Identification of Potential SARS-CoV-2 Inhibitors Using Flexible Docking Based Drug Repurposing of Antivirals

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Abstract

A selection of antiviral compounds from the Drug Repurposing Hub were screened as potential inhibitors against SARS-CoV-2 protein targets using CIFDock, a flexible docking method. CIFDock allows for a fully flexible active site of the protein-ligand complex and retaining of explicit water molecules throughout docking simulations. This method provides a more thorough conformational space search than is attainable by rigid docking methods, and thus a more accurate representation of the binding interactions between these antiviral compounds and the SARS-CoV-2 protein targets. Four proteins critical to viral function were selected as targets of the study: the main protease (Mpro), the papain-like protease (PLpro), the transmembrane protease (TMPRSS2), and the RNA-dependent RNA-polymerase (RdRp). The results reveal potential SARS-CoV-2 viral inhibitors from this library of antivirals, based on favorable Glide scores of the docked protein-ligand poses. The antiviral compounds brecanavir, mozenavir, palinavir, sovaprevir, and telinavir yielded excellent binding scores across all protease targets. Additionally, these particular antivirals have not yet been investigated in clinical trials nor *in vitro* studies regarding COVID-19. Therefore, these compounds can be recommended for further research against SARS-CoV-2, based on extensive docking analysis with relevant protein targets.

Keywords: COVID-19, SARS-CoV-2, flexible docking, antivirals, drug repurposing

INTRODUCTION

Background

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has remained a worldwide crisis claiming the lives of more than 4 million people. Originating in the Hubei province of China in November of 2019, the novel coronavirus has affected more than 200 million people due to its high infectivity rate. Researchers have been locked in a race against time to discover a treatment, be it an antiviral, plasma treatment, etc., to combat the virus. While many antiviral drugs have

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been investigated as a treatment for COVID-19[1–12], there still exists no definitive, effective, and regulatory-appproved treatment for individuals infected with COVID-19 other than management of symptoms. Although effective vaccines have been developed to prevent the disease, vaccine distribution has been sluggish and a significant portion of the world population will remain unvaccinated for the foreseeable future. Even with a vaccine, SARS-CoV-2 variants and breakthrough infections (i.e., those occuring in a vaccinated person) pose a serious health risk to the general population. To this end, researchers are employing virtual screening and other protein docking methods as tools of drug repurposing[13–21] to speed the process of discovering a treatment.

SARS-CoV-2 is in the SARS family of viruses (i.e., beta coronaviruses), encompassing also SARS-CoV-1 (causing the disease known simply as SARS) and MERS (Middle Eastern Respiratory Syndrome).[22] SARS-CoV-2 is a positive strand RNA virus with open reading frames containing 16 non-structural proteins and 4 structural proteins. The SARS-CoV-2 virus is primarily composed of four structural components: the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. The spike protein is important to the binding of the virus to a host cell's ACE2 receptor (the primary mechanism of infection).[23] Two of the open reading frames of the viral RNA code for the chymotrypsin-like (main) protease and the papain-like protease. Both of these are responsible for cleavage of a polyprotein chain, leading to the formation of the additional 16 non-structural protein subunits important to viral function and replication.[24] These non-structural proteins have been one of the main targets of inhibition as they provide necessary functions to the virus such as blocking host immune response. One of many studies virtually screening proteins from the SARS-CoV-2 virus, Castelan-Vega et al. virtually screened thousands of drug molecules against the main protease, returning approved drugs that could possibly be used as treatment of COVID-19, including an antibiotic and chemotherapy compound.[25] Such results brings with it the possibility of other compounds that can then be tested clinically to observe their effect on the SARS-CoV-2 virus.

Strategy

Extensive research is being done to discover a treatment for COVID-19, in the form of clinical trials, screening assays, and virtual screening. In terms of real cost and time required to invest in these endeavors, clinical trials and biological assays are the most demanding, but can lead to more conclusive results as to whether a certain drug inhibits the virus. Virtual screening, on the other hand, incurs a lessened cost while also providing the capability to investigate a larger library of compounds. The goal of virtual screening is to provide experimentalists and clinical researchers a better idea of which drug to investigate first when attempting to treat COVID-19, or in some cases, give them insight into a drug that may inhibit viral function that may not have originally been a candidate. Ideally, virtual screening will reveal a potential drug that has already been approved for use that can then be evaluated as a COVID-19 treatment. Known as drug repurposing, this technique aims to employ previously developed drugs for a new disease.[26] This pandemic has highlighted the need for a treatment of infected individuals, developed quickly and safely. Repurposed drugs often have the added benefit of already passing clinical trials which deem the drug safe to use, decreasing the time between discovery and clinical use.

There exists a multitude of options for effective virtual screening, but typically a rigid docking based method is used. This is often done in order to facilitate high-throughput screening of larger compound databases, but in practice this leads to a non-negligible number of false-positives and

false-negatives. Rigid, and even semi-flexible, docking methods restrict movement of the protein-ligand complex to reduce the computational load required to dock compounds. It is through this movement restriction that a rigid docking method can misidentify poor binding ligands and vice versa. Naturally, this causes problems when using a rigid docking method in the drug repurposing workflow.[27] False-positives that are identified through virtual screening and sent forth for assay or clinical testing can potentially waste valuable time and resources.[28] Potential hits from a rigid docking screening method require additional validation (e.g., with MD) before consideration with experimental analysis.[29] Therefore, this study utilizes a novel flexible docking method, CIFDock, to screen antiviral compounds as potential SARS-CoV-2 inhibitors. CIFDock uses Confab ligand sampling, molecular dynamics, and select active site alanine mutation/backmutation to allow for full flexiblity of the ligand, protein active site, and explicit water molecules. Conformational changes of the ligand can induce conformational changes of the protein through the rigorous workflow implemented in CIFDock. This in turn will provide a more accurate representation of the binding modes of these antiviral compounds than would be gained from a rigid docking method, and can identify potential drug candidates with greater confidence.

METHODS

Protein Selection

The selection of proteins in the SARS-CoV-2 virus for this study was based on the relative importance of the protein to the viral function. For example, it is known that the main protease of the SARS-CoV-2 virus is involved in the production of protein subunits necessary for the viral infection of host cells. [24] As such, it would be prudent to investigate drug targets that have the ability to bind and possibly inhibit this protease. Four protein targets were chosen that are important for the virus to infect and replicate. In particular, two cysteine proteases, Mpro and PLpro, have gained significant attention for their role in releasing various non-structural proteins from a polypeptide chain. After entry into the cell, SARS-CoV-2 uses positive-sense mRNA to produce polyproteins pp1a and pp1ab. Upon cleavage by Mpro and PLpro, all 15 non-structural proteins necessary for replication and transcription are released.[30]

Non-structural protein 3 (the papain-like protease, PLpro) is a multi-domain transmembrane cysteine protease that cleaves four sites along pp1a/pp1ab and releases nsp1, nsp2 and nsp3. PLpro contains a catalytic triad (Cys111, His272, Asp286) which uses Cys111 as a nucleophile, while Asp286 deprotonates His272 to serve as a base to catalyze peptide bond cleavage. PLpro is found in all coronaviruses, is highly conserved, and has been suggested to block host innate immune response.[31] This protease is important for the ability of the virus to use other non-structural proteins that would otherwise remain a part of a larger polyprotein chain. Therefore, inhibition of this protease may lead to the inability of the virus to cleave and unlock these other non-structural proteins, inhibiting its ability to infect and replicate. Non-structural protein 5 (the main protease, Mpro) is another transmembrane cysteine protease that cleaves the remaining non-structural proteins (i.e., nsp5 through nsp15) from pp1a/pp1ab. The Mpro catalytic dyad (Cys145, His41) catalyzes acetylation/deacetylation during the breakdown of peptide bonds in pp1a/pp1ab.[32]

Non-structural protein 12 is a subunit of the trimeric RNA dependent, RNA polymerase (RdRp) for SARS-CoV-2. This protein has the important role of assisting in RNA synthesis by catalyzing the formation of phosphodiester bonds.[33] TMPRSS2, a serine protease, is vital to the infectivity of SARS-CoV-2 into the host cell. TMPRSS2 assists the virion by activating the S (spike) protein via

proteolytic cleavage, which in turn allows for binding to the ACE2 receptor in the host cell.[34] The catalytic triad (Asp345, His296, Ser441) functions in a two-step catalysis, whereby a covalent acyl-enzyme intermediate is formed between ACE2 and Ser441. Then, hydrolysis of the acyl-enzyme intermediates releases both the substrate and the active form of the enzyme.[35]

Protein Preparation

The structures of each protein target, with the exception of TMPRSS2, were taken as crystal structures from the Protein Data Bank[36] (PDB) as 6LU7 (main protease), 7BV2 (RdRp), and 6W9C (papain-like protease). The TMPRSS2 structure was created from homology modeling using SWISSMODEL.[37] Structures were then pre-processed through CHARMMing[38] to determine ionization states with PROPKA, add missing hydrogens, and assign correct bond orders. The protein structures were solvated in a ~50000 count TIP3 water box and neutralized with potassium and chloride ions to a target concentration of 0.15 M. This system was then heated from 110 K to 310 K (biological temperature) across a 40 nanosecond (ns) timescale using molecular dynamics (MD) in CHARMM.[39] After heating, the system was subject to a 20 ns MD simulation to allow time for the temperature and structure of the protein-ligand complex to reach stability. To provide multiple receptor structures of the protein targets for flexible docking, pairwise RMSD clustering was performed on the trajectory of the MD simulation of the final 20 ns equilibration run. Active site residue RMSD was evaluated for all frames of the equilibrium trajectory, and structurally similar conformations (up to a 2 Å cutoff) were "clustered" into groups. Each unique structural group was saved as a discrete structure file representative of that cluster group. The highest RMSD frame was chosen as the representative structure file for each group. This produced seven unique initial receptor structures for Mpro, nine for PLpro, nine for TMPRSS2, and six for RdRp. Each of these unique structures were used as initial receptor conformations for each protein target. In the end, all antiviral compounds were docked into each unique initial receptor structure per target.

