Book Chapter

Therapeutic Strategies against COVID-19 and Structural Characterization of SARS-CoV-2: A Review

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Abstract

The novel coronavirus, SARS-CoV-2 or 2019-nCoV, which originated in Wuhan, Hubei province, China in December 2019, has emerged as a grave threat to public health worldwide. A total of 3,672,238 confirmed cases of coronavirus disease 2019 (COVID-19) and 254,045 deaths were reported globally until May 7, 2020. However, approved antiviral agents for the treatment of patients with COVID-19 remain unavailable. Drug

repurposing of approved antivirals against other viruses such as HIV or Ebola virus is one of the most practical strategies to develop effective antiviral agents against SARS-CoV-2. A combination of repurposed drugs can improve the efficacy of treatment and structure-based drug design can be employed to specifically target SARS-CoV-2. This review discusses therapeutic strategies using promising antiviral agents against SARS-CoV-2. In addition, structural characterization of potentially therapeutic viral or host cellular targets associated with COVID-19 has been discussed to refine structure-based drug design strategies.

Keywords

COVID-19; SARS-CoV-2; 2019-nCoV; Antiviral Agents; Therapeutic Strategies; Crystal Structure

Introduction

In late December 2019, a newly identified coronavirus strain capable of crossing species barrier and infecting humans was first reported in Wuhan, Hubei province, China, and was provisionally termed 2019 novel coronavirus [1,2]. This novel virus was later designated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) owing to its genetic similarity with other coronavirus strains [3]. It is known to cause coronavirus disease 2019 (COVID-19) characterized by influenza-like mild or moderate respiratory symptoms including dry cough, fever, headache, and pneumonia, as well as severe lung injury and multi-organ failure, eventually leading to death [4,5]. The World Health Organization (WHO) officially declared COVID-19 as a pandemic on March 11, 2020 due to the rapid global dissemination of SARS-CoV-2.

According to the WHO, a total of 3,672,238 confirmed cases of COVID-19 and 254,045 deaths were recorded until May 7, 2020 in over 200 countries. Moreover, effective antiviral therapeutic agents or vaccines are not yet available for COVID-19. Repurposing of existing drugs designed for other viruses is the most practical strategy to treat patients with COVID-19 because

they have been tested for their safety. Although *de novo* development of antivirals is a time-, cost-, and effort-intensive endeavor, it is important to generate specific antivirals for SARS-CoV-2 that directly target the viral or host proviral factors [6,7].

With increasing structural data of key proteins of both SARS-CoV-2 and the host, such as the spike glycoprotein (S), the main protease (M^{pro}), RNA-dependent RNA polymerase (RdRp), and human angiotensin-converting enzyme 2 (hACE2), structure-based design of new drugs has emerged as the most promising antiviral strategy. In this review, we have summarized the promising therapeutic potential of pre-existing drugs against COVID-19. In addition, structural characterization of potentially therapeutic viral or host cellular targets associated with COVID-19 has been discussed to refine structure-based drug design strategies.

SARS-CoV-2

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus and belongs to the genus *Betacoronavirus*, which also includes SARS-CoV and MERS-CoV [1,2,8] The genome sequence of SARS-CoV-2 is more closely related with that of SARS-CoV (79% identity) than with that of MERS-CoV (approximately 50%) [1]. Notably, the S protein of SARS-CoV-2 and SARS-CoV are highly homologous with 76.5% amino acid sequence identity [9]. Consequently, SARS-CoV-2 and SARS-CoV are believed to bind to the same host cell entry receptor hACE2 [10,11] instead of human dipeptidyl peptidase 4 (hDPP4), which is used by MERS-CoV [12].

SARS-CoV-2 has club-like spikes on its surface and a distinct replication strategy analogous to other coronaviruses. The life cycle and replication of SARS-CoV-2 is shown in **Figure 1**. Viral infection is initiated by the interaction between S protein and hACE2, followed by subsequent endocytosis or membrane fusion. The S protein comprises two subunits–S1 and S2. The S1 subunit contains the receptor binding domain (RBD) and binds to N-terminal hACE2, while the S2 subunit mediates virus-host

