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Viewpoint

Exploiting Existing Molecular Scaffolds for Long-Term COVID Treatment

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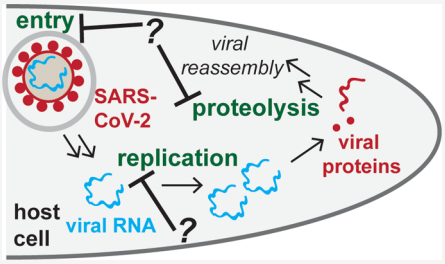
ABSTRACT: Discovery and development of COVID-19 prophylactics and treatments remains a global imperative. This perspective provides an overview of important molecular pathways involved in the viral life cycle of SARS-CoV-2, the infectious agent of COVID-19. We highlight past and recent findings in essential coronavirus proteins, including RNA polymerase machinery, proteases, and fusion proteins, that offer opportunities for the design of novel inhibitors of SARS-CoV-2 infection. By discussing the current inventory of viral inhibitors, we identify molecular scaffolds that may be improved by medicinal chemistry efforts for effective therapeutics to treat current and future coronavirus-caused diseases.



KEYWORDS: *COVID-19, SARS-CoV-2, replication, fusion, protease, antivirals*

Most of the world's initial efforts to treat Coronavirus Disease 2019 (COVID-19) have focused on the repurposing of approved drugs. This approach is warranted, as we seek quick solutions to a pandemic, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The recent announcement of the antiviral remdesivir as the first U.S. Food and Drug Administration (FDA) approved drug for the treatment of patients suffering from severe symptoms of COVID-19 affirms this approach. Anthony Fauci, the director of the National Institute of Allergy and Infectious Disease (NIAID) said that the completed clinical trial with remdesivir proved "that a drug can block this virus", although this drug alone is not a "magic bullet" for COVID-19.

Without the ability to accurately predict a timeline for a vaccine, we need additional clinical candidates to build an arsenal against SARS-CoV infections. Related coronaviruses, SARS-CoV-1 and MERS-CoV (Middle East Respiratory Syndrome Coronavirus), caused serious, albeit less widespread, health epidemics starting in 2002 and 2012. The occurrence of multiple outbreaks further motivates us to annotate therapeutic targets for other coronavirus-caused diseases that might develop in the future. Similar to the treatment of influenza, we would benefit from multiprong defense tactics that include both vaccines and therapeutic agents. Many recent scientific reviews and essays have outlined vaccine efforts, as well as viral and host targets that are the focus of current campaigns aimed at redirecting clinically used compounds for COVID-19.[1]

Here, we outline viral pathways that contain druggable targets currently overlooked and propose general mechanisms to disrupt these pathways. We begin with a short primer on the SARS-CoV-2 viral life cycle and an overview of existing drugs that are the subject of current clinical trials around the world. We then focus on alternative druggable targets largely within the viral replicase machinery, as well as viral entry and proteolytic processing pathways. Our proposed strategies for blocking these pathways are informed by past and current work on inhibitors of SARS-CoV and MERS-CoV, as well as other successful antivirals. We hope to engage the medicinal chemistry community with ideas for novel therapeutic targets in order to develop *new drugs* to specifically disarm coronaviruses in the host.

OVERVIEW OF THE VIRAL LIFE CYCLE

The genome organization of SARS-CoV-2 is similar to that of other members of the β-coronavirus family that also include SARS-CoV-1 and MERS-CoV.[2] The viral RNA-encoded proteins can be divided into two main classes: structural and nonstructural proteins (nsps). The structural proteins (spike, envelope, membrane, nucleocapsid, and hemagglutinin esterase) form the viral particle and play additional functional roles during infection. Viral infection begins with an interaction between the viral spike glycoprotein (S protein) and a receptor, angiotensin-converting enzyme 2 (ACE2), on the host cell surface (Figure 1). ACE2 is expressed in various cell types, including those in the lungs. Host proteases in the membrane are important in this process;[3] namely, a cellular serine protease, called transmembrane serine protease 2 (TMPRSS2) is known to prime the trimer of S protein on

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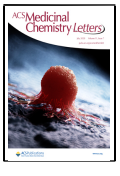

