Identification of drugs targeting multiple viral and human proteins using computational analysis for repurposing against COVID-19

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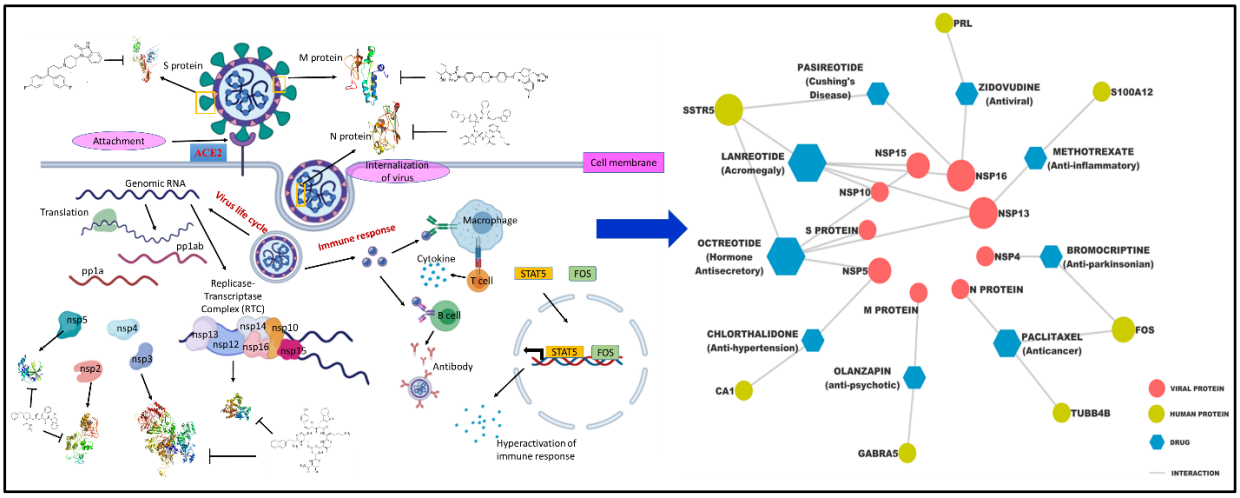
Abstract:

The SARS-CoV2 is a highly contagious pathogen that causes a respiratory disease named COVID-19. The COVID-19 was declared a pandemic by the WHO on 11th March 2020. It has affected about 5.38 million people globally (identified cases as on 24th May 2020), with an average lethality of ~3%. Unfortunately, there is no standard cure for the disease, although some drugs are under clinical trial. Thus, there is an urgent need of drugs for the treatment of COVID-19. The molecularly targeted therapies have proven their utility in various diseases such as HIV, SARS, and HCV. Therefore, a lot of efforts are being directed towards the identification of molecules that can be helpful in the management of COVID-19.

In the current studies, we have used state of the art bioinformatics techniques to screen the FDA approved drugs against thirteen SARS-CoV2 proteins in order to identify drugs for quick repurposing. The strategy was to identify potential drugs that can target multiple viral proteins simultaneously. Our strategy originates from the fact that individual viral proteins play specific role in multiple aspects of viral lifecycle such as attachment, entry, replication, morphogenesis and egress and targeting them simultaneously will have better inhibitory effect.

Additionally, we analyzed if the identified molecules can also affect the host proteins whose expression is differentially modulated during SARS-CoV2 infection. The differentially

expressed genes (DEGs) were identified using analysis of NCBI-GEO data (GEO-ID: GSE-147507). A pathway and protein-protein interaction network analysis of the identified DEGs led to the identification of network hubs that may play important roles in SARS-CoV2 infection. Therefore, targeting such genes may also be a beneficial strategy to curb disease manifestation. We have identified 29 molecules that can bind to various SARS-CoV2 and human host proteins. We hope that this study will help researchers in the identification and repurposing of multipotent drugs, simultaneously targeting the several viral and host proteins, for the treatment of COVID-19.



Keywords: SARS-CoV2, COVID-19, Drug repurposing, Network analysis, Docking.

Introduction:

Novel zoonotic viruses with potential for rapid spread and significant pathology pose a grave threat to humans. During the last few decades many epidemics of viral diseases have occurred such as Ebola, Zika, Nipah, Avian influenza (H7N9), HIN1, Severe Acute Respiratory Syndrome Coronavirus 1 (SARS-CoV1), and Middle East Respiratory Syndrome Coronavirus (MERS-CoV)(1) (2).

In the end of 2019, mysterious pneumonia cases begin to emerge in China's Wuhan city. A novel coronavirus, which was later renamed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) was found to be the causative organism and the disease was termed "coronavirus diseases-19" (COVID-19) (3). COVID-19 is the world's worst pandemic and has so far affected about 5 million people globally (identified cases as on 19th May 2020), with average lethality of ~3%. Infection with SARS-CoV2 results in acute respiratory distress syndrome (ARDS) leading to lung injury, respiratory distress and lethality. Elderly patients and those with comorbidities have been reported to be at risk of higher mortality.

The SARS-CoV2, SARS-CoV1 and MERS-CoV belongs to the family of Coronaviridae and β-coronavirus genus (4). While bats are considered to be the origin of SARS-CoV1 and SARS-CoV2, the intermediate host that led to human transmission of SARS-CoV2 is still unknown. Sequence analysis reveals that SARS-CoV2 is similar to coronavirus identified in Malayan pangolins (Manis javanica) (5). The SARS-CoV2 genome is 29.8 - 29.9kb positive-sense single stranded RNA with 5′-cap and 3′-poly-A tail. Its genome is organised into two segments that encode non-structural (Nsp) and structural proteins. The first segment is directly translated by ribosomal frameshifting into polyprotein 1a (486 kDa) or 1ab (790 kDa) (ORF1a, ORF1ab) which results in generation of non-structural proteins and formation of replication-transcription complex (RTC) (1, 6). Discontinuous transcription of the viral genome results in formation of subgenomic RNAs (sgRNAs) containing common 5′- and 3′- leader and terminal sequences which serve as the template for subgenomic mRNA production (6). The ORF1a/1ab covers the two-thirds of the whole genomic length and encodes for the 16 non-structural proteins (Nsp1-16), which play critical role in various viral processes. The second segment at the 3′-terminus of the genome encodes the four main structural proteins: spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins (6). The life cycle of SARS-CoV2 starts with its entry into the host cell through receptor mediated endocytosis initiated by the binding of its Spike protein to the ACE2 receptor. Subsequently, uncoating of the virus particle releases the genome, which

is translated to generate replication-transcription complex proteins. The viral RTC complex then generates full length negative sense RNA which is subsequently transcribed into full length genome. The viral genome and structural proteins are assembled into virions near the ER and Golgi interface and are transported out of the cell through vesicles by the process of exocytosis (7).

The detailed understanding of the clinical manifestations and the underlying molecular mechanisms that drive disease pathogenesis are still unclear. There is no standard cure for the disease and currently the therapeutic regimen involves symptomatic treatment and previously approved drugs against other viral infections and diseases. Worldwide efforts to develop vaccines and drug against SARS-CoV2 are ongoing. Based on the similarity and information available from other coronaviruses, repurposing of approved drugs is among the best and rapid strategies to identify potential drug candidates (8). In this context, the computational techniques can quickly identify novel molecules that target viral proteins to suggest candidates for repurposing. Hence, during the COVID pandemic a lot of studies have been reported using a variety of such strategies (9).

The *in-silico* studies have identified many drugs that can target viral proteins viz. RNA-dependent RNA polymerase (RdRp), Spike, Membrane, $3CL^{pro}$ and human proteins such as angiotensin converting enzyme 2 (ACE2) which serves as receptor for SARS-CoV2. Among them zanamivir, indinavir, saquinavir, lopinavir, and remdesivir are notable (10, 11). There are many drugs such as baricitinib (12), lopinavir(10), ritonavir (13), remdesivir (14, 15), hydroxychloroquine (16, 17), arbidol (18) etc., that are currently under trial to treat SARS-CoV-2 infection.

However, only a few studies have reported targeting more than one viral protein with a single molecule or using combination therapy. In this study we attempted to identify molecules that can simultaneously bind to multiple proteins of the SARS-CoV2. The strategy to target multiple proteins originates from the fact that individual viral proteins play specific role in multiple aspects of viral lifecycle such as attachment, entry, replication, morphogenesis and egress. Single molecules that can potentially target many viral proteins can perturb viral lifecycle at multiple points and thereby can be highly efficient in curbing SARS-CoV2 infection. In addition, the molecules that simultaneously target multiple viral proteins will have a higher barrier towards emergence of resistant mutants.

In this work, we have used the 3D-structures of the SARS-CoV2 proteins to identify FDA approved drugs that can bind to these proteins using bioinformatics methods. The FDA approved drugs were chosen so that they can be quickly repurposed for treating COVID19.

Additionally, we also analyzed if the identified molecules can affect the host proteins that get differentially expressed as a result of SARS-CoV2 infection. These molecules can be used as modulators of both the SARS-CoV2 and human proteins.

Methods:

Protein structure modelling:

The SARS-CoV2 proteins for which there is no crystal structure reported were modelled using Modeller v9.22(19) (homology modeling) or obtained from I-TASSER server (threading) (20). The modelling template for each protein was identified by performing Delta-BLAST against the PDB database. Proteins were modelled using either single or multiple templates based on the query coverage. The final homology modeling was performed using the modeller9.22. Further, the model stereochemistry and other structural parameters were assessed using standalone PROCHECK tool. The proteins for which suitable templates were not found were obtained from I-TASSER server (https://zhanglab.ccmb.med.umich.edu/I-TASSER/).

Molecular docking of FDA approved drugs in SARS-CoV2 proteins:

The structures of the FDA approved drugs were obtained from the DrugBank (https://www.drugbank.ca/) repository. Ligands were prepared by Schrödinger LigPrep wizard ligands using the default parameters.

The active site of the modelled proteins were identified using either of the two methods 1) the ligand binding pocket, if the co-crystal structures are available or 2) the active site was predicted using sitemap algorithm in Schrodinger v9.3 molecular modelling software (21). The proteins with active site pocket volume of <150 $Å^3$ were removed as smaller pockets may not be amenable to docking. Finally, 13 proteins were selected for docking. The molecular docking was performed using the Glide module of Schrodinger molecular modelling software (www.schrodinger.com/glide). The molecules showing a docking score of -8.5 (roughly corresponding to 1 µm)(22) or better were selected for further analysis.

