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Review

Current Perspective of Antiviral Strategies against COVID-19

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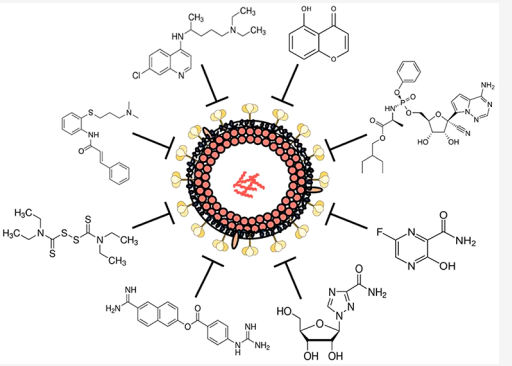
ABSTRACT: COVID-19 was declared a pandemic by the World Health Organization on March 11, 2020. This novel coronavirus disease, caused by the SARS-CoV-2 virus, has resulted in severe and unprecedented social and economic disruptions globally. Since the discovery of COVID-19 in December 2019, numerous antivirals have been tested for efficacy against SARS-CoV-2 *in vitro* and also clinically to treat this disease. This review article discusses the main antiviral strategies currently employed and summarizes reported *in vitro* and *in vivo* efficacies of key antiviral compounds in use.



KEYWORDS: *coronavirus, COVID-19, SARS-CoV-2, antiviral, clinical trials, pandemic*

Coronaviruses (CoVs) are a large family of single strand positive-sense RNA enveloped viruses. These viruses which are approximately 60−140 nm in diameter are classified as such because of the crown-like morphology of the virion under electron microscopy as a result of club-shaped glycoprotein projections.[1,2] CoVs infect animals (cats, bats, pangolin, pigs, and camels) as well as humans.[2−5] In humans, these viruses predominantly infect the respiratory system and cause diseases ranging from a common cold to more severe pneumonias.[2−5] While infection with HCoV-HKU1, HCoV-OC43, HCoV-NL63, and HCoV-229E CoVs have resulted in mild respiratory disease, more severe CoV-related disease has been caused by both severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV)[3] as well as the novel SARS-CoV-2, which has resulted in the current global pandemic that has effectively changed the world as we know it.[6]

CoVs which have resulted in severe human disease have originated as a result of a spill over from their primary animal host, through an intermediate species and finally into humans. In 2002−2003, the spill over of SARS-CoV occurred when the virus jumped from its bat host into an intermediary palm civet cat host and finally into humans and resulted in 8098 infections and 774 deaths before being successfully contained.[7] In 2012, the MERS-CoV spill over emerged from Saudi Arabia and resulted in 2494 infections and 858 deaths before being contained.[8] Here, the bat was the primary host and dromedary camels the intermediate host. By far though, the most successful CoV for human transmission is the novel coronavirus SARS-CoV-2, which was first identified in Wuhan, China in December 2019.[6] While the primary and intermediary hosts remain the subject of huge debates globally, what is true is that despite its lower mortality rate of 6.8% when compared to SARS-CoV or MERS-CoV, which had mortality rates of 9.6 and 34%, respectively, SARS-CoV-2 has by far been the most successful at human transmission. To date, over 5.9 million people have been infected with SARS-CoV-2 globally, and over 365 000 deaths have been reported, and these numbers continue to rise as the pandemic is still not yet contained.[6] To effectively contain this pandemic, many strategies ranging from social distancing to the development of novel antivirals are being employed. This review provides an in depth review of the current antiviral strategies against COVID-19, the disease caused by SARS-CoV-2, as well as current host-directed therapies that are being used to manage the clinical symptoms of this disease. To effectively understand these strategies, it is important to recapitulate what is known about the general virology of this virus and also how it affects the human host.

GENERAL VIROLOGY OF SARS-COV-2

An understanding of the replication cycle and key genomic elements remains essential for the design and development of

