

# The main protease of SARS-CoV-2 as therapeutic target to development specific drugs to treat COVID-19

## Abstract

The pandemic disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) denominated COVID-19 is an important problem of world public health because do not have specific drugs and vaccines available to control the disease progression and the rapid virus spreading. SARS-CoV-2 genome resemble to other SARS-CoV and MERS-CoV coronaviruses genomes, thus drugs developed for SARS and MERS treatment may be used for COVID-19. The aim of this work is discuss the molecular target of SARS-CoV-2, the main protease, a chymotrypsin-like named as 3CL<sup>pro</sup>, for a rational development of specific molecules anti-SARS-CoV-2 with broad-spectrum anti-coronavirus activities. This study is a literature mini review using 47 articles obtained from Public Medline and Science direct mainly from this year 2020. The coronaviruses infection is mediated by spike protein from viral capsid which bind to ACE2 receptor in the host cells which endocytose the viruses RNA, which is translated to proteins that are cleaved by proteases, mainly the 3CL<sup>pro</sup>. This enzyme is crucial to SARS-CoV-2 replication and its inhibition can prevent the virus replication. Thus, it is an important coronaviruses target because the amino acid sequence of 3CL<sup>pro</sup>s from SARS, MERS and SARS-CoV-2 have high similarity. In silico studies have been demonstrated that synthetic small molecules, natural products, peptidomimetic inhibitors, and HIV inhibitors had different docking scores with SARS-CoV-2 3CL<sup>pro</sup>. However, peptidomimetic inhibitors seems to be better inhibitors to this protease and promise drugs to treat COVID-19 and other diseases caused by coronaviruses.

**Keywords:** SARS-CoV-2, Covid-19, therapeutic target, 3CL<sup>pro</sup>Main protease, inhibitors

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**Abbreviations:** E, envelope; M, membrane; N, nucleocapsid, S, spike

## Introduction

On December 31, 2019, Chinese health authorities alerted the World Health Organization (WHO) to several cases of pneumonia without a recognized aetiology in Wuhan City, Hubei province, China. The first cases were reported on December 8, 2019 and on March 11, 2020 COVID-19 gained pandemic status.<sup>1</sup> This pathogen was denominated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the Coronavirus Study Group and the disease was named coronavirus disease 2019 (COVID-19) by the WHO.<sup>2</sup> COVID-19 is affecting 217 countries and territories around the world and 2 international conveyances with more than 9.1 million confirmed cases and about 470 thousand confirmed deaths.<sup>1</sup> Billions of people have been impacted due to mandatory lockdowns, isolations, and quarantines. Thus, the severe effect of the COVID-19 outbreak has imposed major challenges for global health, society and economy. Nowadays, the COVID-19 treatment and control are very difficult because there are no specific antiviral drugs or vaccines available. The prevention and supportive therapies are being implemented to avoid new cases and minimize clinical complications.<sup>3</sup>

## Coronavirus

The current classification of coronaviruses recognizes 39 species in 27 subgenera, 5 genera and 2 subfamilies that belong to the

family Coronaviridae, suborder Cornidovirineae, order Nidovirales and realm Riboviria. Coronaviridae are enveloped single-stranded ribonucleic acid (RNA) viruses that infect humans and animals. The size of positive-sense RNA genome of CoVs is between 26.2 and 31.7 kb. The spike projections of these virions give the appearance of solar corona. The main structural proteins of these viruses are envelope (E), membrane (M), nucleocapsid (N), and spike (S) (Figure 1). Coronaviruses outbreak has been reported three times in the earlier of 21st century namely SARS in 2002 (severe acute respiratory syndrome caused by SARS-CoV), MERS in 2012 (Middle East respiratory syndrome caused by MERS-CoV), and the most recent COVID-19, caused by the new coronavirus, SARS-CoV-2.<sup>2</sup> Seven types of coronaviruses cause human diseases. The four of them (HCoV-NL63, HCoV-229E, HCoV-OC43 and HKU1) induce only mild respiratory diseases, typically only in upper respiratory tract although some of them can cause severe infections in infants, young children, and elderly individuals.<sup>4</sup> The two highly pathogenic and extremely invasive viruses, SARS-CoV and MERS-CoV, cause severe respiratory syndrome in humans and have high mortality rate, about 11 and 35 % for SARS-CoV and MERS-CoV, respectively.<sup>5,6</sup> The transmission rate of SARS-CoV-2 is much higher than SARS-CoV and MERS-CoV and other flu-viruses.<sup>7</sup> The clinical course of SARS and MERS was highly similar, and they also have similar pathogenesis.<sup>8</sup> The coronaviruses attack patient's lower respiratory system by invading the airway epithelial cells, parabronchial epithelial cells, alveolar epithelial cells, vascular endothelial cells and macrophages in the lung. The pathological changes include focal

haemorrhagic necrotising pneumonia with exudative diffuse alveolar damage, which can be fatal. They also affect gastrointestinal system, kidney, liver, heart, and central nervous system.<sup>5-9</sup>

