Identification of potential inhibitors of SARS-CoV-2 Main protease via a rapid in-silico drug repurposing approach

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Abstract

An in-silico drug repurposing study was carried out to search for potential COVID-19 antiviral agents. A dataset of 1615 FDA-approved drugs was docked in the active site of SARS-CoV-2 Main protease. A subset of the top scoring hit compounds was subjected to follow-up molecular dynamics simulations to further characterise the predicted binding modes. The main findings are that the drugs Aliskiren, Capreomycin, Isovuconazonium, emerge as novel potential inhibitors. We also observed that Ceftolozane, Cobicistat, Carfilzomib and Saquinavir are well-ranked by our protocol, in agreement with other recent in silico drug repurposing studies, however MD simulations shows only potential for the three first, as Saquinavir exhibited an unstable binding mode. As many HIV-protease inhibitors has been reported as active and not active, Atazanavir and Lopinavir were included in the data set in order to rationalize the findings. In addition, our protocol ranked favourably Dronedarone suggesting that this recently reported SARS-CoV-2 inhibitor targets SARS-CoV-2 Main protease.

Keywords: SARS-CoV-2, COVID-19, SARS-CoV-2 Main protease, pandemic, repurposed drugs, In-silico drug design tools.

Introduction

The new coronavirus that emerged in China has brought considerable attention from the scientific community around the globe. The rapid spread of the disease has had a significant global impact on health and economics, and there is a pressing need to identify antiviral agents to treat infected patients.[1,2]

Computer-aided drug design methodologies are widely used to support early-stage drug discovery activities.[3] Computational methods are in continuous development, and when used adequately, can efficiently inform the design of experimental studies. Recent work has shown the utility of computational methods to identify potential SARS-CoV-2 antiviral agents.[4,5] SARS-CoV-2 belongs of a family of single-stranded enveloped RNA viruses and it is in the same family as SARS-CoV. Proteases in those viruses are key in the infection to the host and essential for replication into the cell, remaining as an important target for new drugs. The role of these enzymes is to catalyse the cleavage of specific peptide bonds in viral polyprotein precursors or in cellular proteins.[6,7] Protease inhibition has proven effective for new treatments in some other viruses, for example in HIV. The main protease of SARS-CoV-2 seems to share similarities in sequence and function to the HIV protease, which has motivated the use of a number of HIV protease inhibitors in in clinical trials.[8, 17]

Recently a high-resolution structure of SARS-CoV-2 Main protease in complex with a peptidomimetic inhibitor has been solved. The main interactions with the inhibitor occur through hydrogen bonds with PHE140, GLU166, GLN189, THR190 and ASN142.[9] In this report we have analysed by a combination of docking and MD simulations a dataset of FDA approved drugs to identify compounds that could potentially bind to the active site of SARS-CoV-2 Main protease. The findings from this study may be used to inform the design of experiments to identify novel SARS-CoV-2 antiviral agents that could be rapidly used in a clinical setting.