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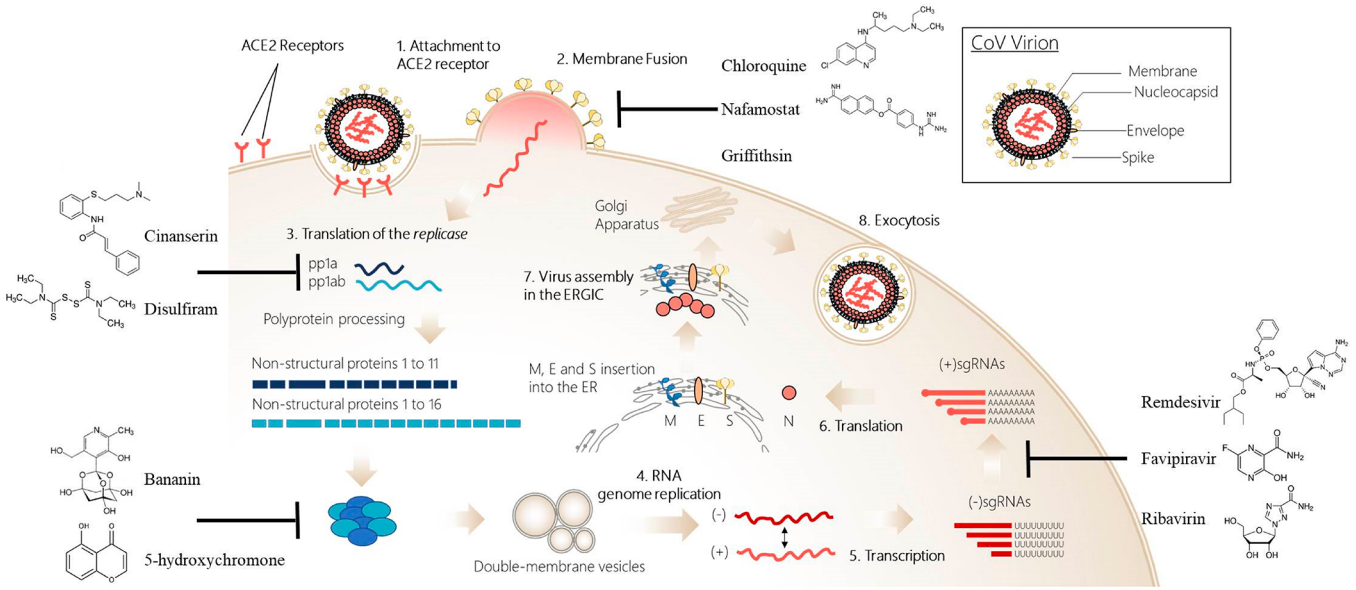

Figure 1. Antivirals targeting the coronavirus replication cycle. The attachment of SARS-CoV-2 virion to the host cell is initiated by the binding between the receptor-binding domain of the S1 subunit of the spike (S) protein and the cellular ACE2 receptor. Compounds present indicate antivirals targeting the various stages of the coronavirus replication cycle. Abbreviations: ACE2, angiotensin-converting enzyme 2; E, envelope; ERGIC, endoplasmic reticulum−Golgi intermediate compartment; M, membrane; N, nucleocapsid; pp1a, polyprotein 1a; pp1ab, polyprotein 1ab; S, spike; sgRNA, subgenomic RNA.

antivirals and vaccines for COVID-19. As with other CoVs, SARS-CoV-2 infections occur mainly through the process of endocytosis upon binding of the virus S1 subunit of the spike (S) protein to the human angiotensin-converting enzyme-2 (ACE2) mammalian cell receptor (Figure 1).[9,10] Given the 89.8% sequence homology of SARS-CoV-2 of the S2 subunit with the SARS-CoV one,[11] it was reasonable to assume that as with SARS-CoV, this subunit played a role in mediating viral surface fusion into mammalian cells through the formation of a six-helix bundle fusion core. Interestingly, structural analyses by Xia and colleagues revealed that, unlike SARS-CoV, the S protein of SARS-CoV-2 possesses a unique S1/S2 furin-recognizable site which plays a key role during the fusion process upon binding of the S1 subunit to the ACE2 receptor.[11] In fact, SARS-CoV-2 results in the formation of syncytium, which is rarely observed during CoV infections, and this could likely be one of the reasons for enhanced viral infectivity and transmissibility of this virus. This site likely allows for the increased capacity of the S protein to mediate infection, as observed in other viruses such as the human immunodeficiency virus (HIV), which possess this syncytium forming ability.[11] Upon receptor binding, the S protein fusion needs to be cleaved using proteases such as furin, trypsin, cathepsins, and transmembrane protease serine 2 (TMPRSS2) to facilitate the membrane melding process, a step referred to as "priming".[10,12] Hoffmann and colleagues found that SARS-CoV-2 recruits the host serine protease TMPRSS2 for this S protein priming, and they speculate that the unique S1/S2 furin-recognizable site may promote virus TMPRSS2-dependent entry into primary cells.[13−15] This finding was confirmed by Ou and colleagues, who found that TMPRSS2 activation did indeed induce receptor-dependent syncytium formation. In addition to this, they discovered that lysosomal cathepsin L was required for fusion activation and also essential for early SARS-CoV-2 endocytosis with the phosphoinositide enzyme phosphatidylinositol 3-phosphate 5-kinase (PIKfyve) and the two pore channel subtype 2 (TPC2), both of which are believed to play a key role in endosomal trafficking and vesicular fusion.[10] Interestingly, they also found that the SARS-CoV-2 S protein may be able to trigger syncytium formation in the absence of priming and speculate that this additional mechanism may also explain the rapid transmission of COVID-19 globally.[10]

