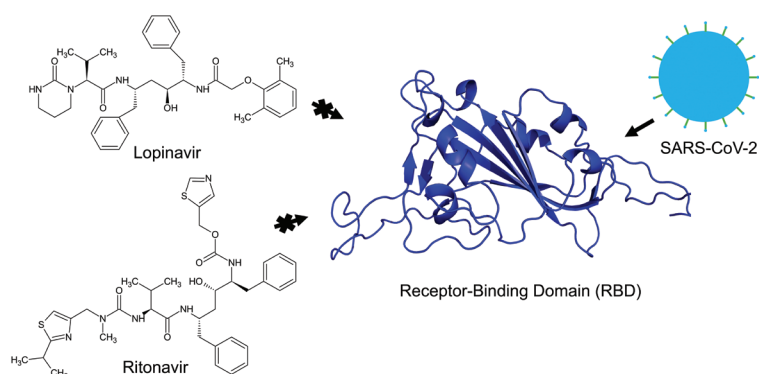


Lopinavir and Ritonavir have High Affinity Toward the SARS-CoV-2 S-protein Receptor-binding Domain Sequenced in Brazil

Graphical abstract



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Highlights

- Lopinavir and ritonavir exhibit strong binding affinities to the Omicron RBD of the SARS-CoV-2 spike protein.
- Specific molecular interactions between these drugs and the virus's receptor-binding domain were identified.
- Molecular dynamics simulations confirmed the stability of the drug-protein complexes.
- A novel structure of the Omicron RBD was developed in this study.
- The Omicron RBD model can serve as a valuable resource for future studies.

In brief

This article presents original research on the interactions between lopinavir and ritonavir with the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein, specifically focusing on the Omicron variant, and highlights their potential role in therapeutic strategies against COVID-19.

Lopinavir and Ritonavir have High Affinity Toward the SARS-CoV-2 S-protein Receptor-binding Domain Sequenced in Brazil

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Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first identified in China in December 2019, rapidly spread worldwide, resulting in the coronavirus disease 2019 (COVID-19) pandemic. Understanding the structural and functional interactions between the virus and host cells is critical for developing therapeutic strategies.

Methods: In this study, we employed *in silico* docking models to investigate the molecular interactions between the receptor-binding domain (RBD) of the SARS-CoV-2 spike glycoprotein, derived from the Brazilian genome sequence, and seven clinically approved drugs: umifenovir, darunavir, lopinavir, ritonavir, remdesivir, pirfenidone, and oseltamivir. The three-dimensional structure of the Omicron RBD model was generated through homology modeling, and potential active site cavities were predicted within the RBD structure.

Results: Among the seven drugs tested, only lopinavir and ritonavir demonstrated significant binding affinities to the RBD. Lopinavir exhibited a binding affinity of -9.8 kcal/mol, forming interactions with residues PHE168, GLY167, SER176, GLN175, GLU166, LEU134, LEU137, TYR171, PHE138, LEU174, and PHE172. Ritonavir showed a binding affinity of -8.9 kcal/mol, interacting with residues ARG148, ASN130, VAL23, SER81, ASN33, PHE29, TYR33, SER31, ASN132, ALA26, ALA30, ALA34, and TYR133. Molecular dynamics simulations confirmed the stability of the complexes formed between lopinavir and ritonavir and the RBD active site.

Conclusion: These findings underscore the potential of these protease inhibitors as therapeutic agents targeting the SARS-CoV-2 spike protein.

Keywords

COVID-19, molecular docking, protease inhibitors, receptor-binding domain, SARS-CoV-2, spike glycoprotein.

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Introduction

Coronavirus disease (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a virus identified in December 2019 in Wuhan, China. COVID-19 is currently recognized as a pandemic by the World Health Organization [1–3].

The first general genome of SARS-CoV-2 in Brazil (GenBank code: MT126808.1) was sequenced on March 1, 2020, and made publicly available at the National Center for Biotechnology Information (NCBI) on the following day [4]. The viral structure includes a spike glycoprotein (S protein), which is divided into S1 and S2 subunits. Together with ACE and TMPRSS2, S protein is important for SARS-CoV-2 entry into host cells [5]. The S1 region contains

the receptor-binding domain (RBD), which interacts with angiotensin-converting enzyme 2 (ACE2) on the surfaces of target cells [6, 7]. This interaction contributes to the stabilization of the S2 domain, a key factor in enabling the fusion between viral and cellular membranes [5, 8]. Because S protein plays a key role in the pathogenesis of COVID-19, several studies have examined the use of monoclonal antibodies targeting this protein; these antibodies' promising neutralizing effects on viral entry into cells *in vitro* support their application potential in COVID-19 prevention [9, 10].

No specific treatments are available for COVID-19. However, since the beginning of the pandemic, scientists have sought effective vaccines or treatments with new compounds or repositioned FDA approved drugs [11]. Given the structural-functional

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relationships between the S protein of SARS-CoV-2 and ACE2—and the importance of the S protein in attachment and entry, resulting in the replication cycle—the S domain might be a promising antiviral drug target. In the present study, we used docking models and conducted an *in silico* analysis of the molecular interactions between FDA approved drugs and the RBD encoded by the Brazilian S Omicron genome sequence, to develop a strategy for functional evaluating drug repositioning.

Methods

Sequence alignment and modeling

Homology modeling is widely applied to create a reliable protein structure by using a protein's own amino acid sequence [12]. The complete genome sequence of the Omicron variant of SARS-CoV-2 in Brazil (ON241705.1) was downloaded from the NCBI nucleotide bank (<https://www.ncbi.nlm.nih.gov/>). The SWISS-MODEL web server (<https://swissmodel.expasy.org>) was used to build the SARS-CoV-2 S protein RBD model by using “Alignment Mode” [13].

The structure obtained (PDB ID: 6W41) was used as a model, showing 99.55% similarity to the RBD homolog sequence. After generating the RBD model using SWISS-MODEL, we evaluated its three-dimensional quality and structural integrity using the Structure Analysis and Verification Server (SAVES v6.0) from the University of California at Los Angeles [14, 15]. To verify the model's validity, we used PROCHECK [16], Verify 3D [17], PROVE [18], and ERRAT [19] software. After model validation, we performed structural minimization in UCSF CHIMERA v1.14 software by using the AMBER ff14SB force field [20] and PROSA [21].

