Molecular Docking and Dynamics Simulations Reveal the Potential of Anti-HCV Drugs to Inhibit COVID-19 Main Protease

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Abstract

Background: Drug repurposing is the fastest effective method to provide treatment for coronavirus disease (COVID-19). Drugs that targeting a closely related virus with similar genetic material such as hepatitis C virus (HCV) and more specifically targeting a similar viral protease would be an excellent choice.

Methods: In this study, we carried out a virtual screening for fifteen anti HCV drugs against COVID-19 main protease using computational molecular docking techniques. Moreover, Velpatasvir (**4**) and Sofosbuvir (**13**) drugs were further evaluated through molecular dynamics simulations followed by calculating the binding free energy using the molecular mechanics generalised born/solvent accessibility (MM-GBSA) approach.

Results: The binding affinity descending order was N3 natural inhibitor (**1**), Velpatasvir (**4**), Sofosbuvir (**13**), Ombitasvir (**3**), Glecaprevir (**2**), Asunaprevir (**8**), Paritaprevir (**10**), Grazoprevir (**11**), Elbasvir (**6**), Ledipasvir (**5**), Daclatasvir (**7**), Pibrentasvir (**9**), Simeprevir (**12**), Dasabuvir (**14**), Taribavirin (**16**) and finally Ribavirin (**15**). Molecular dynamics simulation reveals that Sofosbuvir (**13**) has exciting properties and it was stable within the active site and also showed good MM-GBSA compared to the natural inhibitor N3.

Conclusion: Our results could be auspicious for fast repurposing of the examined drugs either alone or in combinations with each other for the treatment of the COVID-19. Furthermore, this work provides a clear spot on the structure-activity relationship (SAR) for targeting the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) main protease and helps the design and synthesis of new drugs in the future targeting it as well.

Introduction

The recent outbreak of coronavirus pandemic (COVID-19) introduced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) dramatically affected the global public health and economy. As of December 31, 2020, the total confirmed cases of COVID-19 infections is 83,264,560, and the number of confirmed deaths is 1,816,164 among 216 countries, areas, or territories regarding the World Health Organization (WHO).[1] SARS-CoV-2 is a member of the Coronaviridae family and Coronavirinae subfamily, which are enveloped positive-strand RNA viruses having glycoproteins spikes that project from the envelope of the virus, exhibiting a corona-like shape.[2] Among other RNA viruses, coronaviruses have very large genomes with a length ranging from 26 kb to 32 kb.[3] In addition to the encoding structural proteins in the coronavirus genome, the majority part of the genome has been determined, which identifying which proteins important for the replication of the virus or its gene expression.[4]

The main protease (M[pro]) is an important key for coronavirus replication and transcription with almost 306 aa length. It is highly responsible for the conversation of the polypeptide into functional proteins.[4, 5] Both of (M[pro]) and picornavirus 3C-like protease (3CL[pro]) have similar cleavage-site specificity.[6] Accordingly, M[pro] could be an interesting target to explore the efficacy of any drug against coronavirus.

Drug repurposing is the use of an existing drug to treat

a different disease.[7-9] Repurposed drugs can skip the prior screening and could move directly to the clinical trial and approved by the FDA for a recent indication. The drug repurposing approach could be a fast way of drug discovery costing much less than *de novo* drug development.[10] The importance of drug repurposing is currently well-understood especially after the emergence of the pandemic COVID-19.[11] Drugs such as Ivermectin, Ribavirin, Remdesivir, and Sofosbuvir were tested *in silico* and *in vitro* for their potential as a treatment for COVID-19.[12-14] Besides, Lopinavir-Ritonavir, Raltegravir, and Tipranavir were selected from the library of the antiviral drugs approved by FDA to have the most promising binding with the SARS-CoV-2 main protease.[15] Earlier, successful repurposed drugs have been approved such as the use of Aspirin for treatment of coronary artery diseases and the use of Sildenafil to treat erectile dysfunction.[16]

Now, there is an urgent need for effective drugs against COVID-19. One of the fastest ways to search for an effective drug is to examine other effective antiviral drugs (such as anti-HCV drugs).[17] Depending on the close relation and the significant similarities between the hepatitis C virus and coronavirus, some effective anti-HCV drugs can be evaluated against COVID-19.[18]