Upon entry and uncoating of the virus, it is believed that, as with the other CoVs, SARS-CoV-2 dissembles intracellularly to release the nucleocapsid and viral genomic RNA into the cytoplasm for the translation of 5′-proximal open reading frames of the viral genome (ORF1a and ORF1b) and synthesis of two large replicase polyproteins (pp1a and pp1ab).[16] Contrary to the usual rules of translation of ORF1b, a C-terminally extended form of pp1a (pp1ab) is produced via ribosomal frameshifting. Here, the translating ribosome shifts in the −1 direction from ORF1a reading frame into the ORF1b reading frame.[17] The adoption of this ribosomal frameshifting is thought to regulate the ratio of pp1a and pp1ab products.[18] The pp1a and pp1ab proteins are then autoproteolytically cleaved by ORF1a-encoded proteases to produce 16 nonstructural proteins (nsps). The nsp3 papain-like protease PLpro processes the nsp1−4 polyproteins.[19] The remaining cleavage sites are processed by the nsp5 protease that encodes the 3C-like protease (3CLpro) to produce nsps 5−16.[20] Together, these nsps (e.g., the RNA-dependent RNA polymerase (RdRp) (nsp12) and helicase (nsp13)) form the replication−transcription complex (RTC) that is crucial for viral transcription and replication. Following this, the CoV RTC hydrophobic domains attach to the limiting membrane derived from the endoplasmic reticulum (ER) producing CoV replication structures, such as double-membrane vesicles as well as convoluted membranes in the perinuclear region of the infected cell.[21] The formation of the RTC facilitates CoV replication and RNA synthesis of a nested set of subgenomic RNAs, which encodes for several structural and accessory

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Table 1. Clinical Symptoms Associated with COVID-19 Disease Globally

	China[31−41]		Korea[42]		Netherlands[43]		United States[44]	
symptoms	occurrence	%	occurrence	%	occurrence	%	occurrence	%
fever (pyrexia)	2343/2963	79.08	7/28	25.00	46/86	53.49	7/12	58.33
pneumonia	1548/2101	73.68	22/28	78.57			7/12	58.33
cough (pertussis)	1520/2963	51.30	8/28	28.57	66/86	76.74	8/12	66.67
fatigue	535/1488	35.95	3/28	10.71			5/12	41.67
acute respiratory distress syndrome	205/577	35.53						
expectoration	112/351	31.91						
general malaise	47/168	27.98			65/86	75.58		
sputum production	507/1819	27.87	6/28	21.43				
acute liver injury	101/437	23.11						
acute cardiac injury	101/478	21.13						
secondary infection	81/444	18.24						
muscle soreness (myalgia)	438/2963	14.78	7/28	25.00	54/86	62.79		
RNAaemia	6/41	14.63						
shortness of breath (dyspnoea)	362/2482	14.59	1/28	3.57	33/86	38.37	1/12	8.33
chest tightness	50/381	13.12						
acute renal injury	67/543	12.34						
sore throat (pharyngitis)	303/2597	11.67			34/86	39.53	1/12	8.33
headache	297/2560	11.60	7/28	25.00	49/86	56.98	3/12	25.00
pharyngalgia	32/291	11.00						
chills	160/1480	10.81					1/12	8.33
confusion	9/99	9.09						
chest congestion	16/198	8.08						
acute respiratory injury	8/99	8.08						
loss of appetite	18/232	7.76			15/86	17.44		
breathing problems	31/417	7.43						
shock	3/41	7.32						
runny nose (rhinorrhea)	51/833	6.12	6/28	21.43	46/86	53.49	1/12	8.33
nasal congestion	94/1684	5.58	8/28	28.57				
dizziness	24/459	5.23						
diarrhea	142/2963	4.79	3/28	10.71	16/86	18.6	1/12	8.33
GI symptoms (nausea, vomiting, abdominal pain)	100/2209	4.53	1/28	3.57	5/86	5.814	1/12	8.33
pharyngeal discomfort	7/168	4.17						
septic shock	4/99	4.04						
dry throat	5/168	2.98						
chest pain	9/312	2.88			25/86	29.07		
arthralgia	2/168	1.19						
anhelation	2/168	1.19						
ventilator-associated pneumonia	1/99	1.01						
hemoptysis	13/1308	0.99						
conjunctival congestion	9/1099	0.82						
pharyngeal congestion	1/168	0.60						
altered or loss of taste					6/86	6.977		
other					17/86	19.77		

proteins.[22] The full-length positive-strand genomic RNA is then transcribed into a full-length negative-strand template allowing for the synthesis of new genomic RNAs and also overlapping subgenomic negative-strand templates. Subgenomic mRNAs are subsequently synthesized and translated to produce four structural and six accessory proteins. Following translation, virus assembly quickly ensues with the insertion of the membrane (M), envelope (E), and S proteins into the ER.[23] These proteins migrate along the secretory pathway into the endoplasmic reticulum−Golgi intermediate compartment (ERGIC).[24,25] There, the helical nucleocapsid formed by the assembly of the nucleocapsid (N) and genomic RNA multimerizes and interacts with the other structural proteins to form mature viral particle buds.[23] Finally, the virion-containing vesicles fuse with the plasma membrane for release by exocytosis.

