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Potential inhibitors against papain-like protease of novel coronavirus (SARS-CoV-2) from FDA approved drugs

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Abstract

The cases of 2019 novel coronavirus (SARS-CoV-2) infection have been continuously increasing ever since its outbreak in China last December. Currently, there are no approved drugs to treat the infection. In this scenario, there is a need to utilize the existing repertoire of FDA approved drugs to treat the disease. The rational selection of these drugs could be made by testing their ability to inhibit any SARS-CoV-2 proteins essential for viral life-cycle. We chose one such crucial viral protein, the papain-like protease (PLpro), to screen the FDA approved drugs *in silico*. The homology model of the protease was built based on the SARS-coronavirus PLpro structure, and the drugs were docked in S3/S4 pockets of the active site of the enzyme. In our docking studies, sixteen FDA approved drugs, including chloroquine and formoterol, was found to bind the target enzyme with significant affinity and good geometry, suggesting their potential to be utilized against the virus.

Introduction

The recent outbreak of coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 in China has been the cause of major concern for the global community as the number of people infected with the virus has been continuously increasing with a significant geographical spread. As on 15th February 2020, WHO reports 50,580 confirmed cases of infection globally with 1526 deaths. Several attempts are being made to find new therapeutics against COVID-19. China's National Health Commission has recommended using HIV-1 protease inhibitors, lopinavir, and ritonavir as an ad hoc treatment against the infection while Wang et al. have tested seven approved drugs *in vitro* against the clinical isolate of the virus (Wang et al., 2020). The robust research on other aspects of SARS-COV-2, including epidemiology and genome sequencing, has provided useful insights into the new virus (Lu et al, 2020; Chan et al, 2020).

The complete genome sequencing showed that the virus belonged to a large family of coronaviruses and is closely related to Severe Acute Respiratory Syndrome-Coronavirus (SARS-CoV). It encodes for two large polyproteins that are further processed by virally encoded cysteine proteases, namely, the papain-like protease (PLpro) and the 3-chymotrypsin-like protease (3CLpro, also known as the main protease-Mpro). The processing of the viral polyproteins is essential for maturation and infectivity of the virus (Chen et al., 2020). Because of the crucial roles these two proteases play in the viral life-cycle, they are important targets for antiviral drug design.

Recently, SARS-CoV-2 Mpro has been used as a target to screen clinically approved drugs as potential inhibitors (Xu et al., 2020; Liu et al., 2020; Li et al., 2020). Since the safety profile of these FDA approved drugs is well documented and the efficacy of selected few can be quickly tested against a virus culture, the drug repurposing could be an efficient approach to find therapeutics against COVID-19. We have carried out the virtual screening of these drugs against SARS-CoV-2 PLpro to find potential inhibitors of its catalytic domain.

Materials and Methods

Homology modeling

The protein sequence of SARS-COV-2 PLpro was obtained from GenBank (Accession QHD 43415). The catalytic domain of PLpro was delineated by comparing it with the known sequences of other coronaviruses. The homology model of the SARS-CoV-2 PLpro catalytic domain was generated in the SWISS-MODEL workspace (swissmodel.expasy.org/workspace) using SARS-CoV PLpro crystal structure (PDB Id: 3E9S) as a template.

Virtual screening

A total of 2525 FDA approved drugs used for docking studies were downloaded either from the DrugBank database (www.drugbank.ca) or the Zinc15 library (Sterling and Irwin, 2015). Docking was performed using the SEESAR suite of programs from BioSolveIT (www.biosolveit.de/SeeSAR).

Results and Discussion

The SARS-CoV-2 PLpro is responsible for processing three cleavage sites of the viral polyprotein to release mature non-structural proteins 1, 2 and 3. Apart from proteolytic processing, PLpro also has a deubiquitinase and deISGylating activity. There were several earlier attempts to design

inhibitors against SARS-CoV PLpro and they yielded promising results (Ratia et al., 2008; Ghosh et al., 2009, 2010; Baez-Santos et al., 2014). We, therefore, chose SARS-CoV-2 PLpro to virtually screen FDA approved drugs to find potential therapeutics. The multiple sequence alignment of the catalytic domain of PLpro from SARS-CoV-2 with other coronaviruses shows their close similarity as shown in Figure 1.

