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ARTICLE

Virtual screening FDA approved drugs against multiple targets of SARS-CoV-2

Hualou Liang[1] | **Liang Zhao**[2] | **Xiajing Gong**[2] | **Meng Hu**[2] | **Hongbin Wang**[3]

[1]School of Biomedical Engineering, Science and Health Systems, Drexel University, Philadelphia, PA, USA

[2]Office of Research and Standards, Office of Generic Drugs, Center for Drug Evaluation and Research, US Food and Drug Administration, Silver Spring, MD, USA

[3]Center for Biomedical Informatics, College of Medicine, Texas A&M University Health Science Center, Houston, TX, USA

Correspondence
Hualou Liang, School of Biomedical Engineering, Science and Health Systems, Drexel University, 3141 Chestnut Street, Philadelphia, PA 19104, USA.
Email: hualou.liang@drexel.edu

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Abstract

The outbreak of the novel coronavirus severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19) respiratory disease, led to a global pandemic with high morbidity and mortality. Despite frenzied efforts in therapeutic development, there are currently no effective drugs for treatment, nor are there vaccines for its prevention. Drug repurposing, representing as an effective drug discovery strategy from existing drugs, is one of the most practical treatment options against the outbreak. In this study, we present a novel strategy for in silico molecular modeling screening for potential drugs that may interact with multiple main proteins of SARS-CoV-2. Targeting multiple viral proteins is a novel drug discovery concept in that it enables the potential drugs to act on different stages of the virus' life cycle, thereby potentially maximizing the drug potency. We screened 2631 US Food and Drug Administration (FDA)-approved small molecules against 4 key proteins of SARS-CoV-2 that are known as attractive targets for antiviral drug development. In total, we identified 29 drugs that could actively interact with 2 or more target proteins, with 5 drugs (avapritinib, bictegravir, ziprasidone, capmatinib, and pexidartinib) being common candidates for all 4 key host proteins and 3 of them possessing the desirable molecular properties. By overlaying docked positions of drug candidates onto individual host proteins, it has been further confirmed that the binding site conformations are conserved. The drugs identified in our screening provide potential guidance for experimental confirmation, such as in vitro molecular assays and in vivo animal testing, as well as incorporation into ongoing clinical studies.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The pandemic of coronavirus disease 2019 (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a global crisis. Currently, there are no effective drugs for treatment, nor are there vaccines for its prevention. Extant work on drug repurposing has exclusively focused on one single protein target, neither considered multiple proteins at different stages of the virus' life cycle nor accounted for molecular properties important for drug discovery.

WHAT QUESTION DID THIS STUDY ADDRESS?

This work describes a novel drug repurposing strategy for performing in silico molecular modeling screening for potential drugs that interact with multiple proteins of SARS-CoV-2 while at the same time taking into consideration the desirable molecular properties.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

We provide a drug repurposing strategy targeting multiple viral proteins that enables the potential drugs to act on different stages of the virus' life cycle, thereby potentially maximizing the drug potency. Of 29 identified drugs that actively interact with 2 or more target proteins, we find 5 drugs that harbor antiviral activity against all 4 key host proteins of SARS-CoV-2.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

This work identifies key drug repurposing opportunities and dramatically highlights the importance of considering multiple target proteins of SARS-CoV-2 while taking into consideration the desirable molecular properties important for drug discovery.

INTRODUCTION

A novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak has caused a global pandemic resulting in millions of infections and tens of thousands of deaths worldwide. Given the scale and rapid spread of coronavirus disease 2019 (COVID-19), there is an urgent need for treatment options before a vaccine can be produced. Because the emerging virus represents new pathogens that interact with human cells in complicated ways, effective treatment options have been hard to find. Several drug candidates, deemed promising in the early phase of the pandemic, have recently been reported to have no or only moderate efficacy.[1] The need for quickly identifying effective drugs is becoming even more urgent as nations start to ease the lockdowns and vaccines are still months away. In this regard, finding currently approved drugs that could be repurposed against the new virus is a sound strategy.

To devise therapeutic strategies to counteract SARS-CoV-2 infection, it is crucial to understand how this coronavirus hijacks the host during the course of infection, and to apply this knowledge toward repurposing existing drugs. SARS-CoV-2 possesses the typical coronavirus structure with the spike (S) protein and encodes more than 2 dozen proteins, including both structural and nonstructural proteins, some of which are essential to viral entry and replication. The coronavirus begins its life cycle when S protein binds to the cellular receptor called angiotensin-converting enzyme 2 (ACE2) located on the surface membrane of host cells.[2–5] The receptor-binding domain in the spike protein (S-Protein-RBD) is a key target because it initiates the infection process. Upon entrance to the host cells, the viral genome is released as a single-stranded positive RNA. Subsequently, it is translated into viral replicase polyproteins, which are then cleaved into effector proteins by the coronavirus main proteinase (3CLpro) and the papain-like protease (PLpro).[6,7] In order to replicate the RNA genome, the coronavirus encodes a replicase that is an RNA-dependent RNA polymerase (RdRp).[8] These four proteins (RdRp, 3CLpro, PLpro, and S-Protein-RBD) are essential for the pathogenicity of the virus and have been the main targets for drug, either new or repurposing, development efforts.

