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Repurposing nonnucleoside antivirals against SARS-CoV2 NSP12 (RNA dependent RNA polymerase): *In silico*-molecular insight



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ABSTRACT

The pandemic of SARS-CoV-2 has necessitated expedited research efforts towards finding potential antiviral targets and drug development measures. While new drug discovery is time consuming, drug repurposing has been a promising area for elaborate virtual screening and identification of existing FDA approved drugs that could possibly be used for targeting against functions of various proteins of SARS-CoV-2 virus. RNA dependent RNA polymerase (RdRp) is an important enzyme for the virus that mediates replication of the viral RNA. Inhibition of RdRp could inhibit viral RNA replication and thus new virus particle production. Here, we screened non-nucleoside antivirals and found three out of them to be strongest in binding to RdRp out of which two retained binding even using molecular dynamic simulations. We propose these two drugs as potential RdRp inhibitors which need further in-depth testing.

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1. Introduction

SARS-CoV2 RNA dependent RNA polymerase (NSP12) is a 932 amino acids long protein which is divided into two main functional domains: nidovirus RdRp associated nucleotidyl transferase (NIRAN) domain and RNA dependent RNA polymerase (RdRp) domain. NIRAN domain is important in nucleotidyl transfer activity and helps in nucleotide transfer while RdRp domain is involved in the polymerisation activity (Fig. 1) [1]. The N terminal region of the protein contains a beta hairpin structure (28th –50th a.a.) followed by the NIRAN domain (4th –28th, 69th –249th a.a.). The beta hairpin is important in stabilizing the overall structure of the enzyme. The NIRAN domain and the RdRp domain is linked via an interface domain (250th –365th). The RdRp domain is further divided into Finger, Palm and Thumb subdomains. The Finger subdomain (366th –581th a.a long termed as region 1 and 621th –679th a.a. long termed as region 2 in the study) and the Thumb subdomain (816th –919th a.a.) form a closed conformation which is supported by Nsp7 and Nsp8 and one molecule of Nsp8 binds to the Finger subdomain to interact with the Interface domain. Overall, the two subdomains create a particular

conformation to help the Palm subdomain to function. The Palm subdomain (582nd –620th a.a. long termed as region 1 and 680th –815th a.a. long termed as region 2 in the study) possess different motifs (Motif A to E) each performing different functions and form the active site of the enzyme. Motif A (611th –626th) and Motif C (753rd –767th) form the main region of the active site that is important in RNA binding and polymerisation. Motif C possess catalytic residues (759th –767th a.a.) that is found to be conserved in other viral RdRps. The Finger subdomain possess two motifs (Motif F and Motif G) that works together and perform different functions. Motif F (538th –560th a.a.) and Motif G (500th –513rd a.a.) forms a groove through which RNA template enters the active site composed of Motif A and C. Motif F also forms an NTP entry channel for the enzyme. Motif E (810th –821th a.a.) and the thumb subdomain support primer strand during RNA synthesis. The function of Motif B (680th –710th a.a.) and Motif D (774th –796th a.a.) is yet to be elucidated.

The RdRp Complex (NSP12) is composed of NSP12, NSP8 and NSP7 proteins. The NSP7-NSP8 heterodimer stabilise the closed conformation of NSP12 and is important in mediating NSP12-RNA interactions.

RdRp play vital role in viral life cycle by acting as the key molecule for the viral RNA replication. Thus, it can be a potential drug target as inhibition of RdRp should inhibit viral RNA replication and thus new virus particle assembly as well. Discovery of new drugs and testing a newly discovered potential drug molecule in

