Strategic analyses to identify key structural features of antiviral/antimalarial compounds for their binding interactions with 3CLpro, PLpro and RdRp of SARS-CoV-2: in silico molecular docking and dynamic simulation studies

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ABSTRACT
Severe acute respiratory syndrome coronavirus (SARS-CoV-2), a novel member of the betacoronavirus family is a single-stranded RNA virus that has spread worldwide prompting the World Health Organization to declare a global pandemic. This creates an alarming situation and generates an urgent need to develop innovative therapeutic agents. In this context, an in silico molecular docking and molecular dynamics (MD) simulation study on the existing 58 antiviral and antimalarial compounds was performed on 3CLpro, PLpro and RdRp SARS-CoV-2 proteins. The antiviral compounds are best fitted in the binding pockets and interact more profoundly with the amino acid residues compared to antimalarial compounds. An HIV protease inhibitor, saquinavir showed a good dock score and binding free energy with varied binding interactions against 3CLpro and PLpro. While, adefovir, a nucleotide HBV DNA polymerase inhibitor exhibited good dock score and binding interactions against RdRp. Although, the antimalarial compounds showed relatively less dock score but were found to be crucial in displaying essential binding interactions with these proteins. The MD simulation runs for 100 ns on 3CLpro–saquinavir, PLpro–saquinavir and RdRp–adefovir complexes using Desmond revealed fairly stable nature of interactions. This study helped in understanding the key interactions of the vital functionalities that provide a concrete base to develop lead molecules effective against SARS-CoV-2.

Abbreviations: SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2; RNA: Ribonucleic acid; MD: molecular dynamics; 3CLpro: 3C-like proteases; PLpro: papain-like protease; RdRp: RNA-dependent RNA polymerase

Introduction
Novel coronavirus disease (COVID-19) pandemic has resulted in more than 128 million confirmed cases and about 4 million deaths globally till the last week of July (Worldometer, 2021). The second wave of coronavirus has surge through many countries including India. This outcome of infection is mainly due to the severe acute respiratory syndrome caused by the RNA encapsulated SARS-CoV-2 virus. This sudden outbreak of infection is the result of the more transmissible nature of SARS-CoV-2 and had inflicted severe health and socio-economic effects on the lives of millions of people worldwide (Tang et al., 2020). Apart from physical health, psychological health has also been challenged due to the economic conditions and isolations/quarantines this pandemic brings to the peoples. Consequently, this unexpected worldwide viral infectious situation had triggered the process of identification of treatments to find a cure and restrict the transmission of this virus. Since, SARS-CoV-2 belongs to the same class of beta coronaviruses to which the earlier severe acute respiratory syndrome (SARS) virus (SARS-CoV), and Middle East respiratory syndrome virus (MERS-CoV) goes, their infection pattern resembles and found to predominantly attacks the lower respiratory system to cause pneumonia, which is the major cause of mortality (Su et al., 2016; Zhu et al., 2020).

Numerous structural proteins are encoded by the betacoronavirus genome. Amongst them, glycosylated spike (S) protein of both SARS-CoV and SARS-CoV-2 is responsible to mediate host cell invasion via binding to a receptor protein called angiotensin-converting enzyme-2(ACE2) located on the surface membrane of host cells (Du et al., 2009; Hoffmann et al., 2020; Wrapp et al., 2020). Other pivotal nonstructural
proteins encoded by a viral genome are RNA-dependent RNA polymerase (RdRp), coronavirus 3C-like protease (3CLpro) and papain-like protease (PLpro) (Báez-Santos et al., 2015).

Although SARS-CoV-2 and SARS-CoV display only 79% sequence similarity at their genome level, RdRp and 3CLpro proteases of both viruses carry over 95% of sequence similarity at their genome level, RdRp and 3CLpro (4YOG) (Morse et al., 2015). Recent studies revealed that PLpro of both viruses share 95% sequence similarity at their genome level, RdRp and 3CLpro (4YOG). Vascular proteinases viz., 3CLpro and PLpro are required to cleave translated polyproteins which are produced by using host cell protein translation machinery into functional protein fragments (Báez-Santos et al., 2015; Gorbalenya et al., 2000), while, RdRp is a crucial enzyme that catalyses the replication of RNA from an RNA template and has been targeted in various viral infections including hepatitis-C virus (HCV), the Zika virus and the coronaviruses (Elfiky, 2020b). Additionally, it is also reported that PLpro plays a critical role in immune suppression as a result of deubiquitinating certain host cell proteins, like interferon factor 3 and NF-κB (Báez-Santos et al., 2015).

