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ORIGINAL ARTICLE

Direct acting anti-hepatitis C combinations as potential COVID-19 protease inhibitors

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Abstract The coronavirus pandemic could be the most threatening outbreak in the twenty-first century. According to the latest records of world health organization, more than 130 millions have been infected by COVID-19, with more than 2.9 million reported deaths. Yet, there is no magic cure for treatment of COVID-19. The concept of drug repurposing has been introduced as a fast, life-saving approach for drug discovery. Drug repurposing infers investigating already approved drugs for new indications, using the available information about pathophysiology of diseases and pharmacodynamics of drugs. In a recent work, more than 3000 FDA approved drugs were tested using virtual screening as potential antiviral agents for COVID-19. In this work, the top ranked five hits from the previous docking results together with drugs of similar chemical feature and/or mechanistic destinations were further tested using AutoDock Vina. The results showed that anti-HCV combinations could be potential therapeutic regimens for COVID-19 infections.

Keywords Coronavirus pandemic · COVID-19 · AutoDock Vina · Anti-hepatitis C combinations · Direct acting antiviral agent

Introduction

The coronavirus pandemic is a global risk that threatens the lives of millions of people on earth. This pandemic is caused by severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) (also known as coronavirus disease 2019; COVID-19) [1], which was first diagnosed in Wuhan, the capital of china's Hubei province and spread since then to the rest of the world. According to Weekly epidemiological update of the world health organization, more than 130 million reported cases of coronavirus infections were reported worldwide, with number of deaths exceeding 2.9 million. Despite the increasing numbers of cases and deaths, there is no magic cure for SARS-CoV-2 yet. The process of drug discovery takes years of extensive research, and requires millions of dollars to introduce a single drug to the pharmaceutical market [2]. The first stage in the process of drug discovery starts with laboratory testing, which takes in average 3–4 years. Successful molecules in the first stage are tested on experimental animals, a stage that takes around 3 years. Drugs with proven safety and efficacy will be further tested on human through three phases: Phase I (tens of healthy volunteers), Phase II (hundreds of patients), and Phase III (thousands of patients). The real challenge in the drug discovery journey, besides the prolonged timeframe and the high cost, is the low success rate; out of 5000 molecules that undergo laboratory testing, only 250 enter the preclinical stage on experimental animals. Out of these 250 molecules, only 5 molecules are transferred to the clinical trial stage to finally

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launch a single drug. Figure 1 shows the different stages of drug discovery and the estimated timeframe.

The concept of drug repurposing has been recently presented as a shortcut to the expensive long process of drug discovery. Drug repurposing implies investigating already approved drugs for new indications, using the available information about pathophysiology of diseases and pharmacodynamics of drugs. SARS-CoV-2 is a positive RNA strand that shares about 79.6% genome identity with SARS-CoV and about 96% with bat coronavirus [3]. This similarity between SARS-CoV-2 and other coronaviruses can hypothesize how the host immune system may react to this particular virus as well as the virus response [4]. During SARS-CoV viral infection, the trimeric spike glycoprotein is cleaved into two subunits: S1 and S2. The S1 subunit binds to the peptidase domain (PD) of the angiotensin converting enzyme 2 (ACE2) through the receptor binding domain (RBD) [5, 6]. Another critical process for viral infection is the cleavage of S2 by the host protease enzyme [7], which makes this enzyme an attractive target for researchers. Figure 2 shows the life cycle of SARS-CoV-2 and the potential targets for drug development.

In a recent study by Alessandro Contini, more than 3000 FDA approved drugs were tested using virtual screening by PLANTS software for potential use as antiviral agents for SARS-CoV-2. The results showed that a number of the known protease inhibitors could be successful candidates of COVID-19 therapy [8]. As an extension of Contini's work, the top ranked five hits from the docking results [8] were selected for further screening by AutoDock Vina. To widen the scope of our screening, drugs with similar chemical feature and/or mechanistic destinations to the top five hits were also tested and compared with the reported promising drugs of COVID-19, based on the calculated

