Identification of Potential Inhibitors of Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) Main Protease from Non-Natural and Natural Sources: A Molecular Docking Study

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doctors have shown to be promising. Natural chemical substances from plants provide a good source of chemicals for the development of potential novel antiviral drugs against viral pathogens including HIV-1. In January 2020, a new promising target useful for structure-based drug design was elucidated and stored in the Protein Data Bank. In this context, the objective of this study was to determine whether and how a set of both non-natural and natural HIV-1 protease inhibitors could dock to that novel crystallized severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) main protease and, consequently, to identify potential lead compounds to treat COVID-19 infected patients. The results showed that two non-natural compounds, danoprevir and lopinavir, and one compound from plant, corilagin, produced strong interactions with the inhibitor binding site of SARS-CoV-2 main protease. It is expected that this work contributes to validate the use of HIV-1 protease inhibitors against SARS-CoV-2.

Keywords: molecular docking, coronavirus, COVID-19, protease, natural products

Introduction

Coronaviruses (CoVs) are a large family of viruses which may cause illness in animals and humans. Those viruses are known to cause respiratory infections ranging from the common cold to more severe diseases like severe acute respiratory syndrome (SARS). At the end of December 2019, a novel CoV of severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) was identified to be the cause of pneumonia outbreak in China, named COVID-19. This disease is less deadly but much more infectious than common SARS. Until May 25, 5,304,772 people have been infected with the disease, and 342,029 deaths were reported worldwide.

The main symptoms of COVID-19 disease are fever, tiredness, and dry cough. Some patients may have aches and pains, nasal congestion, runny nose, sore throat, and diarrhea. Around 1 out of every 6 people who gets COVID-19 becomes seriously ill and develops difficulty breathing. Elderly people and person with pre-existing medical conditions like high blood pressure, heart diseases, lung diseases, cancer or diabetes are more likely to develop serious illness.

To date, there is neither a vaccine nor a specific antiviral drug to prevent or treat the disease. Previous studies were carried out to investigate the ability of human immunodeficiency virus type 1 (HIV-1) protease inhibitors be used for the treatment of COVID-19 patients. Moreover, a new survey by Genetic Engineering & Biotechnology News (GEN) reveals 35 active drug development programs to fight against COVID-19, which have received public attention in recent days in North America, Europe, and China. Those known drugs including HIV-1 protease inhibitors, such as danoprevir (1), darunavir (2), lopinavir (3), oseltamivir (4), and ritonavir (5) have received considerable attention (Figure 1). Natural products provide a good source of chemicals for the development of potential novel antiviral drugs...
against viral pathogens such as coronavirus, dengue virus, hepatitis B and C virus, herpes simplex virus, influenza virus, and human immunodeficiency virus. Polya published a review regarding protein and non-protein inhibitors from plants, which included potent HIV-1 protease inhibitors (Table 1, and Figure 1).

Coronaviruses contain a genome composed of a long ribonucleic acid (RNA) strand, which acts just like a messenger RNA when it infects a cell and directs the synthesis of two long polyproteins that include the machinery that the virus needs to replicate new viruses. These proteins include a replication/transcription complex that makes more RNA, several structural proteins that construct new virions, and two proteases. The proteases play essential roles in cutting the polyproteins into all of these functional pieces. In January 26, 2020, Liu and co-workers deposited the crystal structure of SARS-CoV-2 main protease (M”) in complex with an inhibitor.

**Figure 1.** Non-natural-HIV-1 protease inhibitors; HIV-1 protease inhibitors from plants; and \(N-[(5\text{-methylisoxazol-3-yl})\text{carbonyl}]\text{alanyl-L-valyl-N-1-}-(\text{1R,2Z})-\text{4-(benzyloxy)-4-oxo-1-}-(\text{3R})\text{-2-oxopyrrolidin-3-yl][methyl]}\text{but-2-etyl-L-leucinamide}.
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Effectively, the crystal structure is currently the only public-domain 3D structure from SARS-CoV-2, and it is organized as a dimer of two identical subunits that together form two active sites (Figure 2). Such a protease is thought to be a promising target for discovery of small-molecule drugs that would inhibit the cleavage of the viral polyprotein and prevent the spread of the infection. \(^{15}\)

To investigate the possibility that non-natural and natural HIV-1 protease inhibitors may interact with the SARS-CoV-2 M\(^{\text{pro}}\) and, consequently, identify potential inhibitors of this enzyme, a molecular docking study was performed.