membrane fusion. S proteins are cleaved by the host cell furin protease and transmembrane serine protease 2 (TMPRSS2) at the S1/S2 boundary and the S2′ position. Proteolytic cleavage at the S1/S2 boundary is thought to promote TMPRSS2-dependent entry into the target cells [10,13,14]. After the release of the viral polycistronic RNA into the cytoplasm, the replicase gene comprising open reading frames (ORFs) 1a and 1ab is directly translated into either replicase polyprotein pp1a (~450 kDa, nsp1-11) or pp1ab (~750 kDa, nsp1-16) by a ribosomal -1 frameshift near the 3′-end of ORF 1a and autoproteolytically cleaved into 16 non-structural proteins (nsp1-16) by two ORF1a-encoded protease domains [15-21]. Furthermore, the main protease M^{pro} (also called $3CL^{pro}$) and papain-like protease (PL^{pro}) participate in this extensive proteolytic cleavage. The large pp1ab polyprotein has no less than 11 conserved cleavage sites that are mediated by M^{pro}, which cleaves at Leu-Gln↓(Ser, Ala, Gly) (arrow indicates the cleavage site) [22,23]. Positive-strand RNA viruses usually form a cytoplasmic enzyme complex called replicase-transcriptase complex (RTC) that can mediate the synthesis of the full-length genome (replication) or discontinuous mRNAs (transcription) [24-26]. Structural and accessory proteins are subsequently translated from these transcripts, and new viruses assemble by budding into the lumen of the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) and are eventually secreted [27,28].

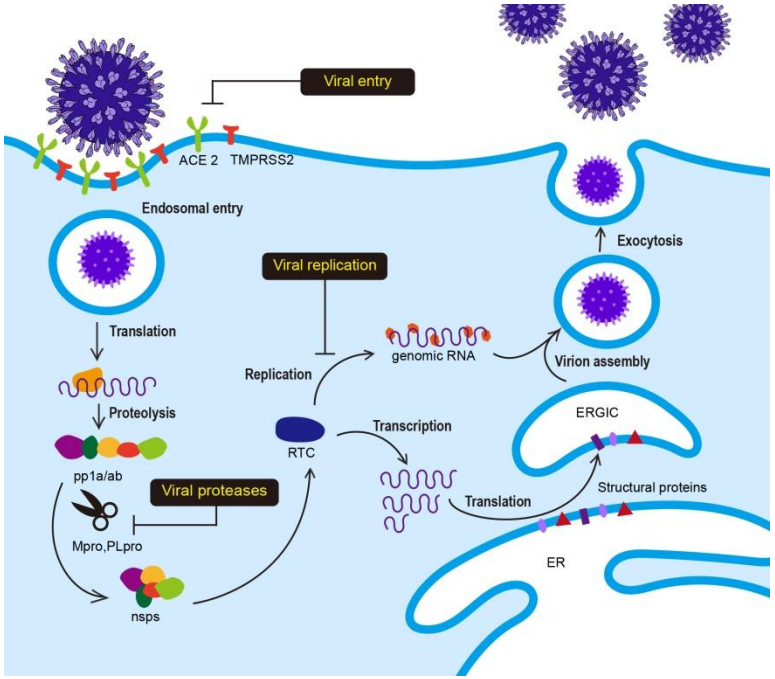

Figure 1: Viral life cycle of SARS-CoV-2. Interaction between the S protein of SARS-CoV-2 and hACE2 initiates SARS-CoV-2 infection. Following receptor binding, the virus enters the cell by acid-dependent proteolytic cleavage of S protein by TMPRSS2 or other proteases. Upon fusion of the viral and host cell membranes, viral genomic RNA is released in the cytoplasm. The viral RNA initiates translation of co-terminal polyproteins (pp1a/ab) by -1 frameshifting. These polyproteins are subsequently cleaved into nonstructural proteins (nsps) by M^{pro} and PL^{pro}. Several nsp proteins interact with nsp12 (RdRp) to form the replicase-transcriptase complex (RTC), which is responsible for the synthesis of full-length viral genome (replication) and sub-genomic RNAs (transcription). The viral structural proteins are expressed and translocated into the endoplasmic reticulum (ER). The nucleocapsid (N) protein-encapsidated genomic RNA translocates with the structural proteins into the ER-Golgi intermediate compartment (ERGIC) for virion assembly. The newly synthesized virions are budded through the cell membrane and exocytosed.