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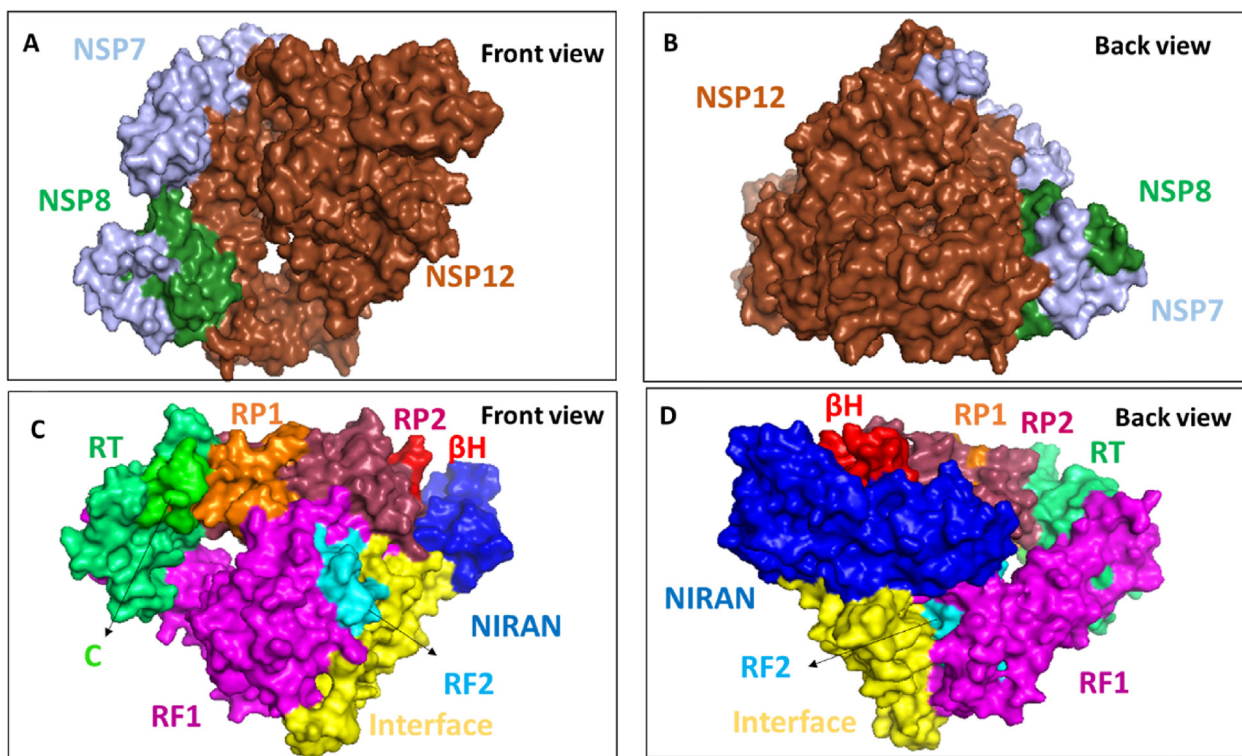


Fig. 1. Representation of the NSP12 and NSP12-NSP7-NSP8 complex. A and B. Represents the front and back surface view of the complex (PDB ID: 6m71). The different proteins are colour coded. C and D represent the different domains of the NSP12 with different colour codes. β H- Beta hairpin region(V31–K50), NIRAN- NIRAN domain (Y69-R249), Interface-Interface region (A250-R365), RF1- NSP12 Finger subdomain region 1 (L366-A581), RF2- NSP12 Finger subdomain region 2 (K621-G679), RP1-NSP12 Palm subdomain region 1 (T582-P620), RP2- NSP12 Palm subdomain region 2 (T680-Q815), RT- NSP12 Thumb subdomain (H816-E919), C- NSP12C terminal region (F920-Q932). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

human system for its stability and safety generally takes long time. Since a large number of approved drugs for human use already exists many of which have already established similar desired roles on other viral systems, screening and repurposing such drugs for activities on a new viral target could be a faster method for drug discovery. SARS-CoV-2 pandemic is a global health concern and there are no specific antiviral therapeutics available to manage the disease severities. In this study, we screened non-nucleoside antivirals against RdRp of SARS-CoV-2 using molecular docking studies followed by molecular dynamic simulations of the shortlisted candidates and have proposed two drugs that could be further explored for their antiviral effects on SARS-CoV-2.

2. Materials and methods

2.1. Molecular docking

The different FDA approved nonnucleoside antivirals were downloaded from the ZINC database in SDF format and was converted to pdbqt file using Autodock tool version 1.5.6. The crystal structure of SARS-CoV2 NSP12-NSP7-NSP8 complex was downloaded in PDB format from the Protein Data Bank (PDB ID: 6m71). Using the Pymol software the NSP12 (RNA dependent RNA polymerase) structure was extracted and coloured as per domains. Initially docking was done using the full protein to screen drugs based on the affinity cut off value of -9 kcal/mol. The positive control used in the study are known approved antivirals Remdesivir [2] monophosphate and Favipiravir ribose monophosphate [3]. The structures were isolated using the crystal structures available in PDB (PDB ID: 7bv2 and 4kn6, respectively). The binding affinity of the two drugs were found to be -6.9 kcal/mol and -7.8 kcal/mol

respectively. As a negative control Cinnamaldehyde and Thymoquinone structure was used due to its known lower affinity toward polymerases of other viruses (ZINC ID: ZINC000001532777 and ZINC000000164367 respectively). The affinity was found to be -4.7 and -5.4 kcal/mol respectively.

The ligand [4] and the protein was prepared in autodock tool [5] and saved in pdbqt format. Blind docking was performed by using Autodock vina tool and the docking result was analyzed using Pymol software and Discovery Studio tool [6]. The COACH tool was also used to identify the binding pockets of the protein [7]. Domain specific analysis was performed by extracting different domains of NSP12 by Pymol software [8] followed by docking with the ligand [9] in autodock tool.

