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ORIGINAL ARTICLE/ ÖZGÜN MAKALE

VIRTUAL SCREENING AND MOLECULAR DOCKING ANALYSIS ON THREE SARS-COV-2 DRUG TARGETS BY MULTIPLE COMPUTATIONAL APPROACH

ÇOKLU HESAPLAMALI YAKLAŞIMLA ÜÇ SARS-COV-2 İLAÇ HEDEFLERİ ÜZERİNDE SANAL TARAMA VE MOLEKÜLER DOKİNG ANALİZİ

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ABSTRACT

Objective: *SARS-CoV-2 is a pandemic virus characterized by upper respiratory tract infection and can range from mild symptoms to severe complications. In this case, drug repurposing and computer-aided studies have become very important to find emergency solutions. In this study, drug-target interactions on three nonstructural protein structures of SARS-CoV-2 of 8820 drug candidates or drug molecules obtained from the DrugBank database were analyzed.*

Material and Method: *Comprehensive virtual screening and molecular docking studies from 8820 drug molecules or candidates obtained from the DrugBank database were performed on the RNA binding protein, 2'-O-methyltransferase, and endoribonuclease of SARS-CoV-2; and potential drug candidates were determined for each target. Virtual screening studies have been done with High-Throughput Virtual Screening (HTVS), Standard Precision (SP), Extra Precision (XP), and Molecular Mechanics Generalized Born Surface Area (MM-GBSA). Also, information about the clinical findings, transmission, pathogenesis, and treatment of SARS-CoV-2 has been given.*

Result and Discussion: *Drug-target interactions on three nonstructural protein structures of SARS-CoV-2 of 8820 drug candidates or drug molecules obtained from the DrugBank database were analyzed. Potential compound recommendations for each drug target were presented. Information was given about key amino acids where active sites of drug target proteins interact with ligands. This study is expected to be useful in target-based drug development studies on the proteins of SARS-CoV-2.*

Keywords: *SARS-CoV-2, molecular docking, virtual screening, DrugBank*

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ÖZ

Amaç: *SARS-CoV-2, üst solunum yolu enfeksiyonu ile karakterize pandemik bir virüstür ve hafif semptomlardan ciddi komplikasyonlara kadar gidebilmektedir. Bu durumda, ilaç yeniden kullanım ve bilgisayar destekli çalışmalar, acil çözümler bulmak için çok önemli hale geldi. Bu çalışmada, 8820 ilaç adayının SARS-CoV-2'nin yapısal olmayan üç protein yapısı DrugBank veri tabanından elde edilen ilaç adayları veya ilaç molekülleri ile olan ilaç-hedef etkileşimleri analiz edilmiştir.*

Gereç ve Yöntem: *DrugBank veri tabanından elde edilen 8820 ilaç molekülü veya ilaç adayının kapsamlı sanal tarama ve moleküler doking çalışmaları, SARS-CoV-2'nin RNA bağlayıcı proteini, 2'-O-metiltransferaz ve endoribonükleaz üzerinde gerçekleştirildi; ve her hedef için potansiyel ilaç adayları belirlendi. Yüksek Verimli Sanal Tarama (HTVS), Standard Precision (SP), Extra Precision (XP) ve Moleküler Mekanik Genelleştirilmiş Doğan Yüzey Alanı (MM-GBSA) ile sanal tarama çalışmaları yapılmıştır. Ayrıca SARS-CoV-2'nin klinik bulguları, bulaşması, patogenezi ve tedavisi hakkında bilgiler de verilmiştir.*

Sonuç ve Tartışma: *Her ilaç hedefi için potansiyel bileşik önerileri sunuldu. İlaç hedef proteinlerinin aktif bölgelerinin ligandlarla etkileşime girdiği kilit amino asitler hakkında bilgi verildi. Bu çalışmanın SARS-CoV-2 proteinleri üzerinde hedef bazlı ilaç geliştirme çalışmalarında faydalı olması beklenmektedir.*

Anahtar Kelimeler: *SARS-CoV-2, moleküler doking, sanal tarama, DrugBank*

INTRODUCTION

World-threatening coronavirus disease-2019 (COVID-19) is a respiratory disease caused by enveloped, positive-polarity, single-chain RNA betacoronavirus, also referred to as SARS-CoV-2 [1]. First, cases of pneumonia of unknown etiology were reported in November 2019 in Wuhan, Hubei Province, China [2]. On January 7, 2020, it was identified that the disease agent was a new coronavirus (2019-nCoV), which had not previously been detected in humans. The name of the disease was later named SARS-CoV-2 because of its close resemblance to SARS-CoV. The World Health Organization (WHO) declared the COVID-19 outbreak an international public health emergency on January 30, 2020, and identified it as a global pandemic on March 11, due to the incidence of COVID-19 in 113 countries outside China [3, 4]. However, much of what we can deduce about the biology of SARS-CoV-2 comes from previous studies on SARS-CoV. Based on these data, the molecular mechanisms underlying this virus's evolution, adaptation, and spread require urgent investigation. Difficulties in controlling COVID-19, the disease's incubation period is due to epidemiological parameters such as infected people becoming infectious and infecting others before they show any symptoms or realize they have the disease. The COVID-19 pandemic has had negative consequences in all areas of life (economy, education, health, social) [5].