Antiviral Strategies against SARS-CoV-2

Antivirals can be broadly divided into two categories: direct-acting antivirals (DAA) and indirect-acting antivirals (IAA). DAAs directly target specific viral components such as viral polymerase or steps in the viral life cycle without affecting other

host cellular processes. The development of DAAs can facilitate the treatment of patients with COVID-19. In contrast, IAAs target host proviral factors and indirectly inhibit viral infection or replication by impeding the function or interaction of these factors. IAAs have an advantage over DAAs because they are not susceptible to viral mutations, which are frequently found in RNA viruses. However, IAAs can alter the host cellular system and are not considered safe. Therefore, DAAs targeting viral entry, proteases, and replication can serve as effective antivirals owing to their enhanced safety features. Drug repurposing of pre-existing antiviral agents is considered one of the most practical strategies because there is no available approved antiviral drug or vaccine for COVID-19. Furthermore, the *de novo* development of drugs typically requires over $1 billion USD and 10–17 years [6,7]. Drug repurposing of several approved antivirals against COVID-19 has progressed into clinical trials (**Table 1**). However, there is a potential risk of drug-resistant mutations with the use of DAA. A combination of repurposed drugs can reduce the time, cost of treatment, and risk of drug-resistance, and increase therapeutic efficacy to facilitate progression into clinical trials [29]. Moreover, due to the existence of crystal structures of viral and host cellular proteins associated with SARS-CoV-2 such as S protein, M^{pro}, RdRp, and hACE2, structure-based drug design can be performed to develop more effective drugs with reduced off-target toxicity [30].

Table 1: Current potential antiviral agents against SARS-CoV-2

	Target	Antiviral agent	Type	Status	Reference
Viral entry	ACE2	HrsACE2	Recombinant protein	-	[74]
	S protein	CR3022	Neutralizing antibody	-	[40]
	Endocytosis	Umifenovir (Arbidol)	Membrane fusion inhibitor	Phase 4	[75,76]
	Endocytosis	EK1C4	Pan-coronavirus fusion inhibitor	-	[77]
	TMPRSS2	Camostat Mesilate	Serine protease inhibitor	Phase 4	[10,76]
	TMPRSS2	Nafamostat	Serine protease inhibitor	Phase 2	[75,78]
	TMPRSS2	Bromhexine hydrochloride	Mucolytic drug	-	[53]
	TMPRSS2	PAI-1	Serine protease inhibitor	-	[79]
	Virus/Cell fusion	Chloroquine	Drug for autoimmune disease	Phase 4	[66]
Viral proteases	M^{pro}	Lopinavir/Ritonavir	Antiviral	Phase 4	[76,80]
	M^{pro}	Darunavir/covicistat	Antiviral	Phase 3	
	M^{pro}	ASC09F/Oseltamivir	Antiviral	Phase 3	[75,81,82]
	M^{pro}	Nelfinavir	Antiviral	-	[82]
	M^{pro}	Baicalein	Antibacterial	-	[83]
	M^{pro}	a-ketoamide	Antiviral	-	[58]
	M^{pro}	Ebselen	Anti-inflammatory	-	[57]
	M^{pro}	Disulfiram	Drug for chronic alcoholism	-	
	M^{pro}	Tideglusib	Kinase inhibitor	-	
	M^{pro}	Carmofur	Anticancer	-	
	M^{pro}	Shikonin	Natural prtoduct	-	
	M^{pro}	PX-12	Anticancer	-	
	M^{pro}	Compound 11a, 11b	Antiviral	-	[56]
Viral replication	RdRp	Remdesivir	Antiviral	Phase 3	[66,75,76]
	RdRp	Ribavirin	Antiviral	Phase 2	
	RdRp	Favipiravir	Antiviral	Phase 4	
	RdRp	Galidesivir	Antiviral	Phase 1	[71]
	RdRp	EIDD-1931	Antiviral	-	[72]
	RdRp	EIDD-2801	Antiviral	-	
	RdRp	Tenofovir disoproxil	Antiviral	Phase 3	[71]

