SARS-CoV-2: Origin, Evolution, and Targeting Inhibition

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused an outbreak in Wuhan city, China and quickly spread worldwide. Currently, there are no specific drugs or antibodies that claim to cure severe acute respiratory diseases. For SARS-CoV-2, the spike (S) protein recognizes and binds to the angiotensin converting enzyme 2 (ACE2) receptor, allowing viral RNA to enter the host cell. The main protease (Mpro) is involved in the proteolytic process for mature non-structural proteins, and RNA-dependent RNA polymerase (RdRp) is responsible for the viral genome replication and transcription processes. Owing to the pivotal physiological roles in viral invasion and replication, S protein, Mpro, RdRp are regarded as the main therapeutic targets for coronavirus disease 2019 (COVID-19). In this review, we carried out an evolutionary analysis of SARS-CoV-2 in comparison with other mammal-infecting coronaviruses that have sprung up in the past few decades and described the pathogenic mechanism of SARS-CoV-2. We displayed the structural details of S protein, Mpro, and RdRp, as well as their complex structures with different chemical inhibitors or antibodies. Structural comparisons showed that some neutralizing antibodies and small molecule inhibitors could inhibit S protein, Mpro, or RdRp. Moreover, we analyzed the structural differences between SARS-CoV-2 ancestral S protein and D614G mutant, which led to a second wave of infection during the recent pandemic. In this context, we outline the methods that might potentially help cure COVID-19 and provide a summary of effective chemical molecules and neutralizing antibodies.

Keywords: SARS-CoV-2, evolution, pathogenic mechanism, structure, targeting inhibition

INTRODUCTION

SARS-CoV-2 is a highly contagious virus that has led to a worldwide epidemic; it causes fever, headache, cough, myalgia, fatigue, sputum production, and hemoptysis (Chen et al., 2020; Huang et al., 2020; Coronaviridae Study Group of the International Committee on Taxonomy of V, 2020; Wong, 2020; Wu and McGoogan, 2020; Xu J. et al., 2020). Most patients infected with SARS-CoV-2 can develop acute respiratory distress syndrome (ARDS) (Zumla et al., 2020). Until April 20, 2021, there have been 142,097,799 confirmed cases and 3,029,811 deaths globally. These statistics are rising rapidly, with approximately 10,000 deaths increasing per day. Some notable SARS-CoV-2 variants probably became dominant in many countries and caused uncontrolled transmission worldwide (Korber et al., 2020; Yurkovetskiy et al., 2020; Plante et al., 2021). According to the World Health Organization’s official report, SARS-CoV-2 has caused one of the worst global health emergencies in history. Until now, scientists are still struggling to prevent the spread of SARS-CoV-2 and have gained
some improvements. In this review, we summarize the origin, evolution, and pathogenesis of SARS-CoV-2. In addition, we show the structures of spike protein, Mpro, and RdRp, as well as the complex structures of these three proteins with their inhibitors. In conclusion, we provide a summary of effective chemical molecules and neutralizing antibodies, which might potentially help cure COVID-19.

ORIGIN AND EVOLUTION OF SARS-COV-2

Numerous studies claim that SARS-CoV-2 belongs to the group of coronaviruses, with a single-stranded genome of about 26-32kb (+ssRNA), which is the largest genome size of RNA virus as known (Woo et al., 2012). After a year of struggles, researchers have learned more about the origin, structure, and inhibition of SARS-CoV-2. Coronaviruses belong to the order Nidovirales, family Coronaviridae and the subfamily Coronavirinae. Coronavirinae consists of α-coronavirus, β-coronavirus, γ-coronavirus and δ-coronavirus (Woo et al., 2012). Coronavirus was first isolated from chickens in 1937. It was not until the SARS outbreak in February 2003 that the coronavirus was considered highly pathogenic to humans (Fouchier et al., 2003). Before that, the coronaviruses that spread among humans mainly caused mild infections in people with a weaker immune system (Vaheri et al., 2013; Su et al., 2016; Zhou et al., 2018). Since 2002, three zoonotic outbreaks have been caused by β-coronaviruses in China, SARS-CoV in 2003 (Fouchier et al., 2003), MERS-CoV in 2012 (Zaki et al., 2012), and the latest outbreak of SARS-CoV-2 at the end of 2019 (Cauchemez et al., 2013; Cui et al., 2019; Huang et al., 2020).

Apart from these, there are other four human coronaviruses: HCoV-229E, HCoV-OC43, HCoV-NL63, and HKU1. These coronaviruses could lead to mild diseases in the upper respiratory of the immune-competent hosts. Regardless, some of viruses could induce serious infections in infants, children, and the elderly (Su et al., 2016). Some people suspect that SARS-CoV-2 directly originated from SARS-CoV, while others believe that it leaked from the laboratory (Andersen et al., 2020). However, the evolutionary analysis of SARS-CoV-2, SARS-CoV, and MERS-CoV by neighbor-joining method showed that SARS-CoV-2 belongs to a new evolutionary branch of coronaviruses (Figure 1) (Cao et al., 2005; Chan et al., 2015; Jiang et al., 2020; Rabaan et al., 2020). Comparing the sequences of SARS-CoV and SARS-CoV-2, the homology is about 90%. Notably, the SARS-CoV-2 spike (S) protein has high sequence homology (up to 98%) with that of bat coronavirus RaTG13 (Li et al., 2003). However, the amino acid sequence homology between MERS-CoV and SARS-CoV-2 related virus is less than 90% (Chan et al., 2015). Apart from the amino acid sequence, the receptor and the host of MERS-CoV are distinct from SARS-CoV-2 and SARS-CoV (Hamming et al., 2007; Chen et al., 2013; Xia et al., 2014; Jiang et al., 2014; Forni et al., 2017; Chafekar and Fielding, 2018; Song et al., 2019; Bleibtreu et al., 2020) (Figure 1).