EFFECT OF SARS-COV-2 ON THE HUMAN HOST

One of the key attributes of a successful pathogen is its ability to survive within a host while still retaining its ability to cause disease. Upon inhalation via aerosolized droplets containing the virus, SARS-CoV-2 binds to the ACE2 receptors of the nasal epithelia.[26,27] This triggers activation of the type I interferon innate immune response and an increase in neutrophils; pro-inflammatory cytokines such as TNF, IL-6, IL-1β, and IL-17 are observed, which ultimately leads to inflammation.[28] Migration of the virus then occurs from the nasal epithelial cells to the lungs utilizing the ACE2 receptors present in blood vessels and alveoli.[29,30] Here, again, the host

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immune system mounts a response to eliminate the virus. In some patients, this cytokine response is severe and results in the formation of a cytokine storm, which has proven to be deadly.[28] If virus containment remains unsuccessful, the virus utilizes the ACE2 receptors of the blood vessels to spread to other organs in the body which possess ACE2 receptors.[29] These include organs such as the heart, liver, kidney, and intestines. The response mounted by the host to combat the virus infection in all of these organs is primarily what results in the pathology and symptoms observed in COVID-19 patients globally (Table 1). In most patients, the median time of incubation period is 4 days, and the median time until development of pneumonia is 3 days after onset. Table 1 summarizes these symptoms reported in 3000 patients across 14 independent studies globally.

CURRENT ANTIVIRAL STRATEGIES AGAINST SARS-COV-2

Numerous antiviral strategies are currently being employed against SARS-CoV-2 and to date over 400 clinical trials registered in ClinicalTrials.gov. While this review is not exhaustive in the description of all currently available antivirals against SARS-CoV-2, the main antiviral strategies currently employed can broadly be divided into two types: strategies directly targeting the virus and strategies indirectly targeting the virus via host modulation. Table 2 lists the main compounds discussed in this review and includes their respective EC50 and IC50 values.

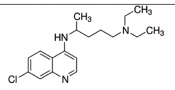
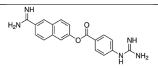
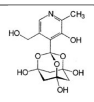
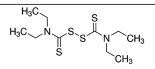
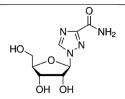
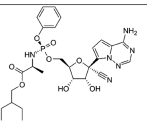
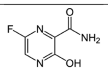
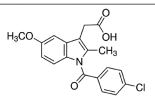
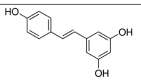
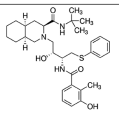
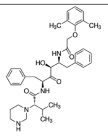
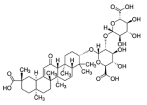
Virus-Directed Therapies. Virus-directed therapies essentially target the eight main steps in the CoV replication cycle (Figure 1). These include drugs that target viral entry, fusion, helicases, proteases, replication, and translation.

Viral Entry Inhibitors. The main inhibitor of viral entry currently being studied is chloroquine, and to date, 43 clinical trials are currently registered on ClinicalTrials.gov.[58] Chloroquine is a derivative of quinine that has been widely used for decades as a form of treatment and prophylaxis against malaria.[59] In addition to its antimalarial properties, chloroquine has shown broad-spectrum antiviral effects against a diverse range of viruses such as the dengue virus,[60] Zika virus,[61] chikungunya virus,[62] and influenza viruses.[63] It also proved to be an effective antiviral *in vitro* against SARS-CoV when introduced prior to or after the establishment of infection.[45,64] More recently, it displayed similar potent antiviral effects against SARS-CoV-2, with an EC_{50} = 1 μM and CC_{50} > 100 μM.[46]

The broad-spectrum antiviral effects of chloroquine can be attributed to multiple potential mechanisms of action. Chloroquine is able to inhibit a pre-entry stage of SARS-CoV viral replication cycle by interfering with the binding of viral particles to their respective cellular surface receptors.[65] Treatment with chloroquine has been shown to inhibit quinone reductase 2, an enzyme involved in the biosynthesis of sialic acids, which are acidic monosaccharides that are crucial components for ligand recognition.[66] This inhibition subsequently resulted in a deficit in the glycosylation of ACE2, ultimately preventing viral binding and infection.[64] Chloroquine is also able to interfere with another early stage of the virus replication cycle, namely the pH-dependent endosome-mediated entry of various enveloped viruses. The presence of chloroquine induces an elevation of the endosomal pH, thus preventing the fusion of viral envelope and the host endosomal membrane, a process that is usually mediated by acidification of the endosome.[62] Given that the viral entry of CoVs into the host cell cytoplasm is also mediated by pH-dependent steps,[67] this could well serve as another mechanism by which chloroquine inhibits CoV infection.

