ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2023. © Pleiades Publishing, Ltd., 2023.

Effective Drug Candidates against Global Pandemic of Novel Corona Virus (nCoV-2019): A Probability Check through Computational Approach for Public Health Emergency

C. G. Bonde[a], J. Gawad[a], R. P. Bhole[b, 1], S. C. Bonde[a], and R. V. Chikhale[c]

[a] *Drug Design Laboratory, Department of Pharmaceutical Chemistry, SVKM's NMIMS School of Pharmacy and Technology Management, Shirpur Campus, 425 405 India*

[b] *Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune, 411018 India*

[c] *UCL School of Pharmacy, London WC1N 1AX, United Kingdom*

Received October 1, 2022; revised October 16, 2022; accepted October 18, 2022

Abstract—The infection of a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) started form Wuhan, Chinais a devastating and the incidence rate has increased worldwide. Due to the lack of effective treatment against SARS-CoV-2, various strategies are being tested in China and throughout the world, including drug repurposing. To identify the potent clinical antiretroviral drug candidate against pandemic nCov-19 through computational tools. In this study, we used molecular modelling tool (molecular modelling and molecular dynamics) to identify commercially available drugs that could act on protease proteins of SARS-CoV-2. The result showed that Saquinavir, an antiretroviral medication can be used as a first line agent to treat SARS-CoV-2 infection. Saquinavir showed promising binding to the protease active site compared to other possible antiviral agents such as Nelfinavir and Lopinavir. Structural flexibility is one of the important physical properties that affect protein conformation and function and taking this account we performed molecular dynamics studies. Molecular dynamics studies and free energy calculations suggest that Saquinavir binds better to the COVID-19 protease compared to other known antiretrovirals. Our studies clearly propose repurposing of known protease inhibitors for the treatment of COVID-19 infection. Previously ritonavir and lopinavir were proved an important analogues for SARS and MERS in supressing these viruses. In this study it was found that saquinavir has exhibited good G-score and E-model score compared to other analogues. So saquinavir would be prescribe to cure for nCov-2019 either single drug or maybe in combination with ritonavir.

Keywords: antiretrovirals, nCoV-2019, global pandemic, respiratory infections, SARS, MERS

DOI: 10.1134/S106816202303007X

INTRODUCTION

In December 2019, outbreak of novel Corona Virus took place in city of Wuhan, Hubai providence of Republic of China. Within few days it has spread across the globe and declared as epidemic [1–3]. European countries, United States of America and most of the countries in rest of the world officially announced this outbreak as National emergency. WHO also declares the outbreak of the new coronavirus as a pandemic [4]. Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV) [5–7].

Coronavirus disease (SARS-CoV-2) is identified as a new strain and was discovered in 2019 and has not been previously identified in humans. Coronaviruses are zoonotic, meaning they are transmitted between animals and people. Detailed investigations found that SARS-CoV was transmitted from civet cats to humans and MERS-CoV from dromedary camels to humans [8, 9]. Several known coronaviruses are circulating in animals that have not yet infected humans [10–12].

Till the end of March 2020, COVID-19 has claimed 700000 patient and more than 30000 deaths. Individual China has reported more than 3000 deaths and Italy reported more than double figures of death as of china due to this outbreak. All the researchers are searching for fast and effective medication/vaccine for COVID-19. Along with India, USA, European countries and most of the provinces are declares lockdown, as the number of corona positive cases are increasing drastically [13–16].

It was noted that, the combination of Ritonavir and Lopinavir was given to two patients in Jaipur Hospital India. Healthcare professional at Jaipur Hospital,

[1] Corresponding author: e-mail: ritesh.bhole@dypvp.edu.in.

declared that by the treatment of this combination patient can be treated [17, 18].

Indian Council for Medical Research (ICMR), Government of India which is India's one of the top medical research organisation has declared Lopinavir and Ritonavir can be used to treat nCoV-2019 [19].

ICMR suggested that Lopinavir and Ritonavir can be used in adults (above the age group of 18 yr). Fixed dose combination can be used to treat this novel infection. As per ICMR, the inclusion of using these antivirals against COVID-19 is based on theirs effectiveness against SARS and MERS outbreak in 2003 and 2012 respectively. A fixed dose combination (Lopinavir/Ritonavir 200 mg/50 mg) to be given twice in a day for two weeks [20, 21].

