Prioritization of potential drugs targeting the SARS-CoV-2 main protease

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Abstract

Since its outbreak in 2019, the acute respiratory syndrome caused by SARS-Cov-2 has become a severe global threat to human. The lack of effective drugs strongly limits the therapeutic treatment against this pandemic disease. Here we employed a computational approach to prioritize potential inhibitors that directly target the core enzyme of SARS-Cov-2, the main protease, which is responsible for processing the viral RNA-translated polyprotein into functional proteins for viral replication. Based on a large-scale screening of over 13, 000 drug-like molecules, we have identified the most potential drugs that may suffice drug repurposing for SARS-Cov-2. Importantly, the second top hit is Beclabuvir, a known replication inhibitor of hepatitis C virus (HCV), which is recently reported to inhibit SARS-Cov-2 as well. We also noted several neurotransmitter-related ligands among the top candidates, suggesting a novel molecular similarity between this respiratory syndrome and neural activities. Our approach not only provides a comprehensive list of prioritized drug candidates for SARS-Cov-2, but also reveals intriguing molecular patterns that are worth future explorations.

Keywords

SARS-Cov-2, drug repurposing, molecular docking, Beclabuvir, neurotransmitters

Introduction

In the end of 2019, a pneumonia caused by a novel coronaviruse SARS-Cov-2 was characterized. After months of transmission, over 10 millions of people have been infected, of which over 500 thousands were dead (end of June, 2020). Given this severe pandemic threaten, it is critical to develop effective vaccines and drugs rapidly. Due to the limited knowledge of the molecular events during viral infection and replication, as well as the insufficient time of clinical development and trials, no effective therapeutics have been achieved yet. Recently, the crystal structures of the main protease (M^{pro}) 3CL of SARS-Cov-2, representing the core enzyme of polyprotein processing and viral replication, have been resolved, both alone and in complex with a ligand [1, 2]. In addition, M^{pro} is known to lack close homologs in human [3, 4]. M^{pro} thus becomes an ideal target for drug development.

Here, we have taken an unbiased computational approach of drug repurposing for M^{pro}. A large-scale virtual drug screening of the comprehensive databases including ZINC15 and DrugBank allows a systematic examination of currently available drugs that potentially targets M^{pro}. Further analyses also reveal intriguing molecular signatures worth further characterizations.

Materials and methods

Protein structure preparation

We downloaded the crystal structure of covid-19 main protease in complex with an inhibition of N3 (PDB: 7QBY) , used the Discovery Studio to remove the water and ligand molecules, and defined the binding site based on the N3 position with a cubic box with radius 30. Then MGLTools was used to add the polar hydrogen atoms, compute Gasteiger charges, and the result was saved as the pdbqt format.

Preparation of the small molecule library

A customized database of over 13, 000 drugs as well as drug-like molecules were compiled from ZINC15 and DrugBank. For multiple-molecule files, OpenBabel was used to split into individual small molecule files. Addition of hydrogen atoms or computation of partial charges were omitted if already present in the structure file. For format conversion, then Python script prepare_ligand4.py was executed to covert from mol2 to pdbqt.

Molecular docking analysis

To perform molecular docking, we used AutoDock Vina to dock each molecule to the given binding site of the main protease, and evaluated the binding affinities. After the computation procedures, all result files were parsed and compiled into a categorized table with all necessary information. Finally, the potential drugs are ranked according to the decrease of binding free energy.

Data analyses and visualization

All data analyses were performed in the R environment (v3.5.0). Structural visualization was accomplished with the PyMOL toolkit. R packages ggplot2 was used for additional data visualization.

Results

Drug repurposing for the main protease of SARS-Cov-2

To comprehensively evaluate all currently available approved drugs as well as drug-like molecules, we have compiled a large-scale molecule database from ZINC15 and DrugBank, comprising of ~13150 ligands. For drug repurposing towards SARS-Cov-2, we followed the standard procedures of molecular docking analysis (Figure 1A). Based on a recently published M^{pro} structure in complex with the N3 ligand, we successfully defined a reasonable binding pocket for potential drug inhibitors. After a long-term docking computation with the AutoDock vina command tool, we were able to compile a comprehensive table of all potential ligands and ranked them according to their affinity scores, representing the free energies in complex with the target protein in the given binding pocket. For each ligand, only the best binding pose or conformation was retained. To eliminate redundancy, a few drugs included were removed if determined to be duplicates of the same molecules.