Herein, some anti-HCV drugs (Figures 1 and 2) were selected for molecular docking studies against M^pro, hoping to repurpose them as a treatment of COVID-19. Lastly, molecular dynamics (MD) simulations were conducted on the obtained complexes from the docking step to get a better understanding of the attraction between the anti-HCVs and the COVID-19 main protease active site. Water was implemented as an explicit solvent, and the system was run for 100 ns to evaluate the constancy of the drugs within the active site of the protein. The Molecular Mechanics/Generalized Born and Surface Area (MM/GB-SA) calculations were carried out to obtain the relative
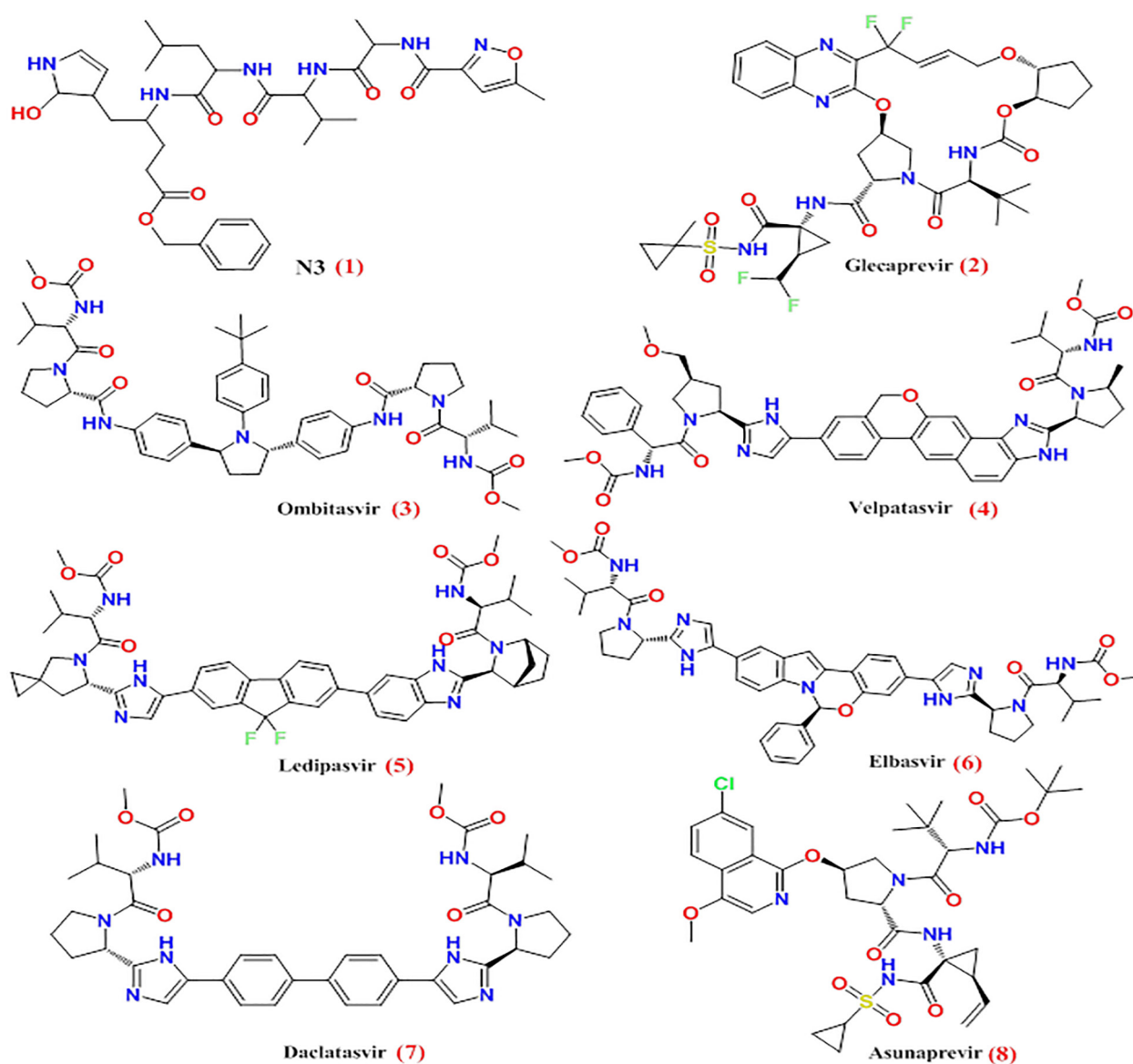

Figure 1. Chemical structures of N3 **1**, Glecaprevir **2**, Ombitasvir **3**, Velpatasvir **4**, Ledipasvir **5**, Elbasvir **6**, Daclatasvir **7**, and Asunaprevir **8**.
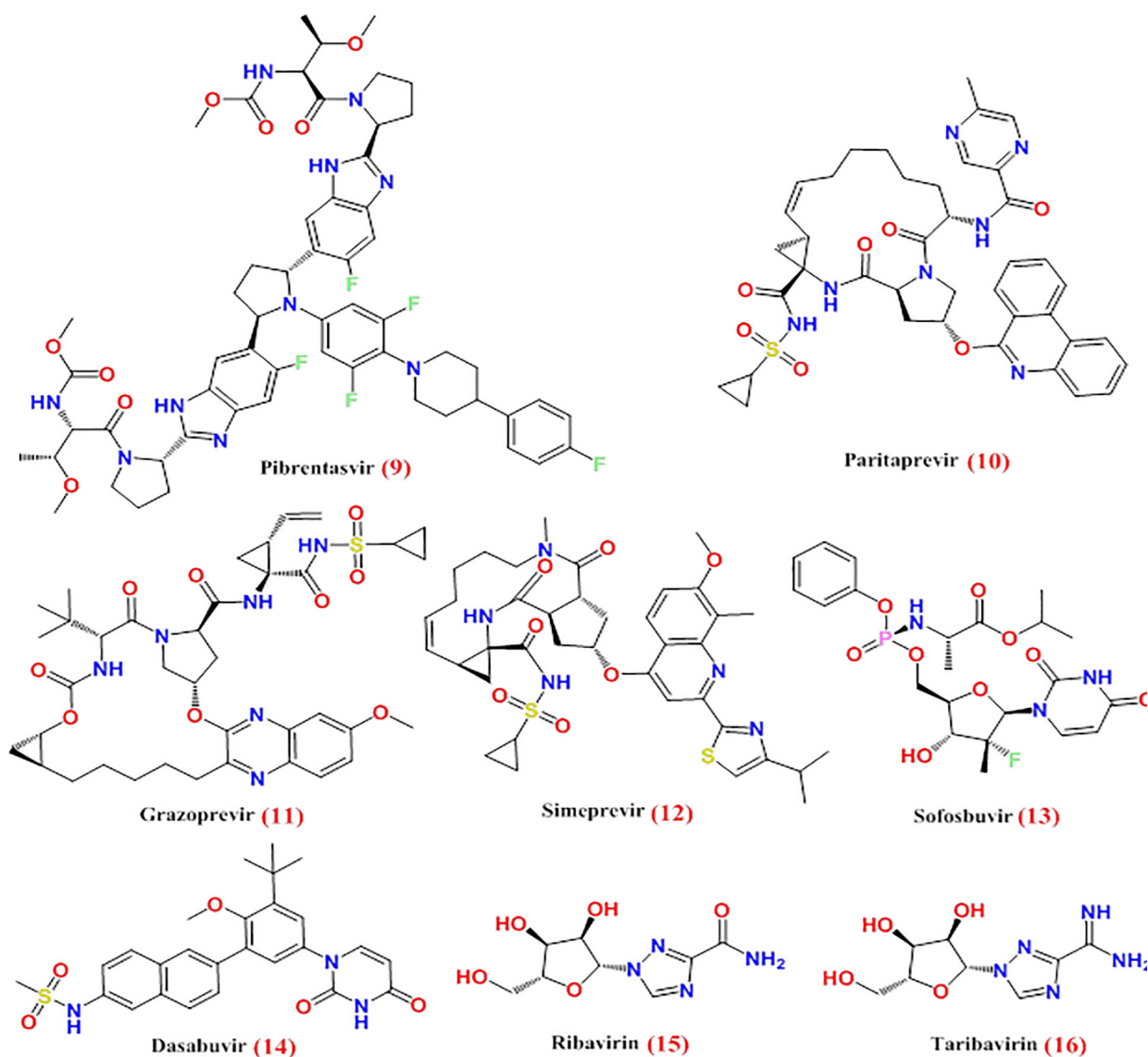
Figure 2. Chemical structures of Pibrentasvir **9**, Paritaprevir **10**, Grazoprevir **11**, Simeprevir **12**, Sofosbuvir **13**, Dasabuvir **14**, Ribavirin **15**, and Taribavirin **16**.

