Prediction of potential inhibitors against SARS-CoV-2 endoribonuclease: RNA immunity sensing

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ABSTRACT

The World Health Organization has classified the COVID-19 outbreak a pandemic which is caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) and declared it a global health emergency. Repurposing drugs with minimum side effects are one approach to quickly respond in attempt to prevent the spread of COVID-19. SARS-CoV-2 encodes several RNA processing enzymes that are unusual and unique for single-stranded RNA viruses, including Nsp15, a hexameric endoribonuclease that discriminates cleaves immediately 3' of uridines. The structure of SARS-CoV-2 Nsp15 is reported to be homologous to that of the Nsp15 endonucleases of SARS-CoV and MERS-CoV, but it exhibits differences that may contribute to the greater virulence of SARS-CoV-2. This study aimed to identify drugs that targeted SARS-COV-2 Nsp15 using a molecular docking-based virtual screening of a library containing 10,000 approved and experimental drugs. The molecular docking results revealed 19 medications that demonstrated a good ability to inhibit Nsp15. Among all the candidate drugs only five FDA approved drugs were used for further investigation by molecular dynamics simulation, the stability of Nsp15-ligand system was evaluated by calculating the RMSD, RMSF, radius of gyration and hydrogen bond profile. Furthermore, MM-PBSA method was employed to validate the binding affinity. According to the obtained results of MD, the complex of Olaparib was showed more stability and lower binding free energy than the control inhibitor during MD simulation time. Finally, we suggest that Olaparib is a potential drug for treating patients infected with SARS-CoV-2 and provide insight into the host immune response to viral RNA.

1. Introduction

Coronavirus disease 2019 (COVID-19) was spreading in 183 countries worldwide as we were preparing this manuscript, causing a pandemic reaching over 3.1 million confirmed cases and 2,24,172 deaths (WHO, 2020; Wu et al., 2020; Zhou et al., 2020) with the numbers continuing to increase. At the same time of facing the current SARS-CoV-2 outbreak, the presence of a natural reservoir has raised serious concerns regarding the potential reemergence of a new generation of SARS-CoV-2 infections (Bhardwaj et al., 2020a). Treatments used during the pandemic include chloroquine alone or combined with azithromycin, even though these treatments have had limitations due to chronic systemic diseases and adverse effects along with questions related to their effectiveness (Peschken, 2020; Yao et al., 2020). This requires thinking differently in order to develop more specific treatment options and better insight into the mysteries of SARS-CoV-2.

SARS-CoV-2 contains single-stranded RNA genomes of positive polarity, similar to that of mRNA (Masters, 2019). The genomic RNA is ∼30 kb in length with a 5' cap structure and a 3' poly(A) tail, which includes genes encoding for several RNA processing enzymes unusual for RNA viruses (Masters, 2019). These RNA-processing enzymes include Nsp15, a hexamer consisting of a dimer of trimers with endoribonuclease activity that preferentially cleaves immediately 3' of uridines. Generally, the replicative structure of nidoviral RNA uridylate-specific endoribonuclease (NendoU) is a unique characteristic of viruses of the order Nidovirales. SARS-CoV-2 is genetically similar to both SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) with 80% and 50% sequence similarity, respectively (Ivanov et al., 2004; Zhou et al., 2020). The functional and structural similarities of the endoribonuclease Nsp15 suggest that endoribonuclease inhibitors may serve as molecules for the development of RNA uridylate-specific endoribonuclease (EndoU) inhibitors.

