Chapter

Evaluation of Drug Repositioning by Molecular Docking of Pharmaceutical Resources to Identification of Potential *SARS-CoV-2* Viral Inhibitors

Fatemeh Hosseini, Mehrdad Azin, Hamideh Ofoghi and Tahereh Alinejad

Abstract

Unfortunately, to date, there is no approved specific antiviral drug treatment against COVID-19. Due to the costly and time-consuming nature of the de novo drug discovery and development process, in recent days, the computational drug repositioning method has been highly regarded for accelerating the drug-discovery process. The selection of drug target molecule(s), preparation of an approved therapeutics agent library, and in silico evaluation of their affinity to the subjected target(s) are the main steps of a molecular docking-based drug repositioning process, which is the most common computational drug re-tasking process. In this chapter, after a review on origin, pathophysiology, molecular biology, and drug development strategies against COVID-19, recent advances, challenges as well as the future perspective of molecular docking-based drug repositioning for COVID-19 are discussed. Furthermore, as a case study, the molecular docking-based drug repurposing process was planned to screen the 3CLpro inhibitor(s) among the nine Food and Drug Administration (FDA)-approved antiviral protease inhibitors. The results demonstrated that Fosamprenavir had the highest binding affinity to 3CLpro and can be considered for more in silico, in vitro, and in vivo evaluations as an effective repurposed anti-COVID-19 drug.

Keywords: bioinformatics, protein–peptide interactions, biological targets, drug development, 3CLpro inhibitor, biological computation, drug design

1. Introduction

The Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (*SARS-CoV-2*), signifies a pandemic threat to international health, with so far nearly 5 million deaths worldwide [1]. Notwithstanding mass vaccination worldwide by emergency approved vaccines such as Pfizer-BioNTech, Janssen, and Moderna, COVID-19 still poses a threat to human health. Furthermore, with the emergence of new mutant strains of SARA-CoV-2 as well as

a significant decrease in the vaccine's efficacies, introducing of new treatment strategies is urgently needed. Therefore, recently many international efforts have been planned for introducing suitable vaccines as well as effective therapeutics [2, 3].

Generally, time is a vital factor in the pandemic condition, so that, rapid detection, vaccination, and treatment methods can significantly reduce mortality. De novo drug discovery and development for lesser-known diseases such as COVID-19 is costly and tedious. Consequently, alternative methods such as the computational drug repurposing approach can accelerate the discovery of new drugs. In this regard, several pipelines have been introduced for in silico drug repositioning against COVID-19. Lately, molecular docking as a popular bioinformatics method has been highly regarded as the core of the most drug repositioning process to achieve effective drug candidates to combat COVID-19 [4–6]. In this chapter, we discussed new advancements and challenges in drug repositioning by molecular docking of pharmaceutical resources to the identification of potential *SARS-CoV-2* viral inhibitors.

2. Origin and pathophysiology aspects of COVID-19

SARS-CoV-2 was firstly discovered in the Huanan Seafood Wholesale market in Wuhan, China on 12 December 2019 [7]. Subsequent to the extensive outbreak of the virus infection, on March 11, 2020, the World Health Organization (WHO) announced the COVID-19 pandemic. As of 27 August 2021, the total number of cases of *SARS-CoV-2* confirmed globally by WHO are 214,468,601 with 4,470,969 reported deaths (https://covid19.who.int/). As per the reports of WHO, the mortality rate of COVID-19 is around 3.7% [8]. Although the host of *SARS-CoV-2* is still indistinct, it is assumed the virus has bats or pangolins origin. However, the main theory suggests that the virus was transmitted to humans from an intermediate host. The virus is mainly transmitted among the individuals through droplet infection, contact routes, and rarely through the feces of the infected patients and mother to child post-childbirth. Fever, cough, fatigue, diarrhea, headache, hemoptysis, dyspnea, acute respiratory distress syndrome, cardiac injury, and lymphopenia are known clinical manifestations of COVID-19. COVID-19 infection can be divided into three phases including the virus replication and appearance of mild signs, the emergence of respiratory symptoms and simulation of the adaptive immune system responses, and the third phase causing hyper-inflammation. Expression of the ACE2 (angiotensin-converting enzyme 2) protein (as the major receptor molecule for the virus) by renal tubular cells, liver cells and testicular cells may the kidney, liver, and testicular tissue damages also observed in the COVID-19 patients [1, 9, 10].

3. Molecular biology of *SARS-CoV-2*

SARS-CoV-2 belongs to beta coronaviruses and has a round or elliptic form, with an approximate diameter of 60–140 nm. The virus genome is an around 30 Kb positive-sense, single-stranded RNA, which encodes four structural proteins including S protein (Spike), E protein (Envelope), M protein (Membrane), and N protein (Nucleocapsid), and several accessory proteins or nonstructural proteins, namely, NSP1 to NSP16 [11]. S protein is 150 kDa, acts as an anchor on the virus envelope, and consists of three domains including the outer N-terminal domain having unit S1 and S2, a cytoplasmic C-terminal domain, and a transmembrane domain. M protein is 25–35 kDa, a transmembrane glycoprotein type III and the most abundant protein on the surface of the virus. Based on the bioinformatics analysis, the protein can play a role in the virus entry into the host cell and its RNA maturation.

Protein name	Length (amino acid)	Role	References
NSP1	180	Host translation inhibitor and also degrade host mRNAs	[1]
NSP2	638	Binds to prohibitin 1 and prohibitin 2	[2]
NSP3	1945	Responsible for release of NSP1, NSP2, and NSP3	[3]
NSP4	500	Viral replication-transcription	[4]
NSP5	306	Cleaves at multiple distinct sites to yield mature	[5]
NSP6	290	Induces formation of ER-derived autophagosomes	[6]
NSP7	83	Forms complex with NSP8 and NSP12 to yield the RNA polymerase activity of NSP8	[7]
NSP8	198	Makes heterodimer with NSP8	[8]
NSP9	198	bind to helicase	[5]
NSP10	139	Unknown	[5]
NSP11	13	Unknown	[5]
NSP12	932	Replication and methylation	[9]
NSP13	932	A helicase core domain	[10]
NSP14	527	Exoribonuclease activity a	[5]
NSP15	346	Mn(2 +)-dependent endoribonuclease activity	[5]
NSP16	298	Methyltransferase	[11]

The role(s) of NSP10 and NSP11 is(are) still not well understood.