binding free energy for all complexes.

Daclatasvir is used by oral route in combination with other medications to manage HCV.[19] It is one of the WHO Lists of Essential Medicines.[20] It inhibits directly the NS5A protein that is essential for the transcription and translation of HCV.[21,22] It was the first NS5A inhibitor to reach the market.[23]

Ledipasvir is a drug for hepatitis C treatment approved as a combination product ledipasvir/sofosbuvir called Harvoni.[24,25] It also inhibits the important viral phosphoprotein, NS5A.[24] Pibrentasvir is an NS5A inhibitor antiviral drug.[26] It was approved for use in the United States and Europe with Glecaprevir for hepatitis C treatment.[27]

Velpatasvir is another NS5A inhibitor used in combination with sofosbuvir to treat all six major genotypes of hepatitis C infection.[28] Elbasvir was approved by the FDA[29] for hepatitis C treatment. It was introduced by Merck and used in combination with Grazoprevir, either in the presence or

absence of Ribavirin.[30] It is a selective and potent NS5A inhibitor of the HCV NS5A replication complex.[31]

Ombitasvir is an antiviral drug against HCV infection. In the US, it is approved by the FDA for use in the treatment of HCV different genotypes in combination with others.[32-34] It is an NS5A inhibitor.[35] Paritaprevir[36] is an NS3-4A serine protease inhibitor[37,38] and used for HCV treatment with promising results.

Asunaprevir (formerly BMS-650032)[39] is an experimental candidate for HCV treatment, and it is in phase III clinical trials.[40] It is an inhibitor of serine protease NS3 of HCV.[41]

Grazoprevir is an HCV protease inhibitor and was approved for the treatment of HCV.[42,43] It was introduced by Merck and passed phase III trials to be used with Elbasvir, either with or without Ribavirin.[42] It has promising activity against many HCV genotypes, including those resistant to most antiviral drugs used nowadays.[44]

Simeprevir is a medication used by the oral route in

combination with others for HCV treatment and is used for genotype 1 and 4.[45] It may be applied in the treatment of patients who also have HIV/AIDS.[46] It is used for the treatment of genotype 1 infected patients, but it was also indicated for type 4 genotype as off-label medical use.[47] It is an HCV protease inhibitor, thus inhibiting protein biosynthesis and preventing the viral maturation as well.[48] Glecaprevir is an HCV NS3/4A protease inhibitor.[49] It is used for the treatment of chronic HCV disease together with Pibrentasvir fighting most genotypes of HCV.[26] Dasabuvir is indicated for the treatment of HCV in combination with Ombitasvir /Paritaprevir/Ritonavir resulting in a cure in more than 90% of people.[50] It works by suppressing NS5B palm polymerase and preventing the genetic replication of HCV.[51]

Sofosbuvir is used to treat HCV with Ribavirin, Simeprevir, Daclatasvir, Ledipasvir, or Velpatasvir.[52] It acts by preventing the NS5B protein.[53] It is one of the WHO List of essential medicines.[20]

Ribavirin is an antiviral drug used for HCV treatment, besides some other viral infections.[54] It is used with others such as Sofosbuvir or Simeprevir for HCV treatment.[55] Its mechanism of action is not fully clear, but a proposed one claiming that it is used to prevent viral RNA synthesis and mRNA capping being a guanosine analog.[56]

Taribavirin is a prodrug of Ribavirin and it is in phase III clinical trials, but not approved till now by the FDA.[57,58] It is active against several DNA and RNA viruses.

