Anti Hepatitis C virus drugs show potential drug repositioning for SARS CoV-2 main protease: an in silico study

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Abstract

Developing effective and safe vaccines/drugs against SARS CoV-2 may take some time. The urgency of the outbreak has led to the usage of broad spectrum of existing anti-viral drugs, across the globe. Among the existing anti-viral drugs, there is still a challenge in identifying the potent drug or the combination of the drugs for the better treatment and faster recovery of the patients. *In silico* molecular docking study aids in the process of drug repurposing and we aimed to identify the binding potential of some of the existing anti-viral drugs and their interactions at the active site of SARS CoV-2 main protease. Results from our study revealed that the drugs Simeprevir, Elbasvir, Paritaprevir, Beclabuvir, Dasabuvir, Teleprevir, Velpatasvir, Ombitasvir, Ledipasvir, Boceprevir, Asunaprevir, Declatasvir, Sofusbuvir which are used in the treatment of Hepatitis C virus infection may act as potent inhibitors for SARS CoV-2 main protease.

Introduction

The toughest situations of the era are currently being faced internationally, with lot of regulations rapidly evolved and changed our way of life in an unprecedented manner to stop the spread of the virus, COVID-19. The Wuhan city of Hubei province in China became the first victim of the virus attack. The World Health Organization (WHO) on January 12, 2020 temporarily named this virus as 2019 novel coronavirus (2019-nCoV) and one month later, WHO renamed it as SARS CoV-2 (Severe acute respiratory syndrome coronavirus-2) as it showed 89.1% nucleotide similarity with SARS CoV (Wu, Zhao, *et al.*, 2020). Coronaviruses infect different species including mammals and aves and can cause chronic diseases that affect the respiratory, neurological and gastrointestinal systems (Bosch *et al.*, 2003). SARS CoV unfolded to five continents within the year 2003 with a death rate of 10%, affecting almost 8000 people and about 800 death for which the host was identified to be bat (Cheng *et al.*, 2007). Later in 2012, the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) broke out in the regions of Arabian Peninsula with a lethal rate of 35% and the host was dromedary (Wu, Liu, *et al.*, 2020). The first whole genomic sequence of SARS CoV-2 was published by Zhang and his team (Wu, Zhao, *et al.*, 2020). SARS CoV-2 belongs to the beta-coronavirus group, sharing its ancestry with the HKU9-1 (Xu *et al.*, 2020; Coutard *et al.*, 2020) bat coronavirus, homologous to SARS CoV. On January 30, WHO declared a Public Health Emergency of International Concern (PHEIC) as it commenced to take the lives of many humans without any precise approved remedy and its quick unfold to many countries worldwide due to human-to-human transmission. COVID-19 has been declared as a pandemic by the WHO on March 11. As of 29th April 2020, the number of confirmed cases are almost 3 million in 213 countries with more than 2,00,000 deaths and the rates are still mounting.

The enveloped coronaviruses belong to genus of the coronaviridae family and subfamily coronavirine and possess a genome with the longest single stranded positive sense RNA of 27–32 kb (van der Hoek, 2004). The virus that infects the host cells act similar to a messenger RNA that helps in the synthesis of various kinds of proteins which includes the ones that help in their replication. The genome of SARS

CoV-2 is identical to other beta-coronaviruses in many ways like the 5'-untranslated region (UTR), a replicase complex (orf1ab) which encodes the non-structural proteins, few structural proteins, 3'-UTR and some unidentified non-structural open reading frames (Zhu *et al.*, 2020). The non-structural proteins involve main protease (Mpro), papain-like protease (PLpro), non-structural protein-12 (nsp-12), nsp-13, nsp-14, nsp-15, nsp-16, and nsp-10. The sub-genomic RNA's are translated into the structural proteins like the spike protein (S protein) containing the receptor binding domain (RBD) which invades the host cell through the cell surface receptor, angiotensin-converting enzyme 2 (ACE-2). The viral fusion protein (S2 of S protein) assists in membrane fusion of virus with the host cells. Further, envelop small membrane protein (E protein) functions as ion channel, membrane protein (M protein) involves in protein-protein interaction that is required for the assembly of coronaviruses and nucleocapsid protein (N protein) play multiple roles in viral replication cycle like modulating the host cell, regulating viral RNA synthesis. The binding of the spike protein with the cell membrane is initiated by enzymes like trypsin, furin, cathepsin L (Hasan *et al.*, 2020).

