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Re-purposing of hepatitis C virus FDA approved direct acting antivirals as potential SARS-CoV-2 protease inhibitors

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ABSTRACT

A new coronavirus strain called as SARS-CoV-2 has emerged from Wuhan, China in late 2019 and it caused a worldwide pandemic in a few months. After the Second World War, it is the biggest calamity observed as there is no specific US Food and Drugs Administration (USFDA) approved drug or vaccine available globally for the treatment. Several clinical trials are ongoing for therapeutic alternatives, however with little success rate. Considering that the time is crucial, the drug repurposing and data obtained from *in silico* models are one of the most important approaches to identify possible lead inhibitors against SARS-CoV-2. More recently, the Direct Acting Antivirals (DAAs) are emerged as the most promising drugs to control viral infection. The Main Protease (Mpro), a key enzyme in the SARS-CoV-2 replication cycle, is found close homolog to the Hepatitis C Virus (HCV) protease and could be susceptible of blocking its activity by DAAs. In the current study, the DAAs were investigated as antivirals using structure based computational approach against Mpro of SARS-CoV-2 to propose them as new therapeutics. In total, 20 DAAs of HCV, including a reference compound O6K were docked against Mpro. The docked structures were examined and resulted in the identification of six highly promising DAAs i.e. beclabuvir, elbasvir, paritaprevir, grazoprevir, simeprevir, and asunapevir exhibiting high theoretical binding affinity to Mpro from SARS-CoV-2 in comparison to other DAAs. Furthermore, the post docking analysis revealed that Cys145, Glu166, His163, Thr26, His41, and Met165 played potential role for the binding of these DAAs inside binding site of Mpro. Furthermore, the correlation between binding energies were found in accord with the results from the reported IC_{50}s for some DAAs. Overall, the current study provides insight to combat COVID-19 using FDA-approved DAAs as repurposed drugs.

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1. Introduction

An acute respiratory disease that is causing progressive lung injury was reported to World Health Organization (WHO) in the late 2019. The disease was first detected from Wuhan (China) and subsequently announced as new coronavirus disease. It was labelled as COVID-19 in February 2020 [20]. The COVID-19 is responsible for more than 10 million infected cases, and more than 450,000 deaths just in few months from 196 countries (the number is still progressing). Therefore, the World Health Organization (WHO) declared SARS-CoV-2 as the new coronavirus as a global pandemic on March 11, 2020 [26]. The world has been battling with this highly infectious COVID-19 disease for more than two years i.e. ~131,965,495 positive cases and 2867,083 deaths (as of April 05, 2021) worldwide. Furthermore, recent studies indicated that COVID-19 not only affects the lung tissues but also exhibits adverse effects on the other organs such as heart, brain and kidneys, thus indicating that potent anti-COVID-19 agent should possess a tendency toward these target organs. Current prevention is based on quarantine to prevent its transmission. However, many promising treatments are yet to complete their clinical trials [1]. Although WHO approved vaccines (i.e. Sinopharm, Sinovac, Pfizer, AstraZeneca and many others) are being used for the time being, still certain side effects (such as blood clotting etc.) are reported related to specific vaccine. Therefore, more therapeutics are urgently required for this fast-moving pandemic so that the world is prepared for next COVID-19 wave. An extensive effort is being performed from researchers around the world to combat this virus.

The SARS-CoV-2 is a positive-sense single-stranded RNA virus belongs to the same family as SARS-CoV, i.e. coronaviridae. It has a largest genome among other known RNA viruses [26]. Its replication mechanism requires a number of key enzymes, notably helicases, main protease (Mpro) and RNA-dependent RNA-polymerase

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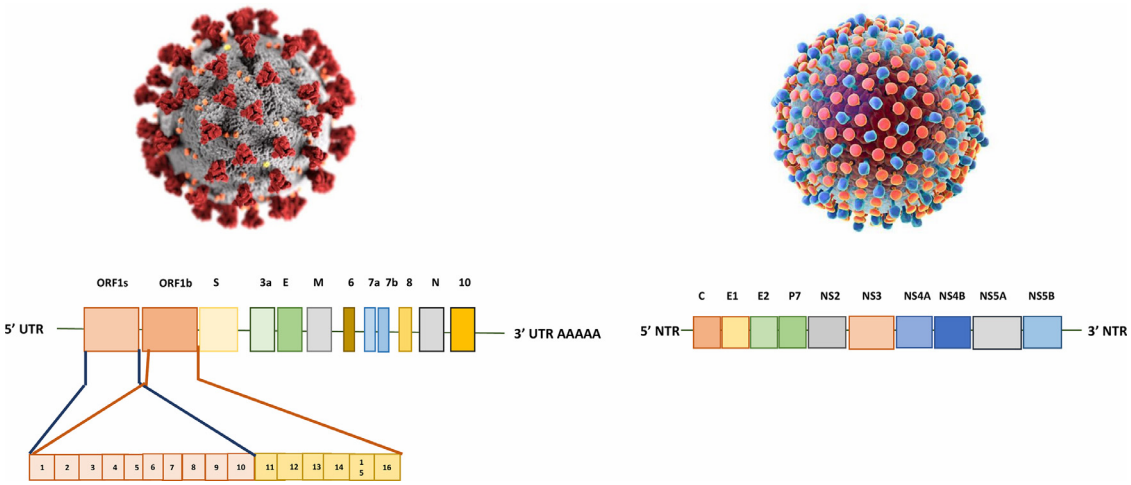

Fig. 1. Schematic representation of SARS-CoV-2 (Left) and Hepatitis C virus (Right) genome. The genome structure represents a ORF1a (Open Reading Frame a) encodes for nsp1–nsp10, ORF1b encodes for nsp1–nsp16, while Spike protein (S), Envelope gene (E), membrane gene (M), Nucleocapsid gene and a poly (A) tail at the 3′UTR (four genes) encode for the Structural proteins. Additionally, the accessory genes are distributed in between the structural genes (i.e. 3a, 7a, 7b, 6, and 8).