The differential gene expression (DEGs) analysis:

The differentially expressed genes were identified by analysing the data from NCBI GEO (GEO ID: GSE-147507) that contains data on cell lines infected with various virus including SARS-CoV-2. The above dataset also included an RNA sequencing study done using lung tissue of two normal and one COVID-19 patients. The DEGs were identified using limma-voom with the criteria of $|\log_2 FC \geq 1|$ and p-value$\leq$0.01. The DEGs were further studied for their involvement in various pathways, processes and diseases using Ingenuity Pathway Analysis (IPA).

Protein-protein interaction network analysis:

The identified DEGs were mapped for their interactions with other human proteins using HIPPIE v2.2 which contains 14855 proteins and 411430 interactions. The reported protein-protein interactions with a minimum score of 0.63 (medium confidence, 2nd quartile)(23) were used for creation of the network using Cytoscape v3.7.2. The largest interconnected component was extracted and connectivity of individual nodes (degree) were calculated to assess their importance. The calculations were performed on the high performance Linux cluster. The flowchart of the methodology is presented in Fig. 1.

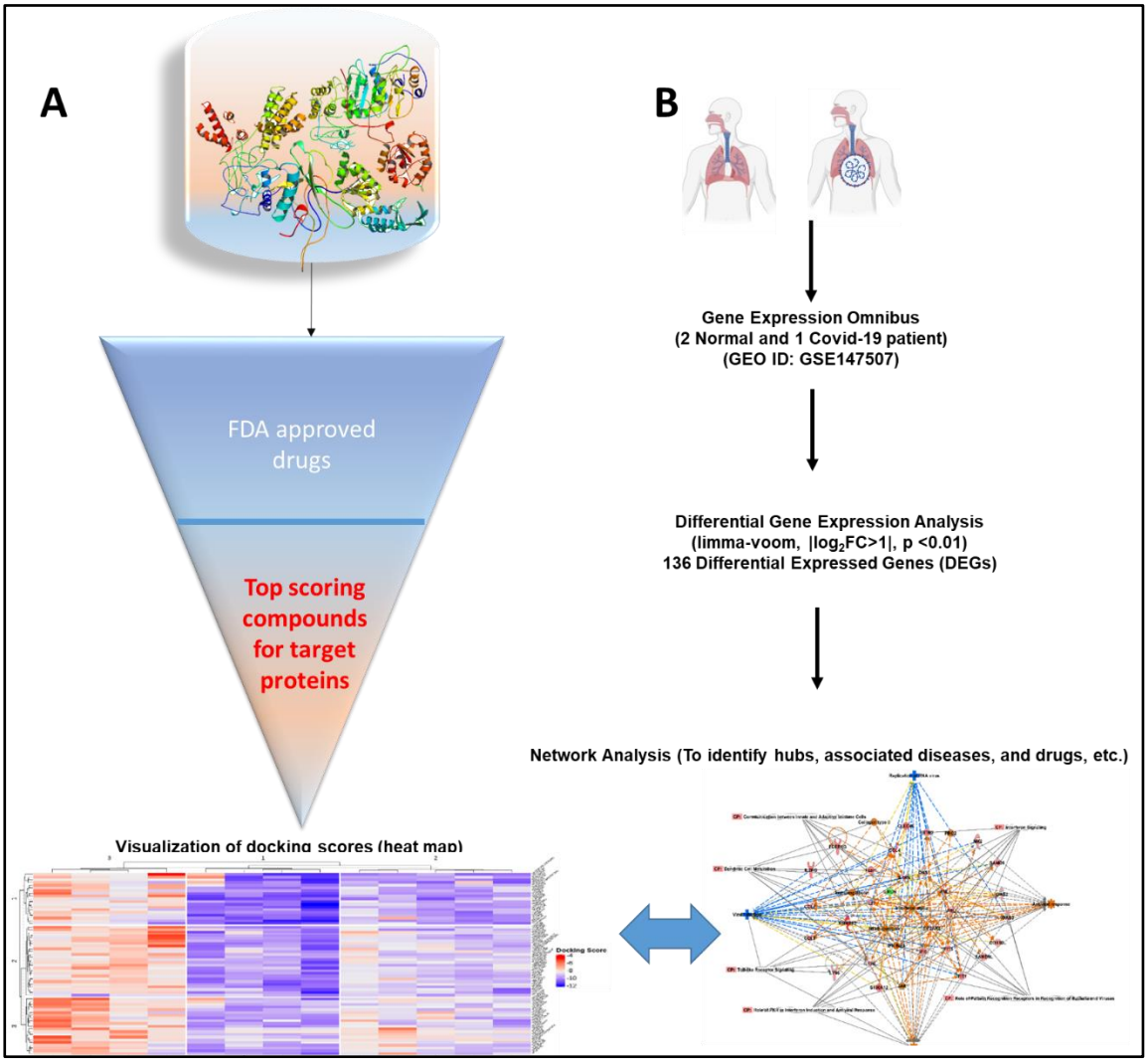


Fig. 1: The flowchart of the screening methodology. (A) The protein structures were either obtained or computationally modelled. The FDA approved drugs were then docked into the protein structures. The drugs showing high affinity (docking score <-8.5) were selected. (B) The differentially expressed genes were identified using analysis of GEO data. Analysis was done to identify important host proteins, enriched pathways, and processes, etc. The DEGs were searched against databases to identify their modulators.

Results and discussion:

As stated earlier a total of 13 viral proteins (Table 1) were selected for molecular docking.

Table 1: The details of the proteins selected for screening.

S. No.	Protein Name	Length*	RefSeq ID	Source[$]
1	Surface glycoprotein	1273	YP_009724390.1	Homology modelled
2	Membrane glycoprotein	222	YP_009724393.1	I-TASSER
3	Nucleocapsid phosphoprotein	419	YP_009724397.2	PDB:6WJI
4	Nsp2	638	YP_009725298.1	I-TASSER
5	PL^{Pro} (Nsp3)	1945	YP_009724389.1	PDB:6W02
6	Nsp4	500	YP_009725300.1	Homology modelled
7	Main protease ($3CL^{Pro}$, Nsp5)	306	YP_009725301.1	PDB:6W63
8	Nsp10	139	YP_009725306.1	PDB:6W61
9	RNA-dependent RNA polymerase (Nsp12)	932	YP_009725307.1	PDB:7BV2
10	Helicase (Nsp13)	601	YP_009725308.1	Homology modelled
11	3'-to-5' exonuclease (Nsp14)	527	YP_009725309.1	Homology modelled
12	EndoRNAse (Nsp15)	346	YP_009725310.1	PDB:6VWW
13	2'-O-ribose methyltransferase (Nsp16)	298	YP_009725311.1	PDB:6W4H

*Number of amino acids. [$]PDB/homology modelling/I-TASSER

The computational analysis of ligands binding to various proteins is a powerful method to quickly identify potential molecules for further analysis. These methods have been successfully used in various studies (24). In the first stage, the molecules were docked into the SARS-CoV2 proteins using Glide module of Schrodinger (www.schrodinger.com/glide) in standard precision (SP) mode. The redocking was done to ensure the appropriate selection of top hits. The molecules were then ranked using Glide score as implied in Schrodinger.

We adapted the following notion for our drug repurposing analysis: **1**. drugs that can inhibit viral entry into host cell by perturbing the function of surface glycoproteins like the spike, membrane and envelope protein. **2.** blocking the functions of viral enzymes that plays a vital role in replication such as 2'-O-ribose methyl transferase, RNA-dependent RNA polymerase, endoRNAse, helicase, 3'-to-5' exonuclease, 3C-like main protease and papain-like protease. **3.** Preventing the function of other non-structural proteins that play accessory role in viral

processes such as Nsp2, Nsp4 and Nsp10. **4.** drugs that can also affect differentially expressed host proteins in COVID-19 along with the viral proteins.

Molecules docking to SARS-CoV2 Structural proteins:

The hallmark feature of coronaviruses is their transmembrane **spike (S) glycoprotein** as this protein is reason for its name “Corona” in Latin meaning, "Crown". The spike protein exists as homo-trimers. Each monomer is about 180kDa and has two distinct subunits S1 and S2. While the receptor binding is mediated by S1 subunit with the help of receptor binding domain (RBD), the fusion between the viral envelope and the host cellular membranes is facilitated by the S2 subunits upon the cleavage of S1-S2 junction by host proteases (25)**.** The S1 subunit of spike protein in SARS-CoV2 has four distinct domains: NTD, CTD1, CTD2 and CTD3, of these the “up” conformation of CTD1 is responsible for binding with ACE2 receptor (26)**.** The sitemap revealed a site that is very close to the receptor binding domain and trimerization interface lined by the residues Ser 46, Leu 48, Leu 303, Lys 304, Ser 305, Glu 309, Thr 732, Thr 734, Asn 758, Thr 827, Leu 828, Phe 833, Gln 836, Tyr 837, Arg 847, Lys 854, Asn 856, Val 860, Gln 949, Val 952, Asn 953, Asn 955, Gln 957, Leu 959, Asn 960, Val 963, and His 1058. Our molecular docking analysis suggest that posaconazole, mefloquine, nebivolol, cangrelor, octreotide, and lapatinib bind to spike protein with appreciable affinity (Fig. 2). Other groups have also predicted the binding of posaconazole to spike protein which further substantiates our analysis (27). Posaconazole is an antifungal agent used in the prevention of invasive fungal infections and is also shown to inhibit the entry of Chikungunya virus (28) and replication of Zika and Dengue viruses by binding to oxysterol-binding protein (sterol transporter) (29). Octreotide is a long-acting somatostatin analogue used for treatment of gastrointestinal tract bleeding, hepatocellular carcinoma and hemorrhage associated with Cytomegalovirus induced colitis (30) (31). Mefloquine is an antimalarial drug used in chloroquine resistant malaria. Nebivolol is an antihypertensive molecule with a very good safety profile in subjects with obstructive respiratory comorbidities (32) and can be an important drug to consider in SARS like diseases. The docking score of -8.5 indicates that nebivolol binds to spike protein with good affinity (Fig. 2).

