Discovery of Therapeutic Targets and Potential Drugs to Fight Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): A Review

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Abstract

SARS-CoV-2, the causative agent of COVID-19, has led to more than two million deaths worldwide. There are currently no specific therapies approved to treat COVID-19 and drug repositioning, rather than new drug discovery, which may be the best short-term option for controlling the pandemic. In this review, the author discusses important viral targets such as the papain-like protease, RNA-dependent RNA polymerase, 3-chymotrypsin-like protease, and spike protein. The information about chemical compounds and targets provided in this review will aid further *in vitro* and *in vivo* studies to discover drugs to treat COVID-19.

Keywords: *COVID-19, drug discovery, SARS-CoV-2.*

Introduction

Infections with coronaviruses have resulted in major outbreaks of pneumonia during the twenty-first century. In 2002, SARS-CoV infections, with a lethality rate of 10%, were found on five continents[1,2]. In 2012, MERS-CoV, which has a lethality rate of 35%, emerged in the Middle East[3,4]. MERS-CoV and SARS-CoV are zoonotic viruses and their primary hosts are camels, and bats or civets, respectively[5,6]. Most recently, a novel coronavirus emerged in late 2019 and, on January 12, 2020, WHO recommended the interim name 2019-nCoV for this new virus. Later, in early February 2020, the International Committee on Taxonomy of Viruses categorized 2019-nCoV as SARS-CoV-2, and named the disease COVID-19[7]. General symptoms of individuals infected with coronaviruses include shortness of breath, cough, fever, and respiratory symptoms. In severe cases, kidney failure, SARS, and pneumonia may develop and lead to death[8]. No specific vaccines or drugs have yet been approved to treat coronavirus infections in humans[9].

Coronaviruses belong to the *Coronaviridae* family and have a positive-sense single-strand RNA genome (Figure 1). Coronaviruses are further classified into four genera, *Alpha-*, *Beta-*, *Gamma-*, and *Delta-coronavirus*. SARS-CoV-2 belongs to the genus *Betacoronavirus*. Coronaviruses contain four structural proteins, the spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N)[10] (Figure 2). Viruses bind to specific receptors on the surface of cells to gain entry. For example, MERS-CoV uses dipeptidyl peptidase-4 as its receptor[11], and SARS-CoV uses ACE2 as its receptor[12]. The spike protein aids attachment and membrane fusion and thus plays a key role in determining host range[7,8,13]. Potential therapeutic interventions against coronaviruses include disrupting viral RNA synthesis by targeting the genetic material of the virus, inhibiting viral replication by targeting enzymes, and blocking binding to cell surface receptors[14]. Blockade of cell signaling pathways needed for viral replication may also have a specific anti-viral effect.

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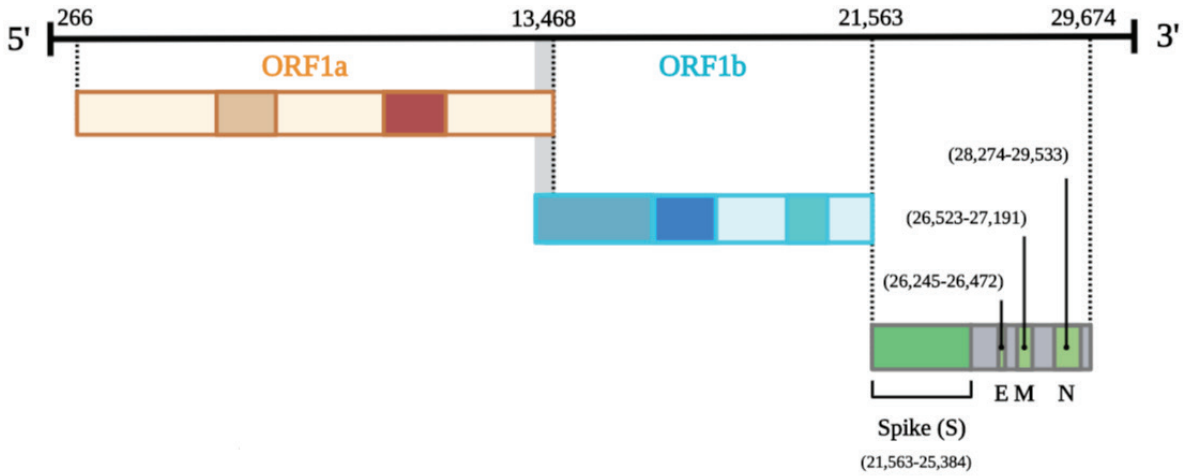

Figure 1: Genome of SARS-CoV-2. This figure created in BioRender.

