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ARTICLE

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

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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 (M^{pro}) 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 (M^{pro}) 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.

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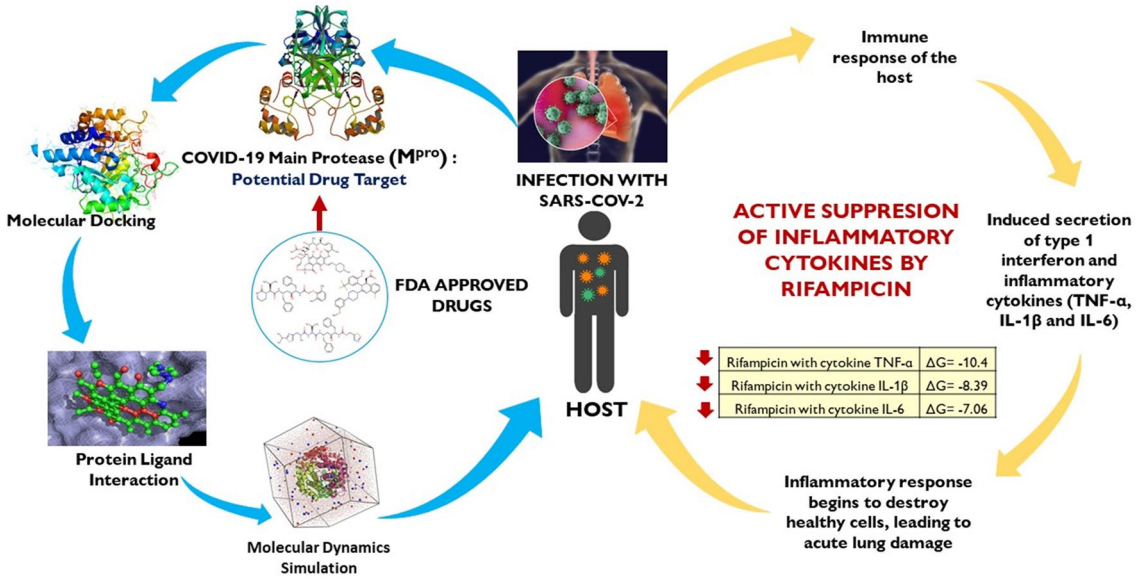
Graphic abstract



Keywords COVID-19 · Cytokine storm · Drug repurposing · Main protease M^{pro} · Molecular docking · Molecular dynamics simulation

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-α, and IL-1β (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-α (PDB ID: 2AZ5),

Table 1 Structural details of SARS-CoV-2 Main Protease M^{pro} [30]

PARAMETERS	COVID-19 main protease in complex with an inhibitor N3 (PDB ID: 6LU7)
Descriptor	Main protease, n-[(5-methylisoxazol-3-yl)carbonyl]alanyl-l-valyl-n ~ 1 ~ -((1r,2z)-4-(benzyloxy)-4-oxo-1-{[(3r)-2-oxopyrrolidin-3-yl] methyl}but-2-enyl)-l-leucinamide
Number of polymer chains	2 CHAINS- A, C
Chain length	A: 306
	C: 6
Formula weight	A: 33,825.5
	C: 680.8
Biological source	Severe acute respiratory syndrome coronavirus 2 (2019-nCoV)

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 M^{pro} (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 gastegier 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