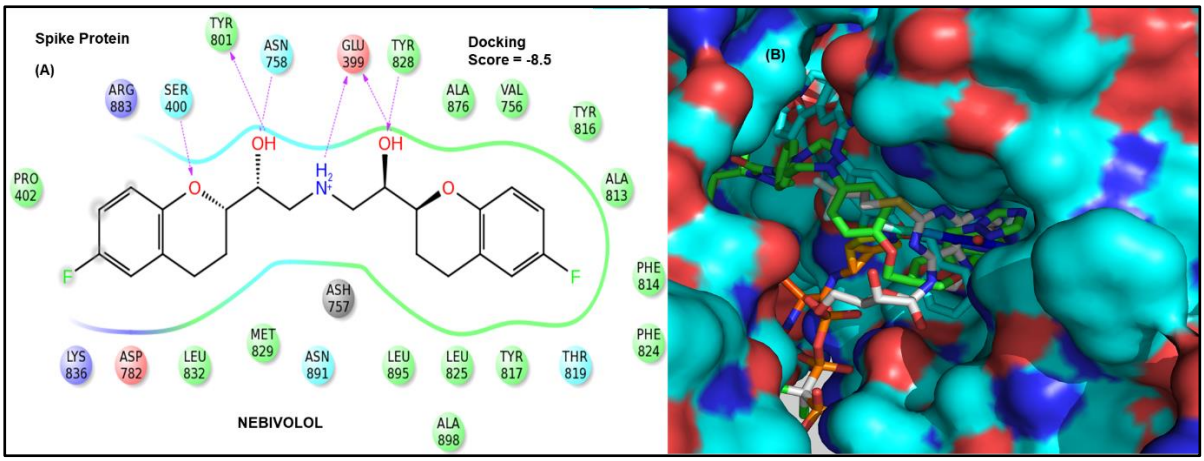


Fig. 2: (A) Binding of nebivolol (docking score -8.5) at the spike protein of SARS-COV2. The molecule is shown in black lines while the receptor residues are shown in coloured circles. The magenta lines indicate the hydrogen bonding interactions between the ligand and the receptor residues. (B) The binding cavity along with the top ligands. The receptor surface is shown in cyan, red and blue colour while the ligands are shown in coloured sticks.

The Membrane (M) protein of coronavirus is a central protein that interacts with other major structural proteins and viral RNA. It is a 22-25kDa transmembrane glycoprotein that contains glycosylated N-terminal domain, three transmembrane domains and C-terminal domain with short hydrophobic tail. It facilitates virion formation and shaping of viral envelope. It stabilizes the nucleocapsid and is involved in viral assembly near the ER-Golgi intermediate compartment (ERGIC) through its interaction with spike protein (33), (4). Antibodies targeting M protein are capable of inducing antibody-dependent complement mediated neutralization (34). Important residues lining the binding site are Ala 21, Asp 22, Ile 23, Ala 38, Ala 39, Asn 40, Lys 44, His 45, Gly 48, Val 49, Ala 50, Asn99, Lys 102, Pro 125, Leu 127, Ser 128, Ala 129, Gly 130, Ile 131, Phe 132, Ala 154, Val 155, Phe 156, Asp 157. Only cangrelor was found to bind to this protein with good affinity (docking score -8.13) (Fig. 3). The sugar and the phosphate group of the molecule have HB interactions with Val 70, Tyr 71, Arg 72, and Gln 185. The long hydrophobic side chain sits in a hydrophobic cavity lined by Leu 67, Trp 75 and Val 143. The trifluoro group is docked in the vicinity of Val 187, Phe 193. Cangrelor is an analog of adenosine triphosphate (ATP) and inhibits platelet adenosine diphosphate receptor (P2Y12) and used in the treatment of percutaneous coronary intervention (35).

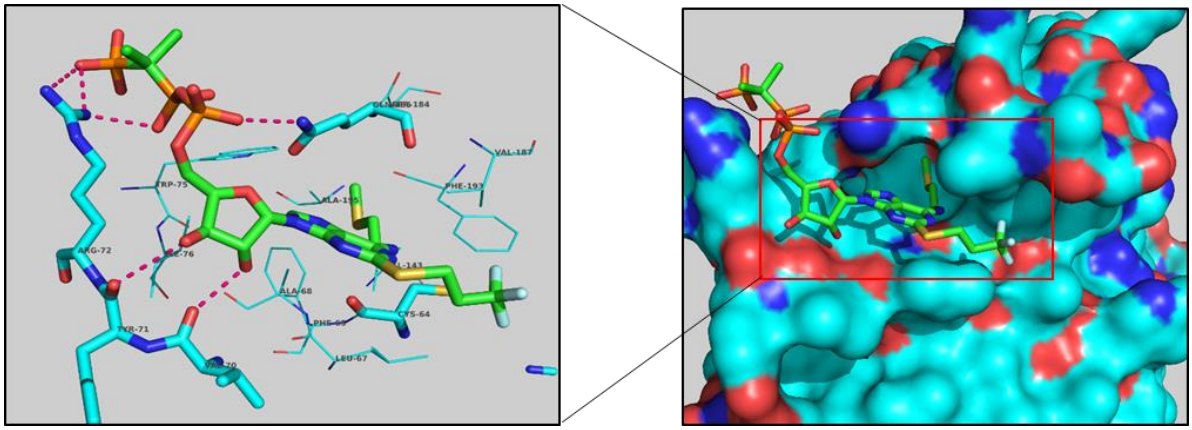


Fig. 3: The docking of Cangrelor (docking score -8.53) in membrane protein of SARS-CoV2. The molecule is shown in black lines while the receptor residues are shown in coloured circles. The magenta lines indicate the hydrogen bonding interactions between the ligand and the receptor residues.

The nucleocapsid (N) protein is crucial for the viral RNA packaging. It is made up of two distinct RNA-binding domains (the N-terminal and the C-terminal domain) linked by serine/arginine-rich (SR-rich) domain (SRD)(36). Previous studies with SARS-CoV1 suggest that N protein inhibits TGF-beta, AP-1, NF-kB signaling and type 1 interferon production but induces apoptosis. The sera of COVID-19 patients shows the presence of IgG, IgA, and IgM antibodies against N protein suggesting its role in eliciting humoral immune response (37, 38). Our study predicts that ribavirin, vasopressin, octreotide, and capreomycin bind to N protein (Fig. 4). Of these, capreomycin, a polypeptide (isolated from Streptomyces capreolus) is used in the treatment of multidrug resistant tuberculosis.

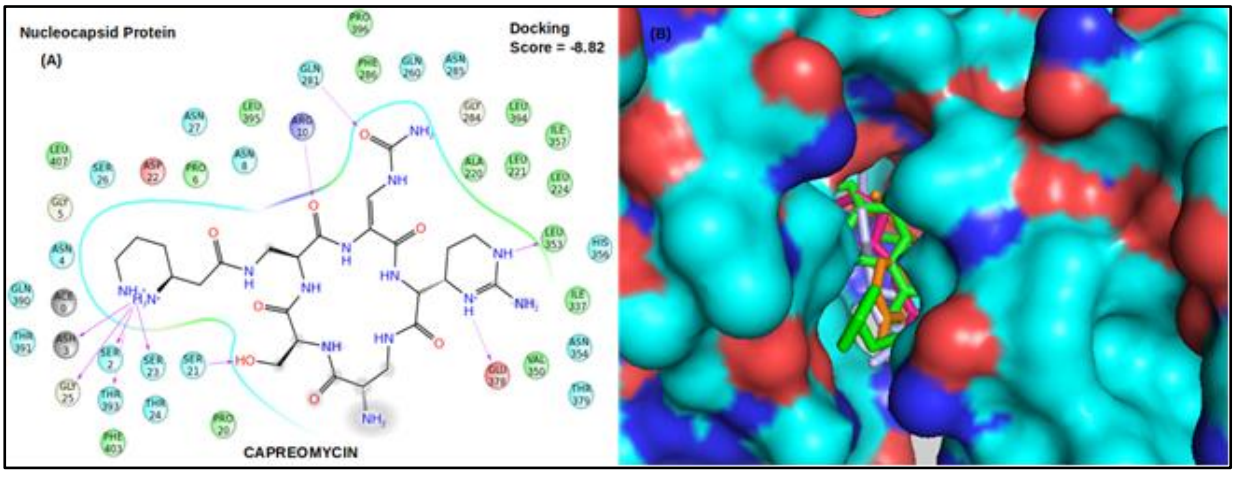


Fig. 4: (A) Binding of capreomycin at the nucleocapsid protein. The molecule is shown in black lines while the receptor residues are shown in coloured circles. The magenta lines indicate the hydrogen bonding interactions between the ligand and the receptor residues. (B) The binding cavity along with the top ligands. The receptor surface is shown in cyan, red and blue colour while the ligands are shown in coloured sticks.

Its mechanism is similar to aminoglycosides and used in the inhalation therapy of pulmonary tuberculosis by spray-drying technology (39, 40). It can be a promising prophylactic agent against SARS-CoV2 using similar application strategy. Capreomycin makes HB interactions with Ser 21, Ser 23, Gln 25, Gln 281, Asn 285, Leu 353, Glu 378, Thr 393.

Molecules docking to SARS-CoV2 enzymes:

2′-O-Methyl Transferase (Nsp16) of SARS-CoV2 belongs to the S-adenosylmethionine-dependent methyl transferase family and is activated upon binding to Nsp10. Capping of viral mRNA at 5′-end is one of the viral strategy for protecting viral transcripts from host 5′ exoribonucleases and escaping the host innate immune response by mimicking as host mRNAs (41). Thus Nsp16 is the potential target for antiviral therapeutics. The binding site was found to be lined by the residues Phe 70, Gly 71, Ala 72, Gly 73, Asp 99, Leu 100, Leu 111, Gly 113, Met 131, Tyr 132, Asp 133, Phe 149, Asp 114, Ala 116, Cys 115, and Val 118. Our study shows that histrelin, ocreotide, lanreotide, pasireotide, capreomycin, zidovudine, triptorelin, venetoclax, folic-acid, and ribavirin binds to Nsp16 with high affinity. Ribavirin makes HB interactions with Asp 99, Try 132, Cys 115, Gly 71, Gly 113 and Ser 98 (Fig. 5). A previous study has also predicted ribavirin as the inhibitor of Nsp16 **(42)**, in addition our analysis also predicts ribavirin as ligand to many of SAR-CoV2 proteins including Nsp16.