Viral Entry

The cryo-electron microscopy (CryoEM) structure of the extracellular domain of the S protein of SARS-CoV-2 revealed a homotrimeric conformation [31]. The binding of RBD—located in the S1 subunit—to hACE2 on the host cell surface initiates interaction between the virus and the host cell; therefore, the switching conformation of RBD is considered an important event for viral entry [32]. CryoEM studies revealed that the RBD in two out of three S proteins binds to the N-terminal domain (NTD) of the neighboring protomer of the S protein. These inter-molecular interactions result in a down (closed) conformation, wherein the hACE2 interaction interfaces are buried inside the structure. Moreover, the RBD in the third S protein forms an up (open) conformation to facilitate binding with the N-terminal region of hACE2 **(Figure 2a)** [31]. The cryoEM study of SARS-CoV-2 S showed that single RBD formed an open conformation in asymmetric trimer. The structural comparisons between S protein of SARS-CoV (PDB ID 6CRZ) and SARS-CoV2 (PDB ID 6VSB) showed that the major structural differences came from RBD in closed conformation. Although the RBD of S from SARS-CoV and SARS-CoV-2 were largely resembled, the SARS-CoV-2 RBD showed higher binding affinity toward hACE2 than SARS-CoV RBD [32,33]. The CyroEM structure of full-length hACE2 revealed a homodimeric conformation, with each monomer of hACE2 binding to one RBD of the SARS-CoV-2 S protein **(Figure 2b)** [34]. The crystal structure of hACE2 in complex with SARS-CoV-2 RBD (PDB ID 6M0J and 6VW1) showed that SARS-CoV-2 RBD binds to the N-terminal region of hACE2 via S19, Q24, T27, F28, D30, K31, H34, E35, E37, D38, Y41, Q42, L45, L79, M82, Y83, Q325, N330, K353, D355, and R357 residues of hACE2 and K417, V445, G446, Y449, Y453, L455, F456, Y473, A475, G476, E484, F486, N487, Y489, Q493, G496, Q489, T500, N501, G502, V503, and Y505 residues of SARS-CoV-2 RBD **(Figure 2c)** [31,32]. Most of these interactions are mediated by α1 of hACE2 **(Figure 2c)**; moreover, an N-glycosylation chain at N90 of hACE2 interacts with SARS-CoV-2 S protein [32].

As mentioned earlier, the S1/S2 junction and S2′ site of the S protein are cleaved by furin and TMPRSS2, to enable efficient entry of SARS-CoV-2 into the host cell **(Figure 2a)**. In addition to trypsin, cathepsin L, and elastase, TMPRSS2 is known to activate the S protein and induce virus-cell membrane fusion [35]. A recent study reported that TMPRSS2 is also essential for SARS-CoV-2 entry into target cells [10,36].

Accordingly, targeting proteins that participate in SARS-CoV-2 entry can be a potential therapeutic strategy. The use of neutralizing antibodies (NAbs) against SARS-CoV-2 S protein is thought to be promising for the treatment of patients with COVID-19 [37]. A Nab—CR3022—known to target SARS-CoV RBD and prevent lung pathology, can also bind to SARS-CoV-2 RBD [38,39]. The crystal structure of SARS-CoV-2 RBD in complex with CR3022 revealed that CR3022 forms a distinct interaction interface with SARS-CoV-2 RBD, and does not overlap with the interaction interface between hACE2 and SARS-CoV-2 RBD **(Figure 2d and 2e)**. Although CR3022 binds to SARS-CoV RBD and SARS-CoV-2 RBD with binding affinities (Kd) of 1 nM and 115 nM, respectively, it is unable to neutralize SARS-CoV-2 *in vitro* largely due to its inability to form the interaction interface and low binding affinity [37,40]. However, continuous efforts being undertaken to identify potent NAbs by collecting plasma from infected individuals has been shown significant progress. The P2B-2F6 from SARS-CoV2 infected patient have overlapping residues G446 and Y449 with higher RBD binding affinity than ACE2/RBD (5.14nM and 4.70nM respectively) [41]. Furthermore, the interaction interface of C105/RBD overlapped with the ACE2 binding region, and B38 share similar binding structure with prominent neutralizing effect [42,43]. Also they showed recent concern of mutation in S (D614G) that might increase SARS-CoV-2 transmission has a rare chance to affect the RBD-binding Mab C105, because of distance between RBD region and D614 [42]. In addition to identifying NAbs targeting SARS-CoV-2 S protein, a pilot trial to use recombinant soluble human ACE2 in COVID-19 patients has been initiated (clinicaltrial.gov #NCT04287686). However, this trial was recently withdrawn as it was not approved by center for drug evaluation (CDE). Because ACE2 can counter

the activation of renin–angiotensin–aldosterone system (RAAS) treatment with ACE2 inhibitors can increase ACE2 expression in some patients to compensate for blocked ACE2 activity [44]. In some animal studies, treatment of RAAS inhibitor resulted in increased expression of ACE2 in specific tissues [45,46]. In this regards, some researchers hypothesized that treatment of RAAS inhibitor might enhance the accessibility of SARS-CoV-2 into cells and therefore increase the risk of severity in patient who carrying COVID-19 [47,48]. However, recent case population study showed that no correlation between use of RAAS inhibitors and increased risk of COVID-19 [49]. The Ramipril, ACE inhibitor showed cardiac protective effect without increased expression of ACE2 [50]. These contradictory results suggested that clinical validations of RAAS inhibitors are need to demonstrate its effectiveness toward COVD-19. The high-resolution X-ray crystal structure of apo-hACE2 and hACE2 in complex with its enzymatic inhibitor MLN-4760 showed that inhibitor binding at the active site of hACE2 can cause large hinge-bending movement [51] **(Figure 2f)**. Furthermore, a structure-based drug discovery study showed that an enzymatic hACE2 inhibitor can prevent SARS-CoV infection [52]. Therefore, hACE2 inhibitors can potentially prevent SARS-CoV-2 infection.