2.2. Molecular dynamics simulations

The best two docked complexes, RdRp-Grazoprevir, RdRp-Ledipasvir along-with a control complex of RdRp-Galidesivir were subjected to molecular dynamics (MD) simulations to understand the conformational and structural changes during protein-ligand complex formation. Simulations also provide information regarding the strength of the interaction and also the binding partners took part in molecular bonding. MD simulations were performed using YASARA, version 15.10.18 [10,11], with the AMBER14 force field [12]. The protein-ligand complex was placed in a water box that is 10 \AA larger than each side of the protein. Hydrogen atoms were added to the protein structure at the appropriate ionizable groups according to the computed pKa in relation to the simulation pH, thus a hydrogen atom will be added if the computed pKa is higher than the pH. The pKa is computed for each residue according to the Ewald method [13,14]. The structure

was then minimized using steepest-descent method followed by simulated annealing. The simulation was performed at pH 7.0 in a 0.9% NaCl solution at 300 K temperature for 50 ns. A cut-off of 7.86 Å was used for van der Waals forces while Particle Mesh Ewald algorithm [15] were used for electrostatic forces. A multiple time step of 1.25 and 2.5 fs were used for intra-molecular and inter-molecular forces respectively. All calculations were carried out on an Intel Core i7 3.00 GHz with 48 GB of RAM.

3. Results and discussion

The drugs listed in Table S3 were screened based on their binding affinities towards the NSP12 protein. Keeping the cut off value of -9 kcal/mol, 3 drugs (Paritaprevir, Grazoprevir and Ledipasvir) were shortlisted which showed affinities above -9 kcal/mol. These drugs were further used to perform domain specific interaction study to identify the interacting domains and their corresponding residues.

Paritaprevir, an inhibitor of HCV NS3-4A serine protease showed a high affinity of -9.2 kcal/mol for NSP12 (RdRp) protein as a whole (Table S3). Also, as the protein associates with other proteins of SARS-CoV2 (NSP7 and NSP8) for its function, we tried to do docking with the whole complex (PDB ID: 6m71) and found it to show a high affinity of -9.5 kcal/mol (Fig. S1). Hence, the drug might interact with RdRp both in single as well as complex state which is important as the protein in its functional state associates with other proteins/co-factors. Next, we tried docking the drug with different domains and subdomains of the NSP12 to identify important interacting residues of the individual domains. Domains that showed an affinity of -9 kcal/mol and above were considered further to identify the interacting amino acids. The affinity of different domains have been listed in Table S4. Affinity of NIRAN (-8.6 kcal/mol) and Interface (-8.2 kcal/mol) domains were found to be lesser than that of RdRp domain (-10.5 kcal/mol) (Fig. S2). Considering the docking results of different subdomains of the RdRp domain, all the three subdomains (Finger, Palm and Thumb) showed a higher affinity, further supporting our initial analysis (Figs. S13–S15). Out of the three subdomains, the Thumb subdomain showed the highest affinity of -9.2 kcal/mol among others (Fig. S15). Also, the RdRp domain based docking showed most of the interacting residues to be of the Thumb subdomain. The interacting residues have been listed in Table S4. Docking analysis of RdRp domain and Thumb subdomain revealed two common residues Ile864 and Tyr867, each showing multiple bonds with the drug (Table S4). Ile864 bound with the drug via Pi Sigma, Pi Alkyl and conventional H bond and Tyr867 showed Pi Alkyl, conventional H bond and Pi Donor H bond. Multiple bond formation indicates the importance of the residue in binding with the drug and it strengthens the interaction. Moreover, H bond being the strongest of all non-covalent interactions, is one of the most important interactions in biology. These residues tend to show H bond interactions with drug which would strengthen the interaction further.

The RdRp Finger subdomain which showed an affinity of -8.8 kcal/mol were further analyzed using Region 1 (L366-A581) and region 2 (K621-G679). Region 1 showed a higher affinity of -10 kcal/mol (Fig. S3) as compared to the region 2 interaction (-6.9 kcal/mol). Also, the docking analysis result of RdRp Finger subdomain as a whole picked up Region 1 amino acids for the interaction. Hence, the drug might interact with the Finger subdomain region 1 along with the Thumb domain. The Palm subdomain analysis showed an affinity of -8.7 kcal/mol and study of two of its regions (Region 1 T582-P620 and Region 2 T680-Q815) revealed a stronger interaction of -11 kcal/mol with the region 2 than with region 1 (-7.5 kcal/mol) (Table S4). Even the Palm

subdomain docking showed interactions with the amino acid residues of Region 2 which further strengthens the observation (Fig. S14). However, no common residues were identified in the docking results of Finger Subdomain and its respective regions as well as Palm subdomain and its respective regions. But the docking result of Palm subdomain and its region 2 identified Met755 and Ala 762 respectively that bound with the drug using Pi Sigma and Alkyl/Pi Alkyl interactions respectively. Both the residues lie in the Motif C of the Palm subdomain that forms the active site of the enzyme where polymerisation takes place. Also, Palm subdomain region 2 based docking showed Glu811 interaction with the drug. The residue lies in the Motif E of the Palm subdomain and is important to support the primer strand during polymerisation (1).