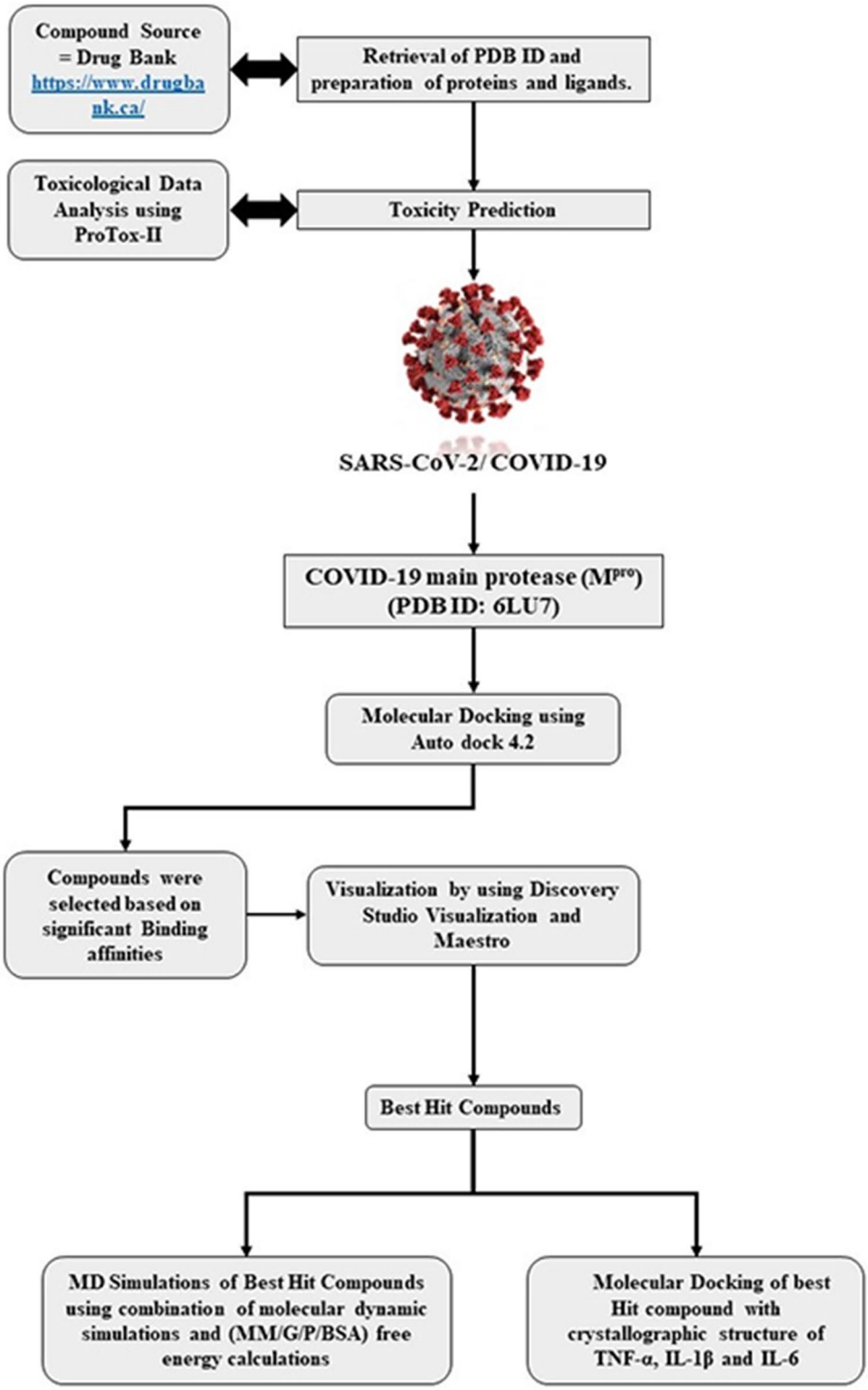


Fig. 1 Schematic representation of the overall workflow utilized in the present study

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.