Materials and Methods

Both docking and molecular dynamics simulation studies using MOE 2019.012 suite[59] and the Desmond simulation package of Schrödinger LLC,[60] respectively, were performed to measure and confirm the binding affinities and modes of the 15 FDA approved anti-HCV drugs towards the main protease enzyme of COVID-19. The N3 co-crystallised inhibitor was applied as a reference.

Docking studies
Preparation of the anti-HCV drugs
The tested HCV drugs were downloaded from the PubChem website and prepared according to the default steps described before.[61] Both the N3 inhibitor and the prepared anti-HCV drugs were saved as an MDB file of a single database to be included in the docking process.

*Preparation of the main protease (M^{pro}) targe*t
The Protein Data Bank (code 6LU7)[62] was used to obtain the X-ray structure of SARS-CoV-2 main protease and it was prepared as described earlier.[63, 64]

Docking of the anti-HCV drugs to the pocket of SARS-CoV-2 main protease
The prepared database was docked following the general methodology applied,[61] and dummy atoms were used as the docking site where dummy atoms surround the co-crystallised ligand binding site showing both the hydrophobic and hydrophilic regions which give the tested compound more free movement and so more reliable results obtained. At the end of the docking process, we selected the best poses having the best scores, binding modes, and rmsd_refine values. Moreover, a validation step was applied at first for the target receptor, and a valid behavior was indicated by low RMSD values between docked and native conformations.[65,66]

Molecular dynamics (MD) simulation
MD was performed using Desmond package (Schrödinger LLC).[60] The normal pressure and temperature (NPT) set with a temperature of 300 K and a pressure of one bar was applied in all runs, while the simulation time was 100 ns with a relaxation time of one ps for all ligands. The parameters for the force field were set to OPLS3,[67] and the cutoff radius was 9 Å in Coulomb interactions. The ligand-protein complex was doped in an orthorhombic periodic box with 10 Å boundaries away from the protein. The solvent was implemented using transferable intermolecular potential with three points (TIP3P) model.[68,69] A 0.15 M NaCl Salt concentration was built using the System Builder panel of Desmond.[70] The pressure control was accomplished using the Martyna–Tuckerman–Klein chain coupling plan with a coupling constant of 2 ps, and the temperature control was implemented using the Nosé–Hoover chain coupling plan[71,72] To calculate the nonbonded forces, the RESPA integrator was used with updating of the short-range forces and the long-range forces every step and every three steps, respectively. The trajectories of the simulations were saved at 20 ps intervals for analysis. The Simulation Interaction Diagram tool was used to analyse the behavior and the interactions of the complex within the trajectories. Also, the RMSD was observed for ligand and protein atoms position during simulation time to monitor the stability of the ligand–protein complex during the simulation.

Molecular dynamics trajectory analysis and calculation of MM-GBSA
Monitoring interactions contribution in the ligand-protein stability was performed through simulation interactions diagram panel of Maestro software. The thermal_mmgbsa.py python script was used to calculate the MM-GBSA, the script use the trajectory file obtained from the simulation, splits that file into individual snapshots, then run MM-GBSA on each frame and output the average binding energies.

Results and Discussion
Docking studies
The catalytic dyad of COVID-19 M^{pro} is a Cys–His one, and the binding pocket is a groove between I and II domains. The N3 inhibitor is located inside the pocket of COVID-19 M^{pro} with an asymmetric view. Molecular docking simulation of Daclatasvir **1**, Ledipasvir **2**, Pibrentasvir **3**, Velpatasvir **4**, Elbasvir **5**, and Ombitasvir **6**, Paritaprevir **7**, Asunaprevir **8**, Grazoprevir **9**, Simeprevir **10**, Glecaprevir **11**, Dasabuvir **12**, Sofosbuvir **13**, Ribavirin **14**, Taribavirin

15, and N3 inhibitor **16** into the main protease pocket was performed. They fitted inside the N3-binding pocket through several different bonds as represented in Figure 1. The selection of the best-docked drugs based on both the binding scores and binding modes. Their binding strength order was: N3 inhibitor (**1**, docked) **>** Velpatasvir (**4**) **>** Sofosbuvir (**13**) **>** Ombitasvir (**3**) **>** Glecaprevir (**2**) **>** Asunaprevir (**8**) **>** Paritaprevir (**10**) **>** Grazoprevir (**11**) **>** Elbasvir (**6**) **>** Ledipasvir (**5**) **>** Daclatasvir (**7**) **>** Pibrentasvir (**9**) **>** Simeprevir (**12**) **>** Dasabuvir (**14**) **>** Taribavirin (**16**) **>** Ribavirin (**15**).

The scores and binding interactions with different amino acids of M^{pro} pocket are represented in Table 1 and supplementary data.

Table 1. The scores and binding interactions of the tested anti-HCV drugs and N3 inside the binding pocket of the main protease.