Ligand Selection

The selection of antiviral drug targets was founded on the principle of targeting similar mechanisms of inhibition. For instance, some of the antivirals selected were developed as HIV treatments, which commonly target the inhibition of the HIV protease. Based on the idea of drug repurposing, such a class of drugs is an ideal candidate for targeting the inhibition of SARS-CoV-2 proteases. Compounding this idea, both existing HIV inhibitors as well as HCV (hepatitis) inhibitors are potentially favorable candidates for the SARS-CoV-2 virus.[40] A selection of protease inhibitors for the three protease targets (Mpro, PLpro, and TMPRSS2), and a selection of polymerase inhibitors for the RNA polymerase (RdRp), were chosen by initial search of the Drug Repurposing Hub database.[41] Both selections include FDA approved drugs, drugs in clinical trials, and investigational drugs. Care was taken to ensure that ligands selected for the docking procedure were already synthesized as drug candidates. Therefore, favorable drug candidates from the *in silico* screening can be immediately used for *in vitro* analysis or clinical trials.

Ligand Preparation

Ultimately, a list of 30 protease inhibitors and 22 polymerase inhibitors comprised the ligands that were docked into the four SARS-CoV-2 protein targets with CIFDock. Final antiviral structures

were downloaded as SDF files from the chEMBL database[42], and the SDF files were converted to MOL2 using OpenBabel[43]. These MOL2 files were uploaded to ParamChem[44] to produce combined topology and parameter stream files necessary to model the ligands correctly with the CHARMM General Force Field (CGenFF).[45] Next, the conformational space of each of these ligands were sampled using Confab (a module of the OpenBabel program), which generates additional ligand conformations by rotating any rotatable bonds of the molecule. A 50 kcal/mol energy cutoff and a 0.5 Å RMSD cutoff were used during Confab conformer generation. The number of ligand conformations generated by this method varied with the number of rotatable bonds, but was capped at 200 conformations to ensure time efficiency of the docking procedure.

Docking Method

CIFDock

The flexible docking portion of this study employs a novel CHARMM-based flexible docking method (CIFDock).[46] CIFDock incorporates induced fit, in which conformational changes of the ligand induce conformational changes in the protein. This method allows for enhanced flexibility of the protein-ligand complex active site, as well as retaining explicit solvent and any co-factors throughout the docking procedure. To achieve this level of flexibility, bulky residues in the active site of a protein that may intrude upon the active site when sampled with dynamics are first mutated to alanine. This allows for a more “open” active site that is better able to accommodate larger and more flexible ligands. This is a feature unqiue to flexible docking methods, and is an important component when considering using molecular docking in drug design and drug repurposing.[27] Modern antiviral compounds are often very large molecules, and without "opening" up the active site, a rigid docking method will struggle to accomodate a large antiviral in a confined active site. Consequently, a rigid docking method may reject that particular protein-ligand docking outright if atoms overlap or steric clashes are too high to find an energy minimum.

Next, the ligand of interest is randomly placed in the protein active site and SGLD[47] (Self-Guided Langevin Dynamics) are run on the ligand to produce a variety of initial conformations. Finally, the alanine residues in the active site are back-mutated to their original residues. During this backmutation, residues are replaced with randomized dihedral angles, providing another element of protein flexiblity to the docking procedure. In the case that a backmutated side chain overlaps with any atoms, a new dihedral angle is assigned until there is no overlap. The active site of the protein-ligand complex is then, again, simulated with SGLD to allow enhanced sampling of the protein-ligand complex conformational space, with possible ligand-induced conformational changes of active site residues. A set of four custom-designed, all-atom scoring functions are combined into an ensemble docking score to evaluate the poses generated by CIFDock. Each scoring function evaluates the intramolecular interactions (e.g., bonds, angles, improper torsions, dihedrals, ligand strain energies), as well as the intermolecular interactions (e.g., solvation free energies, van der Waals, and electrostatic energies). Electrostatic terms were accounted for with a Coulombic potential and van der Waals terms with a Lennard-Jones potential. This procedure was conducted, per target, for all antiviral ligands and all unique protein conformations generated during protein preparation.

Scoring and Analysis

The quality of all final poses generated from the flexible docking of antivirals is ultimately assessed with the Glide scoring function. Glide scores are correlated with the binding free energy

of a protein-ligand complex[48] and have become a widely-accepted format for virtual screening results, and as such all pose scores in this paper are reported as these Glide scores. Final poses generated by CIFDock are first subject to the custom scoring functions, which score all poses generated during the docking and select the top twenty-five poses. These twenty-five poses are then assigned a Glide score using the same Python script that Schrödinger's Maestro program uses to score final poses from its Glide docking method.

The final poses generated from the flexible docking are roughly categorized into three sections based on their Glide scores. Poses with a Glide score greater than -7 are not considered hits against the given protein target, while poses less than -7 but more than -10 are "decent" hits that warrant further *in silico* evaluation. Poses with a Glide score less than -10 are considered promising hits against the given protein target and are good initial candidates for *in vitro* assay analysis and potential subsequent clinical trials. Extremely low-scoring antiviral poses were selected for further analysis by visualizing protein-ligand interactions in Schrödinger's Maestro. The Results section is organized by protein target, with a discussion of these selected extremely-low ranking poses and their interactions with aforementioned targets.

RESULTS AND DISCUSSION

General Overview of Scores

Of the four SARS-CoV-2 protein targets, three of them (in particular, the proteases) achieved Glide scores with highly favorable binding when docked with the selected antiviral drug candidates. Mpro, PLpro, and TMPRSS2 had, on average, a ligand binding score of -10.27, -7.32, and -10.36, respectively. The scores for the RNA polymerase, when compared with the proteases, are less favorable, with an average Glide score of -7.03. An explanation for this will be provided below. For each of these protein targets, certain antiviral compounds bound very well in docking (e.g., a Glide score of -11 and below). For the main protease, lopinavir, telinavir, and saquinavir were the top three ligands, based on the lowest Glide scores of -15.76, -15.69, and -15.20, respectively. For the papain-like protease, voxilaprevir, lopinavir, and sovaprevir were the lowest scoring ligands at -11.02, -10.76, and -10.66, respectively. Finally, the three best ligands for TMPRSS2 included telinavir, glecaprevir, and grazoprevir, with Glide scores of -15.11, -14.03, and -13.96, respectively. Among the low scoring compounds listed above, it is noted that only one of these drugs, lopinavir, has been tested as a treatment for COVID-19.[49] Although the outlook seemed promising based on successful treatment with SARS-1 patients, the drug combination was found to have little useful effect in severe COVID-19 cases. This may be the result of any multitude of factors though, including low bioavailability, dosage, patient reaction, etc. Interestingly, lopinavir is the drug candidate that consistently appeared in the top three lowest Glide scores across all three protease targets. This reinforces the idea that the other top scoring drugs in this study are good initial candidates for further laboratory and potential clinical testing against the SARS-CoV-2 virus. While the initial clinical results of lopinavir as a COVID-19 treatment were disappointing, it serves as a validation of the effort to identify potential initial candidates for drug repurposing. As of writing, all of the drugs discussed in the Results section below remain untested in clinical trials nor had their inhibitory potential measured against the SARS-CoV-2 main protease.

Docking Scores and Inhibition Potential

Not all of the antiviral compounds docked in this study are novel in being screened against SARS-CoV-2 protein targets, and as such an extensive literature search was conducted to evaluate

previous reports on these compounds. Of particular interest was whether or not these compounds had been investigated with *in vitro* assay analysis, an important step in evaluating the effectiveness of a drug inhibiting SARS-CoV-2 viral proteins. Table 1 lists the protease inhibitors screened in this study, along with an indication of whether each compound has been tested in clinical trials, whether it has been screened in an assay with the main protease, and the IC50 value if assay data exists. Two interesting trends emerge from this literature analysis. One is that these antiviral compounds are decent intial leads that should be considered for further analysis as a COVID-19 treatment, given the excellent Glide scores in this study and promising biological assay results from the literature.

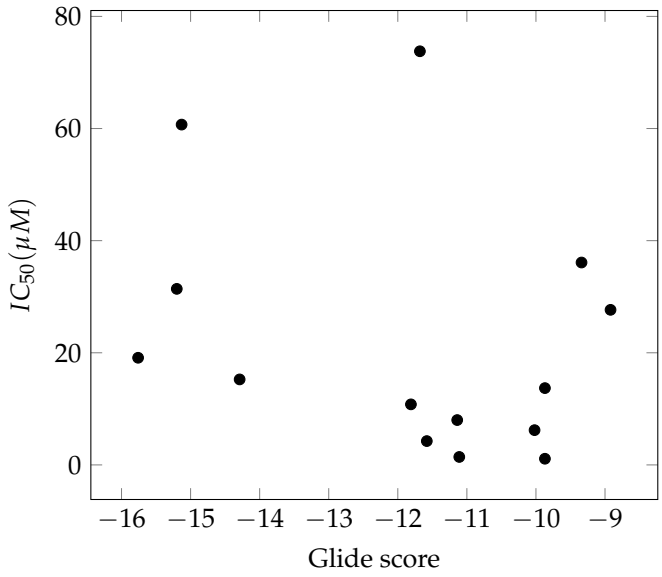

Figure 1: Plot of Glide scores of the antivirals docked into the main protease against the reported IC50 values, if assay results exist.

