Rifampicin and Letermovir as potential repurposed drug candidate for COVID-19 treatment: insights from an in-silico study

Yamini Pathak¹ · Amaresh Mishra¹ · Gourav Choudhir² · Anuj Kumar³,⁴ · Vishwas Tripathi¹

Received: 6 November 2020 / Revised: 20 January 2021 / Accepted: 1 February 2021 / Published online: 10 May 2021
© Maj Institute of Pharmacology Polish Academy of Sciences 2021

Abstract

Introduction Drug repurposing is the need of the hour considering the medical emergency caused by the COVID-19 pandemic. Recently, cytokine storm by the host immune system has been linked with high viral load, loss of lung function, acute respiratory distress syndrome (ARDS), multiple organ failure, and subsequent fatal outcome.

Objective This study aimed to identify potential FDA approved drugs that can be repurposed for COVID-19 treatment using an in-silico analysis.

Methods In this study, virtual screening of selected FDA approved drugs was performed by targeting the main protease (Mpro) of SARS-CoV-2 and the key molecules involved in the 'Cytokine storm' in COVID-19 patients. Based on our preliminary screening supported by extensive literature search, we selected FDA approved drugs to target the SARS-CoV-2 main protease (Mpro) and the key players of cytokine storm, TNF-α, IL-6, and IL-1β. These compounds were examined based on systematic docking studies and further validated using a combination of molecular dynamics simulations and molecular mechanic/generalized/Born/Poisson-Boltzmann surface area (MM/G/P/BSA) free energy calculations.

Results Based on the findings, Rifampicin and Letermovir appeared as the most promising drug showing a very good binding affinity with the main protease of SARS-CoV-2 and TNF-α, IL-6, and IL-1β. However, it is pertinent to mention here that our findings need further validation by in vitro analysis and clinical trials.

Conclusion This study provides an insight into the drug repurposing approach in which several FDA approved drugs were examined to inhibit COVID-19 infection by targeting the main protease of SARS-COV-2 and the cytokine storm.
**Introduction**

A newly identified coronavirus strain (severe acute respiratory syndrome, SARS-CoV-2) was reported in Wuhan, China, in late December 2019 [1]. World Health Organization (WHO) has named the disease caused by this novel coronavirus the coronavirus disease 2019 (COVID-19) (WHO) [2, 3]. According to WHO the global tally of coronavirus cases crossed 40.1 million infections, 1,120,217 deaths, and 30,198,946 cured cases [2, 4]. According to the current situation, this pandemic is still ongoing, lacking efficacious therapeutic options. However, the steps taken to reduce the severity of infection remain limited to supportive strategies intended to avoid further complications of coronavirus infection [5].

Considering the medical urgency of COVID-19, we cannot afford the traditional way of drug discovery as it is a time-consuming process. In this regard, the immediate solution lies in drug repurposing. Drug repurposing (also known as drug repositioning or reprofiling) is a technique to identify new applications for certified or investigational drugs outside the original medical indication. There is increasing evidence that such repurposing medication promises to provide patients with quicker access to drugs while reducing costs in the long and difficult drug development cycle [6]. COVID-19 patients face twin challenges; first, the infection of SARS-CoV-2, its fast transmission, and replication, second, SARS-CoV-2 induced massive production of inflammatory cytokines, known as “cytokine storm”. In recent findings, the Cytokine storm has been linked with acute respiratory distress syndrome (ARDS) [7, 8], disease aggravation, multiple-organ failure, and subsequent fatal outcome in COVID-19 infected patients compared to healthy controls [9]. Thus, a comprehensive strategy needs to be followed in the treatment of COVID-19 patients. Among several proteins of the SARS-CoV-2 virus, main protease (M\(^{pro}\)) (Table 1) [10] is considered an attractive target due to its crucial role in virus replication and transcription [11, 12]. Therefore, taken together with all these findings, in the current study, several FDA approved drugs that exhibit the potential for drug repurposing, e.g., Brivudine, Ciclesonide, Diethylcarbamazine, Elvitegravir, Isoniazid, Loperamide, Letermovir, Lopinavir, Pentoxifylline, Reserpine, Rifampicin, Ritonavir, and Tinidazole (https://www.drugbank.ca/) [13] have been virtually screened for identification of the potential drug candidates which can be repurposed based on binding affinity with coronavirus main protease (M\(^{pro}\)) and the key players of the cytokine storm IL-6, TNF-\(\alpha\), and IL-1\(\beta\) (Fig. 1).