Preparation of ligands

ChemSpider (<http://www.chemspider.com/>) is a chemical database containing more than 67 million structure-based chemistry records [22]. The ligands umifenovir (CSID: 116151), darunavir (CSID: 184733), lopinavir (CSID: 83706), ritonavir (CSID: 347980), remdesivir (CSID: 58827832), pirlfenidone (CSID: 37115), and oseltamivir (CSID: 58540) were obtained from this virtual repository in mol format. All structures were calculated and geometrically optimized with the MMFF94s force field in the GAMESS package [22, 23].

Molecular docking

Before docking, the receptor protein was carefully prepared to ensure the accuracy of the experiments. Active pockets and cavities within the protein structure were identified with the CASTp web server (<http://sts.bioe.uic.edu/castp/index.html>) with a probe radius of 1.4 Å, the standard value for detecting potential active sites [24].

Docking experiments were performed with AutoDock Vina and AutoDock Tools [25]. During preprocessing, all

water molecules and non-essential residues in the RBD of the SARS-CoV-2 Omicron variant were removed to eliminate any interference with the docking process. Polar hydrogen atoms were added to the protein structure to facilitate the formation of hydrogen bonds between the receptor and the ligands. Charges were assigned with the Gasteiger charge model to ensure accurate electrostatic calculations.

The grid box for the RBD was configured to fully encompass the binding site, with the following coordinates: X = −40.0, Y = −39.978, and Z = −6.261. The grid box dimensions were set to X = 80, Y = 80, and Z = 94, thus providing sufficient space for ligands to enable effective exploration of various binding conformations.

The binding affinity of the ligands was assessed according to their Gibbs free energy (ΔG). A threshold of $\Delta G \leq -8.0$ kcal/mol was used to identify candidates with strong binding potential for SARS-CoV-2 treatment. Binding interactions were visualized and analyzed in both 3D and 2D formats with PyMOL v2.0 [26] and LigPlot+ v2.2 [27], respectively. These visualizations highlighted key contacts, including hydrogen bonds and hydrophobic interactions.

Molecular dynamics

All molecular dynamics (MD) simulations were conducted with GRONingen MACHINE for Chemical Simulations (GROMACS) version 5.1.2 [28]. The OPLS-AA/L force field and the TIP3P water model integrated into GROMACS were used for the MD simulations [29]. The peptide topology file was generated with GROMACS, and the ligand topology was obtained with LigParGen. To satisfy minimum image conventions, the system was initially placed in a cubic simulation box with a 2 nm distance between the protein complex and the box boundaries. All bond lengths in proteins and ligands were constrained with the LINCS algorithm, whereas water molecules were constrained with the SETTLE algorithm [30, 31]. A total of 30,000 water molecules were added to a cubic simulation box containing the unbound RBD-Omicron structure. Each system was energy-minimized with the steepest descent algorithm to ensure optimal stability. Short-range and long-range non-bonded interactions were calculated with dual cutoff values of 0.9 nm and 1.4 nm, respectively. The leapfrog algorithm was used to integrate the equations of motion with a time step of 2 fs, and the neighbor list was updated every five steps. Long-range electrostatics were computed with the particle mesh Ewald method with a Fourier grid spacing of 0.15 nm [32].

Periodic boundary conditions were applied in all three spatial dimensions. System equilibration was performed in two main stages: the system was gradually heated to 300 K under constant volume (NVT ensemble) for 10 ns with the v-rescale thermostat, followed by a constant pressure (NPT) ensemble for 5 ns, during which the complexes (RBD-Omicron/ritonavir and RBD-Omicron/lopinavir) were constrained, thus allowing the solvent molecules to relax around them. An additional 10 ns NPT equilibration was performed, during which the constraints on the

complexes were removed. Throughout the equilibration phases, the temperature and pressure remained close to target values. The equilibrated systems were then subjected to production MD simulations for 100 ns, while constant temperature (300 K) and pressure (1 bar) were maintained. We ensured reproducibility by conducting two independent simulations with different initial velocities and equilibration times. Root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF) values were calculated from the MD simulation trajectories to assess system stability and residue flexibility [33].

Results and discussion

On the basis of the sequence identity between SARS-CoV-2 and the RBD, we used SWISS-MODEL to build a valid and high-quality model with 93.85% similarity to the structure of 6W41. **Figure 1A** shows the superposition results. The corresponding regions in the SARS-CoV-2 genome were identified according to the sequence identity and similarity score. **Figure 1B** shows the amino acid sequence between the constructed structure and the model