The second trend concerns the IC50 values that are being reported for these antivirals, which appear to show no correlation with the Glide scores reported from the flexible docking in this study. Figure 1 shows a graph of Glide scores of the antivirals docked into the main protease in this study against the IC50 values of these compounds reported from the literature. A poor trend line, near-zero R-squared value, and a Pearson coefficient of -0.28 indicate no correlation between docking score and IC50, an insight that is becoming prevalent as more assay results are being reported.[50–52] This incongruity of docking analysis with assay results may be a result of myriad factors, especially considering the complexity of the SARS-CoV-2 virus. One hypothesis concerns the nature of the SARS-CoV-2 main protease existing as a homodimer and therefore possessing two active sites. In order to achieve decent inhibition, an antiviral compound may need to bind to a specific active site, or even both simultaneously. Additionally, a compound binding into one of the active sites or the dimer interface may induce conformational changes elsewhere in the main protease, leading to inhibition. This includes the possibility of a compound binding to the Mpro monomer and interfering with the dimerization process, which could lead to inhibition when considering that Mpro function relies on existing as a dimer.[53] Situations such as these are beyond the scope of a molecular docking based screening approach, and would require a longer time scale

MD analysis of the main protease.[54] Alternatively, there may exist hitherto undiscovered cryptic binding sites on the main protease where these antiviral compounds are docking in to achieve high inhibition. These cryptic sites could be located with a variety of well-known methods[55–57], and subsequent docking into these sites could provide more insight into the reason some antivirals have higher inhibition than others. Rationalizing this seeming lack of correlation between traditional docking score and IC50 values will certainly require additional work and is an active area for both computational and experimental scientists.

Analysis of Top Ligands

Mpro

One of the lowest scoring poses from a protein-ligand docking of the main protease comes from lasinavir, an investigational HIV drug. Lasinavir is a peptidomimetic protease inhibitor with a trimethoxybenzene moiety. With a Glide score of -12.92, it constitutes a very favorable binding interaction with the active site of the main protease. While a number of favorable hydrogen bonding interactions with nearby active site waters contributes to this score, a series of active site residue conformations and consequential stabilizing interactions is of far more interest. Figure 2 shows an overlay of the protein conformation of the main protease before docking with the final pose of the protein-ligand complex generated from the docking with lasinavir. When viewed this way, it is much easier to evaluate the movement of active site residues which is vital to the flexible docking method. In the case of lasinavir, there are important conformational changes of certain residues that make this protein-ligand docking such a highly scored pose for the main protease. In particular, the docking of lasinavir results in the movement of catalytic His163 and catalytic His172 into a more favorable conformation that enables a triple π-π stacking interaction with the trimethoxybenze ring on lasinavir.[58] This ultimately results in a four-way π-π stacking network with the nearby Phe140 residue that provides an intensely stabilizing interaction between lasinavir and the main protease. Additional movement of His41 can be seen as the active site of the main protease shifts conformation to accommodate the ligand. Since lasinavir only reached Phase I trials in its investigational study as HIV treatment, it has not been tested clinically against SARS-CoV-2.[59]

Glide scores for the antiviral docking of the main protease were lowest across all protein targets, with an average score of -10.27 and the lowest scored pose of -15.76. Of the 30 antiviral compounds, saquinavir, telinavir and lopinavir scored exceptionally low, indicating a favorable binding affinity to the main protease. When the poses were inspected visually, the lowest scoring final pose of saquinavir exhibits hydrogen bonding with Ser144, Cys117, Tyr118, and His164. Telinavir exhibits a π-π stacking interaction with Phe140 as well as a water-mediated hydrogen bonding interaction with Asn142 and Asp48. Multiple hydrogen bonds can be seen with Glu47, Glu166, Ser144, and Cys145. Lopinavir forms hydrogen bonds with Cys85, His165, Gly146, His41, and a water-mediated hydrogen bond with Thr26.

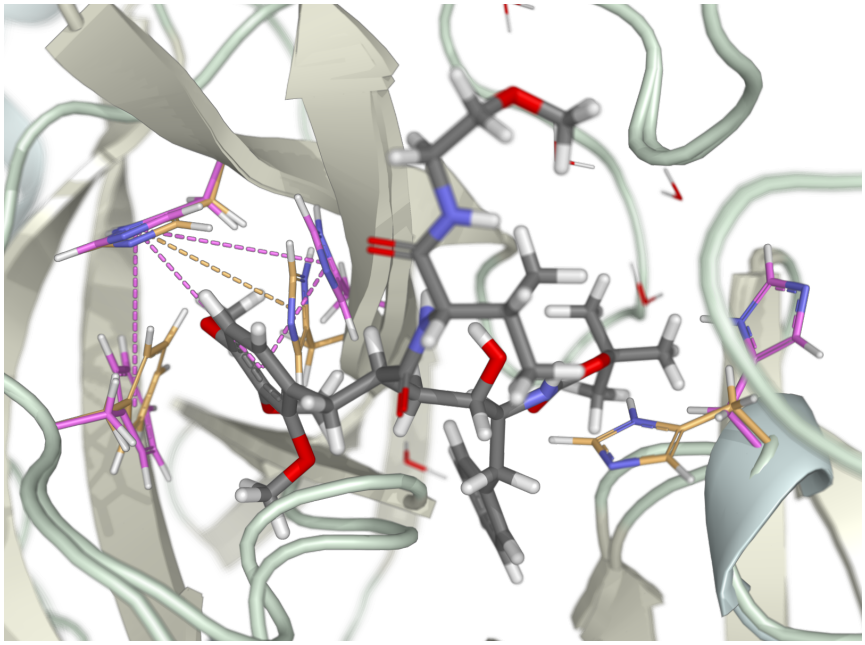

Figure 2: Overlay of the final pose of the docking of lasinavir with the initial structure of the main protease. Residues from the final pose are in purple, while the residues of the initial receptor structure are shown in orange. The movement of His172 can be seen to form a π-π stacking interaction with the ligand, while the movement of His41 can be seen to accommodate the large size of lasinavir in the active site.

PLpro

A top scoring pose from the docking of protease inhibitors with the papain-like protease involves the investigational HIV drug mozenavir. Mozenavir is a non-peptidomimetic substituted aniline compound with an aminobenzene moiety. The final pose had a Glide score of -10.11, with most of that score coming from a highly stabilizing π-π interaction. Figure 3 shows the overlay of the original protein conformation with the final pose of the docked protein-ligand complex. The movement of a critical Tyr262 allows for one of the benzene moieties and one of the aniline moieties of mozenavir to interact with this active site residue to form a dual π-π stacking interaction that is highly stabilizing. In addition, mozenavir forms hydrogen bonds with Gly161 and Gly267. Of particular interest is that mozenavir does not appear often in the current literature, and rarely shows a binding affinity to the papain-like protease in virtual screening. This may be a result of Tyr262 that is protruding into the active site of the protein.[60] Due to the size of the ligand, rigid docking may be ineffective because steric clashes would deem it unsuitable in this active site. As the flexible docking study reveals though, this may be a viable candidate for clinical trials which has hitherto not been considered for SARS-CoV-2.

The average lowest Glide score of the docking of antiviral compounds into the papain-like protease was -7.32, with some antivirals having a lowest score of around -10. Voxilaprevir, sovapreivr, and simperevir were among the best performers, with a Glide score of -11.02, -10.66, and -10.53, respectively. When inspecting the interactions of the final poses visually, voxilaprevir formed a π-π stacking interaction with His270, and hydrogen bonds with Asp162 and Cys268. Sovaprevir

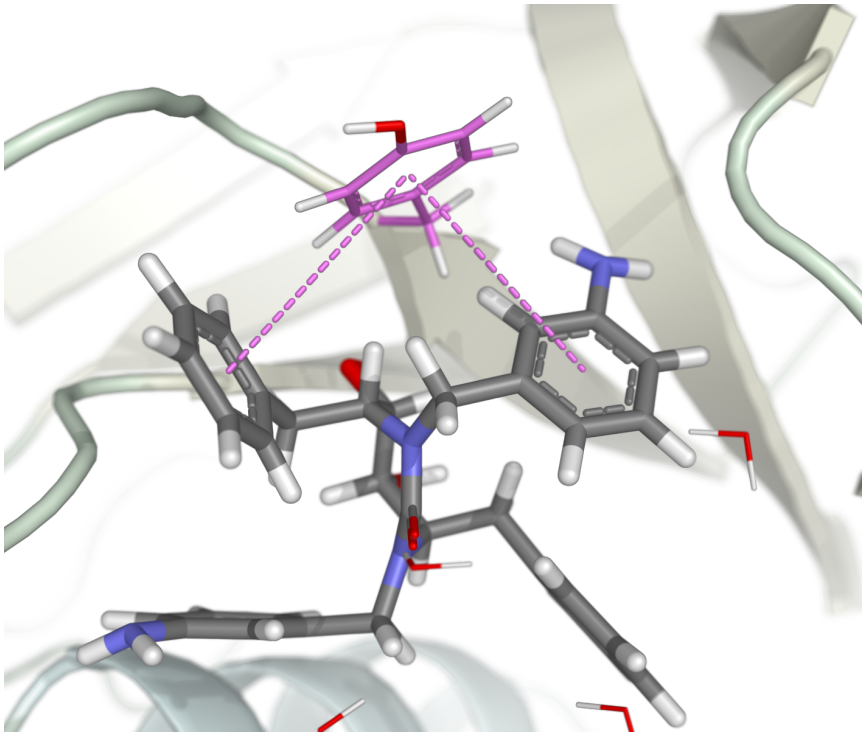

Figure 3: Final pose of the docking of mozenavir with the papain-like protease, showing the dual π-π stacking interaction with active site residue Tyr262.

forms hydrogen bonds with Gly161, Val163, and Thr299. This is particularly interesting, given the large size of sovaprevir and the number of rotatable bonds of the ligand. Simeprevir formed a π-π stacking interaction with Tyr266, as well as hydrgoen bonds with Tyr271 and Val163.

TMPRSS2

Palinavir, with an average Glide score of -9.65 when docked into the active site of TMPRSS2, represents one of the many decent binding ligands for TMPRSS2 in this study. The benefit here, again, is that based on literature searches of virtual screenings and clinical trials with SARS-CoV-2, palinavir has not yet been investigated as a treatment of COVID-19. Palinavir is a peptidomimetic inhibitor with a hydroxyethylamine moiety that was developed for the treatment of HIV type 1. A final pose with a Glide score of -10.65 was selected for further analysis. The topology of palinavir allows for multiple opportunities for hydrogen bonding, of which there are many occurrences in the final complex. This is to the benefit of this particular protein-ligand interaction, as one can see in Figure 4 there is a water-mediated hydrogen bonding interaction of palinavir with catalytic His151.[61] This stabilizing interaction represents a good binding with the transmembrane protease and the potential for further investigation. Additionally, it highlights the importance of modeling explicit waters in docking studies of the SARS-CoV-2 virus, as it can reveal hydrogen bonding interactions with potential drug candidates.