(RdRp). It is consisting of six Open Reading Frames (ORFs) [27]. The first ORF (ORF1a/b) is composed of two-third of RNA genome and encodes for two polypeptides i.e. pp1a and pp1ab while the remaining five ORFs make up of one-third of RNA genome responsible for the production of membrane protein (M), envelope protein (E), nucleocapsid protein (N) and spike protein (S) [12]. Likewise, Hepatitis C virus is also a positive-sense single-stranded RNA virus belongs to the Flaviviridae family. It is classified into eight different major genotypes. The NS3 mediates the production of protease, NS4A/B mediates four polyproteins cleavage events while NS5A/B encodes for the polymerases for viral cycle. It is observed that HCV proteins share the similar genomic construction as SARS-CoV-2 (Fig. 1) [3].

However, the two polypeptides generated from first ORFs (pp1a and pp1ab) encodes for a number of vital Nonstructural Proteins (NSPs), among which is NSP5 (a cysteine 3C-like protease) known as main protease (Mpro). The Mpro protein mediates the production of important NSPs crucial for the viral infection cycle i.e. RNA-dependent RNA polymerase, methyltransferase, and helicase [24]. Hence, the main protease controls the major pathways and replication of viral genome, it can be used as attractive drug target to halt the replication event in the virus life cycle [18]. It is three-domain (domains I to III) cysteine protease having active site between domains I and II. The main protease (NS3) of HCV is a chymotrypsin-like serine protease [2]. Recently developed highly effective therapeutics of HCV are the inhibitors of this protease. The combination of antivirals especially DAAs that directly targets the HCV proteins share the similar genomic construction as SARS-CoV-2 (Fig. 2). These similarities have led to the investigation of existing HCV Direct Acting Antiviral (DAA) against COVID-19 as potential therapies [6]. The DAAs are effective antivirals used against HCV protease and HCV polymerase and RNA-dependent RNA polymerase [11]. Several combination of DAAs are approved by Federal Drug Authorities (FDA) and European Medicine Agency (Mengist et al.) for the HCV as pan-genotypic treatment [21]. Recently, the concept of drug re-purposing has been widely used because of the drugs approved for one disease have considerable advantage in terms of time and cost, pharmacokinetics and toxicity being investigated in humans [4]. The drug re-purposing is one of the promising approaches in recent days to bring about innovative cures by the existing FDA approved drugs [9].

Therefore, the current study was aimed to identify the candidate drugs using a computational approach of molecular docking. For this purpose, a library of FDA-approved Hepatitis *C* DAAs drugs was investigated to find their potential for re-purposing as anti-SARS-CoV-2 drugs against Mpro.

2. Material and methods

In the current study, an *in-silico* based molecular docking approach was applied for the DAAs of HCV for the prioritization of novel drug candidates against SARS-CoV2. The complete work flow of the current study is presented in Fig. 3.

2.1. Protein retrieval

The COVID-19 main protease (PDB ID: 6Y2G) was obtained from the Protein Data Bank [22] in PDB format. The protein contained two chains (A, and B), having a sequence length of 306 amino acids. The 6Y2G protein was incorporated with ligand i.e. ID 06 K, IUPAC Name: ~{tert}-butyl ~{N}-[1-[(2~{S})-3-cyclopropyl-1-oxidanylidene-1-[[(2~{S},3~{R})-3-oxidanyl-4-oxidanylidene-1-[(3~{S})-2-oxidanylidenepyrrolidin-3-yl]-4-[(phenylmethyl)amino]butan-2-yl]amino]propan-2-yl]-2-oxidanylidene-pyridin-3-yl]carbamate.

2.2. Direct Acting Antivirals retrieval

The 20 FDA reported DAAs (beclabuvir, boceprevir, dasabuvir, glecaprevir, paritaprevir, pibrentasvir, ribavirin, ritonavir, telaprevir, velpatasvir, asunaprevir, voxilaprevir, daclatasvir, elbasvir, faldaprevir, omitasvir, grazoprevir, simeprevir, sofosbuvir, and ledipesvir) were obtained from the DrugBank [25] (Fig. 4).The selected dataset of 20 compounds exhibited considerable activity against HCV. Table 1 showed the dataset of selected DAAs.

2.3. Protein preparation

The obtained protein crystal structure was prepared and further refined for molecular docking study. The water molecules were removed while polar hydrogens were added to PDB structure. Kollam united atom force field was used to add charges to protein structure by AutoDock 4.2 [10,19]. Then the structure was saved in a PDBQT format for further molecular docking calculations.

