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Suramin, penciclovir, and anidulafungin exhibit potential in the treatment of COVID-19 via binding to nsp12 of SARS-CoV-2

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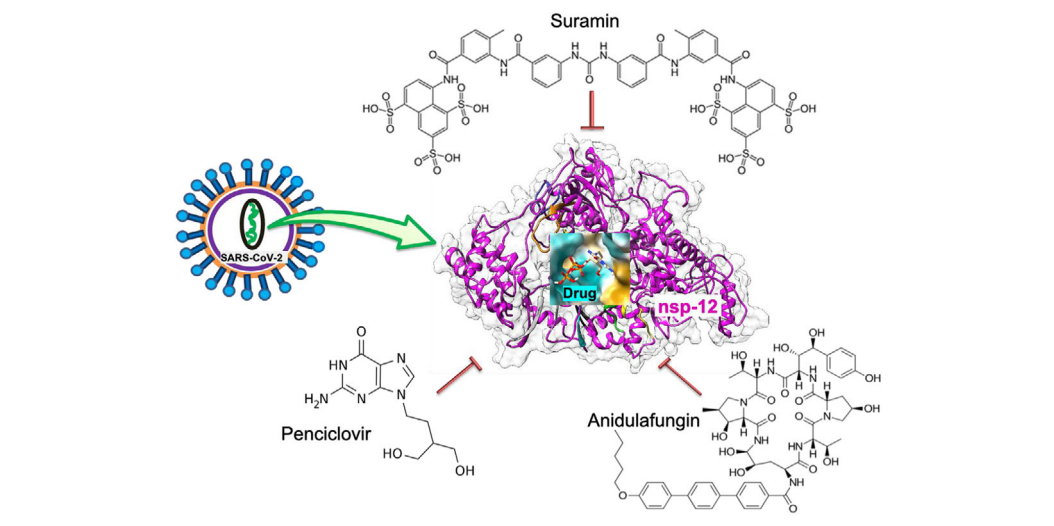
ABSTRACT

COVID-19, for which no confirmed therapeutic agents are available, has claimed over 48,14,000 lives globally. A feasible and quicker method to resolve this problem may be 'drug repositioning'. We investigated selected FDA and WHO-EML approved drugs based on their previously promising potential as antivirals, antibacterials or antifungals. These drugs were docked onto the nsp12 protein, which reigns the RNA-dependent RNA polymerase activity of SARS-CoV-2, a key therapeutic target for coronaviruses. Docked complexes were reevaluated using MM-GBSA analysis and the top three inhibitor-protein complexes were subjected to 100 ns long molecular dynamics simulation followed by another round of MM-GBSA analysis. The RMSF plots, binding energies and the mode of physicochemical interaction of the active site of the protein with the drugs were evaluated. Suramin, Penciclovir, and Anidulafungin were found to bind to nsp12 with similar binding energies as that of Remdesivir, which has been used as a therapy for COVID-19. In addition, recent experimental evidences indicate that these drugs exhibit antiviral efficacy against SARS-CoV-2. Such evidence, along with the significant and varied physical interactions of these drugs with the key viral enzyme outlined in this investigation, indicates that they might have a prospective therapeutic potential in the treatment of COVID-19 as monotherapy or combination therapy with Remdesivir.



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1. Introduction

The novel coronavirus disease 2019 (COVID-19) was declared a global pandemic on the 11th of March 2020 by WHO, barely within three months of the emergence of its first case (Bogoch et al., 2020; Hui et al., 2020). Since that initial instance, it has kept shifting its epicenter through various continents and has now spread to 215 countries and

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territories. It has already infected 235,607,000 people across the globe, claimed more than 48,14,000 lives, gravely impacted the countries socio-economically and even the most advanced healthcare systems have crumbled under its weight. It is a highly contagious pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the symptoms include complications in the gastrointestinal tract, respiratory problems, like cough, cold, shortness of breath, fever, and may even lead to fatality in some cases.

Family of this virus have inflicted the world with similar outbreaks, not the least of which was the Spanish Flu, which during the year 1918-1920 had infected one-fourth of the world's population and killed approximately 50 million (Centers for Disease Control and Prevention, USA). Recent examples of such epidemics caused by the closely related strains include Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS), each one spanning to more than 20 countries and killing approximately 1600 people in their wake, with a fatality rate of 10% and 35%, respectively (Morens et al., 2020). COVID-19 has a global fatality rate of 3.6%, and if the outbreak reaches anywhere near the previous scales of pandemics, this rate would translate to millions of deaths (Morens et al., 2020).

These closely related strains are members of the coronavirus (CoV) family and belong to the class of positive-stranded RNA viruses with a huge 30 kb polycistronic genome. The viral replicase polyproteins namely pp1a and pp1ab are translated by the proximal two-thirds of the 5′ end of CoV genome (ORFs 1a and 1 b) (Kirchdoerfer & Ward, 2019; Courouble et al., 2021). These two polyproteins are cleaved into 16 nonstructural proteins (nsps), which are essential for viral replication and transcription, thus being regarded as a potential virulence factor and drug targets for CoV (Te Velthuis et al., 2010). ORFs near the 3′ end encodes the structural proteins namely S, M, E and N which are the spike, membrane, envelope, and nucleocapsid proteins, respectively (Mirza & Froeyen, 2020; Rizwan et al., 2021). Out of the 16 nsps encoded by the 5′ end, nsp12 houses the RdRp (RNA-dependent RNA polymerase) activity. The central catalytic subunit of the RNA-synthesizing machinery for viral replication and transcription is the RdRp, which has seven catalytic motifs (A-G). Each motif has a particular function from selection and correct positioning to the addition of the newly incorporated NTPs (Nucleoside triphosphates) to the growing chain which is carried out with the help of a set of conserved amino acid residues. RdRp is an essential viral enzyme in the life cycle of RNA viruses and has been targeted in various viral infections, including the hepatitis C virus (HCV), the Zika virus (ZIKV), coronaviruses (CoVs) and others (Elfiky et al., 2015; 2017; Elfiky & Elshemey, 2018; Elfiky & Ismail, 2017; 2018; Ganesan & Barakat, 2017). It has continuously served as a predominant drug target in SARS and MERS research with minimal cytotoxic effects on host cells (Wu et al., 2020). Thus, nsp12 holds potential as an attractive target to develop therapeutic agents against SARS-CoV-2. In addition, human homologs of nsp12 are not known to exist and thus drugs that target nsp12 of COVID-19 are not expected to cause major disruption in physiological processes (Wu et al., 2020).

Of late, attempts had been made to identify small molecules that could bind to nsp12 with the potential of therapeutic intervention for COVID-19 affected patients. Investigations have revealed vitamin B12, valproic acid co-A, Remdesivir, Sofosbuvir, Tenofovir, and Ribavirin to be effective inhibitors of nsp12 (Ahmad et al., 2020; Deng, 2020; Elfiky, 2020; Chien et al., 2020; Narayanan & Nair, 2020; Bhavesh & Patra, 2020). While *in vitro* validation and clinical trial of these molecules, except Remdesivir, are awaited for COVID-19, it is necessary to identify additional molecules with similar objectives to populate the pipeline of potential drug candidates against COVID-19. The previous investigations utilized a homology model of the protein for small molecule screening, and with the experimental (cryo-electron microscopy) three-dimensional structures of the protein having been reported (Gao et al., 2020), the exercise of small molecule screening was necessary to be re-visited.

Novel small molecules would have to undergo validation and toxicity tests and the initial rounds of clinical trials, resulting in a long-drawn process of drug discovery. To address the current pandemic swiftly, drug repositioning is a feasible method to tackle the problem of unavailability of vaccines or therapeutic agents against novel coronavirus. FDA and WHO-essential medicine list 2019, approved drugs, along with some widely prescribed and investigational drugs, provide a safer alternative as there is no requirement to test the toxicity of these drugs. Hence, in this study, we have used molecular docking, MD simulations followed by MM-GBSA to identify and characterize binding interactions of potent drugs capable of inhibiting nsp12 of SARS-CoV-2. The exercise revealed three FDA- approved drugs with the potential to treat COVID-19.

2. Materials and methods

2.1. Sequence alignment

The fasta sequence of nsp12 protein of SARS-CoV (6NUR_SARSCoV) and the newly emerged cryo-EM (6M71) structure of nsp12 of SARS-CoV-2 (6M71_SARSCoV2) were retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) database PDB (Protein Data Bank) and aligned using MultAlin software (Corpet, 1988). Further, graphical enhancement and secondary structure elements were added using ESPript 3.0 (Robert & Gouet, 2014).

2.2. Preparation of the target protein for docking studies

The amino acid sequence of nsp12 of SARS-CoV-2 was retrieved from NCBI (accession number: NC045512.2) and a homology model was built initially based on the cryo-EM structure of RdRp of SARS-CoV (PDB ID 6NUR) using SWISS-MODEL (Waterhouse et al., 2018), since the earlier investigations on drug screening were based on such a homology model. In the course of completion of our manuscript, the cryo-EM structure of nsp12 of SARS-CoV-2 was reported (Gao

et al., 2020) at 2.9 Å resolution in complex with nsp7 and nsp8 proteins (PDB ID 6M71). The coordinates for nsp7 and nsp8 were removed from the PDB file and the resulting PDB file was used for all further analysis of nsp12 protein structure. The structure was further prepared as above for the subsequent docking studies.

2.3. Ligand preparation

A total of 43 diverse arrays of drugs (Table S1) exhibiting medicinal values were downloaded from PubChem databank in .sdf format and converted to .mol2 extension prior to docking using UCSF Chimera (Pettersen et al., 2004).