Although the structure of human TMPRSS2 is not available yet, homology modeling and *in silico* docking studies have demonstrated the molecular mechanisms of camostat mesylate, nafamostat, and bromhexine hydrochloride in inhibiting TMPRSS2 [53]. In this respect, active site-specific inhibitors of TMPRSS2 can be used as potential antiviral agents against SARS-CoV-2.

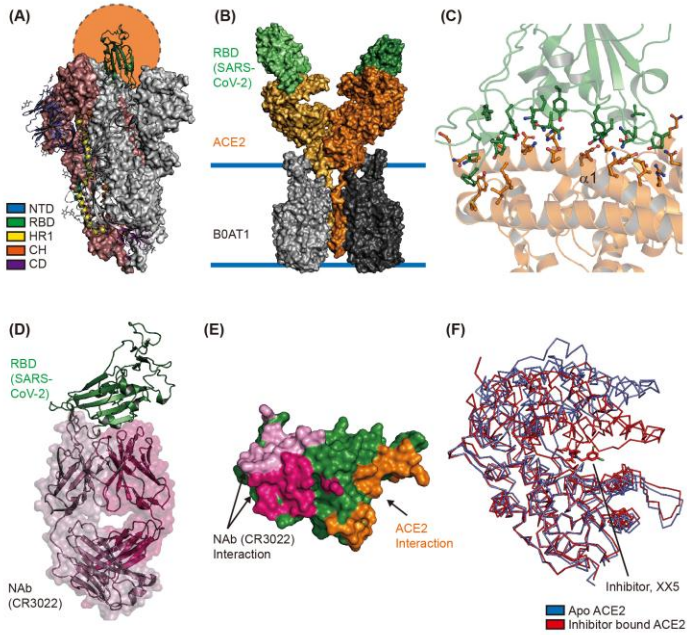

Figure 2: Structural characterization of the interface between ACE2 and SARS-CoV-2. **(A)** Overall structure of the spike glycoprotein (S) of SARS-CoV-2 in its homotrimeric conformation. One up and open conformation of the trimer is shown; the up position of the receptor binding domain (RBD), shown in green, is indicated by the orange circle (PDB ID 6VXX). The N-terminal domain (NTD), RBD, HR1, CH, and C-terminal domain (CD) are shown in blue, green, yellow, orange, and purple, respectively. **(B)** CryoEM structure of human ACE2 in complex with the RBD of SARS-CoV-2 and B0AT1 (PDB ID 6M17). The overall structure reveals that human ACE2 forms a homodimer (orange and light-yellow) with B0AT1 (dark and light grey), which is located in the transmembrane region. The two SARS-CoV-2 RBDs are shown as dark and light green surfaces. **(C)** The interaction interface between RBD and ACE2 in shown (PDB ID 6M0J). The residues involved in the interaction between SARS-CoV-2 RBD and hACE2 are represented with stick models in green and orange, respectively. Alpha helix 1 (α1) of hACE2 is also labeled. **(D)** Overall structure of SARS-CoV-2 RBD in complex with its neutralizing antibody CR3022 (PDB ID 6W41). The Fab regions of the heavy and light chains are shown in hot pink and pink, respectively. SARS-CoV-2 RBD is shown in green. **(E)** Structural comparison of interfaces between SARS-CoV-2 RBD and Nab or hACE2. The interaction interfaces with the light chain of CR3022, heavy chain of CR3022, and hACE2 are shown in pink, hot pink, and orange, respectively. **(F)** Hinge movement of hACE2 upon binding of the enzyme inhibitor. The Apo form (PDB ID 1R42) and inhibitor-bound form (PDB ID 1R4L) are superimposed and shown in blue and red, respectively.