While the success of using chloroquine in the clinic has been largely debated,[65] preliminary results from two clinical trials

Table 2. Compounds Exhibiting Antiviral Properties against CoVs[a]

Compound	Chemical Structure	Virus	EC_{50} (μM; unless stated otherwise)	CC_{50} (μM; unless stated otherwise)	Reference
Chloroquine		SARS-CoV	9 (SARS-CoV)	261 (SARS-CoV)	45
		SARS-CoV-2	1 (SARS-CoV-2)	>100 (SARS-CoV-2)	46
Nafamostat		MERS-CoV	N/R (MERS-CoV) 23	N/R (MERS-CoV)	46
		SARS-CoV-2	(SARS-CoV-2)	>100 (SARS-CoV-2)	46
Griffithsin	-	SARS-CoV	0.05	N/R	47
Bananin		SARS-CoV	< 10	390	48
5-hydroxychromone		SARS-CoV	N/R	N/R	49
Cinanserin		SARS-CoV	25	N/R	50
Disulfiram		SARS-CoV	N/R	N/R	51
		MERS-CoV	N/R	N/R	46
Ribavirin		SARS-CoV	1110	>400	46
Remdesivir		SARS-CoV	0.07 (SARS-CoV)	>10 (SARS-CoV)	52
		MERS-CoV	0.07 (MERS-CoV)	>10 (MERS-CoV)	46
		SARS-CoV-2	0.7 (SARS-CoV-2)	>100 (SARS-CoV-2)	46
Favipiravir		SARS-CoV-2	62	>400	46
Indomethacin		SARS-CoV	50	N/R	53
Resveratrol		MERS-CoV	N/R	N/R	51
Nelfinavir		SARS-CoV	0.05	15	54
Lopinavir		SARS-CoV	N/R	N/R	55
Glycyrrhizic acid		SARS-CoV	100 μg/ mL	N/R	56
Interferon α	-	SARS-CoV	4950-6500 IU/ mL	>10000 IU/ mL	57
Interferon β	-	SARS-CoV	95-105 IU/ mL	>10000 IU/ mL	57
Interferon γ	-	SARS-CoV	1700-2500 IU/ mL	>10000 IU/ mL	57

[a]N/R = not reported.

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have demonstrated the efficacy of chloroquine in reducing SARS-CoV-2 viral load in most patients.[68,69] Caution, however, is still recommended to be exercised as safety data from the use of chloroquine for other diseases has shown that chloroquine can cause severe cardiac ECG QT prolongation and arrhythmias and also prolong QT correction.[70] While most of the SARS-CoV-2 trials specifically excluded patients who were at risk of QT prolongation,[69] two studies which did not raised safety concerns in two COVID-19 trials where an increase in QT prolongation has been observed.[71,72] These findings are currently being reviewed, and it is expected that a final decision on the safety aspects will be made known soon.[73]

Viral Fusion Inhibitors. As discussed above, SARS-CoV-2 enters the host via membrane fusion of the viral envelope and host membrane through the mediation of the S protein and human ACE2 receptor. Using a dual split proteins reporter assay which allows for analysis of membrane fusion, Yamamoto and colleagues performed a high-throughput screen for small molecule inhibitors of MERS-CoV membrane fusion. From this screen, they identified the serine protease inhibitor nafamostat as a potent inhibitor of this S protein-mediated membrane fusion.[54] Further in-depth studies suggested that the antiviral mechanism of nafamostat was mediated via the suppression of the TMPRSS2[74] and that this drug was an effective inhibitor of MERS-CoV infection[74] and also SARS-CoV-2 with an IC_{50} = 23 μM and CC_{50} > 100 μM.[46] Currently, the RACONA trial has been registered to test whether nafamostat can lower lung function deterioration and reduce disease severity.[58]

Another notable inhibitor of CoV protein fusion is the antiviral protein griffithsin. Originally isolated from the red algae *Griffithsia* sp., griffithsin was initially shown to inhibit HIV infection by binding to oligosaccharides on the surface of the viral envelope glycoprotein gp120. Interestingly, while griffithsin does not affect the interaction between the SARS-CoV S protein and the ACE2 receptor, griffithsin exhibits potent antiviral activity against SARS-CoV *in vitro* (IC_{50} = 48 nM)[47] by binding directly to the S protein via interaction with its oligosaccharide moieties.[47] Treatment of SARS-CoV infected mice with griffithsin was extremely promising. All mice treated with a single 10 mg/kg dose survived viral challenge, whereas only 30% of control animals survived. Improved disease outcomes such as prevention of weight loss and improvement of lung pathology were correlated with a reduction in pulmonary viral titers.[47] These potent antiviral effects observed from both *in vitro* and *in vivo* studies therefore suggest that griffithsin warrants further investigation as a potential prophylactic or therapeutic for COVID-19.