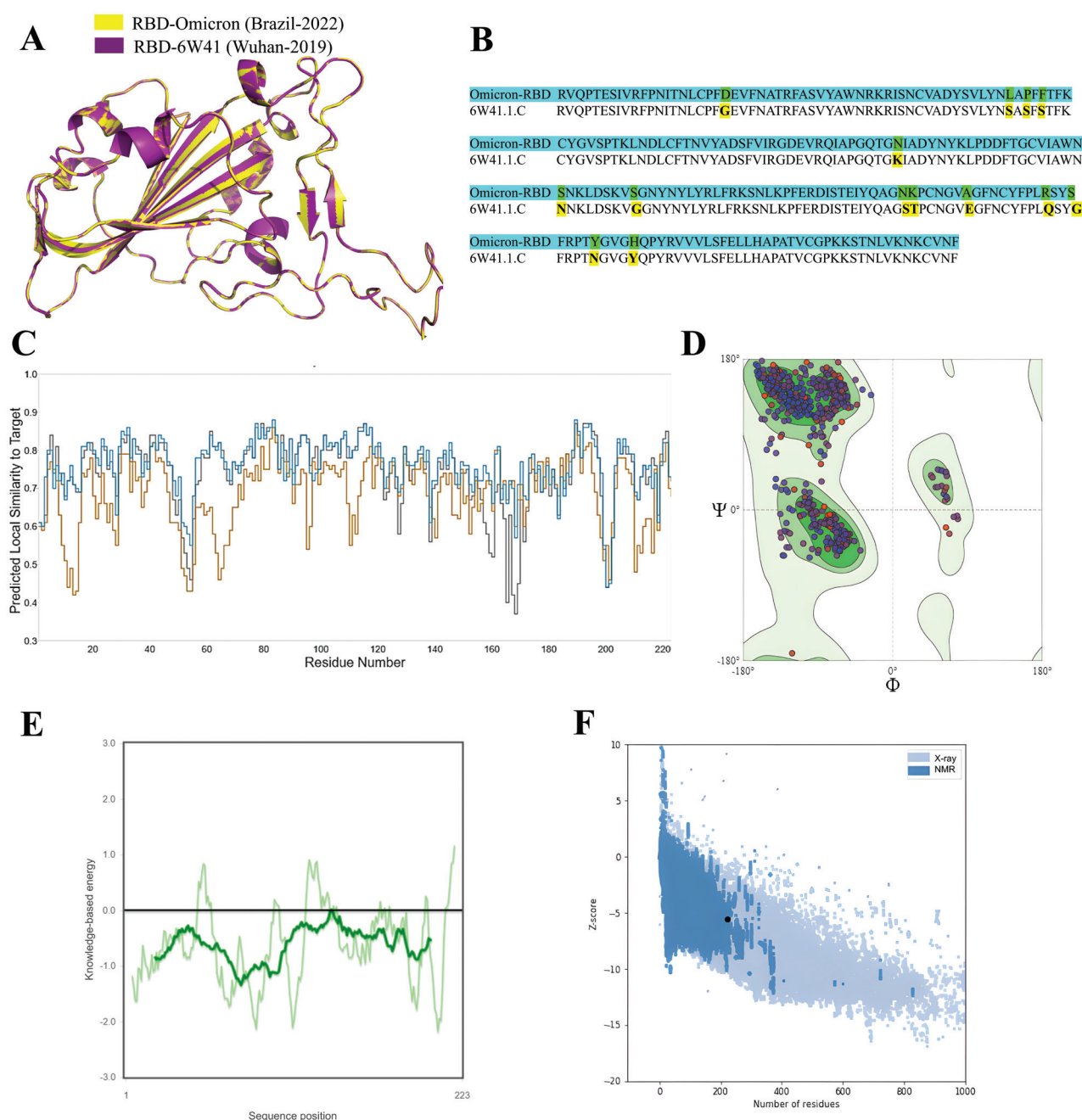


Figure 1 Results of structural modeling according to Omicron RBD structural homology. (A) The Omicron RBD model, built with Swiss-Model is in purple, and the superimposed structure (6W41.1.C) is in yellow. (B) Comparison of sequences of the Brazilian built RBD model (Omicron-RBD) and the structure used in the homologous construction (6W41.1.C). (C) Structural validation chart of the classifier QMEAN-DisCo, referring to RBD-Omicron homology. (D) Ramachandran plot of the RBD-Omicron structure modeled in 3D. (E) Energy prediction results, as compared with the PROSA database structure. (F) Z-score of the RBD-Omicron structure.

example structure (223 amino acids). The 14 mutations (yellow and green) can be seen in the sequence of the RBD of the Brazil-2022 Omicron strain compared with the original strain, Wuhan-2019. As a result of the high sequence similarity, the homology models revealed an unexpectedly conserved overall architecture. The overlap between RBD-BR and the RBD (6W41) of SARS-CoV-2 indicated high structural similarity, with RMSD values of 0.35Å. In addition, the average value of the classifier QMEANDisCo [34] Global was 0.83 ± 0.05 (Figure 1C). The results confirmed the high quality of the model, for which the default score ranged from 0 to 1 [35].

Subsequently, the modeled structure was validated through PROCHECK, Verify 3D, and PROSA II analyses. PROCHECK analysis predicted that the hypothetical model had 89.6% of residues in the favored region, 9.1% of residues in the additionally allowed region, 1.2% of residues in the generously allowed regions, and 0% of residues in the disallowed regions. The quality factor of the residues from the Omicron RBD model, evaluated with Verify3D, indicated that 98.26% of the residues had a favorable average 3D-1D score ≥ 0.2 , suggesting high compatibility of the atomic model (3D) with the amino acid sequence (1D). The ERRAT Ramachandran plot predicted that the final model had 97.8% of residues in favored regions, 2.2% of residues in allowed regions, and 0.0% outlier residues (Figure 1D). A comparative analysis was performed with the ProSA II web algorithm, which predicted that the final structure had a Z-score of -5.91 and therefore was also within the range of scores established for proteins of similar size, with NMR quality (Figure 1E and F).

The viral entry pathway in host cells might be an effective treatment target. In Figure 2, we predicted the conformations of seven therapeutic drugs in complex with the Omicron RBD model, which blocked binding to the putative receptor, human ACE2. The tested drugs in this study were umifenovir, a broad-spectrum antiviral agent used in influenza prophylaxis

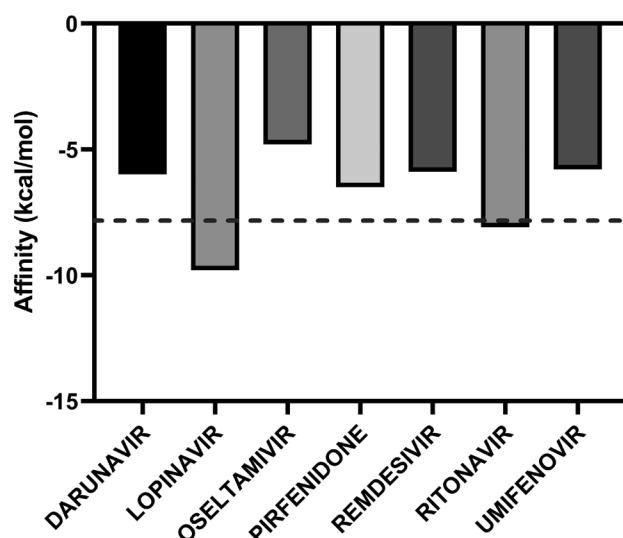


Figure 2 Histogram of binding affinity results (ΔG) between drugs and the modeled RBD. The dashed line shows the limit value of -8 kcal/mol.

and treatment, which also has *in vitro* antiviral effects against SARS-CoV [36, 37]; oseltamivir, a neuraminidase inhibitor used to treat influenza [38]; remdesivir, a nucleotide analog prodrug used to treat Ebola virus [39, 40]; the HIV protease inhibitors darunavir, lopinavir, and ritonavir [41]; and the antifibrotic agent pirfenidone, which is effective against idiopathic pulmonary fibrosis [42, 43].