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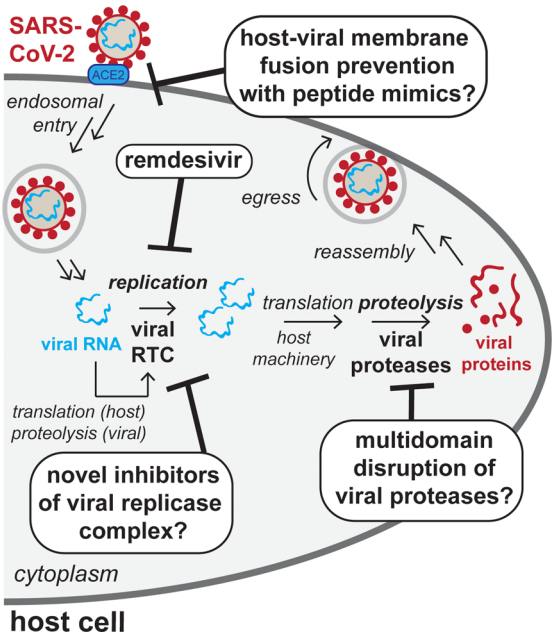

Figure 1. Overview of the proposed viral life cycle of SARS-CoV-2, the infectious agent of COVID-19. Both viral and host machinery are essential for viral infection, replication, reassembly, and egress. Remdesivir, the only FDA approved drug for COVID-19 treatment, blocks RNA replication. Highlighted are points of viral interference that have not been widely exploited: (1) inhibition of additional components of the viral replication−transcription complex (RTC); (2) development of new modes of disrupting viral protease activities; and (3) exploration of peptide-based inhibitors to prevent host−viral membrane fusion.

the viral particle surface prior to cell entry. Cleavage of S protein into two subunits is required for the process of viral and host membrane fusion prior to viral uptake by an endocytic mechanism.

Following engulfment and subsequent release from the endosome, viral genetic material is released into the host cytoplasm prior to translation of the single stranded viral RNA into long polypeptides that contain the nsps. Viral polypeptides are predicted to be cleaved into 16 individual nsps through an autoprocessing mechanism.[4] There are two cysteine-like proteases expressed as part of these polypeptides: one is a papain-like protease (PLP), known as the accessory protease; the other is a chymotrypsin-like protease ($3CL^{pro}$), known as the main protease. $3CL^{pro}$ cleaves itself and processes the remaining polypeptide into nsps 7−16, which make up the RNA replication-transcription complex (RTC). Several components of the RTC aid the RNA-dependent RNA polymerase (RdRP), which is responsible for replicating additional copies of the RNA genome and transcribing multiple mRNA fragments that encode either structural or accessory proteins. Following multiple cycles of replication and translation, the viral particle assembles and exits the cell though a budding mechanism known as scission. It is thought that β-coronaviruses rely on the host cell's endosomal sorting complex required for transport (ESCRT), but the exact method of egress is still not known.[3] Full scission from the host cell releases the virus to infect more cells and continue to replicate.

KNOWN DRUGS IN CLINICAL TRIALS FOR COVID-19 TARGET THE VIRAL LIFE CYCLE

The World Health Organization (WHO) and federal agencies are largely focused on clinical trials for preapproved drugs that are proposed to target some aspect of the viral life cycle described above (Figure 1). The NIH now lists over a thousand ongoing clinical trials for treatments relating to COVID-19. The WHO is currently conducting a worldwide trial ("SOLIDARITY") by focusing on four promising COVID-19 treatments: remdesivir; lopinavir/ritonavir with and without Interferon β-1a (to help stimulate the immune system); and hydroxychloroquine (this last treatment has currently been paused).

The FDA-approved COVID-19 drug, remdesivir, is a nucleotide analog originally developed to treat Ebola infections (caused by another single-stranded RNA virus) and recently shown to inhibit the SARS-CoV-2 RdRP.[5] The FDA has issued an emergency use authorization of hydroxychloroquine and chloroquine, both of which are approved to treat malaria and various autoimmune disorders, and might function by disrupting endosome-mediated entry or egress of the virus.[6] Lopinavir-ritonavir are HIV protease inhibitors that are hypothesized to inhibit SARS-CoV $3CL^{pro}$.[7] In addition to small molecule inhibitor candidates, various clinical trials are exploring the effect of known antibody therapies on COVID-19 progression or plan to test antibodies raised against viral proteins.[1]

VIRAL RNA REPLICASE MACHINERY CONTAINS ALTERNATIVE VIABLE TARGETS

The success of remdesivir in clinical trials, although limited, suggests that inhibition of viral replication is a viable strategy for the treatment of COVID-19. Importantly, it takes more than a single polymerase to replicate the viral genome and ensure pathogenicity. SARS-CoV-2 is thought to involve up to nine nsps in the RTC.[2] Key players include the RdRP (nsp12), helicase (nsp13), and proposed polymerase cofactors/primase(s) (nsp 7, 8). To our knowledge, these proteins are not targets in current COVID-19 clinical trials. The viral RTC proteins are expressed in the cytoplasm and so are more accessible that their host counterparts in the nucleus.