Viral Helicase Inhibitors. Bananins and their derivatives are a class of adamantanes expressing a trioxa-adamantane moiety covalently bound to a pyridoxal derivative that have been identified as inhibitors of the SARS-CoV nsp13 region which encodes for a helicase. Bananins have been shown to interfere with nsp13 unwinding and ATPase activities.[75] Four out of the six members of this class of compounds (bananin, iodobananin, vanillinbananin, and eubananin) have proven to be potent inhibitors of viral helicase activity and are capable of blocking the ATPase activity of the nsp13 (IC_{50} = 0.5−3 μM), with bananin exhibiting antiviral activity against SARS-CoV infected cells (IC_{50} < 10 μM, CC_{50} = 390 μM).[48] Given that adamantane derivatives such as amantadine are currently used clinically as antivirals against influenza A, bananins and their derivatives could represent a novel class of compounds with notable therapeutic agents against SARS-CoV-2.

Another group of compounds that has been reported to exhibit SARS-CoV antihelicase activity through inhibition of ATPase activity are 5-hydroxychromone and its derivatives.[49] While *in vitro* antiviral effects of this class of compounds still remain untested, these could be evaluated as potential as therapeutic agents against SARS-CoV-2.

Viral Protease Inhibitors. 3C-like Protease Inhibitors. The viral 3C-like protease, encoded for by the nsp5 region on the viral genome, is primarily responsible for the proteolytic cleavage of the viral nsp involved in replication from the replicase precursor polyproteins.[76] Given its indispensable role in the CoV replication cycle, the 3C-like protease remains a desirable target for the development of antivirals against SARS-CoV-2 and other CoVs. Cinanserin, a serotonin receptor antagonist, was identified by Chen and colleagues as a potential binding molecule to the catalytic pocket of the SARS-CoV 3C-like protease.[50] This binding resulted in the inhibition of the proteolytic activity of recombinant SARS-CoV 3C-like protease, and cinanserin treatment was shown to significantly reduce SARS-CoV replication (IC_{50} = 25 μM).[50] Although cinanserin has shown no notable side effects in humans when given for short periods of time, prolonged treatment with a high dosage level was associated with the development of hepatotoxicity and malignant hepatoma in dogs and rats, respectively.[50,77] Nonetheless, it may be worthwhile to consider cinanserin as a potential lead molecule for further development of 3C-like protease inhibitors to combat CoV infections.

Papain-like Protease Inhibitors. Similar to the CoV 3C-like protease, the papain-like protease is also responsible for the cleavage of nsp. Unlike 3C-like proteases, it cleaves nsp1−3 as opposed to nsp4−16. Aside from its proteolytic functions, the papain-like protease is also able to deubiquitinate or deISGylate host cell proteins such as interferon factor 3 (IRF3), resulting in the inactivation of the NF-κB pathway of activated B cells, eventually resulting in an immunosuppressive effect on host cells.[78,79] Disulfiram, a drug approved by the United States Food and Drug Administration (FDA) for use in alcohol aversion therapy,[80] has been reported to be a potential inhibitor of CoV papain-like proteases. This is likely due to the fact that disulfiram is a thiol-reactive compound capable of covalently modifying cysteine residues, thus interfering or modifying the catalytic cysteine of the papain-like proteases. Lin and colleagues demonstrated that disulfiram exhibited dose-dependent inhibitory effects on both MERS-CoV and SARS-CoV papain-like proteases with IC_{50} values of 15 and 24 μM, respectively.[51] However, while disulfiram showed a noncompetitive inhibition pattern against MERS-CoV papain-like protease, it instead showed a competitive inhibition pattern against SARS-CoV papain-like protease.[51] This is surprising because the two enzymes are similar in structure and possess identical catalytic triads. It would be interesting to study its antiviral properties and activity against SARS-CoV-2.

Viral Genome Replication Inhibitors. Ribavirin. Ribavirin is a purine nucleoside analogue that exhibits broad-spectrum antiviral activity and is primarily utilized for the treatment of respiratory syncytial virus (RSV) infections as well as in combination with interferon α for the treatment of chronic hepatitis C infections.[81,82] The antiviral properties of ribavirin have been attributed to multiple different mechanisms. Due to its structure, ribavirin primarily acts via the inhibition of

