Drug repurposing commonly against Dengue Virus capsid and SARS-CoV-2 nucleocapsid: An *in silico* approach

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Abstract:

In the middle of SARS-CoV-2 pandemic, dengue virus (DENV) is giving a silent warning as the season approaches nearer. There is no specific antiviral against DENV for use in the clinics. Thus, considering these facts we can potentially face both these viruses together increasing the clinical burden. The search for anti-viral drugs against SARS-CoV-2 is in full swing and repurposing of already 'in-use' drugs against other diseases or COVID-19 has drawn significant attention. Earlier we had reported few FDA approved anti-viral and anti-microbial drugs that could be tested against SARS-CoV-2 nucleocapsid assembly. We explored the possibility of interactions of these shortlisted drugs against SARS-CoV2 with Dengue virus proteins. Here we report three FDA approved drugs (Daclatasvir, Letermovir and Rifampicin) that were seen to be docking onto the SARS-CoV-2 nucleocapsid RNA binding domain, also docking strongly with DENV capsid protein on the RNA binding site and/or the capsid's membrane fusion domain. Thus, the present study proposes these three drugs as common antiviral candidates against both SARS-CoV-2 and DENV.

Introduction:

In the tropical and sub-tropical countries, Dengue virus (DENV) remains a constant threat to human life. According to the world health organisation (WHO), every year 100-400 million cases of dengue virus infections are reported all over the world (1).

Capsid protein, one of the structural proteins of DENV plays a key role in the virus assembly by packaging the viral RNA to form mature virion particle. This 12KDa protein is rich in basic and hydrophobic amino acids which ensures its role in RNA binding and membrane fusion. The protein forms a homodimer conformation with each monomer consisting of four alpha helical chains (α1 -α4) (Figure 1) and the N- terminal unstructured region (2). Helix α1-α3 forms the hydrophobic core of the protein and the longest helix α4 extends out of the core to interact with the negatively charged viral RNA (2). The α1 and α2 helices are mostly composed of uncharged hydrophobic residues with a conserved membrane fusion domain in α2 helix which creates the lipid membrane binding pocket (3, 4). On the other hand, interaction of α4-α4' helix enables nucleic acid binding and stabilise the nucleocapsid core formation (5).

Apart from RNA packaging, the DENV capsid also induces FAS-dependent apoptosis and regulate transcription by binding with histones (6, 7).

Repurposing of clinically approved drugs against the multifunctional capsid protein could be beneficial for treatment/ management of severities of DENV infection. We reported previously that some of the FDA (food and drug administration US) approved antiviral and anti-microbial drugs dock strongly with RNA binding region of SARS-CoV2 nucleocapsid protein and thus might interfere with nucleocapsid-RNA interaction (8). Here, we screened interaction properties of the same shortlisted drugs (Table 1) with DENV capsid protein to

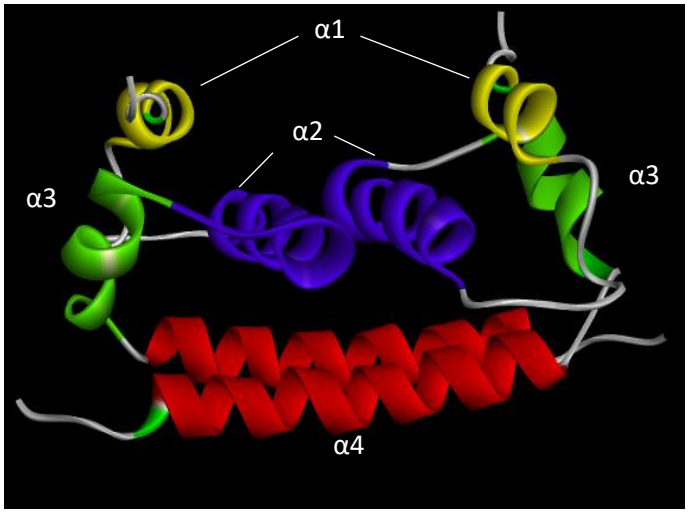

Figure1: Dimeric structure of DENV- capsid protein, helix α1 is yellow, α2 is blue, α3 is green and α4 is red

investigate the possibility of identification of common drug candidate/s that can be used to target both the viruses. This was a blind drug exploration.