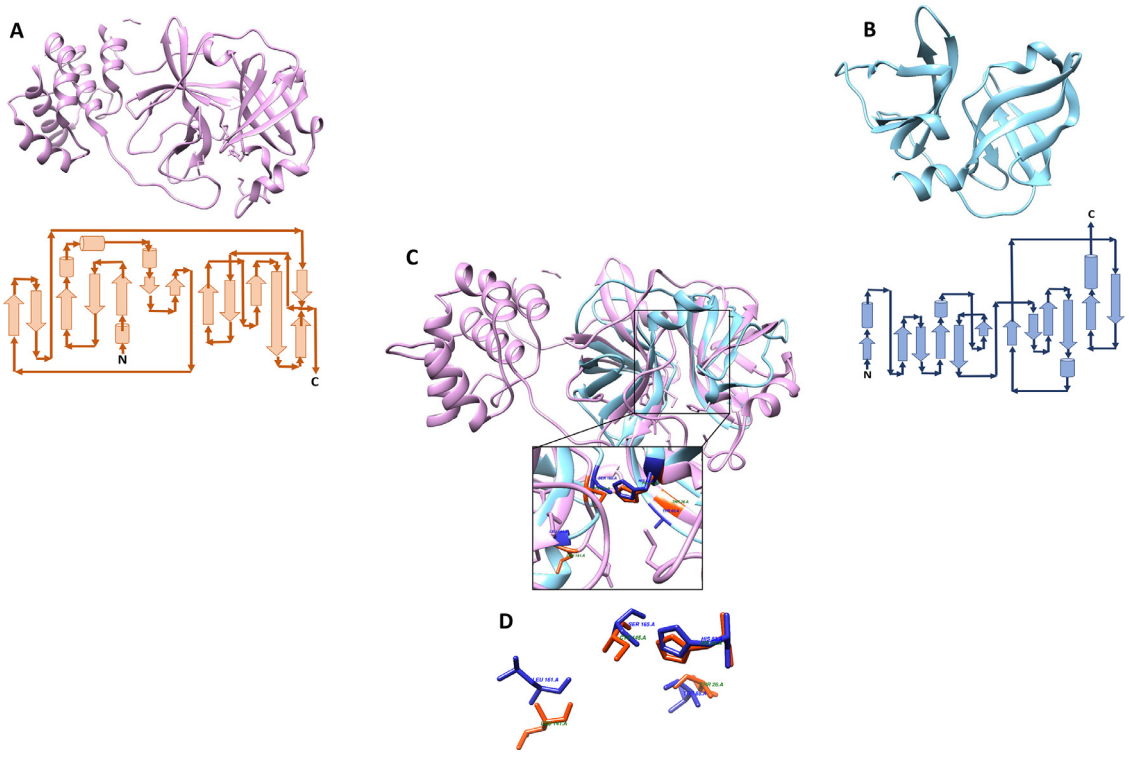

Fig. 2. Structural similarities in main protease of SARS-CoV-2 and HCV. (A) Mpro (PDB ID: 6Y2G) (B) and HCV Protease (C) Superimpose structure of Mpro and HCV protease highlighting the similarities found in both proteases and (D) active residues of both the proteases His41 / Cys145 and His57 / Ser139 of SARS-CoV2 Mpro and HCV protease.

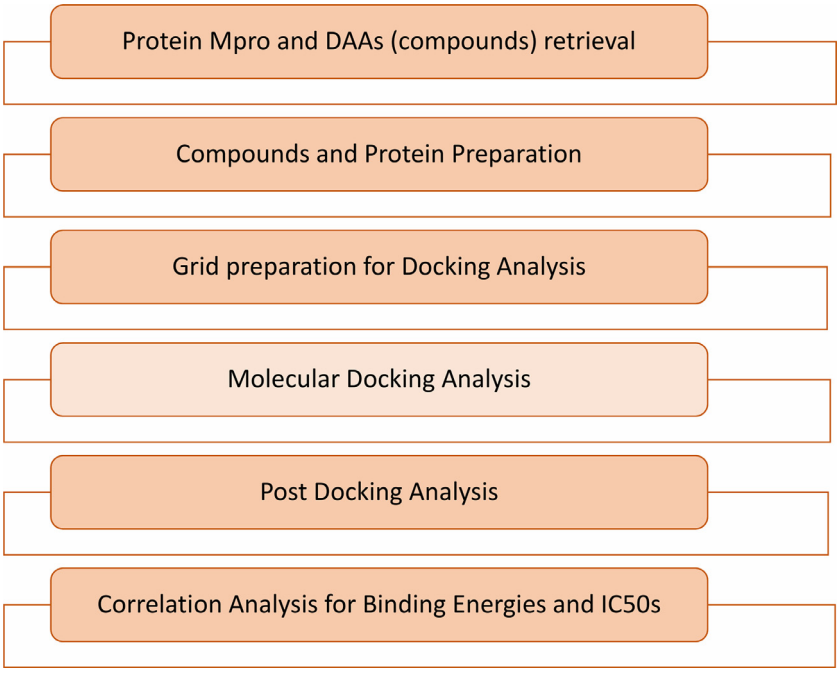

Fig. 3. Complete flowchart of proposed study for docking analysis for DAAs against Mpro protein.

2.4. Ligand preparation

The DAAs library used as ligands for this study was processed and prepared for docking studies. The retrieved PDB structures were energy minimized through Frog2 tool [17]. The minimized 20 DAAs were further analyzed via AutoDock and the gasteiger charges were computed. The saved PDBQT files were used for docking analysis.

2.5. Receptor grid preparation

The grid box for Mpro was generated through AutoDock tool [10,19], around the active site defined by already co-crystalized ligand present in PDB structure with the binding pocket. The grid box dimensions were generated as point 46, 48, and 50 for X, Y, and Z dimensions while the Grid Box center was set as -19.942, -6.232, and -26.819 for *X, Y*, and *Z* center, respectively.