Table 1.
Description of various roles of non-structural proteins from SARS-CoV-2.

N protein is a 43–50 kDa nucleocapsid structural protein and has a vital role in attaching and assembling the virus genome to the matrix of the ribonucleoprotein. The E protein is 8.4–109 kDa and is recognized as a small hydrophobic protein. The protein contributes to viroporin activity, virus assembling, and the virus budding process. Based on the results of several studies, the nonstructural proteins encoded by genes positioned within the 5′-region of the virus genome, have a wide range of roles from host translation inhibition by NS1 to viral replication-transcription NS4 [12, 13]. The main roles of the known nonstructural proteins of *SARS-CoV-2* are summarized in **Table 1**.

4. Antiviral molecular targets and drug development strategies against COVID-19

Generally, a probable antiviral drug target is a molecule (often a protein) with a vital role in the life cycle of the planned virus [14, 15]. Accordingly, to date, several structural and accessory proteins from *SARS-CoV-2* have been subjected to the drug-discovery process. Consistent with the approved information about the *SARS-CoV-2* life cycle, eight steps including virus binding, fusion to host cell, RNA release, translation, proteolysis, replication and translation, viral assembly, and release could be planned to investigate potential anti-COVID-19 drugs. Among the mentioned steps, virus attachment and entry, proteolysis, and replication have received more attention due to more available data about the key proteins in the steps as well as the high similarity of these steps between coronaviruses [16, 17].

In the following sections, the key steps in *SARS-CoV-2* life cycle are discussed in the light of drug development against COVID-19.

4.1 Virus attachment and entry

The trimeric *SARS-CoV-2* spike glycoprotein has a crucial role in the virus attachment and entry. The glycoprotein constituent monomer comprises two subunits, S1 and S2. The S1 encompasses the N-terminal domain (NTD) and the RBD, which is accountable for interacting with ACE2. Therefore, RDB is considered an effective drug target for discovering therapeutic agents such as neutralizing antibodies [18]. In **Table 2**, some anti-RBD antibodies are listed. The results of some studies demonstrated potent therapeutic and prophylactic abilities of anti-RDB antibodies in cell culture or animal model systems. In this regard, Gao et al. demonstrated that a potent COVID-19 antibody, BD-368-2 has significant prophylactic effectiveness in *SARS-CoV-2*-infected hACE2 mice at a dose of 20 mg/kg [24]. Similarly, another study confirmed both prophylactic and treatment activities of CB6 antibody in a dose of 50 mg/kg [25]. The ability of COV2-2130 to reduce the viral burden and levels of inflammation has also been approved [26]. Furthermore, besides the introduced antibodies, several small molecules such as salvianolic acid, arbidol, dri-c23041, cepharanthine, abemaciclib, osimertinib, trimipramine, colforsin, ingenol, and clofazimine have also been considered for in vitro evaluation of their *SARS-CoV-2* entry inhibition activities [27].

4.2 Virus genome replication

Generally, the virus replication directly affects the viral burden and symptom severity in viral infections. Therefore, targeting the key molecules in the *SARS-CoV-2* replication has been highly regarded for drug discovery against COVID-19. Previous studies confirmed that 3-chymotrypsin-like cysteine protease (3CLpro), papain-like protease (PLpro), RNA-dependent RNA polymerase (RdRp), and NSPs involved in the formation of double-membrane vesicles (DMVs) are vital for the replication of *SARS-CoV-2* [28]. Among the mentioned proteins, the 3CLpro is highly regarded as an attractive target for drug development against *SARS-CoV-2* because of its key role in the viral life cycle alongside the absence of closely related homologs in humans. Subsequently, to date, several

Name	EC50 (ng/ml)	References
BD-368-2	15	[12]
CB6	36	[13]
H4	896	[19]
P2B-2F6	410	[20]
B38	177	[19]
COV2-2196	15	[21]
COV2-2130	107	[21]
COV2-2165	332	[21]
CC12.1	22	[22]
C121	1.64	[23]

Table 2.
List of some neutralizing monoclonal antibodies against SARS-CoV-2 S1.

efforts have been made to identify the effective *SARS-CoV-2* 3CLpro inhibitors. The 3CLpro inhibitors are mostly categorized into peptidic and small molecules. Up to now, the efficacies of several 3CLpro peptide inhibitors such as N3, 13b, GC373, and GC376 have been validated. Moreover, some small molecules such as disulfiram, carmofur, ebselen, and tideglusib are known to inhibit 3CLpro from *SARS-CoV-2* [27, 29].

5. Current in use anti-COVID-19 treatments

Unfortunately, to date, there is no specific anti-COVID-19 drug. However, the results of some studies suggested that other anti-viral medicines could be repurposed as effective anti-COVID-19 drugs. Remdesivir, an FDA-approved repurposed antiviral drug, is only in used approved anti-viral therapy against COVID-19 [30]. However, other anti-viral and non-antiviral drugs have also been used for studying their anti-COVID-19 activities. Hydroxychloroquine, an anti-malaria drug with polymerase inhibitory activity, was the first repurposed drug against COVID-19, which was supported by some in vitro effectiveness evidence. However, further clinical trials indicate that there is no association between hydroxychloroquine administration and reduction in the death rate due to COVID-19. Kaletra (a brand name of lopinavir/ritonavir complex) is an approved anti-human immunodeficiency virus (HIV) protease inhibitor, which empirically evaluated for 3CLpro inhibitory activities. Despite, promising in vitro results, clinical trials have not confirmed the significant efficacy of Kaletra in individuals hospitalized with COVID-19. Favipiravir, a purine nucleic acid analog, is another anti-viral drug that is repurposed against mild to moderate COVID-19. The results of clinical trials suggest that Favipiravir has no significant beneficial effect on the mortality rate in patients with COVID-19. Additionally, some other drugs such as colchicine, oseltamivir, ivermectin, tocilizumab, nafamostat, camostat, famotidine, umifenovir nitazoxanide are under evaluation for investigating their probable anti-COVID-19 activities [31–33].