Progress in the development of antiviral therapeutics and preventive agents for SARS-CoV-2 significantly depends on the outcomes of research on SARS and MERS viruses, since viral proteins required for these viruses to enter the host cell and replicate are structurally similar. Therefore, to find the treatment for SARS-CoV-2 infection, researchers are mainly involved in identifying drugs with high target specificity and/or revealing existing drugs to repurpose them. Drugs like lopinavir and ritonavir which targets proteases (3CLpro and PLpro) in other viruses, and remdesivir and favipiravir which blocks RdRp, were studied for their utility to treat SARS-CoV-2 infection, researchers are mainly involved in identifying drugs with high target specificity and/or revealing existing drugs to repurpose them. Drugs like lopinavir and ritonavir which targets proteases (3CLpro and PLpro) in other viruses, and remdesivir and favipiravir which blocks RdRp, were studied for their utility to treat SARS-CoV-2 infection (Elfiky, 2020a; Guo, 2020; Sheahan et al., 2020). Moreover, commonly used antimalarial and autoimmune disease drugs like chloroquine/hydroxychloroquine were found to possess broad-spectrum antiviral properties (Beura & Chetti, 2021; Savarino et al., 2006; Yan et al., 2013). Chloroquine is identified to control virus/cell fusion by increasing endosomal pH and interfering with the glycosylation of cellular receptors of SARS-CoV (Vincent et al., 2005). Besides, some studies also revealed that chloroquine inhibits the replication process of the SARS-CoV (Beura & Chetti, 2021; Keyaerts et al., 2004).

Repurposing the existing antivirals and antimalarials like remdesivir, favipiravir, lopinavir, ritonavir, ribavirin and hydroxychloroquine were found to be logical as suggested by several initial studies (Martinez, 2020a; Weston et al., 2020). However, poor efficacy of these repurposed drugs against SARS-CoV-2 (Martinez, 2020b) highlighted the necessity for continuous and more investigations. Hence, to identify potential drug candidates from compound libraries, computational methods such as docking, molecular dynamics simulation and binding free energy evaluation provide promising choices (Elmezayen et al., 2021). Molecular dynamics with its capability to assess physical movements of atoms and molecules is a powerful tool to predict and analyse the stability of protein–ligand complex. Several researchers establish the rational for repurposing the existing drugs.

### Table 1. Molecular docking results of representative antiviral/antimalarial compounds showing maximum dock score with proteins viz., 3CLpro, PLpro and RdRp.

| S. No. | Proteins (PDB ID) | Compound | Dock score | Glide energy | Atom of ligand | Residues of amino acid | Bond distance (Å) | Hydrophobic interactions |
|--------|-------------------|----------|------------|--------------|---------------|------------------------|------------------|------------------------|
| 1      | 3CLpro (4YOG)     | Saquinavir | −9.163     | −67.689      | OH            | GLU169                 | 2.02             | Phenyl ring and HIE 41 with bond distance 5.26 Å (π–π stacking) |
|        |                   | Tafenoquine | −6.898     | −50.085      | NH            | GLU169                 | 1.86             | Phenyl ring and HIE 41 with bond distance 5.26 Å (π–π stacking) |
|        |                   |           |            |              | O             | GLU169                 | 2.02             | Phenyl ring and HIE 41 with bond distance 5.26 Å (π–π stacking) |
|        |                   |           |            |              | NH            | CYT145                 | 2.47             | –                      |
|        |                   |           |            |              | NH            | LEU170                 | 2.18             | –                      |
|        |                   |           |            |              | NH            | GLU169                 | 1.77             | –                      |
|        |                   |           |            |              | O             | GLU169                 | 1.83             | –                      |
| 2      | PLpro (3E9S)      | Saquinavir | −6.51639   | −59.7326     | NH            | GLU168                 | 2.08             | Phenyl and TYR 269 with bond distance 5.45 Å (π–π stacking) |
|        |                   | Tafenoquine | −6.75206   | −32.4364     | N             | GLN439                 | 2.03             | –                      |
|        |                   |           |            |              | NH            | GLN439                 | 1.86             | –                      |
|        |                   |           |            |              | O             | LEU169                 | 2.47             | –                      |
|        |                   |           |            |              | O             | ARG413                 | 1.86             | –                      |
|        |                   | Mefloquine | −5.607     | −34.94       | NH            | ASP 165                | 2.23             | Quinoline and TYR 269 with bond distance 5.45 Å (π–π stacking) |
|        |                   |           |            |              | O             | TRP417                 | 1.69             | Quinoline and TYR 269 with bond distance 5.45 Å (π–π stacking) |
| 3      | RdRp (4O4R)       | Adefovir  | −7.75206   | −32.4364     | N             | GLN439                 | 2.03             | –                      |
|        |                   | Dihydroartemisin | −5.399    | −28.018      | OH            | GLN439                 | 2.14             | –                      |