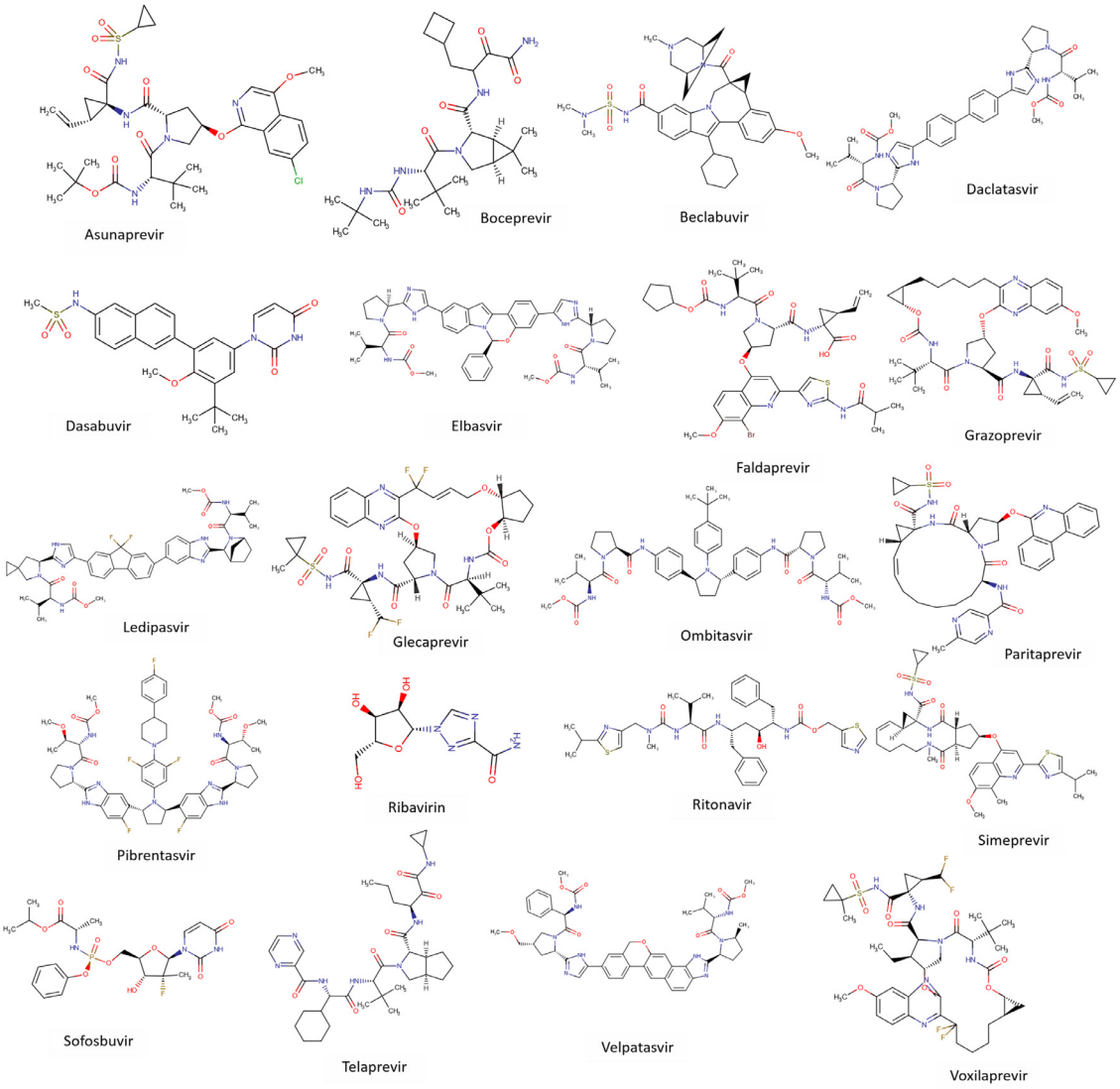

Fig. 4. Structures of 20 DAAs used in current study against Mpro.

Table 1
DAAs Of HCV used in current study.

Inhibitors	DAAs	DrugBank IDs	Status
NS3/4A	Beclabuvir	DB12225	In trials
PRO-	Glecaprevir	DB13879	Recommended for HCV under Brand name Maviret, and Mavyret
TEASE	Paritaprevir	DB09297	Recommended for HCV under Brand name Viekira Pak
INHIBITORS	Boceprevir	DB08873	Recommended mediaction
	Simeprevir	DB06290	2nd Generation Recommended Drug
	Grazoprevir	DB11575	Recommended for HCV under Brand name Epatier
	Faldaprevir	DB11808	Under Investigation
	Voxilaprevir	DB12026	Recommended for HCV under Brand name Vosevi
	Asunaprevir	DB11586	Under Investigation
	Telaprevir	DB05521	Used as Combination with Ribavirin
NS5A	Ledipasvir	DB09027	Recommended for HCV under Brand name Harvoni
inhibitors	Velpatasvir	DB11613	Recommended for HCV under Brand name Epclusa, and Vosevi
	Ribavirin	DB00811	Recommended for HCV under Brand name Ibavyr, Rebetol, and Virazole
	Ritonavir	DB00503	Recommended for HCV under Brand name Kaletra, Norvir, and Viekira Pak
	Daclatasvir	DB09102	Marketed under the name DAKLINZA
	Elbasvir	DB11574	Recommended for HCV under Brand name Zepatier
	Ombitasvir	DB09296	Recommended for HCV under Brand name Viekira Pak
	Pibrentasvir	DB13878	Recommended for HCV under Brand name Mavirat, and Mavyret
NS5B nucleoside polymerase inhibitors (NPIs)	Sofosbuvir	DB08934	Recommended for HCV under Brand name Epculsa, Harvoni, Sovaldi, and Vosevi
NS5B non-nucleoside polymerase inhibitors (NNPIs)	Dasabuvir	DB09183	Recommended for HCV under Brand name Exviera, Viekira Pak

2.6. Molecular docking studies

Molecular docking is commonly used structure-based approach to highlight the binding mode of compounds with selected proteins in terms of Van der Waals, binding affinities, electrostatic noncovalent interactions, ligand conformations in the binding site of the macromolecule and hydrogen interaction of ligand with protein [9]. The predicted binding poses in docking study are ranked as docking scores. The 20 DAAs were used as ligand library for docking studies against Mpro protein. The molecular docking study was performed using AutoDock tool 4.2v with standard docking procedure applied [10,19]. The ligand was docked and implemented by the parameters of 250 times Lamarckian GA settings resulting in 27,000 number of generations. Furthermore, re-docking step was implemented to validate our virtual screening process with co-crystalized ligand present in the Mpro retrieved from PDB. Finally, rigid docking was performed against the active site of Mpro protein while the flexible nature of ligand was set to perform docking without forcing the ligand to the active site only. However, the binding scores and ligand binding probability to the active site of Mpro were used for the evaluation of affinity through the molecular docking study.

2.7. Re-docking process with autodock

The re-docking is performed to assess the performance of any docking program so that it is decided if the docking program is capable of reproducing the same crystal conformation of the bound ligand or not? The re-docking experiment in the case of Mpro was employed by the AutoDock with its co-crystalized ligand.

2.8. Post docking study

The MOE tool was used to investigate the docked DAAs and Mpro complex interaction to visualize the hydrogen and hydrophobic interaction of complex within the range of 5 Å.

2.9. Correlation identification

Moreover, the correlation between IC_{50} and binding affinity was also studied for DAAs and Mpro protein. The IC_{50}s were retrieved from literature mining while the docked binding energies were predicted through AutoDock tool [7].

3. Results

3.1. Molecular docking results

The molecular docking study was carried out to analyze the binding mode of the DAAs against Mpro protein of SARS-CoV-2. The docking analysis highlights the interaction of compounds with crucial amino acids residues present at the active site with binding energies obtained from docking results are given in Table 2. The molecular docking results demonstrated in terms of negative energy and indicating that the lower the binding energy scores, best would be the binding affinity of the ligand to its receptor [13].