**Materials and methods**

**Preparation of protease**

The crystallographic structures of proteins COVID-19 main protease (M\(^{pro}\)) (PDB ID: 6LU7) are represented in the Fig. 2, the crystal structure of TNF-\(\alpha\) (PDB ID: 2AZ5),
IL-1β (PDB ID: 1ITB) [14], and IL-6 (PDB ID: 1ALU), structures were retrieved from RCSB PDB (https://www.rcsb.org/) [15], in.pdb format (Supplementary Table 1). Co-crystallized ligands, as well as crystallographic water molecules, were excluded from the 3D coordinate file of the receptors.

Literature survey and ligand database preparation

A very extensive literature review has been conducted to select the list and structures of FDA approved drugs using PubMed and Google scholar platforms. Based on the findings, we selected potentially effective FDA Approved Drugs for Repurposing were obtained from the drug bank (https://www.drugbank.ca/) [13]. The 11 FDA-approved drug compounds (Table 2) used in the present study were Brivudine, Ciclesonide, Diethylcarbamazine, Elvitegravir, Isoniazid, Letermovir, Loperamide, Pentoxifylline, Reserpine, Rifampicin, and Tinidazole against viral protease that could block SARS-CoV-2 protease. Thereafter, the geometries of the ligands were optimized by Open Babel [16] using force field. The ligands were prepared for docking by using AutoDock 4.2 tools by assigning the charges to all the atoms and storing them as pdbqt.

Toxicity analysis

Toxicity analysis of selected FDA approved drugs were done by the ProTox-II http://tox.charite.de/protox_II/ web server [17]. ProTox-II is an online database in which the small molecule can be analyzed by submitting the SMILES of the same predicts LD50, toxicity class, various toxicity parameters like organ toxicity, Carcinogenicity, Mutagenicity, cytotoxicity, etc. and association of the selected molecule with various adverse pathways based on 33 models. However, in the case of drug repurposing, available toxicity information may be needed to determine whether the repurposed drug supports the proposed clinical use of the new formulation or new route of administration.

Molecular docking

To explain the inhibition mechanism of optimized compounds at the molecular level, a docking study using AutoDock 4.2 was carried out at the interface of COVID-19 main protease Mpro (PDB ID: 6LU7). Molecular docking analysis was done using a local search algorithm to investigate the most preferred binding mode of the selected FDA approved drugs. In addition, we have also used Lopinavir and Ritonavir as a positive control compound, as Lopinavir and Ritonavir have been recently reported as a repositioned drug to treat patients infected with COVID-19 [18]. The Autodock tools were used for preparing the protein for docking, the polar hydrogens, partial charges, and gassteger charges were added using these tools. The protein–ligand interactions were further rendered with the Discovery Studio 2016, Maestro, and Pymol version 1.7.4.5 Edu were utilized for visualization of the docked results. AutoDock4.2 was finally used for blind docking of best hit compounds into the crystallographic structure of TNF-α, IL-1, and IL-6 [19].