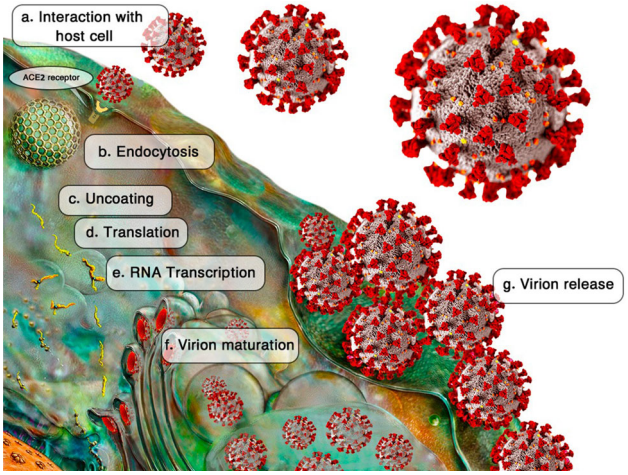

Fig. 2 The life cycle of SARS-CoV-2 showing the possible target site

binding affinity. AutoDock is the most commonly cited docking program in the scientific literature [9].

Materials and method

Protein/macromolecule/receptor

COVID-19 main protease (PDB ID: 6M03) was obtained from the Protein Data Bank [10] in a pdb format with a resolution of 2 Å. The pdb file COVID-19 main protease was a textual format describing the 3D structure of the enzyme. The 6M03 protein was presented in apo form (no ligand bound) and contained one chain (chain A), with a sequence length of 306 amino acids.

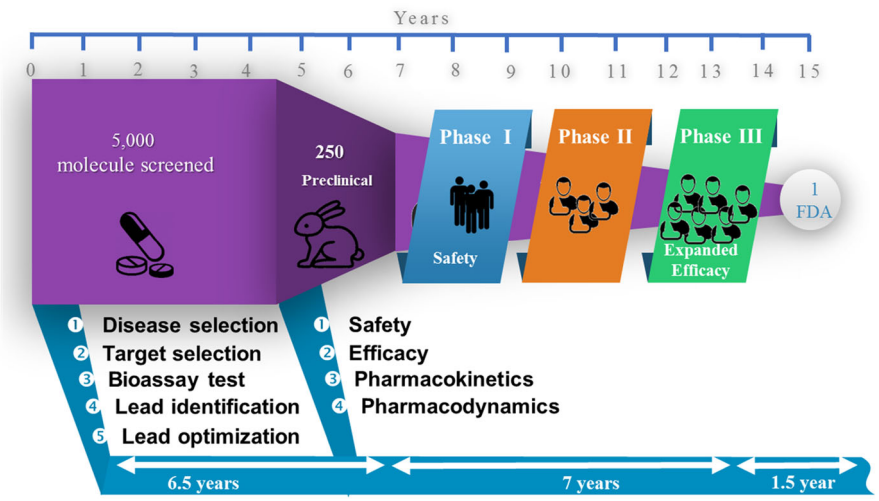

Fig. 1 Overview of drug discovery process showing the timeframe of each stage

Ligands

The top five hits (Angiotensin II human acetate, indinavir, GHRP-2, cabicistat and montelukast), the structurally similar drugs and the other proteases inhibitors were tested. Similar compounds of the top five hits were selected using the Drug Bank with a similarity threshold of 0.7. For sake of comparison, the binding affinity of promising drugs were also calculated. Figure 3 shows the tested drugs and the reason of testing. Drug-like properties were calculated for experimental drugs using "rule of five" which predicts the absorption and the permeation (Table S1). The SMILES (simplified molecular input line entry system) strings of the selected compounds were collected from PubChem database (pubchem.ncbi.nlm.nih.gov) and Drug Bank database (https://databank.worldbank.org), then submitted to UCSF Chimera to generate 3D Structures and for energy minimization.

Molecular docking

The 3D model of COVID-19 protease and the ligands were then prepared in the right format (pdbqt) using Molecular Graphics Laboratory (MGL) tools for conducting docking using Auto Dock Vina [11]. MGL 1.5.6 tools were used to generate grid for the receptor. Discovery Studio Visualizer was used to visualize the results of docking, and the evaluation was based on the binding affinity.