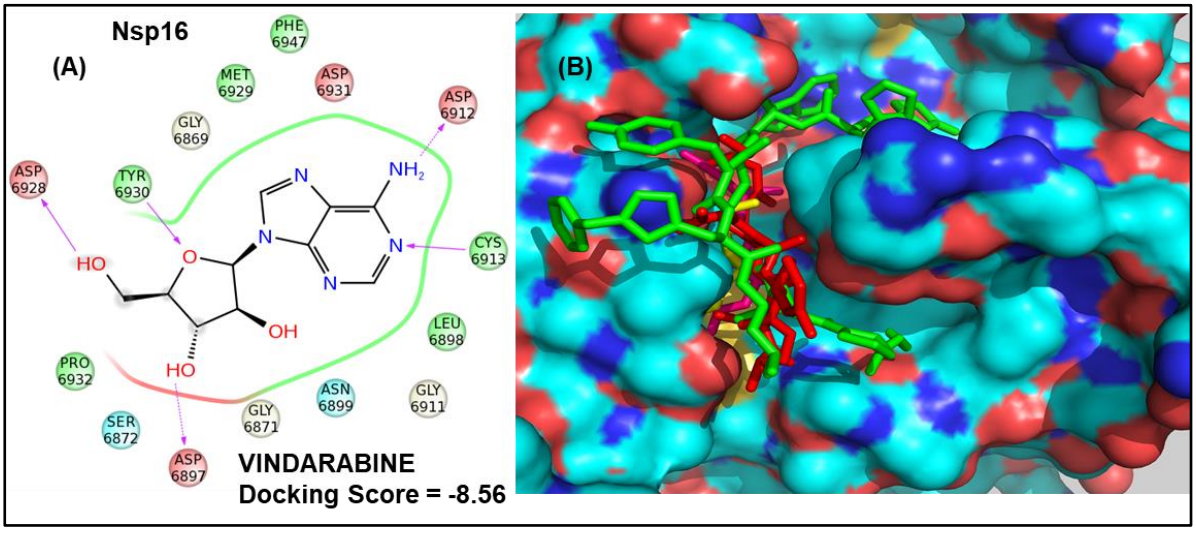


Fig. 5: (A) Binding of vidarabine at the nsp16 protein of SARS-COV2. The drug is shown in black lines while the receptor residues are shown in coloured circles. The magenta arrows indicate the hydrogen bonding interactions (the ligand is acceptor and donor respectively for incoming and outgoing arrows) between the ligand and the receptor residue. (B) The binding cavity along with the top ligands. The receptor surface is shown in cyan colour while the ligands are shown in red, green, and yellow colour.

Zidovudine is used in HIV1 treatment (**21**), histrelin and triptorelin are gonadotropin-releasing hormone analogs used in the treatment of central precocious puberty and endometriosis (43). Lanreotide is a long-acting analog of somatostatin and is used for the management of acromegaly, a condition caused by excess secretion of growth hormone. Octreotide is also a somatostatin analog currently used for the treatment of watery diarrhoea and flushes caused by certain carcinoid tumors. Vidarabine (ara-A) is a purine analog and an antiviral drug used for infections caused by herpes simplex and varicella zoster viruses.

Among all the proteins encoded by SARS-CoV2 genome, **PL^{pro} (papain-like protease) and $3CL^{pro}$ (3C chymotrypsin-like protease)** are two important viral proteases that cleave the two polyproteins (pp1a and pp1ab) into individual functional viral proteins. The $3CL^{pro}$ is a cysteine protease having three domains: β-barrel Domain I (residues 8–101) and II (residues 102–184) and α-helix domain III (residues 201–306) similar in structure to chymotrypsin (44). The functional protease is a dimer that cleaves polyprotein 1ab in 11 regions at its specific cleavage site of Leu-Gln↓(Ser, Ala, Gly). The binding site was defined as residues falling within 5Å of the co-crystallized ligand (PDB: 6W63). The residues Thr 25, His 41, Cys 44, Thr 45, Ser 46, Met 49, Asn 142, Gly 143, Cys 145, His 164, Met 165, Glu 166, Leu 167, Pro 168, Asp 187, Arg 188, Gln 189, and Gln 192 were used for defining the active site. Alatrofloxacin, cangrelor, capreomycin, naldemedine and indinavir are among the drugs predicted to bind to $3CL^{pro}$ (Fig. 6).

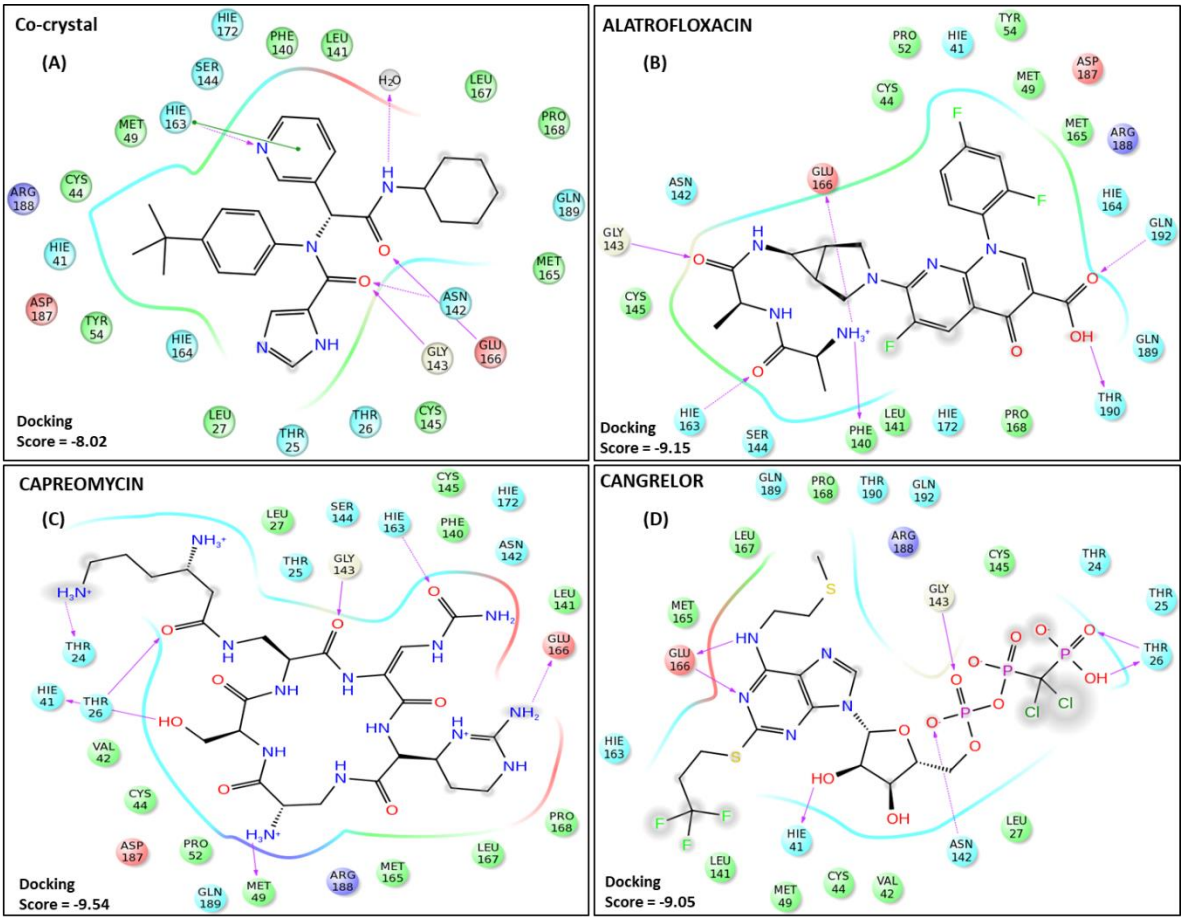


Fig. 6: The docking of drugs in 3CLpro of SARS-COV2. The molecule is shown in black lines while the receptor residues are shown in coloured circles. The magenta lines indicate the hydrogen bonding interactions between the ligand and the receptor residues. (A) Co-crystallized ligand (B) Alatrofloxacin (C) Capreomycin (D) Cangrelor. It can be seen that the docked molecules bind to the receptor in a similar way with a better binding affinity as reflected by the docking score.

Previous studies report α-ketoamides, lopinavir and ritonavir as inhibitor of $3CL^{pro}$ (45, 46). Indinavir is shown to inhibit HIV protease by blocking its active site and leads to immature virus particle formation, however high doses have been linked to lipodystrophy syndrome (47). Naldemedine, is a μ-opioid receptor antagonist used for the treatment of opioid-induced constipation (48). Our study also predicts that tenofovir, nebivolol, ribavirin, nilotinib, lanreotide, ibrutinib, mefloquine, lopinavir, desmopressin, pasireotide, and methotrexate are among top molecules binding to the protease PL^{pro}. An interesting observation is the identification of folic acid as a high affinity ligand of PL^{pro} (Fig. 7).