Some researchers believe that some viruses originated from rodents, such as HCoV-OC43 and HKU1; others originated from bats, which consist of HCoV-NL63, HCoV-229E SARS-CoV, and MERS-CoV (Mittal et al., 2020) (Figure 2). However, the origin of SARS-CoV-2 remains unknown. Some people suspect that bats might be the SARS-CoV-2’s source because the RaTG13 virus isolated from bats has high sequence similarity with SARS-CoV-2 that has come from the same branch. A study reported that bat RaTG13 and SARS-CoV-2 recognize the same receptor ACE2 and have the same ability to infect cells through this receptor. These studies also identified that the ACE2 binding ridge in the bat RaTG13 receptor binding motif (RBM) contains four residues, which is the same as SARS-CoV-2. Moreover,
Leu455, conserved on SARS-CoV-2 and RaTG13, may contribute to the recognition of ACE2. Thus, these findings suggest that SARS-CoV-2 may be derived from RaTG13 or shares a common ancestor with RaTG13 (Li et al., 2003; Shang et al., 2020).

**THE PATHOGENIC MECHANISM OF SARS-COV-2**

The diameter of the SARS-CoV-2 virus particle is approximately 100 nm (Figure 3). It contains structural proteins (the membrane (M), envelope (E), spike (S), and nucleocapsid (N) proteins), an accessory protein, and non-structural proteins (nsps) (NSP1–16) (Cao et al., 2005; Chang et al., 2014; Lu et al., 2020; Yao et al., 2020b; Wu et al., 2020a). The SARS-CoV-2 resembles a corona because of the plenty of S proteins on the envelope. The S protein of coronaviruses, which could help to binding, fuse membrane, enter into the host cells, and induct antibody, is a type I transmembrane (TM) glycoprotein. The accessory and non-structural proteins’ functions are related to virulence because those proteins are involved in viral replication and assembly (Holmes, 2003).

SARS-CoV-2 invades cells in two ways. One is interfering with target cells directly. SARS-CoV-2 occupies ACE2 on the membrane (Kuba et al., 2006; Wu et al., 2011; Hemnes et al., 2018; Gao et al., 2019), blocking signals, disturbing the renin-angiotensin (RAS) system. Another is subsequent immune
system dysfunction (Guo et al., 2008). After SARS-CoV-2 occupies ACE2, a decrease in the number of immune cells is observed, and IL-1 levels are elevated (Chen et al., 2020) (Figure 4).

SARS-CoV-2 employs S protein to infect target cells by fusing the membranes (Tian et al., 2020; Walls et al., 2020; Yan et al., 2020). S protein consists of S1 and S2 subunits. An N-terminal domain (NTD), a receptor-binding domain (RBD), and a C-domain are the main domains of the S1 subunit. Moreover, RBM of the RBD, located in a C-domain’s accessory subdomain, is a hypervariable region for receptor recognition. Another subunit, including two 7-valent element repeats (HR1 and HR2) and a fusion peptide (FP), could fuse to the cell membrane (Bosch et al., 2003; Belouzard et al., 2009; Burkard et al., 2014; Millet and Whittaker, 2014; Millet and Whittaker, 2015; Kirchdoerfer et al., 2016; Park et al., 2016; Walls et al., 2016). In this process, SARS-CoV-2 is more infectious than SARS-CoV because the free energy of binding between RBD and ACE2 of SARS-CoV-2 is remarkably lower than that of SARS-CoV (Wang K. et al., 2020).

After the SARS-CoV-2 entering the host cell, the viral RNA is attached to the ribosome of host, translating two large, coterminal polyproteins. Subsequently, these proteins were digested into components by proteolysis for packaging new virions. Two proteases for this proteolysis are the papain-like protease (PLpro) and the coronavirus main protease (Mpro). Like other known coronaviruses, SARS-CoV-2 also employs RNA-dependent RNA polymerase (RdRp) to replicate the genome of RNA. Above all, the four proteins, spike, Mpro, PLpro, and RdRp, are essential to virus assemble and pathogenesis (Figure 5) (Sheahan et al., 2020).

Given the crucial role of S protein and ACE2 in receptor recognition, as well as the critical role of Mpro and RdRp in the SARS-CoV-2’s replication and transcription, interfering with their functions may provide effective antiviral strategies (Hoffmann et al., 2020). In other words, therapeutics currently targeting structural protein (S protein), non-structural proteins (Mpro, and RdRp) from SARS-CoV-2, and ACE2 from the host are potential treatment options for SARS-CoV-2. Recently, studies on the structural biology of the SARS-CoV-2 and structure-based antibody and drug discovery have appeared. We review these findings in the following sections.

**STRUCTURAL PROTEIN AND ITS INHIBITORS**

**Structure of SARS-CoV-2 Spike Protein**

The structure of S protein was resolved in two states: open and closed state (Walls et al., 2020; Wrapp et al., 2020). In the open state of S protein, one RBD was in the “up” conformation and other RBDs in the “down” conformation. Meanwhile, to fuse the membranes of the virus and host cell, the S protein undergoes a drastic structural rearrangement. In the closed state of S protein, all three RBDs in the “down” conformation are at the interface among protomers (Walls et al., 2020). Compared to the “up” conformation, the “down” conformation in the close state is near the trimer’s central cavity (Figure 6D). Thus, it is expected that the opening of the SARS-CoV-2 RBD is essential for interacting with ACE2 and triggering changes of conformation. The opening of RBD results in cleaving the S2 site, fusing membrane and entering into cells.

After the SARS-CoV-2 pandemic, 12,379 single nucleotide polymorphisms (SNPs) are downloaded in genomic data on June 25, 2020. In these SNPs, four SNPs, C3037U, C14408U, A23403G, and C241U, show high frequency. As for the A23403G, it encodes the mutant D614G in Spike protein.
late January 2020, D614G was first discovered in the viral genomes. Compared with the virus strain provided by Wuhan, Spike mutant D614G is caused by the 23,403 nucleotide mutation of SARS-CoV-2. The G614 form is rare in the world, but it has attracted much attention. However, the transition of spike mutants from D614 to G614 occurred all over the world (Korber et al., 2020). Mutations that increase the chance of viral infection occurred mainly in S protein (Plante et al., 2021). With the sequencing analysis, studies showed that the S mutant D614G is a pseudo-typed virion. Because mutant D614G has association with a lower RT-PCR cycle threshold among patients, the upper respiratory tract has high viral load (Korber et al., 2020). Animal experiments also confirm these results. Hamsters infected with D614G have higher viral titers in the trachea and nasal wash than those in the lungs. This also supports the mutant D614G increase the load of virus in the Covid-19 patients’ upper respiratory tract and might enhance the spread (Plante et al., 2021). Besides, the ratio of the open conformations of mutant D614G increased up to 58% and is in sharp contrast in the ancestral SARS-CoV-2 virus, in which the ratio of RBDS in the open conformation is only 18%. In comparison with the ancestral S protein in the closed state, the NTD and the INT domain of the S protein D614G shift by 4 Å and 6 Å, respectively. Moreover, the NTD of the S protein D614G has a 3 Å shift in the open state (Figure S1) (Yurkovetskiy et al., 2020).