Ritonavir and Lopinavir are HIV protease inhibitor that interferes with the reproductive cycle of HIV. There are ten HIV protease inhibitors are available clinically, either single or combination of the drug. In February, The crystal structure of COVID-19 protease was published in protein data bank [22, 23].

As the more reports are begin for use of HIV protease inhibitors for the treatment of CORONA virus, it was thought worthwhile to made an attempt to study the inhibition of COVID-19 protease enzyme with available HIV-I protease inhibitors in silico. The molecular docking studies with COVID-19 protease complex and clinically used antiretroviral analogues to choose the most effective HIV-I Protease inhibitor for the treatment of COVID-19 infection.

Various reports on use of FDA approved drugs like the antiviral agents specially the HIV protease inhibitors in the treatment of COVID-19 motivated us to perform computational studies. Our previous experience with HIV inhibitors has provided us with an insight in functioning of these antiviral agents [24]. Recent report of COVID-19 protease co-crystallised with a bound peptide (PDB-6LU7) has opened the gates for computational studies on this target.

RESULTS AND DISCUSSION

Molecular Docking and MD Simulation

The molecular docking study was carried out to explore binding modes of these clinically used antiretroviral derivatives with the protease enzyme. The molecular docking simulation studies were carried out by Glide docking tool of Maestro molecular modeling interphase (Schrödinger, USA). The receptor employed here was COVID-19 (PDB code: 6LU7) obtained from RCSB Protein Data Bank (RCSB-PDB). The initial crystal structure consisted of the bound ligand with 6LU7. The bound ligand already bound with the Cys145 with covalent bond, which was a problem during grid generation. So the -SH bond was broken before protein preparation. In the co-crystallized structure, the missing loops were added. The interacting amino acid residues were identified as Hie41, Glu166, Ser144, Hip164, Leu141, Hie41 etc. The binding modes of nine analogues are presented in Figs. 1a–1i. In Saquinavir, Glu166 (red) exhibit dual interaction with nitrogen of bicyclic ring and NH of amido group aliphatic side chain with bond distance 2.55 and 1.80 Å. Other amido –NH forms hydrogen bonds Hip164 with bond distance of 2.22 Å. The –C=O– of amido forms hydrogen bond with Ser144 and Gly143 with bod distance 2.60 and 1.96 Å. The hydrogen of NH_2 forms hydrogen bond with Leu141 with bond distance 1.98 Å. Leu141 showed pi-pi stacking with phenyl ring. Another analogue ritonavir, it has shown single interaction between Gln189 and polar hydroxyl group. Nelfinavir has come up with promising interactions. A single residue Gl166 has shown interactions with nitrogen of quinoline, nitrogen of aliphatic chain and hydroxyl group. Lopinavir has variant types of interaction with residues. Hip164 has formed hydrogen bond with hydroxyl group. Glu166 has interacted with amide, Hydrogen bond is formed between Gln189 and amide group. Other analogues, Darunavir, Indinavir, Fosamprenavir, Atazanavir and Tipranavir has similar kind of interactions between amide, hydroxyl, amine, phenyl ring and Glu166, Gly143, Leu141, Hie41 etc. Glide score and E model score was given in Table 1.

To evaluate the stability of the docked complexes and determine the binding free energies we performed molecular dynamics simulations (MDS) for Saquinavir as this molecule has shown highest bonding affinity with SARS-CoV-2 main protease. Along with SAQ, N3 peptide was also studied in order to observe and compare the binding free energies of the bound ligands and peptide. The details about calculations are mentioned in the material and methods section. All the simulations were performed for 50 ns using explicit solvent model. The last conformation obtained after several steps of NPT and NVT equilibration was subjected for production phase. The first and last step from production calculation was obtained as pdb files by cpptraj tool and further analysed. The MDS trajectories from COVID-19-N3 and COVID-19-SAQ complex calculations were analysed by AMBER-CPPTRAJ module. Ligand RMSD (Fig. 2a) clearly indicates that both the complexes are equally stable during the 50 ns simulation. The initial RMSD increase in N3 is due to conformational changes in the peptide residues which stays between 4–6 Å, SAQ shows a better stability and low deviation from its initial conformation with RMSD between 3–4 Å throughout the simulation. RMSF was calculated for