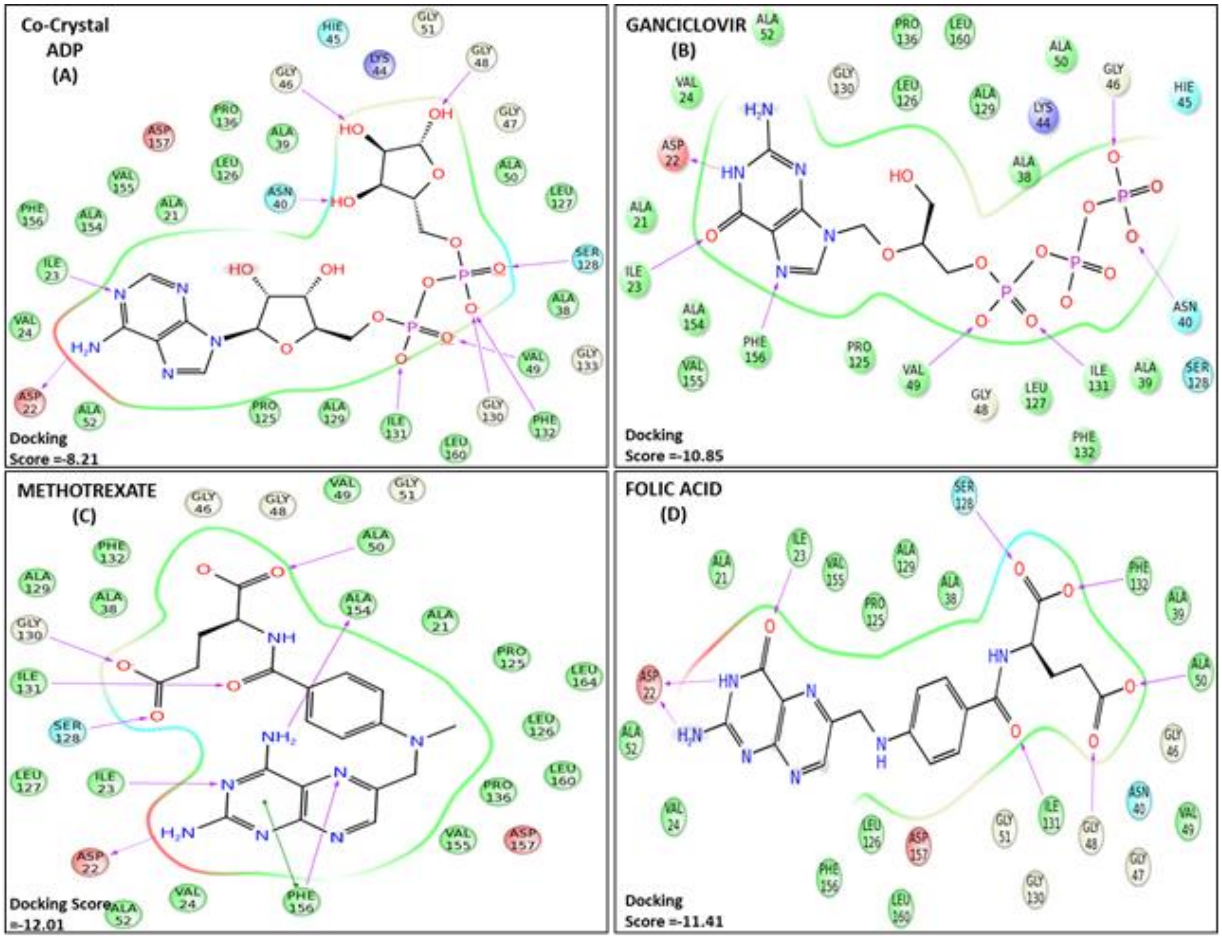


Fig. 7: (A) The docking of top hits in PLpro of SARS-COV2. The molecule is shown in black lines while the receptor residues are shown in coloured circles. The magenta lines indicate the hydrogen bonding interactions between the ligand and the receptor residues. (A) Co-crystallized ligand (B) Ganciclovir (C) Methotrexate (D) Folic acid. It can be seen that the docked molecules bind to the receptor in a similar way with a better binding affinity as reflected by the docking score.

Helicase enzyme (Nsp13) of SARS-CoV2 is motor protein essential for unwinding of both dsDNA and dsRNA and has metal binding (Zn2+) N-terminal and helicase domain (Hel). It is involved in formation of RTC of SARS-CoV2 along with RdRp, which is known to enhance its activity (49). Our analysis shows that lanreotide, methotrexate, octreotide, cangrelor, and pibrentasvir bind to the helicase with high affinity (Fig. 8). Pibrentasvir, is a HCV NS5A inhibitor effective against all HCV genotypes (50). Methotrexate acts as an antimetabolite and thus used as an antineoplastic drug. It is also anti-inflammatory and used in treatment of inflammatory diseases like rheumatoid arthritis. It decreases the de novo synthesis of purines and pyrimidines and forms dimers with thymidylate synthase (TS), hence also used as anti-parasitic drug. Methotrexate is also shown to effectively reduce replication of Zika and Dengue viruses (51).

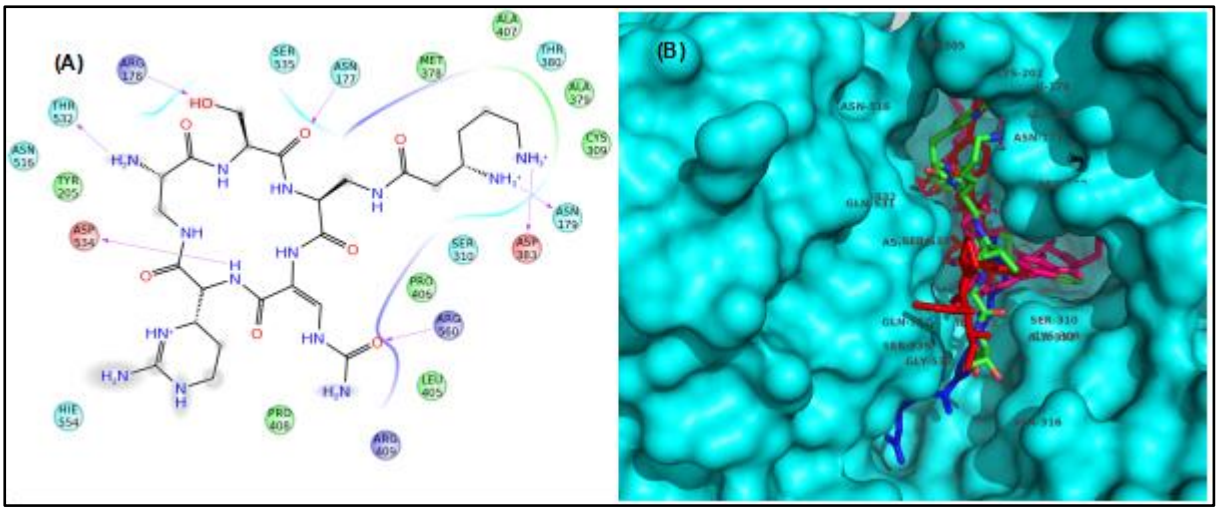


Fig. 8: (A) Binding of lancreotide (docking score -10.34) at the helicase protein of SARS-CoV2. The molecule is shown in black lines while the receptor residues are shown in coloured circles. The magenta lines indicate the hydrogen bonding interactions between the ligand and the receptor residues. (B) The binding cavity alongwith the top ligands. The receptor surface is shown in cyan colour while the ligands are shown in differently coloured sticks.

The most vital enzyme responsible for the replication/transcription of the viral genome is the **RNA-dependent RNA polymerase (RdRp)** also known as Nsp12. The primer for RdRp RNA synthesis is synthesized by Nsp8 (52). Our analysis shows that cobicistat, capreomycin, pibrentasvir, elbasvir, indinavir and remdesivir among others can bind with RdRp (Fig. 9). Cobicistat is known to inhibit the cytochrome-mediated metabolism of HIV protease and was approved in 2012 by FDA as pharmacoenhancer for HIV treatment (53). Other groups have also predicted that cobicistat and capreomycin can inhibit SARS-CoV2 protease (54) (55). Pibrentasvir and elbasvir are HCV NS5A inhibitors and indinavir is potent HIV protease inhibitor (56). The molecules we identified to bind to RdRp can serve as potential alternatives to remdesivir.

The Nsp15 is EndoRNase with endoribonuclease activity. It cleaves the 5′ and 3′ of uridylate residues in RNA by forming 2′-3′cyclic phosphodiester. Its mechanism is similar to that of RNase A, RNAse T1 and XendoU (57). Its NendoU activity can interfere with the host's innate immune response and masks the exposure of viral dsRNA to host dsRNA sensors (58)**.** In our analysis, drugs such as octreotide, desmopressin, macimorelin, and simeprevir were found to target Nsp15 (Fig. 10).

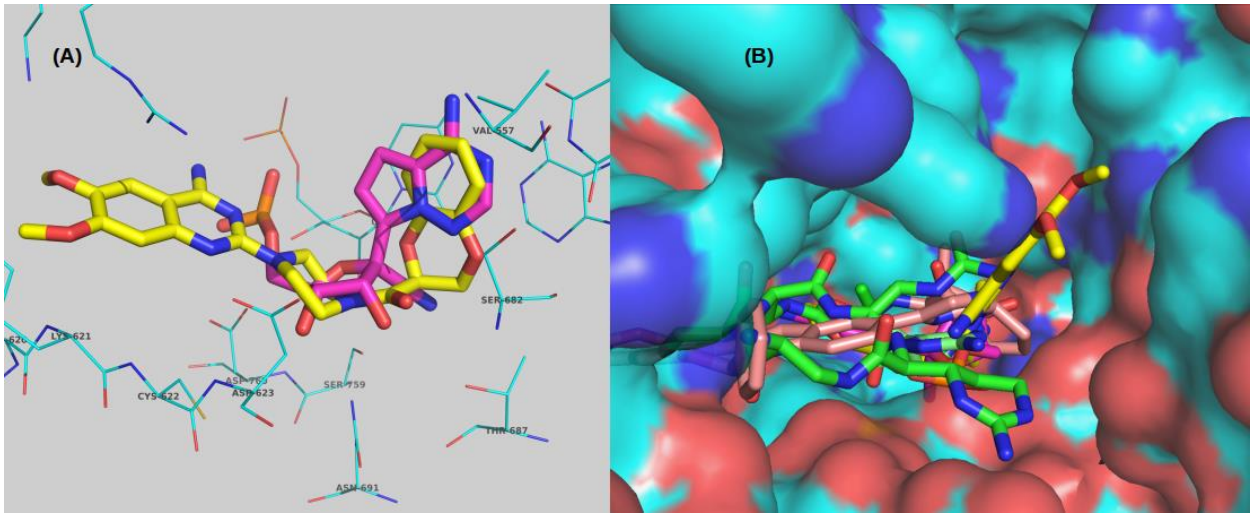


Fig. 9: (A)The binding of doxazocin with RdRp. The doxazocin (yellow) is shown superimposed with remdesivir (magenta) at the active site (cyan) of RdRp. (B) The cavity (surface view) with some of the top molecules in coloured sticks.

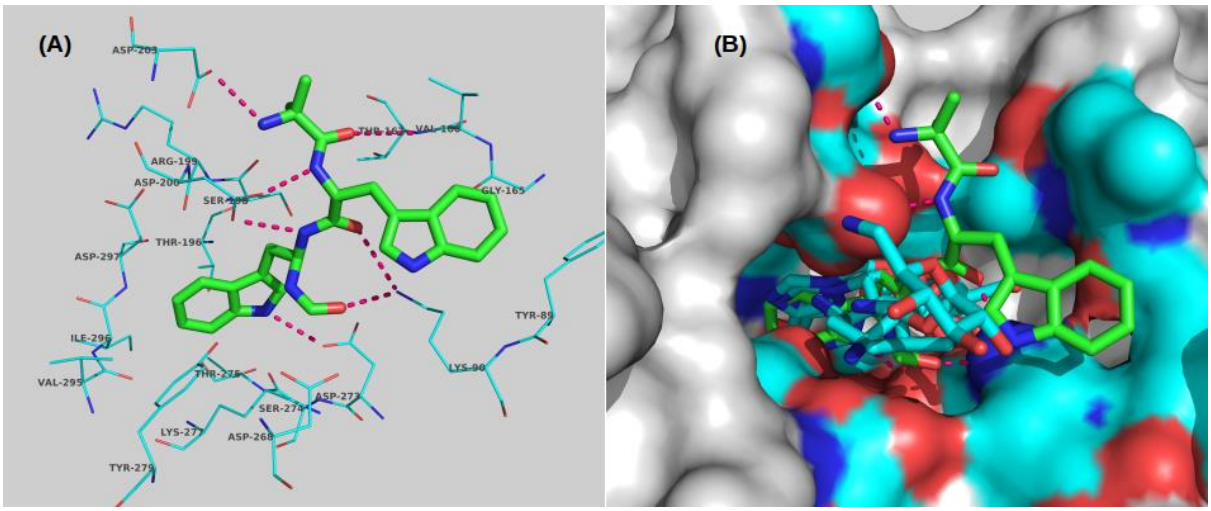


Fig. 10: The binding of macimorelin (docking score -8.5) with nsp15. The drug is shown in green sticks while the receptor is shown in cyan lines. The magenta lines show the hydrogen bonding interactions (B) The cavity (surface view) with some of the top molecules in coloured sticks.