Importantly, SARS-CoV-2 shared approximately 80% of the genome (~30,000 bases) with SARS-CoV and almost all their encoded proteins are homologous and also shows some similarities to MERS-CoV genome.<sup>10</sup> Thus, it will be very important to study the relationship among SARS-CoV, MERS-CoV, and SARS-CoV-2 for developing broad-spectrum antiviral therapies. Accordingly, drugs and other therapies for treating SARS may be used as a reference for COVID-19 treatment. The viral lifecycle steps provide potential targets for drug therapy.<sup>11</sup> Promising drug targets include nonstructural viral proteins because their sequence are found in non-variable region of RNA, such as main 3-chymotrypsin-like protease (3CL<sup>pro</sup>), papain-like protease (PL<sup>pro</sup>), RNA-dependent RNA polymerase, which share homology with other novel coronaviruses.<sup>12,13</sup> Additional drug targets include viral entry, and immune regulation pathways.<sup>13,14</sup>

### Infection and replication

The first step of viral lifecycle is the infection through the binding to a host cell receptor. The glycoprotein spike (S) on the outer surface of coronaviruses and the host transmembrane serine protease 2 (TMPRSS2) are responsible for the attachment to the angiotensin-converting enzyme 2 (ACE2) receptor and entry into the cell targets.<sup>15</sup> This serine protease hydrolyzes specific peptide bonds in S protein and expose the ACE2 receptor-binding domain.<sup>16</sup> Furin, other transmembrane serine protease, can also be associated to ACE2 receptor, cleaves the S protein and facilitate the entry of SARS-CoV-2 in host cell after binding.<sup>17</sup> Lung cells, enterocytes, nasal secretory cells co-express high levels of ACE2 and TMPRSS2.<sup>18</sup> SARS-CoV infection reduces ACE2 expression in lung cells. Because loss of pulmonary ACE2 function is associated with acute lung injury, virus-induced ACE2 downregulation may be important for disease pathology. ACE2 regulates the renin-angiotensin system (RAS). Therefore, a reduction in ACE2 function after viral infection could result in a dysfunction of the RAS, which influences blood pressure and fluid/electrolyte balance, and enhance inflammation and vascular permeability in the airways.<sup>18,19</sup> Following receptor binding, the virus particle uses host cell receptors and endosomes to enter cells. SARS-CoV-2 deliver its nucleocapsid and hijacking the cellular machinery to replicate in the cytoplasm. The RNA genome composes of six to ten open reading frames (ORFs). The virus synthesizes RNA via its RNA-dependent RNA polymerase and encodes two ORFs, ORF1a, that is the longest part of the RNA encodes for the replicases, and ORF1b, which expresses for two large replicative polyproteins (pp) including pp1a and pp1ab comprising about 4000 (~500 kDa) and 7000 (~800 kDa) amino acids.<sup>20</sup> These polyproteins are autocatalytically processed to produce two proteases: a chymotrypsin-like protease serine protease (3CL<sup>pro</sup> Main Protease or 3CL<sup>pro</sup>) and a papain-cysteine proteinases (PL<sup>pro</sup>). These polyproteins are further processed to generate four structural proteins and sixteen non-structural proteins (nsps). The proteolytic processing of the sixteen nsps by 3CL<sup>pro</sup> and PL<sup>pro</sup> is essential for virus replication and maturation because the nsps are involved in downstream binding and replication events including the formation of the replicase complex, which is essential for viral replication and transcription of the genome.<sup>21,22</sup> The 3CL<sup>pro</sup> (33.8 kDa) cleaves the poly protein 1ab at 12 distinct sites in most of peptide bonds involving Leu, Gln↓ (Ser, Ala, Gly↓ marks the cleavage site) to generate nsps 4 to 16 which are essential for