The docking study revealed that, **Ombitasvir** mediates two hydrogen bond interaction with Glu166, one hydrogen bond with Asn142 through nitrogen (NH) and Oxygen atoms of side chain, while Ser46 mediates one hydrogen – arene interaction with the aromatic ring of Ombitasvir as shown in Supplementary Fig. S1. The compound showed good anti-SARS activity with an estimated docking score of -6.85 kcal/mol.

The binding mode of **Beclabuvir** showed significant (highest of all other compounds) docked score i.e. -11.97 kcal/mol. It formed one hydrogen bond with Asn142 residue through its carbonyl oxygen. While Gln192, and His164 residues mediate two hydrogen bonds with oxygen of sulfonamide group. The Glu166 mediates one hydrogen bond with side chain of nitrogen and oxygen of sulfonamide group. Additionally, Met165, Met49, Asp187, and His164 mediate hydrophobic interactions (Fig. 5A).

Furthermore, **Pibrentasvir** compound formed multipe hydrogen bonds, one with Gly143 (backbone hydrogen donor to oxygen of side chain), one hydrogen bond with His164 (hydrogen donor at nitrogen of benzimidazole ring), one hydrogen bond through Cys145 (accept hydrogen from nitrogen of same benzimidazole ring), and one hydrogen bond with Glu166 act as hydrogen acceptor from nitrogen of heterocyclic ring (Supplementary Fig. S2). The estimated docked score of Pibrentasvir was found as -8.31 kcal/mol.

Boceprevir showed good estimated docking score (i.e. -9.33 kcal/mol) and mediated six hydrogen bonds with Cys145, Gly143, His41 and Asn142 amino acids by oxygen atoms of side chain while His164 formed one hydrogen bond with nitrogen atom of heterocycle. Additionally, Met165 mediates hydrogen bond with carbonyl oxygen of side chain (Supplementary Fig. S3).

The estimated binding energy of **Dasabuvir** was found as -9.25 Kcal/mol. It mediates two hydrogen bonds with Asn142, and Phe140 as backbone hydrogen donor and acceptor to oxygen and nitrogen of thiol group present at the side chain of dasabuvir (Supplementary Fig. S4).

Glecaprevir was found to form three hydrogen bonds through oxygen of thiol group with Thr26, Gly143 (as backbone hydrogen acceptor), and Leu27 as side chain hydrogen acceptor. While Cys145 mediates one hydrogen bond with nitrogen of sulfonamide side chain. Whereas His164 mediates one hydrogen bond as backbone hydrogen acceptor from nitrogen of heterocyclic ring (Supplementary Fig. S5). Additionally, 3D interaction showed that Glu166 mediates hydrogen bond with Glecaprevir. The estimated binding energy of -9.64 Kcal/mol was observed.

Paritaprevir set well in the binding side of Mpro protein with an estimated docking score of -11.42 Kcal/mol. It mediated four hydrogen bonds with Glu166 through different nitrogens of side chain. While Pro168 mediates two hydrogen bonds with nitrogen and oxygen, and Phe140 mediates aromatic-hydrogen bond with nitrogen quinolone ring of paritaprevir (Fig. 5B).

The docking study of **Ribavirin** showed the estimated binding energy of -5.35 Kcal/mol. Ribavirin mediates three hydrogen bonds with Thr26, Gly143, and Leu141 as backbone hydrogen donor and

Table 2
Binding energies and K_i values predicted through molecular docking studies.

S. No.	DAAs	Binding Energies	K_i (Jin et al.) predicted (μm)
1	Beclabuvir	-11.97	1.67
2	Boceprevir	-9.33	145.61
3	Dasabuvir	-9.25	164.81
4	Glecaprevir	-9.64	85.37
5	Paritaprevir	-11.42	4.28
6	Pibrentasvir	-8.31	805.13
7	Ribavirin	-5.35	118.94
8	Ritonavir	-8.27	873.55
9	Telaprevir	-9.04	234.71
10	Velpatasvir	-9.4	139.84
11	Voxilaprevir	-8.77	409.85
12	Asunaprevir	-9.99	47.66
13	Daclatasvir	-6.75	11.35
14	Elbasvir	-11.92	1.82
15	Faldaprevir	-7.60	2.68
16	Omitasvir	-6.85	9.57
17	Grazoprevir	-11.37	4.63
18	Simeprevir	-9.07	10.35
19	Sofosbuvir	-9.43	122.22
20	Ledipasvir	-8.46	624.28
21	Test	-9.71	76.50

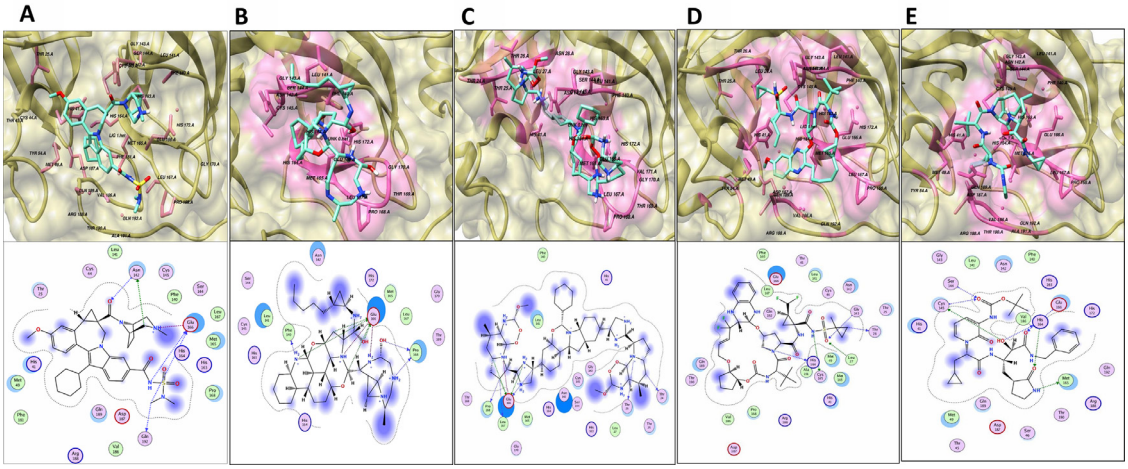

Fig. 5. Analysis of docked compounds showing significant interactions with Mpro active site. (A) Beclabuvir, (B) Paritaprevir, (C) Elbasvir, (D) Grazoprevir, and (E) reference compound (o6k) highlighting the 3D and 2D interactions.

acceptor to nitrogen and oxygen of oxole (furan) ring. Additionally, His41, and Cys145 mediate four hydrogen bonds as side chain hydrogen donor and acceptor with nitrogen of triazole and oxygens of furan ring of Ribavirin (Supplementary Fig. S6).