2.4. Molecular docking

To evaluate the best binding energy and pose of the ligands potentially capable of inhibiting nsp12 of SARS-CoV-2 (both the model and the cryo-EM structure), molecular docking was performed in triplicates with the help of SwissDock (Grosdidier et al., 2011), an automated web-based tool capable of predicting the molecular interactions that may occur between a target protein and a small molecule. Ten best docked poses from each independent docking run for each compound was taken for binding energy calculation while three similar but independent docking for each compound were done using SwissDock. Before initiation of docking both the proteins and the ligands were minimized using CHARMM forcefield (Waterhouse et al., 2018). Docking experiments were executed keeping the accurate default parameters of SwissDock with no prefecture of defined regions (blind docking). After the completion of docking, the protein-ligand complex was examined using the UCSF Chimera (Pettersen et al., 2004). Many of the docking results were also verified using the professional and licensed software Schrodinger (Schrodinger, 2011). Data of ligand binding energy are presented as mean of at least three independent docking experiments ± SEM (Tables S1 and S2). To avoid any false-positive or false-negative results from these docking experiments, we ran MM-GBSA for all of these 43 compounds (along with positive and negative controls) taking best 10 docked poses out of the SwissDock results following methods detailed in Faizul Azam et al., 2020 (Azam et al., 2020) and Celik S et al., 2019 (Celik et al., 2020) using the freely available MM-GBSA calculation server farPPI (Celik et al., 2020; Wang et al., 2019). Based on GAFF2 and ff14SB force field combination and the GB5 procedure using farPPI server, binding energies or re-scored docking energies of the 43 compounds along with controls after MM-GBSA experiments are shown in Table S1 in kcal/mol.

2.5. Molecular dynamics simulations

MM-GBSA-based rescored protein-ligand complexes with the best pose showing lowest binding energy (against the SARS-CoV-2 cryo-EM structure (PDB 6M71)) (Gao et al., 2020), namely, nsp12-anidulafungin, nsp12-penciclovir, and nsp12-suramin were subjected to molecular dynamics simulations using the Molecular Modelling Toolkit or MMTK software (Hinsen, 2000) integrated by V. Munoz-Robles and J. D. Marechal of the Computational Biotech. Chemistry team into UCSF Chimera (Pettersen et al., 2004).

Cryo-EM protein structure of nsp12 (PDB ID: 6M71), was processed by the ProteinPrep wizard of Schrodinger software (Schrodinger, 2011) to complete missing amino acid side chains, proper configuration of N- and C-terminal amino acids and initial energy minimization using OPLS2005 forcefield for the protein (Guvench & MacKerell, 2008; Robertson et al., 2015; Shivakumar et al., 2012). Each protein-ligand complex was individually subjected to molecular dynamics simulations (MD simulations) using the MMTK module of UCSF Chimera. Complexes were pre-processed using the Dock-Prep tool of UCSF Chimera to remove all hydrogens and replaced with AMBER compliant atomic nomenclatures, followed by calculation of charges to all atoms including the ligands using the AMBER ff14sb and AM1-BCC forcefields based on the ANTECHAMBER algorithm (Wang et al., 2006). After adding proper hydrogens and charge calculations, protein-ligand complexes were solvated using three-point TIP3PBOX type water molecules surrounding 10 Å in each of the three directions of the complex forming a cubic box system for simulation. A cubic box system of MD simulation was generated using a 10 Å distance from each side of the protein surface. Noncovalent interactions were measured with a cut off distance of 14 Å. The particle mesh Ewald (PME) (Darden et al., 1993) method was used to calculate electrostatic interactions while Lennard-Jones interaction method (Boulanger et al., 2016) was used to constrain all hydrogen bonds. The system first performed 300 steps of steepest descent with energy minimization. Then, the minimized system was used to perform simulations using an NVT ensemble. The Nosé–Hoover method (Maćkowiak et al., 2017) was used to maintain a fixed temperature while Anderson Barostat (Bereau, 2015) was used to maintain a constant atmospheric pressure (i.e. 1 ATM) using NPT ensemble. The system was heated from 0 K to 298 K using an increment of 10 K/ps time frame. The time step of each simulation was set to 2 fs and finally, production MD was run for 100,000 ps or 100 ns. Visualizations, RMSD, RMSF, Rg, as well as energy calculation and data analysis were performed with UCSF Chimera software (Pettersen et al., 2004). In order to obtain diverse energy parameters including SASA energy of the drugs bound to nsp-12, g_mmpbsa package was utilized (Kuhn & Kollman, 2000; Kumari & Kumar, 2014). 150 snapshots of each complex obtained from the last 10 ns of MD trajectories has been used to compute free energy as well as energy contributions of active site residues to binding energy. The dynamic cross-correlation map for the calculation and plotting of the per residue interaction analysis between motions of backbone α-carbon atoms of the drug bound nsp12 complexes during simulation was conducted by using CHARMM27 all atom forcefield (Vanommeslaeghe et al., 2010) and plotted with the GraphPad Prism software (https://www.graphpad.com/scientific-software/prism/).

2.6. MM-GBSA analysis of each of the best three drugs and Remdesivir bound to nsp12

Molecular mechanics generalized Born surface area (or MM-GBSA) (Wang et al., 2019) are arguably highly accepted thermodynamic methods for binding free energy calculations or prediction since they are more accurate than most scoring functions of molecular docking (and less computationally demanding). Thus, to better understand the drug/ligand binding affinity and binding energies, a MM-GBSA dG Bind analysis was done for the top three docked compounds along with Remdesivir as a positive control. In an MM-GBSA analysis, the binding energy of the receptor and ligands were calculated by the following formula:

Binding energy, a Molecular Mechanics + Implicit Solvent Energy Function represented as kcals/mol = (prime-energy of the optimized complex – prime-energy of the optimized free ligand) – prime-energy of the optimized free receptor. For each of these post-simulation MM-GBSA analysis, we had included final 10 snapshots/projections (i.e. 91st to 100th ns simulated structures) of the aforesaid protein-ligand complexes namely, nsp12-anidulafungin, nsp12-penciclovir, and nsp12-suramin; and compared the same with nsp12-remdesivir complex as well. This analysis was conducted using the Prime-MM-GBSA module of the Schrodinger Inc. where the VSGB solvation model (Li et al., 2011) and OPLS3 force field (Harder et al., 2016) were used for energy minimization and calculations without modifying the original input partial charges of the drugs (Azam et al., 2020). Sampling method was used to minimize the system as well as the drug-receptor interactions. Finally, the results of MM-GBSA experiment were represented as MM-GBSA dG binding energies in kcal/mole (Azam et al., 2020).

2.7. Interaction maps

Three-dimensional interaction maps were prepared using Chimera (Pettersen et al., 2004). Two-dimensional physicochemical interaction maps were prepared using Ligplot + and freely available visualization tool Maestro from Schrodinger Inc. (Schrodinger, 2011).

3. Results and discussion

3.1. Sequence alignment of nsp12 proteins of SARS-CoV and SARS-CoV-2 do not reveal major differences in their key amino acid residues that govern RdRp activity

Recent studies have indicated that SARS-CoV-2 shares ~78% and ~51% genome similarities with SARS and MERS, respectively (Fani et al., 2020; Lu et al., 2020). On the alignment of amino acid sequence of nsp12 of SARS-CoV-2 (PDB ID: 6M71) (Gao et al., 2020) with its closest homologue nsp12 of SARS-CoV (PDB ID: 6NUR) (Kirchdoerfer & Ward, 2019), the RdRp domain comprising three sub-domains (the palm, thumb, finger) and the seven motifs (A-G), as shown in Figures 1 and 2, exhibited high sequence identity within the coronavirus family. Three amino acids namely, Asp760, Asp761, and Arg553 are conserved in RdRp and are reported to be important in rNTP binding and positioning of template overhang substitutions (Zhang et al., 2020). When SARS-CoV-2 RdRp amino acid sequence was compared to that of SARS-CoV, the following mutations were observed: Thr^{614}Asn (motif A) and Ser^{786}Ala (Motif D) (Figure 1). Motifs B, C, and F were, however, 100% conserved. Based on this high sequence conservation of RdRp among different corona virus species and availability of the knowledge about the active site of this enzyme, it was investigated as a key drug target to counter COVID-19. Absence of any homologous protein or a protein with similar structure or sequence in humans is another advantage of using RdRp as a drug target.

3.2. Selection of FDA and WHO-EML 2019 approved drugs for repurposing for COVID-19 treatment

A total of 43 drugs of different physiognomies belonging to various classes like antivirals, antimalarials, antibacterials, antihelminths, antifungals, anticancer, antiarrhythmic, receptor agonist, calcium channel blockers, immunosuppressant, antidiarrheal, anti-asthma and anti-inflammation were selected from the available literature on their previously demonstrated ability to treat diseases that are closely related to COVID-19 like SARS and MERS and other viral diseases (Table S1). The drugs were either FDA or WHO-EML 2019 approved for human use or widely prescribed in different parts of the world or are investigational drugs in clinical trials based on FDA recommendation (Table S1). With the status of the drugs as suitable for human consumption, the need to test toxicity of the compounds do not arise, and the drugs can be suitably repurposed for treatment of COVID-19, provided they bind the target protein with high affinity. The selected drugs were screened *in silico* in search of a potential candidate capable of inhibiting nsp12 of SARS-CoV-2 and, hence restricting the contagion. Remdesivir, a recent investigational antiviral, which entered Phase-3 clinical trial (NCT04292730) to treat COVID-19 on the basis of its ability to inhibit SARS-CoV-2 RdRp (Deng, 2020), was used as a positive control in the docking study as was also done very recently by Simonis A et al. (Simonis et al., 2021) and Kaddoura M et al. (Kaddoura et al., 2020), citing our preliminary publication in PrePrints (Dey et al., 2020). Tetrandrine was chosen as a negative control because while it is known to reduce the expression of Human coronavirus OC43 (HCoV-OC43) spike and nucleoside capsid protein, it is not related with RdRp as of yet.