Result and discussion

Virtual screening of the selected collection of ligands showed marked variations in the Dock score. In this section, only drugs with dock scores higher than the top drugs in Contini's work will be highlighted. The following table shows tested drugs in a descending order according to the calculated dock score.

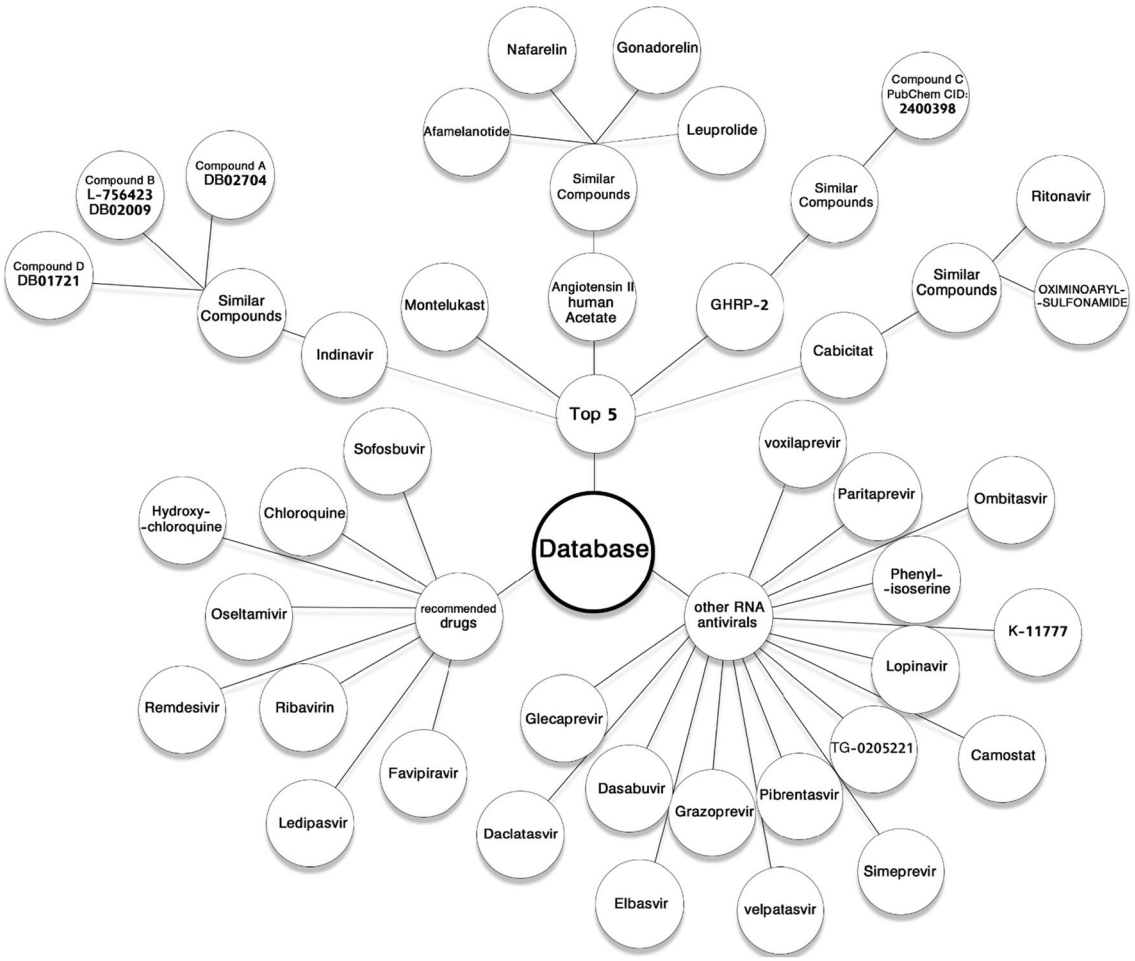

Fig. 3 Data base used for screening for potential COVID-19 protease inhibitor including Contini's top five hits, similar chemical feature and/or mechanistic destinations to the top five hits and compared the reported promising drugs COVID-19