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inosine monophosphate dehydrogenase, which is responsible for the synthesis of guanosine triphosphate.[81,82] Other potential mechanisms include the inhibition of the viral polymerase activity via its 5′ triphosphate metabolite, inhibition of viral capping, and lethal mutagenesis of the RNA genome as possible factors contributing to the antiviral effects of ribavirin.[83] Ribavirin has shown antiviral activity against SARS-CoV *in vitro* in fetal rhesus kidney cells (fRHK-4) at concentrations of about 50 μg/mL.[55] Similarly, ribavirin also exhibited antiviral effects against SARS-CoV-2 (IC_{50} = 110 μM, CC_{50} > 400 μM) in Vero E6 cells.[46] Clinical studies with ribavirin, however, have been met with mixed success. A randomized controlled trial of ribavarin in SARS-CoV patients was proven nonefficacious.[84] This could have been because the concentration of ribavirin required to achieve inhibition was difficult to achieve in clinical settings.[55] Despite this, when used as part of a combination therapy with the HIV-1 protease inhibitor lopinavir, the concentration of ribavirin required to inhibit SARS-CoV was reduced to 6.25 μg/mL.[55] As such, ribavirin may still see success when utilized as a part of combination therapies with other antiviral drugs, interferons, or HIV-protease inhibitors.

Remdesivir. Remdesivir (GS-5734) is an adenosine nucleotide analogue that was originally developed for the treatment of Ebola virus infection. As an adenosine analogue, the antiviral mechanism of remdesivir involves the incorporation of the compound into nascent viral RNA chains, resulting in premature termination of viral RNA replication. Remdesivir exhibits *in vitro* and *in vivo* antiviral activity against both SARS-CoV and MERS-CoV.[52] Treatment of SARS-CoV infected mice with 25 mg/kg remdesivir twice daily mitigated SARS-CoV induced weight loss, reduced SARS-CoV-induced lung pathology, and significantly reduced pulmonary viral antigen levels.[52] The antiviral effects of remdesivir against SARS-CoV and MERS-CoV can be attributed to the inhibition of the viral RNA-dependent RNA polymerase (RdRp), as treatment with remdesivir results in mutations within the nsp12 region of CoVs which encodes RdRp.[85] More recently, remdesivir was shown to be effective against SARS-CoV-2 *in vitro* (IC_{50} = 0.77 μM; CC_{50} > 100 μM),[46] and in novel preprint findings posted onto bioRxiv, remdesivir was shown to significantly reduce COVID-19 disease severity and damage to the lungs of rhesus macaques.[86] In individuals with severe COVID-19, while remdesivir did not show clinical benefits, it significantly reduced time to clinical improvement.[87] Because of these favorable outcomes, including in the first COVID-19 patient in the United States,[88] there are currently nine ongoing remdesivir clinical trials,[58] as it remains important to evaluate these the safety and efficacy of this drug in large COVID-19 patient cohorts.

Favipiravir. Similarly to ribavirin, favipiravir is a nucleoside analogue that has been reported to exhibit broad-spectrum antiviral activity. Favipiravir functions as a selective and potent inhibitor of the influenza viral RNA polymerase and has proven efficacious against RNA viruses other than influenza.[89−91] When administered, favipiravir is first converted to an active phosphoribosylated form, which is subsequently recognized as a substrate by viral RNA polymerases and incorporated into the nascent viral RNA chain, ultimately leading to the premature termination of the RNA chain or lethal mutagenesis via ambiguous base-pairing.[89] While an initial study has shown the efficacy of favipiravir against SARS-CoV-2 (IC_{50} = 62 μM, CC_{50} > 400 μM),[46] further studies are still required to further evaluate its potential as an antiviral against SARS-CoV-2. Currently, there are eight clinical trials to assess the efficacy of favipiravir against COVID-19.[58]

Viral Protein Translation Inhibitors. RNA Interference (RNAi). As RNA viruses, CoVs leverage the host cell machinery for translation of their viral genome. As such, RNA interference (RNAi) provides the possibility of interrupting the CoV replication cycle via the use of short interfering RNAs (siRNAs). siRNAs targeting both the S protein and the replicase 1A region of the SARS-CoV genome have been effective in inhibiting viral gene expression and subsequently viral infection and replication.[92,93] Treatment of SARS-CoV infected rhesus macaques with siRNA inhibitors that targeted the viral genome, S region, and nsp12 coding regions of the virus resulted in a significant reduction in viral loads as well as reduced acute diffuse alveolar damage attributed to the SARS-CoV infection.[94]

Host-Directed Therapies for COVID-19. The second strategy for managing and treating COVID-19 includes modulation of the host immune system through a myriad of ways to enhance the innate immune response or reduce inflammation as a result of SARS-CoV-2 infections.[95] These include the use of repurposed vaccines such as the BCG and MMR vaccines as well as novel vaccines that make use of recombinant viruses, virus spike proteins, convalescent plasma, stem cells, and monoclonal antibodies.[58,95] In addition to these, anti-inflammatory, antihypertensive, antifibrinolytic, antidepressants, and use of oxygen therapies, among others, are also being used for the treatment and management of COVID-19.[58,95] The recent article by Tu and colleagues provides a good summary on the ongoing clinical trials,[95] and the article by Thorlund and colleagues provides "a real-time dashboard of clinical trials for COVID-19".[96] Here, we would like to highlight a few of these compounds, including some with no known targets that have shown immunomodulatory activity against SARS-CoV-2.