A large body of emerging work on repurposing the existing drugs has been exclusively focused on one single protein target (e.g., refs. 9, 10). For example, recently the US Food and Drug Administration (FDA)-approved drug, remdesivir, is an inhibitor of the viral RdRp. Considering the existence of different stages of the virus' life cycle, it is desirable to target multiple viral proteins in that it enables the potential drugs to disrupt the viral infection and replication process in different stages, hence maximizing the drug potency and spectrum. In addition, extant work rarely considers the molecular properties important for drug discovery. It is generally recognized that an ideal drug, besides being pharmacologically active, should additionally possess certain features regarding its bioavailability and its toxicological profile, such as absorption, distribution, metabolization, and elimination/toxicological (ADME-Tox). In this study, we present a novel drug repurposing strategy for performing in silico molecular modeling screening for potential drugs that interact with four target proteins of SARS-CoV-2. To check the impact of the ADME-Tox filtering on the potential drugs identified, we also performed the same screening procedure, but with ADME-Tox

filtering. Overall, we screened all available FDA-approved small molecules, and found five promising candidates with potential therapeutic ability against four key proteins of SARS-CoV-2. Among these five drugs, three possess the ADME-Tox properties.

METHODS

Ligand preparation

The list of the FDA approved drugs was downloaded from the DrugBank database.[11] At the time of writing, the database contains 2632 approved small molecule drugs along with their Simplified Molecular-Input Line-Entry System (SMILES) representations.[12] The docking ligands were then prepared from SMILES strings of the drug candidates using the Pybel,[13] and 3D structures were optimized using the MMFF94 force field.

Protein structure preparation

The structures of the target proteins of SARS-CoV-2 were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB).[14] The 3D protein structure files downloaded include 3CLpro (PDB ID: 6LU7), RdRp (PDB ID: 6M71), S-Protein-RBD (PDB ID: 6LZG), and PLpro (PDB ID: 6W9C). PDB protein structures normally lack hydrogen atoms, which are required for appropriate treatment of electrostatics during docking. As such, hydrogens for pH 7.0 and Gasteiger charges were added to the protein and a pdbqt format file, as required by molecular docking below (AutoDock Vina), was generated by using Open Babel.[15]

ADME-Tox screening

An ideal drug not only must be active against target proteins, but also should possess the appropriate ADME-Tox properties. The ADME in silico screening leads to the widely used Lipinski's rule of five[16] for determining whether it is probable or not for a drug candidate to reach its site of action. These simple rules state that bioavailability is likely to occur if at least 3 of the following rules are obeyed: molecular weight below 500 Daltons, no more than 5 hydrogen bond donors and less than 10 hydrogen bond acceptors; and a calculated logarithm of the partition coefficient of the compound between water and octanol (log P) below 5. Moreover, to ensure good bioavailability, the polar surface area no greater than 140 $Å^2$ and the number of rota S bonds less than 10 are further imposed.[17] The values of these molecular descriptors were obtained using the RDKit Python library.[18]

Considering that the drug toxicity is closely related to its approved dose, we additionally assess the possible toxic effects of drug candidates. We use ProTox[19] to compute the toxic doses, known as median lethal doses (LD50) values in mg/kg body weight. We use the well-defined toxicity classes according to the Globally Harmonized System of classification of labeling of chemicals. The molecule is hence considered toxic when LD50 less than or equal to 300 mg/kg. This additional estimation of toxicity, while complementing the existing toxicity profiles, offers the potential comparison across all products and allows one to get the least toxic.

Molecular docking

We used molecular docking software AutoDock Vina[20] to perform protein-ligand docking analysis, with the above prepared ligands and proteins as docking inputs. To dock drug molecules into the binding sites of the target proteins of SARS-CoV-2, we need to define the 3D search space that encloses the known binding sites and where ligand docking should be attempted.

The 3D search space for each targeted SARS-CoV-2 protein is generated based on its known or inferred active binding sites. For viral protein 3CLPro (6LU7), the center of active site of the box grid was determined according to the position of the N3 ligand in the structure[21] and grabbed using PyMOL.[22] The active site of the COVID-19 virus RdRp domain (6M71) is formed by the conserved polymerase motifs A-G in the palm domain.[23] The search space enclosing the binding sites of S-Protein is similarly determined based on the known structure of the S-Protein-ACE2 complex (6LZG[24]). The published structure of PLpro (6W9C) contains no ligand binding information.[25] Given the fact that PLpro is conserved well between SARS-CoV and SARS-CoV-2, we inferred its spatial binding information by first aligning its structure with SARS-CoV PLpro with known ligand binding information (PDB ID: 4OW0) and then grabbed the corresponding search space using PyMOL.