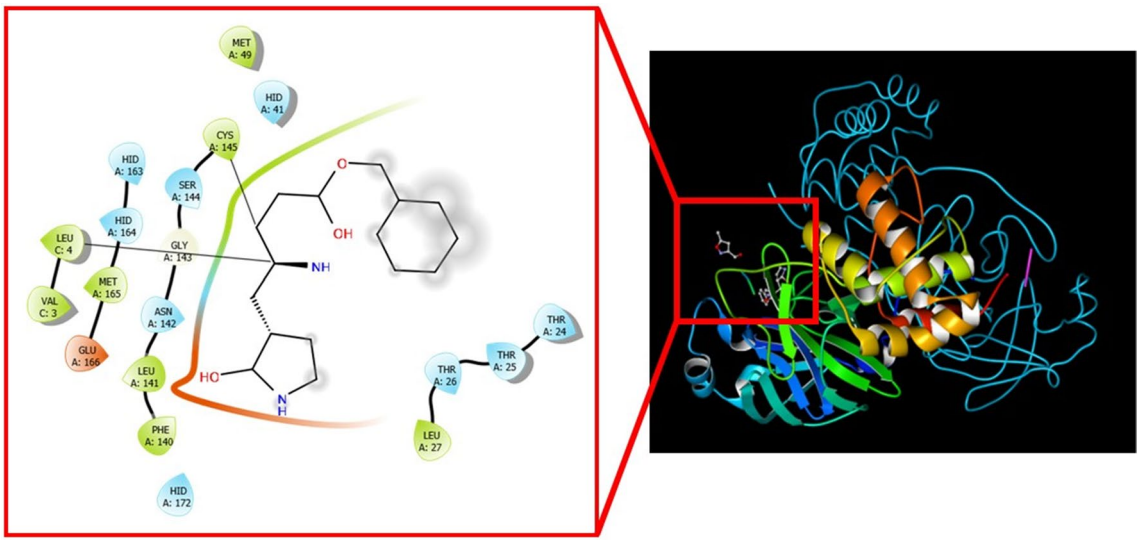


Fig. 2 Cartoon representation of the crystal structure of COVID-19 main protease M^{pro} in complex with an inhibitor N3 showing important interacting residues of the binding pocket of COVID-19 main protease M^{pro} and inhibitor N3

Table 2 List of selected FDA-approved drugs

Drug Bank ID	Drug name
DB03312	Brivudine
DB01410	Ciclesonide
DB00711	Diethylcarbamazine
DB09101	Elvitegravir
DB00951	Isoniazid
DB12070	Letermovir
DB00836	Loperamide
DB00806	Pentoxifylline
DB00206	Reserpine
DB01045	Rifampicin
DB00911	Tinidazole

MM/PBSA free energy calculation

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).

Table 3 Toxicity predictions for selected FDA approved drugs

S.No	Compounds	Toxicity class	LD50 (mg/kg)	Cytotoxicity	Carcinogenicity	Mutagenicity
1	Ritonavir*	4	1000	*Inactive*	*Inactive*	*Inactive*
2	Lopinavir*	5	5000	*Inactive*	*Inactive*	*Inactive*
3	Rifampicin	4	500	*Inactive*	*Inactive*	*Inactive*
4	Letermovir	4	1500	*Inactive*	*Inactive*	*Inactive*
5	Ciclesonide	4	2000	*Inactive*	**Active**	*Inactive*
6	Elvitegravir	4	800	*Inactive*	*Inactive*	*Inactive*
7	Loperamide	4	1190	*Inactive*	*Inactive*	*Inactive*
8	Reserpine	2	50	**Active**	**Active**	*Inactive*
9	Brivudine	6	8400	*Inactive*	*Inactive*	*Inactive*
10	Pentoxifylline	4	780	*Inactive*	*Inactive*	*Inactive*
11	Tinidazole	5	2710	*Inactive*	**Active**	**Active**
12	Diethylcarbamazine	4	660	*Inactive*	*Inactive*	*Inactive*
13	Isoniazid	3	133	*Inactive*	**Active**	Inactive

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-1βin 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, $\Delta G - 43.51, - 34.98$ 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 M^{pro} 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 decently 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 (M^{pro}) (PDB ID: 6LU7)

