SARS-CoV-2 nucleocapsid assembly inhibitors: Repurposing antiviral and antimicrobial drugs targeting nucleocapsid-RNA interaction

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Abstract

SARS-CoV-2 pandemic has become a serious concern due to high transmission of this virus and unavailability of any definitive drugs yet in clinics. While novel antivirals are under investigation stage, scientists are also rigorously trying to use drug repurposing as an option to fight against this highly infectious novel coronavirus. Several drugs are under regular use for other diseases that are getting screened for their usability against SARS-CoV2. In this study we have targeted SARS-CoV-2 nucleocapsid assembly to shortlist FDA approved drugs that could be tested for inhibition of SARS-CoV-2 virus particles inside the host cell. We could shortlist five antiviral and anti-microbial drugs. These showed good fit in docking studies inside the RNA binding cleft of the nucleocapsid protein. Also, these drugs have lipophilic properties suggesting that they have the potential to enter the host cells. We propose that these shortlisted drugs could potentially compete out binding of viral RNA to nucleocapsid and thus inhibit successful virus assembly leading to poor virus progeny levels.

Introduction

SARS-CoV-2 pandemic has led to global search of therapeutics, either novel or repurposed from already existing drugs against other diseases. SARS-CoV-2 is an enveloped RNA virus that undergoes several steps of its life cycle within the host cell. Post entry the virus undergoes RNA translation, replication and virus assembly before new virus particles exit out of the host cells.

Starting from receptor binding on the host cell to virus exit out of the host cell, each of the steps are spearheaded by several viral structural and non-structural proteins.

Thus, functions of these viral proteins could be targeted to inhibit essential steps of the virus life cycle. SARS-CoV-2 spike protein, RdRp and helicase have been widely targeted for therapeutic interventions and drug repurposing. In this study we have targeted the nucleocapsid which assembles and packages the viral RNA inside. Inhibition of virus assembly and RNA packaging could lead to disruption in the virus life cycle and inhibit production of new virus particles thereby reducing the viral load post infection.

Methods:

Nucleocapsid substrate

Crystal structure of RNA binding N-terminal domain of nucleocapsid protein of SARS-CoV-2 was downloaded from Protein data bank (PDB) (1) (PDB entry: 6M3M). The 3D crystal structure consists of four equivalent chains of the RNA binding domains. As the nucleocapsid protein dimerizes during assembly, only two monomers (Chain A and B) were selected to retain the more native conformation for docking studies. The protein structure was converted to a supported file format (pdbqt) after removing the water molecules from the complex by AutoDock Tools 1.5.6 (3). Hydrogen atoms were added back to the side chains which retains an overall positive charge of the protein.

Ligand

In this study, we have screened an array of FDA approved anti-viral and anti-microbial drugs, which have not yet been reported against SARS-CoV2 as potential candidates. The nucleocapsid protein is only accessible in the intracellular environment. Hence, only those drugs have been selected as ligands which have significant lipophilic nature (Table 1). The 3D conformations of the drug molecules were retrieved from Pubchem in SDF format and then exported to PDB file using Pymol software. The ligand was converted to supported format (.pdbqt) by AutoDock Tools 1.5.6 (2,3).

Molecular docking

All the protein-ligand docking experiments were done in Autodock vina software (v1.5.6) (4). We performed a blind docking, keeping the whole protein structure accessible for ligand

binding. Docking was performed keeping the grid centre set at X =8.33 , Y = -17.003, Z = -17.857 and grid-box dimensions at 72Å X 96Å X 80Å with the exhaustiveness value 8. After docking the docked structures were visualized by Discovery Studio Visualizer v20.1.0.19295 (5) and PyMol software (6).

Results and discussion:

In this study FDA approved drugs have been screened for the purpose of drug repurposing against SARS-CoV-2 nucleocapsid assembly. For this virus to undergo successful nucleocapsid assembly, the nucleocapsid protein needs to interact/ bind with the viral RNA at the RNA binding cleft (Figure 1). This is a part of viral RNA packaging. Drugs that inhibit nucleocapsid-viral RNA binding might inhibit successful virus assembly inside the host cell. For a drug to act on virus assembly, it must be able to enter the host cells. Hence, we have picked drugs that showed lipophilic nature. Fifteen anti-viral compounds and fourteen anti-microbial compounds were screened.

After docking each drug, nine possible ligand binding possess were generated. Among those the best docked pose was selected with the higher binding affinity. According to docking score and number of non-covalent bonds made, five anti-viral drugs (Figures 2-6) and five anti-microbial drugs (Figures 7-11) were predicted to be the most suitable binding ligands for the nucleocapsid protein (Table 1).

Best docked ligands were selected by considering two parameters. Firstly, binding affinity that is less than -7 kcal/mol (this is not a documented baseline, however we currently focussed on binding affinity) and the number of non-covalent bonds. Non- covalent bonds enable transient but strong binding between protein and ligand.

The anti-viral compounds shortlisted for future studies have been in use either against HIV (Daclatasvir, rilapvirin, tipranavir, etravirine, raltegravir, etravirin) or HCMV (letermovir) (Table 1,2). These shortlisted antivirals show high affinity for binding with SARS-CoV-2 nucleocapsid. These ligands interact with the residues of the RNA binding cleft.

Our drug screening also revealed that some of the anti-microbial drugs also show significantly high binding ability with nucleocapsid protein of SARS-CoV-2 (Table 1,3) and thus these could be further tested for their possible antiviral activities in *in vitro* and virus culture assays.

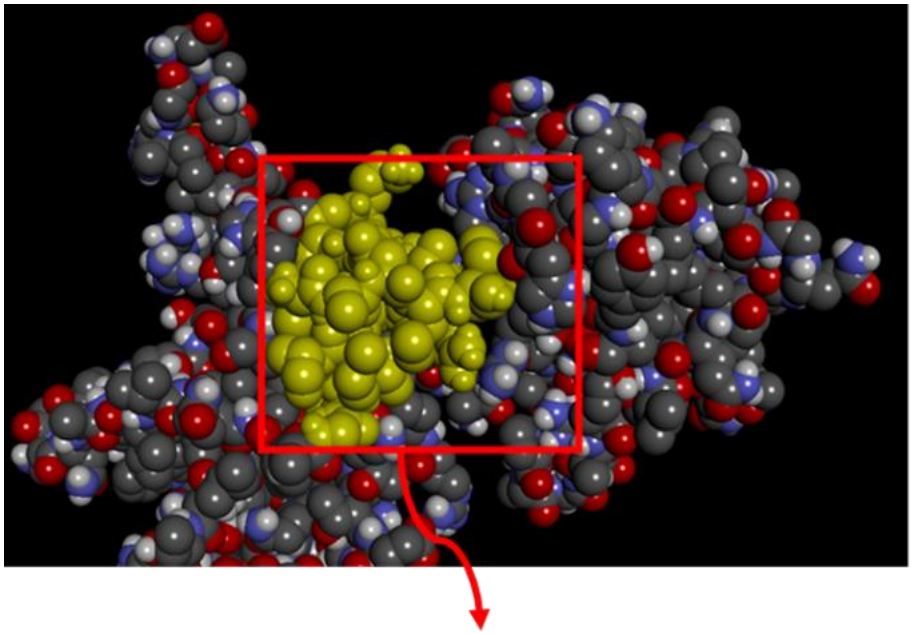


Figure1 : Dimerized nucleocapsid in globular display. Yellow highlighted residues interact with viral mRNA during packaging.