Detailed analyses of the top drug candidates

The top ten candidates were further analyzed for their properties (Table 1, Figures 1-2): Phthalocyanine (DB12983), Beclabuvir (DB12225), Tirilazad (DB13050), Ergotamine (ZINC000052955754), Danicopan (DB15401), Dihydroergotoxine (ZINC000014880002), Cepharanthine (ZINC000030726863), Galicaftor (DB14894), N-1H-indazol-5-yl-2 -(6-methylpyridin-2-yl)quinazolin -4-amine (DB08450), and Petesicatib (DB15297). The binding affinity scores ranged from -11.5 to -9.8, with Phthalocyanine being the top one with a highest score of -11.5. Phthalocyanine is an aromatic macrocyclic compound initially used as a green/blue-colored dye and currently investigated as a

potential photodynamic therapy agent for diseases including skin cancer (https://www.drugbank.ca/drugs/DB12983). Interestingly, the second best candidate Beclabuvir, known as an inhibitor of RNA-dependent RNA polymerase (RdRp) of hepatitis C virus (HCV) [5], is reported to inhibit the RdRp of SARS-Cov-2 as well, according to a recent preprint [6]. Another interesting candidate is Galicaftor, under a clinical trial NCT03540524 for patients with lung cystic fibrosis (https://www.drugbank.ca/drugs/DB14894). It is thus worth further study for whether it might be repurposed for the severe lung virus infection. We also noted that two neurotransmitter-like ligands, Ergotamine and Dihydroergotoxine, are both among the top candidates. Possibly in line with this prediction, a majority of SARS-Cov-2 patients were with taste/olfactory malfunctions [7], and over 1/3 of SARS-Cov-2 patients were with neurologic symptoms (and 45.5% in severe patients) [8]. Indeed, it is recently observed that there is a close connection between nervous system diseases and SARS-Cov-2 [9]. More direct assays are required to verify if there is a novel link between M^{pro} potential inhibitors and neural modulators.

Discussion

In this study, a large-scale ligand screening was performed with in silico computations, which identified top candidates with potentially interesting discoveries. Previously related work are either in a much smaller scale or not relying on drug repurposing [10–12], and therefore our study could be a more comprehensive and up-to-date reference in this important field. Beclabuvir seems a very interesting candidate drug worth experimental verification in the soon future, for its probable activity towards both M^{pro} and RdRp, two core proteins critical for SARS-Cov-2 replication. The lung disease drug under clinical trial, Galicaftor, could be another interesting candidate for this novel lung infection. We also reveal a novel link between neurotransmitter-like modulators and M^{pro} inhibition, which might be a part of the global connection between nervous system diseases and SARS-Cov-2, an intriguing aspect with various independent supportive clues [7–9].

Nonetheless, we have limited ourselves to the M^{pro} target. Further combination analysis of M^{pro} and RdRp could be better in terms of coverage as well as including target/drug interactions. In addition, we did not screen the huge numbers of non-drug-like molecules, such as the lead structures, which are essential in developing novel therapeutics. However, for a drug repurposing study, we have accomplished a most up-to-date large-scale screen of available drug-like structures from

comprehensive databases like ZINC15 and DrugBank, which should be valuable to identify new applications of existing drugs or drug-like molecules.

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Authors' contributions

RD and TZ designed the project. YL, YZ and YH performed the data analyses and interpretation. All authors contributed to and approved the manuscript.

Competing interests

We declare that we have no competing interests.

Data availability

The results from this study are all available in the supplementary materials.

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Tables

Table 1. Top ten drug-like candidates for M^{pro}.

No	Database id	Name	Molecular weight	LogP	Binding affinity (kcal/mol)
1	DB12983	Phthalocyanine	514.552	6.49	-11.5
2	DB12225	Beclabuvir	659.838	2.64	-10.5
3	DB13050	Tirilazad	624.874	6.63	-10.2
4	ZINC000052955754	Ergotamine	581.673	1.991	-10.1
5	DB15401	Danicopan	580.418	2.53	-10
6	ZINC000014880002	Dihydroergotoxine	583.689	2.081	-9.9
7	ZINC000030726863	Cepharanthine	606.719	6.874	-9.9
8	DB14894	Galicaftor	559.47	6.4	-9.9
9	DB08450	N-1H-indazol-5-yl-2-(6-methylpyridin-2-yl)quinazolin-4-amine	352.3919	4.47	-9.8
10	DB15297	Petesicatib	603.54	2.3	-9.8