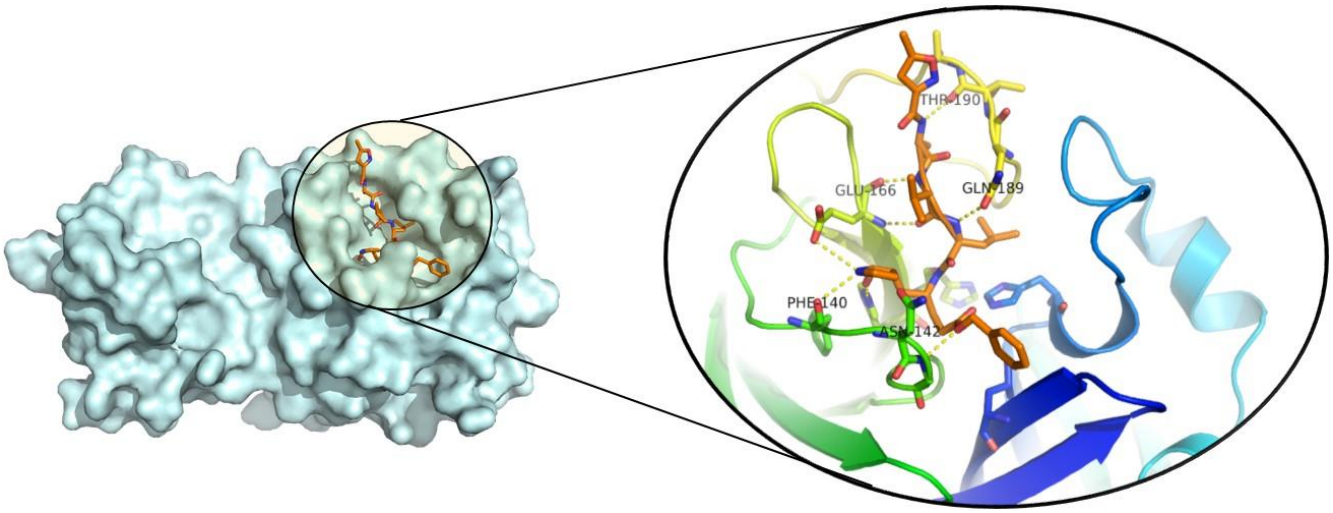

Figure 1. Main protease of SARS-CoV-2 isolated recently in complex with a peptidomimetic inhibitor and its interactions with the protein (PDB ID=6lu7).

Methodology

The main protease of SARS-CoV-2 protein was taken from PDB database (PDB-ID = 6lu7). We selected 1615 compounds extracted from the ZINC database as mol2 files (https://zinc.docking.org/) all of them approved for the FDA for different uses. The protein was stripped from other molecules, such as water, ions and ligands. After that, docking was performed with the software DOCK6 as follow.[10] First global spheres were positioned throughout the protein, and those within 10 Å of the ligand were retained. After that a grid was generated with a 0.4 Å spacing. Once the grid was generated flexible docking was performed using a maximum of 1000 orientations, and an internal energy cut-off of 100 $kcal.mol^{-1}$.

Eleven of the top-ranked molecules were taken from this docking study and subjected to follow up molecular dynamics simulations. Complexes were prepared by taking the most favourable docked pose (best grid score) with a good cluster size. Complexes were solvated in a rectangular box extending at least 10 Å away from the edge of the protein and neutralized with counter-ions (Cl^- and Na^+). The resulting complexes were subjected to a protocol of energy minimization and MD equilibration with the software sander implemented in AMBER.[11] Complex preparation, minimization and equilibration steps were run automatically with the software FESetup1.2.[12] MD production runs were carried out with the software SOMD,[13] for a duration of 500 ns using a timestep of 2 fs. The temperature was set to 300 K and the pressure to 1 atm. Utilities from GROMACS were used for the analysis of the trajectories in order to check stability of the ligands and the protein, and for clustering analysis.[14] Figures and plots were generated with VMD and GRACE respectively.[15]

Results and discussion

The top-scored compounds from the docking calculations indicated a number of diverse candidate ligands for SARS-CoV-2 Main protease as shown in table 1. Several HIV protease inhibitors were identified, in line with previous reports,[16] indeed a number (Ritonavir, Lopinavir) are already under clinical trials.[17]