3.3. Molecular docking and MM-GBSA analyses identified three drugs with the potential to bind to nsp12 with binding energy similar to Remdesivir-TP

Docking, an established strategy for structure-based drug discovery, was utilized to screen the 43 drugs (Tables S1 and S2) for their ability to dock or bind to nsp12 SARS-CoV-2. A drug that binds to the active site of the target protein or interacts with it allosterically by binding at a site away from the active site will be capable of arresting the RdRp activity and thus SARS-CoV-2 spread. Binding energy (in kcal/mol) was used as an

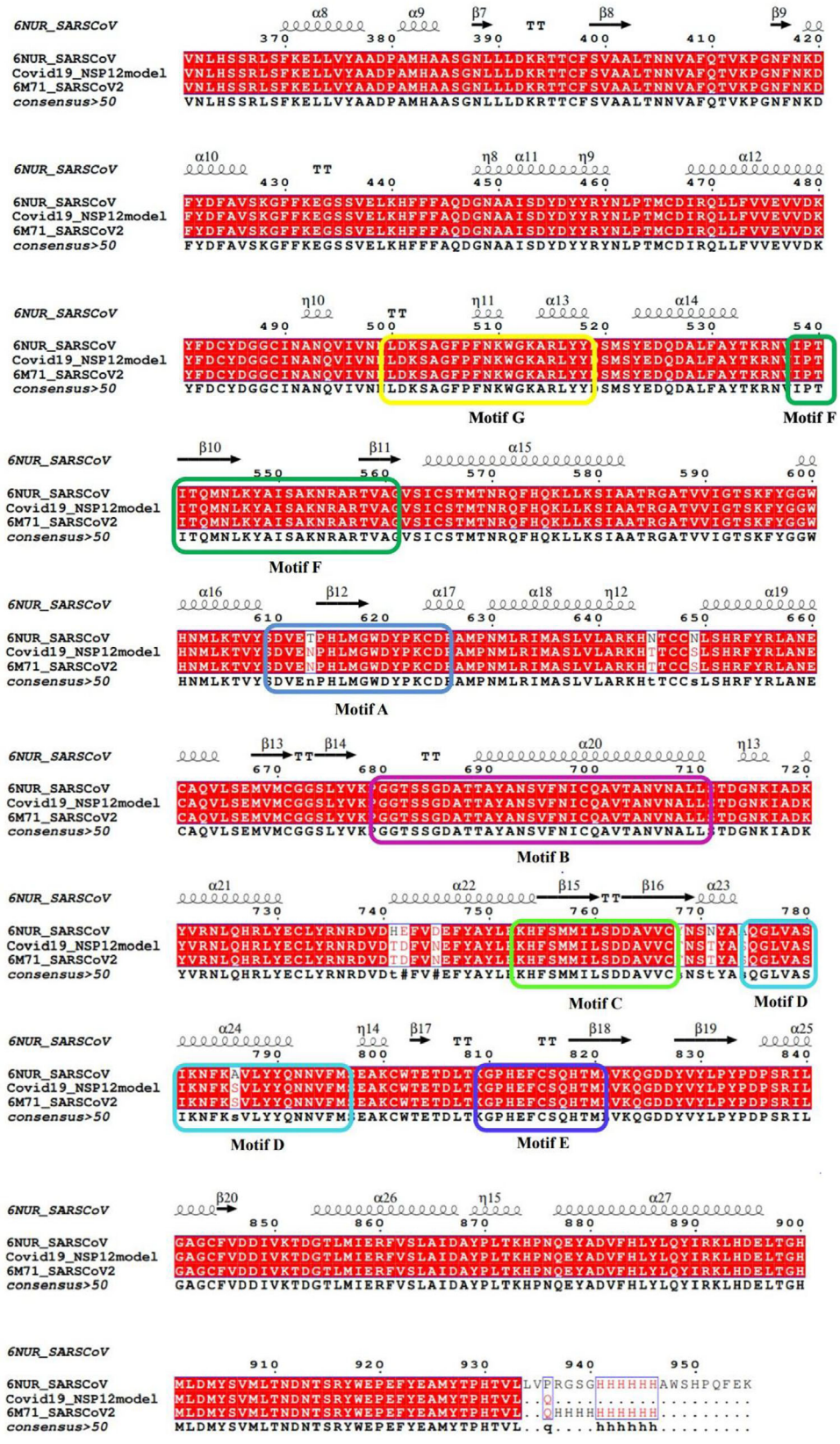


Figure 1. Amino acid sequence alignment of nsp12 protein of SARS-CoV (6NUR_SARSCov) with the sequence used for homology model (COVID19_NSP12model) and that from the cryo-EM (6M71) structure of nsp12 of SARS-CoV-2 (6M71_SARSCov2) using ESPript 3.0. The conserved as well as the non-conserved residues are highlighted in red and white, respectively, and the RdRp motif sequences (Motifs A-G) are highlighted with coloured boxes.

indicator of binary complex formation, with high negative values indicating high affinity binding (Figures 3 and S3). The positive control Remdesivir inhibits the RdRp by mimicking as a nucleoside analogue. De-phosphorylated form of Remdesivir is an inactive prodrug. Remdesivir tri-phosphate (Remdesivir-TP), the active form of the drug, is incorporated into nascent RNA

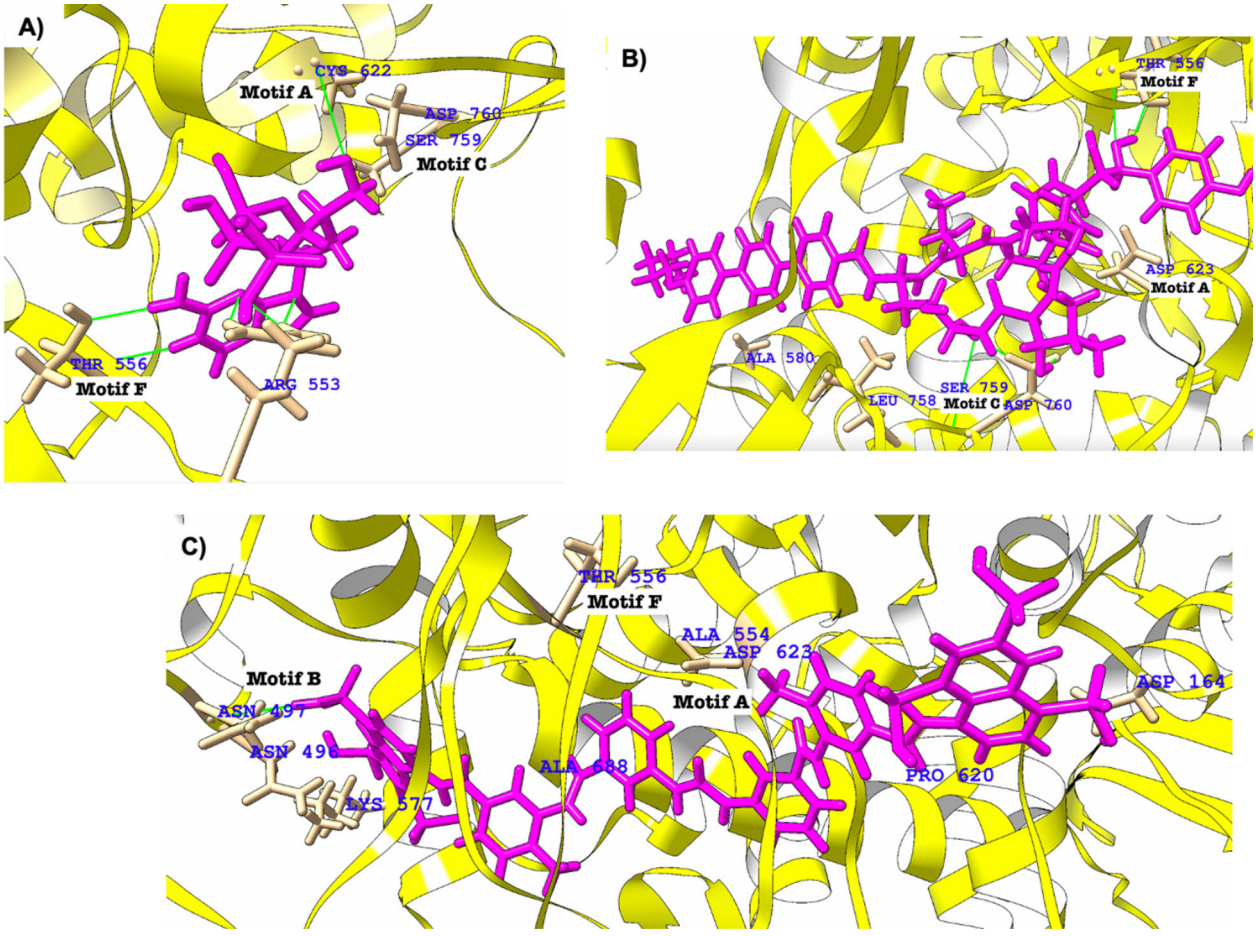


Figure 2. Top three leads docked to the RdRp region of nsp12 (SARS-CoV-2, PDB ID: 6M71). A) Penciclovir, B) Anidulafungin, and C) Suramin.

strand as Remdesivir monophosphate (Rem-MP) while its cleaved pyrophosphate remains close to it and both of them together inhibits the replication (Ruan et al., 2020). Remdesivir-TP is expected to yield a high binding energy, while the negative control Tetrandrine, which is not known to bind to nsp12, is expected to yield low binding energy in the docking experiments. Remdesivir-TP indeed exhibited a high binding energy of of -15.97 ± 0.3 kcal/mol with the homology model as the target protein and -14.22 ± 0.33 kcal/mol with the cryo-EM structure as the target protein. Tetrandrine, on the contrary, exhibited binding energies of -5.59 ± 0.008 and -7.36 ± 0.006 kcal/mol against the homology model and experimental structure, respectively (Figure 3 and S3, and Tables S1 and S2). Thus, drugs with binding energy equivalent to that of Remdesivir-TP were considered as potential drugs, with -10 kcal/mol as the stringent cut off binding energy for consideration as a candidate drug for COVID-19.

Molecular docking of all the 43 drugs to nsp12 SARS-CoV-2 generated the best-fit pose of the ligand and protein and the resulting binding energies are shown in Figure 3 and S3 (and Table 1). Penciclovir (Penciclovir-TP is the active form), an antiviral, Anidulafungin, an antifungal, and Suramin, an antimicrobial, showed binding energies of (mean of $n = 3$) -11.35, -11.66, and -12.86 kcal/mol, respectively (Table 1; Figure 3), as compared to the positive control (-14.22 kcal/mol) considering the cryo-EM structure as the model. The three drugs above are thus expected to inhibit nsp12 of SARS-CoV-2 in a manner similar to Remdesivir-TP. The binding energies of the drugs with the homology model and cryo-EM structures showed differences in their values but the trend overall was similar in both the cases.