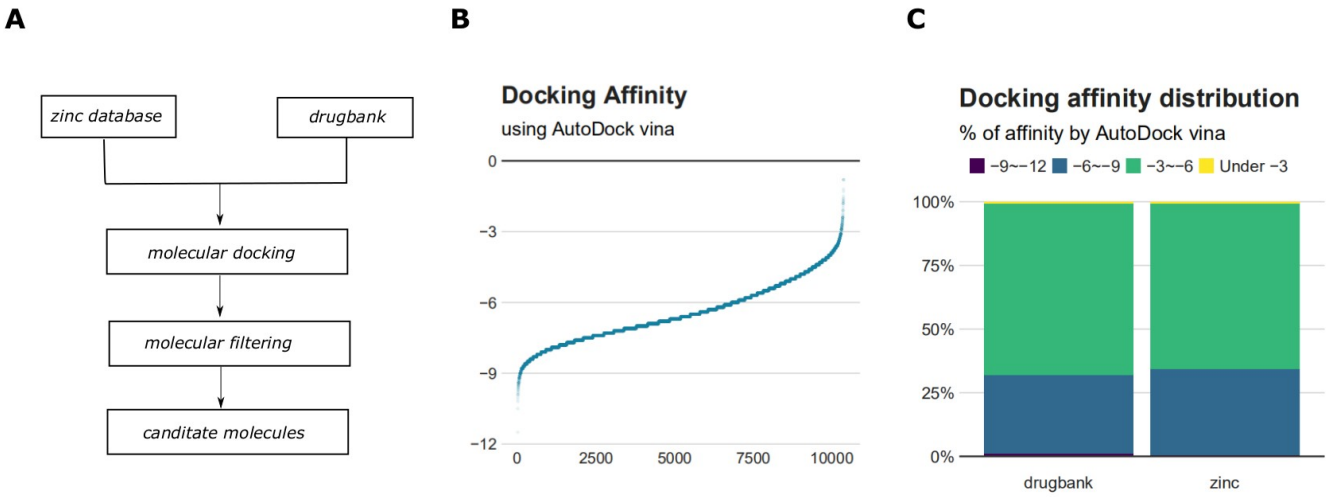
Figure legends

Figure 1. A drug repurposing study designed for SARS-Cov-2. (**A**) A simple schematic flowchart of molecular prioritization. (**B**) Distribution of all drug-like molecules sorted by decreasing binding affinities. (**C**) Distribution of all drug-like molecules categorized by binding affinity intervals.

Figure 2. Structures of the top ten drug-like candidates for the main protease.

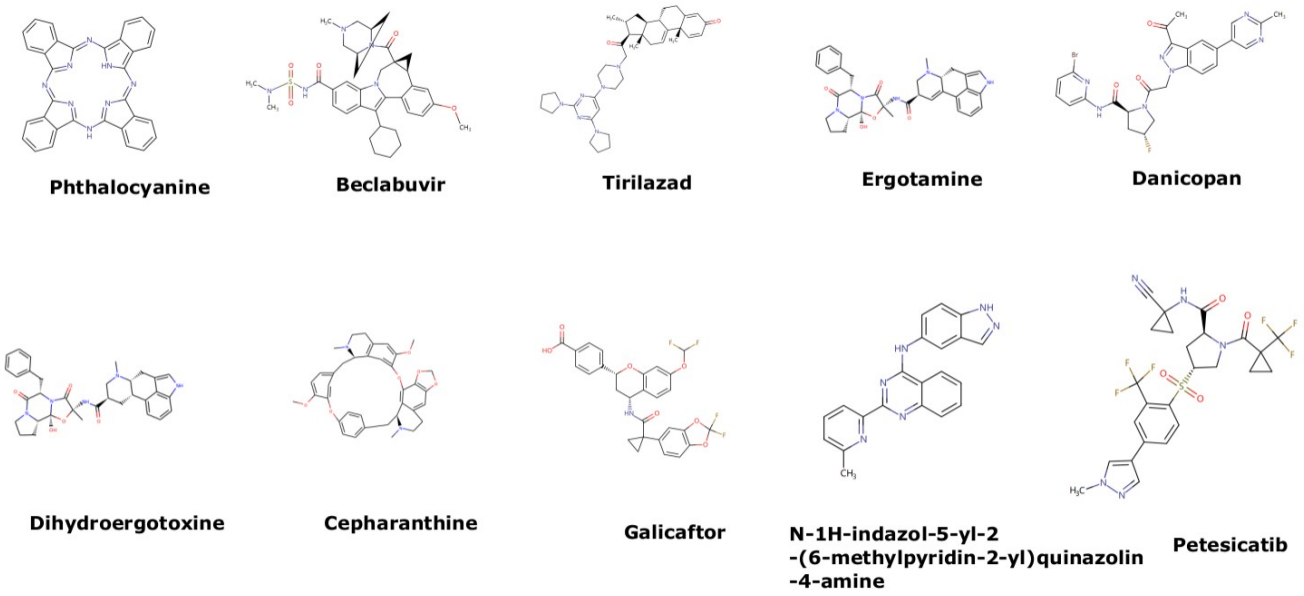
Figure 3. Complex structures of the top ten drug-like candidates docked into the binding site of the main protease.