Coronaviruses belong to the Coronaviridae family and Coronavirinae subfamily, respectively. This subfamily is divided into four types based on their evolutionary relationship and gene sequences: alphacoronavirus, betacoronavirus, gammacoronavirus, and deltacoronavirus. Alphacoronavirus and betacoronavirus species primarily infect mammals, gammacoronavirus, and deltacoronavirus predominantly birds [6]. Along with SARS-CoV-2, the number of coronavirus species causing infection

in humans has been seven. Among these viruses, HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1 circulate in the winter months, causing mild to moderate respiratory infections in adults and children. SARS-CoV and "Middle East respiratory syndrome; MERS"- After CoV, SARS-CoV-2 was the third β-coronavirus that caused severe acute respiratory infections [7].

SARS-CoV first appeared in Guangdong province of China in November 2002 and was recognized as a global threat by the World Health Organization (WHO) in March 2003. This epidemic continued until July 2003, with 8098 confirmed cases and 774 deaths (9.6%) in 26 countries [8]. MERS-CoV emerged in Saudi Arabia in September 2012, and 2494 cases and 858 deaths (35%) were confirmed by laboratory testing from 27 countries. The transmission of MERS-CoV from person to person was limited, and its clinical findings were similar to SARS [9]. Without a specific mortality rate (currently in the range of ~ 1-6%), COVID-19 is believed to be less lethal than SARS (~ 10%) or MERS (~ 35%); however, the number of breeding is estimated to be 2.0-6.5 higher than SARS and MERS [10]. However, the exact measurement of the mortality rate is possible only when the pandemic is over because the total mortality formula is valid only when all cases result.

The zoonotic source of SARS-CoV-2 has not been fully verified, but it is estimated that it is bats according to sequence analysis. A 96% similarity was reported between the gene sequence of the SARS-CoV-2 and the bat coronavirus in a study using the genetic sequencing method. Studies have shown that SARS-CoV-2 shares 79.5% of its genome with SARS-CoV, which is sufficient to be considered different from SARS-CoV [11]. After entering the host, it has been reported that SARS-CoV-2 remains in the respiratory system for several days, an asymptomatic period. Although the incubation period varies between 4.8 and 6.5 days in COVID-19, it is longer in some studies [12]. The virus has been found to last up to 14 days in severe cases and up to 8 days in non-serious cases [13]. It has been observed that the viral load profile peaked at the onset of symptoms and, therefore, can be easily transmitted at an early stage of infection. Severe cases have a high viral load and can potentially be used as markers of case severity and prognosis. The primary mode of transmission is respiratory droplets, and transmission rates seem similar for asymptomatic and symptomatic patients [14].

The size of coronaviruses showing pleomorphic or spherical morphological features varies between 80-220 nm. It has a large envelope of lipid structure with peplomer protrusions on it. Its genome, approximately 26-32 kb in length, surrounds a tubular nucleocapsid with helical symmetry. It has a positive sense of RNA as a 29.9 kb size genetic material that is roughly distributed between adenosines (30%), cytosines (18%), guanines (20%), and resting thymines (32%) [15]. The 5' end of the RNA genome is titled, and the 3' end is polyadenylated. The 5' end of the genome contains an untranslated region, and this replicase complex encodes a total of 27 proteins divided into structural and non-structural proteins (NSP). The structural and auxiliary proteins are encoded with a 10 kb genome

region close to the 3' end. The CoV genome consists of S (Spike), E (Envelope), M (Membrane), and N (Nucleocapsid) structural proteins that make up about 33% of the entire genome and are vital for the viral life cycle. The remaining 67% were distributed among genes encoding 16 different NSPs throughout the ORF [16]. They are involved in virus-cell receptor interactions during viral entry. Sequence studies have shown differences between SARS-CoV-2 and SARS-CoV, such as the absence of the 8a gene or changes in the number of base pairs in the 8b and 3c genes SARS-CoV-2 [17]. These proteins have been extensively researched to design new antiviral agents against COVID-19 because genome and 3D structures show that parent drug binding pockets are probably protected in SARS-CoV-2, SARS, and MERS. It has also been demonstrated that both SARS CoV-2 and SARS-CoV have similar receptor binding domains [18]. SARS CoV has 14 binding residues with the human angiotensin-converting enzyme-2 (ACE-2) receptor. In the new SARS-CoV-2, 8 of these, 14 were observed [11].