The structures of the S proteins between SARS-CoV-2 and SARS-CoV are similar, and the root mean square deviation (RMSD) on 959 Ca atoms is 3.8 Å. The overall structure of SARS-CoV-2 S protein resembles that of SARS-CoV S protein, with the same root mean square deviation (RMSD) of 3.8 Å over 959 Ca atoms (Figure 7A) (Wrapp et al., 2020). For the RMSD value, the structural comparison of S2 subunits, NTDs, subdomains 1 and 2 (SD1 and SD2), and RBDSs are 2.0 Å, 2.6 Å, 2.7 Å, and 3.0 Å, respectively (Figure 7B). The largest discrepancy exists in the RBDSs “down” conformations. The “down” RBD of SARS-CoV is tightly integrated with the NTD of the neighboring chain, while that of SARS-CoV-2 is angled close to the cavity of center.
Moreover, the HR1 domain of SARS-CoV-2 forms a six-helical bundle structure, which has higher helical stability than SARS-CoV. In addition, there are several reports suggesting that SARS-CoV-2 has a better ability to fuse membrane than SARS-CoV.

**Structure of the SARS-CoV-2 S Protein and Its Binding With ACE2**

Recent studies highlight that ACE2 plays a critical role in mediating SARS-CoV-2’s entry. In vitro, the binding affinity of the ACE2 with SARS-CoV-2 RBD is at the nanomolar level, indicating RBD plays important roles in binding (Walls et al., 2020; Tian et al., 2020).

Compared to SARS-CoV, the SARS-CoV-2 RBM has some sequence variations, and the distal end has an obvious change of conformation. Except for that, the structure of the RBD between SARS-CoV-2 and SARS-CoV is similar, and the RMSD on 174 aligned Cα atoms is 1.2 Å (Figures 7C, D) (Lan et al., 2020).

The residues of SARS-CoV-2 RBD are essential for binding to ACE2. The RBD of SARS-CoV-2 forms more contacts with the ACE2’s N-terminal helix, the buried area between the RBD and ACE2 is about 878.4Å², whereas, for SARS-CoV, the buried area of RBD and ACE2 is 839.45Å² (Figure 7G). In addition, there is a four-residue motif Gly-Val/Gln-Glu/Thr-Ser in SARS-CoV-2, instead of a three-residue motif proline-proline-alanine that is seen in SARS-CoV. Also, the Phe486, located in SARS-CoV-2 RBM, is in different directions and stays in the hydrophobic pockets, which consist of Leu79, Met82, and Tyr83 of ACE2 (Shang et al., 2020; Lan et al., 2020). Taken together, these structural changes in the SARS-CoV-2 RBM enhance the interaction for ACE2 binding.

Furthermore, more forces of interaction were formed in the joint surface between ACE2 and the RBD of SARS-CoV-2 than those in SARS-CoV. There are two virus-binding hotspots on ACE2. One hotspot is ACE2lys31, which forms a salt bridge with Glu35 of the RBD from SARS-CoV-2. Another hotspot is ACE2lys331, which forms a salt bridge with Asp38 of the RBD from SARS-CoV-2. The Kd value of ACE2 for binding to the RBDs from SARS-CoV or SARS-CoV-2 is 44.2 nM and 185 nM, respectively. In addition, corresponding to the SARS-CoV RBM’s Leu472, Phe486, located in SARS-CoV-2 RBM, has a different orientation to enhance the interactions between RBM and ACE2 (Figures 7E, F) (Shang et al., 2020).
In conclusion, the changes of structure from SARS-CoV-2 RBM offer more favorable interactions for binding to ACE2 (Benton et al., 2020; Cao L. et al., 2020; Wu et al., 2020b). According to the known structure of SARS-CoV-2 S protein with ACE2 (Benton et al., 2020; Xu C. et al., 2020), many efforts focus on designing inhibitors to disrupt the interaction between S protein and ACE2. These strategies include competitive binding to the RBD or interference with ACE2 binding.

### Inhibitors of SARS-CoV-2 S Protein Neutralizing Antibodies

Among the inhibitors that prevent the interaction between ACE2 and the RBD, neutralizing antibodies against S protein are the most widely studied. The first reported neutralizing antibody is BD-368-2, which was selected from 14 potent neutralizing antibodies purified from patients’ plasma or blood (Cao Y. et al., 2020; Long et al., 2020). The half-maximal inhibitory concentration (IC_{50}) of BD-368-2, against authentic and pseudo SARS-CoV-2, is 15 and 1.2 ng/mL, respectively. BD-368-2 could protect against SARS-CoV-2 in vivo experiments, providing valuable therapeutic effects. The second reported neutralizing antibody is 2B04, which could neutralize wild-type SARS-CoV-2 with remarkable potency in vitro (IC_{50} less than 2 ng/mL) (Alsoussi et al., 2020). 2B04 reduces the viral load of the lung, prevents systemic transmission in a murine model of SARS-CoV-2 infection, and protects challenged...
animals from weight loss. Subsequently, three RBD-specific monoclonal neutralizing antibodies, P2C-1F11, P2C-1A3, and P2B-2F6, from a single B cell were reported (Ju et al., 2020). Their IC50 are 0.03, 0.28 and 0.41 mg/mL, respectively. These monoclonal antibodies can compete with ACE2 for RBD binding, exhibiting robust anti-SARS-CoV-2 neutralizing activity. Thereafter, a series of REGN neutralizing antibodies REGN10933 and REGN10987 showed high clinically therapeutic effects, with IC50 of 42.8 and 40.8 pM, respectively (Baum et al., 2020). The complex ternary of single-particle cryo-EM with a 3.9 Å resolution displays that fab fragments of REGN10933 and REGN10987 can simultaneously bind distinct regions of the RBD. REGN10933 binds at the RBD top. The REGN10987 epitope is located on the RBD side with little overlap with the ACE2 binding site (Figure 8B).