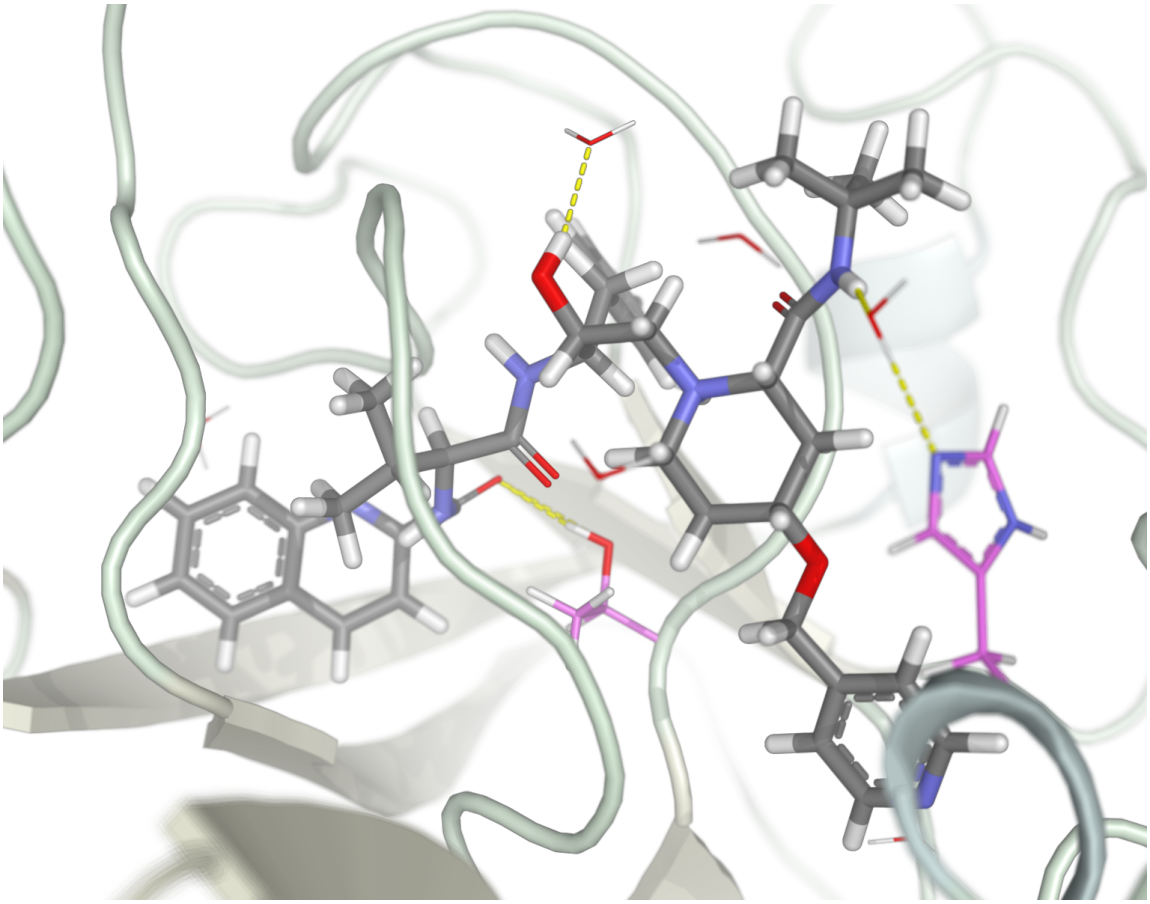

Figure 4: Final pose of the docking of palinavir with the transmembrane protease (TMPRSS2), highlighting the multiple hydrogen bonds between palinavir and the protease, as well as the water-mediated interaction with catalytic His151.

Docking of antiviral compounds into the transmembrane protease provided excellent results, with an average Glide score of -10.36 across all ligands. Atazanavir, narlaprevir, and telinavir, were among the top three ligands with a lowest Glide score of -13.41, -13.56, and -15.11, respectively. Atazanavir formed hydrogen bonds with His151, Gln293, Cys320, and Ser291. Narlaprevir formed numerous hydrogen bonds with Gly298, Ser318, Ser291, Cys292, and Thr314. It also formed two water-mediated hydrogen bonds with Gly317 and Gly294. Telinavir formed a dual π-π stacking interaction between itself and residues Tyr271 and Trp316. It also formed hydrogen bonds with Gly264, Ser291, and Gly319, as well as a water-mediated hydrogen bond with Leu274.

RdRp

As opposed to the three other SARS-CoV-2 protein targets, RdRp inhibition is typically gained through a covalent mechanism. For example, remdesivir functions well because, once it is converted to its active drug form, remdesivir triphosphate, it links covalently to the primer strand of the

template RNA, thus leading to non-obligate RNA chain termination.[62, 63] This mechanism inhibits any further viral RNA replication via the RNA polymerase. Other nucleotide analogues (e.g., galidesivir) have been investigated for inhibition of RdRp, but again these rely on conversion to a triphosphate active form and covalent binding to the RNA primer-template. Because this is an important step of inhibition for the RdRp complex for nucleoside polymerase inhibitors, and because of the nature of the molecular mechanics based dynamics employed during the course of the flexible docking procedure, only the main RdRp subunit (non-structural protein 12) was simulated. This however, allowed for the screening of non-nucleoside polymerase inhibitors as potential inhibitors of the SARS-CoV-2 RNA polymerase.

Non-nucleoside polymerase inhibitors are a class of antiviral compounds widely used to treat infections of the Hepatitis C virus. Unlike nucleoside inhibitors, which compete with nucleotides or the RNA template, non-nucleoside inhibitors bind directly to the RNA polymerase and inhibit conformational changes, thus restricting polymerase activity. Of the 10 non-nucleoside polymerase inhibitors investigated, CIFDock generated final docked poses with Glide scores below -7 for eight out of the ten compounds. Further analysis of these low-scoring final poses was conducted visually to investigate the exact binding of these compounds to the nsp12 domain. One of the lowest scoring polymerase inhibitors, radalbuvir (Glide score = -10.43), was found to form favorable interactions with catalytic Lys465 and Arg475 in the active site. In particular, a cation-π interaction between the thiophene group of radalbuvir and Lys465, as well as a salt bridge between Arg475, the carboxylate moiety of radalbuvir, and Lys465 are both observed. These two residues constitute an important catalytic portion of the active site, with both Lys465 and Arg475 having the role of stabilizing incoming nucleotides in the correct position for catalysis.[64] This suggests that, due to the favorable binding of radalbuvir in the active site, this antiviral compound could potentially act as a competitive inhibitor to the RNA polymerase of SARS-CoV-2.

Overall Glide scores for the docking of antiviral compounds with RdRP are mixed, with lowest Glide scores ranging from -3.69 to -11.32. Of the 20 polymerase inhibitors screened, adafosbuvir, galidesivir, and lomibuvir performed extremely well. Adafosbuvir, with an average lowest Glide score of -9.96, formed a hydrogen bond with Asp604, as well as three water-mediated hydrogen bonds with Asp604, Ser679, and Asp538. Galidesivir, with a score of -9.41, formed a cation-π interaction with Arg473 and hydrogen bonds with Lys471 and Asp543. Galidesivir also formed water-mediated hydrogen bonds with Asp680, Arg544, and Thr476. Lomibuvir, with a score of -7.86, formed a hydrogen bond with Ser679 and water-mediated hydrogen bonds with Asp681, Asp680, and Leu678.

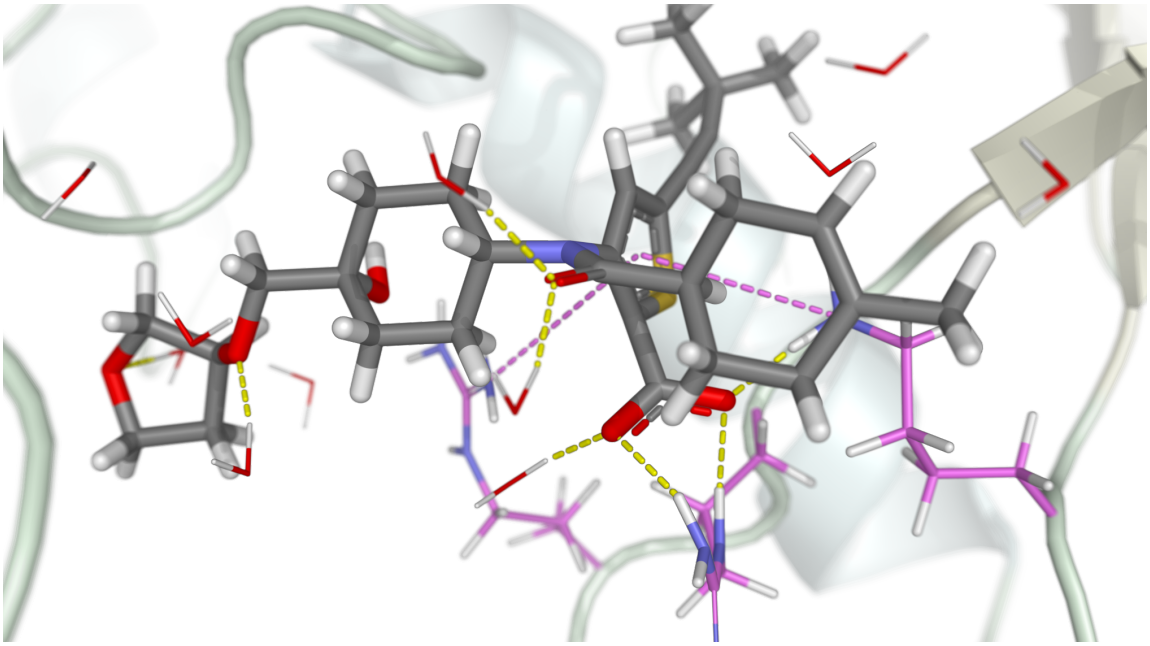

Figure 5: Final pose of the docking of radalbuvir with the RNA-dependent RNA polymerase, highlighting the salt bridge between Lys465, radalbuvir, and Arg475. Hydrogen bonds can be seen with Lys465 and Arg475, which are the residues responsible for stabilizing incoming nucleotides for catalysis. Cation-π interactions are shown in purple, while hydrogen bonds and salt bridges are shown in yellow.