6. Computational drug repositioning

Because of the costly, time-consuming, and complexity of De novo drug discovery, until now all proposed anti-COVID-19 drug candidates are repurposed drugs. Drug repurposing also known as drug re-tasking is a procedure of recognizing new therapeutic application(s) for previously approved, failed, investigational, and or already marketed drugs. Naturally, the drug-repurposing process is based on two fundamental principles including interdependence between different diseases and the confounding nature of drugs. Therefore, drug-repositioning approaches could be categorized into drug-based and disease-based strategies.

The drug-based strategies are vastly based on drug-related data and are used for better understanding the role of pharmacological properties and defining the possibility of defining new pharmaceutical capabilities. Despite the advantages of experimental drug repositioning, the fact that it was time consuming still remained as the main limitation for drug discovery, especially in a pandemic condition. Furthermore, conventional methods use small datasets and biological networks, which may lead to unreliable discoveries.

Nowadays, different computational methods have been introduced that can accelerate the drug-repositioning process [27]. In the next sections, the most common computational approaches for drug repositioning are propounded.

6.1 Molecular target identification and validation in the drug-repositioning process

In a drug discovery project, target identification and validation are key steps that directly affect drug efficacy, as well as probable side effect(s). Theoretically, a drug target molecule can be selected among a wide range of biological entities including proteins, genes, and RNAs. However, an ideal drug target molecule should be drug accessible, efficacious, safe, and meet clinical and commercial requirements [4]. Target identification can be performed by different tools such as analysis of gene modifications, protein overexpression, signaling pathways, protein interactions, and recent bioinformatics evaluations. Regarding antiviral drug discovery, different targets such as envelop proteins, S-adenosyl-L-homocysteine hydrolase, orotidine 5′-phosphate decarboxylase, cytidine triphosphate synthetase, inosine monophosphate dehydrogenase, and DNA/RNA polymerase have been investigated for discovering effective antiviral drugs [34–37]. The identified target molecules can be validated by knocking in/down/out the genes, monoclonal antibodies, and chemical genomics [4, 38]. As mentioned, recently bioinformatics methods, such as ligand-based interaction fingerprint (LIFt), protein-ligand interaction fingerprints (PLIF), and network-based drug discovery, have successfully been used for drug target identification [39].

6.2 Data mining

There are now a large number of diseases- and drugs-linked information such as gene sequences, protein–protein interactions, and drug–protein interactions with increasing rapid growth, which needs effective approaches to quick access and analysis of hidden information. Commonly, text mining is the most applicable method in the majority of data mining–related studies. In the field of computational drug repurposing, text mining has been used to find the gene, drug, and diseases-related data and then categorize the relevant entities. Regarding drug repurposing, text mining has successfully been used in several studies [40, 41]. Brown et al. suggested an online text-mining server with the ability to drug clustering based on the similarity of their physicochemical properties [42]. A text mining-based tool was also introduced by Leaman et al. for identifying disease-related information mentioned in the literature [43]. In another study, Papanikolaou et al. used text mining to recognize biological entities in the Drug Bank database. The retrieved data were then clustered by different algorithms and used for obtaining novel drug–drug relations [44].

6.3 Machine learning (ML)

Machine learning, a crucial subset of artificial intelligence (AI), has been combined into many fields, such as data generation and analytics. Related to drug discovery, ML algorithms may participate in target and lead discovery as well as develop quantitative structure–activity relationships. Briefly, in machine learning-based drug repositioning, different algorithms, such as artificial neural networks (ANNs), support vector machines (SVMs), and random forest (RF), were trained by numerical forms of different features of drugs, diseases, genes, and so on. The trained algorithms can then predict the drug ability of unknown compounds [45]. In this regard, Gottlieb et al. used drug–drug and disease–disease similarity events as grouping features for training a logistic regression classifier and prediction of drug-disease associations [46]. Similarly, Napolitano et al. introduced a SVM model trained by drug-related similarities with the ability to forecast the therapeutic class of United States Food and Drug Administration (FDA)-approved compounds [47]. Aliper et al. introduced a fully connected deep neural network algorithm trained

by gene expression signatures for predicting therapeutic potentials and new drug suggestions [48].

6.4 Network analysis

Biological networks, an outstanding way of modeling biological entities and their interactions, can supply significant insight into the mechanism action of drugs and drug targets and symptoms of diseases. The models can be used to determine informative associations between genes, chemicals, proteins, phenotypes, and any other biological entities by statistical analysis, computational models, and leveraging graph theory concepts. Based on the data sources, network analysis can be classified into metabolic networks, protein–protein interaction networks, drug–drug interaction networks, drug-side effect association networks, disease–disease interaction networks, and gene regulatory networks. Consequently, bionetworks and their analysis can be used to identify potential therapeutic agents and drug repositioning [49–51].

