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A computational drug repurposing approach in identifying the cephalosporin antibiotic and anti-hepatitis C drug derivatives for COVID-19 treatment

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Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused over 1.4 million deaths worldwide. Repurposing existing drugs offers the fastest opportunity to identify new indications for existing drugs as a stable solution against coronavirus disease 2019 (COVID-19). The SARS-CoV-2 main protease (M\textsuperscript{pro}) is a critical target for designing potent antiviral agents against COVID-19. In this study, we identify potential inhibitors against COVID-19, using an amalgam of virtual screening, molecular dynamics (MD) simulations, and binding-free energy approaches from the Korea Chemical Bank drug repurposing (KCB-DR) database. The database screening of KCB-DR resulted in 149 binders. The dynamics of protein-drug complex formation for the seven top scoring drugs were investigated through MD simulations. Six drugs showed stable binding with active site of SARS-CoV-2 M\textsuperscript{pro} indicated by steady RMSD of protein backbone atoms and potential energy profiles. Furthermore, binding free energy calculations suggested the community-acquired bacterial pneumonia drug ceftaroline fosamil and the hepatitis C virus (HCV) protease inhibitor telaprevir are potent inhibitors against M\textsuperscript{pro}. Molecular dynamics and interaction analysis revealed that ceftaroline fosamil and telaprevir form hydrogen bonds with important active site residues such as Thr24, Thr25, His41, Thr45, Gly143, Ser144, Cys145, and Glu166 that is supported by crystallographic information of known inhibitors. Telaprevir has potential side effects, but its derivatives have good pharmacokinetic properties and are suggested to bind M\textsuperscript{pro}. We suggest the telaprevir derivatives and ceftaroline fosamil bind tightly with SARS-CoV-2 M\textsuperscript{pro} and should be validated through preclinical testing.

Keywords: COVID-19; SARS-CoV-2; drug repurposing; virtual screening, molecular dynamics; free energy calculations.
1. Introduction

Coronavirus disease 2019 (COVID-19) is a pandemic viral pneumonia and a threat to global public health. COVID-19 has resulted in over 1.4 million deaths from more than 61.8 million cases worldwide as of Dec 1, 2020 [1]. COVID-19 is caused by a novel coronavirus (nCoV) termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is a member of the larger coronavirus family including severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS CoV) [2-4]. The main protease (M\text{pro}) of SARS-CoV-2 is a non-structural protein that is responsible for processing the polyprotein translated from viral RNA and is a promising therapeutic target for COVID-19 [5-7]. Early evidence in SARS-CoV suggested that M\text{pro} inhibitors may abrogate the viral replication [8]. The atomic-level resolution of the SARS-CoV-2 protease structure must allow rational drug design against COVID-19 [4,9,10]. Drug repurposing (DR) provides the fastest possibilities to find existing drugs that may help treat COVID-19 [11]. Currently, the anti-HIV-1 (human immunodeficiency virus) drugs lopinavir and ritonavir have been repurposed for SARS-CoV-2 M\text{pro} and tested in clinical trials in China [12]. Lopinavir and ritonavir act in combination to abrogate the function of an important enzyme ‘protease’ which is essential for the viral replication. The efficacy and safety of a combination of an anti-HIV-1 drug darunavir and cobicistat are under phase 3 trials for COVID-19 (NCT04252274). Another example of drug repurposing is the Ebola drug remdesivir that has been approved by the U.S. Food and Drug Administration (FDA) for treating COVID-19 patients [13-15]. Chloroquine and hydroxychloroquine have been used to treat malaria and certain inflammatory conditions showed encouraging results against COVID-19 \textit{in vitro} [16-18]. Corticosteroid methylprednisolone (NCT04244591, NCT04273321), favipiravir (NCT04310228), abidol hydrochloride (NCT04254874, NCT04255017), oseltamivir (NCT04255017, NCT04261270), and danoprevir
(NCT04291729) have been subjected to clinical studies in China. Several other antiviral treatments suggested for COVID-19 may include nucleoside analogues, neuraminidase inhibitors, tenofovir disoproxil, lamivudine, and umifenovir [19,20]. These studies reveal the potential of DR drugs in combating COVID-19. Here, we recruited an amalgam of docking-based virtual screening, molecular dynamics (MD) simulations, and binding-free energy approaches to identify suitable existing drugs for the treatment of COVID-19.

2. Materials and methods

2.1. Preparation of molecules

The experimentally determined X-ray crystal structure of SARS-CoV-2 M<sub>pro</sub> (PDB: 6LU7) at a resolution of 2.16 Å was downloaded from RCSB Protein Data Bank (PDB) (https://www.rcsb.org/) [21]. The M<sub>pro</sub> protein complexes with a peptide-like substrate inhibitor N3. Protein was prepared with the Clean Protein functionality of the Discovery Studio 2017 (DS) software. All water molecules were omitted, hydrogen atoms were added, and bond orders were corrected for the protein. The 2D structures of reference inhibitors lopinavir and ritonavir were downloaded from PubChem structures and subsequently energy-minimized using the CHARMm forcefield implemented under the Minimize Ligands module of DS. The resulting 3D structures ensured that they had the correct bond orders, bond lengths, and bond angles for all the ligands. Proteins structures of SARS-CoV (PDB: 2A5I) [22] and SARS-CoV-2 (PDB: 6LU7) [21] main proteases were superimposed using the Superimpose Proteins tool of the DS software. The sequence alignment between two proteins and root mean square deviation (RMSD) value of the protein structure superimposition were obtained.