Molecular dynamics simulation

Molecular Dynamics (MD) simulation studies were performed to find out the stability and/or flexibility of the drug compounds-protease complexes. All simulations were carried out by using the GROMOS96 43a1 force field available in GROMACS 5.1.4 suite [20]. Ligand topology files were generated with the help of the PRODRG server [21]. The prepared protein complexes were solvated in a cubic box of edge length 10 nm along with SPC water molecules. Adequate numbers of ions were added to maintain the system neutrality. To remove the clashes between atoms of the system energy minimization calculations were performed with the convergence criterion of 1000 kJ/mol/nm. PME was utilized to handle the long-range interaction electrostatics [22]. A cutoff radius of 9Åwas used for both van der Waals and Coulombic interactions. Equilibration was completed in two-phases. In the first stage, the solvent and ion molecules were kept unrestrained while in the second stage the restraint
weight from the protein and protein–ligand complexes was gradually reduced, in the NPT ensemble. All hydrogen bonds were kept constrained using the LINCS algorithm [23]. The temperature and pressure of the system were kept at 300 K and 1 atm respectively by using Berendsen’s temperature and Parrinello-Rahman pressure coupling respectively [24]. The production simulation was started from the velocity and coordinates obtained after the last step of the equilibration step. All the systems were simulated for 50 ns and snapshots were taken at every 2 ps interval.
The MM/PBSA (Molecular Mechanics Poisson Boltzmann Surface Area) technique was utilized for the calculation of the binding energy of the protein–ligand complexes. MMPBSA is a collective energy of the system, which is represented by the van der Waal energy, electrostatic energy, SASA energy, and binding energy of the system. In MM-PBSA, the polar part of the solvation energy is calculated by using the linear relation to the solvent accessible surface area. In the present study, the g_mm-pbsa module of GROMACS was applied for the determination of different components of the binding free energy of complexes [25]. Considering the convergence issue associated with MM-PBSA calculations, only the last 10 ns of data were utilized for the analysis. It is to be noted that the entropy calculations were not done in the current study that could change the numerical values of the binding free energy reported for the compounds.

### Results

#### Toxicity evaluation of FDA approved drugs selected in the study

The individual toxicities of FDA approved drugs were predicted by using ProTox-II. Toxicity analysis was performed in order to predict the safety aspects of the FDA approved drug. The major toxicity endpoints were taken into consideration and the drugs which were not following the safety parameters of toxicity endpoints were not taken for further analysis in our priority list. As shown in Table 3, ProTox-II toxicity prediction software gave results mainly associated with three main toxicity aspects cytotoxicity, carcinogenicity, and mutagenicity. According to the toxicological data, most of the selected FDA-approved drugs were not showing any potential cytotoxicity, carcinogenicity, and mutagenicity including the top two hits Rifampicin and Letermovir.

#### Docking analysis

All the 11 compounds and the positive control compound were further docked by Autodock 4.2. The selected 11 compounds obtained from the drug bank were screened based on molecular docking results. Thus among 11 compounds and the positive control compound, 2 hits were found to have good affinities in terms of docking scores (Table 4).
The binding conformation of the drug compounds at the active site of COVID-19 main protease (Mpro) is presented in Supplementary Table 2. The results of our docking study revealed that two drugs Rifampicin and Letermovir showed the best affinity even better than the positive control compound Ritonavir and Lopinavir is represented in the Figs. 3, 4. Thus, docking studies were performed with the reported crystal structure of COVID-19 main protease (PDB ID: 6LU7) to have an idea about consensus docking score and to obtain more insights on the molecular docking of the top hits. Since as per the docking results, rifampicin appeared as the best hit, therefore we further investigated the effect of Rifampicin on the key molecules of Cytokine storm, TNF-α, IL-6, and IL-1β in order to determine whether it can modulate the cytokine storm of the host immune system. Interestingly, our docking results revealed that Rifampicin has a good binding affinity with these main cytokines (TNF-α, IL-6, and IL-1β) (ΔG = -43.51, -34.98 and -29.54 kJ/mol respectively) involved in the Cytokine storm, indicating that Rifampicin may have a poly-pharmacology effect in COVID-19 patients. The results of molecular docking analysis of Rifampicin against Mpro is presented in Supplementary Table 3. Among the selected drugs, the best performers were used for further MD simulation studies.

**Molecular dynamics simulation**

**Root-mean-square deviation (RMSD)**

The recently converged RMSD of the backbone atoms (Fig. 5a) indicates that all the systems were well equilibrated during the 50 ns simulation. RMSD of the ligand atoms (Fig. 5b) indicates the stability of the ligand with respect to the protein and its binding pocket, while Ciclesonide, Letermovir, and Rifampicin showed similar RMSD profiles, remarkably low RMSD was observed for Elvitegravir which implies its better stability in the active site of the protein.