Structure of BD23-Fab in Complex With S Trimer
The cryogenic electron microscopy (cryo-EM)-based structure of BD-23 Fab in complex with the trimer of S protein (s trimer) was resolved at an overall resolution of 3.8 Å (Cao L. et al., 2020). In this 3D reconstruction, a single BD-23 Fab binds the "down" RBD in chain B of S trimer. As for binding to the RBD, the BD-23 Fab’s heavy chain variable domain is involved. In fact, a comparison with the complex structure of RBD-ACE2 shows that BD-23 Fab competes with ACE2 for binding to RBD (Figure 8A).

Structure of Fab Fragments of REGN10933 and REGN10987 in Complex With RBD
In addition to acquiring antibodies from the blood of patients, many studies have attempted to obtain enough antibodies from humanized mice or other mammals and use cocktails to achieve more efficient treatments. In this way, Regeneron Pharmaceuticals developed several prospective antibodies with IC50 at the picomolar level (Baum et al., 2020). The complex ternary’s single-particle cryo-EM with a 3.9 Å resolution displays that fab fragments of REGN10933 and REGN10987 can simultaneously bind distinct regions of the RBD. REGN10933 binds at the RBD’s top. The REGN10987 epitope is located on the RBD side with little overlap with the ACE2 binding site (Figure 8B).
De Novo Protein

Interestingly, D. Baker group designed a series of de novo mini proteins to mimic RBD antibodies (Cao Y. et al., 2020). Applying shape and chemical complementarity strategies, they designed high-affinity mini binders that compete with ACE2 binding. Ten mini proteins (AHB1, AHB2, and LCB1-LCB8) could bind to the RBD, which affinities are ranging from 100 pM to 10 nM. These inhibitors could prevent the entry of SARS-CoV-2 into Vero E6 cells with IC50 from 35 pM to 35 nM. Among them, LCB1 (56 residues) and LCB3 (64 residues) showed stability and are the most potent inhibitors. Their IC50 values are almost six-fold that of the most effective monoclonal antibodies reported so far. Cryo-EM structures determined by the study corresponded to their computational LCB1/RBD or LCB3/RBD models (Figures 8C, D). In a single spike protein, three RBDs can be used simultaneously. These de novo hyper stable mini binders offer good points for new therapeutics of SARS-CoV-2.

Apart from those mentioned above, numerous neutralizing antibodies for SARS-CoV-2 have sprung up (Figures S2, S3). Structural evidence suggests that the stoichiometry of RBD with neutralizing antibodies is different (Figure S2). For instance, S309 Fab (Pinto et al., 2020), H104 Fab (Lv et al., 2020), and C105 Fab (Barnes et al., 2020) could bind two or three RBDs of S protein. The state of the RBD differs when it binds to different Fab fragments. S309 Fab binds RBD of S protein in the closed state (Figures S2A, B). However, H104 Fab and C105 Fab bind RBD of S protein in the open state (Figures S2C–F).

Simultaneously, some structures of neutralizing antibodies with RBD were determined. In these structures, RBD could bind one neutralizing antibody, which includes CR3022 (Yuan et al., 2020b), CC12.1, CC12.3 (Yuan et al., 2020a), and so on (Figures S2A–H). RBD also binds two different neutralizing antibodies (Figures S3I–K) and nanobody (Figure S3M). Here, we summarize inhibitors for S protein or RBD as follows (Table 1) (Toelzer et al., 2020).

Chemical Compounds

There are no effective drugs to prevent the SARS-CoV-2 spread. Thus, therapeutic agents to treat infected individuals are urgently required. Membrane fusion is a crucial step in viral infection. The previously developed coronavirus fusion inhibitors have been shown to prevent virus fusion by targeting the S2 subunit. A pan-coronavirus fusion inhibitor EK1, targeting the subunit S2’s HR1 domain, could inhibit infection of SARS-CoV and MERS-CoV. Subsequently, an EK1 derivative, EK1C4, was found to inhibit the membrane fusion and pseudo-viral infection of SARS-CoV-2 with an IC50 of 1.3 nM and 15.8 nM, respectively (Xia et al., 2020). In addition, IPB02, a lipopeptide fusion inhibitor based on HR2 sequence, showed efficient antiviral activity (Ling et al., 2020; Zhu et al., 2020).
Inhibitors of ACE2

Besides inhibiting the S protein, blocking ACE2 may also be a potential strategy to inhibit the coronavirus infection. Some researchers hope to interrupt the interaction between ACE2 and SARS-CoV-2 by employing existing FDA-approved ACE2 inhibitors (including captopril, enalapril, and lisinopril) (Vaduganathan et al., 2020). In fact, the application of ACE2 inhibitors brought two contradictory impacts on the patients. In some cases, ACE2 inhibitors could reduce inflammation. While in other cases, the inhibitors would improve the entry of viruses. Actually, these ACE2 inhibitors mainly act on the peptidase domain of ACE2, not on the interface for SARS-CoV-2 binding. Moreover, some adverse effects were reported, such as hypertension, heart failure, and these ACE2 inhibitors cause serious side effects in diabetic patients (Bavishi et al., 2020). Therefore, existing FDA-approved ACE2 inhibitors are not an appropriate option for treating SARS-CoV-2 patients (South et al., 2020).

Apart from that, chloroquine (CQ) and hydroxychloroquine (HCQ) were found to be potential therapeutic agents for COVID-19 (Xiu et al., 2020). CQ is a 4-aminoquinoline compound derived from quinine, which has been used to treat malaria, amoebiasis, and SARS-CoV. Studies demonstrated that CQ could increase the endosomal pH value to one which is higher than that required for cell fusion. In addition, CQ could damage the terminal glycosylation of ACE2 receptors, decreasing

