. Next, all heteroatoms and water molecules were removed from the A chain. Finally, both RBD and GBD were used to target through the docking process.

The energy of the A chain structure was minimized via SPDBV software (v4.1) by the GROMOS96 force field (Islam and Iqbal, 2020). Hydrogen atoms were added to the minimized structure. PyRx software (v0.8) was employed to change file type (pdb to pdbqt), add Coleman partial charge, and identify atoms type (Dallakyan and Olson, 2015).

2.2. Ligand Preparation

The ligand library of the study includes 89 high-consumption drugs and Remdesivir as the control. Their pharmacologic classes have not been considered for selecting the drugs and have not been restricted to antiviral drugs. Remdesivir, one of the effective drugs to treat COVID-19 (Beigel et al., 2020), was used as the control. The structures of the ligands were saved in sdf format from the PubChem database (pubchem.ncbi.nlm.nih.gov). The sdf format was converted to pdb by Openbabel software (online version; http://www.cheminfo.org/Chemistry/Cheminformatics/FormatConverter/index.html). Also, if the 3D structures of the ligands were not available, the software changed the 2D structures to 3Ds (Kaushal et al., 2022). PyRx was employed to convert the ligands formats from pdb to pdbqt by adding Gasteiger partial charge and recognizing the rotatable bonds (Dallakyan and Olson, 2015). The ligands with their accession numbers from the PubChem database are available in annexure 1.

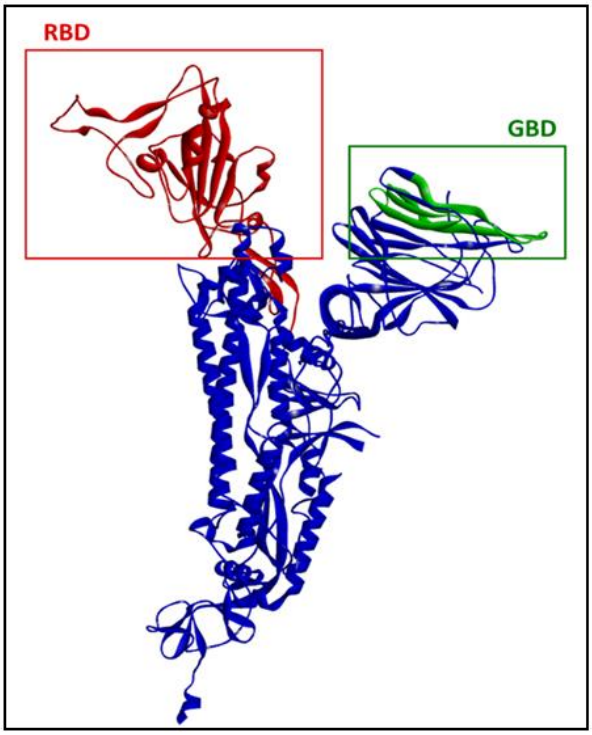

Figure 1. The single-chain structure of the spike protein (blue), RBD (red), GBD (green). PDB ID for spike protein is 6ZGE.

2.3. Docking

AutoDock Vina, associated with PyRx as a graphical software, was employed in a molecular docking process to analyze the pharmacodynamics between the ligand library and the RBD and GBD sites of the target proteins (Dallakyan and Olson, 2015). The main amino acids of

RBD and GBD interacted with ACE2 and gangliosides, respectively (Annexure 2). Although, according to the 6ZGE structure, more amino acids of RBD interacted than GBD, other amino acids of these sites were not considered to increase docking precision because they show high dispersion and larger grid box size than the ligands. The interaction between ligands, with more negative binding energy than control, and both RBD and GBD sites were analyzed by Discovery Studio Visualizer 2021 (Johansson et al., 2012).

3. RESULTS

The ligands and control were docked for both RBD and GBD sites. The results show that Remdesivir binding was 5 and -6.9 kcal/mol at the RBD and GBD sites, respectively. The best conformation and interaction of the Remdesivir were observed at the amino acids Tyr449 and Gly496 of the RBD site (Fig 2a). Also, the amino acids Cys136 and Asn137 of the GBD site showed the best conformation and interaction of the Remdesivir (Fig 2b).