Hence, the drug interacts with RdRp domain as a whole, showing high affinity for the Thumb subdomain, Palm subdomain region 2 and Finger subdomain region 1. The Finger and Thumb subdomains help the palm domain to function and it is very important to further validate a drug that can target all the three subdomains for an efficient inhibition.

Grazoprevir is another HCV NS3/4A protease inhibitor that has showed a reasonable affinity of -9.5 kcal/mol when interacting with the RdRp protein (NSP12) (Table S3). Also, the docking result of Nsp12-Nsp7-Nsp8 complex showed a high affinity of -8.3 kcal/mol hence the drug is predicted to interact with RdRp in its functional state inside the cell (Fig. 2). RdRp domain-based docking showed an affinity of -9.3 kcal/mol which is higher than the affinity of the other domains, -6.8 kcal/mol for NIRAN domain and -7.3 for the interface domain (Table S1).

The docking result of the different subdomains of RdRp showed varying affinities with thumb subdomain showing the highest affinity of -8.2 kcal/mol followed by Palm subdomain and Finger subdomain with an affinity of -7.7 kcal/mol and -7.3 kcal/mol respectively (Figs. S16–18). The analysis of Thumb subdomain revealed Pro868 to be a common residue identified even in the docking result of RdRp domain showing Alkyl/Pi Alkyl interaction (Table S1). Also, half of the interacting residues in the RdRp domain-based docking was found to lie in the Thumb subdomain. Analysis of the Finger subdomain region 1 and 2 showed an affinity of -8.9 kcal/mol and -6.6 kcal/mol respectively (Table S1 and Fig. S6). Hence, the drug is likely to interact with the Finger subdomain region 1 than region 2 as even the docking analysis of RdRp Finger subdomain revealed Region 1 based amino acid residues to interact with the drug (Fig. S16). Docking analysis of Palm subdomain region 1 and region 2 showed an affinity of -6.9 kcal/mol and -10.4 kcal/mol respectively indicating a much higher affinity for region 2 than 1 (Table S1 and Fig. S7). Region 2 docking result revealed two Motif C residues Ala762 and Val764 to interact with the drug via Alkyl/Pi Alkyl and Conventional H bonds (Table S1). Motif C is one of the motifs that form the active site of the enzyme. Amino acid residues of Region 2 of Palm subdomain were found to interact with the ligand when the whole Palm subdomain was used for docking, hence supporting the previous results (Fig. S17). A high affinity of Thumb domain as compared to the other two domains (Finger and Palm) may likely arise because of its small size as compared to the other two domains. But when the individual regions of the Finger and Palm subdomain were studied, it showed a higher binding affinity than the Thumb domain. Hence, it is possible that the drug interacts with the different domains of the protein with different affinities. Also, domain specific docking helps to identify the interacting amino acids more specifically than considering the whole protein. However, the affinity of the drug towards the protein is best determined when the whole protein is considered for docking as that is the natural state of the protein which is accessible to the drug.