ZIND ID	Commercial name	Current use	Grid score	Cluster size	Previous theoretical report	Experimental evidence
49841054	Carfilzomib	Anticancer	-98.58	10	Yes[18]	No
85537014	Cobicistat	HIV treatment enhancer	-98.47	8	Yes[18]	No
29416466	Saquinavir	HIV treatment	-97.62	33	No	No
3830276	Tessalon	Flu symptoms relief	-94.82	27	No	No
4393164	Aliskiren	Hypertension	-92.15	33	No	No

		treatment				
95564694	Naloxegol	opioid-induced constipation treatment	-91.37	11	No	No
29571072	Isavuconazonium	antifungal	-90.51	23	No	No
9164421	Ceftolozane	Antibiotic	-89.67	38	Yes[18]	No
3943297	Pradaxa	Anticoagulant	-88.81	25	No	No
49933061	Dronedarone	cardiac arrhythmias treatment	-88.73	26	No	Yes[19]
150338698	Capreomycin	Antibiotic	-87.42	31	No	No
3941496	Atazanavir	HIV treatment	-83.78	34	Yes[18]	Yes[19]
3951740	Lopinavir	HIV treatment	-79.87	15	Yes[18]	Yes[19]

Table 1. Best molecules binding SARS-CoV-2 protease from Docking results.

Other candidates include antibiotics, anticancer, antifungal, anticoagulants agents (Table 1). The full ranked list of docked compounds is given in the Supporting information.

We selected 11 molecules for further MD simulations along with Atazanavir and Lopiravir as controls (inputs have been deposited at https://github.com/CCPBioSim/Covid-19). All selected molecules have been used previously for systemic oral or intravenous treatments. As SARS-CoV-2 is a systemic disease, it is important to focus on molecules that can reach the specific organs involved such as alveolar lungs tissue. In addition, compounds that also act as antibiotics (Ceftolozane, Capreomycin) could be useful as respiratory diseases often involve complications due to nosocomial infections.[20] Ceftolozane has been reported previously in another repurposing study,[18] but its activity has not been tested experimentally against this virus. Cobicistat (HIV enhancer treatment) was also previously reported as possible inhibitor.[21] Isavuconazonium is an antifungal systemic molecule with good oral bioavailability.[22] Aliskiren targets the renin receptor and is used for treating hypertension.[23] This warrants further discussion because the Renin receptor activates the renin-angiotensin-aldosterone system (RAAS). The virus uses the angiotensin-converting enzyme (ACEs) to enter the cell (pneumocytes for instance) and then this same enzyme causes a local activation of the system RAAS which mediates lung inflammation and injury. Blockade of RAAS with renin inhibitors could help patients recover from the infection. It was therefore interesting to find that Aliskiren was ranked favourably as a SARS-CoV-2 main protease inhibitor, suggesting this molecule could potentially inhibit both virus replication and damage.[24] Our protocol also scored well Dronedarone, a molecule recently reported as an active inhibitor of SARS-CoV-2 virus.[19] However, the mechanism for this activity has not been reported. Our results suggest this molecule targets SARS-CoV-2 main protease. Other well-ranked molecules included Tessalon, Naloxegol and Saquinavir. As additional controls, we evaluated the ranks of Lopinavir and Atazanavir. Lopinavir has been reported to inhibit

SARS-CoV-2, whereas Atazanavir is inactive.[19] Both molecules do not rank very highly. This apparent discrepancy was investigated further below.

We carried out MD simulations to assess further the predicted binding modes from the docking calculations. One parameter useful to evaluate if a pose is stable is measuring the RMSD of the pose during the simulation. We grouped ligands in three categories according to the calculated RMSD values. Type I (Ceftolozane, Cobicistat, Aliskiren, Carfilzomib) include molecules with a very stable pose. Type II (Lopinavir, Pradaxa, Isavuconazonium, Dronedarone, Capreomycin) include molecules that rearrange in a stable binding mode different to that predicted by docking. Type III molecules (Tessalon, Naloxegol, Saquinavir, Atazanavir) do not adopt a well-defined binding mode during the simulations (Figure 2). This analysis suggests indeed that Lopinavir is a more potent inhibitor than Atazanavir, as has been has found experimentally. Saquinavir also does not appear to form a stable binding mode, despite its relatively high rank from the docking calculations. This analysis suggests Aliskiren warrants further investigation, given its potential dual activity on SARS-CoV-2 disease.