Umifenovir has been reported to have antiviral activity *in vitro* [35]. A pilot clinical trial conducted in 36 patients in Wuhan city, using reverse transcriptase-polymerase chain reaction, suggested a potential reduction in both viral load and mortality rates [44]. In addition, a clinical trial using a combination of lopinavir and ritonavir has demonstrated promising results [45]. A phase 4 study is ongoing [46]. However, our molecular docking analysis identified a binding score of -5.7 kcal/mol, indicating moderate affinity for the RBD. This suggests that the positive results observed in initial clinical trials may not be directly related to interactions with this domain. Instead, they could potentially involve immunological mechanisms, such as modulation of the immune response, which require further investigation to be fully understood.

Oseltamivir, a neuraminidase inhibitor commonly used as an anti-influenza drug, has recently been suggested as potentially effective against SARS-CoV-2. This drug is currently being evaluated in a phase 3 randomized controlled trial; however, limited evidence is available regarding its mechanism of action [47]. In our molecular docking analysis, oseltamivir exhibited a binding score of -4.9 kcal/mol with the RBD, indicating a weaker binding affinity compared to other compounds. A previous docking study demonstrated its interaction with both papain-like protease (PLpro) and main protease (Mpro), highlighting these as possible targets. Notably, oseltamivir alone showed a lower binding score (-4.65 kcal/mol) compared to the lopinavir/ritonavir combination, which exhibited a stronger interaction with a score of -8.3 kcal/mol [48]. However, in a separate *in silico* study assessing various SARS-CoV-2-targeting drugs, oseltamivir did not demonstrate significant anti-SARS-CoV-2 potential [49].

We also evaluated remdesivir, a nucleotide analog that inhibits RNA polymerase, which demonstrated a binding affinity of -6.0 kcal/mol. When tested against MERS-CoV, remdesivir was found to interact with the non-structural protein 8 (Nsp8) and Nsp12 domains *in vitro* [50]. Additionally, it has been confirmed that remdesivir associates with both exonuclease (ExoN) and RNA-dependent RNA polymerase (RdRp or Nsp12) [51]. RdRp is a viral enzyme essential for the replication of RNA viruses, including SARS-CoV-2. Elfiky [52] reported that remdesivir binds tightly to RdRp with a binding energy of -7.6 kcal/mol. At the ACE2 binding site, remdesivir exhibited a lower binding energy score of -5.62 kcal/mol [53]. Furthermore, molecular docking studies have identified efficient binding energy of remdesivir toward other targets, including Nsp10, Nsp16, Mpro, the S protein, and other components in their unbound forms [49].

In an animal model of infection, remdesivir has also shown anti-SARS-CoV-1 activity mediated by the viral polymerase and the proofreading exonuclease [54]. Additionally, remdesivir has been found to efficiently

inhibit SARS-CoV-2 in rhesus monkey cell culture [44, 55]. Ongoing clinical trials have demonstrated remdesivir's effectiveness against COVID-19 [55] (phase 3 NCT04292730, March 3, 2020; NCT04292899, March 3, 2020; and NCT04401579, March 3, 2020). On the basis of these findings, the interaction between remdesivir and RBD does not appear to inhibit the receptor-binding domains of SARS-CoV-2. However, its crucial role in RNA synthesis, combined with its ability to interact with ExoN (an enzyme located in the N-terminal domain of Nsp14 that reduces the rate of RNA excision), might help explain its observed efficacy in humans.

The interactions with the best binding affinity (the most negative binding energy values) were observed to fall below

the threshold of the gray score limitation line (-8.0 kcal/mol). Drugs with such scores demonstrated strong binding to the RBD of the S protein, which plays a key role in the molecular replication of SARS-CoV-2. Consequently, these drugs might serve as potential candidates for inhibiting viral replication processes and complementing the ongoing use of other drugs currently in clinical trials that have shown promising results [56]. Among the tested drugs, lopinavir and ritonavir showed the best results in RBD viral protein docking (-9.8 kcal/mol and -8.9 kcal/mol, respectively). Darunavir, another HIV protease inhibitor, was not among the best docked drugs (-5.9 kcal/mol).

Figure 3 shows the best results for molecular docking simulation, residue interaction analysis, and MD simulation.