EndoU enzymes are found in all the kingdoms of living organisms, where they perform various biological functions associated with RNA-processing. All characterized EndoU family members show an RNA endonuclease activity producing 2',3'-cyclic phosphodiester linkage and 5'-hydroxyl ends (Ulferts & Ziebuhr, 2011). EndoU enzymes are present in all coronaviruses, suggesting their activity is important for coronavirus replication in the host (Deng and Baker, 2018;
Nedalkova et al., 2009). Consistent with this is the fact that the administration of Nsp15 inhibitors to cultured cells at different stages of coronavirus infection reduces the infectivity of SARS-CoV (Kang et al., 2007; Ortiz-Alcantara et al., 2010). There is also information regarding coronavirus infections suggesting EndoU/Nsp15 activity is responsible for protein-mediated innate immune sensing and delays activation of the host immune response (Kindler & Thiel, 2014). However, the mechanism of nucleic acid immunity is unknown. Therefore, studies have been conducted to investigate the replication of endoribonuclease-deficient coronaviruses in effort to understand the molecular mechanisms used by coronaviruses to avoid antiviral host responses. Coronavirus replication results in the generation of long double-stranded RNA (dsRNA) intermediates (Masters, 2006) that are recognized by the specific cytoplasmic pattern recognition receptors (PRRs) melanoma differentiation-associated protein 5 (MDAS), oligoadenylate synthetases (OAS), and protein kinase R (PKR), which can activate the type I interferon (IFN) response in macrophages (Kang et al., 2002). However, the activity of EndoU is to cleave polyU sequences from 5'-polyU-containing negative sense (PUN) RNAs, thus limiting the formation of a pathogen-associated molecular pattern (PAMP) and blocking the ability of specific cytoplasmic PRRs to activate the response of innate immune to infection (Hackbart et al., 2020). Inhibiting EndoU activity may serve as a therapeutics approach against the existing and any subsequently emerging SARS-CoV-2 infections. Drug repurposing may be one of the most effective approaches in the fight against COVID-19. The current study virtually screened libraries of approved and experimental drugs to identify those that may potentially inhibit the endonuclease activity of SARS-CoV-2.

2. Materials and methods

2.1. Protein selection

The three dimensional (3D) structure of the SARS-CoV-2 Nsp15 endoribonuclease was obtained from the RCSB protein data bank (PDB code 6vww). All undesired molecules and ions were removed using AutoDockTools (ADT) release 1.5.6. Polar hydrogen atoms and partial charges calculated using the Gasteiger charge method were added to the protein using ADT (Singh et al., 2020a). The active binding site of the SARS-CoV-2 Nsp15 selected for the study was previously identified (Kim et al., 2020).

2.2. Structure-based virtual screening

Virtual screening was performed using two different online docking platforms. The first platform was the Ressource Parisienne en BioInformatique Structurale (RPBS) web portal (https://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py?job s::MtiOpenScreen.C2600172502202708). This platform contains a library of approved, investigational, and experimental drugs (up to 7,000 drugs) and their 3D structures, which was used in docking with PDB 6vww. The second platform was Mcule (https://mcule.com) of which two libraries were used, the drug repurposing library and antiviral compound library. These libraries included up to 3,000 US FDA approved drugs and were both downloaded from the Selleckchem webserver (https://www.selleckchem.com). The grid center was set at −93, 22 and 39 for X, Y, and Z, respectively.

2.3. Molecular docking

The 70 best hits obtained from virtual screening were highlighted and selected for further validation using AutoDock 4.2 (manual docking) to evaluate binding modes and protein-ligand interactions. Each molecular structure was drawn using ChemDraw Ultra 12.0 software. Energy minimization to optimize geometry was achieved using MMFF94. The drawn ligands were converted to 3D structures using structure-data file (SDF) format, which were then converted to Protein Data Bank, Partial Charge (Q), & Atom Type (T) [PDPQT] format using Open Babel. To perform rigid docking with minimization of standard errors (2.85 kcal/mol), all rotatable bonds of the docked ligands were considered non-rotatable. The docking process was performed using default docking parameters. To investigate the best conformations of each ligand, the Lamarckian genetic algorithm was used. The population size and numbers of generations and evaluations were set at 150, 27,000 and 2,500,000, respectively. ADT was also used to prepare the GPF and DPF output parameter files. The best conformations were regarded to have the lowest binding energy. Ten of the most favorable conformations according to binding energy were displayed by ADT. Discovery Studio Visualizer (v.4.5) was employed to visualize the intermolecular interactions between the receptors and ligands.