| TABLE 1 | Summary of inhibitors for spike protein. |
|------------------------------------------|----------------------------------------|
| Type of inhibitor | Target of inhibitor | Inhibitor | IC50 (Pseudovirus) | IC50 (Authentic virus) | Kd | EC50 | ND50 | Reference |
|-------------------|---------------------|-----------|-------------------|----------------------|-----|------|------|-----------|
| **Chemical compounds** | HR1 and HR2 | EK1C4 | 15.8 nM | 36.5 nM | - | - | - | (Xia et al., 2020) |
| | MR2 | IPB02 | 80 nM | - | - | - | - | (Zhu et al., 2020) |
| | COVID-19 | Glycyrrhizinic acid | - | Reduces IC50 by about 10-fold | - | - | - | (Elayi and Varghese, 2020) |
| | ACE2 | Captopril | - | - | - | - | - | (Biyani et al., 2020) |
| | | Enalapril | - | - | - | - | - | (Sharif-Askari et al., 2020) |
| | | ARB | - | - | - | - | - | (Chung et al., 2020) |
| | | Lisinopril | - | - | - | - | - | (Kheisbawi et al., 2020) |
| **Neutralizing antibodies** | Spike | BD-217 | 0.031 µg/ml | 0.84 µg/ml | 0.29 nM | - | - | (Cao Y. et al., 2020) |
| | | BD-218 | 0.011 µg/ml | 0.29 µg/ml | 0.039 nM | - | - | (Cao Y. et al., 2020) |
| | | BD-236 | 0.037 µg/ml | 0.52 µg/ml | 2.8 nM | - | - | (Cao Y. et al., 2020) |
| | | BD-361 | 0.020 µg/ml | 0.760 µg/ml | 1.3 nM | - | - | (Cao Y. et al., 2020) |
| | | BD-368 | 0.035 µg/ml | 1.600 µg/ml | 1.2 nM | - | - | (Cao Y. et al., 2020) |
| | | BD-368-2 | 0.0012 µg/ml | 0.015 µg/ml | 0.82 nM | - | - | (Cao Y. et al., 2020) |
| | | BD-369 | 0.020 µg/ml | 0.270 µg/ml | 0.36 nM | - | - | (Cao Y. et al., 2020) |
| | | 2B04 | 1.46 ng/ml | - | - | - | - | (Alsoussi et al., 2020) |
| | | CA1 | - | - | 4.68 ± 1.64 nM | - | 0.38 µg/ml | (Shi et al., 2020) |
| | | CB6 | - | - | 2.49 ± 1.65 nM | - | 0.036 ± 0.007 µg/ml | (Shi et al., 2020) |
| | | P2C-1F11 | 0.03 µg/ml | 0.03 µg/ml | 2.12 nM | - | - | (Ju et al., 2020) |
| | | P2E-2F6 | 0.05 µg/ml | 0.41 µg/ml | 5.14 nM | - | - | (Ju et al., 2020) |
| | | P2C-1A3 | 0.62 µg/ml | 0.28 µg/ml | 2.47 nM | - | - | (Ju et al., 2020) |
| | | S309 Fab | - | 79 ng/ml | 0.81 nM | - | - | (Pinto et al., 2020) |
| | | H11-H4-Fc | 61 nM | - | 5.5 nM | - | 6 nM | (Huo et al., 2020) |
| | | H11-D4-Fc | 161 nM | - | 10 nM | - | 18 nM | (Huo et al., 2020) |
| | | VH472-Fc | 262 nM | - | 40 nM | - | 0.2 µg/ml | (Huo et al., 2020) |
| | | CR3022 Fab | 347 nM | - | 115 nM | - | - | (Yuan et al., 2020a) |
| | | CR3022 IgG | - | - | 1 nM | - | - | (Yuan et al., 2020a) |
| | | H014 Fab | 3 nM | 35 nM | 0.096 nM | 0.7 nM | - | (Lv et al., 2020) |
| | | REGN10933 | 42.8 pM | 37.4 pM | 3.37 nM | 5.79 pM | - | (Baum et al., 2020) |
| | | REGN10987 | 40.6 pM | 42.1 pM | 45.2 nM | 6.33 pM | - | (Baum et al., 2020) |
| | | REGN10989 | 7.23 pM | 7.38 pM | 3.65 nM | 4.86 pM | - | (Baum et al., 2020) |
| | | REGN10934 | 54.4 pM | 26.3 pM | 4.86 nM | 2.72 pM | - | (Baum et al., 2020) |
| | | C105 Fab | 26.1 ng/mL | - | - | - | - | (Barnes et al., 2020) |
| | | CV30 | 0.03 µg/mL | - | 3.6 nM | - | - | (Hurlburt et al., 2020) |
| | | EY6A | 54 nM | - | 2 nM | - | 0.5 nM | (Zhou et al., 2020) |
| | | CC12.1 Fab | 20 ng/ml | 20 ng/ml | 17 nM | - | - | (Yuan et al., 2020a) |
| | | CC12.3 Fab | 20 ng/ml | 20 ng/ml | 14 nM | - | - | (Yuan et al., 2020a) |
| | | COVA2-39 | 0.036 µg/ml | 0.054 µg/ml | 21 nM | - | - | (Wu N. C. et al., 2020) |
| | | COVA2-04 | 0.22 µg/ml | 2.5 µg/ml | 40 nM | - | - | (Wu N. C. et al., 2020) |
| | | 4A8 | - | 0.39 µg/ml | 92.7 nM | 0.61 µg/ml | - | (Chi et al., 2020) |
| | | Nanobody Ty1 | 54 nM | - | 5–10 nM | - | - | (Hanke et al., 2020) |

IC50, half maximal inhibitory concentration; Kd, dissociation constant; EC50, concentration for half of maximal effect; ND50, half neutralizing dose.
the interaction between viruses and their ACE2 receptor. Under cell culture conditions, CQ can prevent the interaction between the ACE2 and SARS-CoV RBD, for which ED₅₀ value is 4.4 µM (Vincent et al., 2005). CQ could inhibit SARS-CoV-2, which IC₅₀ value is 1.13 µM (Wang M. L. et al., 2020). HCQ is an analogue of CQ, in which one of the N-ethyl substituents of CQ is β-hydroxylated. Hydroxychloroquine has the same mechanism as CQ but shows more tolerability than CQ (Rainsford et al., 2015). The results showed that the IC₅₀ value of hydroxychloroquine is 0.72 µM after 48 h of incubation (Yao X. et al., 2020).

Even though CQ and HCQ show good antiviral activity, safety, and effectiveness, studies found that they may cause QT prolongation (Stas et al., 2008), ventricular arrhythmias (Chen et al., 2006), retinopathy (Yaylali et al., 2013), and other heart-related toxicities which may be harmful to severely ill patients. Overall, the treatment with CQ and HCQ has certain limitations.