Table 1: Mode of action of screened drugs (Drugs shortlisted are highlighted in blue)

Nature of drug	Drug name	Mechanism of action	Drug bank ID
Anti-viral	**Daclatasvir**	Daclatasvir acts against hepatitis C virus by inhibiting D1 domain of NS5A	DB09102
	Letermovir	Letermovir binds to DNA terminase complex of human cytomegalo virus and thus blocks DNA processing required for virus assembly	DB12070
	Tipranavir	Tipranavir binds to HIV-1 protease and inhibit polyprotein processing	DB00932
	Fosamprenavir	Fosamprenavir blockes polyprotein processing HIV-1 by inhibiting protease activity	DB01319
	Elvitegravir	Elvitegravir inhibit integration of HIV-1 genome into host	DB09101

		genome by binding to integrase enzyme	
	Etravirine	Etravirine acts against reverse transcriptase (RT) enzyme of HIV-1	DB06414
	Rilpavirine	Rilpavirine binds to reverse transcriptase (RT) enzyme of HIV-1 and inhibits replication	DB008864
Anti-microbial	**Rifampicine**	Rifampicine targets bacterial DNA dependent RNA polymerase and inhibit function mRNA synthesis	DB01045
	Omadacycline	Omadacycline blocks protein synthesis by binding to bacterial 30S ribosomal subunit	DB12455
	Sarecycline	Sarecycline is targeted against microbial protein synthesis and anti-inflammatory response.	DB12035
	Paromomycin	Paromomycin targets 16S ribosomal RNA and inhibit protein systhesis	DB01421
	Praziquantel	Praziquantel influence worms' muscle contraction by Ca^{2+} influx	DB01058
	Pentamidine	Pentamidine affect nuclear metabolism of DNA, RNA and protein	DB00738
	Sulfadiazine	Sulfadiazine blocks folic acid biosysthesis pathway by inhibiting dihydropteroate synthetase enzyme	DB00359

Methods:

A set of FDA approved anti-viral and anti-microbial drugs (Table 1) were screened to identify potential antiviral candidate/s against DENV capsid protein function.

The three-dimensional crystal structure of Dengue virus capsid protein (PDB ID: 1R6R) was downloaded from Protein Data Bank (PBD) (9). The protein was converted to a readable file format (.pdbqt) using AutoDock Tools 1.5.6 (10), after removing the solvent molecules and protonated with polar hydrogens.

The structures of the drug molecules were retrieved from DrugBank (11) in PDB format and then converted to supported format (.pdbqt) by AutoDock Tools 1.5.6 (10).

All the drug compounds were virtually screened against DENV capsid protein using AutoDock vina (12). A blind docking was performed keeping the grid centre set at X = 2.041, Y = -7.998, Z = 1.282 and grid-box dimensions at 88Å X 62Å X 62Å with the exhaustiveness value 8.

Among all the ligands top three drugs namely Daclatasvir, Letermovir and Rifampicin interacted with significantly high binding affinities (less than -8.0Kcal/Mol) and selected for further analysis using Discovery Studio Visualizer v20.1.0.19295 (13) and PyMol software (14).

Results and discussion:

Total fourteen anti-viral and anti-microbial drugs were screened against DENV capsid and the binding affinities obtained for Daclatasvir, Letermovir and Rifampicin are -9.1Kcal/mol, -8.4Kcal/mol and -9.5Kcal/mol respectively. Binding affinities of all drugs were summarized in Table 2 and the interacting residues for selected ligands is enlisted in Table 3. The common residues of Dengue virus capsid interacting with all the three drugs are Leu 66 and Ile 94.