2.4. Molecular dynamics simulation

The molecular dynamics (MD) simulations were performed on the complex of SARS-CoV-2 endoribonuclease with five FDA approved drugs (Capmatinib, Olaparib, Sorafenib, Risperidone and Brexpiprazol). The MD simulations were carried out by NAMD package version 2.13 (Jiang et al., 2011). The topology files were generated by CHARMM-GUI server. The obtained structures were then solvated in a cubic simulation box containing a complex of protein and ligand by VMD software (William et al., 1996), then sodium and chloride counter-ions were added to neutralize the complexes charge (0.15 mM NaCl). The minimization used the steepest descent method for 5000 steps before transferring the final structure to MD simulation, the equilibration was based on the Berendsen weak thermal coupling method under 100 ps constant temperature (NVT ensemble) (Bhardwaj & Purohit, 2020a), with temperature set at 310K. particle-mesh Ewald (PME) method was applied (Bhardwaj & Purohit, 2020b). The time step was set at 2 fs and final coordinate was saved every 2 ps for all of the simulations performed (Bhardwaj et al., 2020b). After 10ns of simulations, the MD trajectories of the complexes were analyzed using VMD software to obtain RMSD, RMSF, radius of gyration and hydrogen bond profile. According to the preliminary findings of the 10 ns...
simulation time, we were encouraged to extend the simulation time to 100 ns for Nsp15-Olaparib complex.

2.5. Binding free energy

The binding free energy (ΔG_binding) was calculated using CaFE plugin powered by VMD and NAMD softwares (Hui & Tingjun, 2016), and according to MM-PBSA method (Bhardwaj et al., 2020c), this method combines the energies of molecular mechanics with continuum solvent approaches.

\[
\Delta G_{\text{binding}} = G_{\text{complex}} = (G_{\text{receptor}} + G_{\text{ligand}}) \\
G = E_{\text{MM}} + G_{\text{pol}} + G_{\text{non pol}} - T\Delta S \\
E_{\text{MM}} = E_{\text{vdw}} + E_{\text{ele}} \\
G_{\text{non pol}} = \gamma\Delta S \text{ASA} + \beta
\]

where \(E_{\text{vdw}} + E_{\text{ele}}\) (Eq. (2)) represent van der Waals and electrostatic energies, respectively (Bhardwaj et al., 2020d), and \(G_{\text{pol}} + G_{\text{non pol}}\) refers to polar and non-polar solvation energies. By solving the Poisson-Boltzmann linear equation the polar solvation energy was computed (Singh et al., 2020b), while the non-polar solvation energy was obtained by computing the solvent accessible surface area (SASA). In Eq. (4), \(\gamma\) represents a coefficient of surface tension, and \(\beta\) represents a fitting parameter (Hourieh et al., 2019). All energy components in Eq. (2) were calculated utilizing 120 snapshots.

3. Results

3.1. Nsp15 endoribonuclease of SARS-CoV-2

The high resolution crystal structure of the SARS-CoV-2 Nsp15 endoribonuclease was reported March 2020 by Kim and colleagues (Kim et al., 2020). This enzyme is specific for uridine and degrades viral dsRNA and thereby preventing host recognition (Kindler et al., 2017). The enzymatic degradation of the RNA takes place via a transesterification reaction producing a 2‘–3’ cyclic phosphodiester end (Bhardwaj et al., 2006). Previous investigations of various PDB crystal structures of Nsp15 have identified His235, His250, Lys290, Thr341, Tyr343, and Ser294 as the active residues responsible for ligand-protein interactions (Kim et al., 2020; Ortiz-Alcantara et al., 2010). Depending on the similarity of the mutual arrangement of the active sites of Nsp15 and ribonuclease A, it has been proposed that His235, His250, and Lys290 form the catalytic triad (Ricagno et al., 2006).