CONCLUSION

As the COVID-19 pandemic has remained a global health crisis, efforts to investigate potential drug therapies for treatment of the disease must continue. This study employed a flexible docking method (CIFDock) to screen a set of antiviral compounds against four protein targets vital to the function of the SARS-CoV-2 virus: Mpro, PLpro, TMPRSS2, and RdRp. Among the initial set of antivirals, potential inhibitors have been identified by low Glide score poses across an ensemble of protein conformations. A thorough screening has been conducted of these antiviral compounds using flexible ligand - flexible receptor docking, with an in-depth analysis of their interactions with relevant SARS-CoV-2 proteins. The flexible docking conducted in this study provides new insight into the binding modes of antiviral compounds that have been previously screened with rigid docking methods while also providing novel candidates for drug repurposing of antivirals that have not yet been investigated. In particular, the investigational drugs furaprevir, lasinavir, mozenavir, sovaprevir, and telinavir exhibited excellent Glide scores when docked into the three protease targets in this study. In addition, these compounds have not been screened in biological assays (at time of writing) and may prove to be decent candidates for a repurposed drug treatment of COVID-19. Therefore, further research can be recommended into the potential clinical applications of these compounds against the SARS-CoV-2 virus.

Table 1: List of protease inhibitors and the score when docked with the main protease, along with an indication of whether clinical trials have been conducted for said compound and reported IC50 values if main protease assay results exist in current literature.

Protease Inhibitor	Mpro Score	Clinical Trials	IC50 (μM)
amprenavir	-9.75	-	-
atazanavir	-15.13	Phase 2[65]	60.70[66]
boceprevir	-11.14	-	8.00[67]
brecanavir	-11.20	-	-
ciluprevir	-11.20	-	-
danoprevir	-10.99	Phase 4[65]	>50[68]
darunavir	-9.34	Phase 3[65]	36.10[66]
deldeprevir	-11.93	-	-
droxinavir	-12.25	-	-
fosamprenavir	-10.33	-	-
furaprevir	-11.71	-	-
glecaprevir	-10.43	-	-
grazoprevir	-11.81	-	10.80[69]
indinavir	-11.29	-	>50[68]
lasinavir	-12.92	-	-
lopinavir	-15.76	Terminated[65]	19.11[70]
mozenavir	-11.74	-	-
narlaprevir	-9.87	-	1.10[71]
nelfinavir	-11.11	-	1.42[72]
palinavir	-13.90	-	-
paritaprevir	-11.68	-	73.78[73]
ritonavir	-9.87	Terminated[65]	13.70[66]
saquinavir	-15.20	-	31.40[66]
simeprevir	-11.58	-	4.25[69]
sovaprevir	-10.99	-	-
telaprevir	-14.29	-	15.25[71]
telinavir	-15.69	-	-
tipranavir	-8.92	-	27.66[73]
vaniprevir	-10.02	-	6.20[69]
voxilaprevir	-13.04	-	-

Table 2: List of protease inhibitors used in the flexible docking portion of this study. Lowest Glide scores from the docking of each of the ligands in each protein target (Mpro, PLpro, TMPRSS2) are also listed.

Protease Inhibitor	Mpro	PLpro	TMPRSS2
amprenavir	-9.75	-8.70	-10.39
atazanavir	-15.13	-9.31	-13.41
boceprevir	-11.14	-8.63	-11.05
brecanavir	-11.20	-10.51	-11.78
ciluprevir	-11.20	-9.78	-12.90
danoprevir	-10.99	-8.67	-13.32
darunavir	-9.34	-8.73	-11.05
deldeprevir	-11.93	-9.81	-12.65
droxinavir	-12.25	-7.63	-10.76
fosamprenavir	-10.33	-9.31	-10.76
furaprevir	-11.71	-9.68	-12.63
glecaprevir	-10.43	-9.18	-14.03
grazoprevir	-11.81	-9.63	-13.96
indinavir	-11.29	-8.58	-11.22
lasinavir	-12.92	-9.89	-12.69
lopinavir	-15.76	-10.76	-12.88
mozenavir	-11.74	-10.30	-12.90
narlaprevir	-9.87	-10.17	-13.56
nelfinavir	-11.11	-7.77	-12.75
palinavir	-13.90	-7.78	-10.45
paritaprevir	-11.68	-8.38	-12.86
ritonavir	-9.87	-9.50	-12.39
saquinavir	-15.20	-6.68	-11.34
simeprevir	-11.58	-10.53	-13.50
sovaprevir	-10.99	-10.66	-12.26
telaprevir	-14.29	-9.72	-11.60
telinavir	-15.69	-9.99	-15.11
tipranavir	-8.92	-8.39	-9.82
vaniprevir	-10.02	-8.98	-11.57
voxilaprevir	-13.04	-11.02	-12.06

Table 3: List of polymerase inhibitors used in the flexible docking portion of this study. Lowest Glide scores of the final poses of each inhibitor docked into RdRp are also listed.

Polymerase Inhibitor	Glide Score
adafosbuvir	-10.78
beclabuvir	-7.32
dasabuvir	-7.39
filibuvir	-8.45
galidesivir	-11.32
lomibuvir	-9.04
nesbuvir	-5.23
niraparib	-6.75
niraparib tosylate	-6.90
olaparib	-7.33
pamiparib	-5.65
radalbuvir	-10.43
remdesivir	-10.32
rucaparib	-8.59
setrobuvir	-7.24
sofosbuvir	-8.44
talazoparib	-7.46
tegobuvir	-5.66
veliparib	-7.75

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Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary Data

Information on selected binding site residue definitions and antiviral structures for this study are available as supplementary data.

References

[1] B. Sayad, M. Sobhani, R. Khodarahmi, Sofosbuvir as repurposed antiviral drug against covid-19: Why were we convinced to evaluate the drug in a registered/approved clinical trial?, Archives of Medical Research 51 (6) (2020) 577–581. doi:https://doi.org/10.1016/j.arcmed.2020.04.018.
URL https://www.sciencedirect.com/science/article/pii/S0188440920305518

[2] Y. Wang, Y. Liu, L. Liu, X. Wang, N. Luo, L. Li, Clinical outcomes in 55 patients with severe acute respiratory syndrome coronavirus 2 who were asymptomatic at hospital admission in shenzhen, china., J Infect Dis 221 (11) (2020) 1770–1774. doi:10.1093/infdis/jiaa119.

[3] B. A. Ahidjo, M. W. C. Loe, Y. L. Ng, C. K. Mok, J. J. H. Chu, Current perspective of antiviral strategies against covid-19, ACS Infectious Diseases 6 (7) (2020) 1624–1634, pMID: 32485102. arXiv:https://doi.org/10.1021/acsinfecdis.0c00236, doi:10.1021/acsinfecdis.0c00236.
URL https://doi.org/10.1021/acsinfecdis.0c00236

[4] A. Frediansyah, R. Tiwari, K. Sharun, K. Dhama, H. Harapan, Antivirals for covid-19: A critical review, Clinical Epidemiology and Global Health 9 (2021) 90–98. doi:https://doi.org/10.1016/j.cegh.2020.07.006.
URL https://www.sciencedirect.com/science/article/pii/S2213398420301767

[5] U. Agrawal, R. Raju, Z. F. Udwadia, Favipiravir: A new and emerging antiviral option in covid-19, Medical Journal Armed Forces India 76 (4) (2020) 370–376. doi:https://doi.org/10.1016/j.mjafi.2020.08.004.
URL https://www.sciencedirect.com/science/article/pii/S0377123720301581

[6] M. Yousefifard, A. Zali, K. Mohamed Ali, A. Madani Neishaboori, A. Zarghi, M. Hosseini, S. Safari, Antiviral therapy in management of covid-19: a systematic review on current evidence., Arch Acad Emerg Med 8 (1) (2020) e45.

[7] S. De Meyer, D. Bojkova, J. Cinatl, E. Van Damme, C. Buyck, M. Van Loock, B. Woodfall, S. Ciesek, Lack of antiviral activity of darunavir against sars-cov-2, International Journal of Infectious Diseases 97 (2020) 7–10. doi:https://doi.org/10.1016/j.ijid.2020.05.085.
URL https://www.sciencedirect.com/science/article/pii/S1201971220303921

[8] J. S. Khalili, H. Zhu, N. S. A. Mak, Y. Yan, Y. Zhu, Novel coronavirus treatment with ribavirin: Groundwork for an evaluation concerning covid-19, Journal of Medical Virology 92 (7) (2020) 740–746. arXiv:https://onlinelibrary.wiley.com/doi/pdf/10.1002/jmv.25798, doi:https://doi.org/10.1002/jmv.25798.
URL https://onlinelibrary.wiley.com/doi/abs/10.1002/jmv.25798

[9] J. Chen, L. Xia, L. Liu, Q. Xu, Y. Ling, D. Huang, W. Huang, S. Song, S. Xu, Y. Shen, H. Lu, Antiviral Activity and Safety of Darunavir/Cobicistat for the Treatment of COVID-19, Open Forum Infectious Diseases 7 (7), ofaa241 (06 2020). arXiv:https://academic.oup.com/ofid/article-pdf/7/7/ofaa241/33482543/ofaa241.pdf, doi:10.1093/ofid/ofaa241.
URL https://doi.org/10.1093/ofid/ofaa241

[10] M. Migaud, S. Gandotra, H. S. Chand, M. N. Gillespie, V. J. Thannickal, R. J. Langley, Metabolomics to predict antiviral drug efficacy in covid-19, American Journal of Respiratory Cell and Molecular Biology 63 (3) (2020) 396–398, pMID: 32574504. arXiv:https://doi.org/10.1165/rcmb.2020-0206LE, doi:10.1165/rcmb.2020-0206LE.
URL https://doi.org/10.1165/rcmb.2020-0206LE