Nsp14 is the 3'-5'exonuclease that plays a role in proofreading mechanism (59). Nsp14 contains four conserved DE-D-D acidic and a zinc-finger (ZnF) domain (60). Our molecular docking predicted that cangrelor, venetoclax, pimozide, nilotinib, droperidol, nebivolol, indacaterol, ezetimibe, simeprevir, siponimod, lapatinib, elagolix bind to Nsp14 (Fig. 11). Pimozide, a calmodulin inhibitor is shown to inhibit Chikungunya virus secretion (61). Moreover, it binds to the envelope protein of HCV and inhibits infection with many HCV genotypes (62). Ezetimibe is shown to inhibit formation of capsid-associated relaxed circular DNA of Hepatitis B Virus (HBV) (63) and is also shown to inhibit Dengue infection by

interfering in formation of replication complex (64). Indacaterol is the β2-adrenoceptor agonist and used in the treatment of chronic obstructive pulmonary disease (COPD) since it induces bronchodilation effect (65). It is a promising candidate for therapeutics against SARS-CoV2 due to its ability to regulate genes involved in suppressing proinflammatory cytokine production and attenuation of airway hyper-responsiveness (66). However, dose and treatment schedule needs to be evaluated due to its counter effect on the expression of RNase L which is vital for antiviral response.

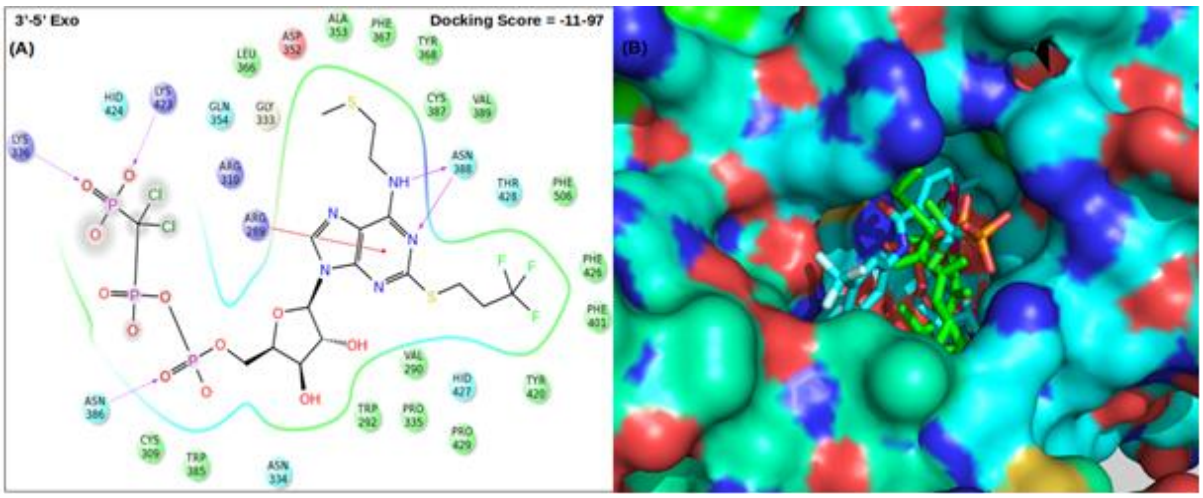


Fig. 11: (A) Binding of cangrelor at the nsp14 protein of SARS-COV2. The molecule is shown in black lines while the receptor residues are shown in coloured circles. The magenta lines indicate the hydrogen bonding interactions between the ligand and the receptor residues. The red lines show cation-pi interactions. (B) The binding cavity alongwith the top ligands. The receptor surface is shown in cyan colour while the ligands are shown in red, blue, green and magenta colour.

Drugs targeting other nonstructural proteins:

Nsp2 gene resides in a region of the coronavirus genome that is relatively nonconserved across coronaviruses. Studies have shown that deletion of Nsp2 region have no effect on viral replication and production of infectious particle. Other reports suggested that Nsp2 interacts with host protein such as PBH1 and PBH2 and alters the host environment to facilitate viral replication (67). Among the top binding molecules for Nsp2 are cangrelor, nilotinib, alatrofloxacin, lomitapide, fosaprepitant, lopinavir, trovafloxacin, saquinavir, daunorubicin (Fig. 12).

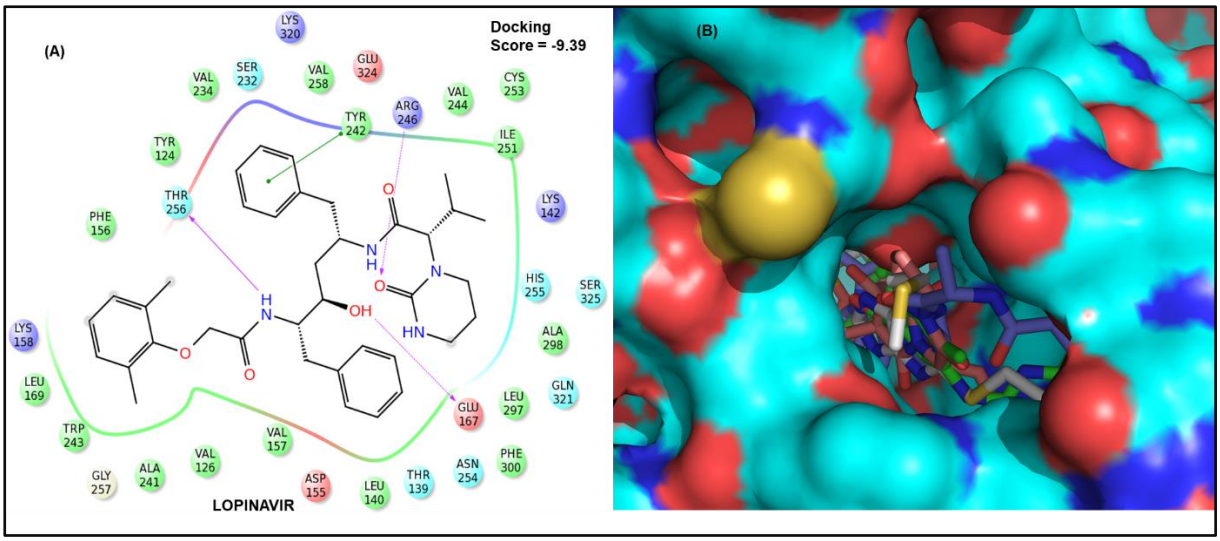


Fig. 12: (A) Binding of lopinavir at the Nsp2 protein. The molecule is shown in black lines while the receptor residues are shown in coloured circles. The magenta arrows indicate the hydrogen bonding interactions (the ligand is acceptor and donor respectively for incoming and outgoing arrows) between the ligand and the receptor residue. The green lines indicate hydrophobic interactions. (B) The binding cavity along with the top ligands. The receptor surface is shown in cyan colour while the ligands are shown in coloured sticks.

Daunorubicin (DNR) is the anthracycline compound used in the Kaposi's sarcoma and lymphomas treatment of HIV-1 infected patients (68). Moreover its derivative N,N-dimethyl daunomycin (NDMD) is used as the inhibitor of Herpes simplex virus (HSV) (69). Saquinavir is a HIV protease inhibitor used in the treatment of both HIV-1 and HIV-2 patients. It produces structurally defective and inactive HIV viral particles by cleaving the gag-pol protein (70). Trovafloxacin belongs to the group of fluoroquinolones and has various activities e.g. antifungal (against *Candida albicans* and *Aspergillus fumigatus)*, antimalarial (against *Plasmodium* sp.), antiparasitic (against of *T. gondii*), and antiviral (71).

The Nsp4 is integral membrane protein with multiple transmembrane domains. It plays crucial role in virus-induced membrane alterations and formation of double membrane vesicles that support viral replication (72). Studies have shown that co-expression of Nsp4 with Nsp3 results in the formation of double-membrane vesicles. The large lumenal loop situated between the first and second transmembrane domains (AA residues 112–164 and 220 to 234) of Nsp4

is vital for induction of membrane rearrangement (73). Nilotinib, cangrelor, lapatinib, alatrofloxacin, bromocriptine were found as top hits for this protein. (Fig. 13)

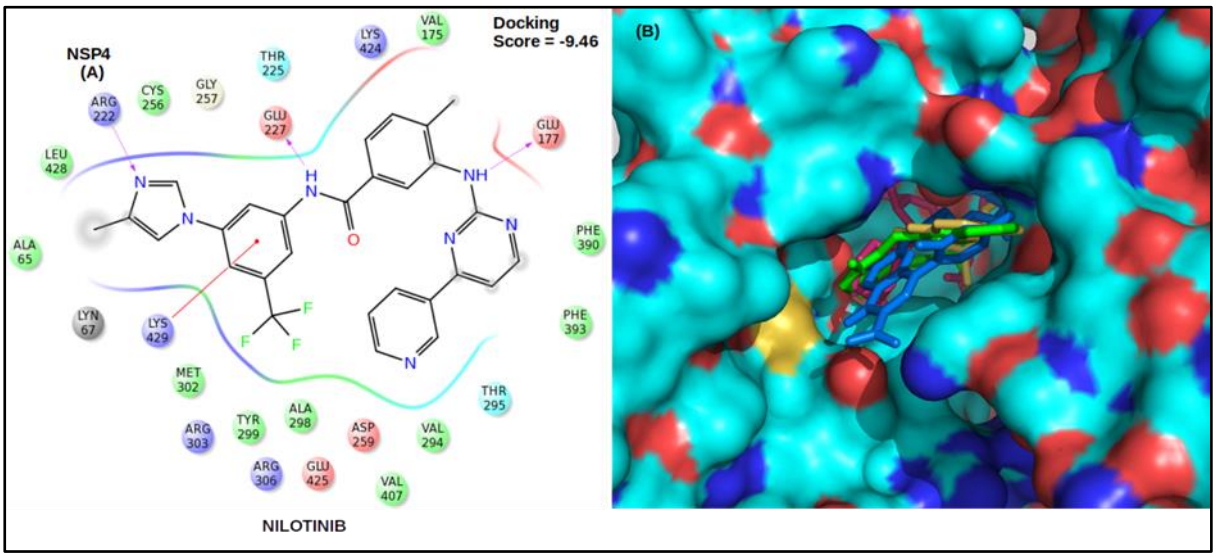


Fig. 13: Binding of nilotinib at the nsp4 protein. The molecule is shown in black lines while the receptor residues are shown in coloured circles. The magenta arrows indicate the hydrogen bonding interactions (the ligand is acceptor and donor respectively for incoming and outgoing arrows) between the ligand and the receptor residue. The red lines indicate cation-π interactions. (B) The binding cavity along with the top ligands. The receptor surface is shown in cyan colour while the ligands are shown in coloured sticks.