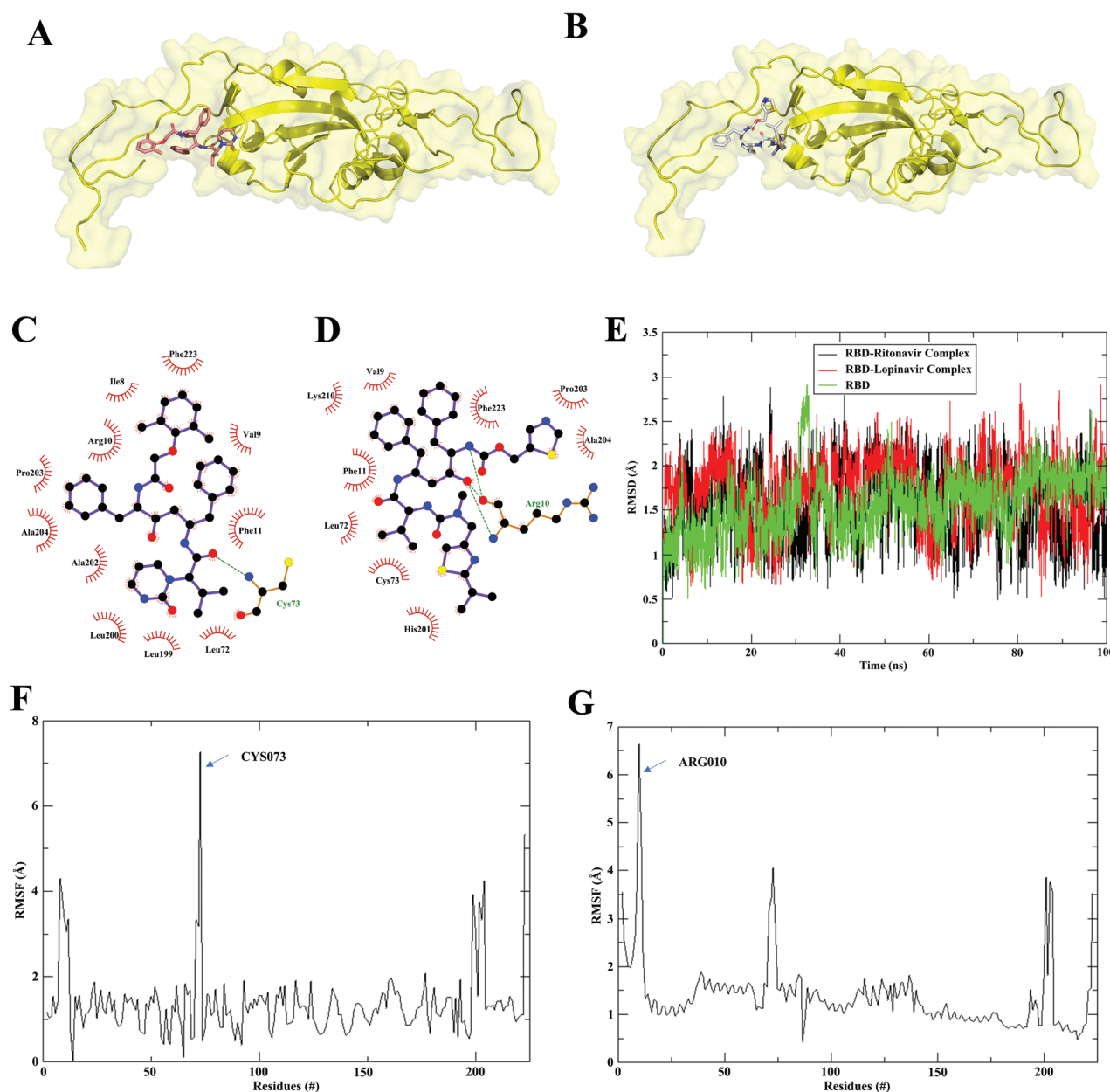


Figure 3 Results of docking and molecular dynamics evaluations. (A) Interaction with lopinavir in the 3D model. (B) Interaction with ritonavir in the 3D model. (C) Lopinavir in the 2D model. (D) Ritonavir in the 2D model. (E) RMSD plot of the complexes and isolated RBD. (F) RMSF of the RBD-lopinavir complex. (G) RMSF of the RBD-ritonavir complex.

The residues involved in hydrophobic interactions with the RBD structure are represented by dashed semicircles around the structure. The H bonds are shown as green dashed lines. Lopinavir (**Figure 3C**) showed 11 interactions: hydrophobic interactions involving ILE8, VAL9, ARG10, PHE11, LEU72, LEU199, LEU200, ALA202, PRO203, ALA204, and PHE 223, and one conventional hydrogen bond to CYS73. Ritonavir (**Figure 3D**), showed ten interactions: hydrophobic interactions involving VAL9, PHE11, ASP71, LEU72, CYS73, HIS201, PRO203, ALA204, LYS210, and PHE 223, and three conventional hydrogen bonds to residue ARG10.

The RMSD quantifies the average positional deviation of C α atoms relative to the initial structure over the course of the simulation, thereby providing insights into the system's structural stability. RMSD values ≤ 2.00 Å are generally considered to indicate a stable protein structure during MD simulations [57]. As shown in **Figure 3E**, the RMSD profiles of the RBD, RBD-lopinavir complex, and RBD-ritonavir complex demonstrated stabilization within the 100 ns simulation timeframe.

Although the RMSD values for the complexes were slightly higher than those for the isolated structure, this difference does not indicate substantial instability, but instead reflects the conformational adjustments and flexibility induced by ligand binding, which are typical and expected phenomena in protein-ligand interactions. This flexibility might enhance the protein's ability to accommodate and stabilize ligands within the binding site.

RMSF analysis indicates residue fluctuations and flexibility during simulation, wherein greater fluctuations are associated with lower stability [58]. This parameter is essential for exploring the roles of individual amino acids in the stability of any binding protein complex. The RMSF of each amino acid in the RBD-lopinavir (**Figure 3F**) and RBD-ritonavir (**Figure 3G**) complexes showed similar fluctuations and only minor variations. Residue CYS073 of the RBD-lopinavir complex was identified in the graph for its fluctuation of 7.23 Å, because of the hydrogen interaction shown in **Figure 3C**, thus confirming the findings from the residue interaction analysis. **Figure 3G** shows the highest fluctuation peak for an ARG010 oscillation, thereby also confirming the hydrogen bond observed in **Figure 3D**.

Because lopinavir and ritonavir are protease inhibitors, several drug repurposing studies have investigated lopinavir and ritonavir alone or in combination. In an *in vitro* evaluation, lopinavir has demonstrated promising results [59], and in combination with ritonavir, it has shown inhibitory effects toward SARS-CoV-2 [60]. Computational studies have focused on evaluating these drugs against proteases present in SARS-CoV-2. Studies using docking and MD have indicated strong affinity of these drugs toward SARS-CoV-2 Mpro, with a free energy of binding of -10.89 kcal/mol (lopinavir) and -14.93 kcal/mol (ritonavir) [61]. In addition, the combination of lopinavir, ritonavir, and oseltamivir improved the free energy values of binding toward Mpro, thus making the system more stable [62]. Efficient binding energy has been observed between ritonavir and

Nsp10, Nsp16, and S protein, and between lopinavir and Mpro/3CLpro, the ADP ribose phosphatase Nsp3, Nsp9, and the ACE2 receptor protein [49].

For clinical use, the lopinavir/ritonavir combination is particularly effective because ritonavir, a strong reversible inhibitor of cytochrome P450 3A4, reduces lopinavir's metabolism, ensuring higher and more sustained plasma levels [63, 64]. Currently, lopinavir/ritonavir are under phase 3 clinical trials (NCT04303299). However, clinical trials with protease inhibitors have not demonstrated sufficient positive interactions for activity against infection. Darunavir and lopinavir/ritonavir might not improve clinical outcomes in the treatment of mild/moderate COVID-19 [64], and darunavir in combination with cobicistat has not achieved clinical improvement over the control [65].

Conclusion

The present study demonstrated that the protease inhibitors lopinavir and ritonavir act by binding the RBD of S protein, an extremely important region for viral entry into cells, according to modeling based on the SARS-CoV-2 genome found in Brazil. This work may support future studies evaluating the mechanisms of action of these drugs against SARS-CoV-2. In addition, it is essential to highlight the significance of research focused on the rational design of drugs, as these molecules can serve as foundational models for further investigations. Understanding potential mechanisms of action at the molecular level is crucial for advancing therapeutic strategies.

Declarations

Availability of data and materials

All data generated or analyzed during this study are included in this article.