Coronaviruses bind to the host cells via the spike (S) protein on the outer surface and enter the cell. S protein recognizes the receptor in the target cell and regulates the virus's entry into the host cell. The virus's life cycle begins by binding the S protein to the angiotensin-converting enzyme 2 (ACE2) receptor on the host cell surface. After binding to the receptor, changing the S protein structure makes it easy for the virus to fuse into the cell and remove its sheath. Then, SARS-CoV-2 RNA is released inside the cell. The following steps are translating RNA into viral replicase polyproteins and cleavage into small pieces by viral proteinases. A series of mRNAs are produced as a result of continuous transcription by a polymerase chain reaction, and translation to viral proteins occurs. Viral proteins and the RNA genome join the virions inside the endoplasmic reticulum and Golgi body and are released out of the cell [19]. The S protein of SARS-CoV-2, a combination of bat SARS-CoV and an unknown β-CoV virus, contains a 3-dimensional receptor-binding domain (RBD) that protects the Van der Waals bonds [20, 21]. Lung epithelial cells are the primary target of the virus. 394 glutamine residues in the RBD region of SARS-CoV-2 are recognized by the lysine residue at the human ACE2 receptor [22].

Although currently, there are no FDA-approved antiviral drugs for the treatment of human coronavirus infections, including COVID-19; currently available inactive virus vaccine (Sinovac), non-replicating viral vector vaccine ChAdOx1 (AZD1222) (AstraZeneca/Oxford), RNA-based virus vaccine BNT162b2 (3LNP-mRNAs) (Pfizer/BioNTech), RNA-based virus vaccine mRNA-1273 (Moderna) vaccine available [23]. Various drug clinical trials are in progress to try possible therapies; the treatment focuses on alleviating symptoms, including a dry cough and fever [24]. Following this, antiviral efficiencies of several FDA-approved drugs have been reported, including Remdesivir and favipiravir, against a clinical isolate of SARS-CoV-2 *in vitro* showed potential inhibition at low-micromolar concentrations [25].

Remdesivir is an adenosine triphosphate analog first described in the literature in 2016 as a potential treatment for Ebola [26]. In 2017, its activity against the coronavirus family of viruses was also demonstrated [27]. Remdesivir is also being researched as a potential treatment for SARS-CoV-2, the coronavirus responsible for COVID-19. Remdesivir was granted an FDA Emergency Use Authorization on 1 May 2020. This is not the same as FDA approval but is currently in clinical trials in several countries as a therapeutic for COVID-19 infections [28]. Remdesivir, a phosphoramidite prodrug containing a 1′-cyano modification of the sugar, is converted into cells into an adenosine triphosphate analog, which was shown to be an inhibitor of the RdRps of SARS-CoV and SARS-CoV-2. Remdesivir triphosphate was incorporated with higher efficiency than ATP by coronavirus RdRps, leading to delayed termination of RNA synthesis, thereby overcoming excision by the viral exonuclease [23, 29].

Favipiravir is a modified pyrazine analog that was initially approved for therapeutic use in resistant cases of influenza. The antiviral targets RNA-dependent RNA polymerase (RdRp) enzymes necessary for the transcription and replication of viral genomes [30]. Not only does Favipiravir inhibits the replication of influenza A and B, and it may be an alternative option for influenza strains that are resistant to neuraminidase inhibitors [31]. Favipiravir has been investigated to treat life-threatening pathogens such as the Ebola virus, Lassa virus, and COVID-19. Favipiravir may have potential antiviral action on SARS-CoV-2, an RNA virus. The preliminary results from a total of 80 patients (including the experimental and control groups) indicated that Favipiravir had more potent antiviral action than lopinavir/ritonavir. No significant adverse reactions were noted in the Favipiravir treatment group [32, 33].

Molnupiravir (Lagevrio®) is an orally administered, small molecule, an antiviral prodrug that inhibits replication of RNA viruses through viral error induction. The drug was developed by Merck and Ridgeback Biotherapy for the prevention and treatment of COVID-19. Severe acute respiratory syndrome coronavirus 2, the causative virus of COVID-19, uses the RdRp complex for replication. Molnupiravir received its first UK approval on 4 November 2021 to treat mild to moderate COVID-19 in adults with a positive SARS-CoV-2 diagnostic test and at least one risk factor for developing a serious illness. The recommended dose of molnupiravir is 800 mg every 12 hours for five days. It should be administered as soon as possible after the diagnosis of COVID-19 and within five days of the onset of symptoms. Molnupiravir has been filed for approval for the treatment of COVID-19 in many countries, including those in the USA, Japan, and the EU, and is authorized for emergency use. In addition, a multinational phase III study is evaluating molnupiravir for post-exposure prophylaxis against COVID-19 [34, 35].

Especially, SARS-CoV-2 proteins can be targeted to interrupt their life cycle [36]. In this article, we aim to perform the discovery of the potential candidates against SARS-CoV-2 through virtual screening of 8820 drug molecules and candidates and build a focused library of novel potential compounds.

