Inhibitors for Novel Coronavirus Protease

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The rapid outbreak of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in China followed by its spread around the world poses a serious global concern for public health. To this date, no specific drugs or vaccines are available to treat SARS-CoV-2. Thus, there remains an urgent need for the development of specific antiviral therapeutics against SARS-CoV-2. To conquer viral infections, the inhibition of proteases essential for proteolytic processing of viral polyproteins is a conventional therapeutic strategy. In order to find novel inhibitors, we computationally screened a compound library of over 606 million compounds for binding at the recently solved crystal structure of the main protease (M^{pro}) of SARS-CoV-2. A screening of such a vast chemical space for SARS-CoV-2 M^{pro} inhibitors has not been reported before. After shape screening, two docking protocols were applied followed by the determination of molecular descriptors relevant for pharmacokinetics to narrow down the number of initial hits. Next, molecular dynamics simulations were conducted to validate the stability of docked binding modes and comprehensively quantify ligand binding energies. After evaluation of potential off-target binding, we report a list of 12 purchasable compounds, with binding affinity to the target protease that is predicted to be more favorable than that of the cocrystallized peptidomimetic compound. In order to quickly advise ongoing therapeutic intervention for patients, we evaluated approved antiviral drugs and other protease inhibitors to provide a list of nine compounds for drug repurposing. Furthermore, we identified the natural compounds (−)-taxifolin and rhamnetin as potential inhibitors of M^{pro}.

Keywords: SARS-CoV-2 ; protease ; Mpro ; virtual screening ; free-energy calculations ; drug repurposing ; shape screening ; docking ; toxicity prediction

1. Introduction

In late 2019, a novel coronavirus, termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was determined as a cause for several cases of respiratory disease in China. Even though most infected patients only suffer from mild symptoms such as fever and cough associated with a good prognosis, the disease can progress into fatal cases of pneumonia and acute respiratory failure, especially in older people with comorbidities [1][2]. The virus rapidly spread from China to over 141 countries worldwide and, up to this date, infected almost two million people claiming more than 100,000 fatalities [3] (as of 14 April 2020), already exceeding the 2003 SARS-CoV epidemic [4]. Most probably, the virus originated from a zoonotic transmission between animals such as bats and humans but progressed to transmit from human to human through common droplet infection [2][5][6]. The World Health Organisation declared the SARS-CoV-2 outbreak a Public Health Emergency of International Concern [7][8] and, based on the 2003 epidemic of SARS-CoV, it will lead to the loss of many lives exerting enormous social impact and economic loss [9]. Clinical treatment of the disease is mainly symptomatic and based on repurposing of already marketed antiviral drugs such as ritonavir and antibiotics to treat secondary infections [1]. Thus, there remains an urgent need for the development of specific antiviral therapeutics and vaccines against SARS-CoV-2 [4][10][11][12].

The inhibition of viral proteases necessary for proteolytic processing of polyproteins has been a successful strategy in the pharmacological treatment of human immunodeficiency virus (HIV) and hepatitis C virus, respectively [13][14], proving the potential of protease inhibitors for the treatment of viral infections. Similarly, the main protease (M^{pro}) of SARS-CoV-2 is thought to be essential for viral replication and, therefore, is regarded as promising target for antiviral pharmacotherapy [10][15]. The crystal structure of the SARS-CoV-2 main protease was recently solved [16] which enables the rational design of specific inhibitory compounds. The close relationship of SARS-CoV-2 to SARS-CoV is reflected by the high sequence identity of 96.1% among their M^{pro} [17][10]. In this regard, it was suggested that compounds developed against SARS-CoV might be effective to treat SARS-CoV-2 [12] and can therefore be considered as template structures for M^{pro} inhibitors. However, these compounds remained in the preclinical or early clinical stage and did not lead to approved therapeutics [18]. Furthermore, their effectiveness for the novel virus might suffer due to differences in individual amino acids [19] as we discuss in the beginning of the next section. Accordingly, the development of specific inhibitors for SARS-CoV-2 main protease remains an urgent necessity in the scientific community [10][11][12] which is reflected by the multiple projects

focusing on this protein. For example, three studies investigating the repurposing of marketed drugs proposed several candidates for SARS-CoV-2 treatment [10][15][20][21]. In another recent study, a deep learning approach based on a fully connected neural network trained on the PDBBind database [22] combined with a homology model of the protease was applied to screen a library of approximately one million compounds including already approved drugs, tripeptides, and natural products [12]. However, the disadvantage of applying therapeutics originally designed for a different target is the risk of undesired pharmacological effects and adverse reactions [23]. Even though vaccine development can be assisted by computational methods [24], this study is focused on the design of a small-molecule enzyme inhibitor.

2. Development

In this study, we screened a large library of over 606 million compounds with the aim to discover novel inhibitors for the SARS-CoV-2 main protease. We designed a protocol consisting of a combination of intensively validated methods that were successfully applied in drug discovery programs either as standalone tools or in combination [25][26][27][28]. In a first step, we performed a shape-based screening with known binders for the SARS-CoV main protease and relevant substructures as template molecules. After the initial shape screening, two different docking protocols were applied followed by the assessment with pharmacokinetic filters to narrow down the number of potential binders. Clustering based on molecular fingerprints was applied to ensure structural diversity of the compounds that were, in the next step, subjected to molecular dynamics (MD) simulations. Based on the obtained trajectories, the binding free energy of the ligands was quantified using Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) post-processing. In the last step, we assessed potential toxic effects of the compounds due to the interaction with 16 known off-targets to make a final selection of 12 compounds. In addition, we report the highest scored natural compounds and a list of marketed drugs that could be repurposed for SARS-CoV-2 treatment. Such a comprehensive exploration of chemical space intending to discover SARS-CoV-2 M^{pro} inhibitors was, to the best of our knowledge, not previously reported.

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