**Methodology**

The crystallographic structure of SARS-CoV-2 M\(^{\text{pro}}\) (PDB ID: 6LU7)\(^{15}\) was used as the biomacromolecular receptor in molecular docking simulations. The three-dimensional structures of both non-natural and natural HIV-1 protease inhibitors were obtained from PubChem.\(^{16}\) Those structures were energy minimized using the universal force field molecular mechanics method.\(^{17}\) The molecular docking simulations were performed with AutoDock Vina 1.1.2 software.\(^{18}\) Molecular graphic representations were performed with PoseView 1.1.2,\(^{19}\) PyMOL 2.1.0,\(^{20}\) and BIOVIA Discovery Studio 2020\(^{21}\) softwares.

**Results and Discussion**

In order to develop a molecular docking protocol, the inhibitor that is co-crystallized with the SARS-CoV-2 M\(^{\text{pro}}\) structure, \(15\), was redocked in its binding cavity. Default parameters of the docking software were used, except the exhaustiveness that was defined as 50. Figure 3 shows that \(15\) was satisfactorily redocked and, consequently, all subsequent docking simulations were performed according to the same protocol.

According to the results of the molecular docking simulations, HIV-1 protease inhibitors interact strongly with the SARS-CoV-2 M\(^{\text{pro}}\). Table 2 shows the results of the calculated inhibitors-protease interaction affinities, as well as the key interacting binding site residues of the SARS-CoV-2 M\(^{\text{pro}}\).

### Table 1. HIV-1 protease inhibitors from plant

<table>
<thead>
<tr>
<th>Compound (class)</th>
<th>Plant source</th>
<th>HIV-1 specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carnosolic acid 6</td>
<td>Rosmarinus officinalis (Lamiaceae)</td>
<td>0.2</td>
<td>7</td>
</tr>
<tr>
<td>Corilagin  7</td>
<td>Phyllanthus amarus (Euphorbiaceae)</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>Kaempferol 8</td>
<td>widespread; Cuscuta reflexa (Convolvulaceae), Pisum sativum (Fabaceae)</td>
<td>7</td>
<td>8-10</td>
</tr>
<tr>
<td>α-Mangostin 9</td>
<td>Garcinia mangostana (Clusiaceae)</td>
<td>5</td>
<td>11.12</td>
</tr>
<tr>
<td>γ-Mangostin 10</td>
<td>Garcinia mangostana (Clusiaceae)</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Oleanolic acid 11</td>
<td>Luffa cylindrica (Cucurbitaceae), Rosmarinus officinalis (Lamiaceae)</td>
<td>8; 22</td>
<td>13</td>
</tr>
<tr>
<td>Uvaol 12</td>
<td>Crataegus pinnatifida (Rosaceae)</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>7-O-Ethylrosmanol 13</td>
<td>semi-synthetic from carnosolic acid</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Rosmanol 14</td>
<td>semi-synthetic from carnosolic acid</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

HIV-1: human immunodeficiency virus type 1; IC\(_{50}\): half maximal inhibitory concentration.
Figure 3. (a) Inhibitor 15; (b) danoprevir; (c) lopinavir; and (d) corilagin docked in the binding site of SARS-CoV-2 M\textsuperscript{pro}. In (a), cyan is the co-crystallized structure; whereas red is the redocked pose.
As expected, the co-crystallized compound (15) presents the best affinity energy for that enzyme (−9.8 kcal mol\(^{-1}\)). Comparing with that compound, two non-natural HIV-1 protease inhibitors, danoprevir (1) and lopinavir (3), showed the best affinity energy for the SARS-CoV-2 M\(^{pro}\) (−8.5 kcal mol\(^{-1}\)). Ritonavir (5), darunavir (2), and oseltamivir (4) showed affinity energies equal to −7.9, −7.6, and −6.0 kcal mol\(^{-1}\), respectively. All HIV-1 protease inhibitors from plants showed better affinity energy for the SARS-CoV-2 M\(^{pro}\) than oseltamivir. Moreover, among those inhibitors, corilagin (7), a chemical isolated from *Phyllanthus amarus*, a plant known in Brazil as “quebrapiedra”, shows the best affinity energy for the enzyme; comparable to danoprevir (1) and lopinavir (3) affinities.

One remarkable finding regarding the simulations is the fact that no ligand-protease interacting pattern was found. The reason is the significant molecular diversity of the studied compounds. Figure 3 shows danoprevir, lopinavir, and corilagin docked into the SARS-CoV-2 M\(^{pro}\) binding cavity. 2D-representations of the key interactions are also shown.

Table 2 shows that the ligands dock in the shallow binding site of SARS-CoV-2 M\(^{pro}\) through polar or hydrophobic interactions. A number of hydrogen bonds acts as “anchors” between carbonyl, hydroxyl, and amino groups of the compounds and the protease (Figure 3, and Supplementary Information section). Two compounds that showed the worst affinity values, oseltamivir and 7-O-ethylrosmanol, did not show significant interactions with the protease.