Coronavirus helicases are motor proteins necessary for unwinding double-stranded RNA, which form secondary structures, in order for replication and translation to occur. Since viral helicases typically share low homology with human DNA helicases, specific helicase inhibitors are unlikely to be toxic to the host.[8] The amino acid sequence of SARS-CoV-1 helicase is identical to that found in SARS-CoV-2; hence, a solved crystal structure of the SARS-CoV-1 helicase provides opportunities for rational drug design.[9] The Lupoli and Zhang laboratories (NYU, Chemistry) are currently using this structure to design small molecule ligands for computationally predicted binding pockets. In addition to a nucleotide binding site, the SARS-CoV helicase contains a zinc binding domain with three zinc fingers, and mutation of residues linking this domain to the helicase core domain were shown to cause a deficiency in nucleic acid unwinding.[9] As a result, there are multiple sites for disruption of helicase activity.

Unique among other RNA viruses, coronaviruses are believed to have two nsps with RNA polymerase activity. The canonical RdRP is primer-dependent for optimal activity, while the second polymerase (nsp8) is hypothesized to be a

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primase.[2] The primary sequences of nsp7, 8 and RdRP are ≥96% identical in SARS-CoV-1 and CoV-2. A solved cryoelectron microscopy structure of the SARS-CoV-1 RTC complex has shown that interactions between RdRP, nsp7 and 8 are essential for activity.[10] Interestingly, the main RdRP also has an N-terminal kinase-like domain that may carry out nucleotidyltransfer reactions. Disruption of this site or any member of this complex represents a novel opportunity for chemical interference.

Inhibition of helicase or primase activities has been pursued as an antiviral approach in the past and has been reviewed in detail elsewhere.[8] Importantly, herpes simplex virus (HSV) helicase UL5 is a member of the same helicase super family 1 (SF1) as SARS-CoV-2 nsp13. In 2002, Boehringer-Ingelheim[11] and Bayer[12] independently reported on inhibitors of the HSV helicase−primase complex that were comparative to approved antivirals for HSV. Boehringer-Ingelheim's aminothiazoylphenyl-based inhibitors of HSV helicase−primase, namely, BILS 179 (Figure 2, compound **1**), exhibit nanomolar antiviral

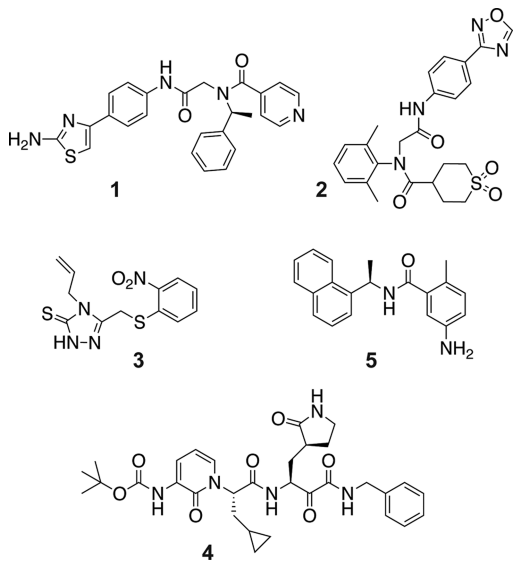

Figure 2. Select chemical scaffolds for viral target disruption. Existing viral helicase inhibitors (**1**−**3**),[8,11,14] including the SARS-CoV-1 helicase binder SSYA10-001 (**2**) and viral protease inhibitors (**4**−**5**).[15,17]

activity and are selective for HSV infection in mouse models. The clinically used amenamevir (**2**), another herpes antiviral, also targets in the helicase−primase at nanomolar concentrations.[8] The most promising inhibitor of SARS-CoV-1 helicase, SSYA10-001 (**3**), which shows micromolar activity against SARS-CoV-1 and MERS-CoV infections in vitro, was awarded a US patent in 2014.[13,14] Given the conservation of helicase sequences in SARS-CoV-1 and CoV-2, this inhibitor will likely serve as a useful starting point for future design of coronavirus helicase modulators.