2.2. High-throughput virtual screening
The Genetic Optimization for Ligand Docking (GOLD v5.2.2) program was used to find possible binders for SARS-CoV-2 M\textsuperscript{pro} [23]. The binding site of the SARS-CoV-2 M\textsuperscript{pro} was determined to be the residues around 10 Å of the bound cocrystal. The automatic genetic algorithm search option of GOLD considers a balance between speed and accuracy for large library screening procedures. The Virtual Screening module of GOLD was used with 30% search efficiency for screening of the Korea Chemical Bank drug repurposing (KCB-DR) database containing 1865 drugs. Further screening of compounds was performed based on the default scoring function Goldscore and a rescoring function Chemscore. Drugs with high Goldscore and low Chemscore binding energy values were selected in comparison with reference inhibitors. A total of 149 drugs binding with M\textsuperscript{pro} active site were screened. The screened compounds were further subjected to more exhaustive conformational searches using the genetic algorithm with 100% search efficiency. Twenty independent docking runs were performed for each molecule with GOLD. Furthermore, the seven top scoring drugs obtained by docking-based virtual screening were tested through MD simulations and binding free energy calculations.

2.3. Molecular Dynamics simulation

MD simulations were performed to further understand the mechanism of protein-drug binding and to get dynamic information about the complex. The filtered hits from molecular docking studies along with reference inhibitors were subjected to MD simulations using the Groningen Machine for Chemical Simulations (GROMACS v5.1.4) package using CHARMM27 forcefield [24]. A separated simulation system was prepared for each selected molecule (Supplementary Table 1). The simulation parameters for all ligands were generated by SwisParam webserver program [25]. Simulations were carried out in dodecahedron boxes with the TIP3P water model, and systems were neutralized by adding counterions. The energy minimization step for each system was conducted using 50000 steps of the steepest
descent algorithm by applying a maximum force of 1000 KJ/mol to avoid steric clashes. The minimized system was then equilibrated under NVT (constant number of particles, volume, and temperature) and NPT (constant number of particles, pressure, and temperature) independently. The NVT ensemble was applied for 500 ps at 300K using a V-rescale thermostat [26]. In the NPT ensemble, each system was equilibrated for 1 ns at 1 bar pressure controlled by Parrinello-Rahman barostat [27]. The LINCS algorithm [28] and Particle mesh Ewald (PME) were used to restrain the bonds of heavy atoms and electrostatic interactions, respectively [29]. All simulations were carried out under periodic boundary conditions to avoid edge effects. Finally, a production run of 50 ns for each equilibrated system was performed under NPT conditions. The results were analyzed with DS, GROMACS, and visual molecular dynamics (VMD) software. GROMACS program ‘gmx rms’ was used for calculating RMSD values of both protein and ligands. This program calculates RMSD values of atoms in a molecule with respect to a reference structure by least-square fitting the structure to the reference structure \((t_2 = 0)\) as given below

\[
\text{RMSD} (t_1, t_2) = \left[ \frac{1}{M} \sum_{i=1}^{N} m_i ||r_i(t_1) - r_i(t_2)||^2 \right]^{\frac{1}{2}}
\]

Here, \(M = \sum_{i=1}^{N} m_i\), and \(r_i(t)\) is the position of atom \(i\) at time \(t\). The protein backbone atoms were selected for calculating protein RMSD values while all ligand atoms were selected for calculating ligand RMSD [30]. The root mean square fluctuation (RMSF) and radius of gyration (Rg) calculations were performed by ‘gmx rmsf’ and ‘gmx gyrate’ utilities of GROMACS program, respectively.

2.4. Binding free energy calculations

Prediction of the binding affinities of small-molecule inhibitors to their biological targets plays an important role in structure-based drug design [31]. In this study, we exploited
a GROMACS compatible program ‘g_mmpbsa’ to predict the protein-ligand binding free energies [32]. For calculating binding free energy, 50 snapshots of protein-ligand complexes were selected evenly from 0 to 50ns of MD trajectories [33]. The protein-ligand binding free energy is calculated as:

\[ \Delta G_{\text{binding}} = G_{\text{complex}} - (G_{\text{protein}} + G_{\text{ligand}}) \]  

(2)

\[ G_x = E_{\text{MM}} + G_{\text{solvation}} \]  

(3)

\[ E_{\text{MM}} = E_{\text{bonded}} + E_{\text{non-bonded}} = E_{\text{bonded}} + (E_{\text{vdw}} + E_{\text{elec}}) \]  

(4)

\[ G_{\text{solvation}} = G_{\text{polar}} + G_{\text{non-polar}} \]  

(5)

\[ G_{\text{non-polar}} = \gamma SASA + b \]  

(6)

where \( x \) may indicate separated ligand or protein or a ligand-protein complex; \( E_{\text{MM}} \) is the molecular mechanics potential energy in the vacuum; \( G_{\text{solvation}} \) is the free energy of solvation; \( SASA \) is the solvent-accessible surface area; \( \gamma = 0.02267 \text{ kJ mol}^{-1} \text{Å}^{-2} \) or \( 0.0054 \text{ kcal mol}^{-1} \text{Å}^{-2} \) is a coefficient related to surface tension of the solvent; \( b = 3.849 \text{ kJ mol}^{-1} \) or \( 0.916 \text{ kcal mol}^{-1} \) is a fitting parameter. The binding interaction between protein and ligand was calculated in three terms such as solvation (\( \Delta E_{\text{sol}} \)), Van der Waals (\( \Delta E_{\text{vdw}} \)), and the electrostatic (\( \Delta E_{\text{elec}} \)) contribution. The final \( \Delta G_{\text{bind}} \) values for protein-ligand complexes were the average values from 0 to 50 ns of MD simulation trajectories.