Visual analysis of a representative pose from MD simulations shows also striking differences in binding modes for Atazanavir and Lopiravir. Atazanavir does not occupy fully the binding pockets occupied by the peptidomimetic ligand reported in the X-ray diffracted structure of SARS-CoV-2 main protease. By contrast Lopinavir fills better this binding pocket. Saquinavir and Pradaxa also occupy partly the binding site, whereas other compounds fill the whole site (Dronedarone, Aliskiren, Isavuconazonium, Capreomicyn, Cobicistat, Ceftolozane) suggesting they may be more effective inhibitors (Figure 3).

Key interactions of some of these molecules with the protein are shown in figure 4. Dronedarone forms hydrogen bonds with THR25, THR26, GLN189 and some hydrophobic interactions with MET165 and PHE181. The deepest cavity in this binding site appears to have a dual hydrophobic and hydrophilic character. This is supported by the binding modes of Aliskiren and Isavuconazonium, with aliphatic and aromatic moieties occupying this cavity. However, in the case of Capreomycin an amide ring occupies this cavity. Aliskiren forms hydrogen bonds with ASN142, SER144 and HIE41. Isavuconazonium forms a hydrogen bonds with VAL186, GLY189, most of the contacts with the protein are hydrophobic in nature. Finally, Capreomycin form hydrogen bonds with ASN142, GLY143, HIE164 in a mostly hydrophilic interaction.