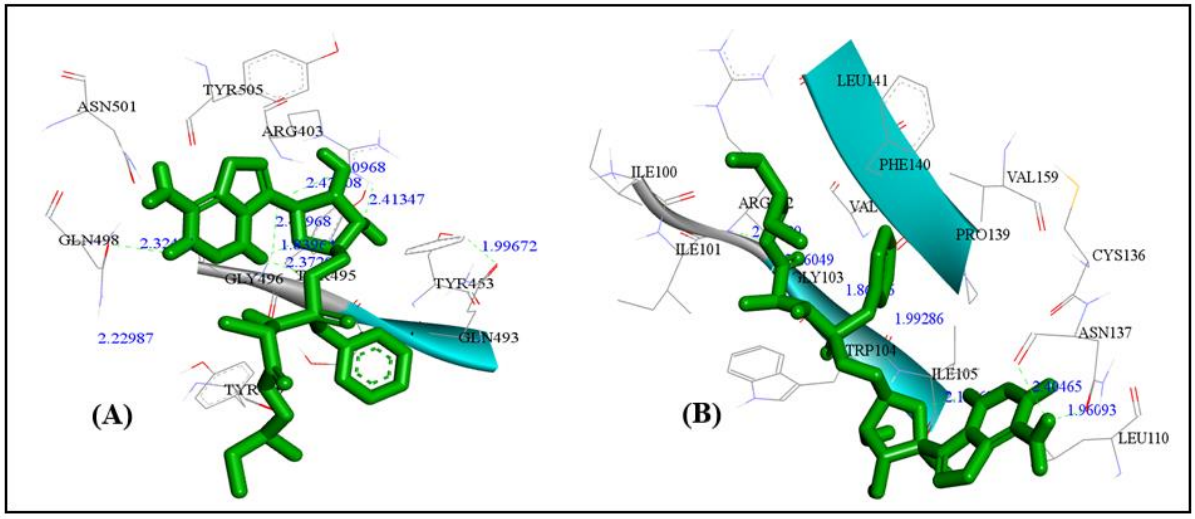

Figure 2. Remdesivir (Control) interacting with S protein (A) RBD, (B) GBD, Black) amino acids, Blue) the length of the hydrogen bonds.

All 89 ligands were compared to the control regarding bindign energy. Since these ligands are FDA-approved, clinically studied, and commercially available, the study ignored the pharmacokinetic prediction. Among these compounds, eight ligands indicated more negative energy than control when interacting with the main amino acids of both sites. The eight repurposed drugs were Ledipasvir, Montelukast, Domperidone, Aprepitant, Folic acid, Losartan, Ticagrelor, and Rivaroxaban. Also, the energy of ligand-GBD binding was generally higher than ligand-RBD binding energy (Annexure 2).

The highest ligand-GBD binding energy was calculated for Ledipasvir with 8.2 kcal/mol. After Ledipasvir, Montelukast and Domperidone have higher binding energies (8

and 7.9 kcal/mol, respectively) than other ligands. The lowest GBD binding energy belongs to Rivaroxaban (-7.2 kcal/mol; Annexure 2). Asn137 was the most key player amino acid in 8 ligand-GBD interactions, except Rivaroxaban. After that, Phen135 cooperated in about half of the ligand interactions, mostly with Asn137. The amino acid individually played a role in Rivaroxaban-GBD binding. Cys137 was engaged in Remdesivir (control) binding to GBD (Fig 3).