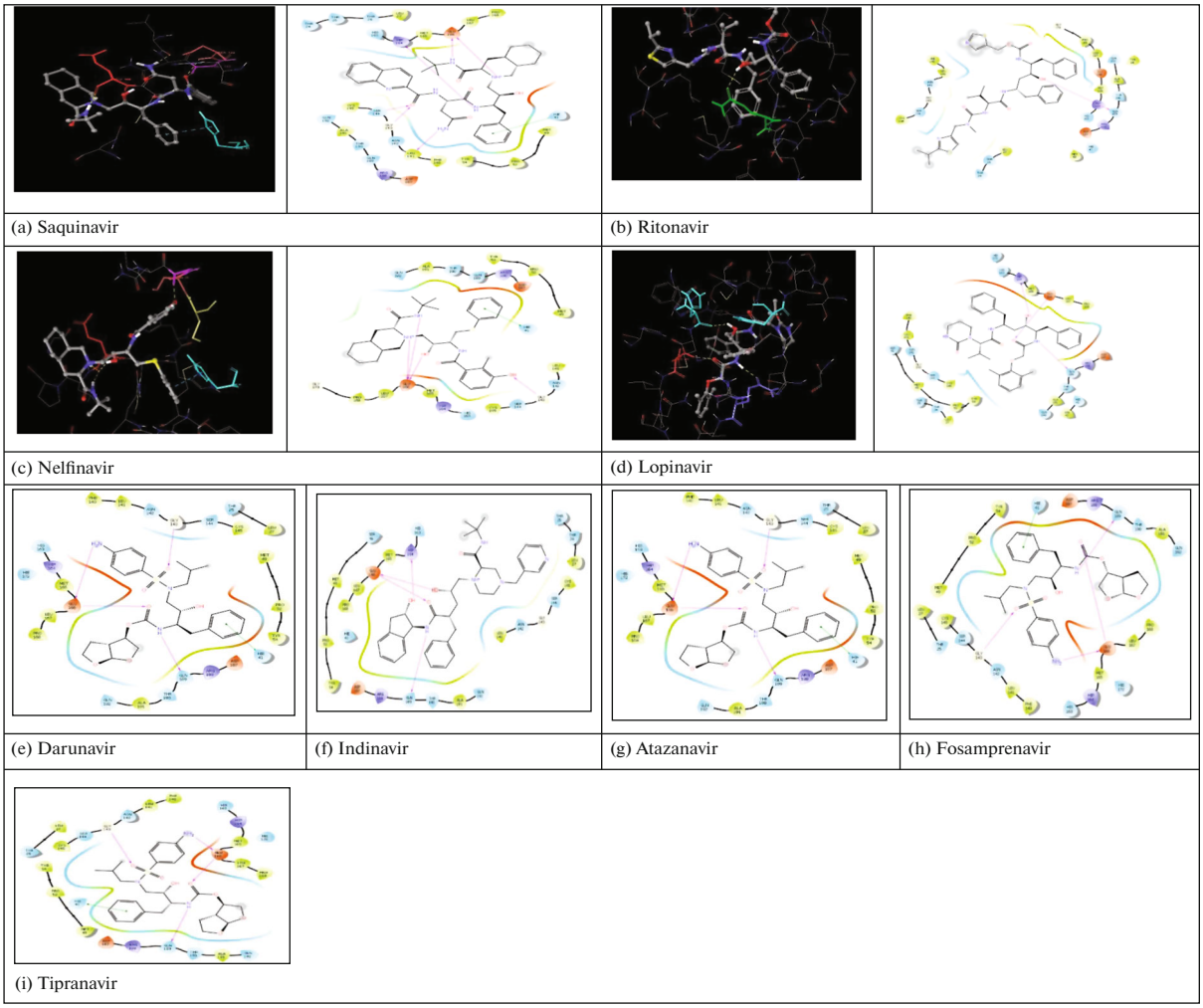

Fig. 1. The COVID-19 binding with (a). Antiviral drugs (a–i). Polar hydrogen are shown in white color, 2D interaction diagram indicates hydrogen bonding (pink arrow), π–π stacking (green line).