We inspected the docking results with PyMOL[22] by visualizing the docks and comparing to the crystal conformation of the ligands. Finally, the candidate molecules were selected by their docking scores that represent the binding affinity to individual protein structures. The docking score is an estimation of the free energy of binding (in kcal/mol), the more negative the value is, the tighter the hit binds to the target.

To illustrate how our drug screening method works for multiple proteins, we used two target proteins (S-Protein-RBD and PLpro) to show the selection process. The rationale was to choose drug candidates that showed higher binding affinity (i.e., low binding free energy) to both

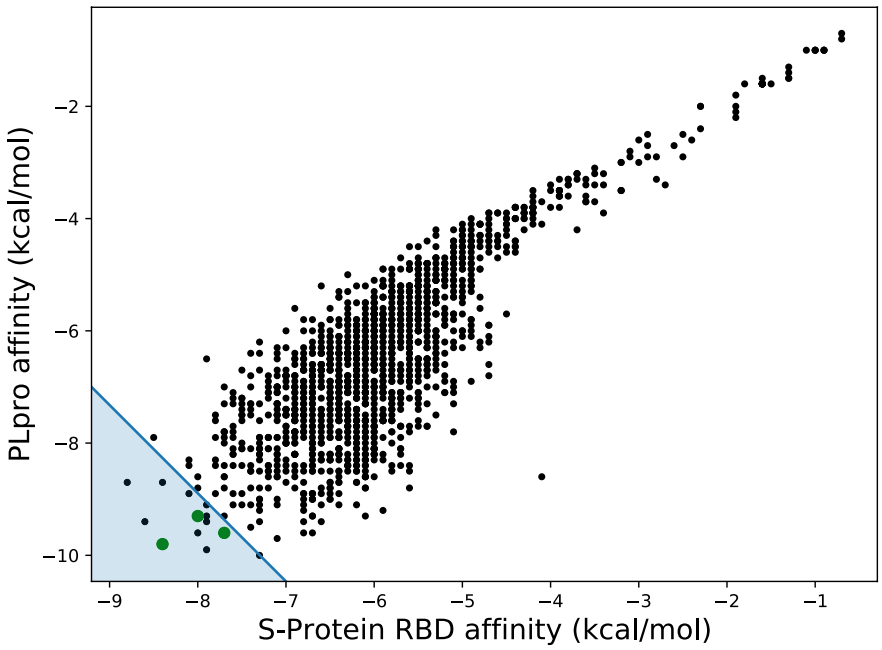


FIGURE 1 Candidate drugs (the shaded area below the line) with low docking free energy for both spike protein receptor-binding domain and papain-like protease (PLpro) are selected for further validation. Highlighted are three common targets (avapritinib, ziprasidone, and bictegravir) for all the four key host proteins (coronavirus main proteinase [3CLpro], RNA-dependent RNA polymerase [RdRp], receptor-binding domain in the spike protein [S-Protein-RBD], and PLpro) while possessing the desirable molecular properties in this study

proteins, as shown in Figure 1. Thus, we defined a simple screening criterion to select top candidates having low docking scores on both proteins. All candidates having scores lower than the screening line (the shaded area) were screened for further consideration. Also shown in Figure 1 are the identified three out of five potential drug candidates (avapritinib, ziprasidone, and bictegravir) binding to all the four key host proteins (3CLpro, RdRp, S-Protein-RBD, and PLpro) while possessing the desirable molecular properties in this study.

Because the docking scores are sensitive to the choice of target structures, we chose the top 40 hits (accounting for 2% drugs) that were ranked based on their docking scores for each protein to make the screening target-dependent. We then identified the overlapping drugs among two, three, and four proteins, respectively. Although how to choose the ranking score cutoff remains a long-standing question in docking, it has been a general practice that has been widely used in drug screening (e.g., refs. 26, 27).

To check the impact of the ADME-Tox filtering on the potential drugs identified, we also performed the same screening procedure above, but with ADME-Tox filtering.

RESULTS

A list of 2631 FDA-approved small molecules was downloaded from DrugBank (version 5.1.6, released April 22, 2020). For some molecules, the SMILES representation could not be resolved correctly and, therefore, were filtered out. We ended up with 2028 molecules for further analysis. After ADME-Tox screening was conducted for all these molecules, thes number of candidates was reduced to 1366.