Amino acid residues in the binding pocket (lys66, Arg 69, Tyr124, Tyr110, Pro152, Pro 68, Arg 150, Ala 51, Pro 68) are maximally involved in the interaction with the shortlisted ligands. Since the screened drug candidates are docking into the RNA binding cleft, we hypothesize that they might potentially compete out the viral RNA and thus inhibit nucleocapsid assembly.

We will further check for in depth docking abilities of the shortlisted drugs with nucleocapsid monomers as well as multimers. Mutational analyses and molecular dynamics simulations will shed light on the involvement of the residues predicted to be engaging in nucleocapsid-drug interactions. The possibilities of contribution of lock and key mechanism in binding of the drug at the RNA binding site needs to be verified.

The flexibility of a molecule and presence of water molecule in cellular environment can significantly alter the interaction properties. Thus, all the screened drug molecules need further validations.

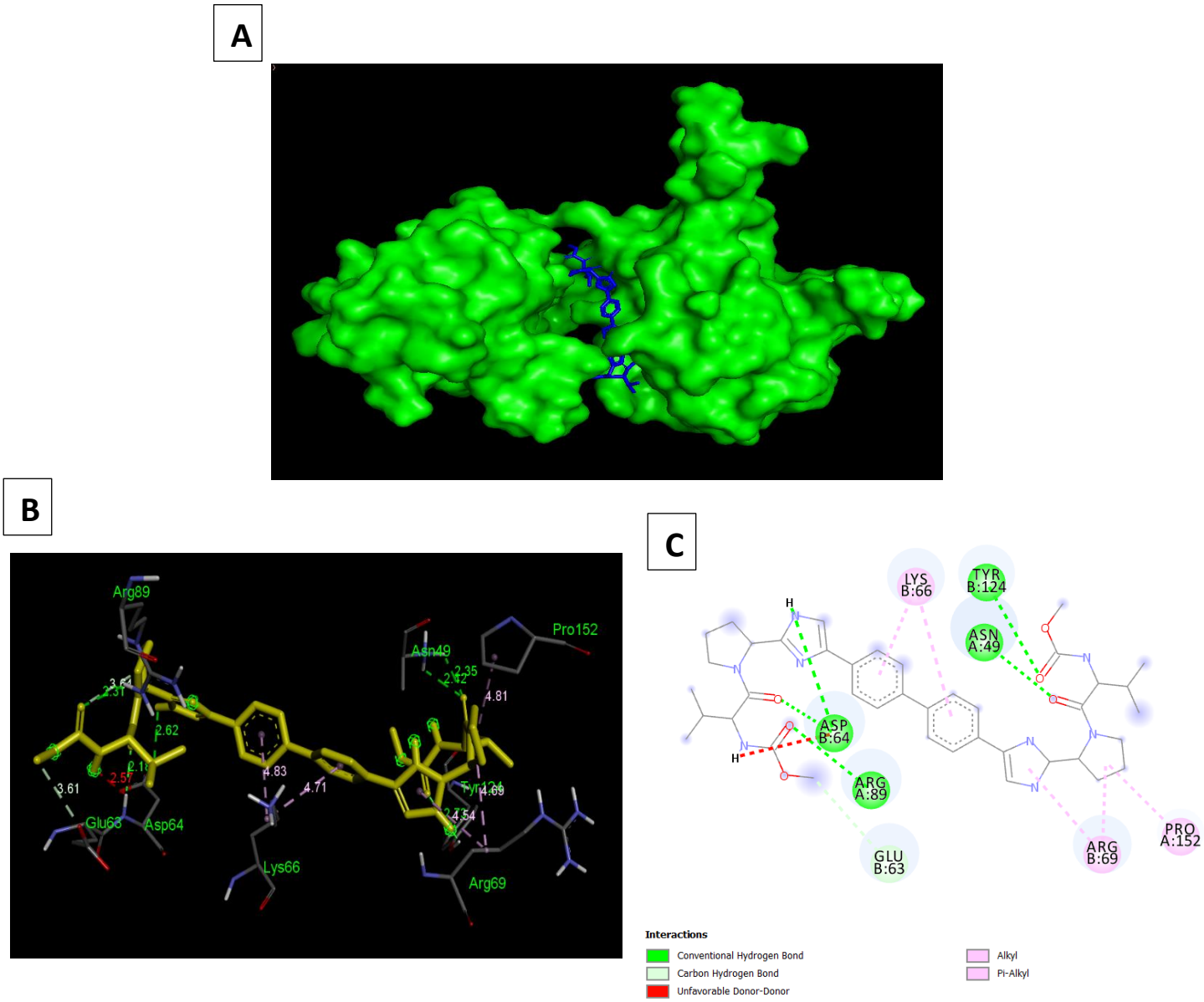


Figure1: SARS- CoV2 nucleocapsid protein docked with anti-viral drug Daclatasvir. A. The best fitted pose of the ligand (blue) in the three-dimensional binding cavity of protein. B. Amino acid residues interacting with the ligand (yellow), bond lengths are indicated. C. The two-dimensional interaction map of the ligand.