viral replication, because they perform function as RNA dependent RNA polymerase, RNA binding proteins, exoribonuclease, helicase, methyl transferase, and unlike structural/accessory protein-encoding genes, such as S, M, E, N proteins, that are located at the 3' end which exhibits excessive variability (Figure 1).<sup>23,24</sup> Among non-structural proteins, most play a vital role in coronaviruses replication. Structural proteins, however, are crucial for virion assembly as well as for infection.<sup>25</sup> The inhibition of the activity of 3CL<sup>pro</sup> would block SARS-CoV-2 replication. The X-ray structures of this protease from SARS-CoV-2, such as PDB:6W63; 6M2N; 6M2Q; 6Y2E; 6Y84; 6YB7, are available on protein databank (Figure 1).

SARS-CoV-2 and SARS-CoV share a very high (96-98%) sequence identity for their 3CL<sup>pro</sup> and is located in a highly conserved region of coronavirus genome. Therefore, it is a potential target for anti-coronavirus inhibitors screening and structure-based activity analyses and high throughput studies have identified potential inhibitors for SARS-CoV and MERS-CoV 3CL<sup>pro</sup>.<sup>26</sup> The structure and activity of SARS-CoV 3CL<sup>pro</sup> has been unveiled and prompted the design of 3CL<sup>pro</sup> inhibitors as novel antivirals. SARS-CoV 3CL<sup>pro</sup> features three domains: I3CL<sup>pro</sup> (comprising residues 8–101), II (comprising residues 102–184), and III (comprising residues 201–301). The active site regions spans domains I and II, which are β-barrel domains, while domain III displays an α-helical organization. The cysteine residue 145 (Cys145) of the active site behaves as the nucleophile, while the histidine residue 41 (His41) functions as the general acid base.<sup>27</sup>

### Drug development anti-SARS-CoV-2

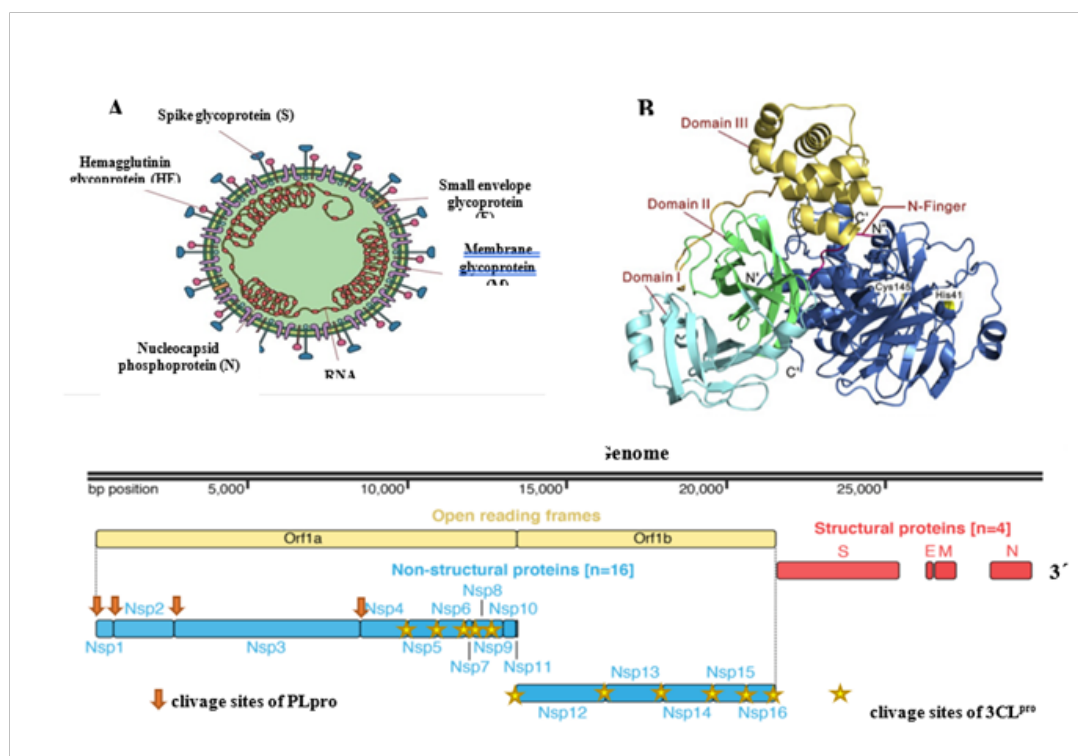
The scientific community is investigating new drugs against SARS-CoV-2 and have come up with three strategies for developing: the first one is assay existing broad-spectrum anti-virals, such as interferons, ribavirin, and cyclophilin that are used to treat coronavirus pneumonia. The great advantage of these treatments is the biochemistry, pharmacologic, pharmacokinetic, dosages and side effects are known and they have been approved for the treating of viral infections. However, they are broad-spectrum and cannot kill coronavirus in a targeted manner.<sup>28</sup> The second strategy is to use existing molecular databases to screen for molecules that may have therapeutic effect on SARS-CoV-2. High-throughput screening makes this strategy possible, and new functions of many drug molecules can be found. The third strategy is directly based on the genomic information and pathological characteristics of different coronaviruses to develop new targeted drugs from scratch. Theoretically, the drugs found through these therapies would exhibit better anti-coronavirus effects.<sup>29</sup>