MATERIAL AND METHOD

Molecular docking studies and other calculations were carried out with the Schrödinger Maestro module. Firstly, NSP9 replicase (PDB: 6W4B), NSP15 endoribonuclease (PDB: 6VWW), NSP16 2'-O-methyltransferase (PDB: 6W75) crystal structures were imported from the protein data bank (http://www.rcsb.org/pdb/) into the 'Protein Preparation Wizard' module. Hydrogens were added, non-bonding command with metals, the formation of disulfide bonds, deletion of water at 5 Å distance from het groups, and preprocess by creating pH: 7.00±2.00 het states using Epik. Subsequently, the appropriate chain was selected. Water molecules and metals contained in protein crystal structures were retained, and molecules other than the protein structure were deleted. pH: 7.00±2.00 regenerate state was created with S2. Finally, H bond determination was optimized using PROPKA pH: 7.00 with water sample orientation, and protein was prepared by minimizing OPLS3 force fields. The 8820 drug molecules and candidates from DrugBank Release Version 5.1.7 database (https://www.DrugBank.ca/releases/latest#structures) were downloaded in 3D SDF file format, and data was entered into the 'Ligprep' module. The OPLS3 force field was preferred, and ionization was carried out using Epik in the range of possible pH: 7.00±2.00; desalinated and tautomer-formed ligand structures were prepared. Data from PDB and scientific literature determined the active site of the SARS-CoV-2 protein structures. Grid determination was run by clicking any atom of the ligand or residue, and the default box was prepared by the 'Receptor Grid Generation' module.

Finally, for the calculation of theoretical ligand-protein interactions, virtual screening was performed with High Throughput Virtual Screening (HTVS), Standard Precision (SP), and Extra Precision (XP) by using flexible ligand options of the 'Glide Ligand Docking' module. Calculation of Molecular Mechanics Generalized Born Surface Area (MM-GBSA) interaction energies was performed to improve docking scores and detect protein-ligand interactions. Results of the Docking score, Glide score, Glide emodel, and MM-GBSA dG Bind was evaluated, and 2D/3D interactions of ligand and protein were determined and exhibited.

RESULT AND DISCUSSION

NSP9 RNA Binding Protein

RNA replicase, as NSP9 is reported as a single-stranded RNA-binding protein, has a dimeric structure [37]. Although the mechanism of binding to nucleic acid and dimerization of this enzyme remains uncertain, it is thought to be located in the Coronavirus RNA replication complex [38]. The dimeric structure of NSP 9 is essential for the pathogenicity of the virus. Thereby, the disruption of the coronaviruses' dimeric structure will be an essential factor in antiviral treatment targets against this structure [39]. The fourth study was performed on the crystal structure of COVID-19 Nsp9 RNA binding

protein (PDB Code: 6W4B). According to the estimated free binding energies, some evaluated drugs could act as SARS-CoV-2 NSP-9 inhibitors (Table 1). In Figure 1, the binding pose of the compound DB04158 in the RNA binding protein active site was shown.

Table 1. The highest interacting compounds in the RNA binding protein active site of SARS-CoV-2 (PDB: 6W4B)

DrugBank ID	Docking score	Glide gscore	Glide emodel	MMGBSA (dG Bind)
DB01661	-10.368	-10.368	-80.994	-37.05
DB02338	-9.724	-9.724	-89.830	-39.94
DB02319	-9.346	-9.346	-89.717	-40.93
DB13755	-9.169	-9.169	-80.215	-94.13
DB03186	-9.048	-9.048	-88.754	-55.64
DB03962	-8.895	-8.895	-79.137	-66.94
DB02498	-8.551	-8.551	-78.372	-47.89
DB01906	-8.517	-8.517	-53.004	-45.71
DB01362	-8.437	-8.437	-64.659	-81.89
DB04983	-8.330	-8.330	-85.622	-45.75
DB04158	-8.044	-8.044	-81.708	-63.06

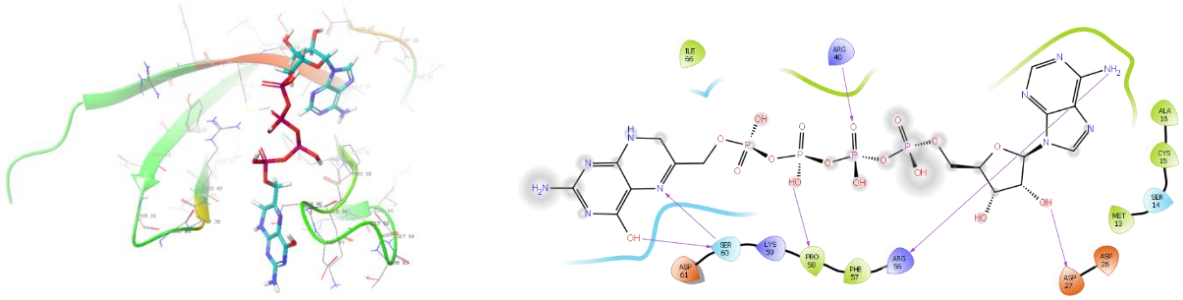

Figure 1. Interaction of SARS-CoV-2 with 6-(adenosine tetraphosphate-methyl)-7,8-dihydropterin [DB04158] in the RNA binding protein active site

CYS74, LEU107, LEU113, 199 ALA108, LEU5, ASN34, LEU98, ASN96, LEU98, PHE41, THR36, ALA9, LEU104, VAL8, 200 ALA108, ASN99, and SER6, are active site residues of essential NSP9 RNA binding protein.