### Table 2. MM-GBSA values for the selected receptor–ligand complex.

| Complex | MM-GBSA dG bind ± S.D. |
|---------|-------------------------|
| 3CLpro (4YOG)–saquinavir | −67.4779 ± 8.91 |
| PLpro (3E9S)–saquinavir  | −73.6161 ± 4.31 |
| RdRp (4O4R)–adefovir    | −38.4075 ± 4.10 |
based on the molecular dynamics studies and identified drug candidates from drug-bank, approved drug list and investigational drugs as a potent inhibitors of SARS-CoV-2 functional proteins (Behera et al., 2021; Keretsu et al., 2020; Tejera et al., 2020; Wang, 2020). Although these studies evaluated the binding affinities of the potent compounds and identified the crucial binding residues of functional proteins, limited information is available about the structural residues/functionalities of ligand molecules which assist in binding. The overall perception prompts us to study the possible binding affinities of available antiviral and antimalarial drugs targeting three significant functional proteins viz., 3CLpro, PLpro and RdRp of SARS-CoV-2. On studying the interaction patterns by molecular docking and dynamic simulations, we could get insight into the requirements of structural features of ligands essential for binding the key amino acid residues in the binding pocket of these crucial proteins that would contribute to establishing the foundations for the future development of leads against SARS-CoV-2.

**Materials and methods**

**Dataset**

Structures of the 58 antiviral and antimalarial compounds were drawn using ChemBioDraw Ultra 14.0 and saved as an SDF file and opened in maestro. The ligand preparation was carried out using the LigPrep tool, which helps to convert the 2D structure of compounds into the 3D molecular structures. The ionization state of the molecules was set to pH 7.0 and subjected to energy minimization by using the LigPrep module (LigPrep, 2017). Since the key functional proteins of
SARS-CoV-2 (3CLpro, PLpro and RdRp) displays similarities with the proteins of SARS-CoV, we opted to select the protein co-crystal structures of 3CLpro (PDB id: 4YOG) (St John et al., 2015), PLpro (PDB id: 3E9S) (Ratia et al., 2008) and RdRp (PDB id: 4O4R) (Croci et al., 2014) from the RCSB protein data bank.

**Molecular docking**

Glide XP docking mode was considered for docking studies using crystal structure of 3CLpro, PLpro and RdRp proteins. The preparation of protein structure was carried out with the Protein Preparation Wizard in Maestro 8.0. After ensuring chemical correctness, water molecules in the crystal structure were deleted and hydrogens were added. The OPLS3 force field was used to minimize the crystal structure of a protein (Harder et al., 2016). The bond orders were assigned, hydrogen atoms were added and the water molecules which did not participate in interactions were removed. The structures were further refined to augment the hydrogen bond network and minimization were done using a standard procedure (Sastry et al., 2013). The binding region (grid) was identified by focusing on the centroid of crystallographic ligand to restrict the centroid of the ligand to be docked. The size of the grid box is so fixed to dock the ligands of the size <\( \leq \) 20 Å. The charge density was evaluated by the OPLS3 force field for the generated binding pocket (Taha et al., 2014). In order to relax nonpolar parts of the receptor, the scaling factor for van der Waals radius and partial atomic charge was set to be 0.8 and 0.15, respectively. For all remaining parameters, the default setting was used.