Ethical approval

Not applicable in this study.

Authors' contributions

All authors contributed to study conception and design. Material preparation, and data collection and analysis were performed by Helyson Lucas Bezerra Braz, Aline Diogo Marinho, Danilo Galvão Rocha, and João Alison de Moraes Silveira. The first draft of the manuscript was written by Aline Diogo Marinho, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that there are no conflicts of interest, financial or otherwise.

Abbreviations

ACE2, Angiotensin-Converting Enzyme 2; COVID-19, Coronavirus Disease 19; MERS-CoV, Middle East Respiratory Syndrome Coronavirus; Mpro, Main Protease; NCBI, National Center for Biotechnology Information; Nsps, Non-Structure Proteins; PDB, Protein Databank; RBD, Receptor-Binding Domain; S Protein, Spike Glycoprotein; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.

References

- [1] Hall DC Jr, Ji HF. A search for medications to treat COVID-19 via in silico molecular docking models of the SARS-CoV-2 spike glycoprotein and 3CL protease. *Travel Med Infect Dis* 2020;35:101646. [PMID: 32294562 DOI: 10.1016/j.tmaid.2020.101646]
- [2] Srivastava K. Association between COVID-19 and cardiovascular disease. *Int J Cardiol Heart Vasc* 2020;29:100583. [PMID: 32715080 DOI: 10.1016/j.ijcha.2020.100583]
- [3] World Health Organization. WHO COVID-19 dashboard. Geneva: World Health Organization, 2020. Available from: <https://covid19.who.int/>. [Last accessed on 16 May 2022].
- [4] de Jesus JG, Sacchi C, Candido DDS, Claro IM, Sales FCS, et al. Importation and early local transmission of COVID-19 in Brazil, 2020. *Rev Inst Med Trop Sao Paulo* 2020;62:e30. [PMID: 32401959 DOI: 10.1590/s1678-9946202062030]
- [5] Samyuktha V, Naveen-Kumar V. Emergence of RBD and D614G mutations in spike protein: an insight from Indian SARS-CoV-2 genome analysis. *Preprints* 2020;2020060032. [DOI: 10.20944/preprints202006.0032.v1]
- [6] Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 2020;367(6483):1260-3. [PMID: 32075877 DOI: 10.1126/science.abb2507]
- [7] Yan R, Zhang Y, Li Y, Xia L, Guo Y, et al. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science* 2020;367(6485):1444-8. [PMID: 32132184 DOI: 10.1126/science.abb2762]
- [8] Istifli ES, Tepe AŞ, Sarikürkcü C, Tepe B. Interaction of certain monoterpene hydrocarbons with the receptor binding domain of 2019 novel coronavirus (2019-nCoV), transmembrane serine protease 2 (TMPRSS2), cathepsin B, and cathepsin L (CatB/L) and their pharmacokinetic properties. *Turk J Biol* 2020;44(3):242-64. [PMID: 32595360 DOI: 10.3906/biy-2005-46]
- [9] Thanh Le T, Andreadakis Z, Kumar A, Gómez Román R, Tollefsen S, et al. The COVID-19 vaccine development landscape. *Nat Rev Drug Discov* 2020;19(5):305-6. [PMID: 32273591 DOI: 10.1038/d41573-020-00073-5]
- [10] Okba NM, Müller MA, Li W, Wang C, GeurtsvanKessel CH, et al. Severe acute respiratory syndrome coronavirus 2- specific antibody responses in coronavirus disease patients. *Emerg Infect Dis* 2020;26(7):1478-88. [PMID: 32267220 DOI: 10.3201/eid2607.200841]
- [11] Poduri R, Joshi G, Jagadeesh G. Drugs targeting various stages of the SARS-CoV-2 life cycle: exploring promising drugs for the treatment of Covid-19. *Cell Signal* 2020;74:109721. [PMID: 32711111 DOI: 10.1016/j.cellsig.2020.109721]
- [12] Elmezayen AD, Al-Obaidi A, Şahin AT, Yelekçi K. Drug repurposing for coronavirus (COVID-19): in silico screening of known drugs against coronavirus 3CL hydrolase and protease enzymes. *J Biomol Struct Dyn* 2020;39(8):2980-92. [PMID: 32306862 DOI: 10.1080/07391102.2020.1758791]
- [13] Biasini M, Bienert S, Waterhouse A, Arnold K, Studer G, et al. SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. *Nucleic Acids Res* 2014;42(W1):W252-8. [PMID: 24782522 DOI: 10.1093/nar/gku340]
- [14] Elfiky AA. Anti-HCV, nucleotide inhibitors, repurposing against COVID-19. *Life Sci* 2020;248:117477. [PMID: 32119961 DOI: 10.1016/j.lfs.2020.117477]
- [15] University of California. SAVES v6: structure analysis and verification server. Available from: <https://saves.mbi.ucla.edu/>. [Last accessed on 30 Jul 2021].
- [16] Laskowski RA, Rullmann JAC, MacArthur MW, Kaptein R, Thornton JM. AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. *J Biomol NMR* 1996;8(4):477-86. [PMID: 9008363 DOI: 10.1007/bf00228148]
- [17] Eisenberg D, Lüthy R, Bowie JU. VERIFY3D: assessment of protein models with three-dimensional profiles. *Methods Enzymol* 1997;277:396-404. [PMID: 9379925 DOI: 10.1016/s0076-6879(97)77022-8]
- [18] Pontius J, Richelle J, Wodak SJ. Deviations from standard atomic volumes as a quality measure for protein crystal structures. *J Mol Biol* 1996;264(1):121-36. [PMID: 8950272 DOI: 10.1006/jmbi.1996.0628]
- [19] Hooft RW, Vriend G, Sander C, Abola EE. Errors in protein structures. *Nature* 1996;381(6580):272. [PMID: 8692262 DOI: 10.1038/381272a0]
- [20] Maier JA, Martinez C, Kasavajhala K, Wickstrom L, Hauser KE, et al. ff14SB: improving the accuracy of protein side chain and backbone parameters from ff99SB. *J Chem Theory Comput* 2015;11(8):3696-713. [PMID: 26574453 DOI: 10.1021/acs.jctc.5b00255]
- [21] Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Res* 2007;35:W407-10. [PMID: 17517781 DOI: 10.1093/nar/gkm290]
- [22] Barca GMJ, Bertoni C, Carrington L, Datta D, De Silva N, et al. Recent developments in the general atomic and molecular electronic