NADPH [DB02338] is the reduced form of NADP+, used in anabolic reactions, such as lipid and nucleic acid synthesis, which require NADPH as a reducing agent. It has a role as a major metabolite and a cofactor. It is a NAD(P)H and an NADP. It is the conjugate acid of an NADPH. Nicotinamide adenine dinucleotide phosphate. A coenzyme composed of ribosylnicotinamide 5'-phosphate (NMN)

coupled with pyrophosphate linkage to the 5'-phosphate adenosine 2',5'-bisphosphate. It is an electron carrier in several reactions, alternately oxidized (NADP+) and reduced (NADPH). Thrombotic complications of COVID-19 may reflect an upregulation of endothelial tissue factor expression that is contingent on the activation of endosomal NADPH oxidase. When single-stranded RNA viruses are taken up into cellular endosomes, they stimulate endosomal formation and activation of NADPH oxidase complexes via RNA-responsive toll-like receptor 7. Therefore, it is proposed that SARS-CoV-2 infection of endothelial cells evokes TF expression, which is contingent on endosomal NADPH oxidase activation [40]. 5,6-Dihydroxy-NADP [DB02319] belongs to the class of organic compounds known as purine nucleotide sugars. These are purine nucleotides bound to a saccharide derivative through the terminal phosphate group. Locarmic Acid [DB13755] is formed when a bridge of adipic acid links two molecules of iothalamic acid (Conray) deprived of an acetyl group. It has been called 'Conray Dimer,' but it is not strictly a polymer as it is not composed only of 2 molecules of Conray but has the additional adipic acid. It is a dibasic acid and therefore combines with two cations. Locarmic acid is a molecule used as a contrast medium. It is one of the biologically active dicarboxylic acid compounds. It has side effects such as hypermotility, diarrhea, and convulsions [41].

Adenylate-3'-phosphate-[[2'-deoxy-uridine-5'-phosphate]-3'-phosphate] [DB03186] belongs to the class of organic compounds known as (3'->5')-dinucleotides. These are dinucleotides where the two bases are connected via a (3'->5')-phosphodiester linkage. Endonuclease catalyzes the cleavage of RNA on the 3' side of pyrimidine nucleotides. Acts on single-stranded and double-stranded RNA. Nicotinamide 8-bromo-adenine dinucleotide phosphate [DB03962] belongs to the class of organic compounds known as (5'->5')-dinucleotides. These are dinucleotides where the two bases are connected via a (5'->5')-phosphodiester linkage. Carba-nicotinamide-adenine-dinucleotide [DB02498] may serve as a stable cofactor for the enzyme-based detection of glucose [42]. Iohexol [DB01362] is an effective non-ionic, water-soluble contrast agent used in myelography, arthrography, nephroangiography arteriography, and other radiographic procedures. Its low systemic toxicity is the combined result of low chemotoxicity and low osmolality. Organic iodine compounds block x-rays as they pass through the body, thereby allowing body structures containing iodine to be delineated in contrast to those that do not contain iodine. The degree of opacity produced by these compounds is directly proportional to the total amount (concentration and volume) of the iodinated contrast agent in the path of the x-rays. After intrathecal administration into the subarachnoid space, diffusion of iohexol in the CSF allows the visualization of the subarachnoid spaces of the head and spinal canal. After intravascular administration, iohexol makes opaque those vessels in its path of flow, allowing visualization of the internal structures until significant hemodilution occurs (Figure 2). Also, iohexol has been used for GFR measurements in pediatric, elderly, and Type I and II diabetic populations [43].