**NON-STRUCTURAL PROTEIN AND ITS INHIBITORS**

**Structure of Mpro and Its Inhibitors**

The main proteinase (Mpro or 3C-like protease) is a cysteine proteinase with a serine proteinase-like structure that can cleave the main part of the polyprotein at 11 conserved sites, releasing RdRp, helicase, and other proteins for viral RNA replication. The functional role of Mpro for the maturation of viral proteins, coupled with the human lack of closely related homology, suggests that Mpro is an attractive target designing antiviral drugs (Ziebuhr et al., 2000; Ziebuhr, 2005; Yang et al., 2005).

**Structure of Mpro With N3**

A Michael acceptor-based peptidomimetic inhibitor N3 designed by computer-aided drug design (CADD) can inhibit Mpro proteases in MERS-CoV and SARS-CoV (Xue et al., 2008). Consistently, N3 could also fit well inside the SARS-CoV-2’s substrate-binding pocket (Jin et al., 2020). In the structure, two Mpro form dimers via 2-fold symmetry axes of crystallography (named protomer A and B) (Figure 9A). Each protomer is formed by three domains (Figure 9B). Antiparallel β-barrel structures are shown in domain I (residues 8–101) and domain II (residues 102–184). Domain III (residues 201–303) consists of 5 α-helices. Mpro has a Cys–His catalytic dyad, and there is a cleft between domain I and II as the N3-binding site.

The electron density indicates that the Cb of the vinyl group on N3 and the Mpro catalytic sites Cys145 form a standard 1.8 Å C–S covalent bond, confirming that the Michael addition has occurred (Figure 9C). Five parts from N3 inserts Mpro active sites as P1, P2, P3, P4, and P5 (Wang et al., 2017). The lactam, located on P1, inserts into the S1 subsite, forming a hydrogen bond with His163 in protomer A. At the P2 site, the Leu’s side chain inserts into the hydrophobic S2 subsite. Val at P3’s side chain is solvent-exposed. The P4 side Ala is encircled by Leu167’s and Phe185’s side chains, and the main chain of Glu189, all of which form a small hydrophobic pocket. At P5, there are van der Waals contacting with Pro168. Through plaque-reduction assay, N3 showed an inhibitory effect on SARS-CoV-2, with individual half-maximal effective concentration (EC₅₀) values of 16.77 µM (Jin et al., 2020).

**Structure of Mpro With 11a and 11b**

By analyzing the structure-activity relationship (SAR) of the reported inhibitor and substrate binding pocket, two compounds 11a and 11b with more effective inhibition than N3 were designed to target SARS-CoV-2 Mpro (Dai et al., 2020). The electron density map in the complex structure clearly shows the extended conformation of 11a in the Mpro substrate-binding pocket (Figure 9D). The Mpro catalytic sites Cys145 forms a covalent bond (1.8-Å) with the aldehyde-based C of 11a (Figure 9E). At P1, the (S)-g-lactam ring of 11a fits well into the S1 site. At P3, the 11a indolyl group is exposed to the S4 position, and Glu166 could stabilize by a hydrogen bond. When binding 11a, multiple water molecules, named W1 to W6, play important roles. Through a 2.9 Å hydrogen bond, W1 has interaction with the amide bond of 11a. As for W2 to W6, the five molecules stabilize 11a in the binding pocket. The 11b shows a similar inhibition binding mode with 11a. (Figure 9F). The biggest difference in binding mode is at P2. The 3-fluorophenyl group in 11b has a downward rotation of about 42.7 degrees relative to the cyclohexyl group in 11a (Dai et al., 2020). The binding modes of 11a and 11b in the Mpro complex structure are similar to the previously reported N3 and other inhibitors (Figure 9F). More water molecules are involved in binding hydrogen bonds in 11a and 11b, and the functional groups on the P2 are involved in some additional interactions, which may be the reason for their enhanced inhibition (Wang et al., 2016; Zhang et al., 2020). Here we summarize inhibitors for Mpro as follows (Table 2).

**Mpro Inhibitors From FDA Approved Library**

For a medical emergency, scientists began to re-screen drugs from all protease inhibitors approved by the FDA, which could inhibit Mpro. The established FRET assay was used to identify potential SARS-CoV-2 Mpro inhibitors by screening all protease inhibitors from the Selleckchem bioactive compound library (Ma et al., 2020).

Noticeable findings are as follows: (1) Boceprevir, an FDA-approved HCV drug, inhibits the Mpro’s enzymatic activity with IC₅₀ of 4.13 µM and EC₅₀ of 1.90 µM against the SARS-CoV-2 virus in the cellular viral cytopathogenic effect (CPE) assay. (Ma et al., 2020). (2) GC-376, an investigational veterinary drug that has the highest enzymatic inhibition against the Mpro with an IC₅₀ value of 0.03 µM (Kim et al., 2016; Pedersen et al., 2018) among candidate inhibitors so far. It showed promising activity against the SARS-CoV-2 virus (EC₅₀ = 3.37 µM). GC-376 can use two different configurations R and S which may explain its high binding affinity to the target Mpro. (3) Calpain/cathepsin inhibitors. Calpain inhibitors II and XII, MG-132, are three potent inhibitors of Mpro with single-digit submicromolar efficacy in the enzymatic assay. Calpain inhibitors II and XII inhibit SARS-CoV-2 with EC₅₀ values of 2.07 µM and 0.49 µM, respectively. MG-132 inhibits SARS-CoV-2 with IC₅₀ values of 3.90 µM (Barnard et al., 2004).
FIGURE 9 | The crystal structure of SARS-COV-2 virus Mpro in complex with inhibitors. (A) Surface representation of the homodimer of Mpro. Protomer A is in green, and protomer B is in pale green. (B) Cartoon representation of one protomer of the dimeric Mpro-inhibitor complex. The protomer is in green. N3 is in red sticks. (C) A zoomed view for the substrate-binding pocket. The key residues, which form the binding pocket, are shown as green sticks, the two waters, assigned as W1 and W2, are shown as red spheres. Four subsites, S1’, S1, S2, and S4, are labeled. P1, P1’, P2, P3, P4, and P5 sites of N3 are shown. Hydrogen bonds are indicated as dashed lines. (D) Close-up view of the 11a binding pocket. The residues involved in inhibitor binding are shown as green sticks. 11a and water molecules are shown as orange sticks and red spheres, respectively. Four subsites, S1’, S1, S2, and S4, are labeled. P1, P1’, P2, and P3 sites of 11a are indicated. Hydrogen bonds are indicated as dashed lines. (E) Close-up view of the 11b binding pocket. 11b and water molecules are shown as cyan sticks and red spheres, respectively. Four subsites, S1’, S1, S2, and S4, are labeled. P1, P1’, P2, and P3 sites of 11b are indicated. Hydrogen bonds are indicated as dashed lines. (F) Comparison of the inhibitor binding modes in SARS-CoV-2 Mpro. Two configurations (S and R) of GC-376, 11a, 11b, N3, and Boceprevir are shown in yellow, blue, orange, cyan, red, and dirty violet, respectively. All structures are drawn by Pymol.