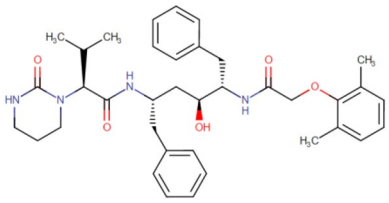
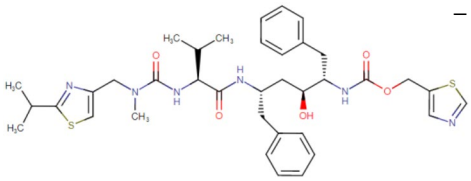
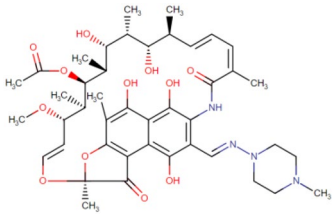
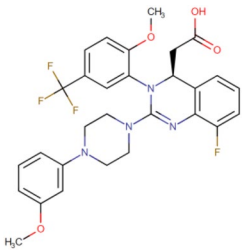
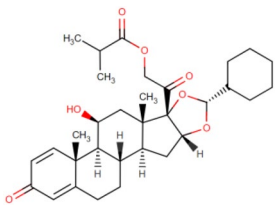
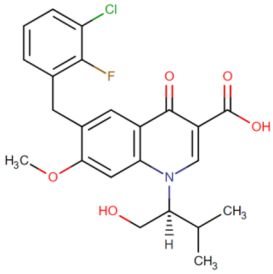
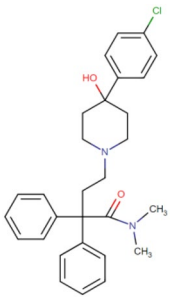
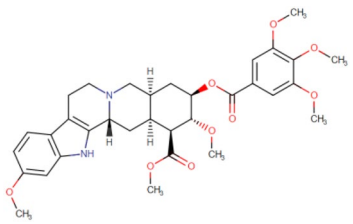
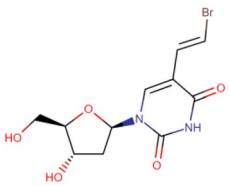
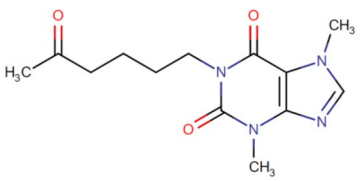
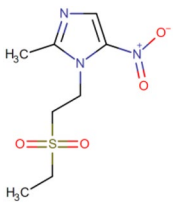
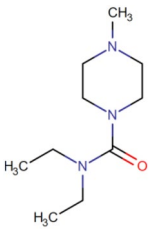
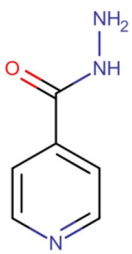
S. No	Drug Name	2D Structure	Affinity (kJ/mol)	Residue Formed Hydrogen Bond Interaction with Compounds
1	Lopinavir*		− 37.61	ASN95, ASP33
2	Ritonavir*		− 35.10	GLN83
3	Rifampicin		− 39.83	CYS145, SER144
4	Letermovir		− 38.95	THR190
5	Ciclesonide		− 36.94	SER144, GLY143, CYS145
6	Elvitegravir		− 31.17	HIS164, THR190, GN192, GLU166

Table 4 (continued)

S. No	Drug Name	2D Structure	Affinity (kJ/mol)	Residue Formed Hydrogen Bond Interaction with Compounds
7	Loperamide		− 30.50	HIS164, CYS145
8	Reserpine		− 27.99	GLN189
9	Brivudine		− 27.70	GLN199, GLU166, THR190
10	Pentoxifylline		− 25.27	GLN192, THR190, GLU166
11	Tinidazole		− 21.04	HIS163, SER144, CYS145
12	Diethylcarbamazine		− 19.33	GLU166
13	Isoniazid		− 19.29	GLU166, PHE140, ASN142, HIS163, GLY143