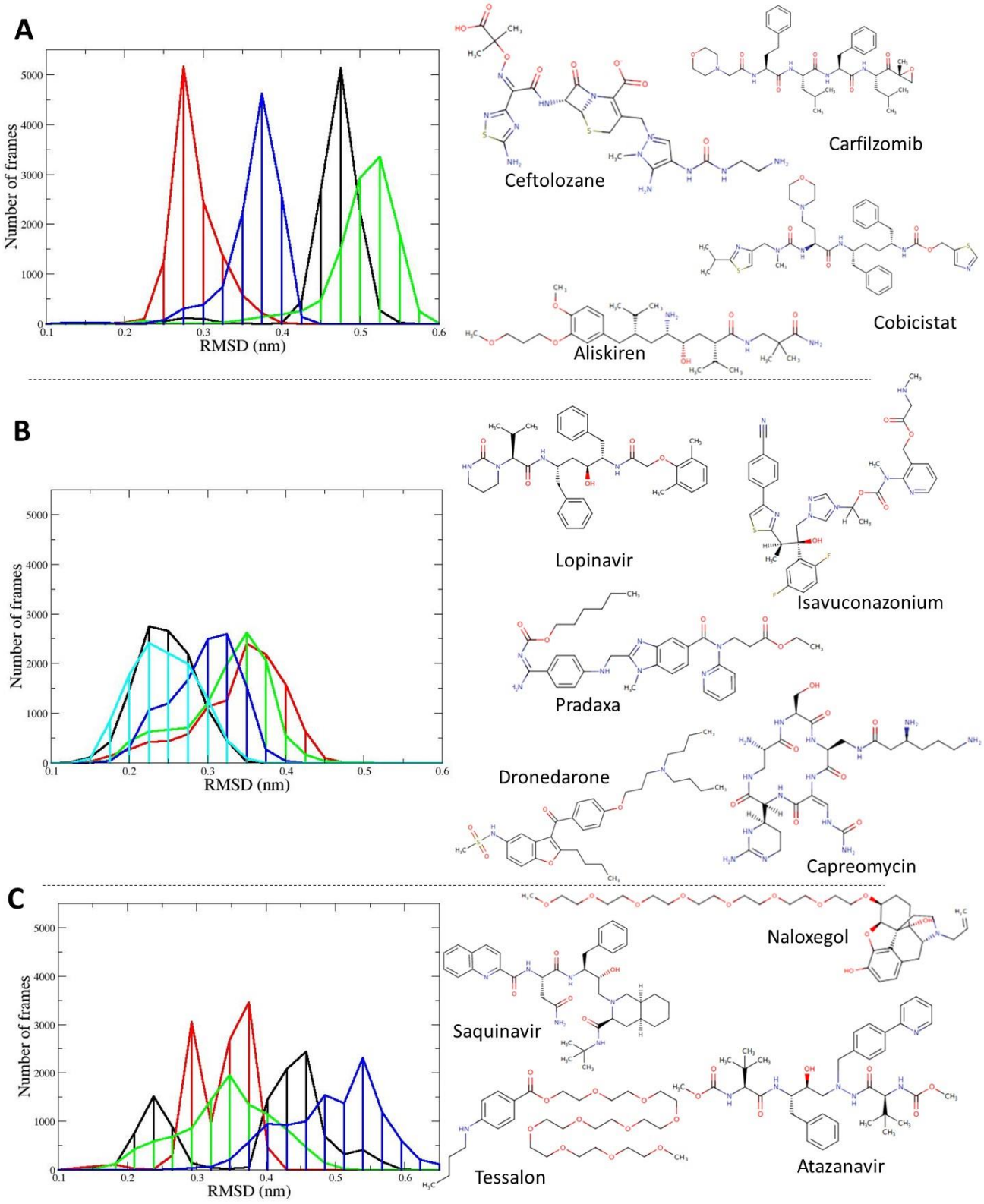

Figure 2. RMSD clustering analysis of 13 molecules from MD simulations. A) Type I (Cobicistat-black, Ceftolozane-red, Carfilzomib-green, Aliskiren-blue), B) Type II (Lopinavir-black, Isavuconazonium-red, Pradaxa-green, Dronedarone-blue, Capreomycin-Cyan). C) Type III (Saquinavir-black, Atazanavir-red, Naloxegol-green, Tessalon-Blue).