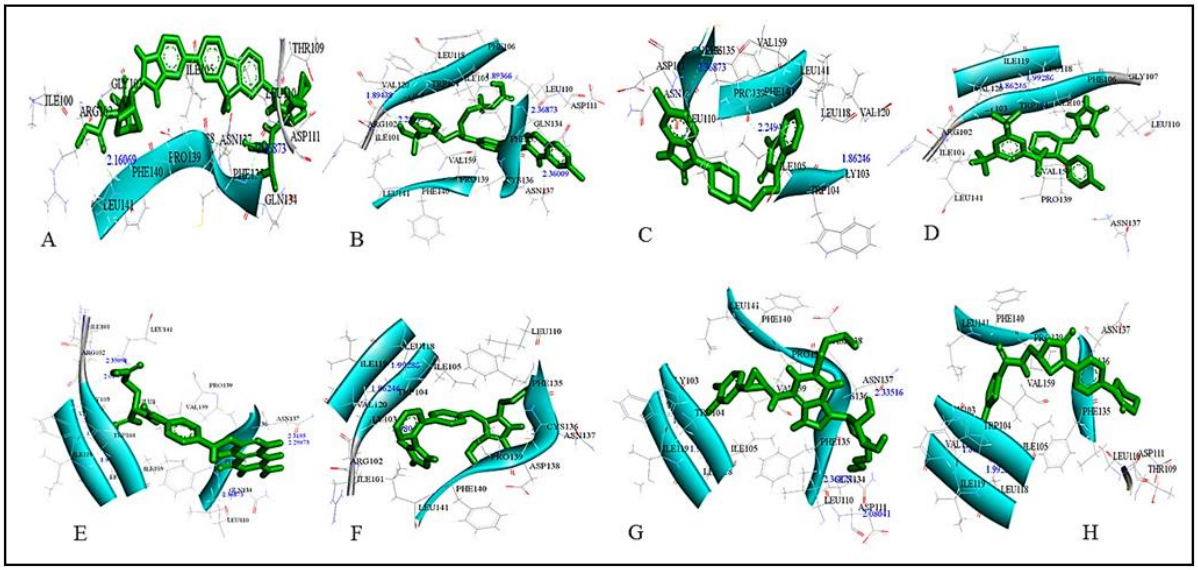

Figure 3. Lignd-GBD interaction; Black) amino acids, Blue) the length of the hydrogen bonds. A) Ledipasvir, B) Montelukast, C) Domperidone, D) Aprepitant, E) Folic acid, F) Losartan, G) Ticagrelor, H) Rivaroxaban.

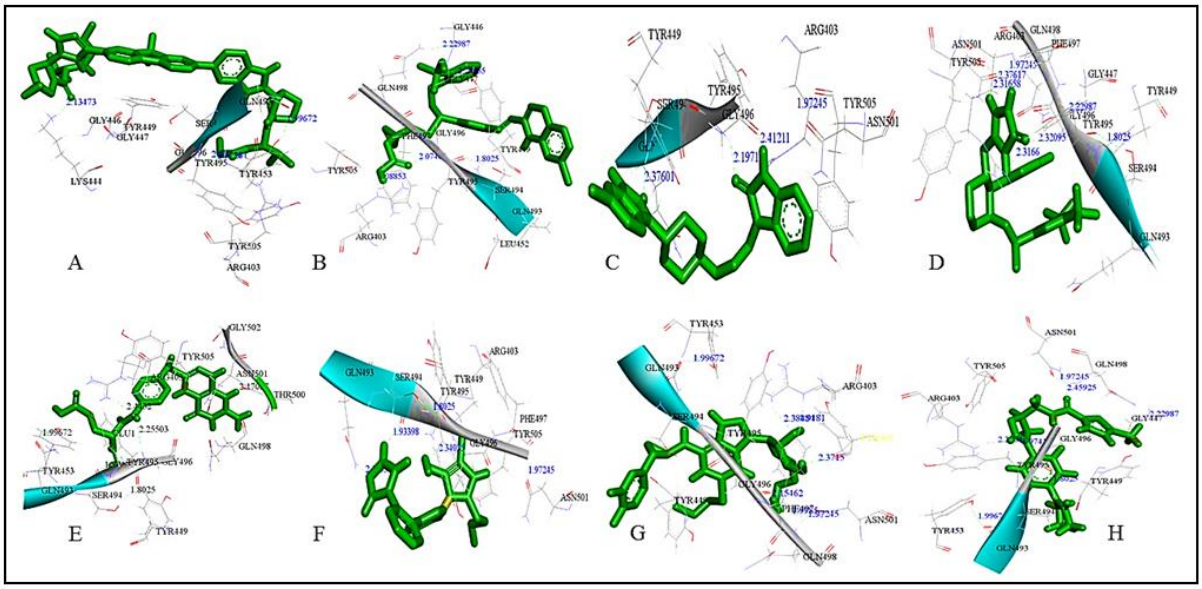

Figure 4. Interaction between Ligand-RBD; Black) amino acids, Blue) the length of the hydrogen bonds. A) Ledipasvir, B) Montelukast, C) Domperidone, D) Aprepitant, E) Folic acid, F) Losartan, G) Ticagrelor, H) Rivaroxaban.