the protein residues in both the complexes during the period of simulations. The active site is formed by residues between 140 to 190. The RMSF (Fig. 2b) shows comparatively higher relative motions in case of N3 compared to SAQ, this cements our understanding about higher stability of COVID-19-SAQ complex. To understand further the ligand-receptor interactions we analysed the initial and final poses of the complexes. The initial pose from MDS of both complexes were aligned and superimposed (Figs. 3a, 3b). N3 is a peptide, its amide linkages provide for hydrogen bonds with His164, Glu166, Gln189 and Thr190. Whereas, SAQ forms hydrogen bonds with the amide groups on decahydroxyquinoline and tertiary butyl with Glu166, hydroxy group of butanamide also forms a hydrogen bond with the Glu166 residue. The phenyl ring forms a Pi interaction with the Met165, these interactions were found to be intact at the end of the 50 ns of MDS. The MMGBSA free energy calculations were per-

Table 1. Clinically used HIV protease inhibitor with their Glide docking and E-Model score on 6LU7

Name of compound	Docking score	E-Model score
Saquinavir	–9.551	–101.666
Nelfinavir	–9.423	–96.373
Lopinavir	–9.357	–104.919
Darunavir	–8.772	–98.886
Indinavir	–8.793	–94.541
Ritonavir	–8.069	–89.56
Amprenavir	–7.210	–79.152
Atazanavir	–6.735	–99.406
Tipranavir	–6.324	–78.902
Fosamprenavir	–5.314	–76.202

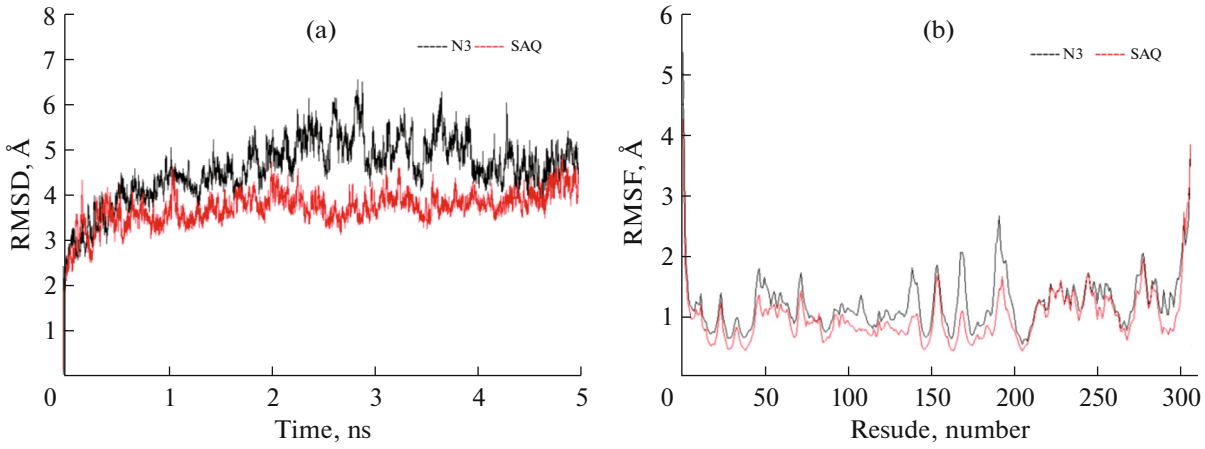

Fig. 2. Molecular dynamics studies on COVID-19 protease bond with peptide (N3) and Saquinavir; (a) RMSD of peptide N3 (black) and antiviral drug Saquinavir (SAQ) for a period of 50 ns; (b) RMSF of protease residues (306 amino acids) for a period of 50 ns.