Daclatasvir acts on non-structural protein 5A of Hepatitis C virus by interrupting its hyperphosphorylation (15). The key interacting residues of Dengue virus capsid with Daclatasvir are Arg 97 (B), Arg 55(A) by conventional hydrogen bonds and Glu 87 (B), Leu 66 (A), Ile 94 (B), Ile 59 (A), Glu 87 (B) by other non-covalent interactions (Figure 2). Arg 55 is located within alpha helix 2 (α2) of the protein that is involved in formation of hydrophobic cleft (α2-α2') that interacts with the lipid membrane (2). Daclatasvir also interacts with Ile 59 which is one of the residues present in conserved membrane fusion domain. Apart from the membrane fusion domain Daclatasvir binds to residues of alpha helix 4 (α4) that are important for Capsid-RNA interactions.

Letermovir which targets DNA terminase complex of human cytomegalovirus (HCMV) (16), creates the highest number of non-covalent bonds with DENV capsid protein consisting maximum Pi-alkyl bonds. Letermovir binds through Arg 22 by hydrogen bond and other non-covalent bonds by Arg 55 (B), Leu 66 (B), Ile 94 (A), Pro 61 (B) (2), Arg 22 (B), Ile 94 (A), Arg 90 (A) (Figure 3). Letermovir also interacts with crucial residues for membrane fusion (Arg 55)

and RNA encapsulation (Ile 94, Arg 90). However, there are unfavourable donor-donor bonds also predicted for both Daclatasvir and Letermovir.