3. Results

An exhaustive computational approach including high-throughput virtual screening, molecular docking, MD simulations, and free energy calculations was used to identify potential drugs against the SARS-CoV-2 main protease enzyme. A workflow of the current study is represented in Figure 1. Anti-HIV protease inhibitors lopinavir and ritonavir are
recommended for the treatment of SARS-CoV and SARS-CoV-2 [4,34,35]. Therefore, we selected the bound cocrystal N3, lopinavir, and ritonavir as the reference inhibitors; these were used as standards for comparing the results obtained from our *in silico* study.

**Figure 1.** A workflow of the computational drug repurposing process for identifying potential drugs ceftaroline fosamil and telaprevir against the main protease of COVID-19.

**3.1. Active site analysis of SARS-CoV-2 M\(^\text{pro}\)**

SARS-CoV and SARS-CoV-2 main proteases are structurally similar and have a RMSD of 0.68 Å and highly conserved sequences with a striking sequence identity of 96.1% (Figure 2) [36]. The close proximity of the inhibitor binding site is highly conserved and the only notable difference is an Ala46Ser mutation whose role is yet to be elucidated. The overall structure of the SARS-CoV-2 M\(^\text{pro}\) contains three domains: domain I (residues 8–101), domain II (residues 102–184), and domain III (residues 201–303). The active site of M\(^\text{pro}\) lies at the interface of domain I and domain II. The active site can further be divided into S1, S2, and S3 subsites [37]. The side chains of residues Phe140, His163, and Glu166, and
main chain atoms of Leu141, Met165, Glu166, and His172 form the S1 subsite. The S1 subsite is a hydrophobic pocket that determines the substrate specificity in M\textsuperscript{pro}. The side chains of His41, Met49, Pro52, Met165, and Gln189 form a hydrophobic pocket at the S2 subsite that accommodates a leucine in the substrate. Further, the side chains of Pro168, Gln189, and Gln192 and main chain atoms of Glu166, Gln189, and Thr190 form the S3 subsite of M\textsuperscript{pro}. The residues His41 and Cys145 form the catalytic dyad required for proteolysis.
3.2. High-throughput virtual screening

The experimentally determined three-dimensional structure of SARS-CoV-2 M\textsuperscript{pro} (PDB: 6LU7) was used for docking-based virtual screening of the KCB-DR database. A total of 149 drugs showed binding with M\textsuperscript{pro} active site (Supplementary Table 2). Of the 149 drug molecules identified in our study, we found that at least six drugs have antiviral effects against other viruses such as human immunodeficiency virus (HIV) and hepatitis C virus (HCV). Fosamprenavir is a prodrug form of amprenavir and an established inhibitor of HIV-1 protease [38]. Moreover, several other HIV-1 protease inhibitors such as nelfinavir, tipranavir, and lopinavir and a HIV-1 integrase inhibitor elvitegravir were also identified in our study. Furthermore, an HCV NS3-4A protease inhibitor telaprevir was found to bind SARS-CoV-2 M\textsuperscript{pro}. Molecular conformations for all 149 drugs were obtained by more exhaustive use of the genetic algorithm search strategy. Docking studies of reference inhibitors N3, lopinavir, and ritonavir showed Goldscore values of 66.03, 60.86, and 48.53, respectively (Table 1). Likewise, Chemscore DG values of -26.64, -26.09, and -25.77 were obtained for N3, lopinavir, and ritonavir, respectively. Furthermore, a high Goldscore value of 66.03 and a low Chemscore value of -26.64 among reference inhibitors were used as the cutoff for candidate drug screening. Further screening was done on the basis of key molecular interactions exhibited by the drugs with the M\textsuperscript{pro} active site. The reference inhibitors formed
hydrogen bonds with one or more residues such as Phe140, Gly143, His164, Glu166, Gln189, and Thr190 (Supplementary Table 3). Drugs having at least one hydrogen bond with the residues above were retained. Consequently, seven drugs were obtained and show good binding properties with M\(^{\text{pro}}\). The screened drugs included ceftaroline fosamil, remikiren, everolimus, atorvastatin, sildenafil, clofazimine, and telaprevir. The drugs in complex with SARS-CoV-2 M\(^{\text{pro}}\) were subjected to MD simulations to study their dynamics of binding at the active site of the virus protein. MD simulations further suggested that only six drugs bind to the active site of the M\(^{\text{pro}}\) and two drugs show better binding energies than reference inhibitors. The details of MD simulations and binding free energy calculation results are discussed in sections 3.3 and 3.4.