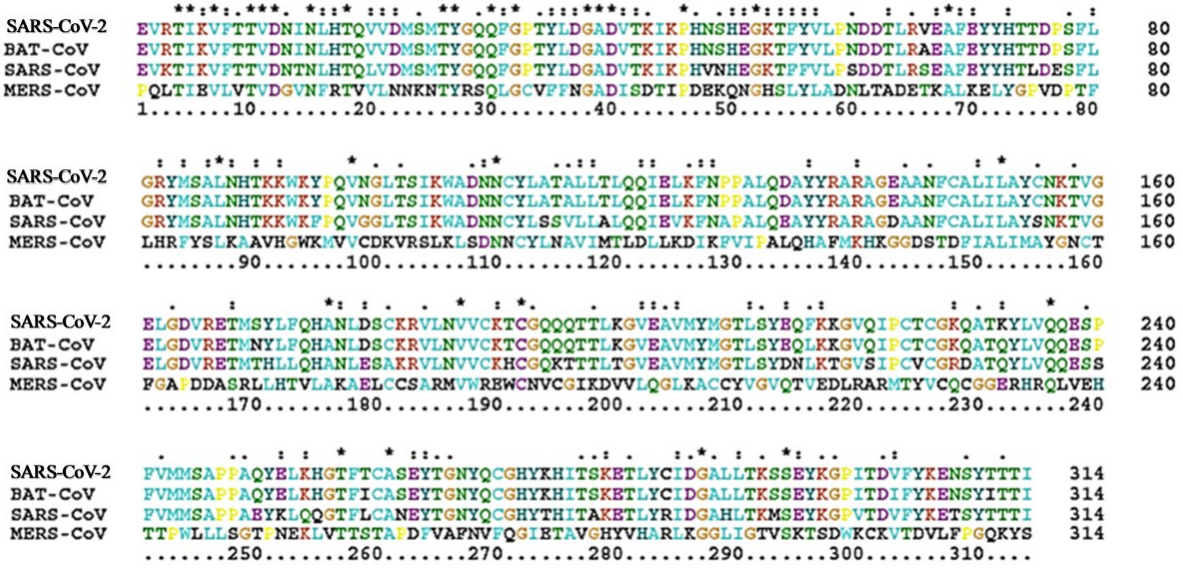

Figure 1: Multiple sequence alignment of the catalytic domains of papain-like proteases (PLpro) from different coronaviruses.

The homology model of SARS-CoV-2 PLpro was generated using the SARS-CoV PLpro crystal structure (PDB Id: *3E9S*) as a template. The homology model was in a closed flap conformation with GMQE and QMean scores (Benkert et al., 2008) of 0.95 and -0.22, respectively. The homology model could accommodate the ligand from the template well. Therefore, the template ligand (an inhibitor of SARS-CoV PLpro) was used to define the binding site for docking. The binding site contained more spacious S3/S4 pockets, rather than the restrictive S1/S2 pockets close to the catalytic residues. The S3/S4 pocket contained residues Asp164, Val165, Arg166, Glu167, Met 208, Ala246, Pro247, Pro248, Tyr 264, Gly266, Asn267, Tyr 268, Gln269, Cys217, Gly271, Tyr273, Thr301 and Asp302. For each ligand, ten poses were generated and docked into the binding site. All these poses were sorted according to their binding affinities. Thirty-four drugs had binding affinity better than 100 μM with no torsional strain, intra- and inter-molecular clashes. These compounds were then inspected visually. Out of thirty-four drugs, the ligand efficiency (LE) value was +2 for ten drugs, +1 for fourteen drugs, and 0 for seven drugs. The remaining drugs had negative LE values and were discarded from further consideration. Among the selected set, there

were several anesthetics, antineoplastic agents, appetite depressants, skin ointments, diagnostic imaging agents and other unsuitable drugs, and hence were removed from consideration. Finally, sixteen drugs were left with an estimated binding affinity within 8 nM to 100 μM range, having no torsional strain, intra- and inter-molecular clashes. These promising inhibitors of SARS-CoV-2 PLpro are listed in Table 1, and their docked poses are shown in Figure 2.

Table 1: Sixteen FDA approved drugs showing the best affinity to SARS-COV-2 PLpro

Name	Binding Affinity	L.E.	Current Application
1. Biltricide	8 nM-8 μM	+	Anthelmintic
2. Cinacalcet	26 nM-3 μM	0	Calcimimetic, to treat hyperparathyroidism
3. Procainamide	30 nM-3 μM	++	Antiarrhythmic
4. Terbinafine	33 nM-3 μM	+	Anti-fungal
5. Pethidine	53 nM - 5 μM	+	Narcotic analgesic
6. Labetalol	113 nM-11 μM	0	To treat hypertension
7. Tetrahydrozoline	137 nM -14 μM	++	Over the counter eye drops and nasal spray
8. Ticlopidine	160 nM-16 μM	+	Inhibitor of platelet aggregation.
9. Ethoheptazine	163 nM-16 μM	+	Opioid analgesic
10.Levamisole	259 nM-26 μM	++	Antihelminthic used for parasitic, viral, and bacterial infections.
11.Amitriptyline	466 nM-46 μM	0	antidepressant with analgesic properties
12.Naphazoline	697 nM-69 μM	+	Decongestant in over-the-counter eye drops and nasal preparations.
13.Formoterol	716 nM-71 μM	0	Management of COPD and asthma.
14. Benzylpenicillin	718 nM-71 μM	0	Narrow-spectrum antibiotic
15.Chloroquine	858 nM-85 μM	0	Antimalarial agent
16.Chlorothiazide	939 nM-93 μM	+	Diuretic