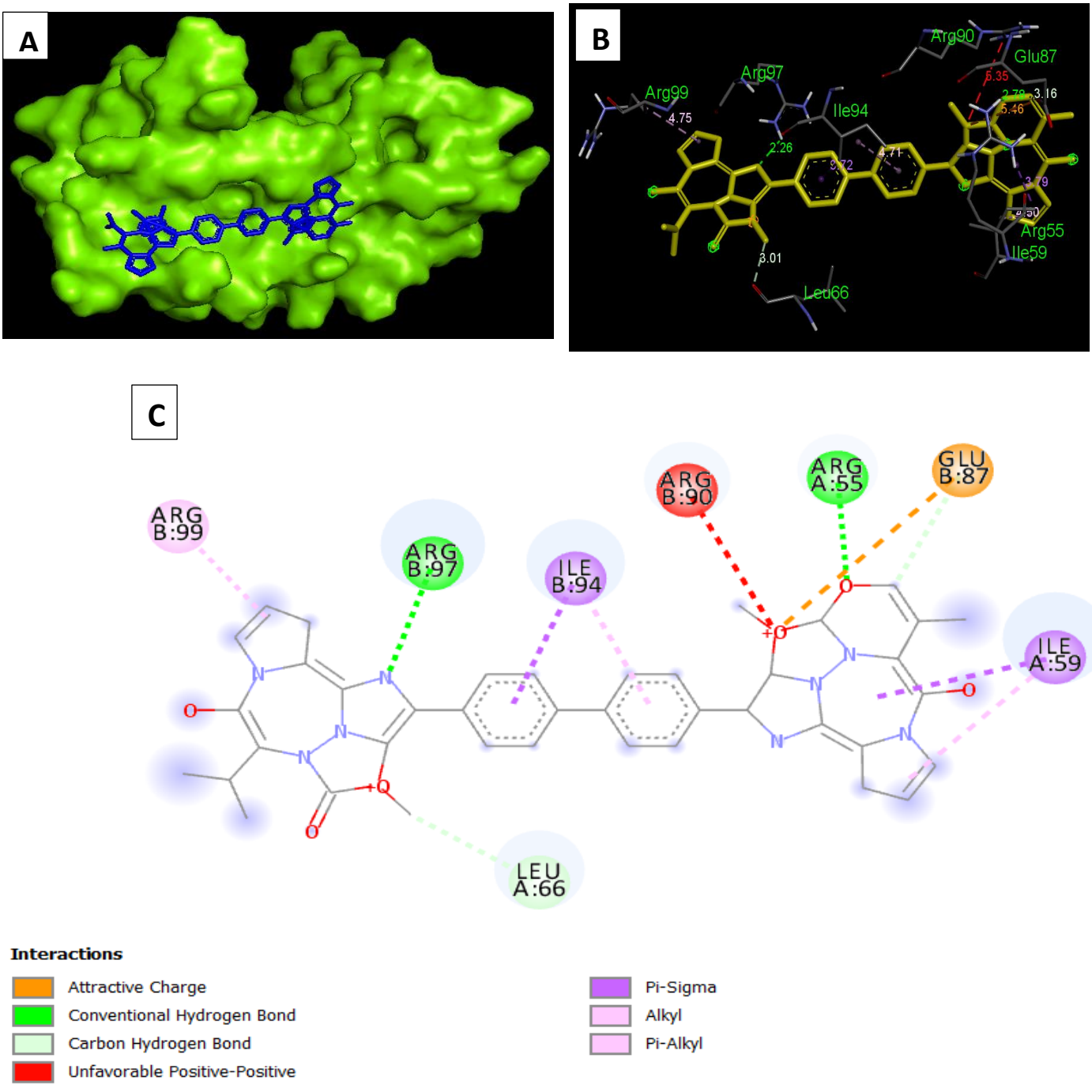

Figure2: DENV capsid protein docked with anti-viral drug Daclatasvir. A. The best fitted pose of the ligand (blue) in the three-dimensional binding cavity of protein. B. Amino acid residues interacting with the ligand (yellow), bond lengths are indicated. C. The two-dimensional interaction map of the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