In addition to the three drugs indicated above with binding energies equivalent to Remdesivir-TP, the drugs Eltrombopag, Ombitasvir, Digitoxin, and Ribavirin also displayed binding energies close to -10 kcal/mol, which may be considered to be significant as well (Figure 3; Table 1). These drugs thus may also be considered for treatment of COVID-19 along with Penciclovir, Anidulafungin, and Sumarin. The fact that Eltrombopag has recently been reported (Warren et al., 2016) as a potent inhibitor of nsp12 validates our study. Additionally, these 43 compounds along with the positive and negative controls were subjected to MM-GBSA analysis or calculation of re-scoring of docking energies and the results are summarized in the Table S1 in kcal/mol with the help of farPPI server.

3.4. The lead drugs interact with nsp12 in the RdRp domain involving multiple motifs, with motifs A and F being common to all the three drugs

Molecular docking provided the best-fit pose of the ligand in the protein structure and this allowed analysis of the interactions of the drugs with the motifs and the amino acid side chains lining the motifs. Such analysis assures that the drugs

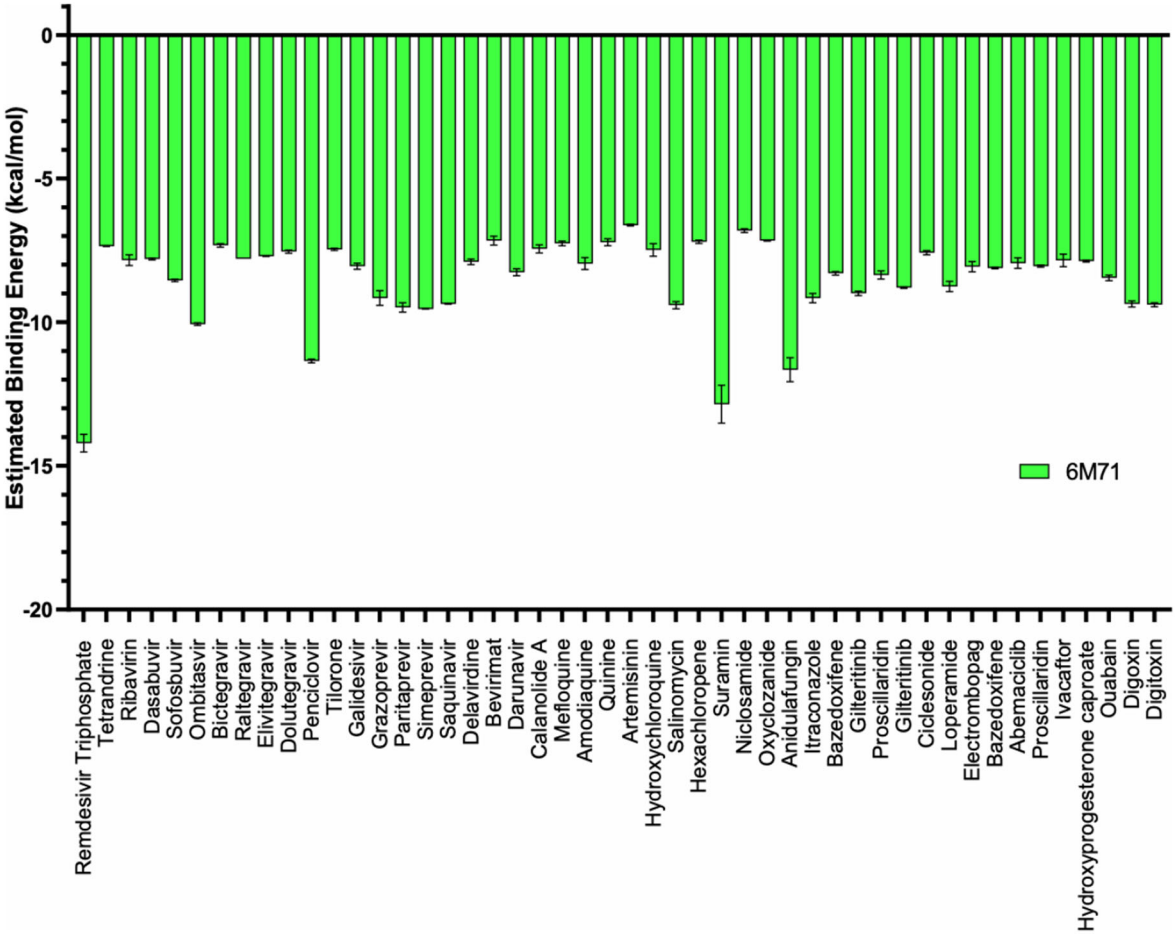
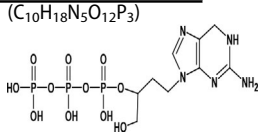
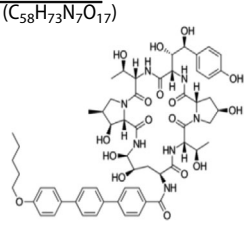
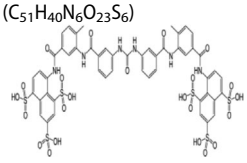


Figure 3. Bar graph representing the binding energies (kcal/mol) of the selected FDA-approved, WHO-EML 2019, investigational and widely prescribed drugs when docked to nsp12 of SARS-CoV-2 cryo-EM structure (PDB ID: 6M71) estimated by SwissDock. Remdesivir-TP and Tetrandrine, FDA investigational antivirals, were used as positive and negative controls, respectively, for the docking experiments *in silico*. Error bars are SEM for each compound as shown in Tables S1 and S2.

will inhibit RdRp activity, if they bind in this domain consisting of seven motifs (Figure S1). The three best drugs indeed bound to the RdRp domain using more than one motif in each case (Figure 2). The side chains that interact with the drug molecules were also identified (Figure 4) indicating the ability of these molecules to fit inside the protein complex comprising of nsp7, 8, and 12. Such interaction analysis would also provide scope for lead optimization in the future, if it is necessary to augment the binding of the drugs to nsp12; however, the optimized molecules would need to be subjected to toxicity tests and clinical trials yet again.

Penciclovir-TP binds to motifs A, C, and F where it forms hydrogen bonds with CYS622, SER759, ASP760, and ASP761 and van der Waals interactions with THR556, TYR689 (Figures 2 and 4; Table 2). This was reported earlier by Zhang et al. (2020) as well, indicating the validity of our investigation (Zhang et al., 2020). Anidulafungin forms hydrogen bonds with ARG555, THR556, SER759, and ASP760 and van der Waals interactions with TYR455, LYS577, TYR619, and SER682, all of which lie in motifs A, C and F (which are involved in positioning of incoming NTPs) (Figures 2 and 3; Table 1). Suramin forms van der Waals interactions with THR556, THR591, SER592, THR687, and SER759 and hydrogen bonds with ASN496, LYS500, CYS813, SER814, TYR680, and SER682, in motifs A, B, and F (enabling the positioning of the ribose moiety of NTPs with H-bond formation with 2′ OH) (Figures 2 and 4; Table 1). However, Remdesivir-TP hydrogen bonds with ASN496, ASN497, LYS500, ARG569, and GLN573 (Figure S4), which are different from the interactions of the three drugs above. It is interesting to note that the motifs A and F are common in interactions of all the three repurposed drugs with nsp12, while B and C vary between the three. None of these drugs, however, interact with motif G (which forms polar interaction with template RNA) as with Remdesivir-TP. Motifs A and F are at the centre of the nsp12 protein and on the top segment of the RdRp domain, while motif G is more toward the surface (Figures S1 and S1). The central non-covalent interactions of the three drugs make them unique compared to Remdesivir-TP and the potential utilization of these drugs individually or in combination with Remdesivir-TP for the successful inhibition of RdRp activity needs to be explored. We thus expect the three drugs to hinder RdRp activity, thus blocking replication and transcription and eventually viral propagation. Examination of docked poses also unveiled substantial information about the non-covalent interactions between the protein and the ligands (Figures 2 and 4; Table 1) and they were varied and different from Remdesivir. This necessitates that the three drugs are also

Table 1. Physico-chemical interactions of the repositioned drugs with SARS-CoV-2 nsp12.

Drug and Its Chemical Structure	Hydrogen bonds with RdRp	Electrostatic interactions with RdRp	van der Waals interactions with RdRp	Hydrophobic interactions with RdRp
Penciclovir- triphosphate ($C_{10}H_{18}N_5O_{12}P_3$) 	CYS622, SER759, ASP760, ASP761	ASP760, ASP761	THR556, TYR689	ILE589, ALA688, LEU758
Anidulafungin ($C_{58}H_{73}N_7O_{17}$) 	ARG555, THR556, SER759, ASP760	ASP452, ARG553, ASP623, ARG624	TYR455, LYS577, TYR619, SER682	ALA554, ALA580, GLY590, LEU758
Suramin ($C_{51}H_{40}N_6O_{23}S_6$) 	ASN496, LYS500, CYS813, SER814, TYR680, SERS682	ARG553, ASP623, ASP760, ASP761, ASP865	THR556, THR591, SER592, THR687, SER759	LEU576, ALA580, ILE589, GLY590, PHE594, GLY683, ALA685, ALA688, LEU758, GLN815

considered for treatment of COVID-19 since they might result in new efficacies hitherto not observed for Remdesivir.

For all of these 43 compounds, best 10 docked poses out of the SwissDock results were taken and following methods detailed in Faizul Azam et al., 2020 (Wang et al., 2019), rescoring of binding energy was performed to avoid any false-positive/false-negative results from these docking experiments using the freely available MM-GBSA calculation server farPPI (Hinsen, 2000). MM-GBSA analysis too depicted Suramin, Anidulafungin, and Penciclovir as the top three compounds with lowest deltaG (or re-scored binding energy) values of −154.73 kcal/mol, −143.26 kcal/mol, and −152.36 kcal/mol, respectively, which are similar to the binding energy of Remdesivir (-157.59 kcal/mol) (Table S1).