Table 1. Docking scores and binding energies of SARS-CoV-2 M\(^{\text{pro}}\) in complex with reference inhibitors and potential drugs shown with respective standard deviations. The reference inhibitors are indicated in italics.

| No. | Name       | Goldscore    | Chemscore | MMPBSA Binding energy (kJ/mol) |
|-----|------------|--------------|-----------|-------------------------------|
| 1   | Ceftaroline fosamil | 82.33 ± 11.72 | -33.62 ± 11.27 | -231.89 ± 36.36 |
| 2   | Telaprevir  | 68.78 ± 10.04 | -29.32 ± 2.31 | -143.62 ± 16.69 |
| 3   | Ritonavir   | 48.53 ± 12.07 | -25.77 ± 2.66 | -122.01 ± 17.88 |
| 4   | Clofazimine | 66.05 ± 1.17  | -33.41 ± 0.35 | -104.63 ± 11.65 |
| 5   | N3          | 66.03 ± 11.76 | -26.64 ± 4.86 | -104.39 ± 20.41 |
| 6   | Lopinavir   | 60.86 ± 8.51  | -26.09 ± 4.46 | -101.17 ± 20.49 |
| 7   | Everolimus  | 79.63 ± 13.78 | -28.02 ± 8.23 | -100.35 ± 45.38 |
| 8   | Sildenafil  | 74.03 ± 7.79  | -28.85 ± 3.81 | -63.71 ± 32.34 |
| 9   | Atorvastatin| 76.06 ± 9.11  | -33.95 ± 5.71 | -59.72 ± 50.01 |
| 10  | Remikiren   | 80.04 ± 11.09 | -26.76 ± 11.34 | 24.68 ± 14.97 |

3.3. Molecular dynamics simulations
MD simulations can simulate the dynamic behavior of molecular systems as a function of time. This approach provides an opportunity to understand the flexibility and dynamics of protein-drug binding. The drugs obtained from virtual screening and reference inhibitors were subjected to MD simulations for 50 ns in each system (Supplementary Table 1).

3.3.1. Stability of simulation systems

The RMSD of protein backbone atoms and potential energy profiles obtained from the simulations indicate the overall stability of the simulated systems (Supplementary Figure S1) [39]. The RMSD values were between 0.15 nm to 0.36 nm for all the protein-drug complexes. Varying RMSD values at the initial few nanoseconds of the simulations indicate the initial adjustment of ligands at the active site of SARS-CoV-2 M\textsuperscript{pro} [39,40,41]. The analysis indicated that each system reached steady-state after convergence, and RMSD values remained within the acceptable value of <0.3 nm except the M\textsuperscript{pro}-Atorvastatin complex (0.36 nm) during the 50 ns simulation run [42].

The stability parameter potential energy (PE) was also analyzed (Supplementary Figure S1). Our analysis found that each simulation system obtained constant average potential energy profiles and remained stable during the simulation period. The RMSF calculations showed comparatively high fluctuations in Glu47 and Tyr154 versus the other residues. These residues are located away from the active site at the periphery of the protein (Supplementary Figure S2). The radius of gyration calculations also showed steady profiles for all complexes, which makes the system compact without any aberrant behavior during the simulations (Supplementary Figure S3). These results suggest that all protein-drug complex systems are stable and reliable for further analysis of binding modes, important molecular interactions, and free energy calculations.
3.3.2. Dynamics of drug binding with SARS-CoV-2 M<sub>pro</sub>

The substrate or inhibitor binding site of SARS-CoV-2 M<sub>pro</sub> can be divided into several subsites such as S1, S1’, S2, and S3 (Figure 2C) [43]. The binding events of reference inhibitors and drugs were assessed at these subsites. The reference inhibitor N3 occupied the S1, S1’, S2, and S3 subsites while lopinavir and ritonavir filled the S1’, S2, and S3 subsites during the simulations (Figure 3). Telaprevir showed similar binding with SARS-CoV-2 M<sub>pro</sub> as N3 inhibitor occupying the S1, S1’, S2, and S3 subsites (Figure 4A). Ceftaroline fosamil, atorvastatin, and clofazimine filled the S1’, S2, and S3 subsites (Figure 4B-D). Sildenafil showed slightly different binding occupying S1, S1’, and S2 subsites while everolimus filled only the S1’ and S2 subsites (Figure 4E&4F). Interestingly, remikiren lost its binding with the SARS-CoV-2 M<sub>pro</sub> active site and moved out of the binding pocket which is also evident through a high binding energy value discussed in the next section (Figure 4G).

**Figure 3.** Binding events of reference inhibitors (A) N3, (B) lopinavir, and (C) ritonavir at S1, S1’, S2, and S3 during 50 ns simulations.
Figure 4. Binding of potential drugs (A) telaprevir, (B) ceftaroline fosamil, (C) atorvastatin, (D) clofazimine, (E) sildenafil, (F) everolimus, and (G) remikiren with the SARS-CoV-2 M\textsuperscript{pro} active site during 50 ns simulations.

3.4. Binding free energy analysis
Structure-based drug design aims to generate small molecules with desirable pharmacological properties and high affinities to their specific protein target. Binding free energy calculations enable a better understanding of how a protein recognizes its biologically relevant ligand or small molecule inhibitor; this ultimately leads to the design of potent therapeutics [44]. An ensemble of molecular conformations generated by MD simulations can be used for free energy calculations to show great promise in drug discovery [45]. Average binding energies of potential drugs against SARS-CoV-2 M\textsuperscript{pro} were calculated by taking multiple conformations at regular intervals from MD simulations [46]. The reference inhibitors showed binding energies of \(-104.39\) kJ/mol, \(-101.17\) kJ/mol, and \(-122.01\) kJ/mol for N3, lopinavir, and ritonavir, respectively (Figure 5). The favorable binding energies for potential drugs were in the range of \(-231.89\) kJ/mol to \(-59.72\) kJ/mol (Table 1). The reference inhibitors ritonavir, N3, and lopinavir showed binding energies of \(-122.01\) kJ/mol, \(-104.39\) kJ/mol, and \(-101.17\) kJ/mol, respectively. The DR drugs ceftaroline fosamil, telaprevir, clofazimine, everolimus, sildenafil, atorvastatin, and remikiren showed binding energies of \(-231.89\) kJ/mol, \(-143.62\) kJ/mol, \(-104.63\) kJ/mol, \(-100.35\) kJ/mol, \(-63.71\) kJ/mol, \(-59.72\) kJ/mol, and \(24.68\) kJ/mol, respectively. Ceftaroline fosamil and telaprevir showed better binding energy scores than reference inhibitors. Only remikiren showed a positive binding energy (\(24.68\) kJ/mol) justifying the loss of binding with SARS-CoV-2 M\textsuperscript{pro} active site; hence, it has little value as a potential drug against COVID-19. We inferred that ceftaroline fosamil and telaprevir can bind with SARS-CoV-2 M\textsuperscript{pro} more tightly than reference inhibitors.
Figure 5. Binding free energy profiles of SARS-CoV-2 M\textsuperscript{pro} in complex with reference inhibitors and potential drugs calculated by the MM-PBSA method.