6.5 Molecular docking

Studying the ligand-protein interactions at the molecular level has a crucial role in pharmaceutical research. Therefore, the scientific community focused on the exploration of the binding phenomenon over the years. Accordingly, some theories, such as lock and key hypothesis, induced-fit theory, and conformational selection were introduced for the interpretation of ligand–protein interactions [52]. Historically, the refinement of a complex structure by optimization of the separation between the partners was the first description of the docking term in 1970. Molecular docking was first being developed in 1980 to predict the best matching binding mode and the molecular interactions of a ligand to a macromolecular partner through the generation of a number of probable orientations of the ligand inside the protein cavity. The method comprises two interrelated steps including orientations sampling and a scoring function, which are responsible for reproducing experimental binding mode and ranking of prepared complexes [52, 53]. Molecular docking can classify into rigid, semi-flexible, and flexible types, according to the degrees of flexibility of the ligand and receptor. In the rigid docking-like to lock-key theory, both ligand and protein are considered rigid entities and hence, there is no internal degree of freedom. Semi-flexible docking is a molecular docking simulation with flexible ligand and rigid receptors. Thus, all degrees of freedom of ligand are explored. Recently, several online and standalone software such as AutoDock, AutoDock Vina, Molegro Virtual Docker, Gold, Surflex-Dock, GLIDE, FlexX, DOCK, FRED, and so on, have been developed for computing different types of molecular docking. Most available software for molecular docking uses flexible ligands and several are trying to model flexible receptor proteins. In recent years, with promising advancements in optimization and the development of new molecular docking algorithms, numerous publications have been planned for comparing the performance of different molecular docking tools. However, it should be stressed that comparison between molecular docking methods is problematic, due to the dependance on docking performance with classes of the subjected targets. The ability of molecular docking methods to reveal the possibility of enzymatic reactions is a compelling reason for various applications related to computational drug design and repurposing, hit identification, lead optimization, binding site prediction, mechanisms of enzymatic reactions, and protein engineering [54–56]. Since the emergence of COVID-19, several molecular docking-based studies [57–62] have been planned to introduce effective anti-COVID-19 drugs by means of drug repositioning. In **Figure 1**, the main steps of a molecular docking-based drug repurposing study are represented.

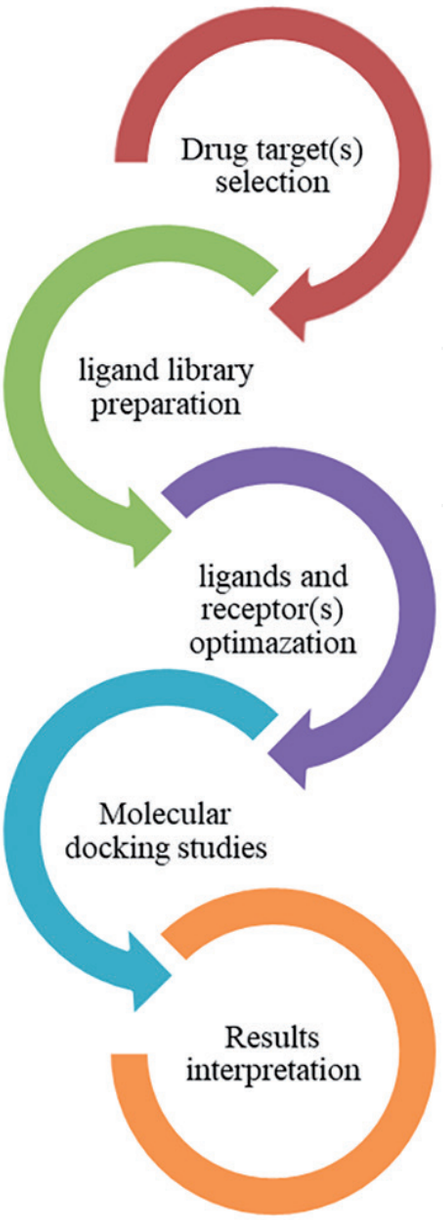

Figure 1.
Schematic representation of the main steps of a molecular docking-based drug repositioning process. Target identification, ligand preparation, and results interpretation are the three main steps.

6.5.1 Recent projects, challenges, and future prospects in molecular docking-based drug repositioning against COVID-19

As a popular bioinformatics method, recently several types of research have been conducted to reposition approved drugs against COVID-19 by means of molecular docking. Despite similar aspects and methodology, the used software, subjected target and ligands can affect the outputs of molecular docking-based drug repositioning [54, 63]. In **Table 3**, some recently published works associated with molecular docking-based drug repurposing are presented. Based on our best knowledge, *SARS-CoV-2* main protease is the most popular target for drug discovery research due to the absence of closely related homologs in humans. Additionally, some host cell proteins such as Angiotensin-converting enzyme 2 (ACE2), Transmembrane Serine Protease 2 (TMPRSS2), Furin, Cathepsin L, Adaptor-Associated Kinase 1 (AAK1), and Two-Pore Channel (TPC2) have also been regarded for drug discovery against COVID-19. However, due to probable side effects, drug repurposing based on host cell targets received less attention.

Subjected target	ligands	Proposed drug or ligand	References
Mpro	FDA-approved drugs	binifibrate and bamifylline	[64]
Mpro	4384-approved drugs	Daunorubicin and eight other compounds	[65]
Mpro	6218-approved drugs	Emodin and blonanserin	[66]
RBD, NSP 10, NSP 16, Mpro, and RdRp	Brazilian Public Health System-approved drugs	penciclovir, ribavirin, and zanamivir	[67]
Mpro	Drug Bank database	levothyroxine, amobarbital and ABP-700	[68]
spike glycoprotein	FDA-approved drugs	Conivaptan and Trosec	[14]
spike glycoprotein	Plant secondary metabolites	Dicaffeoylquinic acid	[15]
Mpro	FDA-approved antiviral drugs	Lopinavir-Ritonavir, Tipranavir, and Raltegravir	[16]
papain like protease	Plant secondary metabolites	I-Asarinin	[17]
Mpro	superDRUG2 database	Binifibrate and Bamifylline	[18]
Mpro	Plant secondary metabolites	ursolic acid, carvacrol and oleanolic acid	[24]
RdRp	FDA-approved anti-viral drugs	remdesivir, ribavirin, sofosbuvir and galidesivir	[25]
Mpro	FDA approved drugs	remdesivir and glycyrrhizin	[26]
Mpro and RdRp	Plant secondary metabolites	cryptomisrine, cryptospirolepine, cryptoquindoline, and biscryptolepine	[27]

The SARS-CoV-2 main protease is the most considered target for drug discovery.

Table 3.
Recently published molecular docking-based drug repositioning research for introducing novel drugs against COVID-19.