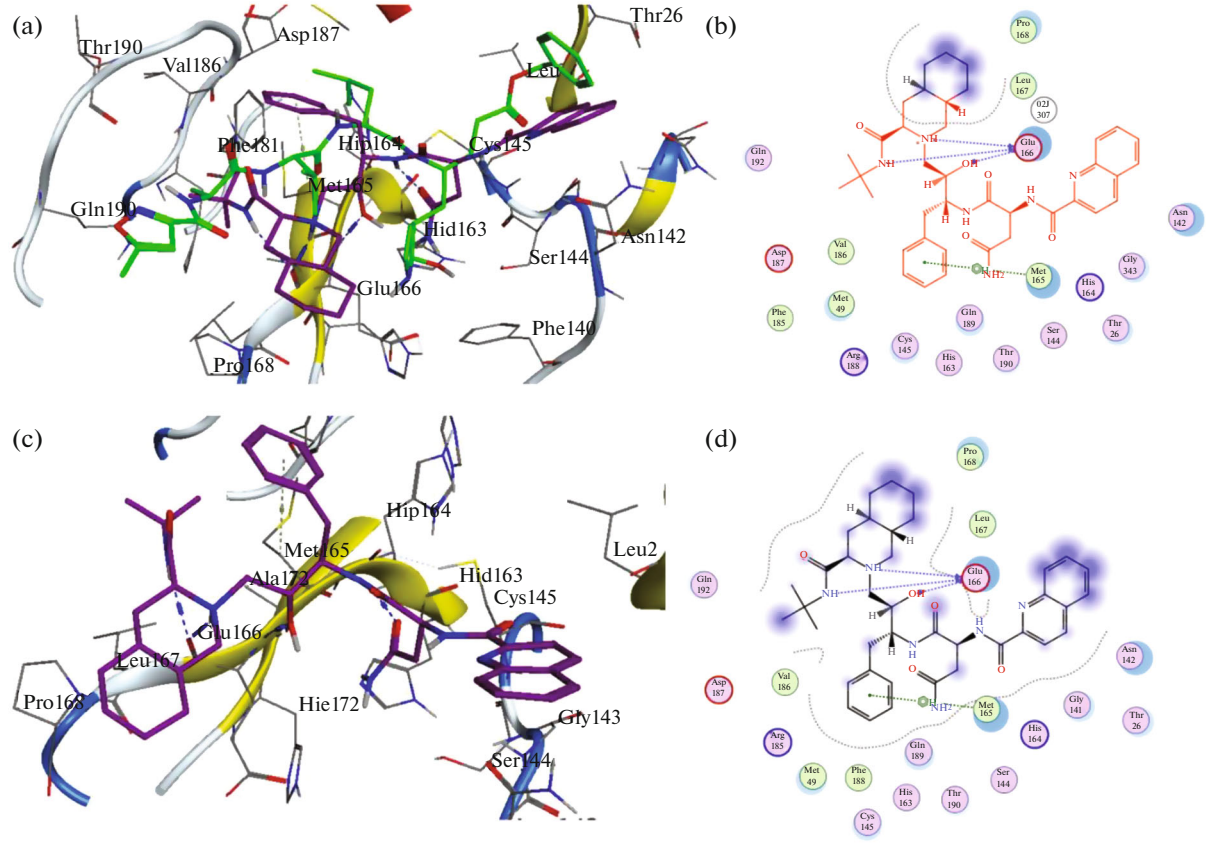

Fig. 3. Binding poses of peptide N3 and SAQ: (a) Interaction of N3 and SAQ with the active site resides of COVID-19 protease post molecular dynamics equilibration and optimisation of ligand binding; (b) 2D representation of SAQ with the COVID-19 protease post molecular dynamics equilibration and optimisation of ligand binding; (c, d) 3D and 2D representation of binding pose of SAQ at the end of MD simulation showing interaction with the binding site residues.

formed on the complete trajectories for both the complexes. The ΔG_{bind} for the COVID-19-N3 and COVID-19-SAQ was found to be −55.0746 and −64.8387 kcal/mol respectively. This also indicates the better stability of the COVID-19-SAQ complex compared to the COVID-19-N3.

EXPERIMENTAL

Molecular Docking Studies

Molecular docking is the most widely used tool for exploring the interactions between an inhibitor molecule and the target protein. The two-dimensional (2D) structures were drawn using ChemDraw Ultra, and converted in mol2 format using marvinsketch. Drawn chemical structures were converted into their three-dimensional (3D) form by using Ligprep tool, of Maestro Molecular Modelling Interphase, Schrödinger (Version 11.8.012). The crystallographic structure was downloaded from the Protein Data Bank, PDB (www.rcsb.org) PDB ID: 6LU7. Before docking, preparation of the receptor was carried out in Protein preparation wizard tool. In our studies, the Glide, Maestro molecular modeling software was used to perform docking calculations. All the ligands were prepared and docked for this study in flexible docking mode and atoms located within a range of 4.0 Å from the amino acid residues were selected in the active site. All the missing hydrogen atoms were included, all water molecules and ligand erased, and the energy minimized. All the default parameters were used. For ligand preparation the pH was 7.0 ± 2.0, force field OPLS2005 was applied. For protein preparation the pH was 7.0 ± 2.0, force field was OPLS2005, ionization was done using Epik [25].