Our study shows that bromocriptine docks to Nsp4 and can be a potential agent against SARS-CoV2. It was previously found to inhibit ZIKV replication by binding to active site of ZIKV-NS2B-NS3 protease (74) and inhibit the translation and replication of Dengue virus by binding to NS3 protease (75)**.** Nilotinib is the inhibitor of platelet-derived growth factor receptor-α (PDGFRα) and used in the antiviral treatment of Human Cytomegalovirus (HCMV)(76). Another molecule monteleukast, a leukotrine inhibitor used as antiasthmatic was also showing moderate affinity towards Nsp4 (docking score -7.56). A previous study suggests that it inhibits Zika virus by disrupting the integrity of the virions (77). Interestingly, Lapatinib which can bind to Nsp4 with high affinity, is a HER2 (ligand-independent receptor tyrosine kinase) inhibitor used in the treatment of HER2-positive breast cancers (78, 79)(66). HER2 inhibition is shown to activate TBK1 through cGAS-STING pathway which is crucial to induce the anti-viral innate immune signaling (80)**.**

Nsp10 stabilizes Nsp16's SAM-binding pocket and extends the substrate RNA-binding groove that activates the 2′-O–MTase activity. It consists of 148 AA residues with two zinc finger domains and often overexpressed along with Nsp14 and Nsp16. It's interacting surfaces are

important for recruiting Nsp14 and Nsp16 to the replication-transcription complex and helps in boosting the ExoN activity of nsp14 and 2′-O-MTase activity of Nsp14 and Nsp16 respectively (41, 81). Lanreotide was the only molecule that showed appreciable binding affinity with Nsp10 (Fig. 14).

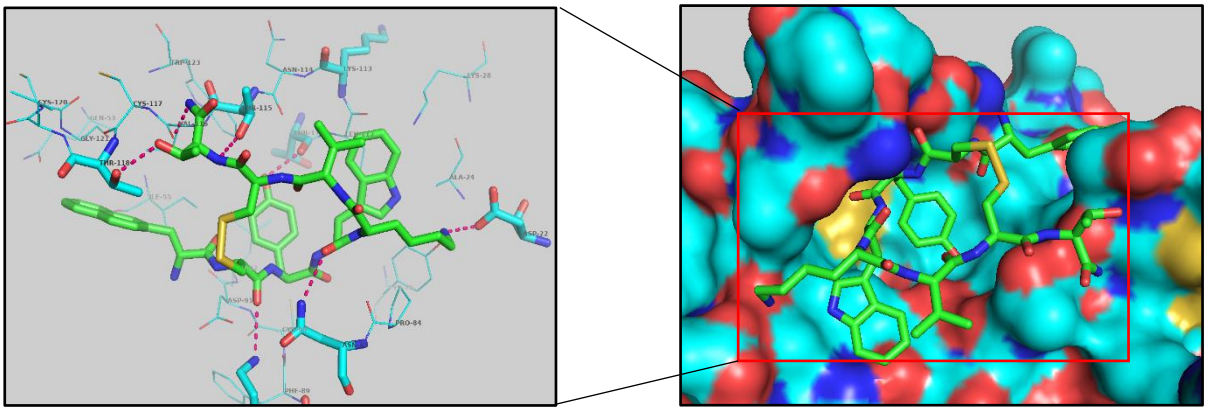


Fig. 14: Binding of Lanreotide at the Nsp10 protein. The molecule is shown in green sticks while the receptor residues are shown in cyan lines. The dashed line indicates the hydrogen bonding interactions. It can be seen that the molecule makes a number of HB interactions with the Nsp10 protein.

Drugs targeting multiple SARS-CoV2 proteins

Cangrelor: is a P2Y12/13 inhibitor and is used as antiplatelet drug. The abnormal blood clotting is an increasingly recognized complication of COVID-19 and adverse prognosis. Therefore, it has been suggested to use thrombolytics/antiplatelet agents in the early stages of infection (82, 83). Importantly in our screening Cangrelor was found to bind to multiple SARS-CoV2 proteins. Among the important targets are the main protease, spike protein, exonuclease, and helicase. Interestingly the target of Cangrelor in humans is P2Y12/13 which is also found to be differentially expressed as a result of SARS-CoV2 infection (Supplementary table 2). Additionally, there are no reported drug interactions between investigational COVID-19 therapies and Cangrelor (97). Therefore, this drug can be important for repurposing to treat the COVID-19. However, it was found to bind with many SARS-CoV2 proteins therefore it has to be seen whether it has a privileged scaffold or it is a promiscuous binder. The short plasma half-life upon intravenous administration may limit its efficacy against SARS-CoV2.

Nilotinib: is a potent tyrosine kinase inhibitor and is used as an anticancer drug. It is found to bind to multiple SARS-CoV2 proteins. It was intriguing to see it binding with two non-structural proteins (Nsp2 and Nsp14) with high affinity. A literature survey showed that it has been reported to exhibit antiviral activity against Human Cytomegalovirus (99). The mechanism is not very clear; however, it has been surmised that it may disrupt the productive

replication of the virus. The SARS-CoV2 coronavirus depends on Abl2 kinase activity to fuse and enter into the cells. The kinase inhibitor imatinib is already under clinical trials for COVID-19 (https://clinicaltrials.gov/ct2/show/NCT04357613). Thus, other kinase inhibitors that are binding to viral proteins can also be potential candidates for repurposing.

Lapatinib: is another potent kinase inhibitor, similar to nilotinib, that is showing good binding with the SARS-CoV2 proteins (S-protein, Nsp4 and Nsp13). Lapatinib is a HER2 (ligand-independent receptor tyrosine kinase) inhibitor used in the treatment of HER2-positive breast cancers (84). Interestingly, HER2 inhibition is shown to activate TBK1 through cGAS-STING pathway which plays a crucial role in anti-viral innate immune signaling (80). Hence, lapatinib can be effective in perturbing SARS-CoV2 replication as well as upregulating anti-viral signaling.

Lancreotide: a long-acting analog of the drug somatostatin widely used in the treatment of Graves' ophthalmopathy, Acromegaly and Endocrine tumors (85),(86),(87),(88), is found to have high binding affinity to multiple SARS-CoV2 proteins such as PL^{pro} (Nsp3), Nsp10, 13 and 16.

Octreotide: another somatostatin analog similar to lancreotide. It is used in the treatment of diarrhoea, pancreatic neuroendocrine tumors and massive hemorrhage caused by cytomegalovirus colitis (89),(90),(40)**.** This drug also binds to the multiple proteins of SARS Cov2 virus which includes the structural proteins S and N and the helicase, endonuclease and methyl transferase. Study suggested, several anticancer drug have potential target of SARS-Cov2 as repurposing drugs (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7239789/).

Capreomycin: a polypeptide isolated from Streptomyces capreolus used in the treatment of multidrug resistant tuberculosis and co-infected (HIV) is also found to bind with 2'OMT, nuclease and RdRp proteins. However, this compound has serious nephrotoxicity and ototoxicity, which has to be taken in into account (39).

Nebivolol: is a selective β1-adrenoceptor blocker. It is used for the treatment of hypertension and cardiac ailments. It directly stimulates endothelial nitric oxide synthase, resulting in increased levels of local nitric oxide. The vasodilation by nebivolol is attributed to enhanced availability of nitric oxide and reduction of cellular oxidative stress (99-101). Nitric oxide is also known to inhibit clot formation, and it has been shown that inhaled nitric oxide decreases the propensity of clotting in ARDS (102, 103). In our study, nebivolol was found to bind with exonuclease Nsp14 and PL^{pro} protease of the SARS-COV2 with high affinity. Its efficacy as an antiviral and the fact that it can induce the production of nitric oxide makes it an ideal candidate for repurposing.

Ribavirin: is a broad spectrum antiviral drug against RNA and DNA viruses currently being used for the treatment of hepatitis C and viral haemorrhagic fevers. It enhances the destabilization of viral RNA and reduces the availability of guanosine. Recent studies suggested that it can be a potent inhibitor of SARS-CoV2 infection (26). Our docking study suggests that ribavirin can target the protease PL^{pro} and Nsp16 of SARS-CoV2.

Desmopressin: a synthetic analog of vasopressin (ADH) used in treatment of vasopressin deficiency, diabetes insipidus, and hemophilia A (91) binds to the PL^{pro} protease and endoR of SARS-CoV2 with high affinity. Interestingly, desmopression is also used in the treatment of Dengue Hemorrhagic Fever/Dengue Shock Syndrome as it reduces vascular leakage by releasing endothelial hemostatic factors and promoting platelets (92).

Pibrentasvir: the hepatitis C virus NS5A inhibitor was found to bind with helicase and RdRp proteins.

Indinavir: HIV protease inhibitor and major component of highly active antiretroviral therapy (HAART) for treatment of HIV/AIDS was found to bind RdRp and $3CL^{pro}$ protease with good affinity.

Venetoclax: is a BCL-2 (antiapoptotic protein) inhibitor is used for the treatment of chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL) (93). It is found to bind with Nsp16 and exonuclease.

Alatrofloxacin a fluroquinolone antibiotic that targets the DNA gyrase enzyme was found to bind with Nsp2 and Nsp4 proteins with good affinity.

A heatmap (Fig. 15) was generated using the docking scores to summarize the binding of important drugs to multiple proteins. The detailed list drugs and their docking scores is given in **supplementary table 1**.