[11] A. Frediansyah, F. Nainu, K. Dhama, M. Mudatsir, H. Harapan, Remdesivir and its antiviral activity against covid-19: A systematic review, Clinical Epidemiology and Global Health 9 (2021) 123–127. doi:https://doi.org/10.1016/j.cegh.2020.07.011.
URL https://www.sciencedirect.com/science/article/pii/S2213398420301810

[12] N. Li, L. Zhao, X. Zhan, Quantitative proteomics reveals a broad-spectrum antiviral property of ivermectin, benefiting for covid-19 treatment, Journal of Cellular Physiology 236 (4) (2021) 2959–2975. arXiv:https://onlinelibrary.wiley.com/doi/pdf/10.1002/jcp.30055, doi:https://doi.org/10.1002/jcp.30055.
URL https://onlinelibrary.wiley.com/doi/abs/10.1002/jcp.30055

[13] N. Pathak, Y.-T. Chen, Y.-C. Hsu, N.-Y. Hsu, C.-J. Kuo, H. P. Tsai, J.-J. Kang, C.-H. Huang, S.-Y. Chang, Y.-H. Chang, P.-H. Liang, J.-M. Yang, Uncovering flexible active site conformations of sars-cov-2 3cl proteases through protease pharmacophore clusters and covid-19 drug repurposing, ACS Nano 15 (1) (2021) 857–872. doi:10.1021/acsnano.0c07383.
URL https://doi.org/10.1021/acsnano.0c07383

[14] K. H. Chowdhury, M. R. Chowdhury, S. Mahmud, A. M. Tareq, N. B. Hanif, N. Banu, A. S. M. A. Reza, T. B. Emran, J. Simal-Gandara, Drug repurposing approach against novel coronavirus disease (covid-19) through virtual screening targeting sars-cov-2 main protease, Biology 10 (1) (2021). doi:10.3390/biology10010002.
URL https://www.mdpi.com/2079-7737/10/1/2

[15] G. Culletta, M. R. Gulotta, U. Perricone, M. Zappalà, A. M. Almerico, M. Tutone, Exploring the sars-cov-2 proteome in the search of potential inhibitors via structure-based pharmacophore modeling/docking approach, Computation 8 (3) (2020). doi:10.3390/computation8030077.
URL https://www.mdpi.com/2079-3197/8/3/77

[16] Y. Kumar, H. Singh, C. N. Patel, In silico prediction of potential inhibitors for the main protease of sars-cov-2 using molecular docking and dynamics simulation based drug-repurposing, Journal of Infection and Public Health 13 (9) (2020) 1210–1223. doi:https://doi.org/10.1016/j.jiph.2020.06.016.
URL https://www.sciencedirect.com/science/article/pii/S1876034120305268

[17] M. Sencanski, V. Perovic, S. B. Pajovic, M. Adzic, S. Paessler, S. Glisic, Drug repurposing for candidate sars-cov-2 main protease inhibitors by a novel in silico method, Molecules 25 (17) (2020). doi:10.3390/molecules25173830.
URL https://www.mdpi.com/1420-3049/25/17/3830

[18] I. Maffucci, A. Contini, In silico drug repurposing for sars-cov-2 main proteinase and spike proteins, Journal of Proteome Research 19 (11) (2020) 4637–4648. doi:10.1021/acs.jproteome.0c00383.
URL https://doi.org/10.1021/acs.jproteome.0c00383

[19] H. Zhang, Y. Yang, J. Li, M. Wang, K. M. Saravanan, J. Wei, J. Tze-Yang Ng, M. Tofazzal Hossain, M. Liu, H. Zhang, X. Ren, Y. Pan, Y. Peng, Y. Shi, X. Wan, Y. Liu, Y. Wei, A novel virtual screening procedure identifies pralatrexate as inhibitor of sars-cov-2 rdrp and it reduces viral replication in vitro, PLOS Computational Biology 16 (12) (2021) 1–20. doi:10.1371/journal.pcbi.1008489.
URL https://doi.org/10.1371/journal.pcbi.1008489

[20] A. Khan, S. S. Ali, M. T. Khan, S. Saleem, A. Ali, M. Suleman, Z. Babar, A. Shafiq, M. Khan, D.-Q. Wei, Combined drug repurposing and virtual screening strategies with molecular dynamics simulation identified potent inhibitors for sars-cov-2 main protease (3clpro), Journal of Biomolecular Structure and Dynamics 39 (13) (2021) 4659–4670, pMID: 32552361. arXiv:https://doi.org/10.1080/07391102.2020.1779128, doi:10.1080/07391102.2020.1779128.
URL https://doi.org/10.1080/07391102.2020.1779128

[21] Y. W. Chen, C.-P. B. Yiu, K.-Y. Wong, Prediction of the sars-cov-2 (2019-ncov) 3c-like protease (3cl (pro)) structure: virtual screening reveals velpatasvir, ledipasvir, and other drug repurposing candidates, F1000Research 9 (2020) 129–129. doi:10.12688/f1000research.22457.2.
URL https://pubmed.ncbi.nlm.nih.gov/32194944

[22] D. Wu, T. Wu, Q. Liu, Z. Yang, The sars-cov-2 outbreak: What we know., Int J Infect Dis 94 (2020) 44–48. doi:10.1016/j.ijid.2020.03.004.

[23] A. A. T. Naqvi, K. Fatima, T. Mohammad, U. Fatima, I. K. Singh, A. Singh, S. M. Atif, G. Hariprasad, G. M. Hasan, M. I. Hassan, Insights into sars-cov-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach., Biochim Biophys Acta Mol Basis Dis 1866 (10) (2020) 165878. doi:10.1016/j.bbadis.2020.165878.

[24] S. Ullrich, C. Nitsche, The sars-cov-2 main protease as drug target, Bio. Med. Chem. Letters 30 (17) (2020) 127377. doi:https://doi.org/10.1016/j.bmcl.2020.127377.
URL https://www.sciencedirect.com/science/article/pii/S0960894X20304881

[25] A. Jiménez-Alberto, R. M. Ribas-Aparicio, G. Aparicio-Ozores, J. A. Castelán-Vega, Virtual screening of approved drugs as potential sars-cov-2 main protease inhibitors, Computational Biology and Chemistry 88 (2020) 107325. doi:https://doi.org/10.1016/j.compbiolchem.2020.107325.
URL https://www.sciencedirect.com/science/article/pii/S1476927120304813

[26] S. Pushpakom, F. Iorio, P. A. Eyers, K. J. Escott, S. Hopper, A. Wells, A. Doig, T. Guilliams, J. Latimer, C. McNamee, A. Norris, P. Sanseau, D. Cavalla, M. Pirmohamed, Drug repurposing: progress, challenges and recommendations., Nat Rev Drug Discov 18 (1) (2019) 41–58. doi:10.1038/nrd.2018.168.

[27] Y. Masoudi-Sobhanzadeh, A. Salemi, M. M. Pourseif, B. Jafari, Y. Omidi, A. Masoudi-Nejad, Structure-based drug repurposing against covid-19 and emerging infectious diseases: methods, resources and discoveries, Briefings in bioinformatics (2021) bbab113doi:10.1093/bib/bbab113.
URL https://pubmed.ncbi.nlm.nih.gov/33993214

[28] T. A. Tummino, V. V. Rezelj, B. Fischer, A. Fischer, M. J. O'Meara, B. Monel, T. Vallet, K. M. White, Z. Zhang, A. Alon, H. Schadt, H. R. O'Donnell, J. Lyu, R. Rosales, B. L. McGovern, R. Rathnasinghe, S. Jangra, M. Schotsaert, J.-R. Galarneau, N. J. Krogan, L. Urban, K. M. Shokat, A. C. Kruse, A. García-Sastre, O. Schwartz, F. Moretti, M. Vignuzzi, F. Pognan, B. K. Shoichet, Drug-induced phospholipidosis confounds drug repurposing for sars-cov-2, Science 373 (6554) (2021) 541–547. arXiv:https://science.sciencemag.org/content/373/6554/541.full.pdf, doi:10.1126/science.abi4708.
URL https://science.sciencemag.org/content/373/6554/541

[29] C. N. Cavasotto, J. I. Di Filippo, In silico drug repurposing for covid-19: Targeting sars-cov-2 proteins through docking and consensus ranking, Molecular Informatics 40 (1) (2021) 2000115. arXiv:https://onlinelibrary.wiley.com/doi/pdf/10.1002/minf.202000115, doi:https://doi.org/10.1002/minf.202000115.
URL https://onlinelibrary.wiley.com/doi/abs/10.1002/minf.202000115

[30] J. Osipiuk, S.-A. Azizi, S. Dvorkin, M. Endres, R. Jedrzejczak, K. A. Jones, S. Kang, R. S. Kathayat, Y. Kim, V. G. Lisnyak, S. L. Maki, V. Nicolaescu, C. A. Taylor, C. Tesar, Y.-A. Zhang, Z. Zhou, G. Randall, K. Michalska, S. A. Snyder, B. C. Dickinson, A. Joachimiak, Structure of papain-like protease from sars-cov-2 and its complexes with non-covalent inhibitors, Nature Communications 12 (1) (2021) 743. doi:10.1038/s41467-021-21060-3.
URL https://doi.org/10.1038/s41467-021-21060-3

[31] D. Shin, R. Mukherjee, D. Grewe, D. Bojkova, K. Baek, A. Bhattacharya, L. Schulz, M. Widera, A. R. Mehdipour, G. Tascher, P. P. Geurink, A. Wilhelm, G. J. van der Heden van Noort, H. Ovaa, S. Müller, K.-P. Knobeloch, K. Rajalingam, B. A. Schulman, J. Cinatl, G. Hummer, S. Ciesek, I. Dikic, Papain-like protease regulates sars-cov-2 viral spread and innate immunity, Nature 587 (7835) (2020) 657–662. doi:10.1038/s41586-020-2601-5.
URL https://doi.org/10.1038/s41586-020-2601-5

[32] H. M. Mengist, T. Dilnessa, T. Jin, Structural basis of potential inhibitors targeting sars-cov-2 main protease, Frontiers in Chemistry 9 (2021) 7. doi:10.3389/fchem.2021.622898.
URL https://www.frontiersin.org/article/10.3389/fchem.2021.622898

[33] W. Yin, C. Mao, X. Luan, D.-D. Shen, Q. Shen, H. Su, X. Wang, F. Zhou, W. Zhao, M. Gao, S. Chang, Y.-C. Xie, G. Tian, H.-W. Jiang, S.-C. Tao, J. Shen, Y. Jiang, H. Jiang, Y. Xu, S. Zhang, Y. Zhang, H. E. Xu, Structural basis for inhibition of the rna-dependent rna polymerase from sars-cov-2 by remdesivir., Science 368 (6498) (2020) 1499–1504. doi:10.1126/science.abc1560.