Overall, this drug shows a strong affinity for the RdRp complex

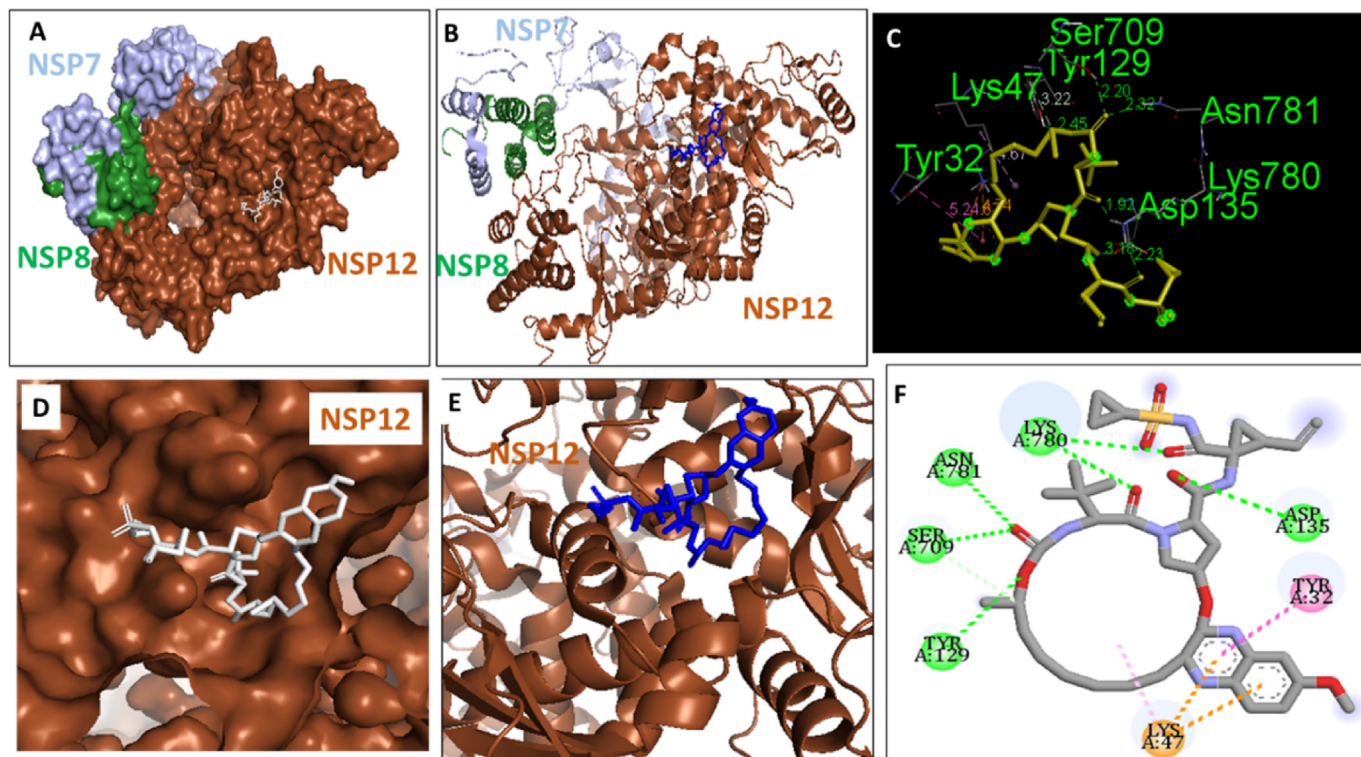


Fig. 2. Representation of the docking result of Grazoprevir with NSP12 complex: A,D. Surface view of the ligand in its best docked position (full and zoomed). B,E. Cartoon view of the ligand in its best docked position (full and zoomed).C. The interaction between the ligand (highlighted in yellow) with the interacting amino acids of the domain. F. The 2D image of the interactions formed between the ligand and the protein. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