No.	Anti HCV drug	S Score[a] (Kcal/mol)	RMSD_Refine[b]	Amino acid bond	Distance (A)
1	N3	-10.29	2.42	Glu166/ H-donor	3.03
				Glu166/ H-acceptor	3.15
				Gln189/H-acceptor	2.86
				Thr190/ H-acceptor	3.02
				His164/ H-acceptor	3.53
				Leu141/ H-acceptor	2.94
				His41/ H-donor	3.29
				His41/ pi-H	4.18
2	Glecaprevir	-9.90	1.94	**Glu166/H-donor**	3.07
				Glu166/H-acceptor	3.27
				Gln189/H-acceptor	2.86
				Thr26/H-acceptor	3.34
3	Ombitasvir	-9.63	2.33	Asn119/H- acceptor	3.07
				Met165/H- acceptor	3.69
				Met165/H- acceptor	3.73
				Pro168/H-donor	3.56
				Asn142/H-pi	4.21
4	Velpatasvir	-9.43	2.37	Thr26/H-donor	3.42
				Thr26/H-acceptor	2.95
				Thr25/H-donor	3.53
				Thr24/H- acceptor	2.92
				Glu166/H-pi	4.55
				Gln189/H-pi	4.09
5	Ledipasvir	-9.40	2.29	Gly143/H-donor	2.89
				Asn119/H-acceptor	2.84
6	Elbasvir	-9.31	2.77	Thr26/H-donor	3.47
				Met49/H- acceptor	3.93
				Glu166/H- acceptor	2.92
				Glu166/H-pi	4.29
				His41/pi-pi	3.98
7	Daclatasvir	-9.05	1.68	Met 49/H-acceptor	4.02
				Glu 166/H-pi	4.20
				Gln189/H-pi	4.02
8	Asunaprevir	-8.97	1.52	**Glu166/ H-donor**	2.87
				Asn142/ H-acceptor	2.99
				Gln189/H-acceptor	3.27
				Met49/H-acceptor	4.36
9	Pibrentasvir	-8.93	2.45	Asn142/H-donor	3.23
				Asn142/H-donor	3.50
				Glu166/H-pi	4.14
				Glu166/H-pi	4.34

Table 1. Continued

No.	Anti HCV drug	S Score[a] (Kcal/mol)	RMSD_Refine[b]	Amino acid bond	Distance (A)
10	Paritaprevir	-8.72	2.06	Asn142/H-acceptor	3.27
				Gly143/H-donor	3.32
				Thr45/H-donor	3.38
				Glu166/H-pi	4.30
				His163/H-pi	4.27
				Met165/H-pi	3.74
				Met165/ H-pi	3.88
11	Grazoprevir	-8.69	1.75	**His164/ H-acceptor**	3.13
				Cys145/H-acceptor	3.67
				Leu141/H-donor	3.34
				His41/pi-H	3.66
12	Simeprevir	-8.68	2.65	**Glu166/H-donor**	2.80
				Asn142/H-donor	3.09
13	Sofosbuvir	-7.93	1.79	**Glu166/H-donor**	3.43
				Cys145/H-donor	3.32
				Cys145/H-acceptor	3.88
				Met165/H-acceptor	3.87
				Gln189/H-pi	4.36
14	Dasabuvir	-7.84	1.47	Asn142/H-donor	3.07
				Met165/H-acceptor	3.50
				Glu166/ H-pi	4.40
				Gln189/H-pi	4.35
15	Ribavirin	-5.91	1.35	Cys145/ H-acceptor	4.25
				Ser144/ H-donor	3.16
				Met165/H-acceptor	3.36
				Met165/ H-pi	3.69
16	Taribavirin	-5.75	1.13	Met165/H-acceptor	3.34
				Met165/H-acceptor	3.61
				Met49/H-acceptor	4.10
				Gln189/H-acceptor	2.91
				His41/ pi-H	3.53

[a]S: The score of a compound inside the protein binding site, [b]RMSD_Refine: The Root Mean Squared Deviation between the predicted pose and the crystal structure, after and before refinement, respectively.

The results of docking studies revealed that Velpatasvir (**4**), and Sofosbuvir (**13**) have the best binding affinities and modes against COVID-19 protease with binding free energies of -9.43, and -7.93 kcal/mol, respectively (Table 1). These energy values were near to that of the docked N3 inhibitor (binding energy = -10.29 kcal/mol). The detailed binding mode of N3 was as follows; the docked N3 moiety occupied the branched pocket of M^{pro}, forming seven hydrogen bonds with Glu166, Gln189, Thr190, His164, Leu141, and His41. Also, it formed one H-pi interaction with His41. Concerning Velpatasvir (**4**), it occupied the pocket of M^{pro}, forming four hydrogen bonds with Thr26, Thr25, and Thr24. Also, it formed two pi-H interactions with Glu166 and Gln189. Moreover, Sofosbuvir (**13**) showed four hydrogen bonds with Glu166, Cys145, and Met165, with additional pi-H interaction with Gln189 (Table 2).

Taking into consideration, the aforementioned facts, MD simulations were conducted on the ligand-potential complex to simulate the interactions of the most promising two candidates (Velapatasvir **4** and Sofosbuvir **13**) selected from docking studies (Table 2) with COVID-19 main protease active site for 100 ns.

Regarding the results of anti-HCV drugs docking compared to N3, achieved an excellent idea about their binding modes. Some showed ideal binding, which indicates high affinity and predicted intrinsic activity. Furthermore, the anti-HCV drugs showed a binding strength order: N3 inhibitor (**1**, docked) > Velpatasvir (**4**) > Sofosbuvir (**13**) > Ombitasvir (**3**) > Glecaprevir (**2**) > Asunaprevir (**8**) > Paritaprevir (**10**) > Grazoprevir (**11**) > Elbasvir (**6**) > Ledipasvir (**5**) > Daclatasvir (**7**) > Pibrentasvir (**9**) > Simeprevir (**12**) > Dasabuvir (**14**) > Taribavirin (**16**) > Ribavirin (**15**).

Table 2. Three-dimensional pictures of the interactions and the positioning between tested anti-HCV drugs and N3-binding pocket within COVID-19 main protease compared to the N3 (Docked). Red dash represents H-bonds and black dash represent H-pi interactions.