*Positive control compounds

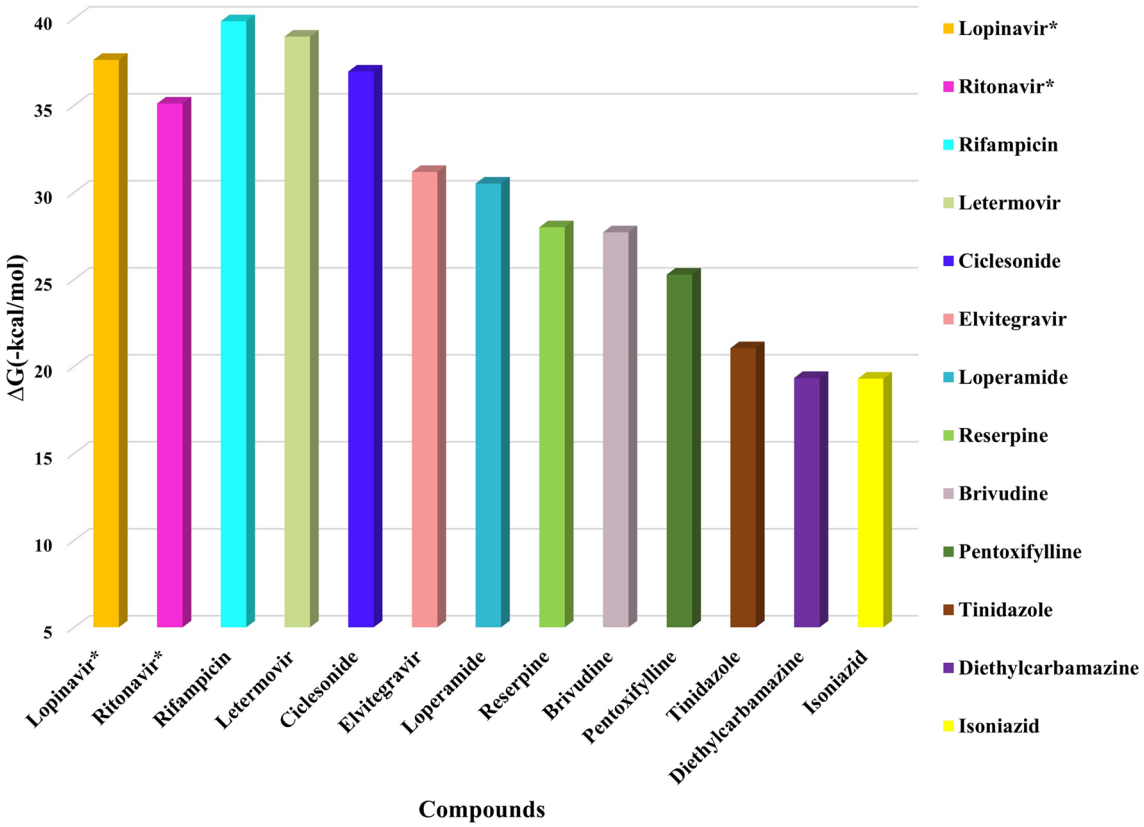


Fig. 3 Histogram showing molecular docking results between COVID-19 main protease M^{pro} (PDB ID: 6LU7) and several drug compounds (the binding energy value ΔG is shown in minus kJ/mol). *Positive control compounds

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 ± 22.985 kJ/mol whereas Rifampicin has less binding energy −116.389 ± 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^{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-α, IL-6, and IL-1β 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^{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^{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 (ΔG_{bind})