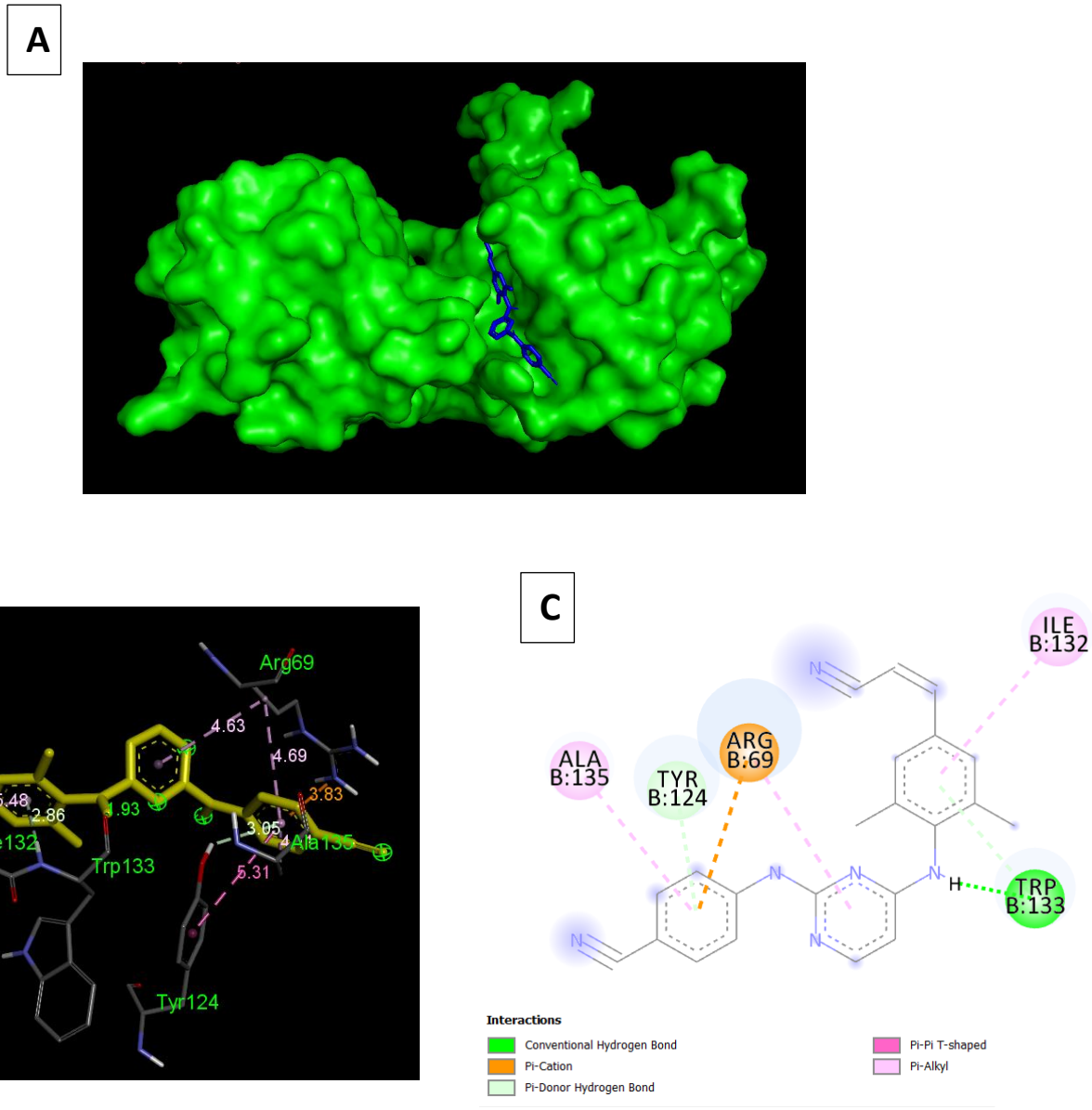


Figure2: SARS- CoV2 nucleocapsid protein docked with anti-viral drug Rilpivirin. A. The best fitted pose of the ligand (blue) in the three-dimensional binding cavity of protein. B. Amino acid residues interacting with the ligand (yellow), bond lengths are indicated. C. The two-dimensional interaction map of the ligand.

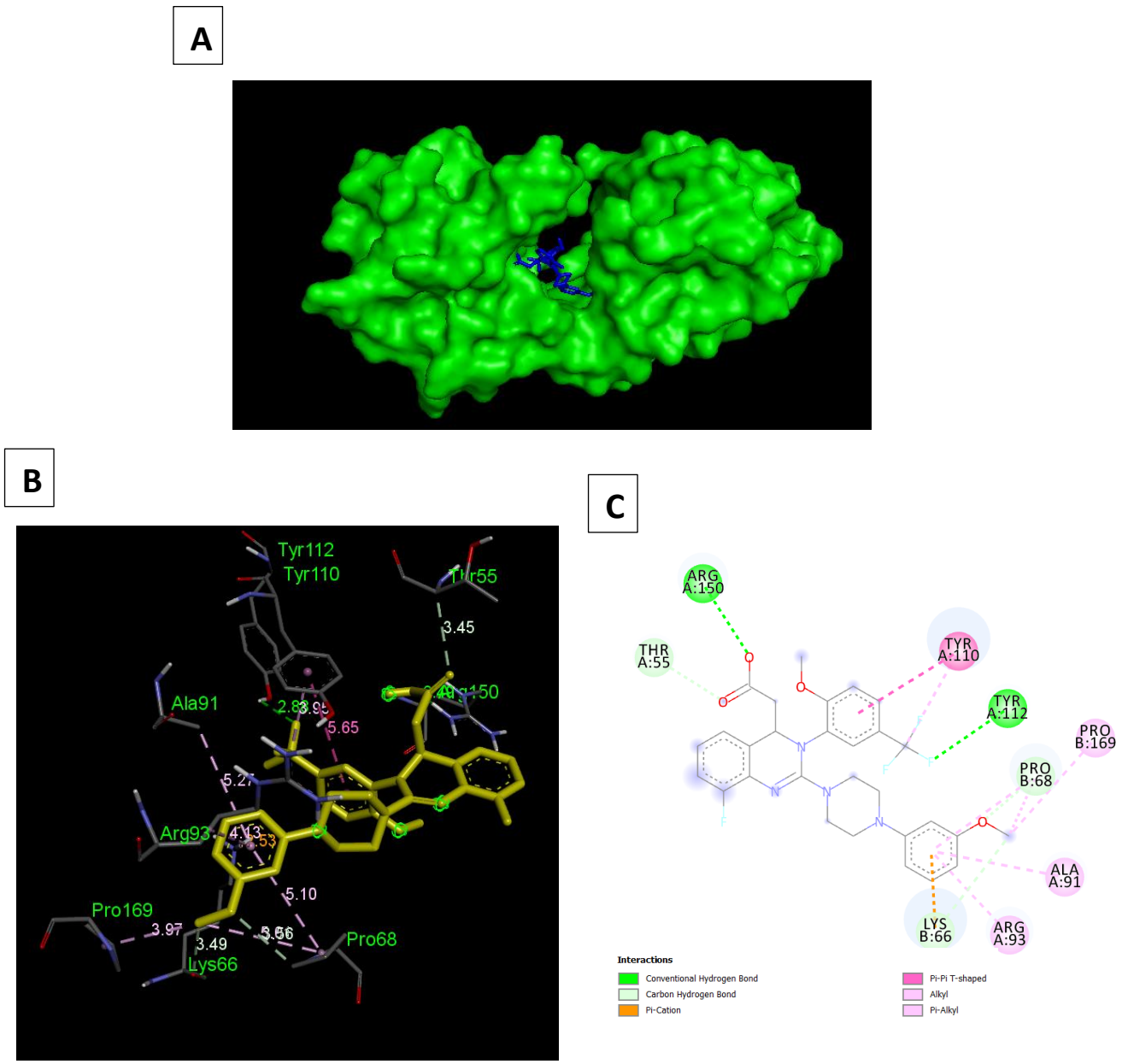


Figure 3: SARS- CoV2 nucleocapsid protein docked with anti-viral drug Letermovir. A. The best fitted pose of the ligand (blue) in the three-dimensional binding cavity of protein. B. Amino acid residues interacting with the ligand (yellow), bond lengths are indicated. C. The two-dimensional interaction map of the ligand.