Viral Proteases

The crystal structure of SARS-CoV M^{pro}—a cysteine protease—consists of domains 1–3. The catalytic processes of M^{pro} are mediated by the non-canonical Cys-His catalytic dyad located between domains I and II [54,55]. The M^{pro} protein is highly conserved among SARS-CoV, MERS-CoV, and SARS-CoV-2, and it shares the common substrate recognition sequence consisting of LQ(S,A,G) [22,23,56]. Among them, the Gln in P1 of the substrate is an important common feature required for their catalytic activity. Human proteases with similar substrate specificity to that of M^{pro} do not exist; therefore, development of M^{pro} inhibitors is one of the potential therapeutic strategies for targeting SARS-CoV-2.

SARS-CoV-2 M^{pro} consists of three domains, analogous to that of M^{pro} from other CoVs **(Figure 3a)** [56-58]. The crystal structure of M^{pro} revealed that it forms homodimers (dimeric protomer) through interaction between domain II of protomer A and N-terminal residues of protomer B **(Figure 3a)** [58]. Homodimerization of M^{pro} is required for its enzymatic activity. Mutational studies on the dimeric interface, as well as crystal structure analysis revealed that the interaction between two protomers is required to form the S1 pocket at the substrate binding site **(Figure 3b)** [54,58,59]. The substrate binding site of SARS-CoV-2 consists of S1′-S1-S2-S4 pockets lined with , H41, S46, M49, Y54, F140, L141, N142, G143, C145, H163, H164, M165, E166, L167, H172, F185, D187, Q189, T190, A191 and Q192 residues **(Figure 3b)** [56-58]. Notably, the S2 pocket of CoVs is typically hydrophobic and can accommodate the bulky P2 fragment **(Figure 3b)**. Several structure-based drug discovery studies have investigated the interaction of inhibitors in the substrate-binding pockets of SARS-CoV-2 M^{pro} **(Figure 3c)** [56-58]. A previous study for developing broad spectrum inhibitors targeting CoV M^{pro} showed that inhibitors of SARS-CoV-2 contain a (S)-γ-lactam ring at P1 position to mimic glutamine and occupy the S1 pocket of SARS-CoV-2 M^{pro} [60]. A total of 103 structures of SARS-CoV-2 M^{pro} in both apo and inhibitor complex forms are available in the protein data bank (PDB) database (https://www.rcsb.org/) until 27 April 2020. Zhang et

al. [58] have developed a peptidomimetic α-ketoamide inhibitors targeting SARS-CoV-2 M^{pro}. They also solved the crystal structure of M^{pro} in complex with α-ketoamide 13b (PDB ID 6Y2G) and showed the presence of a γ-lactam ring at P1 position and cyclopropyl at P2 position **(Figure 3d)**. The biochemical IC_{50} of SARS-CoV-2, SARS-CoV, and MERS-CoV M^{pro} were found to be 0.67, 0.90, and 0.58 μM, respectively [58]. Simultaneously, Dai et al. [56] developed inhibitors with aldehyde substituted compound at warhead for occupying S1 site and thus it covalently bonds catalytic cysteine of SARS-CoV-2 M^{pro} (PDB ID 6LZE and 6MOK) [56] **(Figure 3e)**. These compounds showed high inhibition activity with IC_{50} of 53 and 40 nM *in vitro* and reduced SARS-CoV-2 infection with EC_{50} of 0.53 and 0.72 μM in plaque reduction assay [56]. The crystal structure of SARS-CoV-2 M^{pro} in complex with the inhibitor compound N3 (PDB ID 7BQY), previously designed to inhibit CoV M^{pro}, revealed that N3 occupies the substrate binding pocket and forms a covalent bond with catalytic C145 of SARS-CoV-2 M^{pro}. Consistently, the lactam ring at P1 position of N3 forms a hydrogen bond with H163 of SARS-CoV-2 M^{pro} **(Figure 3f)** [57,61]. X77, a potential inhibitor of SARS-CoV-2 M^{pro}, also occupies the substrate binding pocket; however, it does not form covalent bonds (PDB ID 6W63) **(Figure 3g)**.

In conclusion, M^{pro} of SARS-CoV-2 is a key protein that participates in proteolytic processing of polyproteins and shows no overlapping substrate specificity with any of the known human proteases. Several potent inhibitors share common structural features, including covalent bond formation with catalytic cysteine and a lactam ring at P1 position. Because most inhibitors occupy the substrate binding pocket of SARS-CoV-2 M^{pro}, targeting this pocket could be an efficient and a safe strategy in terms of toxicity.