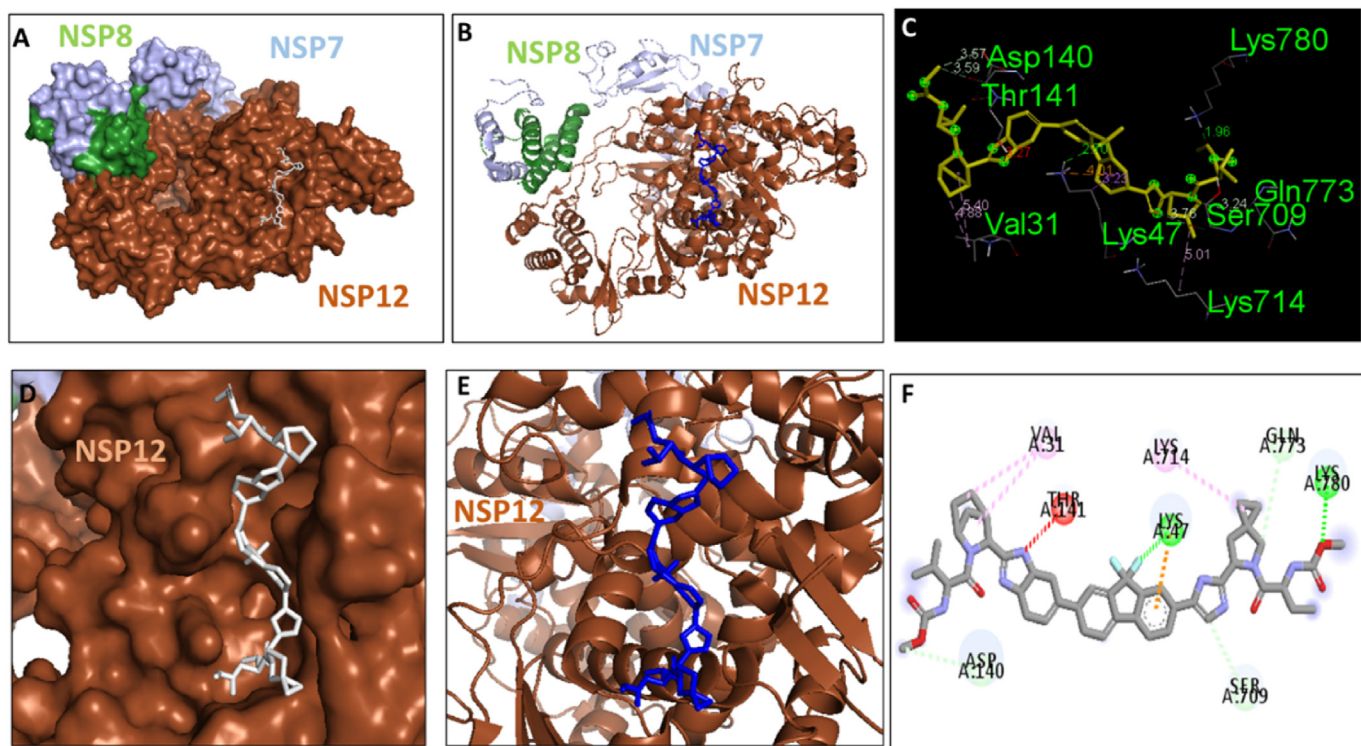


Fig. 3. Representation of the docking result of Ledipasvir with NSP12 complex: A,D. Surface view of the ligand in its best docked position (full and zoomed). B,E. Cartoon view of the ligand in its best docked position (full and zoomed).C. The interaction between the ligand (highlighted in yellow) with the interacting amino acids of the domain. F. The 2D image of the interactions formed between the ligand and the protein. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(NSP12-NSP7-NSP8), RdRp domain as a whole and also for its different domains. Most importantly, it has a high affinity for Palm subdomain region 2 and targets the active site of the enzyme along with its thumb and finger subdomain. Paritaprevir and Grazoprevir share similar structure and hence the docking results were also similar, showing a high affinity for Thumb subdomain, Finger subdomain region 1 and Palm subdomain region 2. It is important to identify drugs that have multiple targets to bind in the protein as it increases its efficiency for binding.

Another drug identified in this study is Ledipasvir which is an HCV NS5A inhibitor. The drug shows a high affinity for NSP12 protein (-9.1 kcal/mol) (Table S3). It also shows a high affinity towards NSP12-NSP7-NSP8 complex, hence the binding sites of the drug is accessible in the complex state of the protein as well (Table S2 and Fig. 3). The analysis of domain specific docking results stated a high affinity of the drug for NIRAN and RdRp domain (-8.4 kcal/mol and -9.1 kcal/mol respectively) (Figs. S8 and S19). Considering the cut off value used for the study (-9 kcal/mol), the RdRp domain was used for further study and subdomain specific docking analysis revealed a higher affinity for Finger and Palm subdomain than with Thumb subdomain ($-8.6, -8.6, -8.2$ kcal/mol

respectively) (Figs. S20–22). Further analysis of the Finger subdomain regions 1 and 2 indicate a higher affinity of Region 1 than region 2 for the ligand (Table S2). The docking results of RdRp domain and Finger subdomain (Figs. S8 and S20) identified most of the interacting residues to be of Finger subdomain Region 1 (Fig. S9) and Leu401 was a common residue identified in the docking results of Finger subdomain Region 1 and Finger subdomain as a whole (Table S2). Hence, the Finger subdomain region 1 could potentially be the specific binding site for the drug when docking of the ligand is performed with RdRp domain and Finger subdomain as a whole. Study of the Palm subdomain identified most of the bonding amino acid residues to belong to the region 2 of the subdomain. Region specific docking revealed Region 2 to show a higher affinity of -9.5 kcal/mol than region 1 and Asp760 is one of the residues detected in case of Palm subdomain region 2 docking (Table S2 and Fig. S10). Asp760 is the catalytic residue that lies in Motif C of the Palm subdomain and is an active site of the Polymerase.

Hence, the drug shows high affinity towards RdRp domain of the enzyme and has a mixed affinity towards different subdomains and regions. Finger subdomain region 1 and Palm subdomain region 2 is