Regarding the subjected ligands evaluation of their anti-COVID-19 potentials, there are several choices, including approved standard drugs, approved natural products, plant secondary metabolites, and under investigation drugs. Due to the time-consuming approval drug process as well as unexpected side effects, drug repurposing based on the approved drugs database is highly recommended [69, 70]. Despite the advantages of in silico drug repositioning against COVID-19, due to differences between natural drug-target micro-environments and drug-target simulations, the discrepancy between the laboratory results and the simulation outputs is expected. Therefore, a recently mixed approach, which is the combination of computational and empirical methods is proposed to fast and accurate drug repositioning [5].

6.5.2 A case study: repurposing FDA-approved antiviral protease inhibitors as SARS-CoV-2 3CLpro inhibitors

As mentioned in Section 3.2, due to the important role in the viral life cycle alongside the absence of closely related homologs in humans, the 3CLpro is considered a proper target for discovering effective antiviral drugs against *SARS-CoV-2*. Therefore, here a molecular docking-based drug-repurposing process was planned to screen the 3CLpro inhibitor(s) among the standard antiviral protease blockers.

6.5.2.1 Retrieval and preparation of ligands and receptor

A small molecule–protein molecular docking study is based on the prediction of probable interactions between the ligand and its receptor. Obtaining the three-dimensional structures of both the ligand and receptor is the first vital step for performing a molecular docking process. Therefore, the raw three structures of a set of FDA-approved antiviral protease inhibitors, as well as 3CLpro from *SARS-CoV-2*, were retrieved from the drug bank database (https://go.drugbank.com/) and protein data bank (https://go.drugbank.com/) respectively. The subjected drugs (**Table 4**) were obtained in the sdf format, and their raw structures were further prepared by adding polar hydrogens, computing Gastieger charge, detecting the root atom, setting the torsion, and the number of torsions. Furthermore, the structure of the 3CLpro was also optimized by deleting water molecules and bound ligands, adding polar hydrogens and Kollman charge using the Python molecule viewer software.

6.5.2.2 Primary screening by blind docking method

Despite primary screening done by the blind docking method, several studies have been conducted to introduce effective 3CLpro inhibitors. However, to date, binding pockets and key amino acids in the enzyme catalytic activity are not well known. Therefore, as primary screening, the blind docking processes through Molegro Virtual Docker 6.0 software were performed between the standard drugs and the 3CLpro to determine the key amino acid(s). In blind molecular docking, the whole surface of a subjected receptor is considered for evaluation of probable interactions with the ligand.

6.5.2.3 Targeted molecular docking

After determining the total affinities of the standard drugs to the 3CLpro as well as more reactive amino acids, targeted molecular docking studies were conducted between the receptor the three top-scoring docked ligands in a grid box, which covers the key amino acid(s) by Autodock 4.2.6 software.

6.5.2.4 Results

The results of the primary screening are presented in **Table 5**. The results demonstrated that Amprenavir, Tipranavir, and Fosamprenavir had a higher

Approved drug	Chemical formula	Accession number
Darunavir	C27H37N3O7S	DB01264
Tipranavir	C31H33F3N2O5S	DB00932
Atazanavir	C38H52N6O7	DB01072
Amprenavir	C25H35N3O6S	DB00701
Fosamprenavir	C25H36N3O9PS	DB01319
Nelfinavir	C32H45N3O4S	DB00220
Ritonavir	C37H48N6O5S2	DB00503
Indinavir	C36H47N5O4	DB00224
Saquinavir	C38H50N6O5	DB01232

Table 4.
Chemical formula and drug bank accession number of nine FDA-approved antiviral protease inhibitors subjected for repurposing against SARS-CoV-2.

Drug	Moldock score (kcal/mol)	Key amino acids
Darunavir	−110.402	THR190,ARG188, ASN142,TYR54,GLN189,ASP187
Tipranavir	−158.307	THR26,ASN142,GLN189,ARG188,GLU166
Atazanavir	−77.870	GLN189,TYR154
Amprenavir	−160.384	TYR154,GLN127,VAL303
Fosamprenavir	−146.601	CYS145,GLN189,SER144,GLY143,LEU141,HIS41,MET165,GLU166
Nelfinavir	−70.32	CYS145,GLN189
Ritonavir	−101.440	GLN189,THR24,CYS145
Indinavir	−98.704	GLN189,GLY143,THR26
Saquinavir	−100.442	GLU166,GLN189,THR26

Table 5.
The results of blind molecular docking between the standard antiviral protease inhibitors and the 3CLpro from SARS-CoV-2. Amprenavir, Tipranavir, and Tipranavir showed high binding affinity to 3CLpro.