Drug	3D interaction	3D protein positioning
Velpatasvir (4)		
Sofosbuvir (13)		
N3 (16)		

Molecular dynamics (MD) simulation

Docking protocols are known to be rapid and rough. However, docking may interface with the reliability of the resulting ligand-protein complexes due to loss of protein flexibility.[73] Thus, more precise molecular dynamics simulations which are more computationally expensive may achieve a superior correlative with docking. Generally, molecular dynamics simulation is used to study the performance of macromolecule, and it is applying Newton's equation of motion to determine the position and speed of each atom of the studied system.[74] Therefore, it provides a more accurate impersonation of protein motion.[75] According to the aforementioned facts, MD simulations were conducted on the drug-protein complex to mimic the interaction of these drugs with COVID-19 main protease active site for 100 ns using the Desmond package.

Protein and ligand RMSD analysis

To track the effect of the drugs on the conformational stability of 6LU7 in the course of simulations, RMSD values of Cα atoms were performed for all the drug-protein structures concerning their initial structure. The obtained results were plotted as a function of the time in Figure 3.
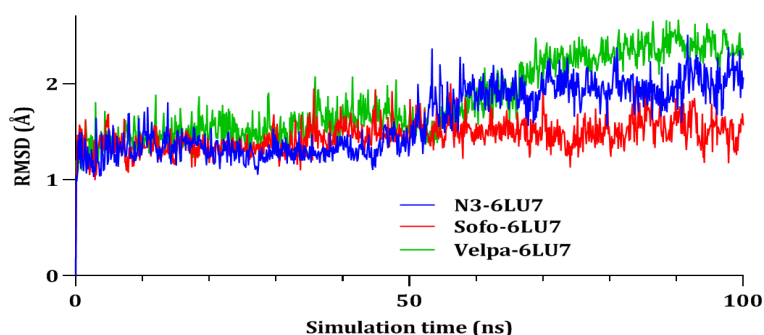
Figure 3. Plots of RMSD for Cα atoms (Å) for the initial structure vs simulation time (ns) for all the complexes.

From the plots, all the drug-protein complexes manage to reach their stable conformation after 10 ns, the protein showed a fluctuation within the acceptable variation regarding RMSD which was less than 3.00 Å indicating the stability of the conformation of the protein.

The RMSD of ligands atoms was also plotted as a function of time, which measure the alignment on its reference atoms conformation inside the active site, Figure 4,

Sofosbuvir (**13**) shows the most stability within the active site, while due to the large size Velpatasvir (**4**) move out of the active site by around 18 Å from its reference place before it reaches equilibrium at about 70 ns in a new place with which is 15 Å from the active site. All complexes reach equilibrium at around 60- 70 ns. A snapshot at 0, 50, and 100ns of Sofo-6LU7 and Velpa-6LU7 complexes are shown in Figure 5.
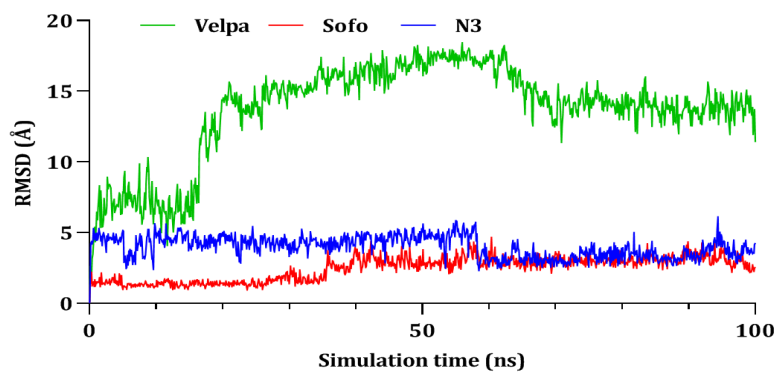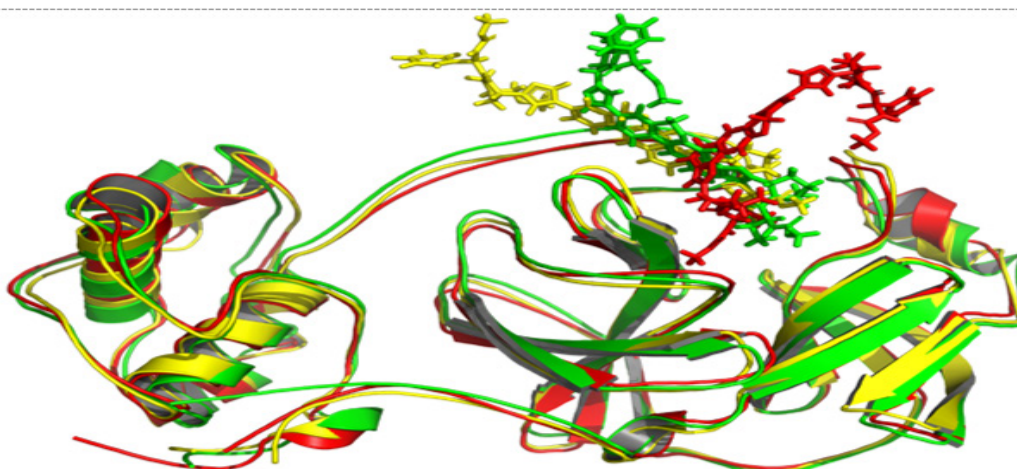