For each molecule, the molecule-protein docking was carried out between the molecule and one of the four target protein structures (6LU7, 6M71, 6LZG, and 6W9C). We applied our screening procedure to pick the top-40 hits for each protein based on the binding affinity. Next, we identified a short list of drug candidates common in combination of two, three, and four proteins, respectively. In total, we identified 29 high-ranked drugs that could actively interact with 2 or more target proteins, with 5 drugs (avapritinib, bictegravir, ziprasidone, capmatinib, and pexidartinib) being common candidates for all 4 key targeted proteins. Table 1 lists the detailed binding affinity between protein-molecule pairs for all the drug candidates specific to each protein target, with the identified five drugs shaded.

As a comparison, we have performed the same screening procedure above, with ADME-Tox filtering imposed. As a result, three out of five drugs (avapritinib, bictegravir, and ziprasidone) were identified. With the ADME-Tox filtering, the resulted top-list drugs are provided in Table S1 and Figure 2 shows the chemical structures of these three identified drugs, with their desirable molecular properties provided in Table 2. The screening results are provided in the Venn diagram Figure 3, showing all possible drug candidates overlapping between/among different host proteins (3CLpro, RdRp, S-Protein-RBD, and PLpro). For example, the identified three core drug candidates (avapritinib, bictegravir, and ziprasidone) are in the intersection among all four proteins, whereas bisoxatin, along with these three core drugs, are the overlap among three proteins (3CLpro, RdRp, and PLpro). We confirmed that all the potential drug candidates selected meet the ADME-Tox screening criteria in Table 2. We note that the drug substance can be delivered as an orally inhaled product or administered via i.v. infusion.

TABLE 1 The binding affinity (the docking score) between the protein-molecule pair, which is calculated as the minimum binding free energy in kcal/mol for all of the molecules specific to each protein target

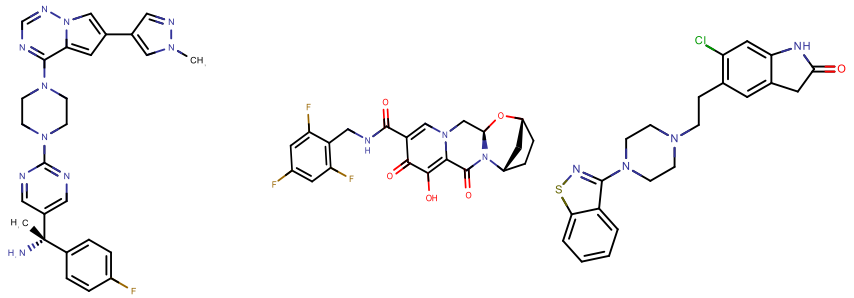
Drug name	3CLpro (6LU7)	RdRp (6M71)	S-Protein-RBD (6LZG)	PLpro (6W9C)
Avapritinib	−7.1	−8.7	−7.7	−9.6
Bictegravir	−7	−8.7	−8.4	−9.8
Capmatinib	−7.1	−8.8	−7.9	−9.3
Pexidartinib	−6.9	−9.2	−9	−9.3
Ziprasidone	−7	−9	−8	−9.3
Bisoxatin	−7.2	−8.8	−6.8	−9.6
Dexamethasone	−5.8	−8.4	−6.2	−9.1
Eltrombopag	−6.9	−8.9	−7.9	−9.1
Enasidenib	−7.1	−8	−8	−8.8
Flibanserin	−6.3	−8.9	−8.6	−9.4
Fluorescein	−7.1	−8.3	−7.1	−9.1
Glimepiride	−7.1	−7.7	−8.2	−8.7
Imatinib	−6.4	−8.3	−7.7	−9.3
Lasmiditan	−7.1	−7.5	−8.4	−8.7
Linagliptin	−5.5	−8.5	−8.1	−8.9
Lumacaftor	−6.5	−8.6	−8	−9.6
Lurasidone	−6.3	−8.4	−7.9	−9.9
Mizolastine	−6.5	−8.5	−7.1	−9.7
NPP	−7.2	−7.5	−8.7	−7.9
Paliperidone	−5.7	−8.5	−7.3	−9.4
Rupatadine	−6.9	−9.2	−7.4	−9.7
Selinexor	−7.2	−8.3	−8.8	−8.7
Sonidegib	−6.5	−8.7	−7	−9.8
Sorafenib	−7.6	−8.5	−7.8	−8.2
Tadalafil	−6.1	−8.4	−6.8	−9.4
Tecovirimat	−6.5	−8	−7.9	−9.4
Tolvaptan	−5.2	−8.5	−8	−8.8
Tucatinib	−5.8	−8.7	−7.6	−9.1
Vemurafenib	−7.1	−7	−8	−8.6

Shaded are the identified five drugs binding to all the four viral proteins.

Abbreviations: 3CLpro, coronavirus main proteinase; PLpro, papain-like protease; RdRp, RNA-dependent RNA polymerase; S-Protein-RBD, receptor-binding domain in the spike protein.

By overlaying docked positions of the selected three drug candidates binding to individual host proteins in Figure 4, we further observed that the binding site conformations are conserved despite docking variation in some of the drugs.