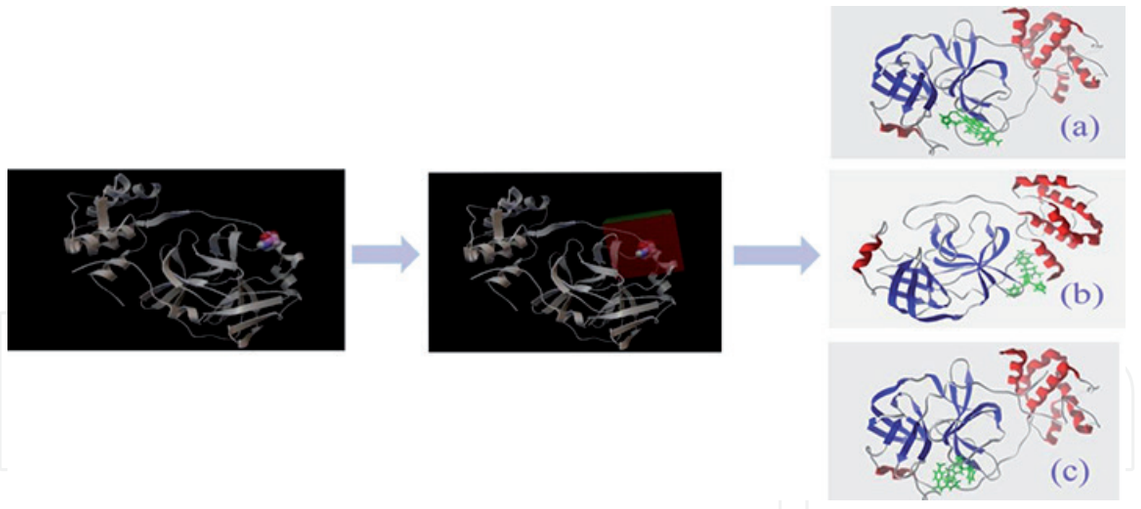

Figure 2.
Graphical representation of the targeted molecular docking between the 3CLpro from SARS-CoV-2 and (a) Fosamprenavir, (b) Amprenavir, and (c) Tipranavir. Fosamprenavir showed the most binding affinity in the subjected docking grid box followed by Amprenavir and Tipranavir respectively.

binding affinity to the 3CLpro than the other tested viral protease inhibitors with Moldock scores of −160.384, −158.307, and −146.601 respectively. Furthermore, it was clear that GLN 189 is a key amino acid in the 3CLpro interactions with different proteases. Therefore, a targeted molecular docking between the three top-scoring standard protease inhibitors (Amprenavir, Tipranavir, and Fosamprenavir) were also performed in a grid box with the center of GLN189. As depicted in **Figure 2**, the subjected standard drugs also showed high affinity to the 3CLpro with binding energies of −5.3, −5.1, and −6.2 kcal/mol respectively. Subsequently, due to the high affinity of Fosamprenavir to the 3CLpro, this antiviral protease inhibitor could be considered for further in silico, in vitro, and in vivo evaluation to develop as a repurposed anti *SARS-CoV-2* treatment.

7. Conclusion

To date, the only approved anti-COVID-19 treatment is a repurposed antiviral drug (Remdesivir). Hence, drug repurposing might be an effective approach for accelerating drug discovery against COVID-19. Computational drug repositioning offers a noteworthy reduction in time and costs of new drug development and increases success rates in comparison to traditional methods. Therefore, to date, different computational methods such as data mining, machine learning, network analysis, and molecular docking have successfully been used for drug repurposing.

Molecular docking is a popular bioinformatics method that recently has been highly regarded for studying the drug ability of biological entities, protein-ligand interactions, mechanism action of drug candidates, and drug repositioning. Retrieval drug candidates from standard databases or previous reports, lead and target optimization, running the molecular docking process, and results analysis are the main steps in molecular docking-based drug repositioning. The binding affinity of a drug candidate to key amino acid(s) of the identified target molecule can be considered a decision factor in the drug repositioning process.

Despite the advantages of computational drug repositioning, studying drug-target interactions by in silico methods is still far from reality.

Author details

Fatemeh Hosseini[1*], Mehrdad Azin[1], Hamideh Ofoghi[1] and Tahereh Alinejad[2]

1 Department of Biotechnology, Iranian Research Organization for Science and Technology, Tehran, Iran

2 Center for Precision Medicine, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China

*Address all correspondence to: fatemeh.hosseini.ap1984@gmail.com

References

[1] Arora G, Joshi J, Mandal RS, Shrivastava N, Virmani R, Sethi T. Artificial intelligence in surveillance, diagnosis, drug discovery and vaccine development against COVID-19. Pathogens. 2021;**10**:1048

[2] Yang J, Marziano V, Deng X, Guzzetta G, Zhang J, Trentini F, et al. Despite vaccination, China needs non-pharmaceutical interventions to prevent widespread outbreaks of COVID-19 in 2021. Nature Human Behaviour. 2021;**5**:1-12

[3] Forni G, Mantovani A. COVID-19 vaccines: Where we stand and challenges ahead. Cell Death and Differentiation. 2021;**28**:626-639

[4] Hughes JP, Rees S, Kalindjian SB, Philpott KL. Principles of early drug discovery. British Journal of Pharmacology. 2011;**162**:1239-1249

[5] Rudrapal M, Khairnar J, Jadhav G. Drug repurposing (DR): An emerging approach in drug discovery. Drug Repurposing Hypothesis Molecular Aspects and Therapeutic Applications. 2020. pp. 93193

[6] Shende P, Khanolkar B, Gaud R. Drug repurposing: New strategies for addressing COVID-19 outbreak. Expert Review of Anti-Infective Therapy. 2021;**19**:689-706

[7] Ronco C, Navalesi P, Vincent JL. Coronavirus epidemic: Preparing for extracorporeal organ support in intensive care. The Lancet Respiratory Medicine. 2020;**8**:240-241

[8] Khachfe HH, Chahrour M, Sammouri J, Salhab H, Makki BE, Fares M. An epidemiological study on COVID-19: A rapidly spreading disease. Cureus. 2020;**12**:e7313

[9] Guo Y-R, Cao Q-D, Hong Z-S, Tan Y-Y, Chen S-D, Jin H-J, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak—An update on the status. Military Medical Research. 2020;**7**:1-10

[10] Greenhalgh T, Jimenez JL, Prather KA, Tufekci Z, Fisman D, Schooley R. Ten scientific reasons in support of airborne transmission of SARS-CoV-2. The Lancet. 2021;**397**: 1603-1605

[11] Alsobaie S. Understanding the molecular biology of SARS-CoV-2 and the COVID-19 pandemic: A review. Infection and Drug Resistance. 2021;**14**:2259

[12] Das A, Ahmed R, Akhtar S, Begum K, Banu S. An overview of basic molecular biology of SARS-CoV-2 and current COVID-19 prevention strategies. Gene Reports. 2021;**23**:101122

[13] Yadav R, Chaudhary JK, Jain N, Chaudhary PK, Khanra S, Dhamija P, et al. Role of structural and non-structural proteins and therapeutic targets of SARS-CoV-2 for COVID-19. Cell. 2021;**10**:821

[14] De Clercq E. Specific targets for antiviral drugs. Biochemical Journal. 1982;**205**:1-13