NOVEL STRATEGIES FOR TARGETING VIRAL PROTEASES

Although many host proteases are deemed essential for late-stage processing of proteins translated from viral RNA, viral 3CL^{pro} (also called M^{pro}) and PLP have emerged as popular targets for COVID-19. HIV protease inhibitors lopinavir and ritonavir, included in the SOLIDARITY trial despite mixed reviews in the clinic, have been predicted to bind SARS-CoV-1 and CoV-2 3CL^{pro} (96% sequence identity) based on computational studies.[7] While HIV proteases are aspartic proteases, coronavirus proteases are members of the cysteine protease family. Given that approximately a quarter of human proteases are predicted to belong to this class, design of specific viral inhibitors remains a challenge. Fortunately, to aid in the rational design of inhibitors, a crystal structure of SARS-CoV-2 3CL^{pro} was reported recently.[15] As part of this work, peptidomimetic compounds (α-keto amides), previously designed against β- and α-coronaviruses, were modified for stability. The most promising mimetic (**4**) inhibited SARS-CoV-2 replication in human lung cells at micromolar concentrations and therefore requires further improvement. Carboxamide and acetamide scaffold structures have been shown to inhibit 3CL^{pro} of SARS-CoV-1, along with other inhibitors that have been reviewed elsewhere.[16] Another viable viral target, PLP, shows lower sequence conservation between SARS-CoV-1 and CoV-2 (76% homology). Notably, PLP possesses additional activities beyond polypeptide processing, such as deubiquitination and cleavage of the ubiquitin-like modifier, ISG15. These PLP-dependent activities contribute to viral immune evasion in the host. Inhibitors of PLP-mediated ISG15 cleavage activity, such as compound **5**, have EC$_{50}$ values of ∼10 uM against SARS-CoV-1 infection in cell culture with no associated cytotoxicity.[17] Structural information and the noted unique features of SARS-CoV cysteine proteases can be exploited for selective inhibition, but existing scaffolds still require optimization to disable SARS-CoV-2 infections in the host.

PEPTIDE-BASED APPROACHES FOR MEMBRANE FUSION INHIBITORS

The S proteins of SARS-CoV-1 and CoV-2 share 76% overall sequence identity, yet the receptor binding domain (RBD) of the latter has 10−20-fold higher affinity to the human ACE2 receptor protein.[18] After the RBD binds ACE2, two heptad repeat domains, HR1 and HR2, interact to form a six-helical bundle, and in so doing bring the viral and host membrane proximal to one another resulting in fusion. This fusion process is essential for viral entry. Design of "coiled coil" heptad repeat-based peptides provides an avenue for drug design that can easily be tailored to respond to mutations that will arise in future S proteins. Using a recently solved crystal structure of the HR1 and HR2 domains of the SARS-CoV-2 S protein, lipidated peptide fusion inhibitors have been designed that inhibit pseudovirus infection of cells with IC$_{50}$ values in the single-digit nanomolar range.[19] Targeting membrane fusion with peptide-based inhibitors offers opportunities and flexibility for entry inhibition in future coronavirus strains, but the cost and possible side effects may be a deterrent for widespread global use.

CONCLUSIONS

We envision that in the coming months and years, there will be three phases of therapeutic intervention for COVID-19: (1) repurposing of existing drugs as treatments and prophylactics; (2) the development of vaccines; and, (3) as the threat of a mutated virus looms large, the development of an arsenal of compounds that are available against other targets to sustain a defense against widespread infection. The reservoir of targets and available compounds against this and related viruses

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described in this perspective is a resource that medicinal chemists are encouraged to explore further.

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Notes

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ABBREVIATIONS

ACE2, angiotensin-converting enzyme 2; COVID-19, Coronavirus Disease 2019; SARS-CoV, Severe Acute Respiratory Syndrome Coronavirus; S protein, spike glycoprotein; nsp(s), nonstructural protein(s); RTC, RNA replication-transcription complex; RdRP, RNA-dependent RNA polymerase; PLP, papain-like protease; $3CL^{pro}$, chymotrypsin-like protease; HR, heptad repeat; RBD, receptor binding domain; WHO, World Health Organization; FDA, Food and Drug Administration

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