[34] M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Krüger, T. Herrler, S. Erichsen, T. S. Schiergens, G. Herrler, N.-H. Wu, A. Nitsche, M. A. Müller, C. Drosten, S. Pöhlmann, Sars-cov-2 cell entry depends on ace2 and tmprss2 and is blocked by a clinically proven protease inhibitor., Cell 181 (2) (2020) 271–280. doi:10.1016/j.cell.2020.02.052.

[35] T. Hempel, L. Raich, S. Olsson, N. P. Azouz, A. M. Klingler, M. Hoffmann, S. Pöhlmann, M. E. Rothenberg, F. Noé, Molecular mechanism of inhibiting the sars-cov-2 cell entry facilitator tmprss2 with camostat and nafamostat, Chem. Sci. 12 (2021) 983–992. doi:10.1039/D0SC05064D.
URL http://dx.doi.org/10.1039/D0SC05064D

[36] H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, I. N. Shindyalov, P. E. Bourne, The protein data bank., Nucleic Acids Res 28 (1) (2000) 235–242. doi:10.1093/nar/28.1.235.

[37] T. Schwede, J. Kopp, N. Guex, M. C. Peitsch, SWISS-MODEL: an automated protein homology-modeling server, Nucleic Acids Research 31 (13) (2003) 3381–3385. arXiv:https://academic.oup.com/nar/article-pdf/31/13/3381/9487150/gkg520.pdf, doi:10.1093/nar/gkg520.
URL https://doi.org/10.1093/nar/gkg520

[38] B. T. Miller, R. P. Singh, J. B. Klauda, M. Hodošček, B. R. Brooks, H. L. Woodcock, Charmming: A new, flexible web portal for charmm, Journal of Chemical Information and Modeling 48 (9) (2008) 1920–1929, pMID: 18698840. arXiv:https://doi.org/10.1021/ci800133b, doi:10.1021/ci800133b.
URL https://doi.org/10.1021/ci800133b

[39] B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan, M. Karplus, Charmm: A program for macromolecular energy, minimization, and dynamics calculations, Journal of Computational Chemistry 4 (2) (1983) 187–217. arXiv:https://onlinelibrary.wiley.com/doi/pdf/10.1002/jcc.540040211, doi:https://doi.org/10.1002/jcc.540040211.
URL https://onlinelibrary.wiley.com/doi/abs/10.1002/jcc.540040211

[40] H. P., S. Preetha, A. Ramasubramanian, L. Prathap, Knowledge and awareness of existing potential treatment approaches for covid-19 among dental students, International Journal of Current Research and Review 12 (2020) 248–253. doi:10.31782/IJCRR.2020.SP32.

[41] S. M. Corsello, J. A. Bittker, Z. Liu, J. Gould, P. McCarren, J. E. Hirschman, S. E. Johnston, A. Vrcic, B. Wong, M. Khan, J. Asiedu, R. Narayan, C. C. Mader, A. Subramanian, T. R. Golub, The drug repurposing hub: a next-generation drug library and information resource., Nat Med 23 (4) (2017) 405–408. doi:10.1038/nm.4306.

[42] M. Davies, M. Nowotka, G. Papadatos, N. Dedman, A. Gaulton, F. Atkinson, L. Bellis, J. P. Overington, ChEMBL web services: streamlining access to drug discovery data and utilities, Nucleic

Acids Research 43 (W1) (2015) W612–W620. arXiv:https://academic.oup.com/nar/article-pdf/43/W1/W612/17435802/gkv352.pdf, doi:10.1093/nar/gkv352.
URL https://doi.org/10.1093/nar/gkv352

[43] N. M. O'Boyle, M. Banck, C. A. James, C. Morley, T. Vandermeersch, G. R. Hutchison, Open babel: An open chemical toolbox, Journal of Cheminformatics 3 (1) (2011) 33. doi:10.1186/1758-2946-3-33.
URL https://doi.org/10.1186/1758-2946-3-33

[44] K. Vanommeslaeghe, E. Hatcher, C. Acharya, S. Kundu, S. Zhong, J. Shim, E. Darian, O. Guvench, P. Lopes, I. Vorobyov, A. D. J. Mackerell, Charmm general force field: A force field for drug-like molecules compatible with the charmm all-atom additive biological force fields., J Comput Chem 31 (4) (2010) 671–690. doi:10.1002/jcc.21367.

[45] K. Vanommeslaeghe, E. Hatcher, C. Acharya, S. Kundu, S. Zhong, J. Shim, E. Darian, O. Guvench, P. Lopes, I. Vorobyov, A. D. Mackerell Jr., Charmm general force field: A force field for drug-like molecules compatible with the charmm all-atom additive biological force fields, Journal of Computational Chemistry 31 (4) (2010) 671–690. arXiv:https://onlinelibrary.wiley.com/doi/pdf/10.1002/jcc.21367, doi:https://doi.org/10.1002/jcc.21367.
URL https://onlinelibrary.wiley.com/doi/abs/10.1002/jcc.21367

[46] S. Vankayala, L. C. Warrensford, A. Pittman, F. Kearns, B. Pollard, H. L. Woodcock, J. Larkin, Cifdock: A novel charmm-based flexible receptor - flexible ligand docking protocol, J. Comp. Chem. 43 (2) (2021) 84–95.

[47] X. Wu, B. R. Brooks, Self-guided langevin dynamics simulation method, Chemical Physics Letters 381 (3) (2003) 512–518. doi:https://doi.org/10.1016/j.cplett.2003.10.013.
URL https://www.sciencedirect.com/science/article/pii/S0009261403017585

[48] R. A. Friesner, R. B. Murphy, M. P. Repasky, L. L. Frye, J. R. Greenwood, T. A. Halgren, P. C. Sanschagrin, D. T. Mainz, Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein ligand complexes, Journal of Medicinal Chemistry 49 (21) (2006) 6177–6196, pMID: 17034125. arXiv:https://doi.org/10.1021/jm051256o, doi:10.1021/jm051256o.
URL https://doi.org/10.1021/jm051256o

[49] B. Cao, Y. Wang, D. Wen, W. Liu, J. Wang, G. Fan, L. Ruan, B. Song, Y. Cai, M. Wei, X. Li, J. Xia, N. Chen, J. Xiang, T. Yu, T. Bai, X. Xie, L. Zhang, C. Li, Y. Yuan, H. Chen, H. Li, H. Huang, S. Tu, F. Gong, Y. Liu, Y. Wei, C. Dong, F. Zhou, X. Gu, J. Xu, Z. Liu, Y. Zhang, H. Li, L. Shang, K. Wang, K. Li, X. Zhou, X. Dong, Z. Qu, S. Lu, X. Hu, S. Ruan, S. Luo, J. Wu, L. Peng, F. Cheng, L. Pan, J. Zou, C. Jia, J. Wang, X. Liu, S. Wang, X. Wu, Q. Ge, J. He, H. Zhan, F. Qiu, L. Guo, C. Huang, T. Jaki, F. G. Hayden, P. W. Horby, D. Zhang, C. Wang, A trial of lopinavir–ritonavir in adults hospitalized with severe covid-19, New England Journal of Medicine 382 (19) (2020) 1787–1799, pMID: 32187464. arXiv:https://doi.org/10.1056/NEJMoa2001282, doi:10.1056/NEJMoa2001282.
URL https://doi.org/10.1056/NEJMoa2001282

[50] J. D. Baker, R. L. Uhrich, G. C. Kraemer, J. E. Love, B. C. Kraemer, A drug repurposing screen identifies hepatitis c antivirals as inhibitors of the sars-cov2 main protease, PLOS ONE 16 (2) (2021) 1–13. doi:10.1371/journal.pone.0245962.
URL https://doi.org/10.1371/journal.pone.0245962

[51] V. Mody, J. Ho, S. Wills, A. Mawri, L. Lawson, M. C. C. J. C. Ebert, G. M. Fortin, S. Rayalam, S. Taval, Identification of 3-chymotrypsin like protease (3clpro) inhibitors as potential anti-sars-cov-2 agents, Communications Biology 4 (1) (2021) 93. doi:10.1038/s42003-020-01577-x.
URL https://doi.org/10.1038/s42003-020-01577-x

[52] S. Zev, K. Raz, R. Schwartz, R. Tarabeh, P. K. Gupta, D. T. Major, Benchmarking the ability of common docking programs to correctly reproduce and score binding modes in sars-cov-2 protease mpro, Journal of Chemical Information and Modeling 61 (6) (2021) 2957–2966. doi:10.1021/acs.jcim.1c00263.
URL https://doi.org/10.1021/acs.jcim.1c00263

[53] A. Douangamath, D. Fearon, P. Gehrtz, T. Krojer, P. Lukacik, C. D. Owen, E. Resnick, C. Strain-Damerell, A. Aimon, P. Ábrányi-Balogh, J. Brandão-Neto, A. Carbery, G. Davison, A. Dias, T. D. Downes, L. Dunnett, M. Fairhead, J. D. Firth, S. P. Jones, A. Keeley, G. M. Keserü, H. F. Klein, M. P. Martin, M. E. M. Noble, P. O'Brien, A. Powell, R. N. Reddi, R. Skyner, M. Snee, M. J. Waring, C. Wild, N. London, F. von Delft, M. A. Walsh, Crystallographic and electrophilic fragment screening of the sars-cov-2 main protease, Nature Communications 11 (1) (2020) 5047. doi:10.1038/s41467-020-18709-w.
URL https://doi.org/10.1038/s41467-020-18709-w