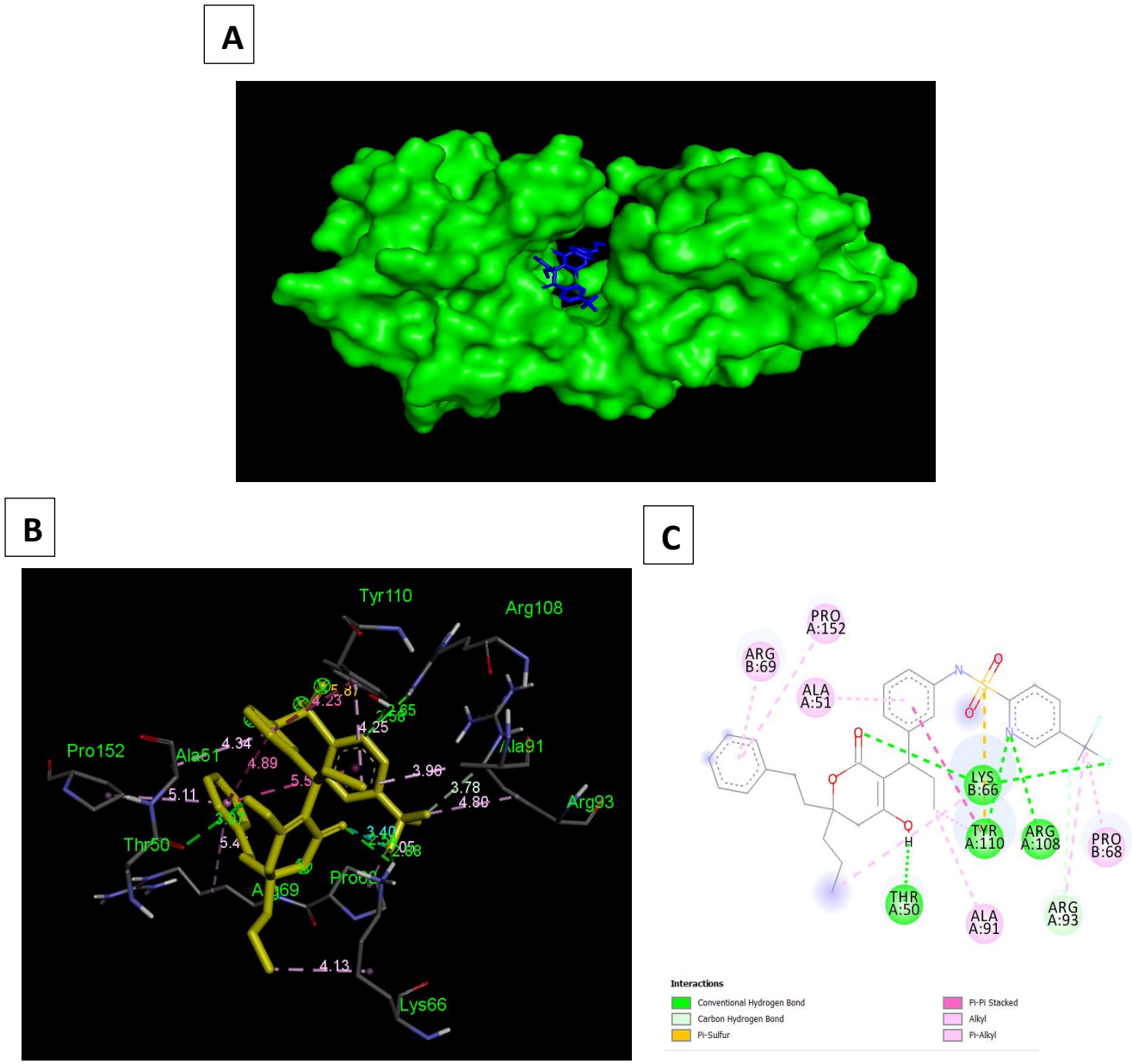
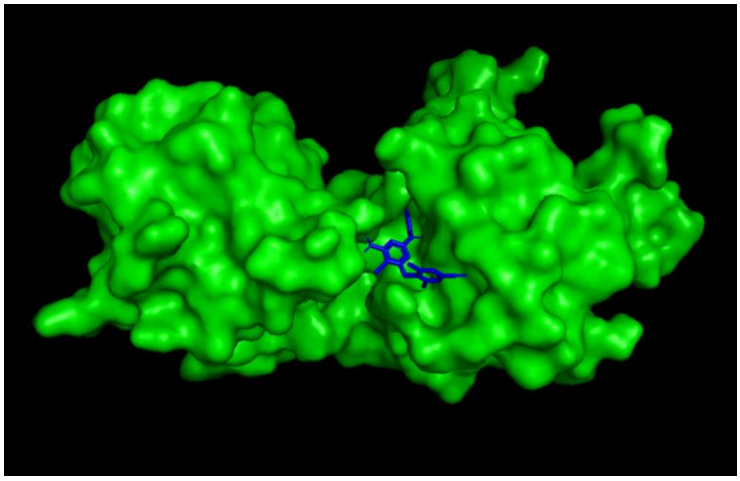
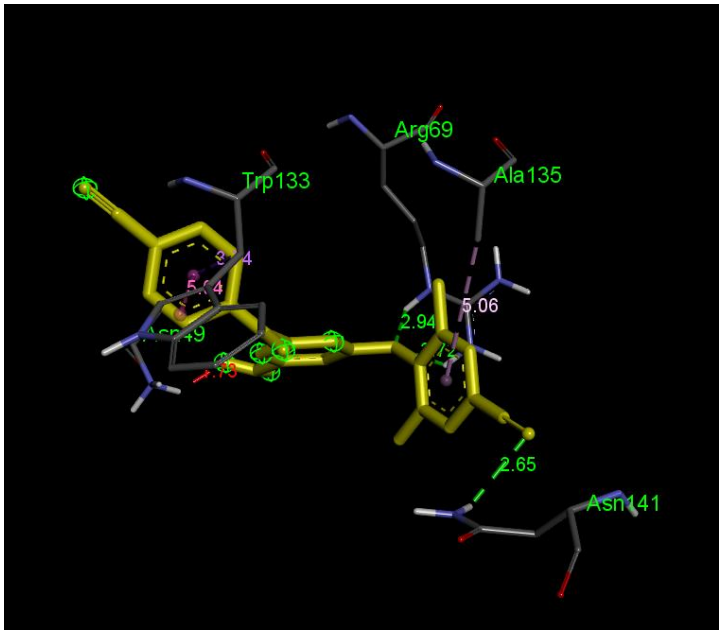


Figure 4: SARS- CoV2 nucleocapsid protein docked with anti-viral drug Tipranavir. A. The best fitted pose of the ligand (blue) in the three-dimensional binding cavity of protein. B. Amino acid residues interacting with the ligand (yellow), bond lengths are indicated. C. The two-dimensional interaction map of the ligand.