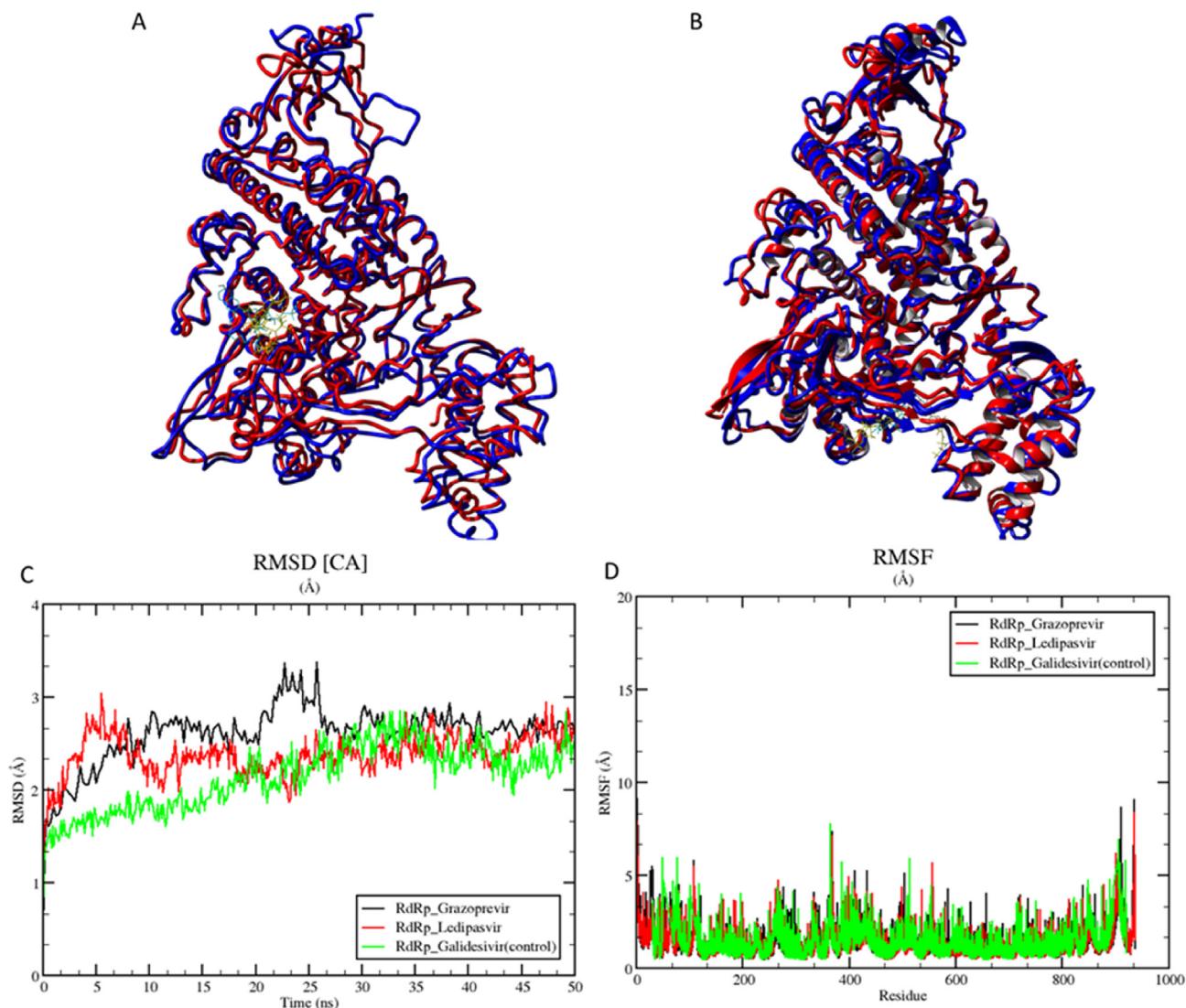


Fig. 4. A-B Conformations of the protein-ligand complexes before and after the MD simulation. A. RdRp-Grazoprevir B. RdRp-Ledipasvir. C-D RMSD and RMSF graphs for RdRp-Grazoprevir, RdRp-Ledipasvir and RdRp-Galidesivir.

likely an important region for the ligand binding.

To predict the binding pockets of NSP12 COACH Tool was used and it detected the binding pockets of the protein to be composed of mostly residues 535–824 which lies in the RdRp domain. The results showed its first four hits with a Confidence score of prediction 0.05–0.09 (higher score indicates a more reliable prediction). The 1st binding pocket encompass the region surrounded by RdRp Finger subdomain and Palm subdomain region 2 with a C score of 0.09, the second detected binding pocket was formed of Palm subdomain region 2 and Thumb subdomain with a C score of 0.07. The third and the fourth binding pocket, composed of RdRp finger subdomain 1 and palm subdomain 2 showed a C score of 0.05. The docking analysis of our study identifies the binding pockets that are predicted by the tool, hence further confirming the reliability of the study. Further studies involving molecular dynamics and *in vitro/ex vivo* studies will further validate the efficacy of the predicted drugs.

3.1. Molecular dynamics simulation

Two protein-ligand complexes obtained from docking were subjected to molecular dynamics simulation studies using mdrun macro of YASARA suite for 50ns time frame. Due to technical difficulties, we could not perform simulation studies with Paritaprevir, Fig. 4A and B demonstrates the difference between the initial and final conformations of the protein-ligand complexes before and after the MD simulation runs of 50ns each. Initial and final poses were aligned and superimposed to understand the dynamicity of the complex. RdRp-Grazoprevir has an RMSD of 1.524 Å over 863 aligned residues with 100.00% sequence identity. RdRp-Ledipasvir has an RMSD of 1.562 Å over 863 aligned residues with 100.00% sequence identity.