**Table 2. Docking results: affinity energy and key binding site residues of SARS-CoV main protease**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Affinity / (kcal mol(^{-1}))</th>
<th>H bond</th>
<th>Hydrophobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-crystallized ligand</td>
<td>−9.8</td>
<td>His-163, Glu-166, Thr-190</td>
<td></td>
</tr>
<tr>
<td>Non-natural-HIV-1 protease inhibitors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Danoprevir</td>
<td>−8.5</td>
<td>His-164, Gln-189</td>
<td>Glu-166</td>
</tr>
<tr>
<td>Lopinavir</td>
<td>−8.5</td>
<td>Thr-26, Glu-166, Gln-189</td>
<td>His-41, Met-49, Asn-142, Cys-145, Met-165</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>−7.9</td>
<td>Ser-46, Gln-189</td>
<td>His-41, Met-49, Cys-145, Met-165, Gln-189</td>
</tr>
<tr>
<td>Darunavir</td>
<td>−7.6</td>
<td>Thr-25</td>
<td>His-41, Met-49, Met-165, Gln-189</td>
</tr>
<tr>
<td>Oseltamivir</td>
<td>−6.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-1 protease inhibitors from plants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corilagin</td>
<td>−8.2</td>
<td>Cys-145, Gln-189, Thr-190</td>
<td></td>
</tr>
<tr>
<td>Uvaol</td>
<td>−7.9</td>
<td>Thr-24, Leu-141</td>
<td>Thr-25, Met-49, Asn-142</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>−7.8</td>
<td>Leu-141, Asp-187, Gln-189</td>
<td>Met-165, Gln-189</td>
</tr>
<tr>
<td>Oleanolic acid</td>
<td>−7.8</td>
<td>Ser-144</td>
<td>Met-49, Cys-145, Gln-189</td>
</tr>
<tr>
<td>γ-Mangostin</td>
<td>−7.6</td>
<td>Thr-190</td>
<td>Met-165, Gln-189</td>
</tr>
<tr>
<td>α-Mangostin</td>
<td>−7.4</td>
<td>His-41, Thr-190</td>
<td>Met-165, Gln-189</td>
</tr>
<tr>
<td>Rosmanol</td>
<td>−7.1</td>
<td>Leu-141, Ser-144, Glu-166</td>
<td></td>
</tr>
<tr>
<td>7-O-Ethylrosmanol</td>
<td>−6.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carnosolic acid</td>
<td>−6.9</td>
<td>Gly-143, Cys-145</td>
<td></td>
</tr>
</tbody>
</table>

HIV-1: human immunodeficiency virus type 1; His: histidine; Glu: glutamic acid; Thr: threonine; Gln: glutamine; Ser: serine; Cys: cysteine; Leu: leucine; Asp: aspartate; Gly: glycine; Met: methionine; Asn: asparagine.

**Conclusions**

Since November 2019, the outbreak of COVID-19 infection has challenged the healthcare systems around the world, and several research approaches have been applied in order to try to identify potential new drugs. One of those strategies was the usage of non-natural HIV-1 protease inhibitors as probable candidates to treat the disease. In this context, one of the proposed hypothesis is the ability that such inhibitors would be able to inhibit the SARS-CoV-2 M\(^{pro}\). Recently, the crystal structure of this specific CoV-2 enzyme was elucidated, and represents a good molecular target for designing new anti-CoV-2 drugs.

Following the line of thought regarding the usage of HIV-1 inhibitors as potential SARS-CoV-2 M\(^{pro}\) inhibitors, in this theoretical project it was verified the probability of some non-natural and natural HIV-1 protease inhibitors, selected from the literature, to be able to dock in the binding site of the SARS-CoV-2 M\(^{pro}\).

It was found that two non-natural compounds, danoprevir and lopinavir, and a compound isolated from *Phyllanthus amarus*, a plant known in Brazil as “quebrapiedra”, corilagin, bind strongly to the binding site of the
protease of CoV-2. Consequently, although subsequent enzymatic experiments must be done, the docking results presented in this report support the hypothesis that perhaps danoprevir, lopinavir, and corilagin may be used as single antiviral agents targeting that enzyme or in combination with other potential therapies for treating COVID-19 patients.

Supplementary Information

Figures of compounds 2, 5, 6, 8, 9, 10, 11, 12, 14 docked into the binding site of SARS-CoV-2 main protease is available free of charge at http://jbcs.sbq.org.br as PDF file.

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References


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