With the rapidly expanding knowledge about SARS-CoV-2, there have been a growing number of registered clinical trials with potential drugs against COVID-19.[28] It is natural to ask how these drugs, albeit not necessarily FDA-approved, are evaluated against our screening method. This also provides us a means of negative control to cross-check our method. Table 3 provides the docking scores (i.e., the binding affinity) with all the four target proteins for select proposed COVID-19 treatments from recent studies (for recent comprehensive reviews, see refs. 29,30). It is generally observed that these drugs have weaker binding affinity than those selected in Table 1. Several factors, either individually or in combination, may contribute to the outcome that none of these drugs makes into our short list: (1) they are investigational drugs (e.g., umifenovir and favipiravir), (2) there is at least one violation of the ADME-Tox screening rule (lopinavir and ritonavir, each has a molecular mass >500 Daltons), and (3) lower binding affinity to meet our screening criteria (e.g., chloroquine and hydroxychloroquine), in addition, both act on the ACE2 receptor (not the target proteins under the current study) as a potential mechanism against SARS-CoV-2. Importantly, it has been recently reported that treatment with an antiviral drug alone may not be sufficient.[1]



Avapritinib Ziprasidone Bictegravir

FIGURE 2 The chemical structures of three identified drug candidates with the absorption, distribution, metabolization, and elimination/toxicological filtering properties: avapritinib, ziprasidone and bictegravir

TABLE 2 The MW (in Daltons), log P, HBDs, and HBA, the number of ROTBs, PSA in Å^2, and predicted toxicity in terms of the LD50 for the top drugs identified in this work

Drug name	MW, Da	logP	HBA	HBD	ROTB	PSA	LD50, mg/kg
Avapritinib	498.56	3.44	8	1	5	106.29	2500
Bictegravir	449.38	1.96	7	2	3	100.87	1600
Ziprasidone	412.94	3.95	5	1	4	76.71	1530
Bisoxatin	333.34	3.51	5	3	2	78.79	600
Dexamethasone	392.46	1.90	7	3	2	94.83	3000
Eltrombopag	442.47	4.14	8	3	5	114.59	5000
Enasidenib	473.37	4.44	8	3	6	108.74	1700
Flibanserin	390.40	3.17	3	1	4	44.27	600
Fluorescein	332.31	3.67	5	2	0	75.99	4738
Lasmiditan	377.36	3.29	5	1	4	62.3	1600
Linagliptin	472.54	1.91	7	1	4	116.86	684
Lumacaftor	452.41	4.82	7	2	5	97.75	1848
Lurasidone	492.68	4.20	8	0	5	84.99	660
Mizolastine	432.49	3.48	5	1	5	70.05	450
Selinexor	443.31	3.85	7	2	5	97.62	1000
Tadalafil	389.40	2.09	7	1	1	74.87	906
Tecovirimat	376.33	2.73	5	1	2	66.48	1000
Tucatinib	480.52	4.68	9	2	5	110.85	3160

Abbreviations: HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; LD50, median lethal dose; log P, octanol-water partition coefficient; MW, molecular weight; PSA in Å^2, topological polar surface area; ROTB, rotatable bonds.

DISCUSSION

In this work, we present a novel drug repurposing strategy for performing in silico molecular modeling screening for potential drug candidates that interact with multiple target proteins of SARS-CoV-2. We additionally conduct the drug screening procedure while considering the desirable molecular properties, such as ADME-Tox. Overall, we screened over 2000 FDA-approved small molecules, and found 5 candidates with potential therapeutic ability against 4 key proteins of SARS-CoV-2. Among these five drugs, three possess the ADME-Tox properties.

The definition of a drug target is crucial to the success of drug discovery.[31] Targeting multiple viral proteins is a novel concept for drug repurposing. In the same vein as drug cocktail or drug combination screening, the rationale for protein combinations is to choose drug candidates that target and block different stages of the virus' life cycle. This is in stark contrast to most existing work for repurposed drugs that exclusively focuses on one single protein target. Therefore, our approach, if successful, has great potential to attack the virus from different angles.