The common symptoms that accompany with the disease are fever, cough, headache, shortness of breath, dyspnea, whereas some acute cases will have pneumonia, kidney failure and even death due to the infection. Differentiation of SARS CoV-2 from the most common symptoms of atypical pneumonia and etiological diagnosis is possible only through laboratory tests. WHO recommended for the screening of the suspected samples with nucleic acid amplification tests (NAAT) like real-time reverse-transcription polymerase chain reaction (rRT-PCR) of the upper respiratory specimens (like nasopharyngeal and oropharyngeal swab) for patients under observation. Patients with severe symptoms are to be tested with the lower respiratory specimens like sputum or endotracheal aspirate (WHO, Interim guidance, 2020). WHO also advised the detection of antigen in the respiratory samples to confirm the presence of viral proteins expressed by SARS CoV-2. This test is used to detect early or at the severe stages of infection as the concentration of serum antibody rises after few days of the infection. Another detection method is based on the host antibody detection which detects the presence of antibodies in the blood samples of people suspected of COVID-19. But this test does not work in the early stage of the infection as the antibodies will develop only after few days or a week. Thus it is mostly possible in the recovery phase.

The main protease or SARS CoV Mpro, also known as chymotrypsin-like protease (3CLpro) cleaves at almost 11 sites (Anand *et al.*, 2003) on the polyprotein to form the non-structural proteins (nsp) which later combine to form the replicase-transcriptase complex (RTC). Inhibitors acting on this enzyme will cease the process of replication of this viral gene (Wu, Liu, *et al.*, 2020). Therefore it is considered to be one of the best choices for drug targets against coronavirus infection. In this study, we performed molecular docking of various known anti-viral drugs in order to predict their potential to be considered as a drug against the SARS CoV-2 infection by looking into the various interactions of these drugs at the active site of the main protease. As a preliminary analysis in the high throughput screening of existing drugs, *in silico* molecular docking studies help in saving time and the cost involved in laboratory and clinical trials. Since the side effects, maximum dosage to be given, and the metabolic characteristics are known for these existing anti-viral drugs, testing them for the new infection should be given an upper hand.

Materials And Methods

The 3D conformers for the ligands were downloaded from Pubchem (Kim *et al.*, 2015) and Chempidder (Harry, 2010) in.sdf and.mol format, respectively. The ligands were converted to their corresponding pdb format using MarvinSketch (<http://www.chemaxon.com>) and OpenBable (O'Boyle *et al.*, 2011) which were read as input for AutoDock 4.2 (Morris *et al.*, 2009) to perform molecular docking studies. AutoDock Tools were used for the preparation of protein and ligands, grid generation, docking and for the analysis of docked conformations. Autodock uses a special file format for the ligand and the receptor called the pdbqt format which is a modified protein data bank format that includes partial charges, Q, atom types, T, and for the ligands; the rotatable bonds information is included. For the conversion from pdb to pdbqt, Autodock uses scripts from the graphical user interface AutoDock Tools package.

Three dimensional structure for SARS CoV-2 (2019-nCoV) was obtained from Protein Data Bank (Berman *et al.*, 2000; Burley *et al.*, 2019) PDB id 6Y2F (Zhang *et al.*, 2020). Heteroatoms were removed from the receptor to make the receptor free of any ligands before starting the docking process. After removing the water molecules; Kollman charges, polar hydrogens and solvation parameters were assigned to the receptor in order to prepare the protein for docking. Ligand preparation steps include addition of Gasteiger charge and merging of non-polar hydrogens. Autodock uses interaction maps or grid maps which were calculated prior to the actual docking run using the Autogrid program incorporated in Autodock 4.2. The interaction energy between each ligand atom type and the receptor molecule is calculated for the entire binding site described using the grid. This reduces the computation time by not calculating the interaction energies during every step of the docking process but just refer the respective grid maps. In the present study, the grid was generated around the binding site described by the amino acid residues interacting with the alpha-ketoamide 13b inhibitor (co-crystal ligand) obtained from the PDB: 6Y2F. The grid was centered on the amino acid residues, His41, Met49, Phe140, Gly143, Ser144, Cys145, His163, His164, Glu166, Pro168, His172, Val186 and Gln189. The grid box had the dimensions 40, 40, 40 Å along x, y and z axes, respectively, with the grid spacing 0.375 Å. The Lamarckian Genetic Algorithm (LGA) was used to pick the best conformers for the ligand with 10 runs for each ligand. After docking, conformations of ligand with receptor were obtained and were ranked on the basis of binding energy. Active site interactions were depicted using Schrödinger (Schrödinger, 2019).