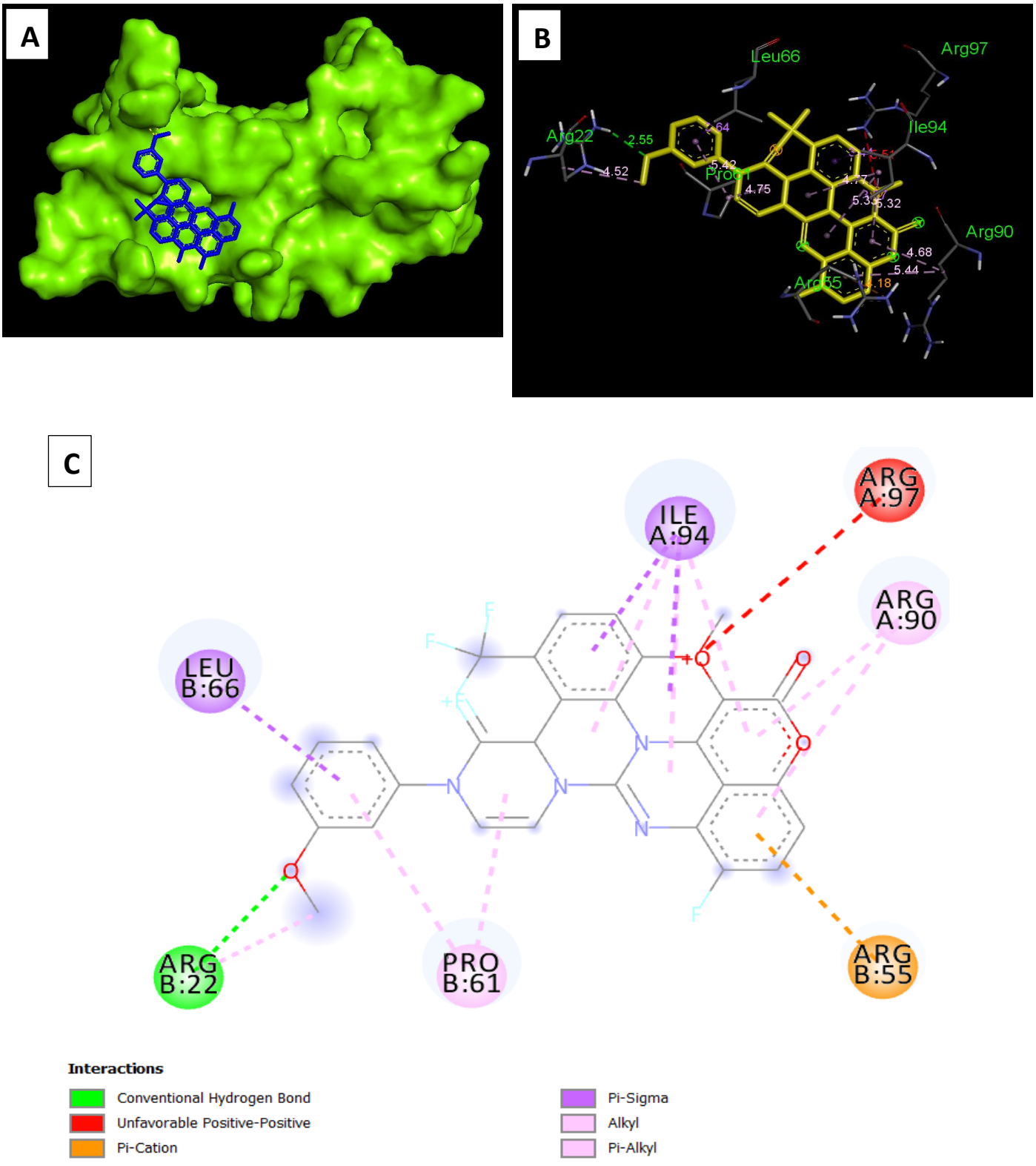

Figure3: DENV capsid protein docked with anti-viral drug Letermovir. A. The best fit pose of the ligand (blue) in the three-dimensional binding cavity of protein. B. Amino acid residues interacting with the ligand (yellow), bond lengths are indicated. C. The two-dimensional interaction map of the ligand.

Rifampicin is widely used as an anti-bacterial against several strains of mycobacterium by forming a stable complex with DNA-dependent RNA polymerase (17). *In-silico* docking predicts rifampicin as an interacting partner with DENV capsid consisting highest binding affinity in this study. Rifampicin interacts with the residues of alpha helix 3 (Lys 67 and Leu 66) and alpha helix 4 (Ile 94 and Ile 97) of capsid protein (Figure 4). Alpha helix 3 creates the hydrophobic core of the protein and acts as a scaffold between α1 and α4. Alpha helix 4 is extended outward of the core protein and possess positively charged residues which facilitates its interaction with negatively charged viral-RNA.