A



B



C

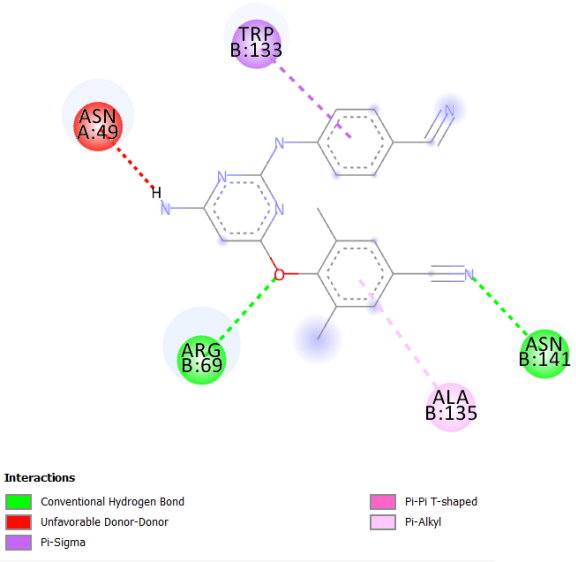


Figure 5: SARS- CoV2 nucleocapsid protein docked with anti-viral drug Etravirin. A. The best fitted pose of the ligand (blue) in the three-dimensional binding cavity of protein. B. Amino acid residues interacting with the ligand (yellow), bond lengths are indicated. C. The two-dimensional interaction map of the ligand.

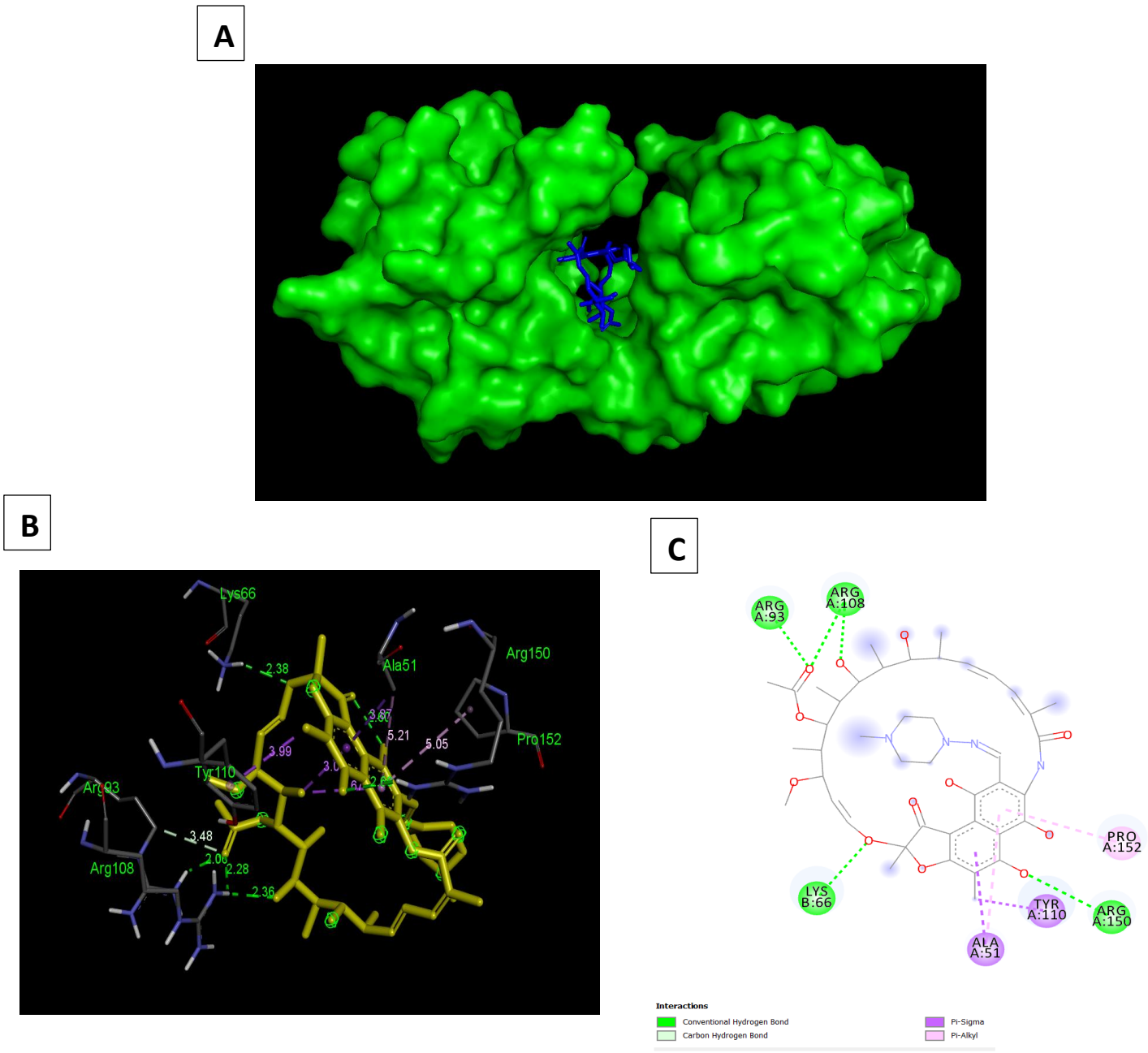
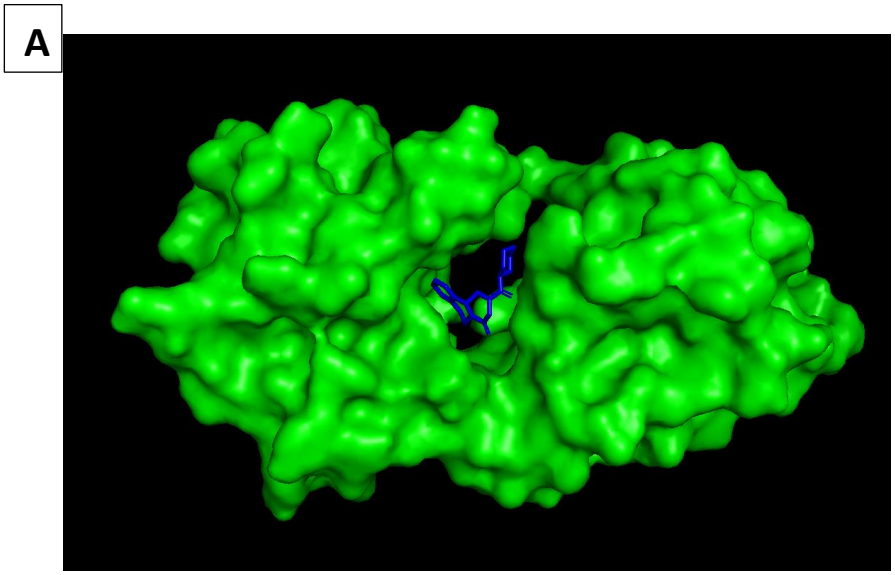
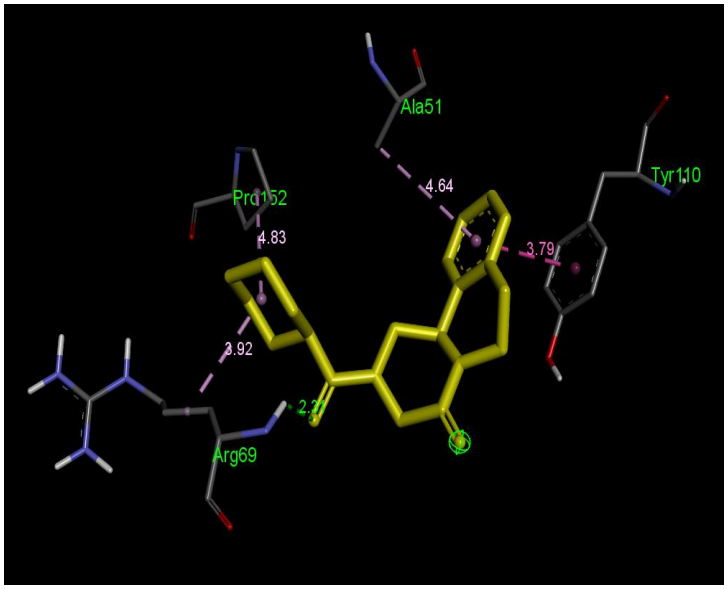