Although the drugs screened in this study are already FDA-approved, they do not have the same safety, quality, and effectiveness assurances. The FDA-approved drugs do not always comply with the "rule-of-five" because they were approved to serve a particular medical need for patients. The trade-offs could have adverse drug reactions and severe side-effects for treatments (such as COVID-19) other than the original

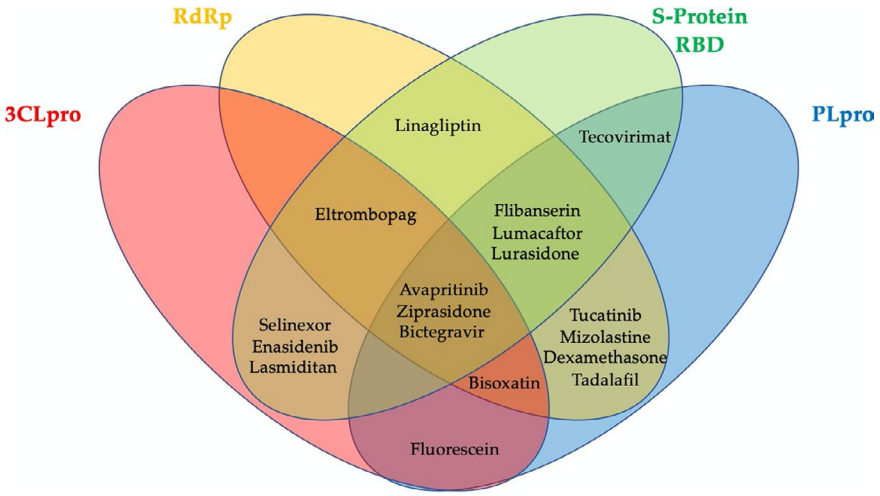


FIGURE 3 The Venn diagram showing all possible drug candidates between different host proteins (coronavirus main proteinase [3CLpro], RNA-dependent RNA polymerase [RdRp], receptor-binding domain in the spike protein [S-Protein-RBD], and papain-like protease [PLpro]). Avapritinib, ziprasidone, and bictegravir in the center, discovered by our screening procedure, are three common targets among all the four key host proteins while having the absorption, distribution, metabolization, and elimination/toxicological filtering properties

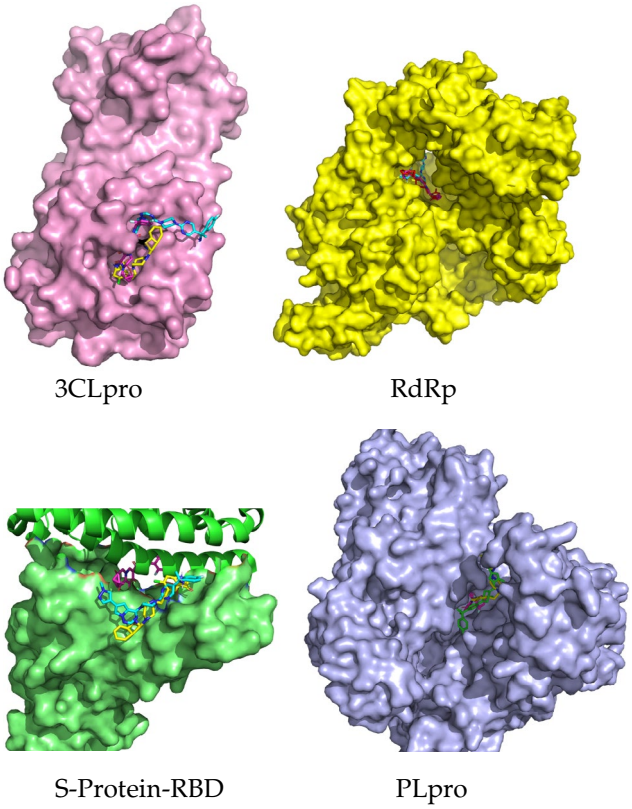


FIGURE 4 Overlaid docked positions of three drug targets (avapritinib, ziprasidone, and bictegravir) binding to individual host proteins: coronavirus main proteinase (3CLpro; PDB ID: 6LU7, pink), RNA-dependent RNA polymerase (RdRp; PDB ID: 6M71, yellow), receptor-binding domain in the spike protein (S-Protein-RBD; PDB ID: 6LZG, green), and papain-like protease (PLpro; PDB ID: 6W9C, purple)

purpose. Modern drug discovery stresses the importance of simultaneous optimization of many physicochemical and biological properties, and incorporation of optimal ADME-Tox properties.[32,33] The drugs that fail to comply with the famous Lipinski's rule of five generally have poor pharmacokinetic properties. Such drugs may show poor absorption, faster rate of metabolism and excretion, unfavorable distribution, and might be toxic in nature. As such, the drug screening with the ADME-Tox properties is an important consideration, particularly for new drug development. Therefore, it is prudent to include ADME-Tox properties, which allow resources to be focused on potential drug candidates. We also caution that a more balanced approach to drug discovery should be more productive than to rely on an overemphasis of rule-of-five compliance.