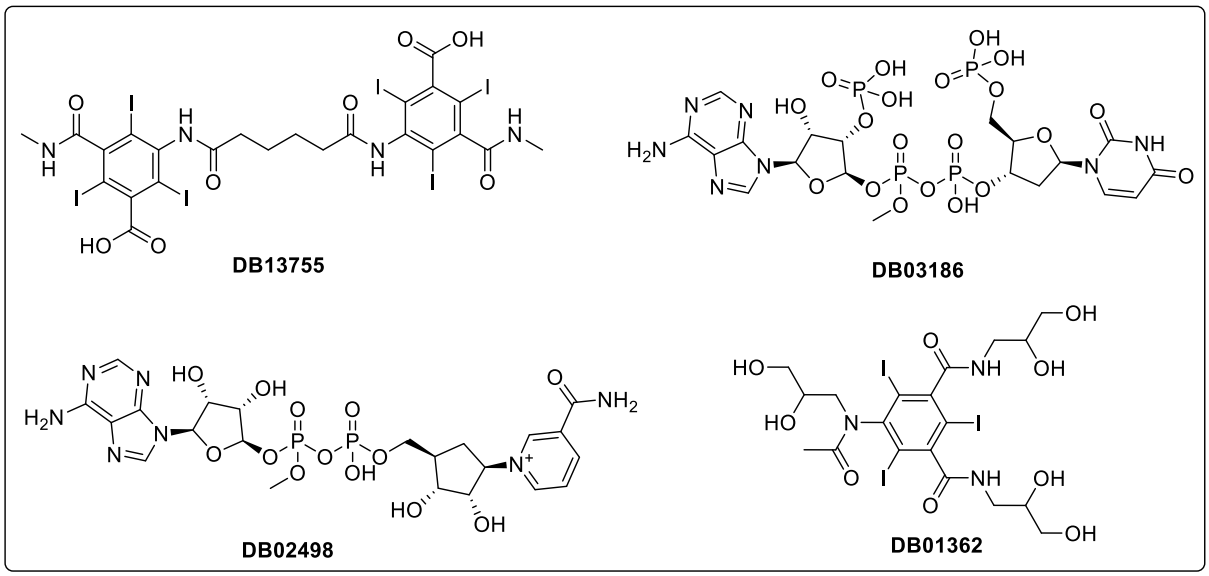

Figure 2. Potential SARS-CoV-2 RNA binding protein inhibitor compounds from the DrugBank database

NSP16 2'-O-methyltransferase

NSP16 in Coronaviruses performs methylation of the cap of the C2'-hydroxyl group of the following nucleotide. This is a necessary process for the virus's lifecycle [44]. NSP10 as a cofactor enhances the activity of NSP16. Although the mechanism of enzymatic action of NSP10 and NSP16 is not known, they have good potential for antiviral activity studies in medicinal chemistry [45]. Because the inhibition of the activity of the O-methyl-transferase can cause a reduction of viral replication and pathogenicity of coronaviruses, it is reported that the interaction of these NSPs may be deactivated by the short peptides derived from NSP10, and this is caused by O-methyl-transferase inhibition [46]. The fifth study was performed on the 1.95 Å resolution crystal structure of the NSP10 - NSP16 complex (PDB Code: 6W75). According to the estimated free binding energies, some evaluated drugs could act as SARS-CoV-2 NSP10-NSP16 inhibitors (Table 2). In Figure 3, the binding pose at the 2'-O-methyltransferase active site of the compound DB03883 was shown.

Important active site amino acids for 2'-O-methyltransferase are CYS6913, ASP6912, ASP6897, GLY6879, ASN6841, ASP6928, GLY6869, LEU6898, and MET6929.

Adenosine-5'-[Beta, Gamma-Methylene]Triphosphate [DB03909] belongs to the class of organic compounds known as purine ribonucleoside monophosphates. These are nucleotides consisting of a purine base linked to a ribose to which one monophosphate group is attached. Adenosine 5'-methylenediphosphate [DB03148] belongs to the class of organic compounds known as purine ribonucleoside monophosphates, like the previous molecule. Adenosine phosphate [DB00131] is the adenine nucleotide containing a phosphate group esterified to the sugar moiety at the 2'-, 3'- or 5'-

position and is also known as adenylic acid. Adenosine monophosphate, AMP, is a nucleotide that is found in RNA. It is an ester of phosphoric acid with the nucleoside adenosine. AMP consists of the phosphate group, the pentose sugar ribose, and the nucleobase adenine. AMP is used as a dietary supplement to boost immune activity and is also used as nutritional supplementation, even for treating dietary shortages or imbalance. NAD, NADP, and the adenylates (i.e., ATP, ADP, and AMP) are the primary regulatory components for any living organism. NAD and NADP are cofactors that act as primary electron carriers in cells. They are reduced by proteins during organic or inorganic matter oxidation and oxidized by proteins involved in respiratory or anabolic processes (Figure 4) [47]. Additionally, according to our study, it was seen that Denufosol [DB04983] could be one of the target molecules for NSP10 - NSP16 as well as NSP-9.