Figure 6: SARS- CoV2 nucleocapsid protein docked with anti-viral drug Rifampicin. A. The best fitted pose of the ligand (blue) in the three-dimensional binding cavity of protein. B. Amino acid residues interacting with the ligand (yellow), bond lengths are indicated. C. The two-dimensional interaction map of the ligand.





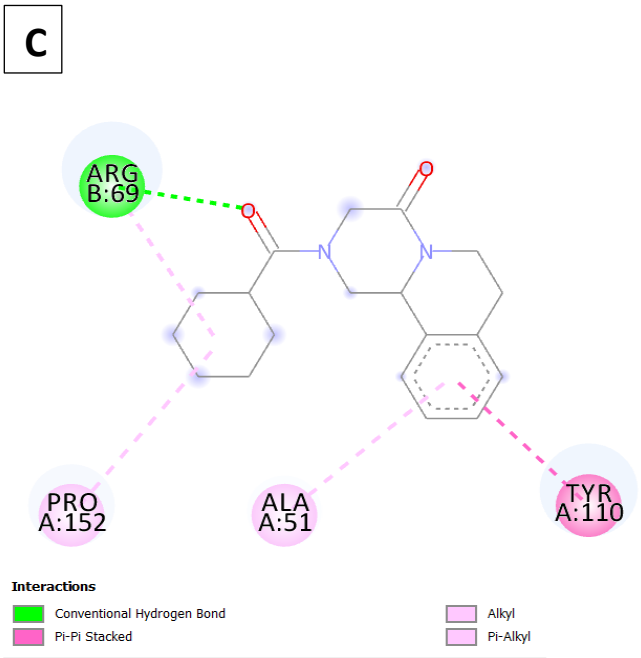


Figure 7: SARS- CoV2 nucleocapsid protein docked with anti-viral drug Praziquantel. A. The best fitted pose of the ligand (blue) in the three-dimensional binding cavity of protein. B. Amino acid residues interacting with the ligand (yellow), bond lengths are indicated. C. The two-dimensional interaction map of the ligand.

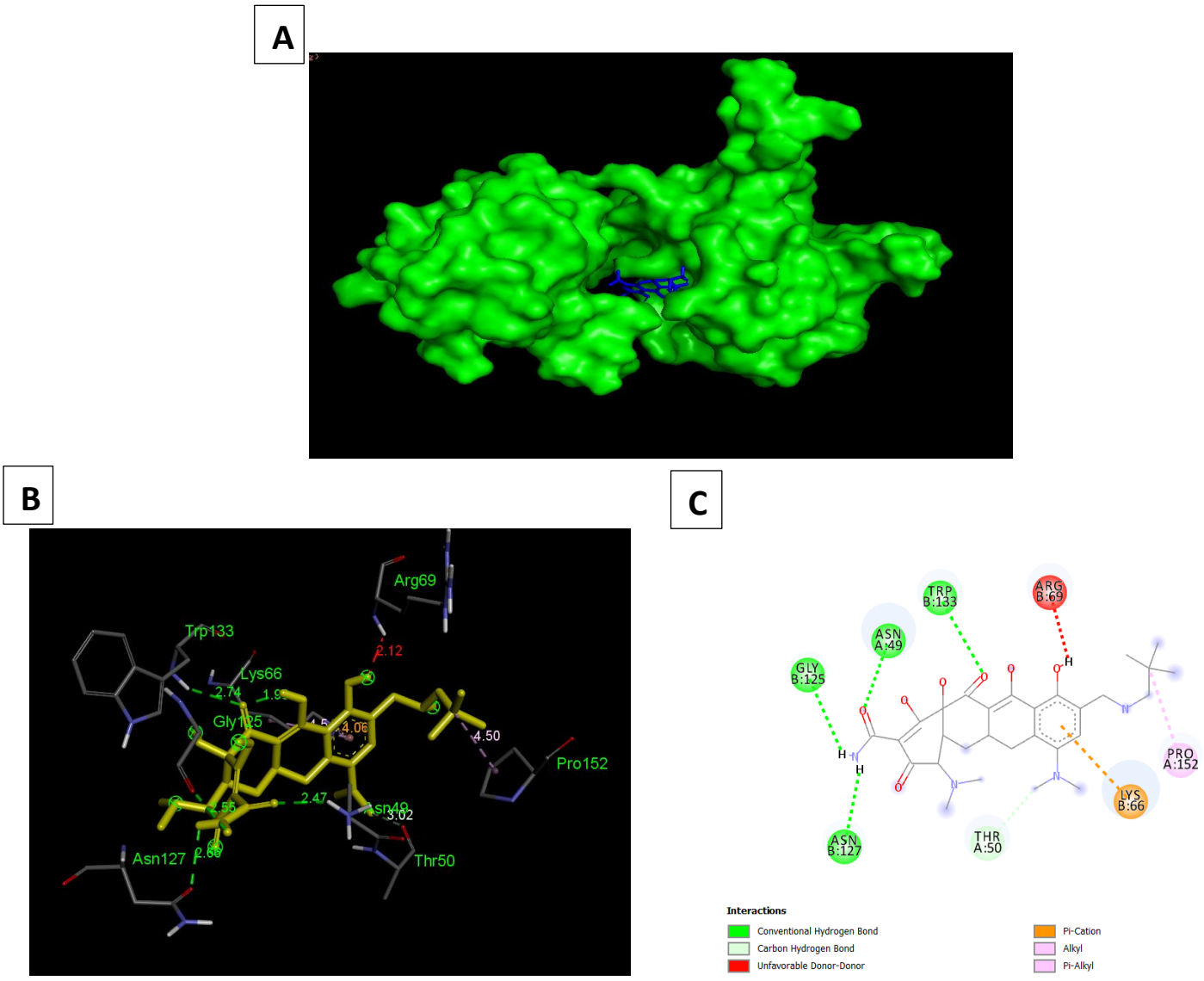


Figure 8: SARS- CoV2 nucleocapsid protein docked with anti-viral drug Omadacycline. A. The best fitted pose of the ligand (blue) in the three-dimensional binding cavity of protein. B. Amino acid residues interacting with the ligand (yellow), bond lengths are indicated. C. The two-dimensional interaction map of the ligand.