Molecular Dynamics Simulation

SARS-CoV-2 main protease (PDB ID: 6LU7) was selected as this crystal structure have a known active site with bound *n*-[(5-methylisoxazol-3-yl)carbonyl]alanyl-l-valyl-*n*~1~-((1*r*,2*z*)-4-(benzyloxy)-4-oxo-1-{[(3*r*)-2-oxopyrrolidin-3-yl]methyl}but-2-enyl)-l-leucinamide also termed as N3 [26]. Saquinavir docked pose was selected for MD study, as this molecule has shown highest bonding affinity with SARS-CoV-2 main protease and simulated using AMBER18 package [27]. Topology and coordinate parameters for SARS-CoV-2 main protease were generated with AMBER99SB force field and ligands were parametrized with general amber force field (GAFF) [28]. The docked complexes were embedded in the TIP3P water model with a margin of 1.0 nm in periodic cubic box. The system was neutralized to pH 7 by adding counter ions and then sufficient number of ions was added to obtain salt concentration of 1.5 M. All the bond lengths were constrained using the SHAKE algorithm and electrostatic interactions were calculated by the Particle Mesh Ewald (PME) method [29]. The solvated system was then subjected to energy minimization using the steepest descent algorithm to eliminate any bad contacts with added water until a tolerance of 1000 kJ mol^{-1} was reached by a series of equilibration protocol. After which the energy minimized system was treated for equilibration by 5 ns NVT simulation at 300 K followed by 5 ns of NPT simulation to achieve proper equilibration of the simulated system. Final production simulations were performed in the isothermal isobaric (NPT) ensemble at 300 K, using an external bath with a coupling constant of 0.1 ps, the total simulation time achieved was 100 ns. The pressure was kept constant (1 bar) by using pressure coupling with the time constant set to 1 ps [30]. The trajectories were stored at every 2 ps during the simulation and trajectories analyzed using built-in commands of AMBER18. The Root Mean Square Deviations (RMSD) for ligands, Root Mean Square Fluctuations of the Protein residues (RMSF) and the Radius of Gyration (RadGyr) were calculated for the protein in each of the protein–ligand complex [31]. Trajectories of docked complexes were used to calculate binding free energies, the molecular mechanics Poisson–Boltzmann surface area (MM-GBSA) method in AMBER18 was used to quantitatively measure the binding strength between the receptor and ligands [32]. The average interactions energies of the Saquinavir and N3 from the crystal structure was calculated for (10000 frames) 100 ns of MD trajectories. Molecular dynamics parameters were depicted in Table 2. The results were processed and graphs were plotted with XMGRACE [33].

Table 2. ΔE_{VDW} = van der Waals contribution from MM; ΔE_{ELE} = electrostatic energy as calculated by the MM force field; ΔG_{GB} = the electrostatic contribution to the solvation free energy calculated by GB; ΔG_{Surf} = solvent-accessible surface area; ΔG_{Sol} = solvation free energy; ΔG_{gas} = gas phase interaction energy; ΔG_{bind} = Binding free energy

Sr. no.	6LU7	
	N3	Saquinavir
	MM/GBSA	
ΔE_{VDW}	−62.3893	−60.8554
ΔE_{ELE}	−26.8010	−98.2661
ΔG_{GB}	51.7751	116.8372
ΔG_{Surf}	−8.0339	−6.8746
ΔG_{bind}	−55.0746	−64.8387
Dock score	−8.915	−9.551

CONCLUSIONS

In summary, we investigated the interaction of clinically used antiretroviral protease inhibitors with COVID-19 protease enzyme using molecular docking and molecular dynamics. During SARS (2003) and MERS (2012) Ritonavir and Lopinavir were played

important role in suppressing the effect of these viruses. Surprisingly it was found that Saquinavir has shown the good G-score (−9.538) and E-model score (−101.66), while other HIV protease inhibitors was ranked below Saquinavir. As some patients with COVID-19 infection is treated with Ritonavir and Lopinavir combination, it will be more effective, if inclusion of Saquinavir as a first line drug for the treatment of COVID-19 positive patents. Other HIV Protease inhibitors also exhibit similar kind of interactions with 6LU7 but on the basis of G-Score and E-Model score it can be concluded that Saquinavir would be effective cure for nCoV-2019 either as a single agent or in combination with Ritonavir.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest.

This article does not contain any studies involving animals or human participants performed by any of the authors.

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