Results And Discussion

SARS CoV-2 main protease is the most potential anti-viral target as it plays a major role in viral gene expression, processing of the polyproteins that are translated from viral RNA and replication. Structure comprises of three domains, domain 1 (residues 10 to 99), domain 2 (residues 100 to 182) and domain 3 (residues 198 to 303). Domains 1 and 2 are made up of anti-parallel beta barrel structures and the substrate binding site (active site) is located in the cleft between these two domains. His41-Cys145 catalytic dyad in the active site is highly conserved among the coronavirus proteases and plays a major role in substrate binding and the activity of the enzyme. Domain 3 is alpha-helical and plays a role in dimerization but only one monomer is active at a time. To battle the present scenario caused by SARS

CoV-2, it is highly essential to repurpose the existing anti-viral drugs targeting SARS CoV-2 main protease, as conventional drug discovery demands time. Several FDA approved anti-viral drugs were used in the molecular docking study against the SARS CoV-2 main protease, in order to elucidate the efficiency of repositioning of these drugs for the pandemic situation being faced currently. Molecular docking results were analyzed based on the binding energy and active site interactions. On the basis of the least binding energies, interactions of ten drugs at the active site of the protease are shown in the Figure 1.

Simeprevir showed least binding energy of -10.70 kcal/mol, followed by elbasvir (-10.24 kcal/mol), paritaprevir (-10.00 kcal/mol), beclabuvir (-9.98 kcal/mol), dasabuvir (-9.75 kcal/mol), telaprevir (-9.36 kcal/mol), velpatasvir (-9.23 kcal/mol), ombitasvir (-9.01 kcal/mol), ledipasvir (-8.85 kcal/mol) and nelfinavir (-8.75 kcal/mol). Of these drugs, simeprevir, elbasvir, paritaprevir, beclabuvir, dasabuvir, telaprevir, velpatasvir, ombitasvir and ledipasvir are the drugs that are used in the treatment of hepatitis C virus infection. Hepatitis C and SARS CoV-2 are positive sense RNA viruses belonging to family Flaviviridae and Coronaviridae, respectively. Other anti-HCV drugs, boceprevir, asunaprevir, daclatasvir, sofosbuvir, analysed in this study also showed good binding energy (Table 1). The binding energy (-8.41 kcal/mol) of the co-crystal ligand, alpha ketoamide 13b, is well comparable with the some of the anti-HCV and anti-HIV drugs. Combination of the least binding energy anti-HCV/anti-HIV drugs reported in Table 1 may also be taken into consideration for further studies to identify their efficacy in the treatment of SARS CoV-2.

Amino acid residues at the active site of the SARS CoV-2 main protease that are involved in the interactions with the anti-viral drugs are depicted in Table 2 and correlates well with the reported co-crystal ligand. From the analysis of drug interactions shown in Table 2, it is very clear that the drugs show interaction with the His41-Cys145 catalytic dyad which is highly an essential indicator for a good inhibitor for SARS CoV-2 main protease. Results revealed that several of the drugs that are used in the treatment of HCV infection shows better binding energy when compared with hydroxychloroquine, liponavir, ritonavir and remdesvir.

Conclusion

Several intense efforts are being vigorously attempted by health practitioners and researchers, all over the world, in order to identify the most promising drug or combination of drugs for the treatment of the patients affected by SARS CoV-2 infection. Drug repurposing plays a vital role in high throughput screening of the existing FDA approved drugs targeting several pathways involved in gene expression and replication of SARS CoV-2. Main protease of SARS CoV-2 is highly targeted for its role in the gene expression and replication process. Designing an inhibitor that binds to SARS CoV-2 main protease may prevent viral replication. We have performed *in silico* molecular docking study to identify the potential inhibitor for SARS CoV-2 main protease from some of the FDA approved anti-viral drugs. Our results strongly suggest that the drugs used in the treatment of Hepatitis C virus shows promising drug repositioning for SARS CoV-2 main protease based on the active site interactions and binding energy.

Further clinical testings of the reported drugs are essential for their use in the treatment of SARS CoV–2 infection.

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Declarations

Competing interests: The authors declare no competing interests

Tables

Due to technical limitations, Tables 1-2 are provided in the Supplementary Files section.

Figures

Figure 1

Active site of SARS CoV-2 main protease depicting the amino acid residues involved in interactions with anti-viral drugs

Supplementary Files

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