3.5. MD Simulation of the lead drugs bound to SARS-CoV-2 nsp12 revealed local conformational changes that might stabilize drug binding

MM-GBSA-based re-scored top three protein-ligand complexes with the best pose showing lowest binding energy (against the SARS-CoV-2 cryo-EM structure (PDB 6M71)) (Chien et al., 2020), namely, nsp12-anidulafungin, nsp12-penciclovir, and nsp12-suramin were subjected to molecular dynamics simulations (Figure 5) to allow conformational changes in the complexes, if any, mimicking such changes in solutions where dynamics reigns supreme and the interactions observed are meaningful. MD simulations of Penciclovir bound to nsp12 indicated an overall minimal structural change indicating that the docked structures were similar to the highest energy minimized conformations on the ns time scale. The longer time needed to stabilize Suramin bound nsp12 structure was probably due to the complex chiral structure of the drug undergoing a number of alternative physico-chemical bond breaking and making to finally obtain its lowest energy structure. Energy parameters after the MD simulation indicated that Suramin-nsp12 interaction was the most stable ($\Delta G = -852.3$ kcal/mol) followed by the interaction of the drugs Penciclovir ($\Delta G = -851.01$ kcal/mol) and Anidulafungin ($\Delta G = -849.95$ kcal/mol) (Table S2). While Suramin displayed the highest van der Waals interactions with nsp12 after MD simulations, Anidulafungin exhibited additional electrostatic interaction, which confirmed and corroborated well with the interaction maps of each of these drugs with the various amino acid side chains of SARS-CoV-2 nsp12 (Figure 5). These interaction maps revealed that Suramin formed two new H-bonds with THR556 and ARG553 (Figure 5). Penciclovir formed four new H-bonds with ASP623, ARG553, ARG555, and THR556 as compared to the pre-simulation docking results. This is a new finding previously not reported by Zhang et al. (Zhang et al., 2020) (Figure 5). Anidulafungin stabilized its binding by H-bonds with ARG555, ARG556, ASP623, CYS622 and electrostatic interactions with ARG553 probably due to the presence of multiple oppositely charged atoms in the structure of the drug near its surrounding protein surface (Figure 5). Overall, the MD simulation results confirmed the stable binding of the three drug candidates with nsp12 and the novel physico-chemical interactions, which may be validated further *in vitro* and *in vivo* and be considered for the treatment of COVID-19.

The root mean square fluctuation, RMSF, is the fluctuation of each atom around its average position. Atomic fluctuations of the Cα atoms of nsp12-bound to either of Suramin, Anidualfungin, or Penciclovir, were measured in the last 10 ns and depicted in Figure 6. It is evident from the figure that overall fluctuations of nsp12 is higher in case of Suramin, than Penciclovir and Anidulafungin. It was obserevd that in case of

Table 2. Repositioned drugs as potential inhibitors of nsp12/RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2 and related information.

Drug	Mechanism of Action	Physical Properties	Dosing Regimen	Side effects	Current application
Penciclovir (**Penciclovir-triphosphate**)	Nucleoside analogue inhibits viral replication	A. Physical State: White to pale yellow solid B. Solubility: i) Penciclovir 0.2 mg/mL in methanol, 1.3 mg/mL in propylene glycol, and 1.7 mg/mL in water ii) Famciclovir (Prodrug) • Soluble in acetone and methanol. • Hygroscopic below 85% relative humidity • Soluble (>25% w/v) in water (25 °C)	• Administration: Oral (tablet form) and cream • Regimen: Herpes liabilis Famciclovir (Prodrug) • Initial episode: 250 mg PO q8hr for 7-10 days • Suppressive therapy: 250 mg PO q12hr for 12 months • Recurrent episodes: 1500 mg PO once. Penciclovir • 0.1%(10 mg/g)-apply q2hr while awake for 4 days	Negligible to normal cells. Some trials suggest headache, oral/pharyngeal edema, and parosmia.	Approved for Herpes Simplex virus (*Herpes liabilis*)
Anidulafungin	Repress fungal cell wall formation by inhibiting 1→3-β-D glucan synthase	A. Physical State: White to off-white powder B. Solubility: Eraxis formulation of Anidulafungin is soluble in 0.9% sodium chloride (saline)	Administration: Intravenous (3.33 mg/mL) Regimen: Day 1: 200 mg IV infusion, Day 2 and thereafter: 100 mg/day IV Continued for 14 days after last positive culture	Not determined extensively. Some reported hepatic and hypersensitive adverse events.	Approved for candedemia
Suramin	Mechanism unknown. Trypanocidal activity; inhibits enzymes involved with the oxidation of reduced NADH.	A. Physical state: A white to off-white powder B. Solubility: Soluble in DMSO, water	Administration: Intravenous Regimen:Trypanosomiasis • 100-200 mg (test dose) IV, then 1 g IV on days 1, 3, 7, 14, 21	Nausea, vomiting and discomfort	Approved for the treatment of African trypanosomiasis and river blindness

nsp12-suramin complex, most dynamic amino acid residues of nsp12 were Asn496, Lys500, Arg553, Thr556, Leu576, Ala580, Ile589, Gly590, Thr591, Ser592, Phe594, Asp623, Tyr680, Sers682, Gly683, Ala685, Thr687, Ala688, Leu758, Ser759, Asp760, Asp761, Cys813, Ser814, Gln815, and Asp865 (Figure 6). Simialrly, for nsp-12-penciclovir and nsp-12-anidulafungin complexes, most dynamic residues were Asp452, Tyr455, Arg553, Ala554, Arg555, Thr556, Lys577, Ala580, Ile589, Gly590, Tyr619, Cys622, Asp623, Arg624, Ser682, Ala688, Tyr689, Leu758, Ser759, Asp760, and Asp761 (Figure 6). These results imply that all three lead compounds/drugs kept continuous interactions with amino acids residues at the vicinity of their binding which are similar to docked poses and this dynamism could be evident from the overall movements of the protein during the 100 ns MD simulation period. This is an expected scenario in case of a ligand-protein binding.

Radius of gyration or R_g values were also calculated to find the extent to which nsp12 maintain its folded structures in presence of the three drugs. Over a period of 100 ns MD simulation, while R_g value of nsp12 diverted by about 0.2 Å in case of nsp12-penciclovir complex, the same changed to a higher extent in case of both nsp12-anidulafungin and nsp12-suramin complexes in the range of 0.25 Å and 0.3 Å, respectively. Nevertheless, this insignificant decrease in the R_g value with an increase in MD simulation time indicates insignificant changes in the side chain conformation of nsp12 protein except the sites that interact with the corresponding drugs. These results indicate that nsp12 will maintain its folded structures in the presence of the three drugs and the interactions are expected to be stable.

The dynamical per residue interaction analysis represented by a residue cross-correlation map for the correlated motions, averaged over a time period of 0.5 ns to 100 ns, is shown in Figure 7. In general, correlations/interaction between mid- to long-range separated portions of the protein, in sufficiently long converged molecular dynamics simulations, decipher the dynamic features brought about by the nature of the protein (nsp12) or its bound ligand/drug like molecule in the present case: Suramin/Penciclovir/Anidulafungin. Usually, positive correlations comprise adjacent groups or amino acid residues, which move as a group. On the other hand, anticorrelated motions, comprise amino acids/motifs at long- and mid-range across the active sites. In Figure 7, the regions colored black to dark blue are sufficiently anticorrelated (please vide the color scale for the amount of correlation or anticorrelations indicated in the plots). The diagrams in the Figure 7a–c are diagonally symmetric. The first three regions of the per residue interaction or correlation map averaged from 0.5 to 100 ns (Figure 7a and b) for nsp12-penciclovir and nsp12-anidulafungin comprises of the anticorrelated motions of residues 750-765 (motif A) with residues Leu758, Ser759, Asp760, and Asp761; and 534-577 (motif F in the catalytic RdRp domain) with residues Arg553, Ala554, Arg555, Thr556, and Lys577; and 604-624 (catalytic motif A) with residues Tyr619, Cys622, Asp623 and Arg624. Similarly, nsp12-suramin complex (Figure 7c) comprises of the anticorrelated motions of residues 680-710 (motif B) with residues Tyr680, Sers682, Gly683, Ala685, Thr687, and Ala688; and 590-623 (motif A in the catalytic RdRp domain) with residues Phe594, and Asp623; and 534-556