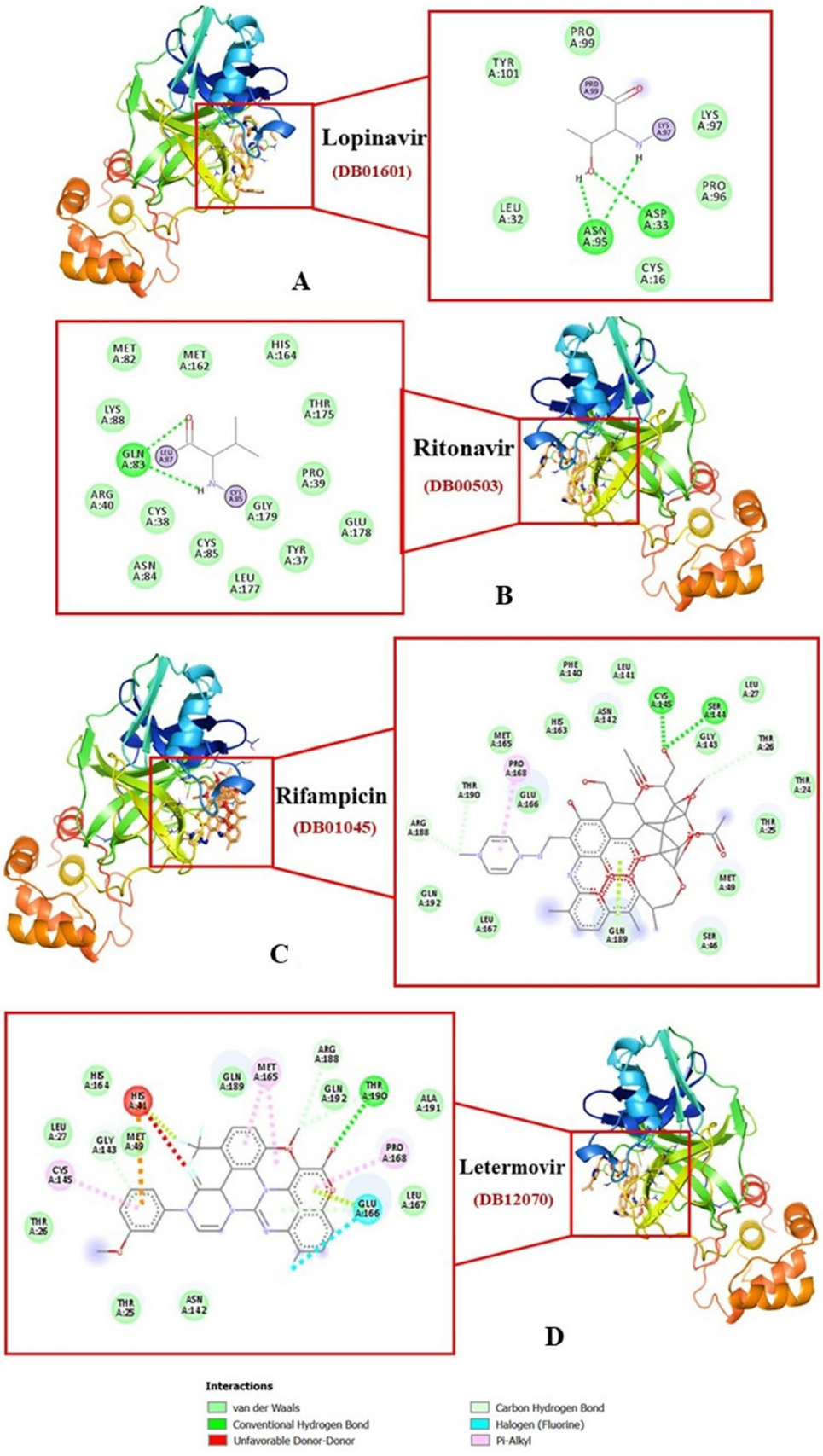


Fig. 4 compounds; (**a**) interaction between M^{pro} and Lopinavir with -37.61 kJ/mol docking energy; (**b**) interaction between M^{pro} and Ritonavir with docking energy −35.10 kJ/mol; (**c**) interaction between M^{pro} and Rifampicin with − 39.83 kJ/mol docking energy; (**d**) interaction between Mpro and Letermovir with − 38.95 kJ/mol docking energy. Interactions were visualized using maestro and pymol