- structure system. *J Chem Phys* 2020;152(15):154102. [PMID: 32321259 DOI: 10.1063/5.0005188]
- [23] Rogério MEF, Chaves HV, Pinto IR, de Sousa NA, Ribeiro KA, et al. ADME-Tox prediction and molecular docking studies of two lead flavonoids from the roots of *Tephrosia egregia* Sandw and the gastroprotective effects of its root extract in mice. *BIO Integr.* 2022;3(2):43-52. [DOI: 10.15212/bioi-2021-0035]
- [24] Tian W, Chen C, Lei X, Zhao J, Liang J. CASTp 3.0: computed atlas of surface topography of proteins. *Nucleic Acids Res* 2018;46(W1):W363-7. [PMID: 29860391 DOI: 10.1093/nar/gky473]
- [25] Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 2010;31(2):455-61. [PMID: 19499576 DOI: 10.1002/jcc.21334]
- [26] SCHRÖDINGER, LLC. PyMOL molecular graphics system (version 2.0) [Software]. 2021. Available at: <https://www.pymol.org>.
- [27] Laskowski RA, Swindells MB. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. *J Chem Inf Model* 2011;51(10):2778-86. [PMID: 21919503 DOI: 10.1021/ci200227u]
- [28] Abraham MJ, Murtola T, Schulz R, Páll S, Smith JC, et al. GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* 2015;1-2:19-25. [DOI: 10.1016/j.softx.2015.06.001]
- [29] Kaminski GA, Friesner RA, Tirado-Rives J, Jorgensen WL. Evaluation and reparametrization of the OPLS-AA force field for proteins via comparison with accurate quantum chemical calculations on peptides. *J Phys Chem B* 2001;105(28):6474-87. [DOI: 10.1021/jp003919d]
- [30] Hess B, Bekker H, Berendsen HJC, Fraaije JGEM. LINCS: a linear constraint solver for molecular simulations. *J Comput Chem* 1997;18(12):1463-72. [DOI: 10.1002/(SICI)1096-987X(199709)18:12<1463::AID-JCC4>3.0.CO;2-H]
- [31] Miyamoto S, Kollman PA. Settle: an analytical version of the SHAKE and RATTLE algorithm for rigid water models. *J Comput Chem* 1992;13(8):952-62. [DOI: 10.1002/jcc.540130805]
- [32] Darden T, York D, Pedersen L. Particle mesh Ewald: an N-log(N) method for Ewald sums in large systems. *J Chem Phys* 1993;98(12):10089-92. [DOI: 10.1063/1.464397]
- [33] Ghosh R, Chakraborty A, Biswas A, Chowdhuri S. Evaluation of green tea polyphenols as novel coronavirus (SARS CoV-2) main protease (Mpro) inhibitors - an in silico docking and molecular dynamics simulation study. *J Biomol Struct Dyn* 2021;39(12):4362-74. [PMID: 32568613 DOI: 10.1080/07391102.2020.1779818]
- [34] Studer G, Rempfer C, Waterhouse AM, Gumienny R, Haas J, et al. QMEANDisCo-distance constraints applied on model quality estimation. *Bioinformatics* 2020;36(6):1765-71. Erratum in: *Bioinformatics* 2020;36(8):2647. [PMID: 31697312 DOI: 10.1093/bioinformatics/btz828]
- [35] Benkert P, Tosatto SC, Schomburg D. QMEAN: a comprehensive scoring function for model quality assessment. *Proteins* 2008;71(1):261-77. [PMID: 17932912 DOI: 10.1002/prot.21715]
- [36] Blaising J, Polyak SJ, Pécheur EI. Arbidol as a broad-spectrum antiviral: an update. *Antiviral Res* 2014;107:84-94. [PMID: 24769245 DOI: 10.1016/j.antiviral.2014.04.006]
- [37] McKee DL, Sternberg A, Stange U, Laufer S, Naujokat C. Candidate drugs against SARS-CoV-2 and COVID-19. *Pharmacol Res* 2020;157:104859. [PMID: 32360480 DOI: 10.1016/j.phrs.2020.104859]
- [38] Uyeki TM. Oseltamivir treatment of influenza in children. *Clin Infect Dis* 2018;66(10):1501-3. [PMID: 29315362 DOI: 10.1093/cid/cix1150]
- [39] Tchesnokov EP, Feng JY, Porter DP, Götte M. Mechanism of inhibition of Ebola virus RNA-dependent RNA polymerase by remdesivir. *Viruses* 2019;11(4):326. [PMID: 30987343 DOI: 10.3390/v11040326]
- [40] Wang M, Cao R, Zhang L, Yang X, Liu J, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* 2020;30:269-71. [PMID: 32020029 DOI: 10.1038/s41422-020-0282-0]
- [41] de Wilde AH, Jochmans D, Posthuma CC, Zevenhoven-Dobbe JC, Van Nieuwkoop S, et al. Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. *Antimicrob Agents Chemother* 2014;58(8):4875-84. [PMID: 24841269 DOI: 10.1128/AAC.03011-14]
- [42] Taniguchi H, Ebina M, Kondoh Y, Ogura T, Azuma A, et al. Pirfenidone in idiopathic pulmonary fibrosis. *Eur Respir J* 2010;35(4):821-9. [PMID: 19996196 DOI: 10.1183/09031936.00005209]
- [43] Noble PW, Albera C, Bradford WZ, Costabel U, Glassberg MK, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. *Lancet* 2011;377(9779):1760-9. [PMID: 21571362 DOI: 10.1016/S0140-6736(11)60405-4]
- [44] Wang Z, Yang B, Li Q, Wen L, Zhang R. Clinical features of 69 cases with coronavirus disease 2019 in Wuhan, China. *Clin Infect Dis* 2020;71(15):769-77. [PMID: 32176772 DOI: 10.1093/cid/ciaa272]
- [45] Deng L, Li C, Zeng Q, Liu X, Li X, et al. Arbidol combined with LPV/r versus LPV/r alone against corona virus disease 2019: a retrospective cohort study. *J Infect* 2020;81(1):e1-5. [PMID: 32171872 DOI: 10.1016/j.jinf.2020.03.002]
- [46] Singh TU, Parida S, Lingaraju MC, Kesavan M, Kumar D, et al. Drug repurposing approach to fight COVID-19. *Pharmacol Rep* 2020;72(6):1479-508. [PMID: 32889701 DOI: 10.1007/s43440-020-00155-6]
- [47] Akram J, Azhar S, Shahzad M, Latif W, Khan KS. Pakistan Randomized and Observational Trial to Evaluate Coronavirus Treatment (PROTECT) of hydroxychloroquine, oseltamivir and azithromycin to treat newly diagnosed patients with COVID-19 infection who have no comorbidities like diabetes mellitus: a structured summary of a study protocol for a randomized controlled trial. *Trials* 2020;21(1):702. [PMID: 32771032 DOI: 10.1186/s13063-020-04616-4]
- [48] Muralidharan N, Sakthivel R, Velmurugan D, Gromiha MM. Computational studies of drug repurposing and synergism of lopinavir, oseltamivir and ritonavir binding with SARS-CoV-2 protease against COVID-19. *J Biomol Struct Dyn* 2020;39(7):2673-8. [PMID: 32248766 DOI: 10.1080/07391102.2020.1752802]
- [49] Deshpande RR, Tiwari AP, Nyayanit N, Modak M. *In silico* molecular docking analysis for repurposing therapeutics against multiple proteins from SARS-CoV-2. *Eur J Pharmacol* 2020;886:173430. [PMID: 32758569 DOI: 10.1016/j.ejphar.2020.173430]
- [50] Gordon CJ, Tchesnokov EP, Feng JY, Porter DP, Götte M. The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus. *J Biol Chem* 2020;295(15):4773-9. [PMID: 32094225 DOI: 10.1074/jbc.AC120.013056]
- [51] Shannon A, Le NT, Selisko B, Eydoux C, Alvarez K, et al. Remdesivir and SARS-CoV-2: structural requirements at both nsp12 RdRp and nsp14 exonuclease active-sites. *Antiviral Res* 2020;178:104793. [PMID: 32283108 DOI: 10.1016/j.antiviral.2020.104793]
- [52] Elfiky AA. Ribavirin, remdesivir, sofosbuvir, galidesivir, and tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): a molecular docking study. *Life Sci* 2020;253:117592. Erratum in: *Life Sci* 2020;258:118350. [PMID: 32222463 DOI: 10.1016/j.lfs.2020.117592]
- [53] Zhang XY, Huang HJ, Zhuang DL, Nasser MI, Yang MH, et al. Biological, clinical and epidemiological features of COVID-19, SARS and MERS and AutoDock simulation of ACE2. *Infect Dis Poverty* 2020;9(1):99. [PMID: 32690096 DOI: 10.1186/s40249-020-00691-6]
- [54] Agostini ML, Andres EL, Sims AC, Graham RL, Sheahan TP, et al. Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exonuclease. *mBio* 2018;9(2):e00221-18. [PMID: 29511076 DOI: 10.1128/mBio.00221-18]
- [55] Williamson BN, Feldmann F, Schwarz B, Meade-White K, Porter DP, et al. Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. *Nature* 2020;585(7824):273-6. [PMID: 32516797 DOI: 10.1038/s41586-020-2423-5]
- [56] National Institutes of Health. ClinicalTrials.gov. Available from: <https://www.clinicaltrials.gov/>. [Last accessed on 4 Jul 2022].