The binding energy of Ticagrelor was the highest in RBD interaction (-6.9 kcal/mol). Montelukast, Domperidone, and Folic acid showed higher and the same ligand-RBD energy

binding (-6.5 kcal/mol). Rivaroxaban has the lowest binding energy with RBD (-5.8 kcal/mol), among the ligands (Annexure 2). Gly496 is the most engaged amino acid in the ligand-RBD interactions. The amino acid can be found in the interaction of all ligands with RBD. Tyr449 is the next more engaged amino acid that plays a role in 6 ligand-RBD interactions that were associated with Gly496. At least, Thr500 was only engaged in Folic acid interaction in association with Gly496 (Fig 4).

Moreover, the hydrogenic bonds were higher among ligand-RBD interactions (28) than ligand-GBD dockings. Also, Remedesevir (control) and Folic acid created the most hydrogenic bonds (8 and 7), particularly when interacted with GBD (4); however, Folic acid-RBD interaction contained lower hydrogenic bonds (3) than Remedesevir-RBD (Annexure 2). These ligands had the highest hydrogenic bonds among all ligand interactions with GBD. The longest hydrogenic bonds with GBD were observed in Montelukast-Asn137 and Folic acid-Leu110 (2.36Å), and the smallest ones were in Domperidone-Lys103 and Losartan-Trp104 (1.86Å; Fig 3). RBD showed the most hydrogenic bonds (4) with Ticagrelor, Rivaroxaban, and the control. The longest and smallest hydrogenic bonds with RBD were respectively measured in Rivaroxaban-Gln498 (2.45Å) and Montelukast-Arg403 (1.08Å; Fig 4). The length of the hydrogenic bonds in the control was the highest for both RBD (Arg102 & G103=2.6Å) and GBD (Arg403=2.47Å; Fig 2).

4. DISCUSSION

Computational biology and virtual screening play a prominent role in accelerating these studies. Many studies have used molecular docking and dynamics to identify effective compounds for treating viral diseases (Nourian et al., 2020; Goudarziasl et al., 2024). During the pandemic, many vaccines have been developed and emergently approved by the FDA. According to many reports, the efficiency of these vaccines is not 100%, and the vaccinated population is not constantly immunized against COVID-19. However, disease severity among vaccinated people is low, and vaccination can reduce the rate of hospitalization or death (Sallam, 2021). Thus, developing specific antiviral drugs against SARS-CoV-2 is crucial. Although virtual screening methods and *in silico* drug discovery can accelerate drug development, pre-clinical and clinical studies are in demand but very time-consuming (March-Vila et al., 2017). Therefore, it could be notable that drug repurposing can be an effective approach to changing the drug development process (Luo et al., 2021). This strategy computationally analyses clinical-assessed and FDA-approved drugs to suppress target

proteins involved in diseases. The approach can effectively save time and budget in developing a treatment for a current disease (Jourdan et al., 2020).

Moreover, although *in silico* drug discovery is significantly a time-saving method to find an effective ligand as a potential drug, it is not yet an emergent solution during a pandemic. Therefore, drug repurposing could be appropriate for struggling with a pandemic (Bayoumy et al., 2020; Mohammadhassan et al., 2020).

In this study, we aimed to repurpose FDA-approved and commercial medications effectively binding to the S protein as the target, potentially inhibiting the protein function and then preventing viral entry into the host cells. The interaction scores, such as binding energy or docking scores, can directly measure and represent the binding intensity between small compounds and the target protein. The interaction score measurement is a common and well-developed approach in molecular docking studies (Singh et al., 2022). The method was widely used as a primary criterion to propose the proper candidates for drug discovery research. The interaction scores are employed to predict the binding affinity and the strength of ligand-protein bonds (Bhagat et al., 2021). The binding affinity is directly correlated with the interaction score, so the best ligand shows a higher interaction score with the target protein. The approach is related to the target protein and is also a standard and efficient metric for screening large chemical spaces in molecular docking studies (Singh et al., 2022).

In addition to the interaction scores, hydrogenic bonds were considered in the study to provide a comprehensive understanding of the ligand-protein interactions. Hydrogenic bonds are considerable in drug repositioning and molecular docking (Bulusu and Desiraju, 2020). They can facilitate the formation of stable complexes between small molecules and target proteins and influence their binding affinity and specificity. If the length of the hydrogenic bond is less than 3.00Å, the interaction is strong (Damongilala et al., 2023). In our study, the longest hydrogenic bond was 2.6 Å for the control and 2.45Å for Rivaroxaban, as a candidate. Generally, the average range of length of the hydrogenic bonds was 2.00-2.5 Å in the present study.