Many compounds have been assayed against SARS-CoV-2 3CL<sup>pro</sup> using combined structure-assisted drug design, virtual drug screening and high-throughput screening<sup>30</sup> and some examples were discussed here. Pyridine-containing α-ketoamides, presented good pharmacokinetic properties in animal model and were detected at high concentrations in lung tissue and broncho-alveolar lavage fluid within 4–24 hours after subcutaneous injections, demonstrating important lung tropism. Besides sub-cutaneous administration, inhalation of nebulized resulted in high and long-lasting (24 h) levels in lung tissue, without any adverse effects, suggesting that these α-ketoamides are potential candidates in COVID-19 therapy.<sup>31</sup>

A mechanism-based synthetic peptidomimetic inhibitor of 3CL<sup>pro</sup>, a Michael acceptor inhibitor N3, was identified by computer-aided drug design that can inhibit the 3CL<sup>pro</sup> of SARS-CoV and MERS-CoV,<sup>32</sup> was shown to form a covalent and irreversible bond with

SARS-CoV-2 3CL<sup>pro</sup>.<sup>33</sup> Furthermore, the high-throughput screening approach for identifying inhibitors of SARS-CoV-2 3CL<sup>pro</sup>, ebselen, an organo selenium compound with anti-inflammatory, anti-oxidant

and cytoprotective properties, was assayed with N3. They had expressive antiviral and host cell protection activities demonstrating their potential against SARS-CoV-2.<sup>33</sup>



**Figure 1** SARS-CoV-2 structure: SARS-CoV-2 capsid showing major proteins and single-stranded RNA (A), the model of 3CL<sup>pro</sup> a SARS-CoV-2 drug target (B) and (C) genome of SARS-CoV-2 and the large replicase polyprotein P1ab. The figure A, B and C were adapted from MoonchakV Projecto, references 29 and 47 respectively.

The described inhibitors from different coronaviruses 3CL<sup>pro</sup>s can be employed as anti-SARS-CoV-2, due to the high similarity of this protease among coronaviruses, as stated before. The majority of these inhibitors are bindcovalently active-site cysteine, including  $\alpha$ -halocarbonyl, acrylamides, sulfonyl chlorides, aldehydes, isatines, or  $\alpha$ -ketoheteraromates.<sup>34</sup> However, they did not fit very well into the active site, because they are not peptide substrate of the protease. Interestingly, some compounds noncovalently binding to the active site of the 3CL<sup>pro</sup> seems to be a better strategy. A high-throughput screening identified pyrazolidinones, nitroanilides and  $\alpha$ -aminoacylamides as SARS-CoV-2 3CL<sup>pro</sup> inhibitors with good antiviral inhibitory. But these molecules suffer from extensive metabolism and rapid clearance. Nevertheless, they are a promising starting point for further drug development.<sup>35</sup>

The anti-coronaviruses activity has been investigated in many plant-derived natural products and flavonoids, with diverse biological activities, have been shown to inhibit the 3CL<sup>pro</sup>.<sup>29</sup> Quercetin, a flavonoid compound, is widespread in fruits and vegetables, displays a great diversity of biological activities including anti-inflammatory, anti-oxidant, anti-viral, anti-allergic, anti-cancer as well as vasoprotective.<sup>36</sup> Studies have found that quercetin exhibits antiviral properties against a variety of viruses, including Influenza A Virus, Hepatitis C Virus, Enterovirus, and SARS-CoV.<sup>37</sup> It has been confirmed that quercetin showed a good inhibitory effect on SARS-CoV-2 3CL<sup>pro</sup>, about 82 %, expressed in *Pichia pastoris*.