3.5. Binding mode analysis of potential drugs

Binding modes of ceftaroline fosamil and telaprevir were assessed using average structures calculated from MD simulation trajectories. Superimposition of complexes of SARS-CoV-2 M\textsuperscript{pro} with reference inhibitors and drugs indicated that they bind at the same substrate/inhibitor binding cavity of the protein (Supplementary Figure S4). Ceftaroline fosamil formed four hydrogen bond interactions with Thr24, Thr25, His41, and Thr45 (Figure 6). Hydrogen bonding interaction of ceftaroline fosamil with Thr24 and Thr25 was further supported by recently published crystal structures of SARS-CoV-2 M\textsuperscript{pro} with inhibitors Z1348371854 (PDB: 5RF6), PCM-0102535 (PDB: 5RG0), and NCL-00025412 (PDB: 5RG3). Additionally, the binding of ceftaroline fosamil with SARS-CoV-2 M\textsuperscript{pro} was facilitated by hydrophobic interactions with residues such as Cys44, Ser46, Met49, Met165,
Arg188, Gln189, Thr190, Ala191, and Gln192 (Table 2). Van der Waals interactions with residues such as Thr24, Cys44, Thr45, Met49, His164, Met165, Glu166, Pro168, Val186, Asp187, Arg188, Gln189, Ala191, and Gln192 were also observed in the case of ceftaroline fosamil. Telaprevir formed five hydrogen bonds with residues such as Gly143, Ser144, Cys145, and Glu166. Binding site residues such as His41, Met49, Asn142, His163, His164, Met165, Arg188, Thr190, and Gln192 showed hydrophobic interactions with telaprevir. Van der Waals interactions with residues such as Thr25, Thr26, Leu27, His41, Met49, Phe140, Leu141, Asn142, His163, His164, Met165, Leu167, Pro168, His172, Arg188, Gln189, Thr190, and Gln192 were also identified.

We further calculated the distance between hydrogen bond acceptor and donor atoms throughout the 50 ns simulations. The distances are represented as graphs which indicate that the average distance always remained less than the acceptable limit of 3.5 Å (Supplementary Figure S5). The reference inhibitor N3 has extensive hydrogen bonding interactions with eight bonds with SARS-CoV-2 M\textsuperscript{pro} active site (Table 2). Lopinavir and ritonavir formed two and three hydrogen bonds with M\textsuperscript{pro}, respectively. Moreover, the inhibitors N3, lopinavir, and ritonavir formed hydrophobic interactions with residues such as Leu27, His41, Met49, Asn142, Gly143, Cys145, His164, Met165, Leu167, Pro168, Phe181, Val186, and Ala191 as well as van der Waals interactions with Thr25, Thr26, Leu27, His41, Val42, Ser46, Met49, Leu50, Tyr54, Cys85, Leu141, Asn142, Gly143, Cys145, His163, Met165, Glu166, Leu167, Pro168, Val186, Asp187, Arg188, Gln189, Thr190, Ala191, and Gln192. Ceftaroline fosamil and telaprevir exhibit several important molecular interactions with SARS-CoV-2 M\textsuperscript{pro} active site that are comparable with reference inhibitors. Our results demonstrate that ceftaroline fosamil and telaprevir bind tightly to SARS-CoV-2 M\textsuperscript{pro} and may inhibit its activity through several molecular interactions such as hydrogen bonds, hydrophobic interactions, and Van der Waals interactions.
Figure 6. Binding modes of top hit drugs (A) ceftaroline fosamil and (B) telaprevir in the active site of SARS-CoV-2 M\textsuperscript{pro}. The top panel indicates the 2D structures of the drugs. The middle panel represents the binding of drugs at subsites S1, S1’, S2, and S3 of SARS-CoV-2 M\textsuperscript{pro}. Ceftaroline fosamil and telaprevir are shown in stick form in green and orange, respectively. The lower panel demonstrates the 2D diagram of various molecular interactions exhibited by the drugs with SARS-CoV-2 M\textsuperscript{pro}. Ligands are represented as purple sticks while interacting amino acids are orange sticks. Hydrogen bond interactions are indicated in dotted green lines with a distance measure between the hydrogen bond acceptor and donor atoms.
Table 2. Molecular interactions exhibited by potential drugs with SARS-CoV-2 M\text{pro}. The reference inhibitors are shown in italics.