Drugs with binding affinity higher than ivermectin

As shown in Table 1, three of the tested drugs (Simeprevir, Ledipasvir and pibrentasvir) showed binding affinity higher than ivermectin. Interestingly, the three drugs are antiviral for hepatitis C infection with different modes of action. Simeprevir is a hepatitis C virus (HCV) NS3/4A protease inhibitor indicated for the treatment of chronic hepatitis C virus (HCV) infection in patients with HCV genotype 1 or 4 [12]. Simeprevir was approved by the FDA in November 2014 and is marketed under the brand name Olysio® as oral tablets. Ledipasvir is a direct acting antiviral (DAA) and prescribed in combinations to treat chronic HCV genotypes 1, 4, 5, and 6. Although ledipasvir's mechanism of action is unknown, it is postulated to prevent hyperphosphorylation of NS5A (Non-structural protein 5A), which is required for viral protein reproduction, RNA replication and assembly of HCV virions [13]. Pibrentasvir inhibits HCV NS5A; a protein that is essential for replication, assembly and maturation of infectious viral proteins. Pibrentasvir is indicated for patients with chronic hepatitis C virus (HCV) genotype 1, 2, 3, 4, 5 or 6 infection with or without compensated cirrhosis [14]. Ivermectin was first used against worms (except tapeworms), but, in 2012 it was approved for the treatment of head lice infestations in patients 6 months of age [15]. Ivermectin is mainly used for the treatment of intestinal strongyloidiasis resulting from *Strongyloides stercoralis* and onchocerciasis resulting from *Onchocerca volvulus* and scabies caused by an infestation of the skin by *Sarcoptes scabiei* [16]. Ivermectin showed ability to induce ~5000-fold reduction in SARS-CoV-2 RNA within two days [17]. The mechanism of action of ivermectin on in SARS-CoV-2 RNA is not confirmed, but the calculated binding affinity speculates the possibility of competitive binding to viral protease [17].

Drugs with binding affinity higher than the top five hits

As shown in Table 1, seven drugs showed binding affinities higher than the five top hits, but less than ivermectin. These drugs included compound (A), glecaprevir, paritaprevir, elbasvir, daclatasvir, velpatasvir and compound (B). Compound (A) -(2R,3R,4R,5R)-3,4-Dihydroxy-N,N'-bis[(1S,2R)-2-hydroxy-2,3-dihydro-1H-inden-1-yl]-2,5-bis(2-phenylethyl)hexanediamide- showed high binding affinity to COVID-19 protease. This ligand is not approved yet as an FDA drug, and it is a synonym for diol-based HIV-1 protease inhibitor 4. The compound computed properties are compared to the properties typically used for druglikeness and leadlikeness (Table S1) according to the "rule of five", which helps optimize pharmacokinetic properties of drug molecules. Glecaprevir is a direct acting antiviral agent and HCV NS3/4A protease inhibitor, and is indicated for adult patients with chronic HCV genotype 1, 2, 3, 4, 5 or 6 with compensated cirrhosis or infection without cirrhosis [18]. Paritaprevir is used in combination with other drugs for treatment of HCV genotype 1a, 1b and

Table 1 Binding affinity score with COVID-19 protease (6M03)

Drug name	Dock score	Drug name	Dock score
Simeprevir	− 9.5	Afamelanotide	− 7.4
Ledipasvir	− 9.4	Sofosbuvir	− 7.3
Pibrentasvir	− 9.4	Camostat	− 7.1
Ivermectin	− 8.7	Lopinavir	− 7.1
Compound (A)	− 8.6	Indinavir	− 7.0
Glecaprevir	− 8.4	Ritonavir	− 7.0
Paritaprevir	− 8.2	Gonadorelin	− 6.7
Elbasvir	− 8.2	Leuprolide	− 6.7
Daclatasvir	− 7.9	Remdesivir	− 6.5
velpatasvir	− 7.9	TG-0205221	− 6.4
Compound (B)	− 7.9	GHRP-2	− 6.4
Angiotensin II human Acetate	− 7.9	Oximinoaryl sulfonamide	− 6.2
voxilaprevir	− 7.8	Cabicistat	− 6.0
K-11777	− 7.7	Ribavirin	− 5.6
Grazoprevir	− 7.6	Chloroquine	− 5.5
Dasabuvir	− 7.5	Oseltamivir	− 5.3
Compound (C)	− 7.5	Phenylisoserine	− 5.2
Compound (D)	− 7.5	Favipiravir	− 5.1
Ombitasvir	− 7.4	Montelukast	− 4.6
		Hydroxychloroquine	− 4.4