In addition, enzyme inhibition assays in vitro also showed that quercetin had inhibitory activity against SARS-CoV-2 3CL<sup>pro</sup>.<sup>38</sup> The 3CL<sup>pro</sup> sequence of SARS-CoV-2 is highly similar to that of SARS-CoV,<sup>28</sup> thus, quercetin might have antiviral effects on SARS-CoV-2. This flavonoid was docked to 3CL<sup>pro</sup> and was a good binder with energy of -5.6 kcal/mol. It also binds to S protein, ACE2, RNA-dependent RNA polymerase and PL<sup>pro</sup> indicating good potential against SARS-CoV-2.<sup>39</sup> Quercetin has a wide range of sources with relatively low cost, so it is worth testing its efficacy against SARS-CoV-2 infection. Besides, many other plant derived compounds from Chinese herbal medicine and common use such as andrographolide (labdane diterpenoid from *Andrographis paniculata*), glycyrrhizin (glycosylated saponin from *Glycyrrhiza glabra*), baicalin (glycosyloxyflavone obtained from *Scutellaria baicalensis*), luteolin (3',4',5,7-tetrahydroxyflavone common flavonoid), tanshinone and derivatives (abietane diterpenes isolated from *Salvia miltiorrhiza*), kampferol (natural flavonoid), hesperidin (flavanone glycoside found in citrus fruits), and betulinic acid (naturally occurring pentacyclic triterpenoid) was also docked to 3CL<sup>pro</sup> and demonstrated to be very good binders of this protease. However these compounds were not specific to this protease and interacted to other viral targets, thus, they can be considered excellent adjuvants in the treatment of COVID-19.<sup>29,37,39,40</sup>

The approved HIV protease inhibitors were previously repurposed



for the treatment of the SARS outbreak in 2002 to 2004.<sup>41</sup> So, they were hypothesized to inhibit the SARS-CoV-2 3CL<sup>pro</sup>. These drugs were docked inside the SARS-CoV-2 3CL<sup>pro</sup>. Importantly ritonavir, lopinavir, and darunavir had very good docking score of -8.878, -8.358 and -7.208, respectively. These three inhibitors are being tested against SARS-CoV-2.<sup>42</sup> However, HIV protease is an aspartic protease and differs considerably from 3CL<sup>pro</sup>, but it also shares some common elements, such as a tetrahedral transition state and receptor pockets to recognize the amino acid side chains of the substrates.<sup>42,43</sup>

Peptidomimetic seems to be more potent inhibitors to 3CL<sup>pro</sup>. They have been used to treat many diseases like cancer, diabetes, autoimmune and viral and have high success rates in commercial development.<sup>44</sup> These peptide inhibitors mimic a peptide bond and are better inhibitors than the others compounds. They bind to active site of SARS-CoV-2 3CL<sup>pro</sup> by multiple points in a combination of hydrophobic, hydrophilic and charged residues holding with hydrogen bonds. Peptide-like molecules provide a basic pharmacophore for the design of SARS-CoV-2 main protease inhibitors. The amide linkage backbone gives them the flexibility to fit comfortably inside the binding site. Peptides may be the best alternative for small molecules for COVID-19 treatment, because they have great structural diversity, are easy to synthesize and are much less toxic when compared to synthetic and natural small molecules.<sup>45,46</sup>

## Conclusion

A great diversity of compounds, synthetics or naturals, new or repurposed, have been extensively investigated against different targets of SARS-CoV-2 to develop drugs and pharmaceutical formulations in the shortest possible time to face the current pandemic. The 3CL<sup>pro</sup> seems to be one of the best targets in coronaviruses, because the amino acid sequence of this protease is very similar to the 3CL<sup>pro</sup>s from SARS and MERS, coronaviruses that infect humans and cause lethal diseases. Besides, the 3CL<sup>pro</sup> inhibitors, specially the peptidomimetics, would be more specific to viral protease than the host enzymes, because 3CL<sup>pro</sup>-like proteases are not common in the human host. Thus, 3CL<sup>pro</sup>-based drugs for the treatment of COVID-19 would be expected to be more tolerable for humans due to the fewer side effects. However, these questions will only be answered after biological and clinical studies.

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## Conflicts of interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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