| Compound | Hydrogen bond interactions | Van der Waals interactions |
|----------|-----------------------------|-----------------------------|
|          | Ligand atom | Amino acid | Amino acid atom | Length (Å) | Hydrophobic interactions |                                  |
| Ceftaroline fosamil | O2 | Thr24 | OG1 | 3.21 | Cys44, Ser46, Met49, Met165, Arg188, Gln189, Thr190, Ala191, Gln192 |
|          | N5 | Thr25 | OG1 | 2.92 | Thr24, Cys44, Thr45, Met49, His164, Met165, Glu166, Pro168, Val186, Ala191, Gln192 |
|          | O18 | His41 | NE2 | 3.14 |                        |
|          | O3 | Thr45 | OG1 | 3.24 | Asp187, Arg188, Gln189, Ala191, Gln192 |
| Telaprevir | O1 | Gly143 | N | 3.11 |                                  |
|          | O1 | Ser144 | N | 2.97 | Thr25, Thr26, leu27, His41, Met49, Phe140, Leu141, Asn142, His163, His164, Met165, Leu167, Pro168, His172, Arg188, Gln189, Thr190, Gln192 |
|          | O1 | Cys145 | N | 3.25 |                                |
|          | O3 | Glu166 | O | 3.26 |                                |
|          | O3 | Glu166 | N | 2.81 |                                |
|          | O7 | Gly143 | N | 2.92 |                                |
|          | O7 | Ser144 | N | 3.21 |                                |
| N7 | O8 | His164 | O | 3.61 | Leu27, Met49, Cys145, Met165, Pro168, Ala191 |
|          | O8 | Glu166 | OE1 | 2.89 |                                |
|        | O     | Glu166 | N     | 2.78 |
|--------|-------|--------|-------|------|
| N      | Glu166| O      | 2.77  |      |
| N      | Gln189| O      | 2.94  |      |
| N2     | Gln189| O      | 3.35  |      |
| Lopinavir | N3    | His41  | O     | 2.78 |
|         | N4    | Gln189 | OE1   | 1.73 |
|         |       |        |       |      |
| Ritonavir | N6    | Ser46  | N     | 2.41 |
|         | O1    | Glu166 | N     | 1.91 |
|         | N3    | Glu166 | O     | 1.98 |

Lopinavir: Met49, His164, Met165, Leu167, Pro168, Phe181, Val186

Ritonavir: Thr25, Thr26, Leu27, Val42, Ser46, Met49, Leu50, Cys85, Gly143, Cys145, Glu166, Leu167, Pro168, Val186, Asp187, Arg188, Thr190, Ala191, Gln192

His41, Thr45, Leu50, Asn142, Gly143, Met165, Leu167, Pro168, Asp187, Arg188, Gln189, Thr190, Ala191, Gln192
3.6. Substructure search

A substructure search in PubChem database was performed using telaprevir as an initial structure for identifying compounds having similar structures with different functional groups. We obtained 231 compounds that were further assessed for their good pharmacokinetic properties. The procedure of Kumar et al. 2015 was utilized for measuring the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the compounds using DS software [39]. A total of 14 compounds were obtained with desirable pharmacokinetic properties such as good aqueous solubility, non CYP2D6 binding, low hepatotoxicity, high intestinal absorption, and inability to cross Blood Brain Barrier (BBB). These compounds were further tested for their binding with SARS-CoV-2 M\textsuperscript{pro} active site through molecular docking. The compounds exhibited a range of docking Goldscores: 51.36 to 79.87; Chemscores were -36.75 to -22.24 (Supplementary Table 4). Finally, 11 compounds having better docking scores when compared to the parent compound telaprevir and reference inhibitors, N3, lopinavir, and ritonavir were retained. Besides the telaprevir forming hydrogen bonds with residues Gly143, Ser144, Cys145, and Glu166, its derivatives also showed hydrogen bonds with other important active site residues such as Asn142, His164, and Gln189 (2D interactions, Supplementary Table 4). These results indicate that telaprevir derivatives have better binding properties and safer pharmacokinetic profiles than telaprevir. The details of compounds with their 2D structures are shown in Table 3.

Table 3. Telaprevir derivatives identified by a substructure search in the PubChem database.

| No. | PubChem CID | IUPAC Name | 2D structure |
|-----|-------------|------------|--------------|

Journal Pre-proof
(3S,3aS,6aR)-N-[(3S)-1-(cyclopropylamino)-1,2-dioxohexan-3-yl]-2-[(2R)-3,3-dimethyl-2-[(2S)-2-phenyl-2-(pyrazine-2-carbonylamino)acetyl]amino]butanoyl]-3,3a,4,5,6,6a-hexahydro-1H-cyclopenta[c]pyrrole-3-carboxamide

(3S,3aS,6aS)-2-[(2S)-2-[(2S)-2-cyclohexyl-2-(pyrazine-2-carbonylamino)acetyl]amino]-3,3-dimethylbutanoyl]-N-[(3S)-1-(cyclopropylamino)-1,2-dioxohexan-3-yl]-6-methylidene-1,3,3a,4,5,6a-hexahydrocyclopenta[c]pyrrole-3-carboxamide

(1R,2S,3S,6R,7S)-4-[(2S)-2-[(2S)-2-cyclohexyl-2-(pyrazine-2-carbonylamino)acetyl]amino]-3,3-dimethylbutanoyl]-N-[(3S)-1-(cyclopropylamino)-1,2-dioxohexan-3-yl]-4-azatricyclo[5.2.1.02,6]dec-8-ene-3-carboxamide