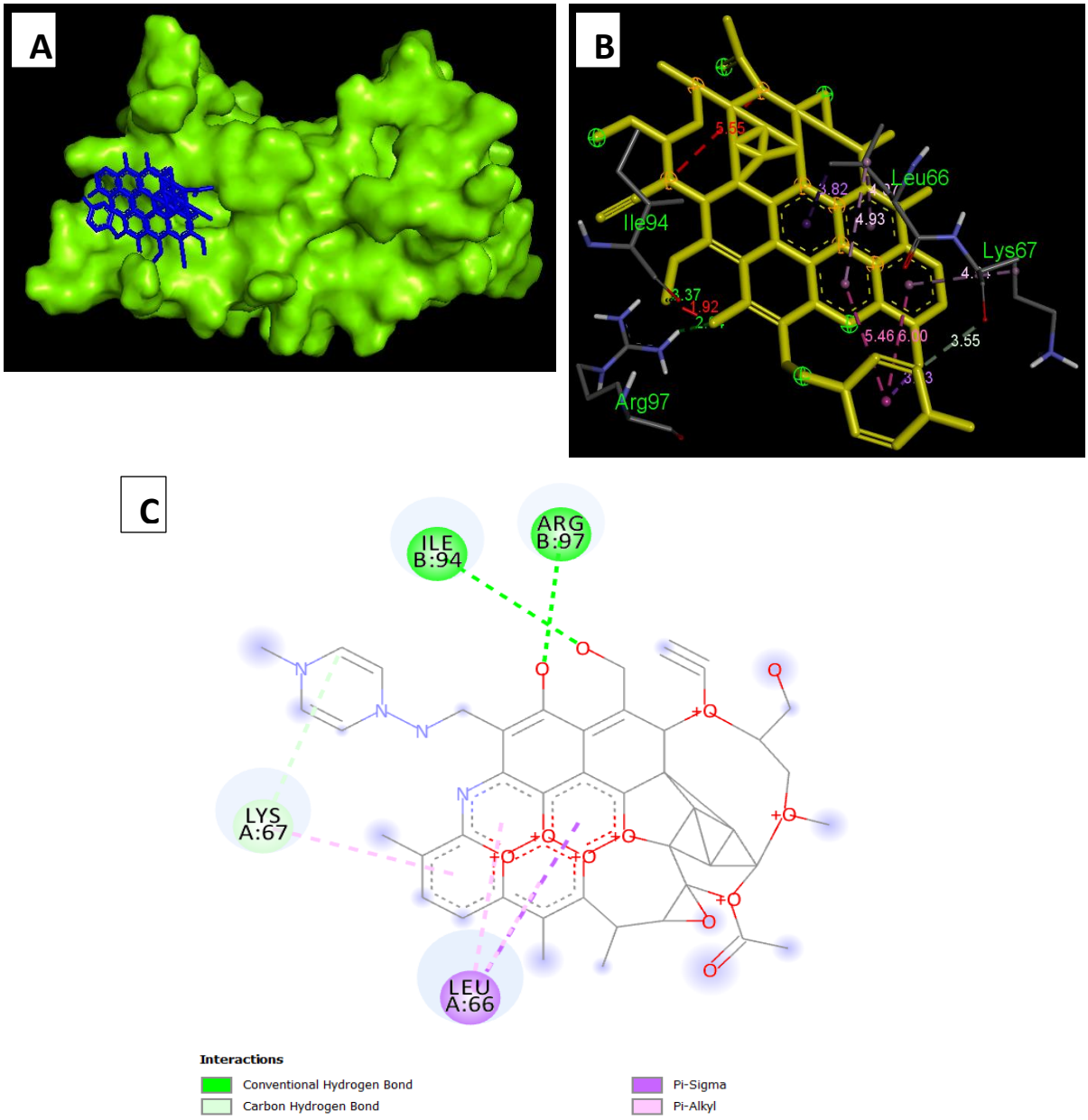

Figure4: DENV capsid protein docked with anti-viral drug Rifampicin. A. The best fit pose of the ligand (blue) in the three-dimensional binding cavity of protein. B. Amino acid residues interacting with the ligand (yellow), bond lengths are indicated. C. The two-

Summary:

Capsid protein is around 80% conserved among all four DENV serotypes (18) hence it might be a strong target for anti-viral drugs. The present study shed light on three potential drug candidates which were previously described as potential inhibitors of SARS-CoV2 nucleocapsid-RNA interaction with highest binding affinities. The drug candidates Daclatasvir, Letermovir and Rifampicin have been predicted to have the potential of intervening with DENV capsid protein membrane integration and viral-RNA packaging which in turn affects mature virion particle formation (Figure 5). However, ligands are complex chemical structures with several reactive atoms, so the chance of creating bonds is significantly high. Hence, to extend this study and further confirm, we will perform molecular dynamics studies. The inhibitory properties of suggested drugs need validations using *in-vitro* and *ex-vivo* assays.