In the fight against coronaviruses, there are three approaches for developing new drugs. The first strategy requires testing existing broad-spectrum anti-viral agents. The second strategy involves using molecular databases to identify molecules with the potential to act as therapeutic agents against coronaviruses. The third strategy uses the genomic data and pathological characteristics of different coronaviruses[14]. This review will provide information about chemical compounds and targets for further *in vivo* and *in vitro* studies to identify drugs to treat COVID-19.

Potential Drug Targets for SARS-COV-2

Drug targets for coronaviruses can be divided into different categories based on therapeutic pathways: (1) action on the host's specific enzymes or receptors to prevent the virus from entering the host's cells, (2) production of virulence factors to restore the host's innate immunity, (3) action on structural proteins of the virus, and (4) action on functional proteins or enzymes that are critical to the virus. The target proteins include Nsps, such as Nsp1, Nsp7-Nsp8 complex, Nsp9-Nsp10, and Nsp14-Nsp16, 3CLpro, Nsp3 (Nsp3b, Nsp3c, Nsp3e, and PLpro), ORF7a, E-channel, helicase, CRBD, TMPRSS2, ACE2, NRBD, RdRp, and spike protein[14] (Figure 2).

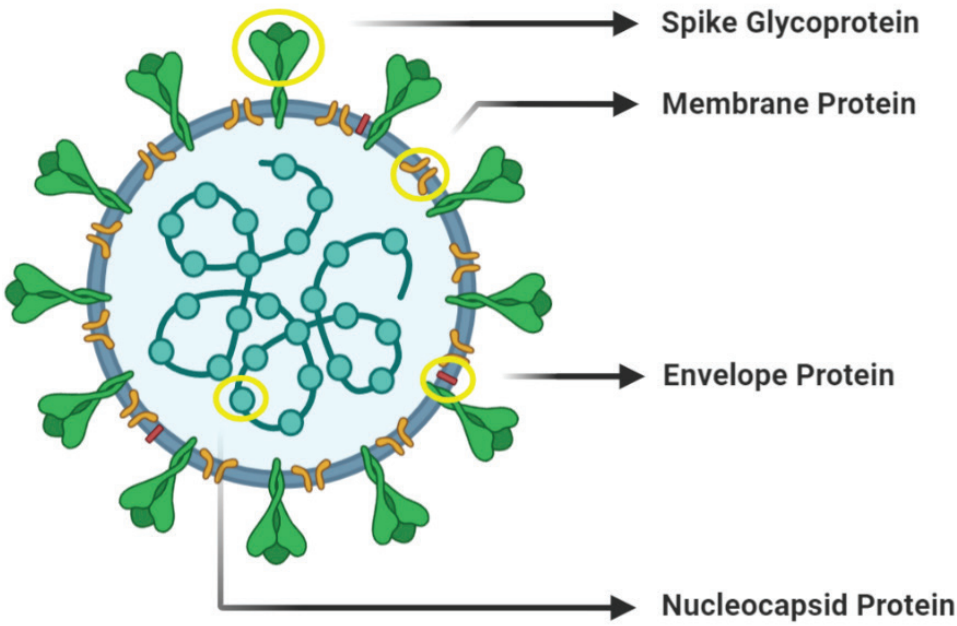

Figure 2. Structure of SARS-CoV-2. This figure created in BioRender.

Targets Preventing Viral RNA Replication and Synthesis

Nsps are important functional proteins of coronaviruses and are involved in RNA transcription, protein synthesis, translation, processing and modification, infection of host cells, and viral replication. The helicase, RdRp, PLpro, and 3CLpro are the most important targets for the development of inhibitors[14].

Papain-Like Proteinase

PLpro is important for the release of nonstructural proteins, such as Nsp3, Nsp2, and Nsp1, and for correcting the replication processes of SARS-CoV-2[15]. PLpro has also been confirmed to trigger host innate immunity[16]. Since PLpro is indispensable for SARS-CoV-2 replication and infection of the host, inhibitors of PLpro would likely be effective in combating SARS-CoV-2. Unfortunately, there are no approved inhibitors of PLpro[14].

Table 1. Potential PLpro Inhibitors.