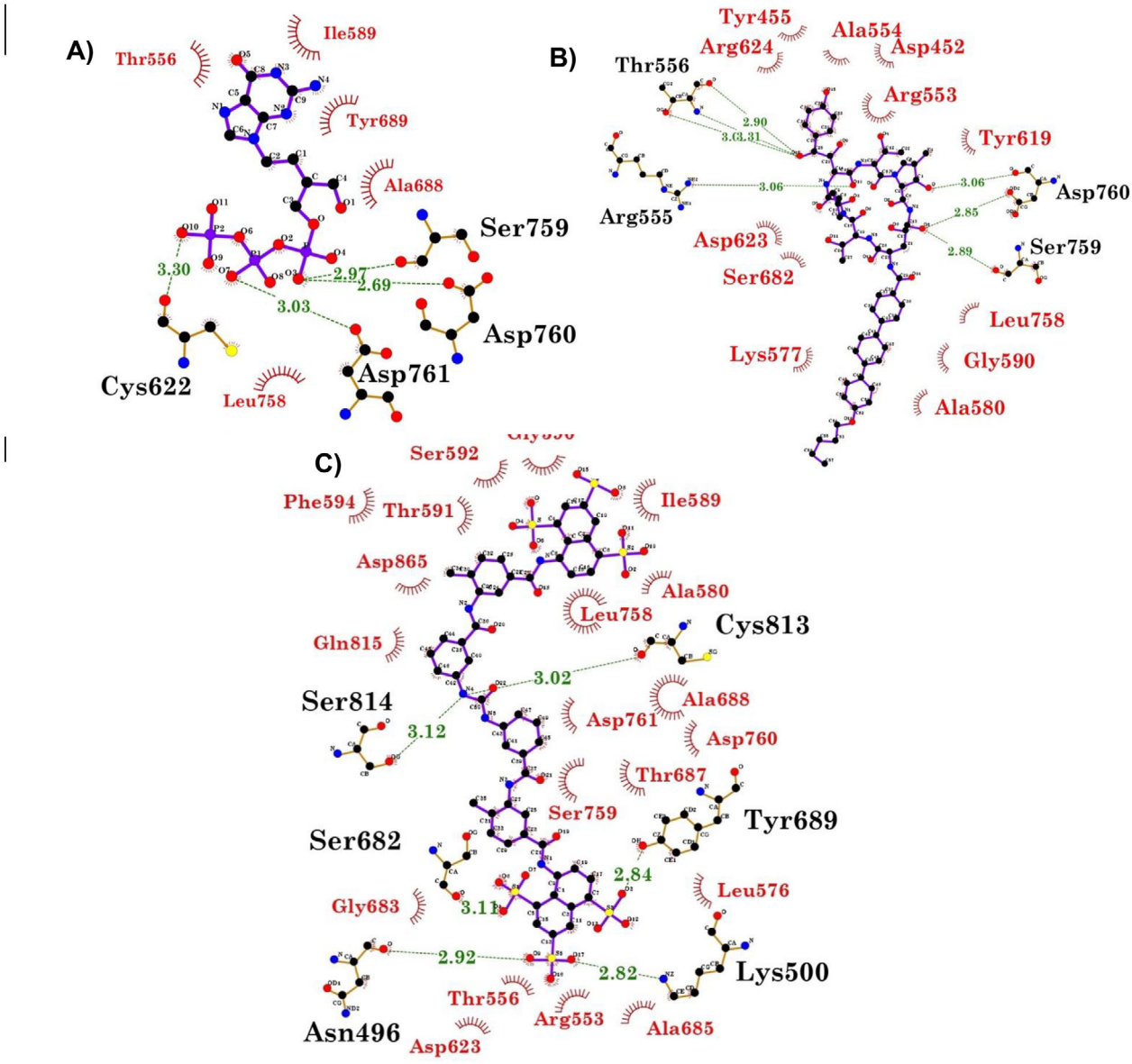


Figure 4. Interaction maps of A) Penciclovir, B) Anidulafungin, and C) Suramin with SARS-CoV-2 nsp-12. Green lines depict hydrogen bonds and eye shaped residues exhibits hydrophobic interactions.

(catalytic motif F) with residues Arg553, and Thr556. These anti-correlated amino acid residues traverse the active site and involve most of the RdRp domain of the nsp12 protein.

3.6. MM-GBSA analysis of the best three drugs bound to nsp12 complexes reveals similar binding affinities of Suramin, Anidulafungin and Penciclovir in comparison to Remdesivir

Molecular mechanics generalized Born surface area or MM-GBSA (Wang et al., 2019) analysis of nsp12 complexes of the best three compounds namely, Suramin, Anidulafungin and Penciclovir indicated similar binding energies like that of Remdesivir (Supplementary Table S3). For each of these post-simulation MM-GBSA analysis, we had included final 10 snapshots/projections (i.e. 91st to 100th ns simulated structures) of the aforesaid protein-ligand complexes namely, nsp12-anidulafungin, nsp12-penciclovir, and nsp12-suramin; and compared the same with nsp12-remdesivir complex as well. The PRIME energies for Remdesivir, Suramin, Anidulafungin, and Penciclovir were revealed to be −37504, −37129, −37055, and −36944 cal/mol which were almost similar (Table S4). However, the prime-MMGBSA dG binding energies for Suramin (-89 cal/mol) was far better than Anidulafungin (-49 cal/mol) and Penciclovir (-30 cal/mol), but similar to Remdesivir (-93 cal/mol) (Table S4). These results are more reliable than just docking scores and further increases reliability when presented in addition to MD simulations. Their sufficiently low binding energies confirm strong interactions of Suramin, Anidulafungin, and Penciclovir with nsp12, like Remdesivir.

In addition to hydrophobic, and electrostatic interaction, it was observed that the stability of Anidulafungin, Suramin, and Penciclovir in the RdRp domain of the nsp12 was also partially supported by the SASA energy with almost similar extent for all three drugs as calculated by the g_mmpbsa analysis. SASA energy for drug bound nsp12 are as follows: −8.09 ± 1.42 KJ/

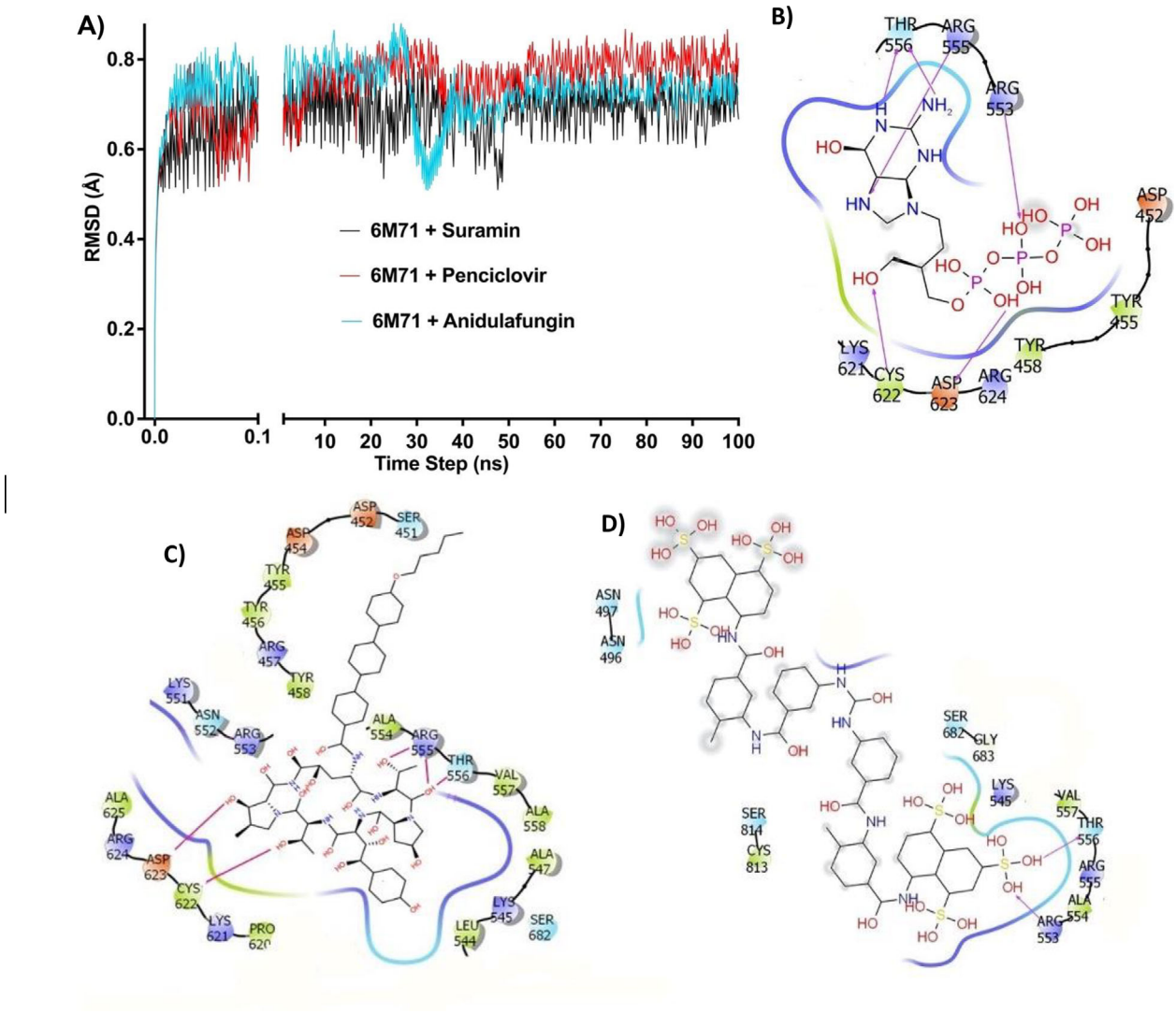


Figure 5. The RMSD of the backbone atoms of SARS-CoV-2 nsp12 structure (PDB ID: 6M71) relative to their energy minimized complex structures as a function of time and interaction maps of the three leads with SARS-CoV-2 nsp12 after 100 ns MD simulation depicting the amino acid residues in nsp12 that physico-chemically interact with the drugs. [A] RMSD plot from initial MD trajectory of 0 ns to final trajectory at 100 ns showing almost similar but a low total change in RMSD of ~0.8 Å for complexes of each of the three leads (Suramin (black), Penciclovir (red) and Anidulafungin (blue)) against SARS-CoV-2 nsp12. [B-D] Interaction maps of the three leads with SARS-CoV-2 nsp12 after 100 ns simulation depicting the amino acid residues in nsp12 that interact with the drugs. Magenta lines depict hydrogen bonds. [B] Anidulafungin, [C] Suramin, and [D] Penciclovir interacting with SARS-CoV-2 nsp12.

mol for nsp12-penciclovir complex, −7.83 ± 1.83 KJ/mol for nsp12-anidulafungin complex, −8.13 ± 2.04 KJ/mol for nsp12-suramin complex, and −8.01 ± 2.12 KJ/mol for nsp12-remdesivir complex.