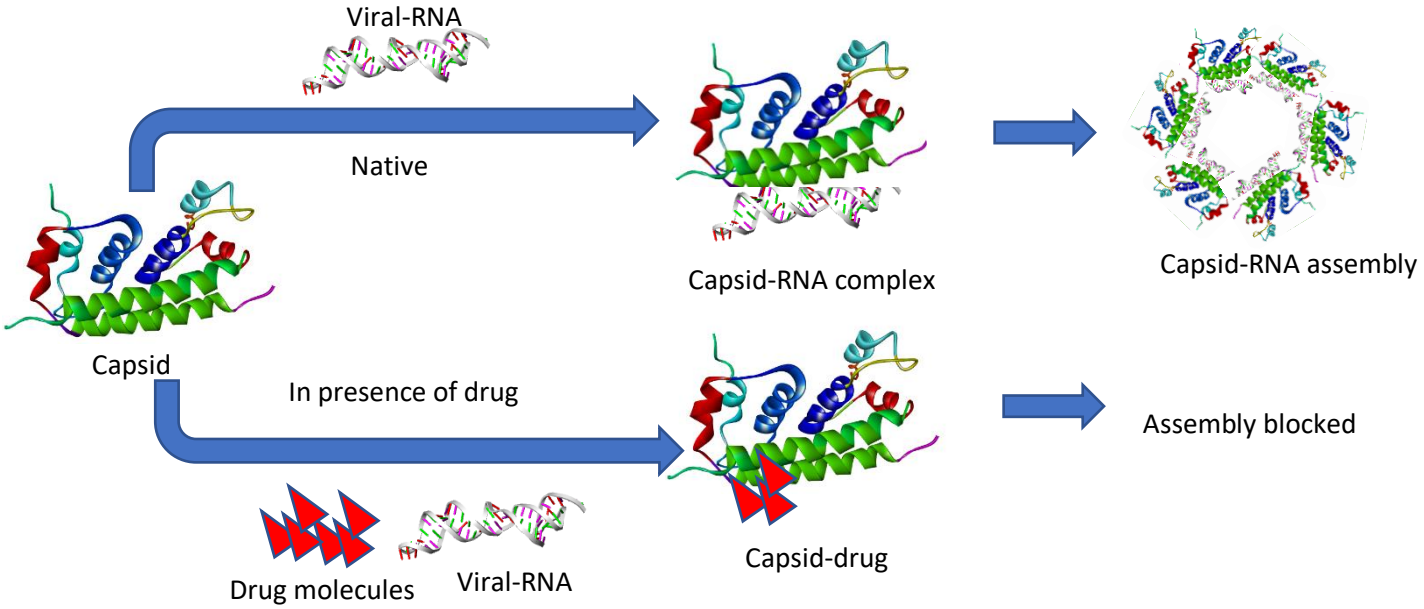

Figure 5: Schematic diagram capsid interaction. In native condition viral RNA binds to helix α4 (green) and assemble as to mature virion. In presence of drug molecules binding site is occupied by drug and RNA-capsid interaction is blocked.

Table 2: Binding Affinities of all screened drugs (shortlisted drugs are coloured in blue)

Nature of drug	Drug name	Binding affinity (Kcal/mol)
Anti-viral	**Daclatasvir**	**-9.1**
	Letermovir	**-8.4**
	Tipranavir	-7.6
	Fosamprenavir	-7.3
	Elvitegravir	-7.2
	Etravirine	-7.0
	Rilpavirine	-6.8
	Rifampicine	**-9.5**
	Omadacycline	-7.5

Anti-microbial	Sarecycline	-6.9
	Paromomycin	-6.3
	Praziquantel	-6.0
	Pentamidine	-5.5
	Sulfadiazine	-5.2

Table 3: Interacting amino acids of Daclatasvir, Letermovir and Rifampicin

Nature of drug	Drug name	Binding affinity (Kcal/mol)	Types of bond	Interacting amino acids	Bond length (Å)
Anti-viral	**Daclatasvir**	-9.1	H-bond	Arg 97 (B) Arg 55 (A)	2.2 2.7
			Attractive charge	Glu 87 (B)	5.4
			C-H bond	Glu 87 (B) Leu 66 (A)	3.1 3.0
			Pi-sigma	Ile 94 (B) Ile 59 (A)	3.7 3.7
			Pi-alkyl	Ile 94 (B) Glu 87 (B)	4.7 4.5
	Letermovir	-8.4	H-bond	Arg 22 (B)	2.5
			Pi- cation	Arg 55 (B)	4.1
			Pi-sigma	Leu 66 (B) Ile 94 (A) (2)	3.6 3.4, 3.7
			Pi-alkyl	Pro 61 (B) (2) Arg 22 (B) Ile 94 (A) (3) Arg 90 (A) (2)	5.4,4.7 4.5 4.7, 5.3, 5.3 4.6, 5.4
Anti-microbial	**Rifampicin**	-9.5	H bond	Ile 94 (B) Arg 97 (B)	3.3 2.6
			C-H bond	Lys 67 (A)	3.5
			Pi-sigma	Leu 66 (A)	3.8
			Pi-alkyl	Lys 67 (A) Leu 66 (A) (2)	4.9 4.9,4.0

Acknowledgements

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