No	Compound/Drug (Source)	Mechanism	Reference
1	Chloramphenicol	Antibacterial	14
2	Baicalin (*Scutellaria baicalensis*)	Antiinflammatory, Antiviral, Antibacterial, Antitumor	14
3	Ribavirin	Antiviral	14
4	Piceatannol (*Vitis vinifera*)	Antiviral	14
5	Sulfasalazine	Antibacterial	14
6	Rosmarinic acid (*Salvia verticillata*)	Antioxidant, Antiviral	14
7	Tigecycline	Antibacterial	14
8	Phaitanthrin D (*Isatis indigotica*)	Antiviral	14
9	Valganciclovir	Antiviral	14
10	Sugetriol-3,9-diacetate (*Cyperus rotundus*)	Anti-HSV-1, Anti-HBV	14

3-Chymotrypsin-Like Cysteine Protease:

3CLpro is first autoclaved from viral poly-proteins to generate mature enzyme, which then further cleaves downstream Nsps at 11 sites to deliver Nsp4-Nsp16[17]. 3CLpro is thus important in the SARS-CoV-2 life cycle and a study of the catalytic mechanism and structure of 3CLpro established this enzyme as an appealing target for anti-SARS-CoV-2 drug discovery[18]. Inhibitors of SARS-CoV 3CLpro are typically small molecules and peptides but, again, there are no approved inhibitors[14].

Table 2. Potential 3CLpro Inhibitors.

No	Compound/Drug (Source)	Mechanism	Reference
1	Oxytetracycline	Antibacterial	14
2	Andrographiside *(Andrographis paniculata)*	Antiinflammatory, Antiviral	14
3	Amprenavir	HIV-1 protease inhibitor	14
4	Theaflavin 3,3′-di-O-gallate *(Camellia sinensis)*	Antiviral, Antioxidant, Antitumor	14
5	Phenethicillin	Antibacterial	14
6	Phyllaemblinol *(Phyllanthus emblica)*	Antiviral	14
7	Cefpiramide	Antibacterial	14
8	Cosmosiin *(Scutellaria baicalensis)*	Anti-HIV, Antiinflammatory, Antioxidant	14
9	Demeclocycline	Antibacterial	14
10	Hesperidin *(Citrus aurantium)*	Antioxidant, Antiinflammatory	14

RNA-Dependent RNA Polymerase

Nsp12-RdRp, a conserved protein in coronaviruses, is a vital component of the replication or transcription complex[19]. Inhibitors of Nsp12-RdRp should not cause significant side effects or toxicity to host cells and this enzyme was an important drug target for MERS-CoV and SARS-CoV. Unfortunately, no specific inhibitors of Nsp12-RdRp were identified[20].

Table 3. Potential RdRp Inhibitors.

No.	Compound/Drug (Source)	Mechanism	Reference
1	Baicalin *(Scutellaria baicalensis)*	Antibacterial, Antiinflammatory, Antiviral, Antitumor	14
2	Idarubicin	Antitumor	14
3	Sugetriol-3,9-diacetate *(Cyperus rotundus)*	Anti-HBV	14
4	Novobiocin	Antibacterial	14

Cont... **Table 3. Potential RdRp Inhibitors.**

5	Phyllaemblicin B *(Phyllanthus emblica)*	Antiviral	14
6	Atovaquone	Antimalarial	14
7	Betulonal (*Cassine xylocarpa*)	Anti-HIV-1	14
8	Gniditrin (*Gnidia lamprantha*)	Antitumor	14
9	Itraconazole	Antifungal	14
10	Chlorhexidine	Antibacterial	14

Targets Inhibiting Virus Structural Proteins

The most important structural protein of coronaviruses is the spike protein, which binds to host cell receptors to mediate cell entry and determines host tropism[21]. The spike protein is cleaved into S1 and S2 subunits by a host cell protease. The main purpose of the S1 subunit is to bind to host cell surface receptors, and the S2 subunit then mediates virus-cell and cell-cell membrane fusion. Cleavage activation and structural integrity of spikes also play important roles in virulence and invasiveness[22]. Agents that prevent SARS-CoV-2 from entering cells by targeting the spike protein or specific receptors on the surface of host cells are thus likely to be effective antiviral therapies.

It has been widely reported that ACE2 is the unique receptor for the RBDs of both SARS-Co-V and SARS-CoV-2 spike proteins[23]. A recent study showed that the sequences of both spike RBDs are similar, and that there are critical interactions between several key amino acid residues of ACE2 and the RBDs[24]. ACE2 is thus a candidate host target for drugs to block SARS-CoV-2 from entering host cells[9].

Conclusion

In summary, this review provides information about chemical compounds and targets for further *in vivo* and *in vitro* studies to identify drugs to treat COVID-19.

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