A couple of findings strongly support that targeting RdRp, 3CLpro, PLpro, and S-Protein-RBD in combination is a viable strategy for repurposed drugs. First, among the five drugs (avapritinib, bictegravir, ziprasidone, capmatinib, and pexidartinib) identified being common candidates among the four key host proteins considered, bictegravir is an antiviral drug that has been reported as one of the best drugs for SARS-CoV-2.[34] For two drugs (capmatinib and pexidartinib) that do not meet the ADME-Tox properties, both remain unreported for treating COVID-19, thus deserve attention for potential repurposing against SARS-CoV-2. Other drugs on our short list, such as bisoxatin and selinexor are also recently identified as potent repurpose drugs to develop new chemical libraries for inhibiting SARS-CoV-2 entry into the host.[35,36] Of particular note is dexamethasone on our list, which is a corticosteroid medicine with predominantly anti-inflammatory glucocorticoid effects. The mechanism of action of dexamethasone is more on the modulation of human immune response rather than direct inhibition of viral replication.[37] It has been recommended in patients with COVID-19 on

TABLE 3 The binding affinity, represented as the minimum binding free energy in kcal/mol, for select drug candidates from recent studies against four key proteins (RdRp, 3CLpro, PLpro, and S-Protein-RBD) of SARS-CoV-2

Drug name	3CLpro (6LU7)	RdRp (6M71)	S-Protein-RBD (6LZG)	PLpro (6W9C)
Hydroxychloroquine	−4.5	−5.7	−6.2	−6.2
Chloroquine	−4.4	−6.0	−5.7	−6.5
Remdesivir	−5.5	−7.5	−6.3	−7.2
Lopinavir	−4.7	−7.8	−7.3	−8.8
Ritonavir	−5.9	−6.9	−6.3	−7.9
Umifenovir	−4.8	−6.0	−5.8	−7.2
Favipiravir	−5.6	−4.9	−5.2	−4.9

Note: that none of these drugs makes into our short list.

Abbreviations: 3CLpro, coronavirus main proteinase; PLpro, papain-like protease; RdRp, RNA-dependent RNA polymerase; SARS-CoV-2, severe acute respiratory syndrome-coronavirus 2; S-Protein-RBD, receptor-binding domain in the spike protein.

oxygen or mechanical ventilation by multiple government agencies, such as the National Institutes of Health (NIH), and recently by the World Health Organization (WHO) and the European Medicines Agency (EMA). The use of dexamethasone, however, is only recommended in adults and adolescents who require supplemental oxygen therapy, as supported by recent data.[37] Second, our molecular docking analysis reveals that some drug candidates do not solely interact with one single protein target, instead, they actively bind to multiple viral proteins. Take a widely publicized example of the antiviral drug, remdesivir, we confirmed that it indeed has the strongest binding affinity (−7.5 kcal/mol) to RdRp among the four proteins we considered, which is consistent with the recent experimental data.[38] Therefore, our method not only is useful for selection of candidate drugs, but also can be utilized for identification of protein binding sites.

We note, however, that remdesivir, albeit just FDA-approved, does not make the cut for our short list. It has at least two reasons: (1) its molecular weight (602.58 Daltons) is greater than 500 Daltons, which may limit pulmonary drug delivery following oral route,[39] and (2) its binding affinities to other three proteins (3CLpro, PLpro, and S-Protein-RBD) are, respectively, −5.5, −7.2, and −6.3 kcal/mol, which do not meet our screening criteria. Caveat should be given that all of the reasons are purely rooted from the proposed computational approach and may not be consistent with the actual drug performance in the clinic. For instance, a product's clinical performance can be different if there are factors overlooked or not sufficiently addressed by the current approach and/or the drug is given through a different delivery route (e.g., through oral inhalation). This exception, while providing a negative control over our method, also raises the question about the limitation of our screening method, which is primarily based on the binding affinity. The additional evaluation of toxicity should complement the existing toxicity profiles, allowing one to compare across all products, including investigational drugs and get the least toxic. Although the drugs selected by our screening procedure show excellent binding affinity to the target proteins, other drugs that do not possess the binding affinity as strong as ours and/or do not meet the ADME-Tox screening criteria can be potential candidates if additional knowledge of the molecular details of SARS-CoV-2 infection is considered. As such, it should be pointed out that drugs that have not been identified through our screening process may still have beneficial effects.

We note that a pH 7.0 is used in this study to estimate the binding affinity (i.e., binding free energy) with the molecular docking software AutoDock Vina.[20] It has been known that pH that prevails in the human body is ~ 7.4. However, it may vary across different tissues. For lung tissues, it has been reported that pH is around 6.6 for epithelial fluid and 6.7 for lung tissues and low/high airways.[40] Although physiologically normal intracellular pH is most commonly between 7.0 and 7.4, there can be variations across different organelles that can span from around 4.5 to 8.0.[41] In addition, pH can be more acidic for extra-thoracic, thoracic, bronchiolar, and alveolar-interstitial tissues. Recent studies[42] used a pharmacokinetic modeling approach to show that the changes in lung pH can affect lung exposures in patients with COVID-19. Based on these understandings, we calculated the binding affinity for the top-listed 3 drugs with a pH range of 5.0–8.0 to check the potential impact of the pH values on the binding affinity for all 4 proteins. The results are shown in Table 4, where we observe that the variations in the affinity are rather small when the pH values vary, indicating a relatively insensitivity of our screening method to the pH values. Although we found that the binding free energy is quite robust to the variations in pH values, we acknowledge that the use of pH of 7.0 may not be optimal given multiple viral proteins involved and various stages of the viral dynamic cycle.