In a docking study, Marinho et al. (2020) used an interaction score system for virtually repurposing six common inhibitive medications, including Azithromycin, Baricitinib, Chloroquine, Hydroxychloroquine, Quinacrine, and Ruxolitinib, to inhibit main protease (MPro) of SARS-CoV2. The best affinity and inhibition were observed by Ruxolitinib, although all studied drugs were effective in inhibiting the MPro. In another *in silico* molecular docking, interaction scores were utilized to analyze antiviral and antimalarial drugs, including Oseltamivir, Ritonavir, Remdesivir, Ribavirin, Favipiravir, Chloroquine, and

Hydroxychloroquine, to suppress MPro. The binding energy and interaction score indicated a higher inhibitive effect of Ritonavir against MPro (Narkhede et al., 2020). Talluri (2021) also employed interaction scores to study antiviral and antimalarial medications to inhibit MPro via an *in silico* docking approach. The results showed that the highest interaction score and binding affinity were predicted by Danoprevir and Darunavir. In addition to interaction scores, hydrogenic interaction was considered in the above-mentioned studies; however, interaction scores play centeral roles, followed by hydrogenic interactions, in these *in silico* analyses. For instance, Narkhede et al. (2020) only mentioned the number of hydrogen bonds between the ligands and the target protein, as well as the names and positions of the amino acids involved in these hydrogen bonds.

Therefore, we focused on the interaction scores as a standard and effective approach, and hydrogenic bonds, for repurposing common and FDA-approved drugs, both antiviral and non-antiviral, to inhibit the S protein of SARS-CoV2. RNA-dependent RNA polymerase (RdRp) is a key enzyme in regulating SARS-CoV-2 replication (V'kovski et al., 2021). This enzyme can be inhibited by analog nucleoside drugs such as Remdesivir, which was introduced as a potential treatment for Ebola in 2016 (Warren et al., 2016). The effect of Remdesivir on the Coronaviridae family was first identified in 2017 on the MERS virus, then during the COVID-19 pandemic. Remdesivir functions as a non-obligatory chain terminator for the RdRp enzyme (Kokic et al., 2021). So, Remdesivir was employed as the control in the study.

The results indicated that these 8 candidate ligands could bind to GBD of S protein more strongly than RBD. During viral infection, GBD needs to bind to the host phospholipid membrane (HPLM) to facilitate RBD binding to ACE2 (Fantini et al., 2020). Thus, these studied ligands can make strong interactions with GBDcausing them to disrupt GBD-HPLM binding. As a result, RBD-ACE2 binding is not stable, so the host cell is not infected by SARS-CoV2 (Fantini et al., 2020). However, these ligands can effectively inhibit RBD of S protein, too. The 8 ligands will be introduced as the following.

Ledipasvir is an effective drug for treating chronic hepatitis C and can inhibit the nonstructural protein (NSP) 5A of the hepatitis C virus (HCV). This protein plays a role in genomic RNA replication and assembly of the virus particle (Buggisch et al., 2018). In addition, low side effects of the drug, commonly fatigue and headache, can be considered significant benefits of Ledipasvir compared with other anti-HCV drugs (Markcus et al., 2018). The FDA approved Ledipasvir in 2014 and 2015. It was also beneficial for treating simultaneous HCV- and HIV-infected patients (Maugeri et al., 2018). In a computational

study, molecular docking was employed to analyze the inhibitory effect of Ledipasvir on the main protease of SARS-CoV2 (Pirzada et al., 2021). Also, a clinical study has demonstrated that the SARS-CoV2-infected patients receiving Ledipasvir and Sofosbuvir showed a faster therapeutic response than the control group (Markcus et al., 2018). In the present study, Ledipasvir could significantly bind the S protein of SARS-CoV2.

Montelukast, or Singular (Merck, German), is a leukotriene receptor antagonist. The drug was approved by the FDA in 1998 and is commonly used with corticosteroids to treat asthma (Dixon et al., 2022). The over-consuming side effects include abdominal pain, somnolence, thirst, headache, vomiting, psychomotor hyperactivity, and convulsions with shallow frequency (Kovesi, 2019). Montelukast has been effectively used against MERS-CoV. In an *in silico* study, this drug could bind the RBD of the S protein of the SARS-CoV2, preventing the virus from entering the cell. It has been indicated that Montelukast could also reduce virus infection (Gan et al., 2021). In another molecular docking study, Montelukast could more significantly bind the main protease of SARS-CoV-2 than Remdesivir (Copertino et al., 2021).