In RMSF graph, area near residue number ~380 and ~900 shows higher fluctuations (Fig. 4). These fluctuations may address an important event (in the form of interaction) which takes place. Different energy contributors like Bond, Angle, Dihedral, Planarity, Coulomb, VdW to make up the total potential energy (Fig. S23) seems to be quite equilibrated and normal during the whole 50ns course of simulation run. Only certain small change in energy can be recorded in RdRp-Grazoprevir complex near 7 and 41 ns time frames. This change corresponds to the intermediary states while attaining stable conformation of the complex.

Apart from these graphs for solvent accessible surface area (SASA) (Fig. S24A), radius of gyration (Rg) (Fig. S24B), number of H-bonds in solute and solute-solvent states (Fig. S25) and secondary structure content (Fig. S26) were also recorded.

Ligand conformation (Fig. S27A) and ligand movement (Fig. S27B) showed that ligands are displacing from their initial conformation and increasing the time-frame may give more insight. Ligand binding energy (Fig. S28) also demonstrate a decrease in the binding energy of the ligands with the protein molecule. Around 18ns Ledipasvir binds to RdRp with an energy of > -400 kJ/mol but again decreased to -100 kJ/mol.

Overall RMSD for C-alpha atom and RMSF per residue based in all five complexes are shown in Fig. 4. RdRp-Grazoprevir complex shows non-equilibrated increasing RMSD till 30ns and seems to get stabilized till the end of simulation run of 50ns. Although stabilization has been observed, equilibration can be attained with an extended simulation run. Similar patterns were observed in case of RdRp-Ledipasvir and RdRp-Galidesivir.

Taken together, this study indicates that the Ledispavir and Grazoprevir showed interaction with RdRp. Longer simulation studies combined with binding assays would help in further screening of these drugs and determining possibility of using these for repurposing against SARS-CoV-2 RdRp. Since, Ledispavir and

Grazoprevir showed binding with RdRp, the same drugs could also be used as templates for QSAR based drug designing or pharmacophore modelling.

Declaration of competing interest

Authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbrc.2021.07.050>.

Authors' contributions

FB performed and analyzed experiments and wrote the manuscript. AKS helped in proof reading the manuscript. For MD simulation FB was guided and helped extensively by Dr. Sanjit Kumar, Assistant Professor, Center for Bioseparation Technology, Vellore Institute of Technology (VIT) University, Vellore, Tamil Nadu, India. UR conceptualized the work, analyzed the data and wrote the manuscript.

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References

- [1] Y. Gao, et al., Structure of the RNA-dependent RNA polymerase from COVID-19 virus, *Science* 368 (2020) 779–782.
- [2] W. Yin, et al., Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir, *Science* 368 (2020) 1499–1504.
- [3] L. Naesens, et al., Role of human hypoxanthine Guanine phosphoribosyltransferase in activation of the antiviral agent T-705 (favipiravir), *Mol. Pharmacol.* 84 (2013) 615–629.
- [4] E. Clercq, et al., Approved antiviral drugs over the past 50 years, *Clin. Microbiol. Rev.* 29 (2016) 695–747.
- [5] O. Trott, et al., AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, *J. Comput. Chem.* 31 (2010) 455–461, <https://doi.org/10.1002/jcc.21334>.
- [6] Dassault Systèmes BIOVIA, Discovery Studio Modelling Environment, Dassault Systèmes, San Diego, 2016. Release 2017.
- [7] J. Yang, et al., Protein-ligand binding site recognition using complementary binding specific substructure comparison and sequence profile alignment, *Bioinformatics* 29 (2013) 2588–2595.
- [8] W.L. DeLano, PyMOL, DeLano Scientific, San Carlos, 2002.
- [9] T. Sterling, et al., Zinc 15 – ligand discovery for everyone, *J. Chem. Inf. Model.* 55 (11) (2015) 2324–2337.
- [10] E. Krieger, G. Vriend, New ways to boost molecular dynamics simulations, *J. Comput. Chem.* 36 (13) (2015 May 15) 996–1007.
- [11] E. Krieger, K. Joo, J. Lee, J. Lee, S. Raman, J. Thompson, et al., Improving physical realism, stereochemistry, and side-chain accuracy in homology modeling: four approaches that performed well in CASP8, *Proteins* 77 (Suppl 9) (2009) 114–122.
- [12] Cornell WD, Cieplak P, Bayly CI, Gould IR. A second generation force field for the simulation of proteins, Nucleic Acids, and Organic Molecules. ACS Publications.
- [13] Krieger E, Nielsen JE, Spronk C. Fast Empirical pKa Prediction by Ewald Summation. Elsevier.
- [14] Cheatham T, Miller JL, Fox T. Molecular dynamics simulations on solvated biomolecular systems: the particle mesh Ewald method leads to stable trajectories of DNA, RNA, and Proteins. ACS Publications.
- [15] U Essmann, L Perera, ML Berkowitz, A smooth particle mesh Ewald method, *J. Chem. Phys.* 103 (1995) 8577.