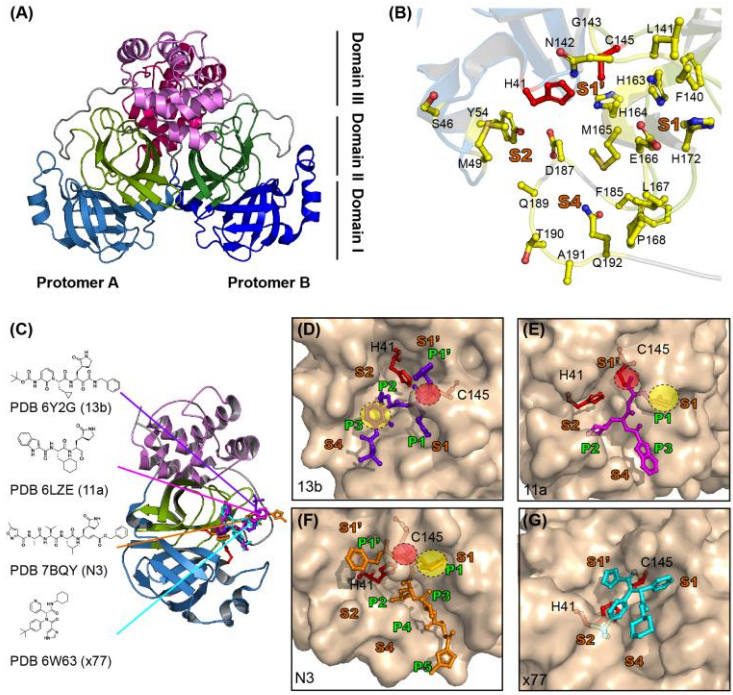

Figure 3: Structure of SARS-CoV-2 viral M^{pro} and its complex with inhibitors. **(A)** Crystal structure of SARS-CoV-2 M^{pro}. M^{pro} is a cysteine protease that consists of three domains and two protomers. Protomer B is shown in darker colors than protomer A and each domain is shown in different colors (sky blue, split pea, and violet color represent domains 1, 2, and 3, respectively). **(B)** Substrate binding site of SARS-CoV-2 M^{pro}. The substrate binding site of M^{pro} is subdivided into S1, S1′, S2, and S4 (shown in bold orange). The inhibitors bind to 17 residues shown as yellow sticks (H41, S46, M49, Y56, F140, L141, N142, C145, H164, M165, E166, L167, H172, Q189, F185, T190, and Q192). The cysteine-histidine dyad (C145-H41) between domains 1 and 2 is shown in red. **(C)** SARS-CoV-2 M^{pro} in complex with its inhibitors. The structures of SARS-CoV-2 M^{pro} in complex with 13b (PDB ID 6Y2G, purple sticks), 11a (PDB ID 6LZ2, magenta sticks), N3 (PDB ID 7BQY, orange sticks), and x77 (PDB ID 6W63, cyan sticks) are shown. The molecular interaction of each inhibitor in the active site is shown as a surface and stick complex (**D, E, F,** and **G** are 13b, 11a, N3 and x77). The ɤ-lactam ring that plays an important inhibitory role is shown in the yellow circle, and C-S covalent bonds with Cys145 are shown in the red circle.

Viral Replication

Replication of SARS-CoV-2 genomic RNA is mediated by a multiprotein complex consisting of several non-structural proteins, such as nsp7, nsp8, nsp12, and nsp14. The functional core of this multiprotein complex consists of RNA-dependent RNA polymerase (RdRp, also called nsp12) [62]. SARS-CoV-2 RdRp plays an important role in the replication and transcription of viral genomic RNA **(Figure 1)** and its catalytic residues are highly conserved among CoVs [62,63]. It is because of this reason that the nucleotide analog remdesivir (GS-5734, Gilead) was treated to target RdRp of MERS-CoV, SARS-CoV, and SARS-CoV-2 [64-66]. Although the viral RdRp is a core component of viral replication, nsp7 and nsp8 are still required for full-fill transcriptional activity of RdRp [63,67-69]. The cryoEM structure of nsp12 revealed an N-terminal β-hairpin (aa 31–50), extended nidovirus RdRp-associated nucleotidyl-transferase domain (NiRAN, aa 117–250), interface domain (aa 251–365), and RdRp domain (aa 366–920) consisting of finger, palm, and thumb subdomains [69,70] **(Figure 4a)**. Structural studies have demonstrated that nsp12 can recognize the RNA template in a sequence-independent manner, suggesting that the enzymatic activity of RdRp is largely sequence independent. The cryoEM structure of SARS-CoV-2 RdRp in complex with an RNA template or its small molecule inhibitor remdesivir **(Figure 4b)** revealed the molecular inhibitory mechanism of remdesivir [70]. Remdesivir monophosphate interacts with the primer strand and uridine of the template strand by base stacking and hydrogen bonding, respectively, at the center of the catalytic active site of RdRp [70] **(Figure 4c)**. The covalent incorporation of remdesivir monophosphate into the primer strand blocks the entry of nucleotide triphosphates to the active site, and terminates the transcriptional activity of RdRp [70] **(Figure 4b)**. Other nucleotide analog compounds such as favipiravir, ribavirin, EIDD-1931, and EIDD-2801 may exhibit a similar mechanism of action as remdesivir to inhibit RdRp with non-obligate RNA chain termination [71-73]. Although the U.S Food and Drug Administration issued an emergency use authorization for remdesivir on May 1, 2020 for the treatment of suspected or laboratory-confirmed COVID-19 in adults and children