4 by inhibition the of NS3/4A serine protease [19]. Elbasvir is part of combination therapy for treatment of chronic Hepatitis C. It is a direct acting antiviral by targeting HCV non-structural protein 5A with inhibition effect, the role of the protein is unknown but it is obvious that the protein is essential for viral replication and virion assembly [20]. Angiotensin II human acetate showed the highest binding affinity in Contini's work. The drug is authorized for use in the European Union (Giapreza) and indicated for the treatment of refractory hypotension in adults with sepsis [21]. The compound has interacted with the target with a binding affinity score of − 7.9 (kcal/mol), but Angiotensin II human acetate is a natural substrate for protease. It is here worth mentioning that three drugs (Velpatasvir, daclatasvir and compound B) showed binding capacity equivalent to angiotensin II human acetate. Daclatasvir is a direct-acting antiviral agent used for the treatment of HCV genotype 1 and 3. The target of daclatsvir is Nonstructural protein 5A [22]. Velpatasvir is an HCV NS5A inhibitor used in combination with other drugs for the treatment of patients with chronic HCV genotypes 1, 2, 3, 4, 5 or 6 infection without cirrhosis or with compensated cirrhosis, or in combination with ribavirin if associated with decompensated cirrhosis [23]. Compound B (L-756423) is still experimental, and completed phase 2 in treatment of human immunodeficiency virus (HIV) Infections by targeting gag-pol polyprotein [24]. The ligand protein interaction score was -7.9 (kcal/mol). So, the ligand might have the ability to inhibit Covid-19 protease. Figure S1 shows the binding sites of the drugs with highest dock scores in this study.

Other potential protease inhibitors

As Table 1 shows, the other tested drugs exhibited lower dock scores than angiotensin II human acetate. In this section, the indication and the mode of action of the highest 14 these drugs are briefly discussed. K-11777 is a cysteine protease inhibitor that acts as a broad-spectrum antiviral, by targeting cathepsin-mediated cell entry. K-11777 was observed to inhibit SARS-CoV virus entry in the sub-nanomolar range. So, potential inhibitory effect of K-11777 on COVID-19 protease is possible. Grazoprevir is used for treatment of chronic hepatitis C in combination with other drugs as a direct acting antiviral medication. The drug is used for genotypes 1a, 1b, or 4 infection in adults [23], by the inhibition of NS3/4a protease. Which leads to the inhibition of HCV viral replication [25]. Dasabuvir is indicated for the treatment of patients with HCV genotype 1a or genotype 1b in combination with other drugs, including those with compensated cirrhosis. Dasabuvir Inhibits HCV RNA-dependent RNA polymerase, the enzyme is encoded by the NS5B gene, and is essential for the replication of viral genome [26].

Compound (C) (2S)-N-Benzyl-2-acetamido-3-(1H-indol-3-yl)propanamide interacted with the target with binding affinity of − 7.5, but still not approved as therapeutic drug. The compound has good computed properties not only for drug likeness criteria but also for leadlikeness criteria in accordance with the "rule of five" (Table S1). Compound (D) (N-[2-hydroxy-1-indanyl]-5-[(2-tertiary-butylaminocarbonyl)-4(benzo[1,3]dioxol-5-ylmethyl)-piperazino]-4-hydroxy-2-(1-phenylethyl)-pentanamide) is similar compound to indinavir with a similarity score of 0.789 and it is targeting gag-pol polyprotein [27], but the action of the compound is not available. The ligand interacted with the target with a score of − 7.5 kcal/mol.