The docking glide score, hydrogen bonding and \( \pi-\pi \) interactions with the surrounding amino acids were calculated and best-suited conformations of ligands with maximum dock score were studied precisely.

**Molecular dynamic simulations**

The MD simulation was performed using Desmond (Schrodinger LLC) for three systems namely 3CLpro–saquinavir, PLpro–saquinavir and RdRp–adefovir complexes, which allows to understand the binding of a ligand–protein complex in a simulated physiological solvent-based environment. The procedure for MD simulation study was carried out as reported (Vijayakumar et al., 2018, 2019). All ligand–enzyme complex system were solvated by water model SPC with an orthorhombic box with a dimension of 10 Å each side and the system was naturalized with the addition of Na\(^+\) and Cl\(^-\) ions. The water layer thickening was set at 10 Å. The systems were minimized with a maximum

![Figure 2. Key interactions of functional groups of antiviral and antimalarial compounds with amino acid residues of 3CLpro pocket.](image-url)
iteration of 2000 steps and each system after equilibration for about 1 ns, were submitted for the development of MD runs for 100 ns MD simulations. The OPLS 2005 force field parameters were used in all simulations. Temperature and pressure were set to 300 K and 1.01325 bar, respectively, using Isothermal-isobaric (NPT) ensemble. For Coulomb interactions, the cut-off ratio was set to 9 Å and a long-range method of smooth Particle Mesh Ewald was used. The MD simulation trajectories were saved at every 100 ps intervals to obtain approximately 1000 frames for analysis. Desmond’s simulation interaction diagram option was used to generate detailed information such as protein and ligand Root Mean

Figure 3. MD simulations for 3CLpro–saquinavir complex: (a) Root Mean Square Deviation (RMSD) as a function of simulation time; (b) Root Mean Square Fluctuation (RMSF) for 3CLpro residues. RMSF is useful for characterizing local changes along the protein chain.
Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF) and ligand interaction profile from the simulation trajectory. The stability of MD simulations was monitored by viewing the RMSD of the ligand and protein atom positions in time.

**Binding free energy (MM-GBSA) calculations of the complexes**

The relative free energy of the binding for the docked complexes viz., 3CLpro–saquinavir, PLpro–saquinavir and
RdRp–adefovir, was estimated using Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) embedded in Schrodinger, NY, 2019 (Release, 2017). The MM-GBSA calculations used an OPLS3 force field, a VSGB 2.0 solvation model and default prime protocol (Genheden & Ryde, 2015; Harder et al., 2016; Li et al., 2011). The MM-GBSA method is efficient, reliable and broadly used to calculate accurate relative change in binding free energies (Chen et al., 2020; Forouzesh & Mishra, 2021).

Results and discussion

Glide score, binding free energy and docking pose of a total 58 number of antiviral (31 compounds) and antimalarial (27 compounds) were studied against 3CLpro (PDB id: 4YOG), PLpro (PDB id: 3E9S) and RdRp (PDB id: 4O4R) of SARS-CoV-2. The results of the molecular docking study is depicted in Table 1 and Supporting Information Tables S1–S6. It was observed from protein–ligand interaction diagram that co-crystallized ligand (4F5 in PDB 4YOG) was found to prominently interact with GLU169 and HIS41 residues of 3CLpro.
While, co-crystallized ligand (TTT in PDB 3E9S) had shown binding interactions with ASP165, TYR269, GLN270 residues of PLpro and interactions of co-crystallized ligand (2OV in PDB 4O4R) with TYR341, ARG392, ARG413, GLN414, LYS166 of RdRp was observed. During the docking study, the amino acid residues that were found to interact with the co-crystallized ligand in their respective PDB were chosen as constraints. The best two compounds from each class with docking against 3CLpro, PLpro and RdRp were selected based on their high docking score and interaction with selected amino acid residues.

3CLpro

Molecular docking

The docking results of these compounds in the active site of 3CLpro (PDB id: 4YOG) revealed that saquinavir, an HIV protease inhibitor, possess the highest docking score of $-9.163$ with the binding free energy of $-67.689$ (Table 1), followed by indinavir (an HIV protease inhibitor) and remdesivir (a nucleotide analog) to generate dock score of $-6.974$ and $-6.963$, respectively. Telaprevir showed similar scores as that of remdesivir.