Domperidone is a dopamine antagonist and facilitates gastric emptying. Therefore, the drug is used as an antiemetic. Although Domperidone can be partially transferred across the blood-brain barrier, there is no adverse event on the neural system (Field et al., 2019). Thus, the properties of Domperidone can support safety if used in the long term. In the United States, it is also used to treat gastroparesis and any chronic condition causing nausea and vomiting (Hale et al., 2018). In a study, the effect of Domperidone on SARS-CoV-2 was analyzed. Then the drug was experimentally studied on different cell lines. As a result, Domperidone could negatively influence the interaction between the virus and the host (Mirabelli et al., 2021).

Aprepitant is the first drug to be introduced from the antagonists of the neurokinin 1 (NK1) receptor antagonists, and it was approved by the FDA in 2006. The drug is usually used to prevent nausea and vomiting caused by chemotherapy (Muñoz and Coveñas, 2020). Aprepitant is well-tolerated by patients. However, the most reported side effects of the drug are weakness and fatigue. Anorexia, constipation and diarrhea are also observed as adverse events caused by Aprepitant (Zhang et al., 2020). In addition, many *in silico* studies report the inhibitory effect of this drug on different proteins of SARS-CoV2, particularly the main and the NSP 15 (Mahmud et al., 2021).

Folic acid, as a cofactor, can participate in many intracellular reactions. Folic acid, known as vitamin B9 (folacin), is converted into Folic acid in the body. Folic acid plays a

role in carbon transfer reactions as a carbon source. Although plants and bacteria can synthesize Folic acid, this substance is not made by the human and animal body, so it is considered an essential vitamin (Froese et al., 2019). Folic acid deficiency is associated with many disorders, including megaloblastic anemias, neurological disorders, neural tube defects, vascular diseases, and thrombosis (Pope et al., 2019; Marawne et al., 2022). However, many studies have demonstrated no side effects for Folic acid (Zarou et al., 2021). Folic acid can negatively influence COVID-19 through ferroptosis as a cell death pathway. Ferroptosis is an iron-dependent and reactive oxygen species (ROS)-reliant cell death (Mou et al., 2019). It has been indicated that SARS-CoV2-infected cells undergo ferroptosis stress. As a result, it is necessary to add vitamin B6, Folic acid (vitamin B9), and vitamin B12 in the treatment process of COVID-19 to suppress the effects of the virus-mediated changes in the host cell metabolism. In addition to the S protein, the effects of Folic acid on the main protease have also been studied by molecular docking (Kumar et al., 2021).

Losartan is an angiotensin type I receptor antagonist, blocking angiotensin II activities more entirely than renin drugs or ACE inhibitors. Losartan is a relatively conventional drug to reduce blood pressure (Costantino et al., 2021). However, some side effects, such as headache, malaise, edema, and upper respiratory tract infections, have been reported in 5 to 10% of patients (Al-Majed et al., 2015). In an *in vitro* study, Vero E6 cells were treated with Losartan and then infected by SARS-CoV-2. As a result, the virus replication decreased by about 80% and 70% pre- and post-exposure. In addition, Losartan significantly suppressed cell destruction (Nejat et al., 2020).

Ticagrelor can prevent platelets from sticking, resulting in heart attack and sudden death. This drug is a P2Y12 receptor antagonist and reversibly binds the receptor. In patients suffering from acute coronary syndrome, Ticagrelor is administrated to prevent atherothrombotic events (Kubisa et al., 2018). The reported side effects of Ticagrelor include dyspnea, bruising, and various types of haemorrhages such as gastrointestinal, nasal, subcutaneous, dermal, and procedural sites (Guerbaai et al., 2019). Since COVID-19 can cause thrombotic effects and Ticagrelor is an anticoagulant drug, many studies indicate the drug can reduce the complications caused by COVID-19 (Kubisa et al., 2018). In a recent *in silico* study, Ticagrelor could significantly bind the main protease and S protein of SARS-CoV-2. Losartan, with a smaller structure than other ligands (Abosheasha et al., 2022), can interact with fewer amino acids (Gly496 and Asn137 from RBD and GBD, respectively), as indicated in the *in silico* study.