The docking study for **Ritonavir** showed better alignment at the active site of Mpro with an estimated binding energy of -8.27 Kcal/mol. It formed total of four hydrogen bonds with significant active site residues. It mediates two hydrogen bonds as acidic and basic hydrogen acceptor from Asn142, and Cys145 through side chain thiazole ring. The Glu166 amino acid mediates one hydrogen bond as basic through nitrogen of side chain aromatic ring while Phe140 mediates one hydrogen bond as basic acceptor (Supplementary Fig. S7).

Docking study highlights that **Telaprevir** mediates four hydrogen bonds with Mpro protein showing significant binding energy score of -9.04 Kcal/mol. The Met165 mediates one hydrogen bond with oxygen while Glu166 mediates two hydrogen bonds as backbone hydrogen acceptor to oxygen and nitrogen of cyclopropane ring, respectively (Supplementary Fig. S8).

It was observed through docking analysis that **Velpatasvir** formed interactions with an estimated binding energy of -9.4 Kcal/mol. It mediates multiple hydrogen bonds as backbone hydrogen donor to Gly143, Thr26 and Glu166. Additionally, it has made an arene – H interaction between the heterocyclic aromatic ring of Velpatasvir and the Thr26. (Supplementary Fig. S9).

The docking study of **Voxilaprevir** revealed that it mediates two hydrogen bonds with Mpro protein. It was found that Gln189 and His41 act as hydrogen bond partners with the Voxilaprevir atoms. The docking score calculated for voxilaprevir was -8.77 Kcal/mol (Supplementary Fig. S10).

Asunaprevir mediates ten hydrogen bonds with an estimated binding energy of -9.99 Kcal/mol. The docking study highlights that Glu166 formed two hydrogen bonds between quinolone ring as a hydrogen donor (side chain hydrogen donor). The same Glu166 mediates one hydrogen bond with side chain oxygen of pyrrole ring as backbone hydrogen donor with a distance of 2.95 Å. While His41, Cys145, and Asn142 interact with oxygens of sulfonamide group, nitrogen found at side chain and chlorine of quinolone ring through hydrogen bonds having a bond distance of 3.16 and 3.05 Å, respectively. Furthermore, Ser144 and Gly143 mediate three hydrogen bonds as backbone hydrogen donor to oxygen of sulfonamide group with the bond distance of 3.04, 2.81, and 2.95 Å, respectively (Supplementary Fig. S11).

The docked structure of **Daclatasvir** showed estimated binding energy as -6.75 Kcal/mol. The study showed it mediates one hydrogen bond with Mpro protein through Leu27 interacting with imidazole ring (Supplementary Fig. S12).

Elbasvir drug facilitates significant interaction with Mpro protein resulting in the second highest estimated binding energy of -11.92 Kcal/mol. It mediates multiple hydrogen bonds with different active site residues. Thr26 acts as backbone hydrogen acceptor, mediating one hydrogen bond with nitrogen. Glu166 was found to mediate three hydrogen bonds with imidazole ring as side chain and backbone hydrogen acceptor to Elbasvir (Fig. 5C).

The docked structure of **Faldaprevir** showed estimated binding energy of -7.60 Kcal/mol. It mediated two hydrogen bonds as side chain and backbone acceptor with Cys145. Glu166 was found to intermediate one hydrogen bond with oxygen of quinolone ring, while Gln189 formed one hydrogen bond as side chain hydrogen acceptor to carboxylic oxygen adjacent to cyclopropane ring (Supplementary Fig. S13).

Grazoprevir exhibited one of the most significant binding energy estimated as -11.37 Kcal/mol. The docking study revealed that Grazoprevir mediate multiple hydrogen bonds as hydrogen acceptor and donor (from backbone) with Gly143, His164 and Cys145 residues to the oxygen of sulfonamide group (Fig. 5D).

Simeprevir exhibited the estimated binding energy of -9.07 Kcal/mol resulting in the most stable interaction with Mpro protein. It mediates multiple hydrogen bonds with different significant active side residues. Met165 and Gln189 are mediating at least two hydrogen bonds with oxygen of sulfonamide group. On the other hands, Cys145 is making a close proximal interaction with the nitrogen atom of the ligand Simeprevir (Supplementary Fig. S14).

Furthermore, **Sofobusvir** was also found to mediate multiple hydrogen bonds with Mpro proteins showing binding energy of -9.43 Kcal/mol. It was observed that Cys145 and Gly143 mediate hydrogen bonds with oxygen of heterocyclic ring as backbone hydrogen donor while Phe140 and His163 mediate hydrogen bond as side chain hydrogen donor to heterocyclic oxygen, whereas, Ser144 mediates hydrogen bond as side chain hydrogen acceptor to nitrogen of heterocyclic ring. Additionally, Glu166 and Met165 mediate hydrogen bonds with oxygen of phosphate group of Sofobusvir (Supplementary Fig. S15).

Nevertheless, **Ledipasvir** drug mediates two hydrogen bonds as side chain hydrogen acceptor with Gln186 through oxygen, and

Table 3
Identified IC_{50} through literature for eight compounds.

S.No	DAAs	Binding Energies	IC_{50} identified through Literature Mining (μm)	References
1	Boceprevir	-9.33	19.6	[3]
2	Paritaprevir	-11.42	73.38	[18]
3	Ritonavir	-8.27	13.4	[14]
4	Telaprevir	-9.04	15.25	[3]
5	Asunaprevir	-9.99	15	[3]
6	Omitasvir	-6.85	75.49	[18]
7	Grazoprevir	-11.37	10.8	[3]
8	Simeprevir	-9.07	4.25	[3]

Table 4
Detail analysis of molecular docking studies.