(1S,7R)-4-[(2S)-2-[(2S)-2-cyclohexyl-2-(pyrazine-2-carbonylamino)acetyl]amino]-3,3-dimethylbutanoyl]-N-[(3S)-1-(cyclopropylamino)-1,2-dioxohexan-3-yl]-4-azatricyclo[5.2.1.02,6]dec-8-ene-3-carboxamide
(3S)-N-[(3S)-1-(cyclopropylamino)-1,2-dioxohexan-3-yl]-2-[(2S)-3,3-dimethyl-2-[[{2S}-2-phenyl-2-(pyrazine-2-carbonylamino)acetyl]amino]butanoyl]-3,3a,4,5,6,6a-hexahydro-1H-cyclopenta[c]pyrrole-3-carboxamide

(1S,3S,7R)-4-[(2S)-2-[[{2S}-2-cyclohexyl-2-(pyrazine-2-carbonylamino)acetyl]amino]-3,3-dimethylbutanoyl]-N-[(3S)-1-(cyclopropylamino)-1,2-dioxohexan-3-yl]-4-azatricyclo[5.2.1.02,6]dec-8-ene-3-carboxamide

(3S,3aS,6aR)-2-[(2S)-2-[[{2S}-2-cyclohexyl-2-(pyrazine-2-carbonylamino)acetyl]amino]-3,3-dimethylbutanoyl]-N-[(3S)-1-(cyclopropylamino)-1,2-dioxohexan-3-yl]-6-oxo-1,3,3a,4,5,6a-hexahydrocyclopenta[c]pyrrole-3-carboxamide

(3S)-N-[(3S)-1-(cyclopropylamino)-1,2-dioxohexan-3-yl]-2-[(2S)-3,3-dimethyl-2-[[{2S}-2-phenyl-2-(pyrazine-2-carbonylamino)acetyl]amino]butanoyl]-3,3a,4,5,6,6a-hexahydro-1H-cyclopenta[c]pyrrole-3-carboxamide
4. Discussion

The COVID-19 pandemic continues to spread chaos globally. The current situation compels researchers to find a fast and stable solution in terms of potent inhibitors against SARS-CoV-2. We used a computational drug repurposing approach to find existing drugs against COVID-19. Our docking-based virtual screening of KCB-DR database results suggested 7 drugs as potential binders. The screened compounds included approved drugs...
such as the cephalosporin antibacterial ceftaroline fosamil, remikiren (effective in the
treatment of hypertension), antineoplastic drug everolimus, blood cholesterol-lowering drug
atorvastatin, sildenafil effective in erectile dysfunction and pulmonary arterial hypertension,
the anti-leprosy drug clofazimine, and the HCV NS3-4A protease inhibitor telaprevir.

Sildenafil has been under Phase 3 clinical trials in China (NCT04304313). Remikiren
has been reported as a potential drug against COVID-19 by Nguyen et al. [47]. Telaprevir is
predicted to bind effectively to SARS-COV-2 papain-like protease (PLpro) [48]. However,
other potential drugs in our study have yet to be evaluated against COVID-19. The potential
candidates were screened through exhaustive in silico approaches employing molecular
docking, MD simulations, and free energy calculations. Our results suggested that ceftaroline
fosamil and telaprevir have better binding energies than reference inhibitors. Ceftaroline
fosamil occupies the S1’, S2, and S3 subsites while telaprevir filled the S1, S1’, S2, and S3
subsites of SARS-CoV-2 Mpro active site. Moreover, ceftaroline fosamil formed a close
contact with Ser46 residue which is mutated (Ala46Ser) in SARS-CoV-2 Mpro versus SARS-
CoV (Figure 2D). SARS-CoV Mpro inhibitors may provide critical information about the
molecular interactions necessary for targeting SARS-CoV-2 Mpro due to their high sequence
and structure similarities (Table 4) [49]. This analysis suggested that complexes of SARS-
CoV and bound inhibitors exhibit hydrogen bond formation with residues such as His41,
Phe140, Asn142, Gly143, Ser144, Cys145, His163, His164, Met165, Glu166, Gln189,
Thr190, and Gln192. Further, the SARS-CoV-2 Mpro binding site can accommodate a variety
of inhibitors as evident from recent crystal structure complexes reported in the PDB database.
The small size inhibitors such as carmofur (PDB: 7BUY) [50] and 2-Methyl-1-tetralone
(PDB: 6YNQ) [51] may bind only at S1 and S2 subsites. Medium size inhibitors like
boceprevir (PDB: 7BRP) [52], 7j (PDB: 6XMK) [53], and feline (PDB: 6WTJ) [54] may bind
to S1’, S1, and S2 subsites. Some large inhibitors may bind to S1’, S1, S2, and may extend
through S3 subsites, for example N3 (PDB: 6LU7) [21], alpha-ketoamide (PDB: 6Y2F) [43],
and X77 (PDB: 6W63) [55]. These compounds have dissimilar structures and sizes, yet they
bind with and inhibit the activity of SARS-CoV-2 M\textsuperscript{pro}. Therefore, a potential future
perspective lies in the structure-activity relationship (SAR) studies which should provide
greater insights into the selectivity of inhibitors of SARS-CoV-2 M\textsuperscript{pro}.

Ceftaroline fosamil formed four hydrogen bond interactions including residues Thr24,
Thr25, and His41 as observed in recently determined crystal structures of SARS-CoV-2 M\textsuperscript{pro}
(Table 4). Interestingly, telaprevir formed hydrogen bond interactions with important residues
such as Gly143, Ser144, Cys145, and Glu166 suggesting its relevance as a potential SARS-
CoV-2 M\textsuperscript{pro} inhibitor. Telaprevir was previously used as a combination therapy against
Hepatitis C virus (HCV) infection that was paired with ribavirin, peginterferon alfa-2a, and
peginterferon alfa-2b [56]. Some side effects of telaprevir combination therapy were skin
rashes and anemia in adverse events of treatment [57]. To avoid the side effects of telaprevir,
analogs with better pharmacokinetic properties can be further synthesized and explored as
M\textsuperscript{pro} inhibitors.