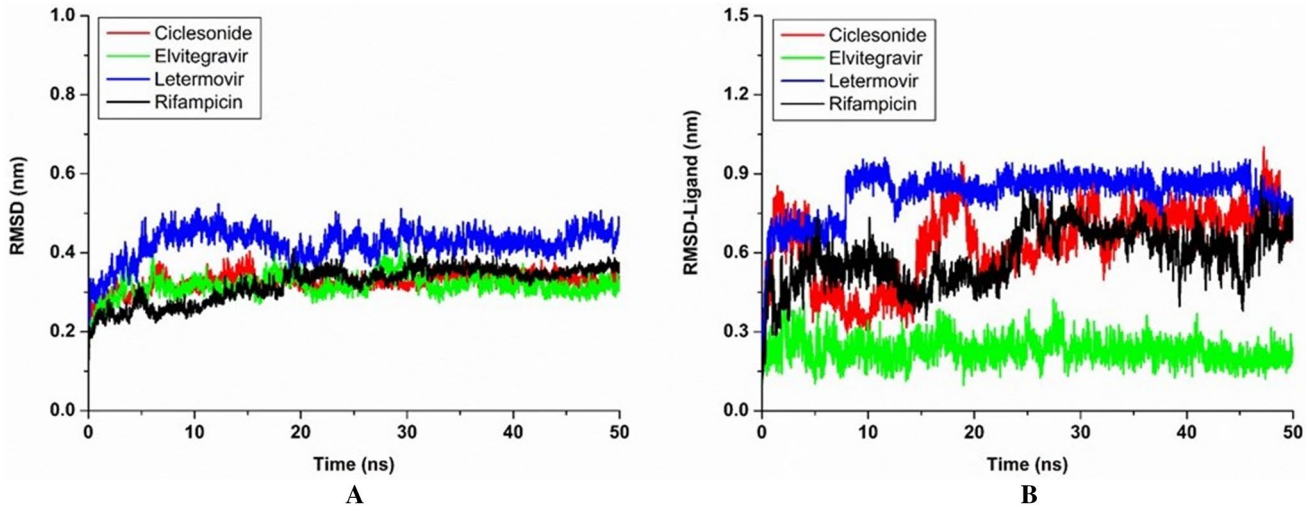


Fig. 5 **a** Root mean square deviation (RMSD) backbone; (**b**) RMSD ligand

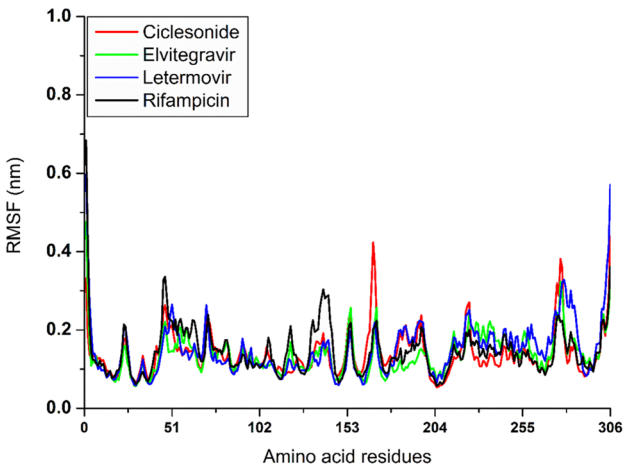


Fig. 6 Root mean square fluctuation (RMSF)