147 Figure 1.



149

150 Figure 2.



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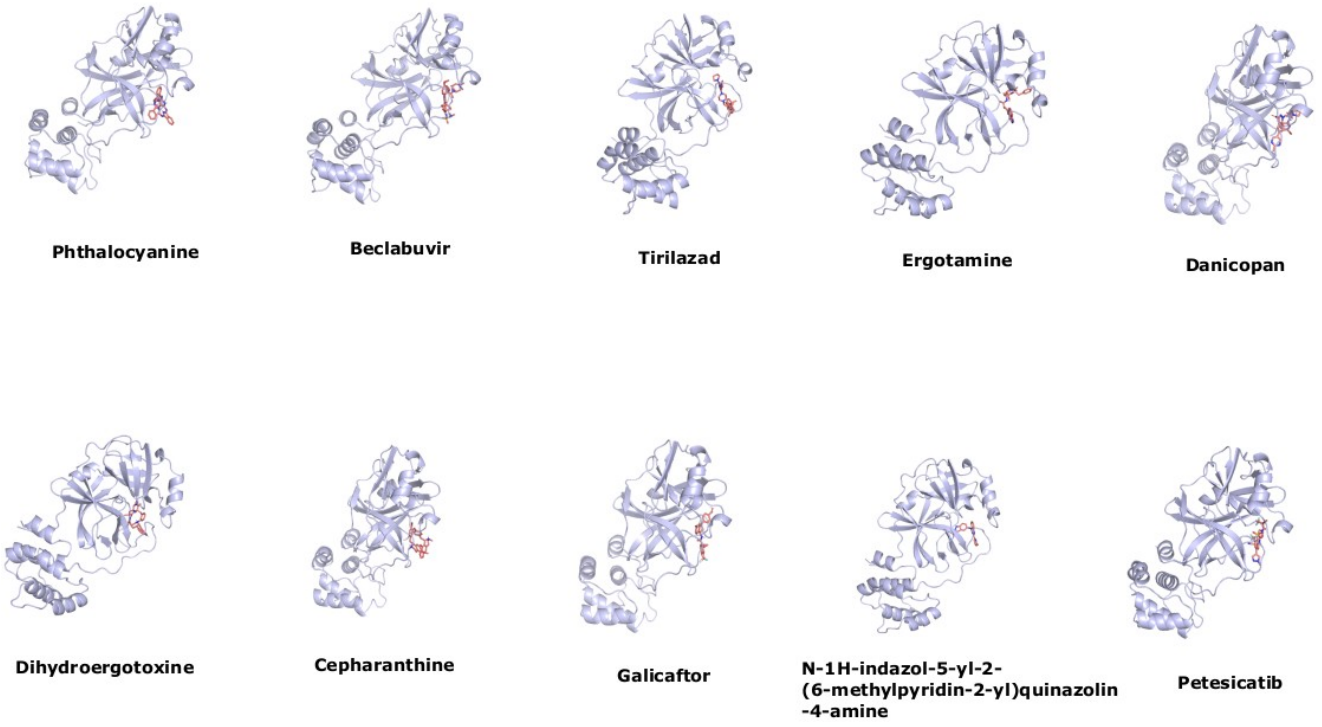
153 Figure 3.


Phthalocyanine
Beclabuvir
Tirilazad
Ergotamine
Danicopan
Dihydroergotoxine
Cepharanthine
Galicaftor
N-1H-indazol-5-yl-2-(6-methylpyridin-2-yl)quinazolin-4-amine
Petesicatib