TABLE 2 | The summary of recently reported Mpro inhibitors.

| Compounds | SARS-CoV-2 Mpro inhibition (µM) | SARS-CoV-2 Antiviral activity (µM) | Development Stage |
|-----------|---------------------------------|-----------------------------------|------------------|
| N3        | Kobs/µM=11,300 ± 800 M⁻¹ S⁻¹     | EC50 = 16.77 ± 1.7                | Preclinical; not tested in animals models |
| Ebselen   | IC50 = 0.67 ± 0.09               | EC50 = 4.67 ± 0.80               | In clinical trials |
| 11a       | IC50 = 0.053 ± 0.005             | EC50 = 0.53 ± 0.01               | Preclinical; Favorable PK in rats and low toxicity in rats and dogs |
| 11b       | IC50 = 0.040 ± 0.002             | EC50 = 0.72 ± 0.09               | Preclinical; Favorable PK in rats |
| Boceprevir| IC50 = 4.13 ± 0.61 K=1.18        | EC50 = 1.31 ± 0.58               | FDA-approved |
| GC-376    | IC50 = 0.03 ± 0.008 K2/Ki=40,800 M⁻¹ S⁻¹ | EC50 = 3.37 ± 1.68 | Preclinical; Tested in felines |
| MG-132    | IC50 = 3.80 ± 1.01               | NT.                              | Preclinical; Tested in mice |
| Calpain inhibitor II | IC50 = 0.97 ± 0.27 K=0.40 | EC50 = 2.07 ± 0.76 | Preclinical; not tested in animals models |
| Calpain inhibitor XII | IC50 = 0.45 ± 0.06 K=0.13 | EC50 = 0.49 ± 0.18 | Preclinical; not tested in animals models |
In summary, different potent SARS-CoV-2 Mpro inhibitors had been found with potent antiviral activity. Based on these hits, further development may lead to clinical application towards SARS-CoV-2 infections.

**Structure of RdRp and Its Inhibitors**

Remdesivir is an adenosine analogue that converts intracellularly into active drugs in the form of triphosphate (RTP), which binds to the nascent viral RNA chain, leading to premature termination (Velthuis et al., 2011; Ahn et al., 2012; Subissi et al., 2014; Kirchdoerfer and Ward, 2019; Wang M. L. et al., 2020). (including SARS/MERS/CoV/Ebola) Remdesivir is considered to be a promising antiviral drug against various RNA viruses (including SARS/MERS/Ebola virus) (Mulangu et al., 2019). Evidence showed that Remdesivir also efficiently inhibited SARS-CoV-2 infection through RdRp (van Hemert et al., 2008; Gong and Peersen, 2010; Sheahan et al., 2017).

Cryo-EM structures of RdRp provide insights into the inhibition mechanism of remdesivir (Yin et al., 2020) (Figures 10B, C). Different from the SARS-CoV RdRp, nsp12 of SARS-CoV-2 RdRp has an additional β-hairpin at the N-terminus (residues 31–50), as well as an extended nidovirus RdRp-associated nucleotidyl-transferase domain, which has three β-strands and seven helices (NiRAN, residues 115–250) (Lehmann et al., 2015; Gao et al., 2020). An interface domain (residues 251–365) is following the NiRAN domain. The nsp12 RdRp domain shows the canonical cupped right-handed configuration with the thumb subdomain. NiRAN, Interface, Fingers, Thumb, Palm, nsp7, nsp8 are shown in orange, gray, hot pink, green, blue, cyan, and violet, respectively. (D) The conserved zinc-binding motifs in the apo structure rendered in ribbon are highlighted. The residues are shown as green sticks, and zinc is shown as red spheres. All structures are drawn by Pymol.

**FIGURE 10** | The structure of the apo RdRp complex. (A) The schematic diagram for the components of the RdRp complex, which contains nsp7, nsp8, and nsp12. The polymerase motif (A-G) and β-hairpin are highlighted because it is unique to the SARS-CoV-2. (B, C) Two views structure of the apo nsp12-nsp7-nsp8 complex. nsp12 consists of an N-terminal β-hairpin (residues 31–50) and an extended nidovirus RdRp-associated nucleotidyl-transferase domain (NiRAN, residues 115–250). An interface domain (residues 251–365) is following the NiRAN domain. The nsp12 RdRp domain shows the canonical cupped right-handed configuration with the thumb subdomain. NiRAN, Interface, Fingers, Thumb, Palm, nsp7, nsp8 are shown in orange, gray, hot pink, green, blue, cyan, and violet, respectively. (D) The conserved zinc-binding motifs in the apo structure rendered in ribbon are highlighted. The residues are shown as green sticks, and zinc is shown as red spheres. All structures are drawn by Pymol.
activity does not require specific sequences during the elongation step. At the 3’ end of the primer strand is RMP, covalently bound to the primer strand at the +1 position. Additional nucleotides at the +2 and +3 positions of the template strand interact with residues at the back of the finger subdomain (Figure 11E). As observed in the structure, only one RMP was assembled into the primer strand though the presence of excess RTP in a complex assembly (Tchesnokov et al., 2019; Gordon et al., 2020).

The RMP position is at the center of the active catalytic site (Figure 11D). RMP interacts with the upstream bases from the primer chains to form base stacking and forms two hydrogen bonds with the uridine groups from the template chains. In addition, RMP interacts with the side chains of K545 and R555. There were two magnesium ions near the bound RMP. Both magnesium ions interact with the diester phosphate backbone to form a part of the active catalytic site (Figure 11D).