Figure 4. Plots of RMSD for ligand atoms (Å) for the initial atom position vs simulation time (ns) for all drug-protein complexes.
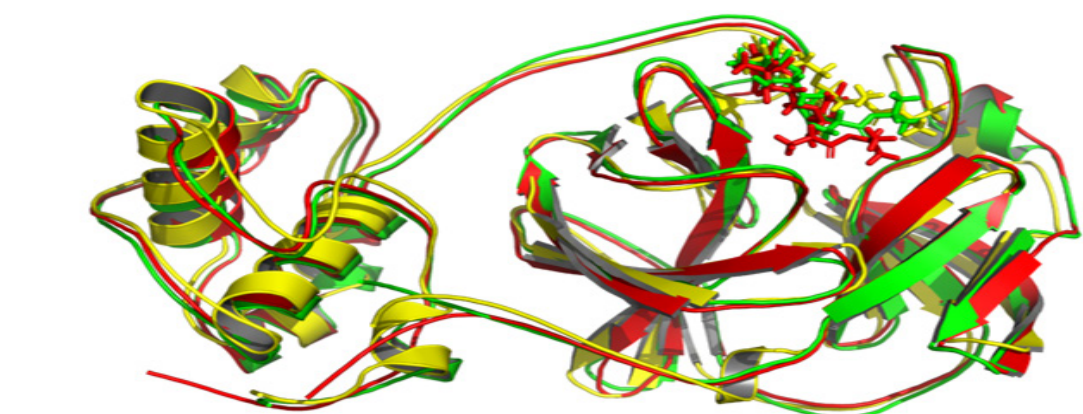

Sofo-6LU7

Velpa-6LU7

Figure 5. The aligned structures of Ligands-6LU7 during simulation; green 0ns, yellow 50ns, red 100 ns.

The simulation interaction diagram panel was used to analyse the interaction of the 6LU7 with both Velpatasvir (**4**) and Sofosbuvir (**13**). Figure 6, shows the interactions of the active site with both ligands, Velpatasvir (**4**) could not keep a consistent hydrogen bonding during simulation, the best it could form was with Asp187 during 33% of simulation time followed by Gln189 which kept contact during 20 % of simulation time, water bridged hydrogen bond play important rule to retain the Velpatasvir (**4**) within the active site, a water bridge hydrogen bond was formed with Cys145, His164, and Glu166 during 44 %, 49 %, and 43% of the time, respectively. Hydrophobic bonds such as Van der Waals (VdW) interaction and pi-pi
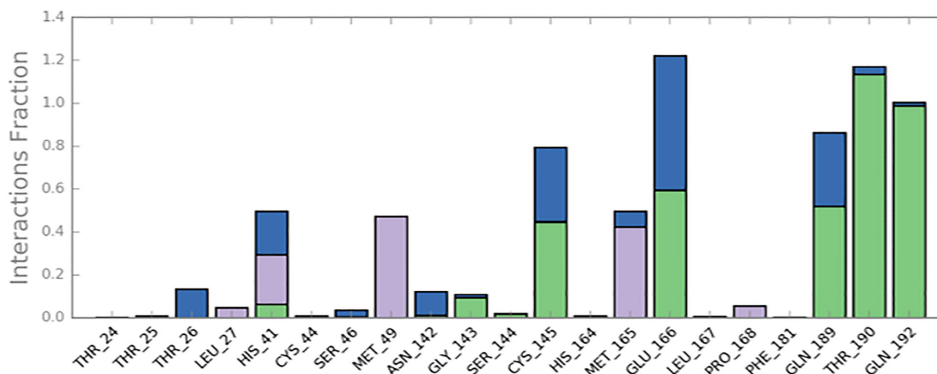
interactions also play a role in the stability of the ligand within the active site, Pro168, Leu167, and Ala191 were able to keep contact during 30 – 40 % of the time.

Sofosbuvir (**13**), on the other hand, was able to tight bind to the active site through multiple hydrogen bonds, two with Thr190 (113 %), one H-bond with Gln192 (98 %), Glu166 (59 %), Gln189 (51 %), and Cys145 (44 %), respectively. The pyrimidine moiety plays an essential role in the hydrogen bonding interactions through its amide NH and carbonyl groups. Water bridge H-bonds were also formed between Cys145, Glu166, and Gln189 and ligand during the simulation. VdW was created with Met49 and Met165, the CH3-S- moiety of the methionine amino acid
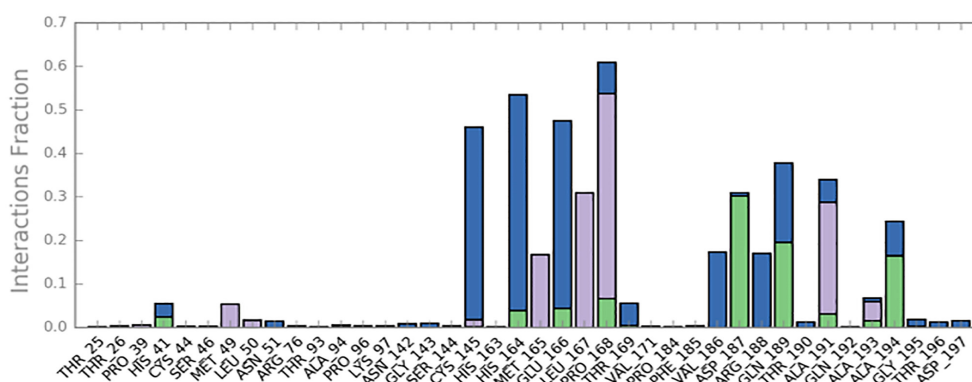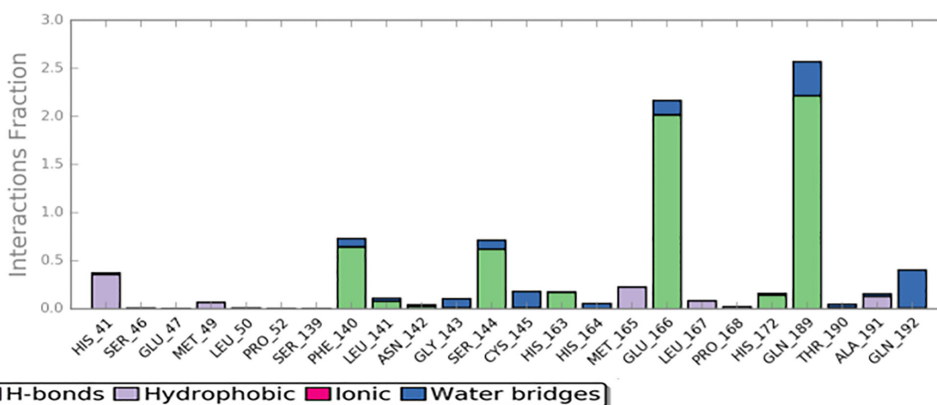