Table 2. The highest interacting compounds in the 2'-O-methyltransferase active site of SARS-CoV-2 (PDB: 6W75)

DrugBank ID	Docking score	Glide gscore	Glide emodel	MMGBSA (dG Bind)
DB03909	-16.385	-16.385	-149.900	-90.39
DB03148	-14.124	-14.124	-140.407	-82.91
DB03883	-13.968	-13.968	-106.783	-74.04
DB03708	-13.587	-13.587	-134.589	-84.40
DB02902	-13.467	-13.467	-127.326	-85.23
DB04983	-13.350	-13.350	-111.547	-57.35
DB03458	-13.349	-13.349	-131.126	-86.46
DB04366	-13.332	-13.332	-132.399	-82.68
DB03464	-13.213	-13.213	-104.830	-69.22
DB00131	-13.115	-13.115	-108.655	-60.46

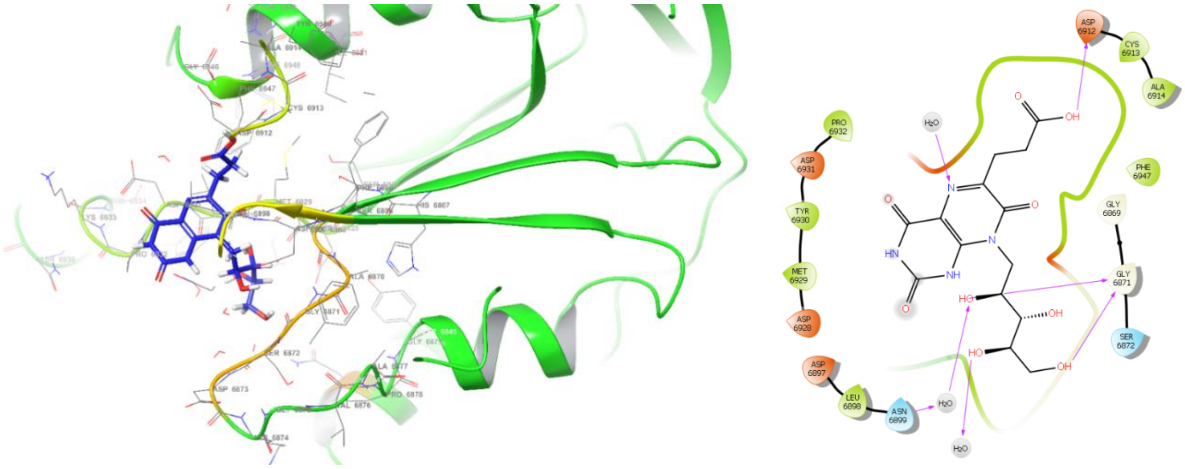

Figure 3. Interaction of SARS-CoV-2 with Carboxyethyllumazine [DB03883] in the 2'-O-methyltransferase active site

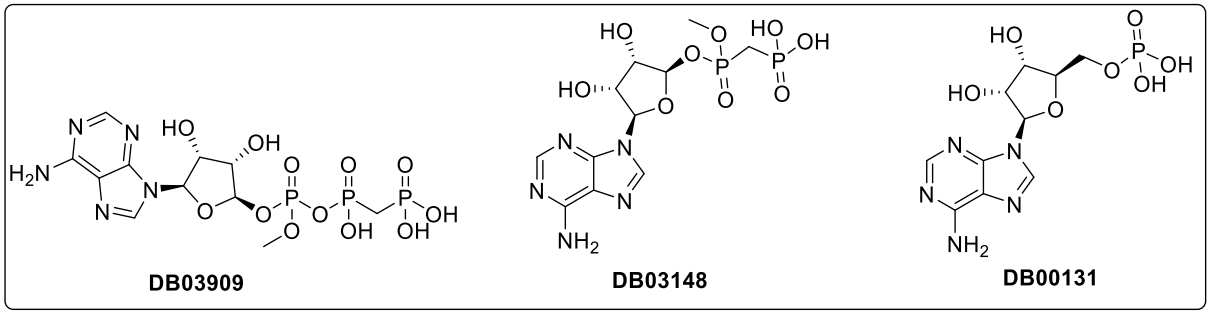

Figure 4. Potential SARS-CoV-2-2'-O-methyltransferase inhibitor compounds from the DrugBank database.

NSP15 Endoribonuclease

NSP15, as nidoviral uridylate specific endoribonuclease (NendoU), is a member of the EndoU family that plays a role in viral RNA processes. Enzymatic activity of this protein is reported to be increased with Mn^{+2} [48]. This protein is conserved in nidoviruses (coronaviruses and arteriviruses), and its function is to cleave 3' ends of uridylates of RNA molecules [49]. Although the presence of NendoU in the viral replication is essential, coronaviruses that do not have this protein are reported to be replicated. The ninth study was performed on the structure of the COVID-19 NSP15 endoribonuclease (PDB Code: 6VWW). According to the estimated free binding energies, some evaluated drugs could act as SARS-CoV-2 NSP15 endoribonuclease inhibitors (Table 3). Figure 5 shows the binding pose at the endoribonuclease active site of DB04663.

Table 3. The highest interacting compounds in the NSP15 Endoribonuclease active site of SARS-CoV-2 (PDB: 6VWW).