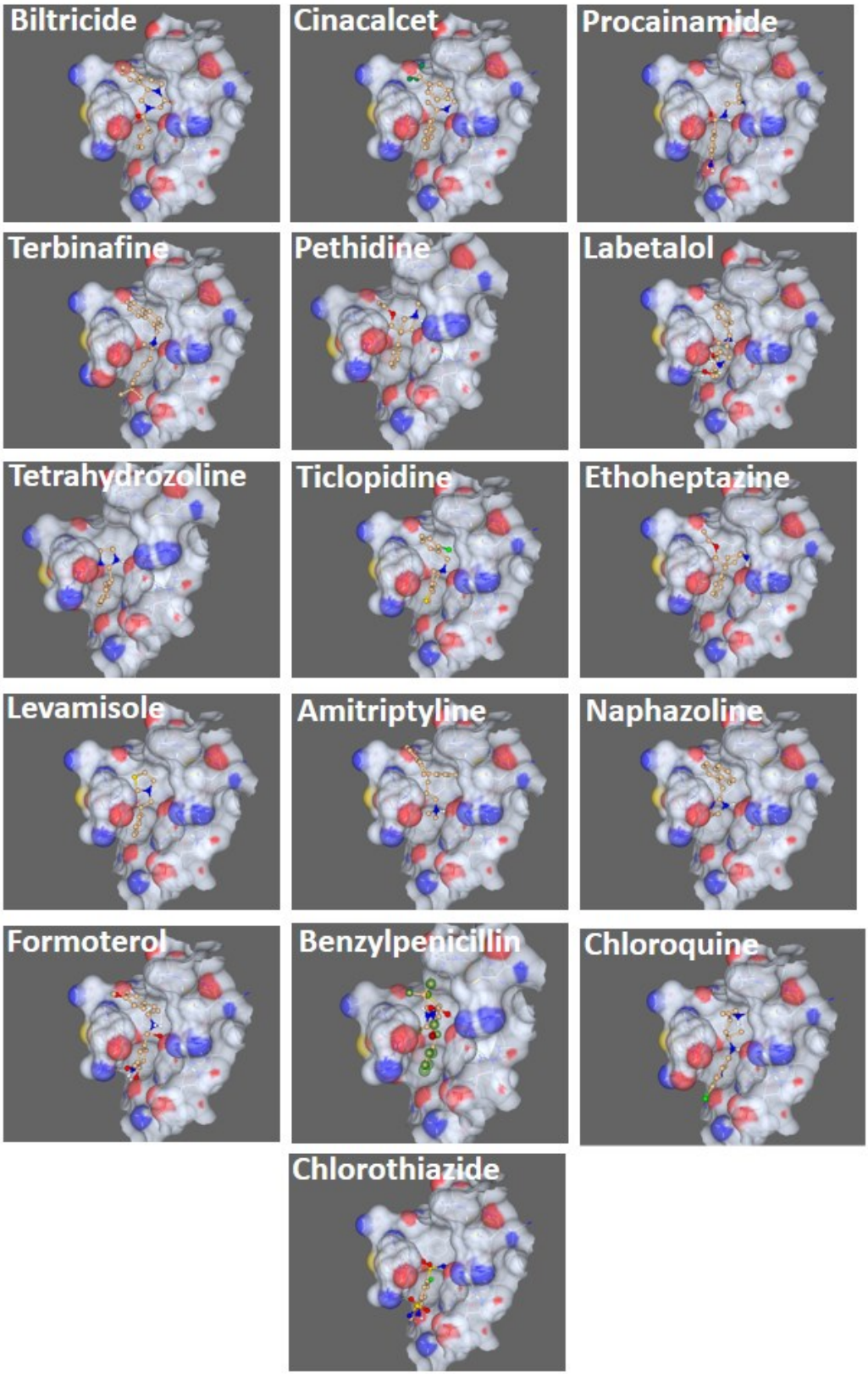

Figure 2: Docked poses of selected FDA approved drugs in the S3/S4 binding site of SARS-COV-2 PLpro. The drugs are shown as ball-and-stick models inside the binding site (shown as semi-transparent protein surface). The figures were drawn in Seesar (www.biosolveit.de/SeeSAR).

Interestingly, our analysis picked up the anti-malarial drug, chloroquine, as a potential inhibitor of viral PLpro. The anti-viral activity of chloroquine has been reported earlier (Savarino et al., 2006 & Yan et al., 2013). Recently, China has launched a few trials of the drug in patients after it was shown to block the SARS-CoV-2 infection at a low micro-molar concentration (EC_{50}: 1.13 μM) in cell culture experiment (Wang et al., 2020). It was found that chloroquine works against the viral infection at the entry-level, as well as post-entry stages. It is likely that the effect of chloroquine during post-entry stages might be manifested through its inhibition of the crucial viral protein, PLpro. The other interesting molecule picked up in our analysis is formoterol, which relaxes muscles in the airways to improve breathing and is used as a bronchodilator in the management of chronic obstructive pulmonary disease (COPD) and asthma. The drug will have a synergistic effect in treating patients if it also inhibited the activity of viral PLpro.

While the search for new therapeutics against COVID-19 continues, it will take time for a novel molecule to reach clinics. Therefore, exploring the potential of existing drugs in a rational way assumes significance. Our *in silico* study shows that sixteen FDA approved drugs have the potential to inhibit SARS-CoV-2 PLpro, and they should be evaluated in virus cultures to assess their effectiveness.

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