S. No.	DAAs	No of Hydrogen Bonds	Residues of Active Site
1	Beclabuvir	6	Gln192, Asn142, and His164
2	Boceprevir	6	Cys145, Gly143, His41, Met165 and Asn142
3	Dasabuvir	2	Asn142, and Phe140
4	Glecaprevir	6	Thr26, Leu27, Cys145, Glu166, and Gly143
5	Paritaprevir	5	Gly143, Asn142, His164, and Cys145
6	Pibrentasvir	5	Gly143, Asn142, His164, Cys145, and Glu166
7	Ribavirin	7	Thr26, Gly143, Cys145, His41, and Leu141
8	Ritonavir	4	Glu166, Asn142, Cys145, and Phe140
9	Telaprevir	4	Asn142, Met165, and Glu166
10	Velpatasvir	3	Thr26, Gly143, and Glu166
11	Voxilaprevir	2	His41, and Gln189
12	Asunaprevir	10	Glu166, His41, Cys145, Ser144, Gly143, and Asn142
13	Daclatasvir	2	Leu27, and Thr25
14	Elbasvir	6	Thr26, Thr24, Pro168, Leu167, and Glu166
15	Faldaprevir	4	Cys145, Glu166, and Gln189
16	Omitasvir	3	Glu166, and Asn142
17	Grazoprevir	4	Thr26, Gly143, Asn142, and Cys145
18	Simeprevir	3	Gln189, Met165, and Asn142
19	Sofosbuvir	8	Cys145, Gly143, Phe140, His163, Ser144, His14, Glu166 and Met165
20	Ledipasvir	5	Cys145, Met49, Gln186, Phe140, Met165, and Glu166
21	Test	7	Ser144, Cys145, Met165, and His164

with Cys145 through oxygen of side chain, while two hydrogen-arene (aromatic) bonds were formed with Glu166 and Met165 amino acid with aromatic and imidazole ring of Ledipasvir, respectively. Additionally, Phe140 mediates one hydrogen bond with oxygen atom of side chain. The bonding energy calculated for Ledipasvir against Mpro was estimated as -8.46 Kcal/mol (Supplementary Fig. S16).

Simultaneously, the **redocking** study was performed to validate our docking results for DAAs against Mpro protein. The docking analysis for **o6k** (as test compound) showed significant interaction with active site residues of Mpro with -9.71 Kcal/mol binding energy. It has mediated total of seven hydrogen bonds with active amino acids. Ser144 formed one hydrogen bond with oxygen of side chain, Cys145 formed two hydrogen bonds with oxygen as hydrogen acceptor and donor, Met165 formed one hydrogen bond with nitrogen of pyrrole ring, while His164 formed one hydrogen bond with oxygen of pyrrole ring and the other with hydroxyl group (Fig. 5E).

The docking study revealed that compounds Beclabuvir, Paritaprevir, Asunaprevir, Elbasvir, Grazoprevir, and Simeprevir have shown high binding energies i.e. from -9.9 to -11 Kcal/mol (i.e. greater than test compound). The complete binding interactions of DAAs with Mpro proteins are presented in Table 4.

3.2. Correlation identification between IC_{50} and binding energies

In the current study, the correlation between IC_{50} (identified through literature mining) and calculated binding energies was also investigated. The IC_{50}s for eight compounds out of twenty DAAs against Mpro enzyme were found in whole cell culture. The Table 3 reported the IC_{50} of each compound and the corresponding source reference. The correlation predicted for these eight compounds along with their binding energies was estimated to be 0.725 (Fig. 6). This high correlation classified this study as significant. The correlation identification helped to find a relation that *lower the binding energy (*i.e. *higher negative value), lower will be the IC_{50} value*. The significant relationship between the IC_{50} and binding energies provides an insight for further prediction of IC_{50} of unknown compounds.

4. Discussion

The current pandemic coronavirus disease 2019 (SARS-CoV-2, previously known as 2019-nCoV) is classified as serious global threat to humans resulting in 54 million confirmed cases, growing at rates of up to 600,000 new cases/day while over 181,000 deaths [8](as of 24 April 2020) with an estimated fatality rate of 3% (Worldometer 2020, https://www.worldometers.info/coronavirus/). This pandemic emerged as deadly disease that is damaging the alveoli, resulting in respiratory failure and subsequent death. Due to barely accessible affected patient's report of biopsy and autopsy, the pathology of COVID disease is unclear. Despite numerous approaches (such as personal protection, social distancing, and disinfection), all treatments measure that employed anti-inflammatory and antivirals are supportive to only treat the symptoms of COVID disease, while no specific antiviral drugs have been confirmed to be effective yet [6]. The greatest challenge today is to effectively control the spread of the pandemic until a successful vaccine is available [16].

The Direct Acting Antivirals (DAAs) are newly emerged class of antivirals used for the treatment of HCV [15]. The DAAs inhibit the NS3/4A protease, the NS5A protein, and the NS5B polymerase proteins of HCV providing an effective treatment approach. The DAAs have revolutionized the HCV treatment based on their favorable