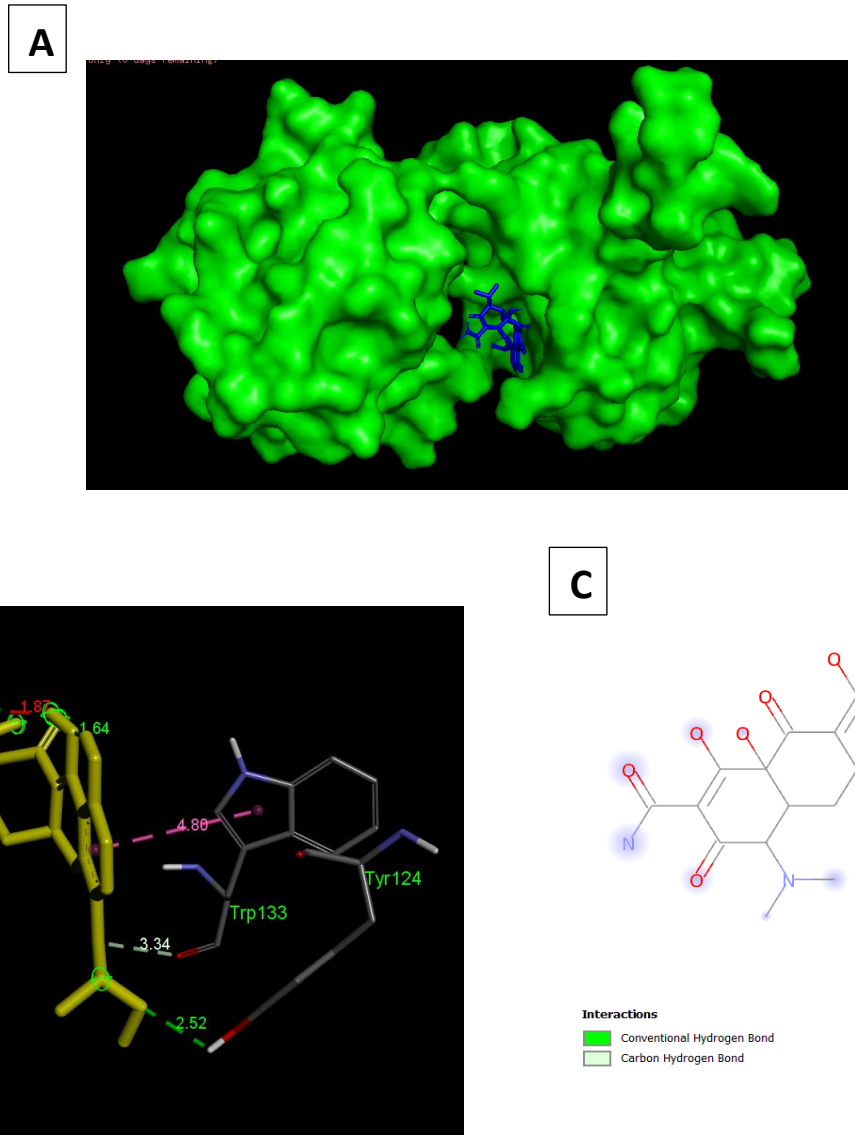


Figure 9: SARS- CoV2 nucleocapsid protein docked with anti-viral drug Sarecycline. A. The best fitted pose of the ligand (blue) in the three-dimensional binding cavity of protein. B. Amino acid residues interacting with the ligand (yellow), bond lengths are indicated. C. The two-dimensional interaction map of the ligand.

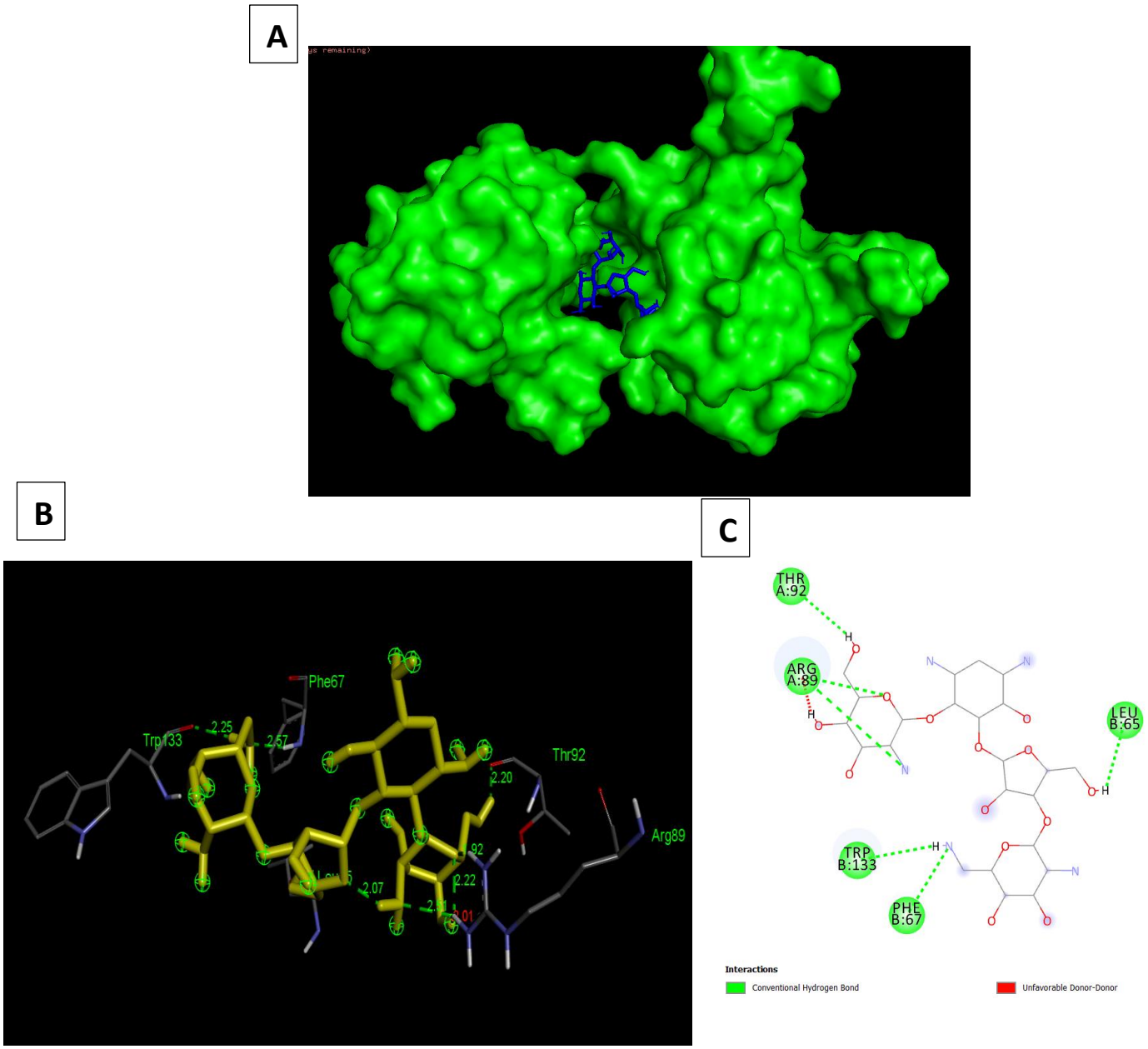


Figure 10: SARS- CoV2 nucleocapsid protein docked with anti-viral drug Paromomycin. A. The best fitted pose of the ligand (blue) in the three-dimensional binding cavity of protein. B. Amino acid residues interacting with the ligand (yellow), bond lengths are indicated. C. The two-dimensional interaction map of the ligand.