[15] Dinesh DC, Tamilarasan S, Rajaram K, Bouřa E. Antiviral drug targets of single-stranded RNA viruses causing chronic human diseases. Current Drug Targets. 2020;**21**:105-124

[16] Mummed Y. Molecular targets for COVID-19 drug development: Enlightening Nigerians about the pandemic and future treatment. Biosafety and Health. 2020;**2**(4):185-187

[17] Tian D, Liu Y, Liang C, Xin L, Xie X, Zhang D, et al. An update review of emerging small-molecule therapeutic options for COVID-19. Biomedicine and Pharmacotherapy. 2021;**137**:111313

[18] Su H, Xu Y, Jiang H. Drug discovery and development targeting the life cycle of SARS-CoV-2. Fundamental Research. 2021;**1**(2):151-165

[19] Huang C, Lokugamage KG, Rozovics JM, Narayanan K, Semler BL, Makino S. SARS coronavirus nsp1 protein induces template-dependent endonucleolytic cleavage of mRNAs: Viral mRNAs are resistant to nsp1-induced RNA cleavage. PLoS Pathogens. 2011;**7**:e1002433

[20] Cornillez-Ty CT, Liao L, Yates JR III, Kuhn P, Buchmeier MJ. Severe acute respiratory syndrome coronavirus nonstructural protein 2 interacts with a host protein complex involved in mitochondrial biogenesis and intracellular signaling. Journal of Virology. 2009;**83**:10314-10318

[21] Lei J, Kusov Y, Hilgenfeld R. Nsp3 of coronaviruses: Structures and functions of a large multi-domain protein. Antiviral Research. 2018;**149**:58-74

[22] Sakai Y, Kawachi K, Terada Y, Omori H, Matsuura Y, Kamitani W. Two-amino acids change in the nsp4 of SARS coronavirus abolishes viral replication. Virology. 2017;**510**:165-174

[23] Cottam EM, Whelband MC, Wileman T. Coronavirus NSP6 restricts autophagosome expansion. Autophagy. 2014;**10**:1426-1441

[24] Cao Y, Su B, Guo X, Sun W, Deng Y, Bao L, et al. Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells. Cell. 2020;**182**:73-84

[25] Shi R, Shan C, Duan X, Chen Z, Liu P, Song J, et al. A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. Nature. 2020;**584**:120-124

[26] Zost SJ, Gilchuk P, Case JB, Binshtein E, Chen RE, Nkolola JP, et al. Potently neutralizing and protective human antibodies against SARS-CoV-2. Nature. 2020;**584**:443-449

[27] Xiang R, Yu Z, Wang Y, Wang L, Huo S, Li Y, et al. Recent advances in developing small-molecule inhibitors against SARS-CoV-2. Acta Pharmaceutica Sinica B. 2021;**11**(11):100-110

[28] Cheung NN, Lai KK, Dai J, Kok KH, Chen H, Chan K-H, et al. Broad-spectrum inhibition of common respiratory RNA viruses by a pyrimidine synthesis inhibitor with involvement of the host antiviral response. Journal of General Virology. 2017;**98**:946-954

[29] Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, et al. Structure of M pro from SARS-CoV-2 and discovery of its inhibitors. Nature. 2020;**582**:289-293

[30] Beigel J, Tomashek K, Dodd L. Remdesivir for the Treatment of Covid-19—Final Report. 2020

[31] Idda ML, Soru D, Floris M. Overview of the first 6 months of clinical trials for COVID-19 pharmacotherapy: The most studied drugs. Frontiers in Public Health. 2020;**8**(Aug):1-7

[32] Hassanipour S, Arab-Zozani M, Amani B, Heidarzad F, Fathalipour M, Martinez-de-Hoyo R. The efficacy and safety of Favipiravir in treatment of COVID-19: A systematic review and meta-analysis of clinical trials. Scientific Reports. 2021;**11**:1-11

[33] Serafin MB, Bottega A, Foletto VS, da Rosa TF, Hörner A, Hörner R. Drug repositioning is an alternative for the treatment of coronavirus COVID-19. International Journal of Antimicrobial Agents. 2020;**55**:105969

[34] Gashaw I, Ellinghaus P, Sommer A, Asadullah K. What makes a good drug target? Drug Discovery Today. 2011;**16**: 1037-1043

[35] Zdrazil B, Richter L, Brown N, Guha R. Moving targets in drug discovery. Scientific Reports. 2020;**10**:1-15

[36] Saxena SK, Mishra N, Saxena R. Advances in Antiviral Drug Discovery and Development. Part I: Advancements in Antiviral Drug Discovery. London: Future Medicine Ltd.; 2009

[37] Saxena SK, Mishra N, Saxena R. Advances in Antiviral Drug Discovery and Development: Part II: Advancements in Antiviral Drug Development. London: Future Medicine Ltd.; 2009

[38] Smith C. Drug target validation: Hitting the target. Nature. 2003;**422**: 342-345

[39] Katsila T, Spyroulias GA, Patrinos GP, Matsoukas M-T. Computational approaches in target identification and drug discovery. Computational and Structural Biotechnology Journal. 2016;**14**:177-184

[40] Lu Z. PubMed and beyond: A survey of web tools for searching biomedical literature. Database. 2011;**2011**:36

[41] Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. Proceedings of the National Academy of Sciences. 2001;**98**:5116-5121

[42] Brown AS, Patel CJ. MeSHDD: Literature-based drug-drug similarity for drug repositioning. Journal of the American Medical Informatics Association. 2017;**24**:614-618

[43] Leaman R, Islamaj Doğan R, Lu Z. DNorm: Disease name normalization with pairwise learning to rank. Bioinformatics. 2013;**29**:2909-2917

[44] Papanikolaou N, Pavlopoulos GA, Theodosiou T, Vizirianakis IS, Iliopoulos I. DrugQuest-a text mining workflow for drug association discovery. BMC Bioinformatics. 2016;**17**:333-341