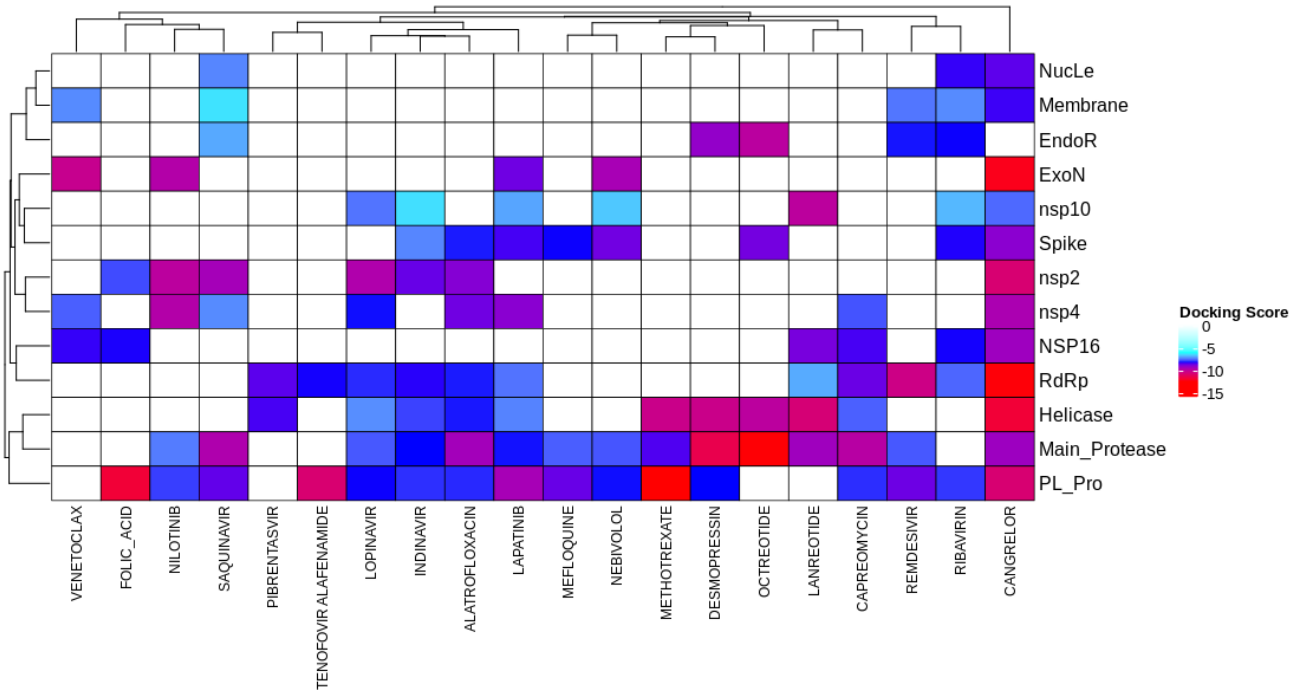


Fig. 15: Drugs binding to multiple proteins. The drugs are shown along the X-axis while the proteins are shown along Y-axis. The white-red colour spectrum shows increasing binding affinity.

Analysis of the transcriptome data and drug interactions of few differentially expressed genes:

As stated earlier, the differentially expressed genes were identified by analyzing the data from NCBI GEO (GEO ID: GSE-147507) using limma-voom with the criteria of $|\log_2 FC \geq 1|$ and p-value≤ 0.01. The differentially expressed genes were then analyzed using IPA and Cytoscape. Details are given in Supplementary Table 2. A protein-protein-interaction network analysis was done using Cytoscape to identify the network hubs based on their interactions with other proteins using Degree centrality. The giant component was extracted from the network with 1446 nodes containing 1770 interactions. Top 5% of the proteins (total 72) were selected as hubs based on the degree centrality for further analysis.

A search for the DEGs (136) at drug-gene interaction database (DGIdb) resulted in the identification of 352 drugs. (Supplementary table 3). Further, we wanted to know if some of them can also target viral proteins. An intersection with drugs identified early revealed 29 drugs that can target both viral and human proteins. It is worth to mention that among the human proteins many of them (e.g. IL2RG, DAPP1, CCL7, IFIT1, MMP8, FOS) are hubs i.e. very important proteins in the generated protein-protein-interaction network. Therefore, the drugs with multi-targeting ability against these proteins as well as SARS-CoV2 proteins can have a

552 significant therapeutic utility for COVID-19 which is a novelty of this study (Supplementary
553 Table 4).
554
555 The analysis indicated that some of proteins upregulated during SARS-CoV2 infection are also
556 targeted by the drugs identified to bind to viral proteins in our analysis. TUBB48 (Tubulin Beta
557 4A Class IVa) is upregulated during SARS-CoV2 infection. Previous study suggests that drug-
558 induced microtubule depolymerization results in reduction of infectious virus particle release
559 due to defect in spike protein incorporation into the virions. Paclitaxel, which targets the N
560 protein, is a cytoskeletal drug which stabilizes the microtubule polymer formation and protects
561 it from disassembly(94). The transcription complex Activator protein 1 (AP1) is composed of
562 homo/hetero dimers of Fos, Jun, CREB and others ATFs. The studies on the SARS-CoV1
563 infection in the Vero and Huh7 cell shows that nucleocapsid protein is the potent activator of
564 (AP-1)(95). Interestingly, asthmatic patients show higher expression of c-fos in their epithelial
565 cells. It is also observed that TNF-α induced ROS and intracellular glutathione depletion in the
566 airway epithelial cells induces the production of AP-1 and leads to the pulmonary fibrosis (96,
567 97). Our analysis suggests that paclitaxel and bromocriptine which dock with nucleocapsid and
568 Nsp4 proteins can also effectively bind to c-Fos and thereby would be beneficial in inhibiting
569 the SARS-CoV2 as well as in alleviating lung injury observed in COVID19. The transcriptome
570 analysis revealed that S100/calgranulin is upregulated during SARS-CoV2 infection.
571 Calgranulin is polypeptide released by the activated inflammatory cells such as leukocytes,
572 PBMC phagocytes and lymphocytes and is accumulated at the sites of chronic inflammation.
573 It is the ligand for RAGE receptors and is the major initiator of cascading events in
574 inflammation amplification (98). Interestingly, the differential gene expression analysis of
575 PBMCs from SARS patients shows the higher expression of this calgranulin families (97) and
576 this protein is found in higher quantity in the Bronchoalveloar Lavage Fluid (BALF) and
577 sputum of patients with inflamed lungs, COPD, and ARDS (99). Our analysis suggests that the
578 anti-inflammatory agent methotrexate which has high affinity to the Nsp13 protein of SARS-
579 CoV2 also shows appreciable binding to calgranulin and can thereby be useful to curtail
580 systemic inflammation in lungs observed during COVID19 in addition to its inhibitory effect
581 on SARS-CoV2. Transcriptomic analysis also suggests increased expression of endogenous
582 prolactin, which leads to prolactin induced STAT5 activation and its pathways. Prolactin has a
583 dual role in human physiology functioning as a hormone (secreted from anterior pituitary
584 gland) and cytokine (secreted by immune cells). It causes anti-apoptotic effect and induces
585 proliferation in immune cells in response to antigens leading to increased production of

immunoglobulin, cytokines, and autoantibodies(100). We envisage that prolactin may be one of the significant player in trigger of cytokine storm implicated in COVID19. Interestingly, our study suggests that zidovudine which target the O'-methyl transferase (Nsp16) can also bind to prolactin and can be of high significance in management of COVID19 due to dual ability to affect Nsp16 and prolactin.

Conclusions

The overall goal of this study was to identify molecules that can dock with multiple SARS-CoV2 proteins that play vital role(s) in the viral lifecycle. Our study predicted several promising drug candidates with high binding affinity towards many of SARS-CoV2 proteins. These drugs will be very effective than drugs that target single viral proteins due to their ability to affect multiple aspects of viral lifecycle and enhance the barrier towards the evolution of drug-resistant mutants, a usual phenomenon observed in RNA viruses. For instance, drugs like Cangrelor that binds to both Spike and Membrane proteins can be very effective inhibitor of SARS-CoV2 entry. Similarly, Lanreotide and Octreotide which can bind to both the structural and non-structural proteins can effectively inhibit viral entry as well as the post entry events like viral genome replication and transcription. Capreomycin is also a promising candidate to inhibit SARS-CoV2 replication as it binds with high affinity to RdRp, methyl transferase and nucleocapsid proteins that play a pivotal role in viral replication and transcription. Our analysis also predicted drug candidates that interact with the viral protein and have a positive effect on the host signalling pathways vital to control viral infection or disease manifestation. Nebivolol a β-adrenoreceptor blocker, which stimulates nitric oxide production by endothelial nitric oxide synthase (59, 60) is found to bind to PLpro protease and exonuclease of SARS-CoV2. Interestingly, nitric oxide has been used to reverse pulmonary hypertension and improve severe hypoxia in SARS-CoV1 (72) and SARS-CoV2 patients. Hence, nebivolol can be a promising therapeutic strategy to curb SARS-CoV2 infection and reverse severe clinical manifestation observed in critical Covid-19 patients. Lapatinib binds to Nsp4, a viral protein crucial for promoting membrane rearrangement and establishment of viral replication complex. Lapatinib is a HER2 inhibitor which can also trigger TBK1 activation that plays a crucial role in anti-viral innate immune signalling (67). Thereby, lapatinib has the dual advantage of inhibiting SARS-CoV2 replication as well as upregulating anti-viral signaling. Similarly, indacaterol which targets the exonuclease is also a promising agent due to its ability to regulate genes involved in suppressing pro-inflammatory cytokine production and attenuation of airway hyper-responsiveness (71). We also observed that the molecules that bind to Nsp4, 13, 16 and

N proteins can also bind to human proteins that play a pivotal role in disease pathogenesis by promoting inflammatory signalling leading to cytokine storm thereby suggesting that the molecules such as a paclitaxel, methotrexate, and zidovudine can have a dual beneficial effect in the management of COVID19. Overall our study predicts promising agents with potential to inhibit crucial viral processes, upregulate anti-viral host response and alleviate severe lung disease condition thereby providing attractive avenues for design of potential and multipronged therapeutic strategies against COVID 19.

Author Contribution: The work is designed and conceptualized by SK, AD, GA and GHS. Data generation and work performed by SK, PK, and AD. The manuscript was prepared by SK, AD, PK, GA, PVK, SHK and GHS.

Acknowledgment: All authors acknowledge Director, Institute of Life Sciences (ILS), Bhubaneswar and Dept. of Biotechnology, Govt. of India for providing necessary facilities for this work. SK and PK are thankful to the University Grant Commission (UGC) for fellowship. GA is thankful to DST for fellowship (No. SR/WOS-A/CS-129/2016). GHS is thankful to DBT-Wellcome trust India alliance for fellowship.

Conflict of Interest: Authors has no conflict of interest.

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