**FIGURE 11** | The structure of the template-RTP RdRp complex. (A) The sequence of the RNA duplex with a 5’ U10 overhang as a template for extending primer and assembling the RdRp-RNA complex. (B) Cryo-EM Structure of the Remdesivir and RNA bound RdRp complex. Template and Primer are shown as yellow and cyan. (C) The conserved RdRp motifs (A to G) of the RNA-bound complex overlap with the apo structure, and color in gray, with a close view at the active site. Motifs (A to G) are shown in light orange, orange, blue, light blue, aquamarine, warm pink, and pale green, respectively. (D) A close view of the RdRp active site, showing the covalently bound RMP, magnesium ions, and pyrophosphate. Key residues and bases interacting with Remdesivir are shown. (E) Remdesivir-bound RdRp complex and protein-RNA interactions in the RNA. All structures are drawn by Pymol.
Seven conserved motifs construct the active catalytic site of the nsp12 RdRp from A to G (Figures 10C and 11C). Motifs ABCD are from the palm subdomain forming the active catalytic center (Figure 11C). Regarding the two magnesium ions in the catalytic center, both D760 and D761 are involved in the coordination (Figure 11D) (te Velthuis, 2014).

Interesting differences between the apo and complex structures were revealed by structural comparison (Figure 11C). Firstly, nsp7 moved toward the RdRp, resulting in the interface’s rearrangement and lead to a weaker association of the second nsp8 in the complex. Secondly, the loop, connecting the first and second helix of the thumb subdomain, moved outward to adapt the binding of the double-stranded RNA helix. Thirdly, residues K500 and S501 from motif G moved to accommodate the template strand RNA’s binding. Except for these changes, nsp12 in the apo and the RNA-complex are very similar (Venkataraman et al., 2018). In particular, the structural elements of the active catalytic site can be accurately superimposed, suggesting that RdRp is a relatively stable enzyme that can function as a replicase.

Besides remdesivir, several nucleotides, including galidesivir, ribavirin, favipiravir, and EIDD-2801, inhibit SARS-CoV-2 replication efficiently (Gordon et al., 2020). Especially, EIDD-2801 showed 3 to 10 folds more effective than remdesivir in preventing SARS-CoV-2 replication.

DISCUSSION

As the third widespread coronavirus that threatens human beings, SARS-CoV-2 has brought great disaster to the world (Yao et al., 2020a; Liang et al., 2020). Unfortunately, the SARS-CoV-2 variant D614G is more infectious than the ancestral SARS-CoV-2 (Plante et al., 2021). The analysis shows that the destruction of the interaction between D614 and T859 and the increase in the proportion of open RBD may be the reason for the increase in SARS-CoV-2 infection. Recently, other mutations, such as E484Q and L452R, have emerged and have decreased the effect of the vaccine. Fortunately, some patients who contracted SARS-CoV-2 recovered quickly after receiving cocktail therapy (Baum et al., 2020). The cocktail’s formula includes Regeneron’s REGN10933 and REGN10987 antibodies, zinc, vitamin D, famotidine, melatonin, and aspirin. Current drug candidates mainly inhibit S protein and some non-structural proteins (Mpro, PLpro, helicase, and RdRp). Small molecule inhibitors and neutralizing antibodies are potential therapies for SARS-CoV-2 infection.

For small molecule inhibitors, some FDA-approved compounds for clinical use have shown efficacy against SARS-CoV-2. Remdesivir, an inhibitor developed for the Ebola virus, inhibits SARS-CoV-2 by binding RNA replicase RdRp. Compared to small molecules, the neutralizing antibodies exhibit robust inhibition against SARS-CoV-2 infection. The neutralizing antibodies BD-368-2 (Cao L. et al., 2020), BD23-Fab (Cao L. et al., 2020), and P2B-2F6 (Ju et al., 2020) against S protein showed good IC50 values and could effectively compete with ACE2 binding. The IC50 values of neutralizing antibodies REGN10933 and REGN10987 can be as low as a few picomoles, which play an essential role in successful cocktail therapy. Besides this antibody cocktail, two de novo mini proteins designed by CADD with high affinity to S protein, LCB1 and LCB3, show effective inhibition to the invasion of SARS-CoV-2 to host cells (Cao L. et al., 2020). The structure of the neutralizing antibodies and SARS-CoV-2 S protein complex provides a robust basis, further promoting the understanding of antibody-based therapy. In fact, many patients have saved their lives based on the application of antibodies.

To date, human beings have experienced several epidemic outbreaks, and each episode has had varying degrees of negative impacts on health, economy, psychology, and human behavior. When it comes to coronavirus, researchers have worked hard to discover its replication and pathogenesis and have gained some achievements in this regard. As we all know, many viruses have been present in natural reservoirs for a long time. Owing to human activities, viruses spread from natural hosts to humans or other animals. Unfortunately, people still cannot predict when the virus will arrive and what impact the virus will have. In this review, we provide the origin and evolution of SARS-CoV-2 to show that people should maintain the barrier between natural reservoirs and human society. After this worldwide epidemic, finding effective drugs or inhibitors is imminent. Although antibodies have shown positive effects, the defects are still obvious and not easy to overcome. In particular, it is impossible and costly to extract enough neutralizing antibodies from convalescent patients for countless clinical treatments. In this review, we summarize almost all the existing neutralizing antibodies or drugs and display their structures. Comparing or incorporating their characteristics is important to optimize or design more effective drugs. In the future, de novo recombinant protein may overcome the bottleneck of antibodies, improving the binding affinity and yield. It is theoretically feasible to minimize the effective antibodies or design de novo antibodies based on the structural data. In the case of Ebola, zmPep, consisting of three chimeric MBS, was successfully used to cure two Ebola patients in 2014 (Lyon et al., 2014). Therefore, the commercial antibody will bring hope to SARS-CoV-2 patients.

In addition to the above two treatments, the vaccine’s clinical application will effectively prevent the spread of SARS-CoV-2 (Silveira et al., 2020). For emergency use, the whole virus inactivated vaccine and mRNA vaccine (Park et al., 2020) have shown the prospect of prevention (Calina et al., 2020). In the future, more in-depth research on SARS-CoV-2 will bring new therapeutic schemes for its prevention and treatment.

AUTHOR CONTRIBUTIONS

SN and BY were the first authors with equal contributions, and YW and FW were the corresponding authors with equal contributions. All authors contributed to the article and approved the submitted version.
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SUPPLEMENTARY MATERIAL

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