**Root-mean-square fluctuation (RMSF)**

The RMSF profiles were very similar for all the complexes which evince the structural stability of the protein during the simulation is represented in the Fig. 6.

**Radius of gyration (Rg)**

The radius of gyration profiles was very similar for all the complexes which evince the structural stability of the protein during the simulation is represented in the Fig. 7.

**Solvent accessible surface area (SASA)**

The solvent-accessible surface area (SASA) profiles were very similar for all the complexes which evince the structural stability of the protein during the simulation is represented in the Fig. 8.

**Hydrogen bond**

The strength of a hydrogen bond can be inferred from the distance between the donor and the acceptor atoms. The distribution of the hydrogen bond distances is represented in the Fig. 9a concerning the donor-acceptor distance was in the following order: Rifampicin, Ciclesonide followed by Letermovir and Elvitegravir where lower hydrogen bond distances were observed in case of Rifampicin is represented in the Fig. 9b. The number of hydrogen bonds was also relatively more for Rifampicin.
Table 4  Molecular docking analysis of several compounds against COVID-19 main protease (Mpro) (PDB ID: 6LU7)

| S. No | Drug Name  | 2D Structure | Affinity (kJ/mol) | Residue Formed Hydrogen Bond Interaction with Compounds |
|-------|------------|--------------|-------------------|--------------------------------------------------------|
| 1     | Lopinavir* | ![Lopinavir](image1) | −37.61            | ASN95, ASP33                                           |
| 2     | Ritonavir* | ![Ritonavir](image2) | −35.10            | GLN83                                                 |
| 3     | Rifampicin | ![Rifampicin](image3) | −39.83            | CYS145, SER144                                         |
| 4     | Letermovir | ![Letermovir](image4) | −38.95            | THR190                                                |
| 5     | Ciclesonide| ![Ciclesonide](image5) | −36.94            | SER144, GLY143, CYS145                                |
| 6     | Elvitegravir| ![Elvitegravir](image6) | −31.17            | HIS164, THR190, GN192, GLU166                         |
| S. No | Drug Name   | 2D Structure | Affinity (kJ/mol) | Residue Formed Hydrogen Bond Interaction with Compounds |
|-------|-------------|--------------|-------------------|--------------------------------------------------------|
| 7     | Loperamide  | ![Loperamide 2D Structure](image1.png) | −30.50           | HIS164, CYS145                                          |
| 8     | Reserpine   | ![Reserpine 2D Structure](image2.png) | −27.99           | GLN189                                                  |
| 9     | Brivudine   | ![Brivudine 2D Structure](image3.png) | −27.70           | GLN199, GLU166, THR190                                  |
| 10    | Pentoxifylline | ![Pentoxifylline 2D Structure](image4.png) | −25.27           | GLN192, THR190, GLU166                                 |
| 11    | Tinidazole  | ![Tinidazole 2D Structure](image5.png) | −21.04           | HIS163, SER144, CYS145                                 |
| 12    | Diethylcarbamazine | ![Diethylcarbamazine 2D Structure](image6.png) | −19.33           | GLU166                                                  |
| 13    | Isoniazid   | ![Isoniazid 2D Structure](image7.png)  | −19.29           | GLU166, PHE140, ASN142, HIS163, GLY143                 |

*Positive control compounds*
Free binding energy analysis/Poisson – Boltzmann surface area (MM-PBSA)

The MMPBSA calculation of the last 10 ns showed that Letermovir has maximum binding energy $-267.430 \pm 22.985$ kJ/mol whereas Rifampicin has less binding energy $-116.389 \pm 16.260$ (Table 5).