3.2. Structure-based virtual screening and molecular docking

For virtual screening, molecular docking is a useful computational technique for predicting the binding affinity of ligands and proteins, which helps to predict the ability of compounds to inhibit ligands and to conceptualize their mechanism of action. In the current study, the downloaded Nsp15 target enzyme (PDB code: 6vww) was virtually screened against three libraries, two of which included FDA approved drugs while the third contained approved, investigational, and experimental drugs. Uridine-5‘-triphosphate (UTP), the substrate of Nsp15 endoribonuclease, was used as a reference (docking score −6.2). Congo red was used as an investigational inhibitor control (docking score −7.9) as it has been previously reported to be a potent inhibitor of SARS-COV Nsp15 (Ortiz-Alcantara et al., 2010). The virtual screening results revealed 70 drugs that had binding energies greater than both the Congo red and UTP. For further validation, manual molecular docking was performed by using AutoDock 4.2 and Discovery Studio Visualizer was used to investigate the binding modes of the docked drugs according to the visualized results. The drugs exhibiting unpromising binding were excluded from further analysis. Drugs prescribed for external use were also excluded. As a result, only 19 of the 70 drugs were selected for consideration (Table 1). As shown in Table 1, the drug with the best score for binding with Nsp15 was diosgenin glucoside (−11.2 kcal/mol), but each of the final drugs showed binding affinities better than that of the control inhibitor Red Congo. Evaluation of the docked drugs revealed that they each fit well in the binding pocket of Nsp15 and formed convenient hydrogen-bond interactions with active residue, such as His235, His250, Lys290, Ser294, Thr341, and Trp343. In addition to the hydrogen bonding, Van der Waals, Pi-Pi stacking, Pi-Cation, Pi-Anion, and Pi-Sigma interactions were also observed (Figure 1).

3.3. Molecular dynamics simulation analysis

To confirm the binding affinity and stability of the Nsp15-ligand complexes obtained from molecular docking analysis, MD simulations were carried out on five complexes of Nsp15 with the FDA approved drugs Capmatinib, Olaparib, Sorafenib, Risperidone and Brexpiprazol as well as the control inhibitor (Congo Red). However, since the simulation time (>100 ns) is computationally expensive and time consuming, we initially conducted 10 ns simulation time, then the simulation time was extended to 100 ns for the most stable complex (Nsp15-Olaparib). The stability of the Nsp15-ligand complexes was estimated in terms of RMSD (for both ligand and backbone atoms), RMSF and radius of gyration (Figures S1–S5, supplementary material). The RMSD plot of backbone atoms suggests that the complex containing Olaparib (Figure S1) showed less mobility among the other Nsp15-ligand complexes and control inhibitor indicating that this complex is the most stable one. Moreover, RMSD plot of ligands was showed that Olaparib (Figure S2), has less fluctuations and smaller RMSD values (<1.57 Å) than the control inhibitor (<2.85 Å) and other ligands, this indicates that Olaparib has a stable conformation. On the other hand, RMSF was analyzed to obtain information about the flexibility of the complexes residues. The average RMSF values (Figure S3) were found to be 0.94, 0.87, 0.89, 0.91, 0.98 and 0.94 Å for complexes of Nsp15 with Capmatinib, Olaparib, Sorafenib, Risperidone, Brexpiprazol and control inhibitor, respectively. Indicating that the residues of Nsp15-Olaparib complex have the lowest fluctuations. The radius of gyration (Rg) is defined as an indication of the structure compactness and stability of a protein (Bhardwaj & Purohit, 2020c; Hourieh et al., 2019). Therefore, values of Rg for all complexes were calculated during MD simulations. A similar Rg behavior is achieved for all of Nsp15-ligand complexes except for...
Nsp15-Brexipyrazol (Figure S4), the later was showed a relatively less compactness as compared to the other four systems and control inhibitor. It’s obvious that the binding affinity of the ligands is affected by number of hydrogen bonds, where, the more number of H-bonds formed between ligand and key residues can contribute to increasing the binding affinity. Therefore, H-bonds number was calculated during all simulation time to investigate their stability in the protein–ligand

Table 1. Virtual screening and molecular docking of select drugs onto the SARS-CoV-2 Nsp15 endoribonuclease.