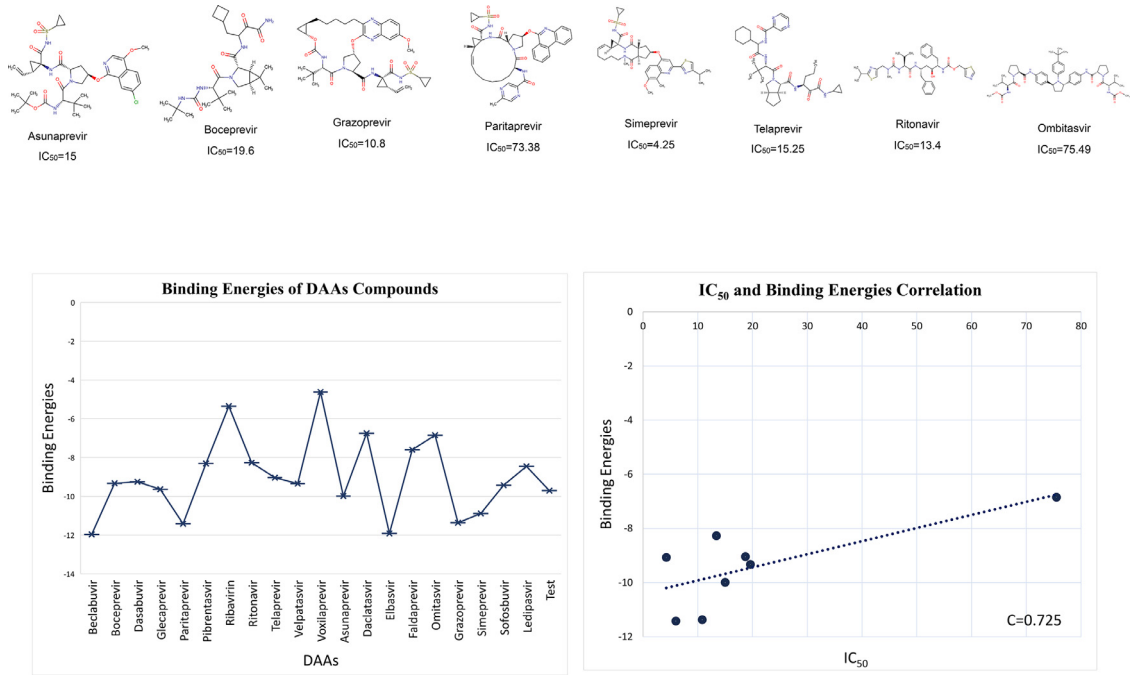

Fig. 6. Correlation analysis between IC_{50} and binding energies.

side effects profiles and enhanced rates of sustained virological response. The Mpro (Main Protease) is classified as one of the key enzymes responsible for the replication and transcription of coronavirus. Accordingly, Mpro could be an interesting target to explore the efficacy of any drug against coronavirus. Due to the similarities found in HCV and SARS-CoV2 genomics and proteomics, the drug repurposing of DAAs against Mpro of COVID was applied in the current study [4].

This study was aimed to consider DAAs (due to the striking activity against the HCV protease) against Mpro from SARS-CoV-2 and proposed them to be evaluated against COVID-19. The repurposed drugs can skip the prior screening and could move directly to the clinical trials and approved by the FDA for an immediate indication [5]. The DAAs of HCV such as Beclabuvir, Boceprevir, Dasabuvir, Glecaprevir, Paritaprevir, Pibrentasvir, Ribavirin, Ritonavir, Telaprevir, Velpatasvir, Asunaprevir, Voxilaprevir, Daclatasvir, Elbasvir, Faldaprevir, Omitasvir, Grazoprevir, Simeprevir, Sofosbuvir, and Ledipasvir were tested *in silico* for their potential as a treatment for COVID-19. Earlier, successful repurposed drugs have been approved such as use of Aspirin for the treatment of coronary artery diseases and the use of Sildenafil to treat erectile dysfunction [23].

Herein, the docking study of selected twenty DAAs was conducted against Mpro through AutoDock along with the crystallized ligand as reference compound to identify novel compounds against Mpro and validate the current study. The docking study revealed that all compounds were computationally predicted with the lowest binding energies ranging from ∼-4 to -11 Kcal/mol, and formed stable interactions through intermolecular hydrogen bonds. It was found that six compounds i.e. Beclabuvir, Paritaprevir, Asunaprevir, Elbasvir, Grazoprevir, and Simeprevir showed standard minimum binding energies of -9.9 to -11.9 kcal/mol compared with the reference compound (i.e. -9.7 Kcal/mol) and they exhibited tight interactions against Mpro with stable docking and higher binding energies. Paritaprevir, Asunaprevir, Grazoprevir, and Simeprevir are protease inhibitors (for HCV), Beclabuvir is a non-nucleoside polymerase inhibitor of the hepatitis C virus (HCV) nonstructural protein 5B (NS5B), while Elbasvir is hepatitis C virus nonstructural protein 5A inhibitor [25].

To get insight in to the intermolecular interactions, the molecular docking studies were further analyzed through MOE tool. The post docking analysis of these compounds showed that most of the hydrogen bonds formed between DAAs and Mpro mediates the involvement of GLU166, CYS145, MET165, ASN142, HIS41, THR26, and HIS164 active site residues reported as most significant amino acids present at the active site of Mpro enzyme. In addition, our findings may help in understanding the structure and activity relationship requirements for Mpro targeting drugs. For example, the essential role of the pyrimidine moiety and imidazole moiety in the six prioritized compounds were found as mediating the most significant hydrogen bonds with the crucial amino acids of the active site through its azole and pyrrolidine rings. This finding may help to innovate the new drug candidates against COVID-19. Furthermore, the docked binding energies and IC_{50} through literature survey were examined to find the correlation among them. It was observed through correlation analysis that the higher negative binding energy value resulted in lower IC_{50} value.

Finally, this study found a correlation among the affinities of the tested anti-HCV drugs against COVID-19 protease. Accordingly, we suggest such drugs for the repurposing pathway to reach an effective therapy for pandemic COVID-19. Furthermore, these drugs could be used either alone or in combination with each other's or with interferons as in the case with the Hepatitis C virus.

5. Conclusion

The emergence of COVID-19 variants is a major bottle-neck in the discovery of a successful molecular therapeutic. Therefore, understanding of the molecular basis of the available FDA approved drugs is a key to find new treatment options. The successful application of DAAs against HCV grabs the attention to try them against COVID-19 also. Hence, twenty DAAs of HCV were subjected to molecular docking against SARS-CoV-2 main protease. The docking analysis revealed the variable affinities of these DAAs

towards SARS-CoV-2 Mpro compared to the O6K reference compound. However, the six shortlisted DAAs are recommended for further clinical testing against COVID-19. Nevertheless, before clinical recommendations of these six shortlisted DAAs against COVID-19, it is highly recommended to conduct larger, dose determining, randomized, and prospective controlled clinical trial.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Reaz Uddin: Conceptualization, Methodology, Software, Visualization, Investigation, Supervision, Writing – review & editing. **Khurshid Jalal:** Data curation, Writing – original draft. **Kanwal Khan:** Data curation, Writing – original draft. **Zaheer ul-Haq:** Visualization, Investigation.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.131920.

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