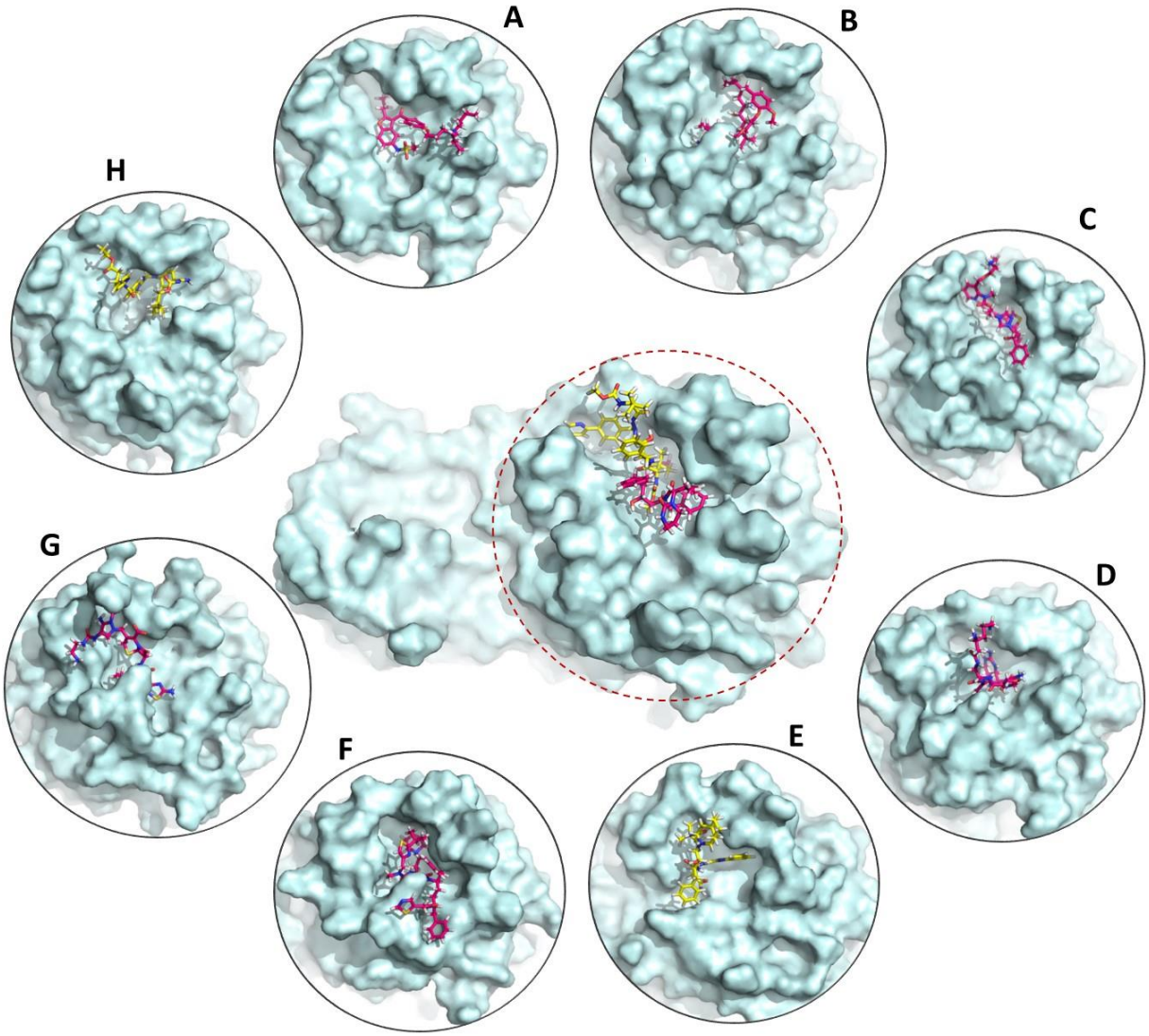

Figure 3. Representative snapshot from the MD simulations of main protease of SARS-CoV-2 in complex with different molecules. Central structure is the protein in complex with Atazanavir (yellow) compared with Lopiravir (red). The other molecules are depicted in yellow or red depending on whether they adopt a binding mode more similar to Atazanavir or Lopiravir. A) Dronedarone B) Aliskiren c) Isavuconazonium d) Capreomicyn e) Saquinavir f) Cobicistat g) Ceftolozane h) Pradaxa.