Discussion

The novel coronavirus, SARS-CoV-2 has posed a global threat due to the lack of any specific treatment. Considering the fast rate of transmission of this virus and subsequent severe inflammatory response by the host immune system (Cytokine storm) leading to the multiple organ failure and finally the fatal condition, mainly, the two culprits can be identified, [26] virus key proteins and severity of host immune response in the form of the cytokine storm. Therapeutic targeting should address these two crucial aspects. This would be a comprehensive approach to the treatment of COVID-19 patients. Therefore, in this study, we targeted the key virus protein, the main protease (M\textsuperscript{pro}) of SARS-CoV-2, which helps the virus in replication and transcription. Another major concern in the COVID-19 patients is the release of many cytokines in the form of cytokine storm which is now considered as one of the major causes of multiple organ failure. Thus, in the proposed study we have also targeted key inflammatory cytokines TNF-\alpha, IL-6, and IL-1\beta involved in the cytokines storm to modulate the immune system’s hyperactive systemic response. Lopinavir and Ritonavir are well-established proteases inhibiting drugs for HIV [27]. In several studies, both drugs were also proposed to treat SARS and Middle East respiratory syndrome (MERS) [28]. This combination has also been used in COVID-19 patients in order to control COVID-19 infection [29]. Therefore, in this study, we have taken these drugs as a standard reference to compare the efficacy of the binding of our selected FDA approved drugs. After identification of the active sites of COVID-19 main protease M\textsuperscript{pro} (PDB: 6LU7), we further performed a docking study of our selected compounds Rifampicin, Letermovir, Ciclesonide, Elvitegravir, Loperamide, Reserpine, Brivudine, Pentoxifylline, Tinidazole, Diethylcarbamazine, and Isoniazidas potential inhibitors of the COVID-19 main protease M\textsuperscript{pro}. The binding energies obtained from docking 6LU7 with selected FDA approved drugs showed inhibition potential of these drugs in the order, ranked by binding affinity ($\Delta G_{\text{bind}}$).
Rifampicin and Letermovir as potential repurposed drug candidate for COVID-19 treatment:

Fig. 4 compounds; (a) interaction between Mpro and Lopinavir with -37.61 kJ/mol docking energy; (b) interaction between Mpro and Ritonavir with docking energy -35.10 kJ/mol; (c) interaction between Mpro and Rifampicin with -39.83 kJ/mol docking energy; (d) interaction between Mpro and Letermovir with -38.95 kJ/mol docking energy. Interactions were visualized using maestro and pymol.
i.e., Rifampicin, Letermovir, Ciclesonide, Elvitegravir, Loperamide, Reserpine, Brivudine, Pentoxifylline, Tinidazole, Diethylcarbamazine, and Isoniazid was $-39.83$, $-38.95$, $-36.94$, $-31.17$, $-30.50$, $-27.99$, $-27.70$, $-25.27$, $-21.04$, $-19.33$, and $-19.29$ kJ/mol respectively. Intriguingly, among the selected FDA-approved drugs, two drugs Rifampicin and Letermovir were giving binding affinity even better than the reference drugs. Furthermore, Rifampicin also showed a good binding affinity with inflammatory cytokines TNF-α, IL-6, and IL-1β indicating it may be a potential drug for repurposing in immune modulation during cytokine storm. Therefore, the current study was a two-pronged approach to target the virus main protease and cytokine storm by modulating the severity of the host immune system. To sum up, in our current study based on in-silico analysis, Rifampicin and Letermovir appeared as the most promising potential drug which can be repurposed to target the main protease of
Rifampicin and Letermovir as potential repurposed drug candidate for COVID-19 treatment:…

SARS-CoV-2 and modulate the cytokine storm of the host immune system to protect COVID-19 patients from viral infection progression and multiple organ failure. However, our findings need further validation by clinical trials.

Conclusion

Drug repurposing is an attractive option for the rapid identification of potential therapeutics for COVID-19. This study aimed to examine several FDA-approved drugs that could be repurposed to inhibit COVID-19 infection by targeting the main protease of SARS-COV-2 and the cytokine storm caused by the host immune system. Therefore, the results of this study indicate that Rifampicin, a well-established medicine for the treatment of tuberculosis has a stronger binding affinity for COVID-19 main protease Mpro and the key molecules of Cytokine storm namely TNF-α, IL-6, and IL-1β, in comparison to the other drugs taken in this study. To sum up, our in-silico findings suggest that Rifampicin and Letermovir may be used as a repurposed drug for the treatment of COVID-19. However, it is pertinent to mention here that these findings warrant further in vitro and clinical trials in order to precisely conclude our findings.