| Rank | Drug name                          | Chemical structure | Virtual docking score (Kcal/mol) | Manual docking score (Kcal/mol) | Nsp15 binding site residues involved in intermolecular interactions with the drug |
|------|------------------------------------|--------------------|---------------------------------|---------------------------------|----------------------------------------------------------------------------------|
| 1    | Diosgenin glucoside (Diaglucide)   | ![Chemical structure image](image1) | -11.2                           | -8.83                           | His235, His250, Lys290, Val292, Thr341, Tyr343, Lys345, and Leu346               |
| 2    | Alpha-Ergocryptine                 | ![Chemical structure image](image2) | -10.9                           | -8.38                           | His235, His250, Lys290, Val292, Thr341, Glu340 and Tyr343                       |
| 3    | Capmatinib                         | ![Chemical structure image](image3) | -10.3                           | -8.25                           | His250, Lys290, Val292, Glu340 Thr341, Tyr343, Lys345 and Leu346                |
| 4    | Lixivaptan                         | ![Chemical structure image](image4) | -10.3                           | -8.56                           | His235, Lys290, Val315, Lys335, Glu340, Thr341, Tyr343 and Leu346               |

(continued)
| Rank | Drug name         | Chemical structure | Virtual docking score (Kcal/mol) | Manual docking score (Kcal/mol) | Nsp15 binding site residues involved in intermolecular interactions with the drug |
|------|------------------|--------------------|----------------------------------|-------------------------------|----------------------------------------------------------------------------------|
| 5    | Zoliflodacin     | ![Chemical structure](image1.png) | -10.1                            | -8.31                         | His235, Glu248, His250, Lys290, Val292, Tyr343 and Leu346                      |
| 6    | Olaparib         | ![Chemical structure](image2.png) | -10.0                            | -8.83                         | His235, Glu248, Lys290, Val292, Ser294, Thr341, Tyr343, Pro344, Lys345, and Leu346 |
| 7    | Pranlukast       | ![Chemical structure](image3.png) | -9.4                             | -8.22                         | His235, His250, Lys290, Val292, Thr341, Tyr343 and Leu346                      |
| 8    | Sorafenib (Nexavar) | ![Chemical structure](image4.png) | -9.4                             | -8.3                          | Gly230, His235, Glu248, His250, Lys290, Ser294, His338, Glu340, Thr341, Tyr343, Pro344, Lys345 and Leu346 |
| 9    | Risperidone      | ![Chemical structure](image5.png) | -9.3                             | -8.42                         | His235, Glu248, His250, Lys290, Val292, Met331, Trp333, Tyr343, Lys345, and Leu346 |
system. The hydrogen bonds profile is illustrated in Figure S5, which shows that Nsp15-Olaparib system has a higher number of hydrogen bonds in comparison with the other four Nsp15-ligand systems and control inhibitor, suggesting the higher stability of Nsp15-Olaparib system complex. In case of the most stable complex (Nsp15-Olaparib), the simulation time was extended to 100 ns. Then, the RMSD values for the backbone atoms were calculated for the entire simulation time (Figure 2). The RMSD was <0.5 nm for all the simulation time, except for the short period ranged 72-76 ns which showed a