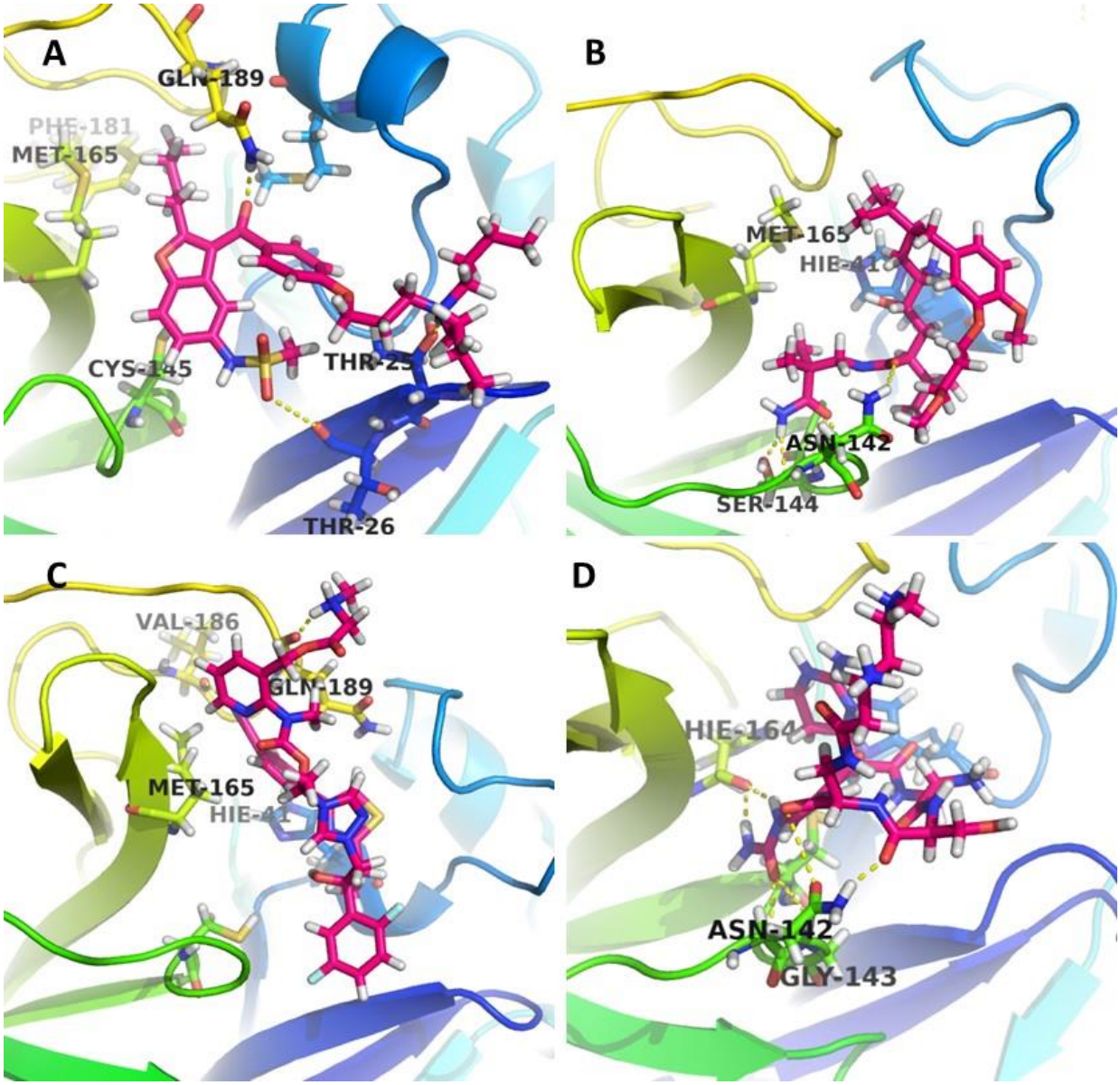

Figure 4. Interactions of some molecules with SARS-CoV-2 main protease from a representative snapshot taken from the MD simulations. A) Dronedarone, B) Aliskiren, C) Isavuconazonium, D) Capreomycin.

Conclusions

We have identified candidate compounds that could be evaluated as SARS-CoV-2 Main protease inhibitors. Some of those molecules were also reported in recent studies.[18] In addition to Cobicistat, Ceftolozane and Carfilzomib, other molecules such as Aliskiren, Capreomycin and Isovucanazonium appears to be potential binders of SARS-CoV-2 main protease. If Aliskiren is confirmed as inhibitor, it could have a dual activity against SARS-CoV-2, which could be beneficial for treating patients. We also proposed that the recently identified SARS-CoV-2 inhibitor Dronedarone could be an inhibitor of the Main protease.

There is urgency in finding treatment for the new SARS-CoV-2 disease as at the time of writing this report there is no drug of choice available. Drug repurposing studies such as the one conducted here may help identify antiviral agents among molecules already approved for human use. However, we stress that such findings should be considered preliminary until safety and efficacy has been demonstrated by adequate clinical studies.

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MD inputs for various ligand-protein complexes have been deposited at https://github.com/CCPBioSim/Covid-19.

Supporting information: full ranked ligands docked in SARS-CoV-2 Main protease.

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