There are many studies reporting an association between COVID-19 and hematological biomarkers, particularly hypertension, hyperglycemia, platelets, ferritin (a blood iron-contained protein), and D-dimer, fibrin produced during blood clot fibrinolysis (Nokhostin et al., 2020). Mahmoud et al. (2022) showed that all patients suffering from severe COVID-19 have high levels of ferritin, platelets and D-dimer. They also found that hypertension and diabetes are two independent, crucial risk factors causing to be infected by SARS-CoV2. In another clinical study, Sana and Avneesh (2022) demonstrated that severe COVID-19 is significantly correlated with hypercoagulable conditions caused by high levels of platelets and D-dimer. Also, they reported that there is a correlation between high levels of ferritin and the severity of COVID-19. Thus, it seems that some repurposed ligands, including Folic acid, Losartan, and Ticagrelor, can influence hematological agents to suppress SARS-CoV2, in addition to S protein inhibition.Rivaroxaban is an anticoagulant drug that suppresses the formation of blood clots and, consequently, atherosclerosis. This drug can bind the factor as a competitive inhibitor or antagonist of coagulation factor X. A high dosage can also lead to some types of haemorrhage (Bonaca et al., 2020). An *in silico* study has indicated that the drug can considerably bind both the main protease and S protein of the virus, causing COVID-19 (Omarjee et al., 2020). Also, a clinical study has reported that Rivaroxaban can effectively inhibit thrombotic events caused by COVID-19. Rivaroxaban is also a small drug Ticagrelor (Lopez et al., 2021), like Losartan, so it can interact with a few amino acids (Gly496 and Phe135 from RBD and GBD, respectively) of the targeted receptor.

This study introduced eight effective ligands from 89 commercial drugs to inhibit the S protein of SARS-CoV2. Ledipasvir and Losartan have shown the highest efficiency and inhibitory effects among these eight ligands. Ledipasvir is an antiviral drug with a higher potential effect on other viruses. There is also no such side effect derived from Ledipasvir. Losartan has also been studied *in vitro* with few side effects (Costantino et al., 2021) and functions as an angiotensin receptor antagonist. Ledipasvir has the best binding energy, although the binding energies of these 8 ligands are so close together. So, Ledipasvir could more effectively bind the sites, but Rivaroxaban was not more efficient than other ligands.

5. CONCLUSIONS

In the study, 89 FDA-approved medications were analyzed against the S protein of SARS-CoV2 by molecular docking. The binding energy of each ligand was measured when they were bond RBD and GBD of the receptor. As a result, Ledipasvir, Montelukast,

Domperidone, Aprepitant, Folate, Losartan, Ticagrelor and Rivaroxaban can firmly bind S protein. However, Ledipasvir and Losartan showed the most effective inhibitory effects against S protein. As the study is *in silico*, furhter stuies, such as *in vitro*, *in vivo* and clinical, are highly recommended.

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7. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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Annexure 1. All ligands used for molecular docking, along with Remedesevir (control).