Table 5 Binding free energy calculation of four stable complexes during simulation

| Name of molecules | Van der waal energy (KJ/mol) | Electrostatic energy (KJ/mol) | Polar solvation energy (KJ/mol) | SASA energy (KJ/mol) | Binding energy (KJ/mol) |
|-------------------|-------------------------------|-------------------------------|-------------------------------|----------------------|------------------------|
| Ciclesonide       | $-231.571 \pm 14.141$         | $-12.783 \pm 7.504$           | $72.648 \pm 13.093$          | $-18.364 \pm 1.134$ | $-190.070 \pm 13.003$  |
| Elvitegravir       | $-266.868 \pm 13.659$         | $-28.820 \pm 9.003$           | $114.401 \pm 15.866$        | $-17.952 \pm 1.041$ | $-199.239 \pm 15.563$  |
| Letermovir        | $-325.169 \pm 31.257$         | $-16.882 \pm 5.047$           | $97.118 \pm 15.786$         | $-22.498 \pm 1.404$ | $-267.430 \pm 22.985$  |
| Rifampicin        | $-177.790 \pm 17.341$         | $-49.032 \pm 26.129$          | $127.435 \pm 32.818$        | $-17.003 \pm 1.660$ | $-116.389 \pm 16.260$  |

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s43440-021-00228-0.

Author contributions VT: conceived the idea, drafting the article, and supervised the entire study and final approval of the version to be submitted. YP, AM, and GC: contributed to the research tool and analysis and interpretation and drafting of the manuscript. AK: contributed to the final version of the manuscript and done the critical analysis. All authors discussed the results and contributed to the final manuscript.

Funding None.

Compliance with ethical standards

Conflict of interest All authors have no conflict of interest to report.

References

1. Paraskevis D, Kostaki EG, Magiorkinis G, Panayiotakopoulos G, Sourvinos G, Tsiodras S. Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. Infect Genet Evol. 2020;79:104212.
2. World Health Organization (WHO). Novel Coronavirus (2019-nCoV). WHO Bull. 2020;3:1–4.
3. World Health Organization (WHO). Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV). Geneva, Switz. 2020. pp. 1–6.

4. Worldometer. Worldometer for coronavirus cases. Worldometer. 2020.

5. Hai ZD, Lun WuK, Zhang X, Qiong DS, Peng B. In silico screening of Chinese herbal medicines with the potential to directly inhibit novel coronavirus. J Integr Med [Internet] Shanghai Chang-hai Hospital. 2019;2020(18):152–8. https://doi.org/10.1016/j.jimj.2020.02.005.

6. Parvathaneni V, Kulkarni NS, Muth A, Gupta V. Drug repurposing: a promising tool to accelerate the drug discovery process. Drug Discov Today [Internet] Elsevier Ltd. 2019;24:2076–85. https://doi.org/10.1016/j.drudis.2019.06.014.

7. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel Coronavirus in Wuhan, China. Lancet. 2020;395(10223):497–506.

8. Meduri GU, Kohler G, Headley S, Tolley E, Stentz F, Postlethwaite A. Inflammatory cytokines in the BAL of patients with ARDS: Persistent elevation over time predicts poor outcome. Chest. 1995;108(5):1303–14.

9. Li X, Geng M, Peng Y, Meng L, Lu S. Molecular immune pathogenesis and diagnosis of COVID-19. J Pharm Anal. 2020;10(2):102–8.

10. Tahir U, Qamar M, Alqahtani SM, Alamri MA, Chen LL. Structural basis of SARS-CoV-2 3CLpro and anti-COVID-19 drug discovery from medicinal plants. J Pharm Anal. 2020;10(4):313–9.