Although docking scores of ritonavir (HIV protease inhibitor), rupintrivir (HRV protease inhibitor) and nelfinavir (HIV protease inhibitor) are lesser as compared to saquinavir, these protease inhibitors have produced comparable binding free energy in the range of $-60$ to $-63$ when compared with saquinavir (Supporting Information Table S1). Among 27 antimalarial compounds studied, tafenoquine was able to produce a docking score of $-6.898$ and binding free energy of $-50.085$, somewhat better than hydroxychloroquine (dock score $-6.814$). Other antimalarial compounds showed dock score below $-6.5$ and lesser binding free energy (Supporting Information Table S2).

Key interactions of saquinavir in the binding pocket of 3CLpro were found with amino acid GLU169. Three hydrogen bond interactions were observed between amido -CO, alcoholic -OH and decahydroisoquinolinium –NH of saquinavir with GLU169. Similarly, amide -NH bonded to quinoline residue showed hydrogen bonding with CYT145 of the pocket. Phenyl ring and HIE41 were found to have $\pi-\pi$ stacking interaction (Figure 1(a,b)). In the case of indinavir, alcoholic -OH group and amide –NH located on dihydroidindene showed hydrogen bond interaction with LEU170 of 3CLpro pocket, while, GLU169 and CYT145 showed H-bond interactions with –OH group of piperazinium side chain and amide –NH, respectively. $\pi-\pi$ stacking interactions were observed between pyridine and phenyl ring with HIE41 and HIS194 residues (Supporting Information Figure S1(a,b)). Again, regarding remdesivir, GLU169 residue was able to produce two H-bonds with $=\text{O}$ (oxy) group of
phosphoramidate. One more H-bond was observed between the primary amino –NH and PHE143. Similar, π–π stacking interactions were observed between HIE41 and phenyl ring alike indinavir (Supporting Information Figure S2(a,b)).

Docking poses of antimalarial compounds viz., tafenoquine (Figure 1(c,d)), hydroxychloroquine (Supporting Information Figure S3(a,b)) and clindamycin (Supporting Information Figure S4(a,b)) revealed similar H-bond interactions with 3CLpro as in the case of antiviral compounds. Predominantly, GLU169, LEU170, LYS191 and CYT145 interacted with amino –NH, alcoholic –OH and ethereal ‘‘O’’ of these compounds. While HIE41 was found in π-cation
interaction with quaternary nitrogen –N of hydroxychloroquine. Therefore, in 3CLpro, GLU169 and CYT145 are the most vital amino acid residues along with LEU170, PHE143 and LYS191 to instigate important H-bond interactions with structural features like an alcoholic –OH, amide and amino –NH of the ligands. Whereas, HIE41 could play its role in p-p stacking revealed as p-p and p-cation type of interactions.

Based on the results obtained, an illustration has been designed (Figure 2) revealing the key interactions of crucial functional groups of antiviral and antimalarial compounds which played a significant role in binding with amino acid residues of 3CLpro pocket.

**MD simulation of 3CLpro–saquinavir complex**

With the best docking score, saquinavir was subjected to MD simulation study with 3CLpro protein. MD simulation was done using Desmond program to confirm the binding mode of 3CLpro–saquinavir complex. The plotted trajectory data for RMSD and RMSF is shown in Figure 3(a,b), respectively. RMSD was calculated based on the atom selection after all 3CLpro frames were aligned on the reference frame backbone. The simulation was found to be moderately equilibrated since changes around 4.0 Å for 3CLpro and saquinavir were observed during 100 ns run time. Therefore, saquinavir was found to be fairly stabilized in the binding pocket of 3CLpro protein. Furthermore, protein RMSF data also indicates stability since overall fluctuations of the residues of protein were seen in the range of 0.6–2.4 Å. This lesser limit of fluctuations for active site residues and its backbone atoms attributed to small conformational changes.