No.	***Ligand***	***CID***	***No.***	***Ligand***	***CID***	***No.***	***Ligand***	***CID***
1	Rivaroxaban	9875401	31	Naproxen	156391	61	Esomeprazole	9568614
2	Sofosbuvir	45375808	32	Nortriptyline	4543	62	Lansoprazole	3883
3	Ledipasvir	67505836	33	Gemfibrozil	3463	63	Levofloxacin	149096
4	Gliclazide	3475	34	Fluoxetine	3386	64	Domperidone	3151
5	Linezolid	441401	35	Enalapril	5388962	65	Aprepitant	135413536
6	Temozolomide	5394	36	Gliclazide	3475	66	Caffeine	2519
7	Sitagliptin	4369359	37	Fluvoxamine	5324346	67	Valaciclovir	135398742
8	Dimethyl Fumarate	637568	38	Cetirizine	2678	68	Duloxetine	60835
9	Venlafaxine	5656	39	Fluoxetine	3386	69	Ibuprofen	3672
10	Valsartan	60846	40	Risperidone	5073	70	Phenylephrine	6041
11	Metformin	4091	41	Sotalol	5253	71	Solifenacin	154059
12	Escitalopram	146570	42	Carvedilol	2585	72	Rizatriptan	5078
13	Atorvastatin	60823	43	Chlordiazepoxide	2712	73	Rosuvastatin	446157
14	Clobazam	2789	44	Propranolol	4946	74	Folic acid	135398658
15	Citalopram	2771	45	Gemfibrozil	3463	75	Ferrous sulfate	24393
16	Lisinopril	5362119	46	Clotrimazole	2812	76	Hydrochlorothiazide	3639
17	Losartan	3961	47	Loratadine	3957	77	Pregabalin	5486971
18	Theophylline	2153	48	Lovastatin	53232	78	Ranitidine	3001055
19	Alendronate	2088	49	Metronidazole	4173	79	Apixaban	10182969
20	Ketotifen	3827	50	Naproxen	156391	80	Lacosamide	219078
21	Codeine	5284371	51	Gabapentin	3446	81	Levetiracetam	5284583
22	Acetaminophen	1983	52	Fexofenadine	3348	82	Lenalidomide	216326
23	Enalapril	5388962	53	Pantoprazole	4679	83	Teriflunomide	54684141
24	Iodoquinol	3728	54	Clopidogrel	60606	84	Abiraterone	132971
25	Diazepam	3016	55	Montelukast	5281040	85	Oxcarbazepine	34312
26	Sodium Valproate	16760703	56	Fenofibrate	3339	86	Ticagrelor	9871419
27	Alprazolam	2118	57	Pramipexole	119570	87	Desloratadine	124087
28	Bupropion	444	58	Zonisamide	5734	88	Everolimus	6442177
29	Sertraline	68617	59	Ropinirole	5095	89	Favipiravir	492405
30	Lorazepam	3958	60	Risedronate	5245	90	Remdesivir (Control)	121304016

Annexure 2. The binding energy and hydrogen bonds of the effective ligand-protein interaction and involved amino acids.

No.	***Ligand***	***Binding domain***	***Binding Energy (kcal/mol)***	***Amino acids***	***Hydrogenic Bonds (Amin oacid [Length (A°)])***
1	Ledipasvir	RBD	-6.4	Tyr449, Gly496	Lys444[2.13], Gly496[2.13], Tyr495[2.35]
		GBD	-8.2	Phe135, Asn137	Leu141[2.16], Phe135[2.26]
2	Montelukast	RBD	-6.5	Tyr449, Gly496	Gly447[2.28], Gly496[2.07], Arg403[1.08]
		GBD	-8	Asn137	Arg102[2.25], Ile105[1.89], Asn137[2.36]
3	Domperidone	RBD	-6.5	Tyr449, Gly496	Gln493[2.37], Gly496[2.41/2.19]
		GBD	-7.9	Asn137	Pro139[2.24], Lys103[1.86]
4	Aprepitant	RBD	-6.1	Tyr449, Gly496	Tyr505[2.31/2.31], Gly496[2.32]
		GBD	-7.8	Asn137	Gly133[1.88], Cys136[2.13]
5	Folic acid	RBD	-6.5	Gly496, Thr500	Tyr495[2.25], Arg403[2.44], Asn501[2.17]
		GBD	-7.6	Phe135, Asn137	Arg102[2.35], Ly103[2.01], Leu110[2.36], Asn137[2.31/2.25]
6	Losartan	RBD	-6.2	Gly496	Gln493[2.15], Ser494[1.93], Gly496[2.34]
		GBD	-7.4	Asn137	Ile119[1.99], Trp104[1.86], Gly103[1.88]
7	Ticagrelor	RBD	-6.9	Tyr449, Gly496	Arg403[2.38], Tyr505[2.37], Phe497[1.99], Gly496[2.15]
		GBD	-7.3	Phe135, Asn137	Asn137[2.33]
8	Rivaroxaban	RBD	-5.8	Gly496	Tyr495[1.97], Arg403[2.37], Gln498[2.45], Gly447[2.22]
		GBD	-7.2	Phe135	Val120[1.88]
9	Remdesivir (control)	RBD	-5	Tyr449, Gly496	Gln498[2.32], Gly496[2.4/1.83], Tyr495[2.37], Arg403[2.47/2.41]
		GBD	-6.9	Cys136, Asn137	Asn137[2.4/1.96], Ile105[2.16], Arg102[2.6], Gly103[2.6]