hospitalized with severe symptoms, the clinical efficacy of remdesivir against SARS-CoV-2 is not known yet. Moreover, no significant clinical benefits of remdesivir against SARS-CoV-2 were observed in a recent randomized, double-blind, placebo-controlled, multicenter clinical trial (ClinicalTrials.gov, NCT04257656) [73].

Taken together, compounds that target SARS-CoV-2 RdRp are largely nucleotide analogs because of their ability to form covalent bonds with the viral template RNA and block the catalytic active site of RdRp.

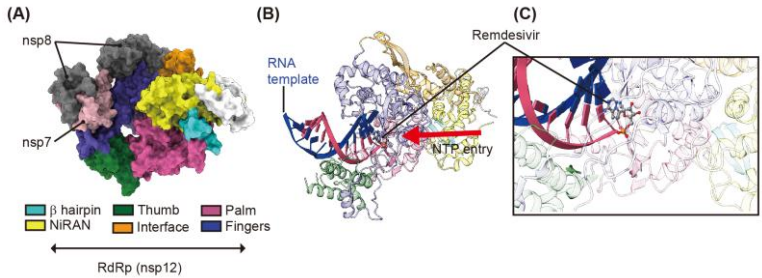

Figure 4: CryoEM structure of RdRp in complex with cofactors (nsp7 and nsp8), RNA template, and remdesivir. **(A)** Surface representation of the CryoEM structure of SARS-CoV-2 RdRp in complex with its cofactors (two nsp8 and one nsp7) (PDB ID 6M71). nsp7 and nsp8 are shown in grey and pink, respectively. The β-hairpin, NiRAN, interface, thumb, palm, and finger of SARS-CoV-2 RdRp are shown in cyan, yellow, green, orange, purple, and blue, respectively. **(B)** A cartoon representation of the overall structure of SARS-CoV-2 RdRp in complex with the RNA template and its inhibitor remdesivir (PDB ID 7BV2). The RNA template and primer strand are shown in blue and red, respectively. The red arrow indicated the direction of NTP entry. **(C)** magnified view of remdesivir monophosphate binding region. Remdesivir covalently binds to the primer RNA strand and interacts with the template RNA.

Conclusions

Zoonotic coronavirus outbreaks such as COVID-19 can not only affect public health but also have a major impact on civil societies and the global economy. Therefore, global cooperation among academic institutions, governments, and pharmaceutical companies is necessary to overcome COVID-19.

Despite intensive worldwide efforts undertaken by researchers to contain the spread of SARS-CoV-2, COVID-19 has attained pandemic status. Considering that the development of an effective vaccine and new therapeutics are still in the early stages, repurposing FDA-approved and well-characterized drugs might be a pragmatic approach. Consequently, some of these drugs, such as remdesivir, have been approved for emergency use and some are being tested in clinical trials. In addition, combination treatment might be one of certain approaches to achieve synergistic effects and reduce the risk of drug-resistant mutation.

A few studies have showed that some pre-existing drugs are effective for the treatment of patients with COVID-19. In this review, we described the ongoing therapeutic strategies targeting various components of the SARS-CoV-2 life cycle (Table 1). In addition, we provided structural insights into the mechanism of action of well-characterized drugs targeting the interaction between hACE2 and the spike protein of SARS-CoV-2 for viral entry, as well as M^{pro} and RdRp for viral replication. We believe that structural characterization can aid in developing an effective therapeutic strategy not only against COVID-19 but also other viral outbreaks in the future.

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