[45] Patel L, Shukla T, Huang X, Ussery DW, Wang S. Machine learning methods in drug discovery. Molecules. 2020;**25**:5277

[46] Gottlieb A, Stein GY, Ruppin E, Sharan R. PREDICT: A method for inferring novel drug indications with application to personalized medicine. Molecular Systems Biology. 2011;**7**:496

[47] Napolitano F, Zhao Y, Moreira VM, Tagliaferri R, Kere J, D'Amato M, et al. Drug repositioning: A machine-learning approach through data integration. Journal of Cheminformatics. 2013;**5**:1-9

[48] Aliper A, Plis S, Artemov A, Ulloa A, Mamoshina P, Zhavoronkov A. Deep learning applications for predicting pharmacological properties of drugs and drug repurposing using transcriptomic data. Molecular Pharmaceutics. 2016;**13**:2524-2530

[49] Lotfi Shahreza M, Ghadiri N, Mousavi SR, Varshosaz J, Green JR. A review of network-based approaches to drug repositioning. Briefings in Bioinformatics. 2018;**19**:878-892

[50] Somolinos FJ, León C, Guerrero-Aspizua S. Drug repurposing using biological networks. Processes. 2021;**9**:1057

[51] Jarada TN, Rokne JG, Alhajj R. A review of computational drug repositioning: Strategies, approaches, opportunities, challenges, and directions. Journal of Cheminformatics. 2020;**12**:1-23

[52] Salmaso V, Moro S. Bridging molecular docking to molecular dynamics in exploring ligand-protein recognition process: An overview. Frontiers in Pharmacology. 2018;**9**:923

[53] Salmaso V, Sturlese M, Cuzzolin A, Moro S. Combining self-and cross-docking as benchmark tools: The performance of DockBench in the D3R Grand Challenge 2. Journal of Computer-Aided Molecular Design. 2018;**32**:251-264

[54] Kumar S, Kumar S. Molecular docking: A structure-based approach for drug repurposing. In: Silico Drug Design. Amsterdam, Netherlands: Elsevier; 2019. pp. 161-189

[55] Agarwal S, Mehrotra R. An overview of molecular docking. JSM Chem. 2016;**4**:1024-1028

[56] Chaudhary KK, Mishra N. A review on molecular docking: Novel tool for drug discovery. Database. 2016;**3**:1029

[57] Shawky E, Nada AA, Ibrahim RS. Potential role of medicinal plants and their constituents in the mitigation of SARS-CoV-2: Identifying related therapeutic targets using network pharmacology and molecular docking analyses. RSC Advances. 2020;**10**: 27961-27983

[58] Dutta M, Nezam M, Chowdhury S, Rakib A, Paul A, Sami SA, et al. Appraisals of the Bangladeshi medicinal plant calotropis gigantea used by folk medicine practitioners in the management of COVID-19: A biochemical and computational approach. Frontiers in Molecular Biosciences. 2021;**8**:481

[59] Azim KF, Ahmed SR, Banik A, Khan MMR, Deb A, Somana SR. Screening and druggability analysis of some plant metabolites against SARS-CoV-2: An integrative computational approach. Informatics in Medicine Unlocked. 2020;**20**:100367

[60] Rahman MR, Banik A, Chowdhury IM, Sajib EH, Sarkar S. Identification of potential antivirals against SARS-CoV-2 using virtual screening method. Informatics in Medicine Unlocked. 2021;**23**:100531

[61] Patil R, Chikhale R, Khanal P, Gurav N, Ayyanar M, Sinha S, et al. Computational and network pharmacology analysis of bioflavonoids as possible natural antiviral compounds in COVID-19. Informatics in Medicine Unlocked. 2021;**22**:100504

[62] Parida PK, Paul D, Chakravorty D. Nature's therapy for COVID-19: Targeting the vital non-structural proteins (NSP) from SARS-CoV-2 with phytochemicals from Indian medicinal plants. Phytomedicine Plus. 2021;**1**: 100002

[63] Luo H, Mattes W, Mendrick DL, Hong H. Molecular docking for identification of potential targets for drug repurposing. Current Topics in Medicinal Chemistry. 2016;**16**: 3636-3645

[64] Te Velthuis AJ, Van Den Worm SH, Snijder EJ. The SARS-coronavirus nsp7+ nsp8 complex is a unique multimeric RNA polymerase capable of both de novo initiation and primer extension. Nucleic Acids Research. 2012;**40**:1737-1747

[65] Shi Z, Gao H, Bai X-c, Yu H. Cryo-EM structure of the human cohesin-NIPBL-DNA complex. Science. 2020;**368**:1454-1459

[66] Subissi L, Posthuma CC, Collet A, Zevenhoven-Dobbe JC, Gorbalenya AE, Decroly E, et al. One severe acute respiratory syndrome coronavirus protein complex integrates processive RNA polymerase and exonuclease activities. Proceedings of the National Academy of Sciences. 2014;**111**: E3900-E3909

[67] Jang K-J, Jeong S, Kang DY, Sp N, Yang YM, Kim D-E. A high ATP concentration enhances the cooperative translocation of the SARS coronavirus

helicase nsP13 in the unwinding of duplex RNA. Scientific Reports. 2020;**10**:1-13

[68] Decroly E, Debarnot C, Ferron F, Bouvet M, Coutard B, Imbert I, et al. Crystal structure and functional analysis of the SARS-coronavirus RNA cap 2′-O-methyltransferase nsp10/nsp16 complex. PLoS Pathogens. 2011;**7**: e1002059

[69] Gil C, Ginex T, Maestro I, Nozal V, Barrado-Gil L, Cuesta-Geijo MÁ, et al. COVID-19: Drug targets and potential treatments. Journal of Medicinal Chemistry. 2020;**63**:12359-12386

[70] Hassanzadeganroudsari M, Ahmadi AH, Rashidi N, Hossain MK, Habib A, Apostolopoulos V. Computational chemistry to repurposing drugs for the control of COVID-19. Biologics. 2021;**1**:111-128