3.7. Penciclovir binds near the incoming nucleotide binding sites while Anidulafungin and Suramin bind near the palm of the RdRp revealing differential binding modes of these three drugs to nsp12

The nsp12 protein is composed of a NiRAN (N-terminal nidovirus associated nucleotidyl transferase) domain followed by an interface and a C-terminal RdRp domain (Figure 8A). RdRp is the main catalytic house of nsp12 consisting of a finger, palm, and a thumb domain which have relatively conserved motifs. For example, G and F are conserved motifs of finger domain while A to D including some part of E forms a conserved motifs of palm domain (Figure 8) (Gao et al., 2020). Our docking analyses showed that all three hit molecules interacted with key amino acids such as ASP760 and ASP761 which are part catalytic centre of palm domain, thus, they may interfere with catalytic activity or metal ion-chelation. Anidulafungin made electrostatic interaction with ARG553 and ASP623. These amino acids are vital for template position in rNTP binding and sugar recognition domains (Figure 8). Interestingly, the structural comparison of RdRp-suramin and RdRp-remdesivir complex revealed that first Suramin molecule inhabits the −1 to −3 position of RNA template strand while the second Suramin occupies −4 to +1 place of primer strand. These findings indicate that two Suramin molecules can hinder interaction of RNA template-primer formation as well as effectively block the NTP entry at the palm domain (Figure 8) (PDB Id: 7D4F).

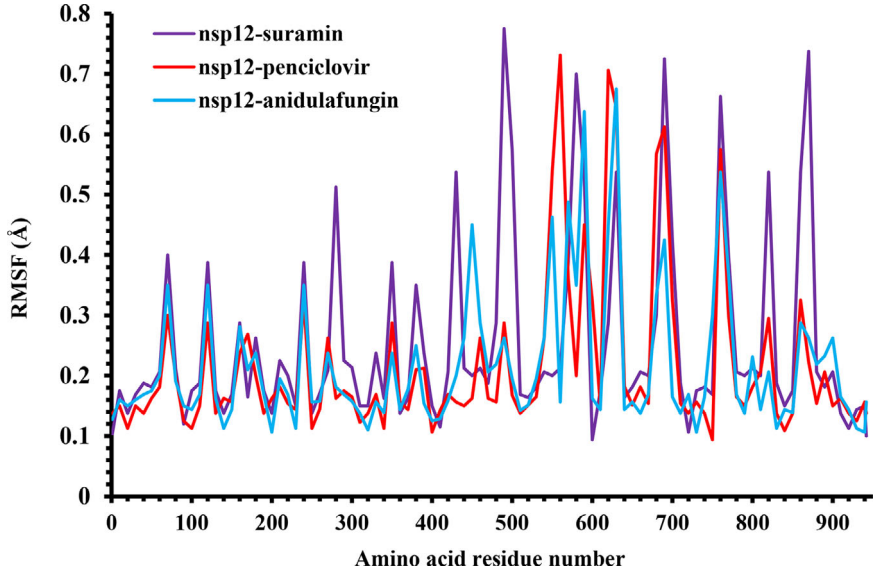


Figure 6. The per-residue RMSF plots for the energy minimized complexes of the top three drugs (Suramin, Anidulafungin, and Penciclovir) with the nsp12 after 100 ns MD simulation depicting the key amino acid residues fluctuating during the corresponding simulation.

3.8. FDA or WHO-approved drugs Penciclovir, Anidulafungin and Suramin are potential inhibitors of nsp12 and suitable for clinical trials for COVID-19 treatment

Penciclovir, Anidulafungin, and Suramin, identified in the present study, have immense potential for the inhibition of SARS-CoV-2 RdRp activity as evident from the above findings. The former two are FDA approved and the latter (Suramin) is listed as a drug in the WHO Essential medicine list-2019. These drugs can thus be tested directly in humans inflicted with COVID-19. Various characteristics of the drugs along with their dosage regimen are summarized in Table 2 for due consideration by the scientific and medical community. Penciclovir (trade name-Denavir) (Selisko et al., 2018) is a synthetic nucleoside analogue, an acyclic guanine derivative. It is an antiviral drug whose active form Penciclovir-TP is used for the treatment of various herpesvirus infections. In its active form, Penciclovir binds with a much higher affinity to viral polymerase than human polymerase, thus impairing viral replication. This accounts for the low cytotoxicity of healthy host cells (National Center for Biotechnology Information).

Anidulafungin (trade name-Eraxis and Ecalta) is a synthetic antifungal drug, generally used for the treatment of *Candida* infections. It disrupts fungal cell wall synthesis by inhibiting the enzyme complex 1,3-β-D-glucan synthase which is involved in the synthesis of 1,3-β-D-glucan, a component of the fungal cell wall. This leads to cell death and inhibition of fungal growth (Bacon et al., 2003).

Suramin (trade names −309 F, Antrypol, Bayer 205, Belganyl, Fourneau 309, Germanin, Moranyl, Naganin, Naganol, Naphuride) is a synthetic sulphated naphthylamine with multiple biological effects. In the 1920s it was found as a treatment for African trypanosomiasis (African sleeping sickness), wherein it acts by inhibiting the enzymes of energy metabolism of the parasite. It can inhibit many other enzymes and proteins like RNA polymerase, reverse transcriptase, thymidine kinase, dihydrofolate reductase, urease, hexokinase and others. Hence, the precise mechanism of action remains unclear (Kathryn & Gumbo, 2008). Suramin has been identified with various other biological functions, for example, it is used in the treatment of cancer (Stein, 1993) as well as an antiviral agent against HIV (Cheson et al., 1987; Li et al., 2015).

4. Conclusion

The nsp12 of SARS-CoV-2 seems to be a potential drug target due to its highly conserved architecture and catalytic functions. The present study identified three potential drugs having similar binding energies when compared to nsp12-remdesivir-TP, which recently got FDA approval and is currently under extensive use for COVID-19 treatment, and which have unique interactions with the protein as compared to Remdesivir-TP. These three drugs in the current study may inhibit SARS-CoV-2 RdRp activity as evident from the findings above and are thus expected to be of use in the treatment of COVID-19 as well. Penciclovir (Denavir) (Selisko et al., 2018) is a synthetic guanine nucleoside analogue (Table 2). Anidulafungin (Eraxis) is a synthetic antifungal drug that disrupts fungal cell wall synthesis, generally used for *Candida* infections (Bacon et al., 2003). Suramin is a synthetic sulphated naphthylamine with multiple biological effects (Cheson et al., 1987; Kathryn & Gumbo, 2008; Li et al., 2015; Stein, 1993). While, the first two are FDA approved and the latter (Suramin) is listed as a drug in WHO Essential medicine list-2019. These drugs can thus be tested directly in humans inflicted with COVID-19. We have summarized various characteristics of the drugs along with their dosage regimen in Table 2 for due consideration for the scientific and medical community. It also opens up avenues for lead optimization, based on the detailed interactions presented here, in the search of highly potent novel entities to bind specifically to

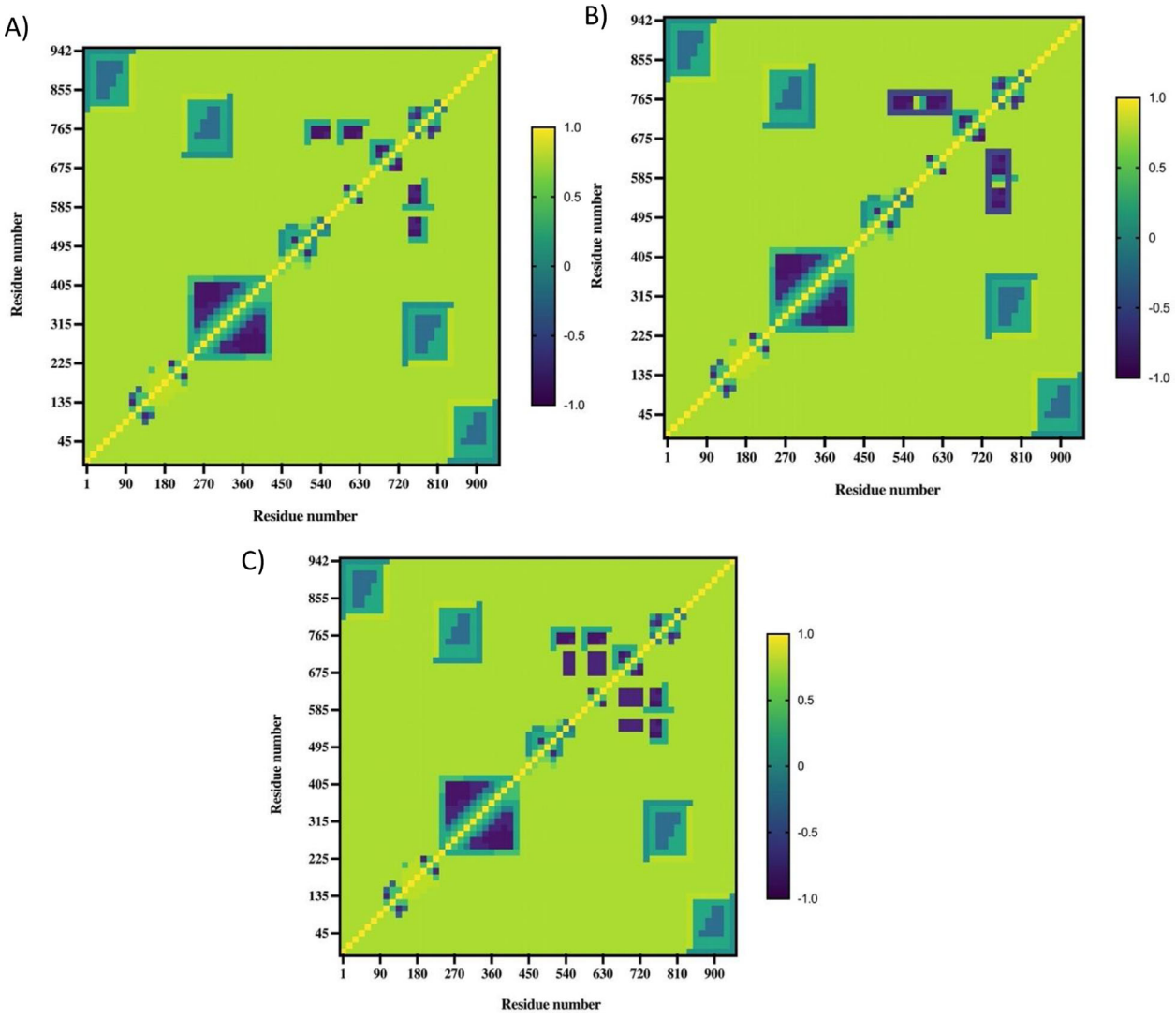


Figure 7. Calculated residue–residue-interaction-based correlated motions (dynamic cross-correlation map) within nsp12 from 0.5 ns to 100 ns molecular dynamic simulation of the nsp12-pencilcovir/nsp-12-anidulafungin/nsp12-suramin complex. (A) nsp12-pencilcovir, (B) nsp-12-anidulafungin, and (C) nsp12-suramin complex.

nsp12. The lead optimized drugs, if any, however, will need to be subjected to toxicity evaluation and clinical trials.