DrugBank ID	Docking score	Glide gscore	Glide emodel	MMGBSA (dG Bind)
DB04663	-10.704	-10.704	-50.680	-43.84
DB01661	-10.483	-10.483	-97.611	-51.32
DB03797	-10.337	-10.337	-88.501	-64.81
DB02393	-10.208	-10.208	-51.170	-48.75
DB02738	-10.177	-10.177	-88.432	-55.18
DB03725	-10.125	-10.125	-78.298	-45.37
DB04023	-10.080	-10.080	-73.818	-64.10
DB02312	-10.065	-10.065	-50.544	-42.02
DB03161	-9.772	-9.772	-78.517	-57.45
DB03532	-9.723	-9.723	-83.812	-46.42

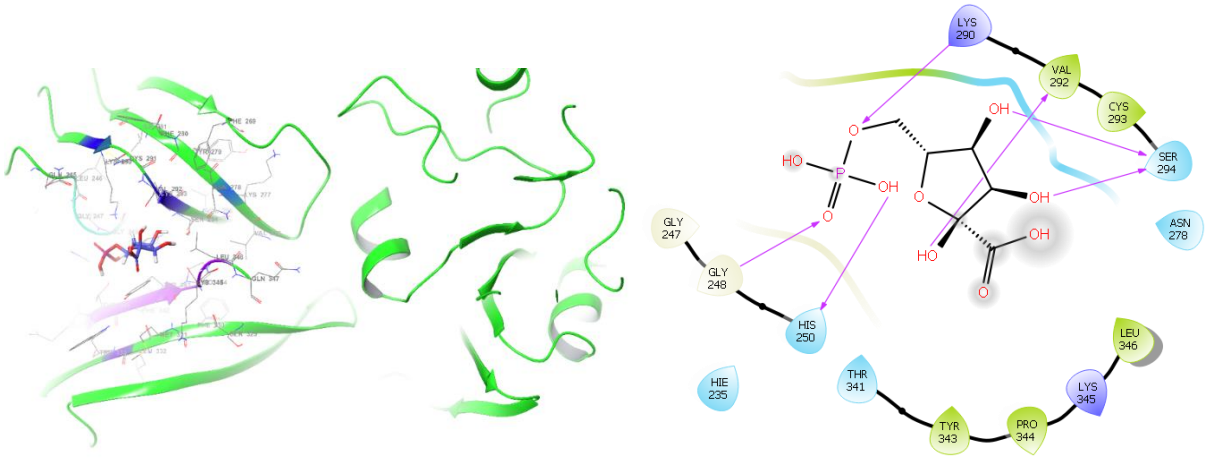

Figure 5. Interaction of SARS-CoV-2 with 2-Keto-6-Phosphate-D-Gluconic Acid [DB04663] in the endoribonuclease active site

ASN 278, THR 341, SER 294, GLN 245 HER 338, ALA 232, CYS 291, VAL 292, LYS 345, LYS 335, LYS 290, HIP 235, and GLN 245 contain important active site NSP15 Endoribonuclease residues.

2-Keto-6-Phosphate-D-Gluconic Acid, Alpha-Furanose Form [DB04663] belongs to the class of organic compounds known as pentose phosphates. These are carbohydrate derivatives containing a pentose substituted by one or more phosphate groups (Figure 6). According to our study, Adenosine-5'-Pentaphosphate [DB02738] and 1-(5-Phospho-D-ribosyl)-ATP [DB01661], described in other sections above, may be one of the target molecules for NSP15 endoribonuclease.

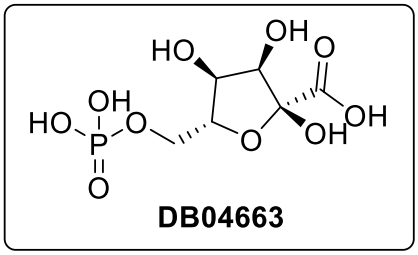

Figure 6. Potential SARS-CoV-2 endoribonuclease inhibitor compounds from the DrugBank database.

Consequently, in this study, drug-target interactions on three nonstructural protein structures of SARS-CoV-2 of 8820 drug candidates or drug molecules obtained from the DrugBank database were analyzed. Potential compound recommendations for each drug target were presented. Information was given about key amino acids where active sites of drug target proteins interact with ligands. The latest updated information on the clinical findings, transmission, pathogenesis, and treatment of SARS-CoV-

2 has also been presented. This study is expected to be useful in target-based drug development studies on the proteins of SARS-CoV-2.

AUTHOR CONTRIBUTIONS

Conception: *İ.Ç., M.E.;* Design: *İ.Ç., M.E.;* Supervision: *İ.Ç., M.E.;* Resources: *İ.Ç., M.E., E.U., U.İ.;* Materials: *İ.Ç., M.E.;* Data Collection and/or processing: *İ.Ç., M.E.;* Analysis and/or interpretation: *İ.Ç., M.E;* Literature search: *İ.Ç., M.E., E.U., U.İ.;* Writing manuscript: *İ.Ç., M.E., E.U., U.İ.;* Critical review: *İ.Ç., M.E., E.U., U.İ.;* Other: *E.U., U.İ.*

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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