11. Xu Z, Peng C, Shi Y, Zhu Z, Mu K, Wang X, et al. Nelfinavir was predicted to be a potential inhibitor of 2019-nCov main protease by an integrative approach combining homology modelling, molecular docking and binding free energy calculation. biorXiv. 2020. https://doi.org/10.1101/2020.01.27.921627.

12. Sheahan TP, Sims AC, Leist SR, Schäfer A, Won J, Brown AJ, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020;11(1):1–4.

13. Bank DRUG. Drug Bank. Metamphetamine: Amphetamine MDMA; 2005.

14. Vigers GPA, Anderson LJ, Caffes P, Brandhuber BJ. Crystal structure of the type-1 interleukin-1 receptor complexed with interleukin-1β. Nature. 1997;386(6621):190–4.

15. Protein Data Bank, RCSB PDB: homepage. Rscb Pdb. 2019;2:24.

16. O’Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR. Open Babel. J Cheminform. 2011;3:1–14.

17. Banerjee P, Eckert AO, Schrey AK, Preisser R, ProfFox-II: a web-server for the prediction of toxicity of chemicals. Nucleic Acids Res. 2018;46(W1):W257–63.

18. Stover H. Lopinavir–ritonavir in severe COVID-19. Nat Med. 2020;26(4):465.

19. Wu J, Qu Y, Deng JX, Liang WY, Jiang ZL, Lai R, et al. Molecular docking studies of kirenol a traditional Chinese medicinal compound against rheumatoid arthritis cytokine drug targets (TNF-α, IL-1 and IL-6). Biomed Res. 2017;12:9.

20. Van Der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, Berendsen HJC. GROMACS: Fast, flexible, and free. J Comput Chem. 2005;26(16):1701–18.

21. Schüttelkopf AW, Van Aalten DMF. PRODRG: A tool for high-throughput crystallography of protein-ligand complexes. Acta Crystallogr Sect D Biol Crystallogr. 2004;60(8):1355–63.

22. Abraham MJ, Gready JE. Optimization of parameters for molecular dynamics simulation using smooth particle-mesh Ewald in GROMACS 4.5. J Comput Chem. 2011;32(9):2031–40.

23. Hess B, Bekker H, Berendsen HJC, Fraaije JGEM. LINCS: a linear constraint solver for molecular simulations. J Comput Chem. 1997;18(12):1463–72.

24. Berendsen HJC, van der Spoel D, van Drunen R. GROMACS: A message-passing parallel molecular dynamics implementation. Comput Phys Commun. 1995;91(1–3):43–56.

25. Kumari R, Kumar R, Lynn A. G-mmpbsa -A GROMACS tool for high-throughput MM-PBSA calculations. J Chem Inf Model. 2014;54(7):1951–62.

26. Delia RV, Harrison K, Oyston PC, Lukaszewski RA. Targeting the “Cytokine Storm” for therapeutic benefit. Clin Vaccine Immunol. 2013;20(3):319–27.

27. Israr M, Mitchell D, Alam S, Dinello D, Kishel JJ, Meyers C. The HIV protease inhibitor lopinavir/ritonavir (Kaletra) alters the growth, differentiation and proliferation of primary gingival epithelium. HIV Med. 2011;12(3):145–56.

28. Chu CM, Cheng VCC, Hung IFN, Wong MML, Chan KH, Chan KS, et al. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. Thorax. 2004;59:252–6.

29. Lim J, Jeon S, Shin HY, Kim MJ, Seong YM, Lee WJ, et al. The Author’s response: case of the index patient who caused tertiary transmission of coronavirus disease 2019 in Korea: the application of lopinavir/ritonavir for the treatment of COVID-19 pneumonia monitored by quantitative RT-PCR. J Korean Med Sci. 2020;35:1–6.

30. Kinjo AR, Bekker GJ, Suzuki H, Tsuchiya Y, Kawabata T, Ikegawa Y, et al. Protein data bank Japan (PDBj): updated user interface, resource description framework, analysis tools for large structures. Nucleic Acids Res. 2017;25:962.

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.