| Rank | Drug name | Chemical structure | Virtual docking score (Kcal/mol) | Manual docking score (Kcal/mol) | Nsp15 binding site residues involved in intermolecular interactions with the drug |
|------|-----------|--------------------|---------------------------------|---------------------------------|---------------------------------------------------------------------------|
| 10   | Talniflumate | ![Chemical structure](image1) | -8.9 | -8.18 | His235, Gln245, Glu248, His250, Lys290, Cys291, Ser294, His338, Glu340, Thr341 and Tyr343 |
| 11   | Brexpiprazole | ![Chemical structure](image2) | -8.4 | -7.72 | His250, Lys290, Val292, Met331, Pro344, Glu340, Thr341, Tyr343 and Lys345 |
| 12   | Cabozantinib | ![Chemical structure](image3) | -8.4 | -7.43 | His235, Gln245, Gly247, His250, Lys290, Cys291, Tyr343 and Lue346 |
| 13   | Dicumarol | ![Chemical structure](image4) | -8.4 | -7.0 | His235, Gln248, Lys290, Val292, Ser294, Thr341, Tyr343, Pro344 and Lue346 |
| 14   | Enzastaurin | ![Chemical structure](image5) | -8.2 | -8.79 | His235, Lys290, Val292, Cys293, Ser294, Thr341, Lys345 and Leu346 |
| Rank | Drug name                 | Chemical structure | Virtual docking score (Kcal/mol) | Manual docking score (Kcal/mol) | Nsp15 binding site residues involved in intermolecular interactions with the drug |
|------|---------------------------|--------------------|----------------------------------|---------------------------------|---------------------------------------------------------------------------------|
| 15   | Loratadine                | ![Loratadine](image) | 8.1                              | 7.49                            | His235, Glu248, His250, Lys290, Cys291, Val292, Thr341 and Tyr343              |
| 16   | Chidamide (Tucidinostat)  | ![Chidamide](image) | 8.0                              | 8.65                            | His235, Glu248, His250, Ser294, Glu340, Thr341 and Tyr343                      |
| 17   | Amenamevir                | ![Amenamevir](image) | 8.0                              | 8.82                            | His235, Glu248, His250, Lys290, Val315, Cys334, Lys335, Glu340, Thr341 and Tyr343 |
| 18   | Apalutamide               | ![Apalutamide](image) | 8.0                              | 7.4                             | His235, His250, Val315, Trp333, Lys335, Glu340, Thr341 and Tyr343              |
| 19   | Deserpidine               | ![Deserpidine](image) | 9.1                              | 7.52                            | His235, Glu248, His250, Lys290, Val292, Met331, Trp333, Tyr343, Lys345 and Leu346 |
| 20   | Congo Red (Inhibitor Control) | ![Congo Red](image) | 7.9                              | 7.31                            | Glu248, His250, Lys290, Val315, Trp333, Lys335, Asp336 and Tyr343              |
Table 1. Continued.

| Rank | Drug name                  | Chemical structure | Virtual docking score (Kcal/mol) | Manual docking score (Kcal/mol) | Nsp15 binding site residues involved in intermolecular interactions with the drug |
|------|----------------------------|--------------------|----------------------------------|---------------------------------|--------------------------------------------------------------------------------|
| 21   | UTP (Reference Control)    | ![UTP Structure](image) | -6.2                             | -5.62                           | His235, Leu246, Glu248, His250, Lys290, Val292, Ser294, Tyr343, Pro344 and Leu346 |

Figure 1. Two-dimensional representation of the binding modes of drug candidates with the SARS-CoV-2 Nsp15 endoribonuclease. The candidate drugs include Diosgenin glucoside (1), Alpha-Ergocryptine (2), Capmatinib (3), Lixivaptan (4), Zoliflodacin (5), Olaparib (6), Pranlukast (7), Sorafenib (8), Risperidone (9), Talniflumate (10), Brexpiprazole (11), Cabozantinib (12), Dicumarol (13), Enzastaurin (14), Loratadine (15), Chidamide (16), Amenamivir (17), Apalutamide (18), and Deserpidine (19). The binding of Congo red (20) and UTP (21) are also shown. The core structures of the docked drugs occupy the UTP binding site.
higher deviation. This indicates the stability of the selected Nsp15-Olaparib system. Besides, the RMSF values of the binding site residues (His235, His250, Lys290, Ser294, Thr341 and Tyr343) showed less fluctuation than other residues which indicate that binding of Olaparib was accompanied by decreasing the flexibility of these key residues (Figure 3). Additionally, the average of hydrogen bonds number of Nsp15-Olaparib complex was found to be 2.54 (Figure 4).
3.4. Binding free energy analysis

Further validation of the binding energy obtained by molecular docking was achieved through utilization of molecular mechanics Poisson–Boltzmann surface area approach (MM-PBSA). As listed in Table S1 (supplementary material), the calculated binding free energies ($\Delta G_{\text{binding}}$) of...
the selected five drugs and control inhibitor were $-58.38$, $-59.63$, $-46.81$, $-41.20$, $-38.89$ and $-44.98$ kcal/mol for Nsp15 complex with Capmatinib, Olaparib, Sorafenib, Risperidone, Brexpiprazol and control inhibitor, respectively. For complexes of Capmatinib, Olaparib, Sorafenib, and Risperidone, the binding free energies were lower than those
of Brexpiprazol and control inhibitor. However, Olaparib was showed relatively lower binding free energy, while Brexpiprazol was showed the higher binding free energy in comparison with the control inhibitor, moreover, \( \Delta G_{\text{Binding}} \) of Nsp15-Olaparib complex was recalculated for the 100 ns simulation time and it was found to be equal to \(-45.35\) kcal/mol. These results are compatible with the previously reported stability behavior of Nsp15-ligand complexes during MD simulation time.