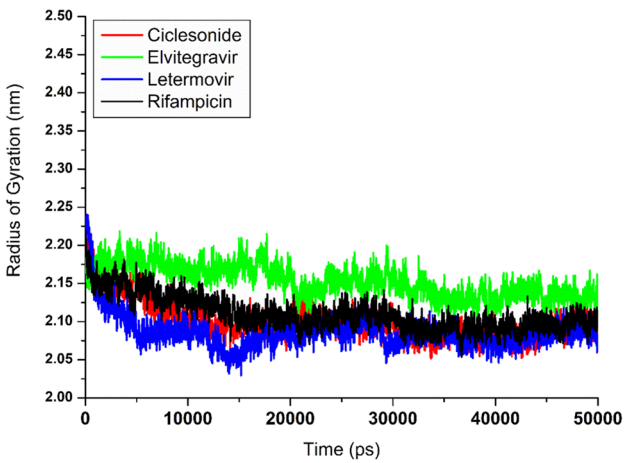


Fig. 7 Radius of gyration for all four complexes over the 50 ns simulations

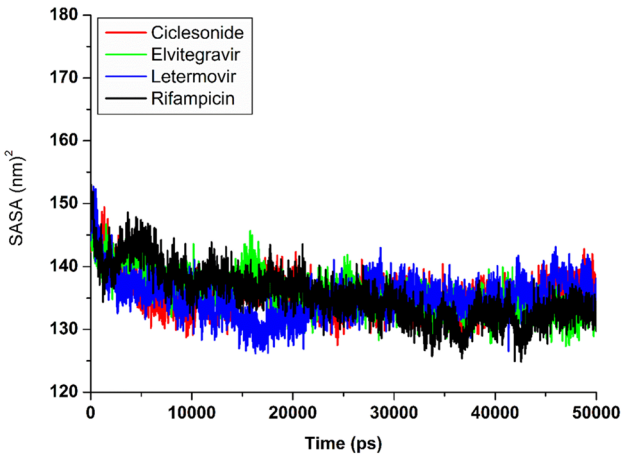


Fig. 8 Solvent accessible surface area (SASA)

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

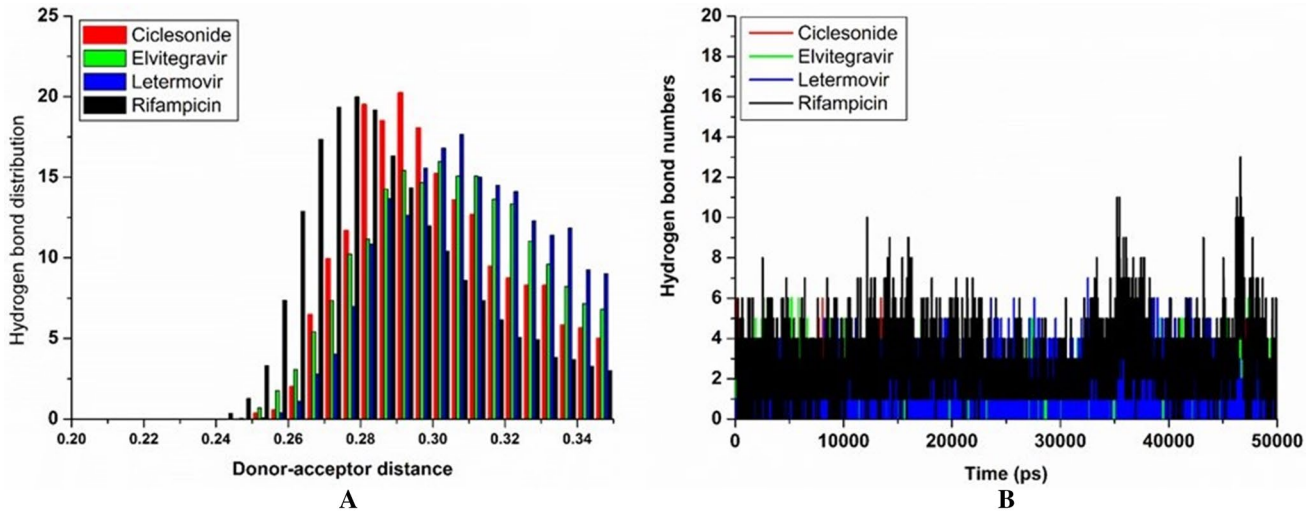


Fig. 9 **a** Hydrogen bond numbers; (**b**) Hydrogen bond distribution for all four complexes during MD simulations on 50 ns

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 ± 14.141	− 12.783 ± 7.504	72.648 ± 13.093	− 18.364 ± 1.134	− 190.070 ± 13.003
Elvitegravir	− 266.868 ± 13.659	− 28.820 ± 9.003	114.401 ± 15.866	− 17.952 ± 1.041	− 199.239 ± 15.563
Letermovir	− 325.169 ± 31.257	− 16.882 ± 5.047	97.118 ± 15.786	− 22.498 ± 1.404	− 267.430 ± 22.985
Rifampicin	− 177.790 ± 17.341	− 49.032 ± 26.129	127.435 ± 32.818	− 17.003 ± 1.660	− 116.389 ± 16.260

SARS-CoV-2 and modulate the cytokine storm of the host immunes 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 M^{pro} 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.

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Compliance with ethical standards

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

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