Ombitasvir is a direct acting antiviral used for the treatment of patients with genotype 4 chronic HCV infection without cirrhosis, by the inhibition of HCV non-structural protein 5A, which required for viral replication, but the main role is not clear yet. Afamelanotide is currently the only approved drug therapy used in the management of erythropoietic protoporphyria, and has greater affinity for its target and a longer biological half-life [26]. The drug interacted with the target with a score of − 7.4 kcal/mol. Despite afamelanotide is peptide analogue of the endogenous alpha melanocyte-stimulating hormone (α-MSH), the drug is more resistant to degradation by serum and proteolytic enzymes [28], so it is expected to resist degradation by COVID-19 protease. Sofosbuvir (Sovaldi®) is a direct anti-viral used to treat the infectious liver disease caused by HCV by preventing viral replication through binding to the two Mg^{2+} ions present in HCV NS5B polymerase's GDD active site motif. The drug is used in combination with other drugs [29]. Sofosbuvir showed a binding affinity with a score of − 7.3 kcal/mol in the ligand protein interaction with COVID-19 protease. Camostat is a potent serine protease inhibitor, which is expected to have the ability to inhibit protease enzyme. Clinical trials on the drug has been performed with Estimated Enrollment of 180 participant [30]. Lopinavir isan antiviral, used in combination with other drugs in the treatment of HIV infection, and the drug is HIV protease inhibitor, which is responsible for the cleavage of gag polyprotein [31]. Lopinavir is now under study for repurposing for the treatment of COVID-19 in combination with ritonavir umifenovir [32]. Indinavir is an antiretroviral drug for the treatment of HIV infection, by targeting HIV type 1 protease [33]. Indinavir approved for medical use in 1996 [34]. The drug docking score of the interaction between the drug and COVID-19 protease was − 7.0 (kcal/mol). Ritonavir is an HIV protease inhibitor, and indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection [35]. Ritonavir was first approved

on March, 1996 by FDA [36]. The drug targets are nuclear receptor subfamily 1 group I member 2 and HIV type 1 protease. The action of ritonavir on both targets is activating and inhibiting respectively, so it is expected that the drug might inhibit COVID-19 protease as in ligand protein interaction with dock score − 7.0 (kcal/mol). Gonadorelin is a synthetic decapeptide gonadotropin-releasing hormone (GnRH). Gonadorelin is indicated for increasing the functional capacity and response of the gonadotropes of the anterior pituitary and after removal of a pituitary tumor by increasing residual gonadotropic function of the pituitary. Gonadorelin has agonist action on both gonadotropin-releasing hormone receptor and putative gonadotropin-releasing hormone II receptor [37], thus it might have inhibiting effect on COVID-19 protease as the score of ligand protein interaction with the target is − 6.7 (kcal/mol).

Hepatitis C combination drugs

Hepatitis C infection is caused by hepatitis C virus which can deteriorate from chronic inflammation to fibrosis, cirrhosis and even to hepatocellular carcinoma [38]. Combination of HCV infection was recommended to increases treatment efficacy. These combinations include ledipasvir/sofosbuvir (Harvoni®) dasabuvir/ombitasvir/paritaprevir/ritonavir (Viekira PAK®), elbasvir/grazoprevir (Zepatier®), ombitasvir/paritaprevir/ritonavir (Technivie®) sofosbuvir/velpatasvir (Epclusa®), glecaprevir/pibrentasvir (Mavyret®). The pharmacokinetic and the pharmacodynamic profiles of these combinations have proven safety. Using these combinations as antivral agents for COVID-19 based on the results of the docking score could also guarantee synergistic effects without any unexpected drug-drug interactions. As shown in Table 1, Mavyret® combination is the most recommended. Harvoni® and Zepatier® combinations are also recommended due to the high dock score and the reported safety.

The current outbreak of coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still threatening a large number of people worldwide. This study aimed to examine several compounds that may be used to inhibit the disease. Antiviral drugs for HCV infection showed the highest binding affinity (13 drugs out of the top 20 drugs). The binding affinity of ivermectin is high compared with other drugs so, we the binding affinity of Ivermectin as a benchmark. Simeprevir, pibrentasvir and ledipasvir were the most promising candidates for COVID-19 protease but further research and investigation are required to confirm the efficiency of these compounds.

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