[54] Y. Miao, Y.-m. M. Huang, R. C. Walker, J. A. McCammon, C.-e. A. Chang, Ligand binding pathways and conformational transitions of the hiv protease, Biochemistry 57 (9) (2018) 1533–1541. doi:10.1021/acs.biochem.7b01248.
URL https://doi.org/10.1021/acs.biochem.7b01248

[55] W. Yu, S. K. Lakkaraju, E. P. Raman, L. Fang, A. D. MacKerell, Pharmacophore modeling using site-identification by ligand competitive saturation (silcs) with multiple probe molecules, Journal of Chemical Information and Modeling 55 (2) (2015) 407–420. doi:10.1021/ci500691p.
URL https://doi.org/10.1021/ci500691p

[56] A. Kuzmanic, G. R. Bowman, J. Juarez-Jimenez, J. Michel, F. L. Gervasio, Investigating cryptic binding sites by molecular dynamics simulations, Accounts of Chemical Research 53 (3) (2020) 654–661. doi:10.1021/acs.accounts.9b00613.
URL https://doi.org/10.1021/acs.accounts.9b00613

[57] F. Comitani, F. L. Gervasio, Exploring cryptic pockets formation in targets of pharmaceutical interest with swish, Journal of Chemical Theory and Computation 14 (6) (2018) 3321–3331. doi:10.1021/acs.jctc.8b00263.
URL https://doi.org/10.1021/acs.jctc.8b00263

[58] Z. Jin, X. Du, Y. Xu, Y. Deng, M. Liu, Y. Zhao, B. Zhang, X. Li, L. Zhang, C. Peng, Y. Duan, J. Yu, L. Wang, K. Yang, F. Liu, R. Jiang, X. Yang, T. You, X. Liu, X. Yang, F. Bai, H. Liu, X. Liu, L. W. Guddat, W. Xu, G. Xiao, C. Qin, Z. Shi, H. Jiang, Z. Rao, H. Yang, Structure of mpro from sars-cov-2 and discovery of its inhibitors, Nature 582 (7811) (2020) 289–293.

doi:10.1038/s41586-020-2223-y.
URL https://doi.org/10.1038/s41586-020-2223-y

[59] S. Durdagi, B. Aksoydan, B. Dogan, K. Sahin, A. Shahraki, Screening of clinically approved and investigation drugs as potential inhibitors of covid-19 main protease: A virtual drug repurposing study, ChemRxiv (03 2020). doi:10.26434/chemrxiv.12032712.v1.

[60] T. Klemm, G. Ebert, D. J. Calleja, C. C. Allison, L. W. Richardson, J. P. Bernardini, B. G. Lu, N. W. Kuchel, C. Grohmann, Y. Shibata, Z. Y. Gan, J. P. Cooney, M. Doerflinger, A. E. Au, T. R. Blackmore, G. J. van der Heden van Noort, P. P. Geurink, H. Ovaa, J. Newman, A. Riboldi-Tunnicliffe, P. E. Czabotar, J. P. Mitchell, R. Feltham, B. C. Lechtenberg, K. N. Lowes, G. Dewson, M. Pellegrini, G. Lessene, D. Komander, Mechanism and inhibition of the papain-like protease, plpro, of sars-cov-2, The EMBO Journal 39 (18) (2020) e106275. arXiv:https://www.embopress.org/doi/pdf/10.15252/embj.2020106275, doi:https://doi.org/10.15252/embj.2020106275.
URL https://www.embopress.org/doi/abs/10.15252/embj.2020106275

[61] N. Singh, E. Decroly, A.-M. Khatib, B. O. Villoutreix, Structure-based drug repositioning over the human tmprss2 protease domain: search for chemical probes able to repress sars-cov-2 spike protein cleavages, European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences 153 (2020) 105495. doi:10.1016/j.ejps.2020.105495.
URL https://europepmc.org/articles/PMC7384984

[62] J. H. Beigel, K. M. Tomashek, L. E. Dodd, A. K. Mehta, B. S. Zingman, A. C. Kalil, E. Hohmann, H. Y. Chu, A. Luetkemeyer, S. Kline, D. Lopez de Castilla, R. W. Finberg, K. Dierberg, V. Tapson, L. Hsieh, T. F. Patterson, R. Paredes, D. A. Sweeney, W. R. Short, G. Touloumi, D. C. Lye, N. Ohmagari, M.-D. Oh, G. M. Ruiz-Palacios, T. Benfield, G. Fätkenheuer, M. G. Kortepeter, R. L. Atmar, C. B. Creech, J. Lundgren, A. G. Babiker, S. Pett, J. D. Neaton, T. H. Burgess, T. Bonnett, M. Green, M. Makowski, A. Osinusi, S. Nayak, H. C. Lane, Remdesivir for the treatment of covid-19 - final report., N Engl J Med 383 (19) (2020) 1813–1826. doi:10.1056/NEJMoa2007764.

[63] G. Kokic, H. S. Hillen, D. Tegunov, C. Dienemann, F. Seitz, J. Schmitzova, L. Farnung, A. Siewert, C. Höbartner, P. Cramer, Mechanism of sars-cov-2 polymerase stalling by remdesivir, Nature Communications 12 (1) (2021) 279. doi:10.1038/s41467-020-20542-0.
URL https://doi.org/10.1038/s41467-020-20542-0

[64] A. Shannon, B. Selisko, N.-T.-T. Le, J. Huchting, F. Touret, G. Piorkowski, V. Fattorini, F. Ferron, E. Decroly, C. Meier, B. Coutard, O. Peersen, B. Canard, Rapid incorporation of favipiravir by the fast and permissive viral rna polymerase complex results in sars-cov-2 lethal mutagenesis, Nature Communications 11 (1) (2020) 4682. doi:10.1038/s41467-020-18463-z.
URL https://doi.org/10.1038/s41467-020-18463-z

[65] Nih clinical trials website (2021).
URL clinicaltrials.gov

[66] M. Mahdi, J. A. Mótyán, Z. I. Szojka, M. Golda, M. Miczi, J. Tőzsér, Analysis of the efficacy of hiv protease inhibitors against sars-cov-2 main protease, Virology Journal 17 (1) (2020) 190. doi:10.1186/s12985-020-01457-0.
URL https://doi.org/10.1186/s12985-020-01457-0

[67] L. Fu, F. Ye, Y. Feng, F. Yu, Q. Wang, Y. Wu, C. Zhao, H. Sun, B. Huang, P. Niu, H. Song, Y. Shi, X. Li, W. Tan, J. Qi, G. F. Gao, Both boceprevir and gc376 efficaciously inhibit sars-cov-2 by targeting its main protease, Nature Communications 11 (1) (2020) 4417. doi:10.1038/s41467-020-18233-x.
URL https://doi.org/10.1038/s41467-020-18233-x

[68] S. Liu, C. Z. Lien, P. Selvaraj, T. T. Wang, Evaluation of 19 antiviral drugs against sars-cov-2 infection, bioRxiv (2020). arXiv:https://www.biorxiv.org/content/early/2020/05/07/2020.04.29.067983.full.pdf, doi:10.1101/2020.04.29.067983.
URL https://www.biorxiv.org/content/early/2020/05/07/2020.04.29.067983

[69] K. Bafna, K. White, B. Harish, R. Rosales, T. A. Ramelot, T. B. Acton, E. Moreno, T. Kehrer, L. Miorin, C. A. Royer, A. García-Sastre, R. M. Krug, G. T. Montelione, Hepatitis c virus drugs that inhibit sars-cov-2 papain-like protease synergize with remdesivir to suppress viral replication in cell culture, Cell Reports 35 (7) (2021) 109133. doi:https://doi.org/10.1016/j.celrep.2021.109133.
URL https://www.sciencedirect.com/science/article/pii/S2211124721004721

[70] B. Ellinger, D. Bojkova, A. Zaliani, J. Cinatl, C. Claussen, S. Westhaus, J. Reinshagen, M. Kuzikov, M. Wolf, G. Geisslinger, P. Gribbon, S. Ciesek, Identification of inhibitors of sars-cov-2 in-vitro cellular toxicity in human (caco-2) cells using a large scale drug repurposing collection, Research Square (2021). doi:10.21203/rs.3.rs-23951/v1.
URL https://doi.org/10.21203/rs.3.rs-23951/v1

[71] J. D. Baker, R. L. Uhrich, G. C. Kraemer, J. E. Love, B. C. Kraemer, A drug repurposing screen identifies hepatitis c antivirals as inhibitors of the sars-cov2 main protease, PLOS ONE 16 (2) (2021) 1–13. doi:10.1371/journal.pone.0245962.
URL https://doi.org/10.1371/journal.pone.0245962

[72] J. Rodon, J. Muñoz-Basagoiti, D. Perez-Zsolt, M. Noguera-Julian, R. Paredes, L. Mateu, C. Quiñones, I. Erkizia, I. Blanco, A. Valencia, V. Guallar, J. Carrillo, J. Blanco, J. Segalés, B. Clotet, J. Vergara-Alert, N. Izquierdo-Useros, Identification of plitidepsin as potent inhibitor of sars-cov-2-induced cytopathic effect after a drug repurposing screen, bioRxiv (2021). arXiv:https://www.biorxiv.org/content/early/2021/01/04/2020.04.23.055756.full.pdf, doi:10.1101/2020.04.23.055756.
URL https://www.biorxiv.org/content/early/2021/01/04/2020.04.23.055756

[73] V. Mody, J. Ho, S. Wills, A. Mawri, L. Lawson, M. C. C. J. C. Ebert, G. M. Fortin, S. Rayalam, S. Taval, Identification of 3-chymotrypsin like protease (3clpro) inhibitors as potential anti-sars-cov-2 agents, Communications Biology 4 (1) (2021) 93. doi:10.1038/s42003-020-01577-x.
URL https://doi.org/10.1038/s42003-020-01577-x