### 4. Discussion

The novel coronavirus SARS-CoV-2 is believed to have jumped from a bat to humans in Wuhan, China on and has managed to cause a pandemic that continues to grow very broadly worldwide. Efforts are currently underway to identify the ancestry of this virus and better understand its behavior. Since the outbreak of COVID-19, there has been a rapid spread of SARS-CoV-2 in most countries of the world. Unfortunately, there are currently no antiviral drugs known to directly inhibit the virus or trigger an early immune response to stop the progression of the virus infection inside the body. Therefore, identifying effective antiviral agents that reduce the effects of the novel coronavirus remains a significant need. With inadequate time during the storm of the pandemic to develop novel treatments, the repurposing of agents with minimal side effects that are listed in drug libraries is a promising approach. As previous studies have determined that EndoU activity has an important function regarding virus replication in infected hosts (Deng and Baker, 2018; Nedialkova et al., 2009), we targeted the action of the SARS-CoV-2 Nsp15 endoribonuclease in our current study.

In coronaviruses, PUN RNAs can form stem-loop structures that by binding to cytoplasmic receptors of viral recognition may be recognized by hosts as dsRNA, thereby stimulating an innate immune response. The function of EndoU during virus replication is to cleave PUN RNAs, which prevents the accumulation of PUN RNA and limits the potential for generating PAMPs (Hackbart et al., 2020). Therefore, coronaviruses may have evolved unique mechanisms to escape early recognition by the immune system (Hackbart et al., 2020). There are previous studies that support the concept that RNA viruses have developed unique protective mechanisms to prevent early detection by host immune sensing. For instance, endoribonuclease PA-X-deficient influenza viruses induce potential innate immune responses in birds and mammals (Gao et al., 2015; Gong et al., 2017; Hu et al., 2016; Xu et al., 2017), suggesting a role in immune evasion. In addition, endoribonuclease activity in hepatitis C virus (HCV) acts to reduce levels of polyU-rich RNA substrate and helps hide the virus RNA from host immune response sensing (Kato et al., 2011; Neufeldt et al., 2016). Polioviruses use unique the action of a PolyU sequence attached to viral protein genome-linked (VPg) protein with that linkage possibly contributing to the escape virus from the immune response (Steil et al., 2010).

In the current study, homology structures of endoribonuclease SARS-CoV-2 were evaluated based on the analysis of
5. Conclusions

In the current study, molecular docking-based virtual screening and MD were employed for prediction of potential inhibitors of SARS-CoV-2 endoribonuclease. The recently released crystal structure of the SARS-CoV-2 Nsp15 (PDB 6vww) was utilized as a target. Nsp15 is one of the vital enzymes required for SARS-CoV-2 replication and is involved in evading host immune sensing. 19 drugs which have binding affinities greater than those of the investigational inhibitor Congo red and normal substrate UTP were selected. Furthermore, molecular dynamics simulation was performed on the complexes of Nsp15 with five FDA approved drugs (Capmatinib, Olaparib, Sorafenib, Risperidone and Brexpiprazole) for further investigation by molecular dynamics simulation (10 ns). Based on the preliminary findings of MD simulation (10 ns), the simulation time was extended to 100 ns for Nsp15-Olaparib complex. However, RMSD, RMSF, radius of gyration and hydrogen bond profile were calculated to validate the stability of Nsp15-ligand system. Also, MM-PBSA method was employed to validate the binding affinity. According to the obtained results of MD, the complex of Olaparib showed more stability than the control inhibitor during simulation time. Furthermore, the estimated binding free energies were revealed that this drug has a higher binding affinity than the control inhibitor.

in vitro and in vivo evaluations are needed to confirm and expand our findings.

Disclosure statement

The authors declare that they have no conflict of interest.

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Data availability statement

All data generated or analyzed during this study are included in this published article and its supplementary material files.

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