On studying the 3CLpro–saquinavir contacts obtained by trajectory analysis of MD simulation (Figure 4(a,b)), it is observed that GLU169 residue of 3CLpro is still most vital for interaction with saquinavir. But some changes are seen in the H-bonding pattern when compared with molecular docking data. H-bonding of saquinavir with amidic atom 45 (N, 95%) and decahydroisoquinolinium atom 8 (–NH, 93%) with GLU169

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**Figure 9.** PLpro–saquinavir contacts analysis via MD simulations: (a) Types of PLpro–saquinavir interactions monitored throughout the simulation. Green bar-H bonds, Light purple bar-Hydrophobic, Dark pink bar-Ionic and Blue bar-Water bridges, (b) A timeline representation of the interactions and contacts: Top panel indicates the total number of specific contacts throughout the trajectory. The bottom panel shows the type of residues interacting with the ligand in each trajectory frame.
is the most crucial as it is also reflected by molecular docking. But H-bonding of alcoholic -OH of saquinavir with GLU169 is not observed in simulation as was revealed in docking. Also, GLU169 has shown one more H-bond with amido atom 27 (O, 39%) via water bridge. Instead of H-bond formation with CYT145 as was observed in the docking study, the residues GLN192 & GLY146 were found to possess minor H-bonding interaction with saquinavir. While phenyl ring and HIS41 were involved in π-π stacking interaction (Figure 5(a,b)).

**PLpro**

**Molecular docking**

Docking studies of antiviral compounds with PLpro (PDB ID: 3E9S) revealed that these compounds are less tending to produce better scores when compared with 3CLpro. Still, saquinavir showed a docking score of −6.516 and binding free energy of −59.732 (Table 1). Saquinavir is followed by valganciclovir and famciclovir. Although the docking score of ritonavir and rupintrivir (protease inhibitor) is less, these compounds were able to show comparable binding free energy as that of Saquinavir (Supporting Information Table S3). Antimalarial compounds mefloquine and halofantrine were found to generate docking scores of −5.607 and −5.077 with lesser binding free energy (Supporting Information Table S4).

Binding poses of saquinavir in the active site of PLpro revealed that amino acid GLU168 is in three H-bond interactions with amide -NH, alcoholic -OH and decahydroisoquinolinium –NH of saquinavir. Two π-π stacking interactions were observed between quinoline and phenyl rings with TYR269.

Figure 10. H-bond and π-π stacking interactions of saquinavir with PLpro active site residues after MD simulation in the selected trajectory 0.00 through 100 ns run.
Valganciclovir showed four H-bond interactions. ASP165 was found to involve in two H-bond interactions with amino –NH and alcoholic –OH, while, other two H-bond interactions were shown by GLN270, GLY267 with carbonyl oxygen C=O and amino –NH, respectively. Purine residue of valganciclovir and TYR269 are caught up in two π-π stacking interactions (Supporting Information Figure S5(a,b)). Additionally, very weak π–cation interaction is seen between amino –NH and TYR265. With famciclovir, residues GLY267 and TYR274 of PLpro pocket formed H-bonds with two ester carbonyl oxygen C=O and GLN270 showed H-bond interaction with an amino –NH group. Purine ring of famciclovir and TYR 269 were found to be involved in π-π stacking interaction (Supporting Information Figure S6(a,b)).

Docking poses of antimalarial compounds mefloquine (Figure 6(c,d)) and halofantrine (Supporting Information Figure S7(a,b)) have shown fewer H-bond interactions and lesser docking scores, as only ASP165 residue of PLpro pocket interacted with amino –NH of these compounds. Major π-π stacking was observed with quinoline and phenanthrene structures with TYR 269. Hence, the PLpro amino acid residues contributing to the H-bond interactions with both antiviral and antimalarial compounds are GLU168, ASP165, GLN270, GLY267 and TYR274, while TYR269 is important for π-π interactions. To visualize the resides and functional groups involved in each type of interaction we have summarized the results showing the interactions of important structural features of antiviral and antimalarial compounds with amino acid residues of PLpro pocket (Figure 7).
MD simulation of PLpro–saquinavir complex

Since saquinavir was found best fitted with PLpro during docking studies, it was considered for MD simulation. The plotted trajectory data for RMSD and RMSF (Figure 8(a,b)) in totality falls in the same line as revealed by docking studies about saquinavir’s lesser docking score and binding free energy with PLpro as compared with 3CLpro. Even if, the simulation was found to be equilibrated during 100 ns run time with changes upto 2.8–4.0 Å for PLpro and saquinavir, respectively, the RMSF data pointed out fluctuations for some residues which exceed 3.0 Å. Nonetheless, fluctuation of most of the residues were within the limit of 2.4 Å.