Thus, we performed a substructure search in the PubChem database to identify
telaprevir derivative compounds. The 11 resulting compounds had desirable pharmacokinetic
properties such as good aqueous solubility, non CYP2D6 binding, low hepatotoxicity, high
intestinal absorption, and inability to cross the BBB besides good binding properties with
SARS-CoV-2 M\textsuperscript{pro} active site. Therefore, we demonstrate that telaprevir derivative
compounds identified by our \textit{in silico} study have potential against COVID-19 and may bind
to the SARS-CoV-2 main protease.

5. Conclusion
Our computational drug repurposing study identified ceftaroline fosamil and telaprevir as putative inhibitors against a critical COVID-19 drug target SARS-CoV-2 main protease. Our inhibitors formed hydrogen bonds with important residues such as Thr24, Thr25, His41, Gly143, Ser144, Cys145, and Glu166; these are consistent with the crystal structures of complexes of inhibitors bound with SARS-CoV and SARS-CoV-2 main proteases. A previously unexplored hydrogen bond interaction was identified in the case of ceftaroline fosamil with Thr45 located near the S1’ subsite of SARS-CoV-2 M\textsuperscript{pro}. Considering the side effects of the telaprevir, its derivative compounds with better pharmacokinetic properties are suggested as potential antiviral candidates against COVID-19. We recommend telaprevir analogs and ceftaroline fosamil identified in our study for \textit{in vitro} testing and further validation through randomized clinical trials before being used in COVID-19 patients.
Table 4. Hydrogen bond formation between SARS-CoV M\textsuperscript{pro} active site residues and bound inhibitors of the selected complexes.

| PDB Entry | Thr24 | Thr25 | Thr26 | His41 | Phe140 | Asn142 | Gly143 | Ser144 | Cys145 | His163 | His164 | Met165 | Glu166 | Gln189 | Thr190 | Gln192 |
|-----------|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 5RF6*     | ✓     |       |       |       |        |        |        |        |        |        |        |        |        |        |        |       |
| 5RG0*     | ✓     | ✓     | ✓     | ✓     | ✓      |        |        |        |        |        |        |        |        |        |        |       |
| 5RG3*     | ✓     | ✓     |       |       |        |        |        |        |        |        |        |        |        |        |        |       |
| 6LU7*     |       | ✓     | ✓     | ✓     | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      |       |
| 6Y2F*     | ✓     | ✓     | ✓     | ✓     | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      |       |
| 6Y2G*     | ✓     | ✓     | ✓     | ✓     | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      |       |
| 1UK4      | ✓     | ✓     | ✓     | ✓     | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      |       |
| 1WOF      |       | ✓     |       |       |        |        |        |        |        |        |        |        |        |        |        | ✓      |
| 2A5I      | ✓     | ✓     | ✓     | ✓     | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      |       |
| 2A5K      | ✓     | ✓     | ✓     | ✓     | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      |       |
| 2ACF      |       |       |       |       |        |        |        |        |        |        |        |        |        |        |        |        |       |
| 2ALV      | ✓     |       |       |       |        |        |        |        |        |        |        |        |        |        |        |        |       |
| 2AMD      | ✓     | ✓     |       |       |        |        |        |        |        |        |        |        |        |        |        |        |       |
| 2AMQ      | ✓     | ✓     | ✓     |       |        |        |        |        |        |        |        |        |        |        |        |        |       |
| 2D2D      | ✓     | ✓     |       |       |        |        |        |        |        |        |        |        |        |        |        |        |       |
| 2GTB      | ✓     | ✓     | ✓     | ✓     | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      | ✓      |
| 2GX4      | ✓     | ✓     |       |       |        |        |        |        |        |        |        |        |        |        |        |        |       |
|    | 2GZ7 | 2GZ8 | 2HOB | 2OP9 | 2Z3C | 2Z3D | 2Z3E | 2ZU4 | 2ZU5 | 3ATW | 3AVZ | 3AW0 | 3IWM | 3SN8 | 3SNA | 3SNB | 3SNC | 3SND | 3SNE | 3SZN | 3TIT |
|----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
|    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    |
|     | 3TIU | 3TNS | 3TNT | 3V3M | 3VB4 | 3VB5 | 3VB6 | 3VB7 | 4MDS | 6Y7M |
|-----|------|------|------|------|------|------|------|------|------|------|
|     | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    | ✓    |

* Recently determined SARS-CoV-2 M\textsuperscript{pro} structures
Author Contributions

R.K., V.K., and K.W.L. conceived the idea of the project. R.K. and V.K. performed the experiments and compiled the manuscript. R.K. and K.W.L. analysed the results. All the authors have read and approved the manuscript for submission.

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Conflicts of interests

The authors declare no conflicts of interest.
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Highlights

- 1865 drugs from KCB-DR database were screened to search potential inhibitors for COVID-19.
- Drugs were screened by molecular docking, MD simulations, and binding free energy calculations.
- Telaprevir and ceftaroline fosamil were identified as potential M\(^{pro}\) binders.
- Telaprevir derivatives having good ADME properties bind well with M\(^{pro}\).
- A new hydrogen bond interaction was discovered with ceftaroline and Thr45 of M\(^{pro}\).
Conflict of Interest

The authors declare no conflicts of interest.