- [57] Ahmed MZ, Zia Q, Haque A, Alqahtani AS, Almarfadi OM, et al. Aminoglycosides as potential inhibitors of SARS-CoV-2 main protease: an in silico drug repurposing study on FDA-approved antiviral and anti-infection agents. *J Infect Public Health* 2021;14(5):611-9. [PMID: 33866129 DOI: 10.1016/j.jiph.2021.01.016]
- [58] Zarezade V, Rezaei H, Shakerinezhad G, Safavi A, Nazeri Z, et al. The identification of novel inhibitors of human angiotensin-converting enzyme 2 and main protease of Sars-Cov-2: a combination of in silico methods for treatment of COVID-19. *J Mol Struct* 2021;1237:130409. [PMID:33840836 DOI:10.1016/j.molstruc.2021.130409]
- [59] Anastasiou IA, Eleftheriadou I, Tentolouris A, Tsilingiris D, Tentolouris N. In vitro data of current therapies for SARS-CoV-2. *Curr Med Chem* 2020;27(27):4542-8. [PMID: 32400323 DOI: 10.2174/0929867327666200513075430]
- [60] Kang CK, Seong MW, Choi SJ, Kim TS, Choe PG, et al. In vitro activity of lopinavir/ritonavir and hydroxychloroquine against severe acute respiratory syndrome coronavirus 2 at concentrations achievable by usual doses. *Korean J Intern Med* 2020;35(4):782-7. [PMID: 32460458 DOI: 10.3904/kjim.2020.157]
- [61] Nutho B, Mahalapbutr P, Hengphasatporn K, Pattarangoon NC, Simanon N, et al. Why are lopinavir and ritonavir effective against the newly emerged coronavirus 2019? Atomistic insights into the inhibitory mechanisms. *Biochemistry* 2020;59(18):1769-79. [PMID: 32293875 DOI: 10.1021/acs.biochem.0c00160]
- [62] Duangchaemkarn K, Reisfeld B, Lohitnavy M. A pharmacokinetic model of lopinavir in combination with ritonavir in human. *Annu Int Conf IEEE Eng Med Biol Soc* 2014;36:5699-702. [PMID: 25571289 DOI: 10.1109/EMBC.2014.6944921]
- [63] Oldfield V, Keating GM, Plosker G. Enfuvirtide: a review of its use in the management of HIV infection. *Drugs* 2005;65(8):1139-60. [PMID: 15907147 DOI: 10.2165/00003495-200565080-00007]
- [64] Li Y, Xie Z, Lin W, Cai W, Wen C, et al. Efficacy and safety of lopinavir/ritonavir or arbidol in adult patients with mild/moderate COVID-19: an exploratory randomized controlled trial. *Med* 2020;1(1):105-13. [PMID: 32838353 DOI: 10.1016/j.medj.2020.04.001]
- [65] Chen J, Xia L, Liu L, Xu Q, Ling Y, et al. Antiviral activity and safety of darunavir/cobicistat for the treatment of COVID-19. *Open Forum Infect Dis* 2020;7(7):ofaa241. [PMID: 32671131 DOI: 10.1093/ofid/ofaa241]