Drug repurposing is an effective strategy for identifying new therapeutic purposes from existing drugs, which could shorten the time and reduce the cost compared with de novo drug discovery. Among various drug repurposing strategies, this work represents our effort to identify additional

TABLE 4 The binding affinity, represented as the minimum binding free energy in kcal/mol, for the top-listed 3 drugs at the selected pH value ranging from 5.0 to 8.0 with a step of 0.5 for all 4 key proteins (RdRp, 3CLpro, PLpro, and S-Protein-RBD) of SARS-CoV-2

		Binding affinity		
Protein	**pH**	**Avapritinib**	**Bictegravir**	**Ziprasidone**
3CLpro (6LU7)	5.0	−7.1	−6.8	−7.1
	5.5	−7.1	−6.8	−7.1
	6.0	−7.1	−6.8	−7.1
	6.5	−7.1	−6.8	−7.1
	7.0	−7.1	−7.0	−7.0
	7.5	−7.1	−7.0	−7.0
	8.0	−7.1	−7.0	−7.0
RdRp (6M71)	5.0	−8.7	−8.6	−8.8
	5.5	−8.7	−8.6	−8.8
	6.0	−8.7	−8.6	−8.8
	6.5	−8.7	−8.6	−8.8
	7.0	−8.7	−8.7	−9.0
	7.5	−8.7	−8.7	−9.0
	8.0	−8.7	−8.7	−9.0
S-Protein-RBD (6LZG)	5.0	−7.3	−8.4	−8.0
	5.5	−7.3	−8.4	−8.0
	6.0	−7.3	−8.4	−8.0
	6.5	−7.3	−8.4	−8.0
	7.0	−7.7	−8.4	−8.0
	7.5	−7.7	−8.4	−8.0
	8.0	−7.7	−8.4	−8.0
PLpro (6W9C)	5.0	−9.6	−9.8	−9.4
	5.5	−9.6	−9.8	−9.4
	6.0	−9.6	−9.8	−9.4
	6.5	−9.6	−9.8	−9.4
	7.0	−9.6	−9.8	−9.3
	7.5	−9.6	−9.8	−9.3
	8.0	−9.6	−9.8	−9.3

Shaded are the binding affinities calculated at the pH value of 7.0, as reported in this study. Note that that the binding affinity is quite robust to the variations in pH values.

Abbreviations: 3CLpro, coronavirus main proteinase; PLpro, papain-like protease; RdRp, RNA-dependent RNA polymerase; SARS-CoV-2, severe acute respiratory syndrome-coronavirus 2; S-Protein-RBD, receptor-binding domain in the spike protein.

unanticipated therapeutic options with accelerated evaluation for the treatment of COVID-19. The elucidation of additional candidate therapies would greatly enhance the probability of rapidly identifying safe and efficacious treatment options. As such, it would mitigate the potential drug shortage during the current pandemic outbreak, and further provide an opportunity to develop generic drug products with equivalent therapeutic effect. Therefore, it is critical that multiple therapeutic options that demonstrate efficacy against SARS-CoV-2 are available to mitigate potential emergence of drug resistance and drug shortages.

The use of repurposed drugs relies on the assumption that the benefits outweigh associated risks (adverse drug reactions). One key consideration to using repurposed agents is the propensity of these agents to cause acute toxicity, which has not yet been carefully vetted by drug repurposing methods currently available. This acute toxicity, particularly for combination therapy ("drug cocktail"), may outweigh the undefined benefit of a specific antiviral agent. This is of upmost concern in patients at high risk for toxicity and in situations where adverse events may preclude entry into investigational trials. Therefore, toxicity of the potential candidates should be properly assessed, as we have done in this work.

In conclusion, in silico screening the FDA approved drugs against multiple proteins of SARS-CoV-2 can provide valuable insights to fast-track clinical trials for drugs with an established safety profile. Several top hits from our short list, including the five drug candidates actively binding to all four key host proteins, could be beneficial for treatment of coronaviral infections. The targets identified in this paper provide new candidates for future research studies and clinical intervention protocols. Additionally, we propose a novel screening strategy targeting multiple viral proteins, which may provide guidance in screening antiviral drugs from other drug databases.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

All authors wrote the manuscript. H.L. and H.W. designed the research. H.L., L.Z., X.G., M.H., and H.W. performed the research. H.L. and H.W. analyzed the data.

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The opinions expressed in this manuscript are those of the authors and should not be interpreted as the position of the US Food and Drug Administration.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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