As a result of a slightly higher limit of changes in PLpro, saquinavir was found to be lesser stable with PLpro as compared to 3CLpro protein.

According to the docking study, the only three H-bond forming residues of PLpro is GLU168. While the MD simulation trajectory analysis of PLpro–saquinavir complex revealed several H-bond forming residues (Figure 9(a,b)). Saquinavir’s hydroxyl atom 11 (O) was found to form H-bonding with GLU168 (34%) via water bridge and ASP165 (91%), while residues TYR274 (69%), ARG167 (45%), ASP303 (91%) and VAL166 (54%) were observed in water bridge H-bonding with amide atom 27 (O). The amido atom 26 (N) had formed H-bonds with ASP303 (98%), ALA247 (94%) and THR302, whereas the amide atom 22 (O) was observed in water bridge H-bonding with TYR269 (33%). Prominent π-π stacking interactions with TYR269 residue are reflected in simulation data as observed in docking analysis (Figure 10).

RdRp

Docking studies

Dock score and binding free energy of antiviral compounds with RdRp (PDB ID: 4O4R) showed entirely different results when compared and analysed. Adefovir (a nucleotide HBV DNA polymerase inhibitor) (Table 1), tenofovir (a nucleotide reverse-transcriptase inhibitor) and valganciclovir (a nucleotide analog) were found to possess best dock scores in the range of $-6$ to $-7$. While, HIV protease inhibitors ritonavir, indinavir and nucleotide analog remdesivir were able to show good binding energy in the range of $-50$ to $-60$ (Supporting Information Table S5). Docking results of antimalarial drugs with RdRp in terms of docking glide scores and binding free energy are poor as compared to antiviral drugs. Dihydroartemisinin, amodiaquine and tafenoquine showed dock score in the range of $-5.2$ to $-5.3$ and binding free energy in the range of $-28$ to $-38$ (Supporting Information Table S6).

When we looked at the binding poses of antiviral compounds with maximum dock scores against RdRp, several H-
bond interactions were observed but lacked \( \pi \)-type of connections. Adefovir (Figure 11(a,b)) and tenofovir’s (Supporting Information Figure S8(a,b)) phosphonic acid moiety have shown H-bond interactions with ARG413 and LEU169. Phosphonic acid moiety was also found to show H-bond interaction with ARG392, while purine rings of these compounds formed H-bonds with GLN439. Now, valganciclovir (Supporting Information Figure S9(a,b)) and ganciclovir (Supporting Information Figure S10(a,b)) lack phosphonic acid moiety, but valganciclovir possesses a larger side chain comprising of ether, ester, alcoholic \(-OH\) and primary \(-NH_2\) features. ASP167, GLU168 and LEU169 of RdRp formed H-bonds with an alcoholic \(-OH\) and primary \(-NH_2\) functions. Purine moiety was found to interact with ARG413, TRP417 and GLN439. Although, ritonavir (Supporting Information Figure S11(a,b)), indinavir (Supporting Information Figure S12(a,b))
S12(a,b)) and remdesivir (Supporting Information Figure S13(a,b)) produced fewer H-bond interactions, pyrrole and phenyl rings of these compounds have instigated π-cation interactions with ARG393 and ARG392 of RdRp. It may be assumed that this π-cation interaction might be contributing to better binding energies with RdRp.

Binding poses of antimalarial compounds dihydroartemisinin (Figure 11(c,d)), amodiaquine (Supporting Information Figure S14(a,b)) and tafenoquine (Supporting Information Figure S15(a,b)) showed H-bond interactions with TRP417, GLN439, ARG413 and LYS219 residues of RdRp. Dihydroartemisinin and amodiaquine possess alcoholic and phenolic–OH group, respectively, which showed H-bonds with TRP417 and GLN439. While Quinoline –N of amodiaquine and ethereal oxygen C–O of tafenoquine was observed to show H-bond interactions with ARG413 and LYS219 in the order. The π-cation binding with ARG392 of amodiaquine and tafenoquine was observed with quinoline and phenyl ring, respectively. Thus, crucial H-bond forming amino acid residues of RdRp are ARG413, GLN439, GLU168, TRP417, LEU169, ARG392, ASP167 and LYS219. At the same time, π-cation interactions of compounds with ARG393 and ARG392 are also
found to be essential. For better understanding the binding affinities of structural features with amino acid residues of the binding pocket of RdRp, an artistic impression has been created and illustrated (Figure 12).