Type of drug	Name of drug (Drug Bank ID)	Lipophilicity (logP)	Binding affinity (kcal/Mol)	Types of bond	Interacting amino acids	Bond length (Å)
Anti-viral	**Daclatasvir** (DB09102)	4.67	-8.1	H bond C-H bond Pi-alkyl	Arg 89 (A) Asp 64 (B) (2) Asn 49 (A) (2) Tyr 124 (B) Glu 63 (B) Lys 66 (B) (2) Arg 69 (B) (2) Pro 152 (A)	2.3 2.6, 2.1 2.3, 2.4 2.7 2.7 4.8, 4.7 4.5, 4.6 4.8
Anti-viral	**Rilpivirine** (DB08864)	3.8	-7.8	H bond Pi-cation Pi-donor H bond Pi-Pi T shaped Pi-alkyl	Trp133 (B) Arg 69 (B) Tyr 124 (B) Tyr 124 (B) Ala 135 (A) Arg 69 (B) (2) Ile 132 (B)	1.9 3.8 3.0 5.3 4.1 4.6, 4.6 5.4
Anti-viral	**Letermovir** (DB12070)	4.58	-7.6	H bond C-H bond Pi-cation Pi-Pi T shaped Pi-alkyl	Arg 150 (A) Tyr 112 (A) Tyr 55 (A) Lys 66 (B) Pro 68 (B) Lys 66 (B) Tyr 110 (A) Tyr 110 (A) Pro 68 (B) (2) Pro 169 (B) Ala 91 (A) Arg 93 (A)	2.4 2.8 3.4 3.4 3.5 2.5 5.6 3.9 5.0, 5.1 3.9 5.2 4.1
Anti-viral	**Tipranavir** (DB00932)	6.29	-7.5	H bond C-H bond Pi-Sulfur Pi-Pi stacked Pi-alkyl	Thr 50 (A) Tyr 110 (A) Arg 108 (A) Lys 66 (B) (2) Arg 93 (A) Tyr 110 (A) Tyr 110 (A) Arg 69 (B) Pro 152 (A)	3.0 2.5 2.6 2.5, 2.8 3.7 5.8 4.2 5.4 5.1

					Ala 51 (A) Lys 66 (B) Ala 91 (A) Arg 93 (A) Pro 68 (B)	4.3 4.1 3.9 4.8 4.0
Anti-viral	**Etravirine** (DB06414)	3.6	-7.0	H bond Pi-sigma Pi-Pi T shaped Pi-alkyl	Arg 69 (B) (2) Asn 141 (B) Trp 133 (B) Trp 133 (B) Ala135 (B)	2.4, 2.7 2.6 3.7 5.0 5.0
Anti-bacterial	**Rifampicin** (DB01045)	3.85	-8.6	H bond C-H bond Pi-sigma Pi-alkyl	Arg 93 (A) Arg 108 (A) (2) Lys 66 (B) Arg 150 (A) Arg 93 (A) Ala 51(A) Tyr 110 (A) Ala 51(A) Pro 152 (A)	2.0 2.2, 2.3 2.3 2.6 3.4 3.8 3.9 5.2 5.0
Anti-bacterial	**Praziquantel** (DB01058)	2.42	-7.3	H bond Pi-Pi stacked Pi-alkyl	Arg 69 (B) Tyr 110(A) Ala 51(A) Pro 152 (A) Arg 69 (B)	2.3 3.7 4.6 4.8 3.9
Anti-bacterial	**Omadacycline** (DB12455)	0.94	-7.2	H bond C-H bond Pi-cation Pi-alkyl	Asn 127 (B) Gly 125 (B) Asn 49 (A) Trp 133 (B) Thr 50 (A) Lys 66 (B) Pro 152 (A)	2.6 2.5 2.4 2.7 3.0 4.0 4.5
Anti-bacterial	**Paromomycin** (DB01421)	2.9	-7.0	H bond	Thr 92 (A) Arg 89 (A) (3) Trp 133 (B) Phe 67 (B) Leu 65 (B)	2.2 1.9, 2.2, 2.5 2.2 2.5 2.2

Anti-bacterial	**Sarecycline** (DB12035)	0.17	-7.1	H bond C-H bond	Tyr 124 (B) Trp 133 (B) Trp 133 (B)	2.5 3.3 4.8

Table1: Details of shortlisted drugs

Name of drug	Approved against	Mode of Action	Drug bank ID
Daclatasvir	HCV	Daclatasvir binds to the N-terminus of the D1 domain of NS5A of hepatitis C virus This domain interacts with various host cell proteins and membranes during replication complex formation.	DBSALT001166
Rilpivirine	HIV	Rilpivirine binds to reverse transcriptase (RT) enzyme of HIV-1 and blocks replication in a non-competitive manner.	DB08864
Letermovir	HCMV	DNA terminase complex of human cytomegalovirus is required to cut the DNA before virus assembly. Letermovir blocks the DNA terminase complex thus inhibiting DNA processing.	DB12070
Tipranavir	HIV	Tipranavir inhibit the formation of functional viral protein by binding to viral protease.	DB00932
Etravirine	HIV	Etravirine directly binds and inhibits reverse transcriptase enzyme activity HIV -1	DB06414

Table2: Mode of action of shortlisted anti-viral drugs

Name of drug	Approved against	Mode of Action	Drug bank ID
Rifampicin	Bacteria	Rifampin blocks RNA synthesis in bacterial cells by inhibition of DNA- dependent RNA polymerase.	DB01045
Praziquantel	Parasites	Pentamidine acts against parasite worms' muscle by creating muscle contraction with rapid Ca^{2+} influx	DB01058
Omadacycline	Bacteria	Omadacycline blocks the 30S ribosomal subunit of bacteria and inhibit protein synthesis.	DB12455
Paromomycin	Enteric bacteria	Paromomycin inhibit bacterial protein synthesis by binding to the 16S ribosomal RNA	DB01421
Sarecycline	Bacteria	Sarecycline targets microbial protein synthesis and affect the anti-inflammatory response	DB12035

Table3: Mode of action of shortlisted anti-microbial drugs

Acknowledgements

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