Existing experimental and clinical studies fortify our findings. Amongst the selected drugs, Suramin, the only reported non-nucleoside inhibitor of norovirus RdRp (Croci et al., 2014), was recently shown to exhibit antiviral activity against the SARS-CoV-2 cell lines (VERO E6) by targeting initial stages of replication in the viral life cycle (Salgado-Benvindo et al., 2020). The clinical benefits of Suramin against COVID-19 infection in humans are being explored in clinical trials as well (ChiCTR2000030029). Furthermore, we demonstrated that Penciclovir potentially binds to the catalytic center of the SARS-CoV-2 RdRp and it has been previously established that it reduces the COVID-19 infection but with a higher half-maximal effective concentration (EC_{50}=95.96 μM) compared to that of Remdesivir (0.77 μM). This indicates that it might require high concentrations of the nucleoside analog to reduce the viral infection; nonetheless, this drug provides an option for treating the deadly disease (Wang et al., 2020). Recent reports by Simonis A et al. (Simonis et al., 2021) and Kaddoura M et al. (Kaddoura et al., 2020) have also endorsed our claims for Penciclovir. Finally, experimental evidences have also revealed that Anidulafungin inhibits viral infection with lower half maximal inhibitory concentration (IC_{50}=4.64 μM) compared to that of Remdesivir (11.41 μM) against SARS-CoV-2 and is a potential candidate (Jeon et al., 2020). Though claims above are promising but the results predicted and discussed in the present investigation are solely based on *in silico* methods and it would require further experimental evaluation into their molecular mode of action. Nonetheless, similar studies have been performed recently using other protein targets of SARS-CoV-2, such as nsp15 and Mpro (viral main protease) (Bhardwaj et al., 2021; Sharma et al., 2021). This justifies an optimal procedure to discover bioactive molecules to inhibit the virus. For instance, similar computational analyses have shown the inhibitory potential of acridinedione analogues (Bhardwaj et al., 2021) as well as various polyphenols and phytochemicals extracted from *Broussonetia papyrifera* and *Torreya nucifera*, respectively, against the Mpro of SARS-CoV-2 (Bhardwaj et al., 2021; Ghosh et al., 2020). Studies also have been deployed by Bhardwaj et al., 2020 where they docked 65

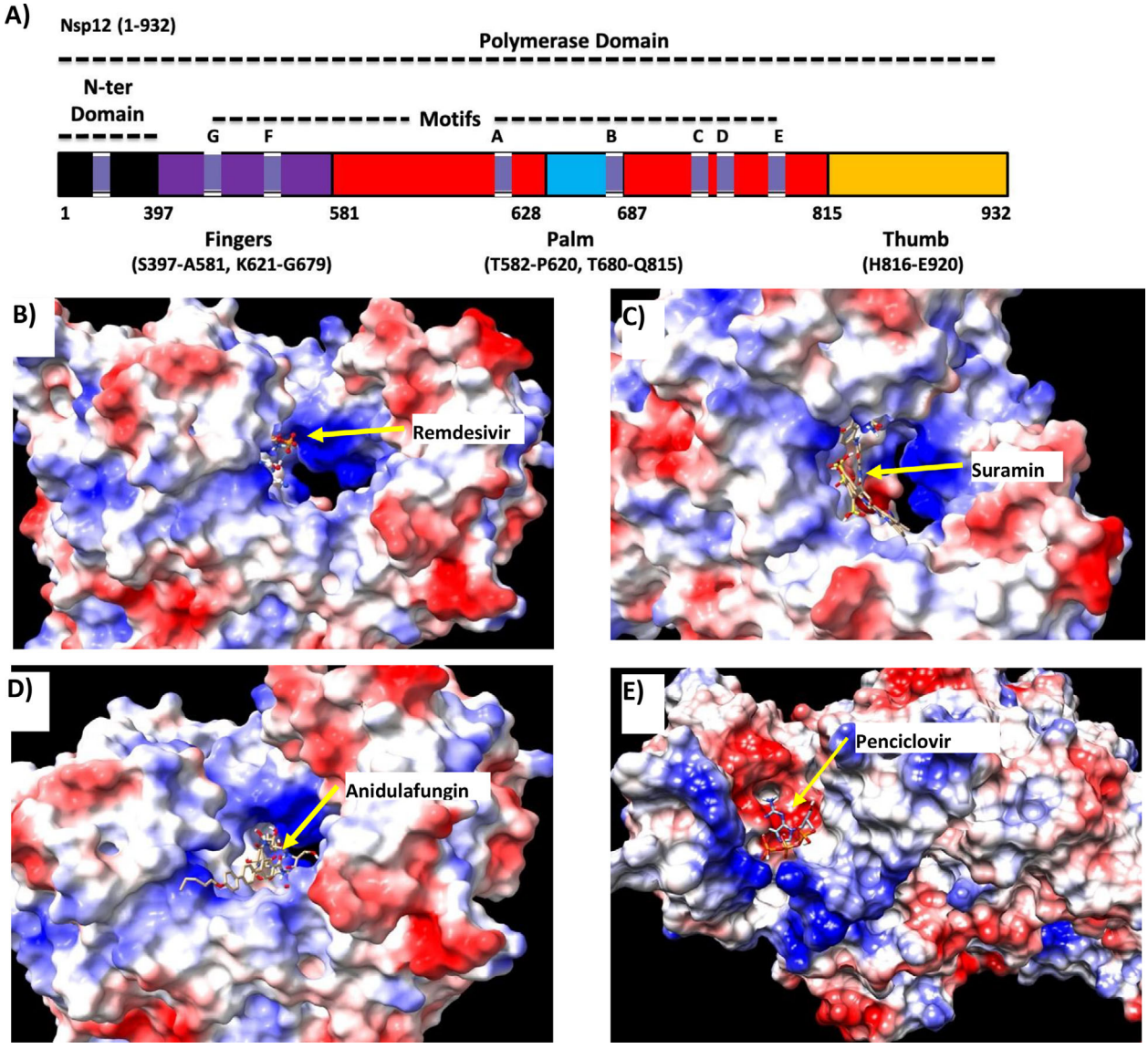


Figure 8. Detailed structural analysis of complexes formed between nsp12 and each of the top three leads and Remdesivir as a control highlighting electro-positive (blue surface) and electro-negative (red surfaces) regions of the protein favorable for interactions with oppositely charged atoms of the small drug molecules. A) Domain architecture of nsp12 with an emphasis on residue numbers, nsp12 complexes bound to B) Remdesivir, C) Suramin, D) Anidulafungin, and E) Penciclovir.

bioactive molecules of tea plant against Mpro (Bhardwaj et al., 2021). Ghosh et al., 2020 also followed similar chronology of steps and came up with three polyphenols, which can be used as potential inhibitors against SARS-CoV-2 Mpro (Ghosh et al., 2020). Yet another study used *in silico* structure based method to target approved and investigational drugs to the interface of nsp12 and nsp8 (Mutlu et al., 2020). This further validates that though experimental analysis and clinical trials are needed for a candidate drug but *in silico* approach can help ease the long-drawn procedure of drug discovery by providing us promising leads in a less resource dependent manner.

It is to be noted that while all the existing reports targeting nsp12 utilized experimental cryo-EM structure for docking studies, ours is the first demonstrating dynamics of the protein utilizing MD simulation and that too on a long-time scale of 100 ns, with such structures being subjected to docking of small molecules. We have additionally performed MM-GBSA for the top three drug molecules along with Remdesivir as a control. This has further increased the reliability of our *in silico* analysis, unlike others. Our MD simulation studies revealed that Penciclovir formed four new H-bonds with ASP623, ARG553, ARG555, and THR556 as compared to the pre-simulation docking results. This is a new finding previously not reported by Zhang et al. (Zhang et al., 2020) (Figure 7) and indicates potential specificity of Penciclovir for nsp12. Current study has also evaluated all possible non-covalent bonds including H-bonds, van der Waals forces, electrostatic interactions and hydrophobic interactions between the drugs and the amino acid residues of nsp12 in the vicinity. This will help future lead optimization, if any, for not only the leads we identified but also for Remdesivir to identify potent inhibitor of RdRp.

The key implications of the study are manifold. Prior to our study only Remdesivir had been experimentally shown to bind RdRp of SARS-CoV-2 proteome, while after uploading

the current work and its findings as a preprint (Dey et al., 2020), a cryo-EM structure (PDB Id: 7D4F; Yin, W et al., 2020) is solved showing RdRp bound to suramin thus validating our *in silico* findings. We expect similar findings with Anidulafungin and Penciclovir in the near future. Also, our study has not only included FDA approved compounds but also WHO suggested compounds, thereby increasing the scope for drug-repurposing. Drug repurposing will allow direct human trials and help avoid delay in conducting PK-PD studies first, since these drugs are already approved for human consumption. This can speed up the process of therapeutic development against SARS-CoV-2. This is the only study so far which could identify three small molecules (i.e. Suramin, Anidulafungin and Penciclovir) with similar binding energies to Remdesivir indicating that they can be equally potent inhibitors of RdRp. Moreover, the recently evolved SARS-CoV-2 strains that are 58 times more infectious in the UK (VOC 202012/01, a.k.a. B.1.1.7), South Africa (501Y.V2, a.k.a. B.1.351) and Nigeria (B.1.207), have mutations mainly in the S protein (including N501Y) thus rendering the S protein less amenable as drug target. Thus, our approach with nsp12 as target holds promise against COVID-19 UK, SA and Nigeria strains as well. The three leads identified in the current study can at least be used as alternate monotherapies or combination therapies along with Remdesivir to combat COVID-19.

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Disclosure statement

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