Figure 6. The histogram of Velpatasvir -6LU7, Sofosbuvir -6LU7, and N3-6LU7 contact throughout the trajectory.

Table 3. Total energy and potential energy of the complexes in kcal/mol.

	Total energy (kcal/mol)	Potential energy (kcal/ mol)
N3	-99760.524	-121909.445
Sofosbuvir	-99883.206	-122041.919
Velpatasvir	-102040.977	-124693.094

Table 4. Prime MM-GBSA energies for ligands binding at the active site of COVID-19 main protease.

	ΔG Binding	Coulomb	Covalent	H-bond	Packing	Lipo	Solv_GB	VdW
N3	-80.00	-43.59	4.67	-3.45	-16.27	-2.25	45.02	-64.12
Sofosbuvir	-69.17	-31.03	4.16	-2.37	-14.32	-1.74	32.05	-55.90
Velpatasvir	-63.01	-16.49	4.18	-0.73	-22.39	-1.05	34.25	-60.78

was able to interact with the aliphatic side chain, while a pi-pi interaction was formed between the imidazole ring of the histidine and the phenyl ring of Sofosbuvir (**13**).

Examination of the natural inhibitor N3, we quickly note that the inhibitor entirely depends on two amino acid residuals to stick into the active site, Gln189 and Glu166, these residuals can form up to three hydrogen bonding during the simulation. Phe140 and Ser144 were both able to form a one hydrogen bond with the inhibitor during 100 ns trajectories. Van der Waals's interactions with His41 and Met164 were maintained about 40 – 50 % of simulation.

The potential energy of the complexes along with the total energy over the period of the simulation are reported in Table 3. As we can see from Table 3, Sofosbuvir (**4**) shows good binding energy compared to the natural inhibitor ~ 10 kcal/mol difference. On the other hand, Velpatasvir (**13**) binds to the new site firmly with 63.01 kcal/mol binding energy, Velpatasvir (**13**) also shows better Van der Waals binding energy.

MM-GBSA study

Schrodinger software provides a python script called thermal_mmgbsa.py which was used to calculate the MM-GBSA to trajectories and extract the average binding energies, this includes the average MM-GBSA binding energy, average Coulomb energy, average Covalent binding energy, average Van der Waals energy, average Lipophilic energy, average Generalized Born electrostatic solvation energy, and average Hydrogen-bonding energy. All the obtained energies are shown in Table 4.

Finally, our study confirmed 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 combinations with each other's or with interferons as the case with the Hepatitis C virus.

Conclusion

Fifteen anti-HCV drugs targeting Hepatitis C protease were subjected to molecular docking against COVID-19 main protease. The tested drugs showed variable affinities toward COVID-19 protease compared to N3 inhibitor in the order of N3 inhibitor (**1**, docked) > Velpatasvir (**4**) > Sofosbuvir (**13**) > Ombitasvir (**3**) > Glecaprevir (**2**) > Asunaprevir (**8**) > Paritaprevir (**10**) > Grazoprevir (**11**) > Elbasvir (**6**) > Ledipasvir (**5**) > Daclatasvir (**7**) > Pibrentasvir (**9**) > Simeprevir (**12**) > Dasabuvir (**14**) > Taribavirin (**16**) > Ribavirin (**15**). Molecular dynamics simulation reveals that Sofosbuvir (**13**) has exciting properties; it was very stable within the active site, and it also showed good MM-GBSA compared to the natural inhibitor N3. However, Sofosbuvir (**13**) is recommended for further clinical testing against COVID-19. It may be examined either alone or with other candidates. Also, our findings may help in understanding the SAR essential for M^{pro} targeting drug, like the essential rule of the pyrimidine moiety of Sofosbuvir (**13**) in the hydrogen bonding with the crucial amino acids of the active site through its amide NH and carbonyl groups, to help the design and synthesis of new drugs targeting COVID-19 in the future.

Author Contributions

Conceptualization: AAA; Data curation: AAA and RA; Formal Analysis: AAA, RA, and MMD; Investigation: AMM and IHE; Methodology: AAA, RA, and MMD; Project administration: AAA and IHE; Resources: AAA and IHE; Software: AAA and RA; Supervision: AAA and IHE; Validation: MMD and AAM; Visualization: AAA and RA; Writing – original draft: AAA and RA; Writing– review and editing: AAA, RA, and IHE. All authors approved the final version of the manuscript.

Conflict of Interest

The authors did not have any conflict of interest.

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