**MD simulation of RdRp–adefovir complex**

The plotted trajectory data for RMSD (Figure 13(a)) of RdRp–adefovir complex showed initial changes in the conformation of the complex which becomes stable later during the simulation period to get equilibrated. The overall changes found in the RMSD plot for RdRp is upto 2.4 Å and 4.00 Å for adefovir. RMSF data indicated major fluctuations for a few residues upto 3.00–4.00 Å. Otherwise, 2.0 Å is the maximum range of fluctuations for most of the residues of RdRp (Figure 13(b)).

As per the trajectory analysis of RdRp–adefovir complex (Figure 14(a,b)), ARG392 and ARG413 interacted through three H-bonds with adefovir. ARG392 has donated two H-bonds to adefovir’s phosphonic acid atom 13 (O, 79%) and atom 12 (O, 52%), while ARG413 has formed H-bond with atom 12 (O, 57%). SER 410 was also found to form H-bond through water bridge with atom 12 (O, 42%). π-cation interaction was observed between LYS419 and purine ring of adefovir. But H-bonding with residue LEU169 as seen in docking data was not observed with simulation results (Figure 15(a,b)).

**MM-GBSA binding free energies**

The MM-GBSA calculations were performed to estimate binding free energy or affinity (dG Bind) to revalidate the inhibitor affinity for the receptor–ligand complexes viz., 3CLpro–saquinavir, PLpro–saquinavir and RdRp–adefovir, predicted by the docking simulation studies. The MM-GBSA binding energy data indicates that the dG bind values for the 3CLpro–saquinavir, PLpro–saquinavir and RdRp–adefovir complexes was found to be –67.4779, –73.6161 and –38.4075, respectively. The results are tabulated in Table 2. The net binding energy of the complexes predicted by MM-GBSA was found significant as more negative value indicating stronger system stability.

**Conclusion**

In the present study, the binding pattern of 3CLpro–saquinavir, PLpro–saquinavir and RdRp–adefovir complexes were investigated using molecular docking, molecular dynamics as well as MM-GBSA free energy calculations. Molecular docking studies revealed that the structural scaffold and functionalities of saquinavir displayed better dock scores and binding energy with pivotal proteases 3CLpro and PLpro. While, adefovir exhibited a good dock score and glide energy against RdRp. Saquinavir blocks the residues GLU169, CYT145 through intermolecular hydrogen bonding and formed π–π stacking with HIE41 of 3CLpro protein. It was also observed to be involved in H-bond interactions with GLU168 and π–π stacking with TYR269 of PLpro protein. While adefovir was found to block GLN439, LEU169 and ARG413 through intermolecular hydrogen bonding with the active site of RdRp protein. The RMSD, RMSF and percentage binding interaction data of 3CLpro–saquinavir, PLpro–saquinavir and RdRp–adefovir complex systems showed that these complexes remained stable throughout the trajectories. Almost the same amino acid residues were observed in binding interactions during MD analysis at 100 ns of the three complex systems as were involved in docking studies. Additional water bridges were observed between saquinavir and ASP165, TYR274, ARG167, ASP303, ALA247, THR302, VAL166 in the PLpro–saquinavir complex.

The present study has identified the crucial structural features of saquinavir and other studied antiviral/antimalarial compounds required to interact with the key amino acid residues in the binding pocket of 3CLpro, PLpro and RdRp proteins. Therefore, for future drug development, these structural features can be utilized to design new leads for the inhibition of vital proteins of SARS-CoV-2.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Authors’ contribution**

Saurabh C. Khadse: Conceptualization, Methodology, Investigation and Interpretation, Writing-original draft, Writing-review and editing. Nikhil D. Amnerkar: Methodology, Editing-original draft, Writing-review and editing, Validation. Ashish M. Dhote: Methodology, Docking studies, Data collection and analysis. Vikas R. Patil: Docking studies, Data collection and analysis. Deepak K. Lokwani: Methodology, MD simulation studies, Interpretation. Vinod G. Ugale: Visualization, Validation, Resources. Nitin B. Charbe: Visualization, Validation, Resources. Bhoomendra A. Bhongade: Critical revision, Editing, Validation.

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