ACE2 Inhibitors. Given that ACE2 is critical for virus infection, it follows that inhibition of this receptor could be a good antiviral strategy against SARS-CoV-2 infections. Recently, Monteuil and colleagues showed that human recombinant soluble ACE2 (hrsACE2) effectively blocks SARS-CoV-2 infections in a dose-dependent manner in Vero E6 cells as well as human capillary and kidney organoids and is able to significantly reduce viral loads.[30] These findings pave the way for the use of ACE2 as a treatment strategy for COVID-19 to prevent the spread to other organs in the body.

Chloroquine. In addition to its direct antiviral activity discussed above, chloroquine also modulates the host immune system.[65] Specifically, it inhibits p38 mitogen-activated protein kinase (MAPK) as well as caspase-1 to inhibit virus replication and it reduces pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNFα), IL-1, and IL-6 which are significantly raised during the SARS-CoV-2 induced cytokine storm.[65,97,98]

Interferons. Both type I and II interferon treatment have been reported to provide prophylactic protection and therapeutic potential against SARS-CoV replication *in vitro*, with interferon β notably being significantly more efficacious than interferon α and γ with IC_{50} = 95−105 IU/mL and CC_{50} > 10 000 IU/mL.[57] MERS-CoV infected marmosets treated with interferon-β1b experienced less severe disease outcomes and had overall lower mean viral loads in necropsied lung and extrapulmonary tissues when compared to untreated animals.[99]

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Similarly, MERS-CoV infected rhesus macaques treated with a combination of interferon-α2b and ribavirin experienced improved clinical parameters.[100] A clinical trial of severe MERS-CoV patients receiving a combination of ribavirin and pegylated interferon-α2a revealed that this treatment was associated with improved survival at 14 days.[101] While this observation was not sustained over a longer period of 28 days,[101] it is possible that a similar treatment against COVID-19 can lead to improved survival of severe COVID-19 patients as well as rapid recovery of milder COVID-19 cases. There are currently 28 clinical trials to assess the beneficial effects of interferon treatment in COVID-19 patients.[58]

Indomethacin. Indomethacin, a nonsteroidal anti-inflammatory drug (NSAID) that primarily acts as an inhibitor of the cyclooxygenase (COX) family of proteins,[102] has been shown to possess antiviral activity against SARS-CoV (IC_{50} = 50 μM).[53] While treatment with indomethacin did not have any effect on virus infectivity, binding, or entry into host cells, evidence strongly suggests that it acts by inhibiting viral RNA synthesis via a novel pathway independent from the cyclooxygenase pathway.[53] Currently, there is one clinical trial evaluating the efficacy of indomethacin in patients with mild COVID-19 symptoms.[58]

Resveratrol. Resveratrol is a naturally occurring stilbenoid derivative found in a variety of plants and fruits such as grapes (*Vitis vinifera*) and cranberries (*Vaccinium macrocarpon*) that is commonly known for its antioxidant properties.[103] Resveratrol exhibits potent inhibition against MERS-CoV *in vitro* and also significantly reduced viral-induced cell death.[51] It is likely that its anti-CoV activity is as a result of either activation of cellular survival factors and DNA repair in response to DNA damage via activation of the ERK1/2 signaling pathway or prevention of virus-induced apoptosis via down-regulation of FGF-2 signaling. While the exact mechanism still warrants further investigation, the observations suggest that the antiviral effects against CoV were primarily due to the promotion of cellular survival and prevention of virus-induced apoptosis.[51]

HIV-1 Protease Inhibitors. HIV-1 protease inhibitors are another class of small molecules that have also demonstrated the ability to inhibit CoV infection. Nelfinavir and lopinavir have both been reported to inhibit the viral replication of SARS-CoV *in vitro*.[54,55] In fact, HIV-1 protease inhibitors have had favorable outcomes in clinical trials against CoVs, with multiple studies reporting that patients given a formulation of lopinavir combined with ritonavir resulted in a lower overall mortality rate and improved clinical conditions such as respiratory distress.[55,99] As such, there are currently 30 clinical trials assessing the efficacy of lopinavir/ritonavir for COVID-19.[58] Of note are two trials that did not show improved clinical outcomes in COVID-19 patients.[95] However, in MERS-CoV and SARS-CoV clinical trials, patients treated with lopinavir/ritonavir also received ribavirin as part of the standardized treatment protocol. Given the synergistic antiviral effects observed from the combination of ribavirin with HIV-1 protease inhibitors in these cohorts, HIV-1 proteases are not being ruled out,[55] and there are in fact three trials testing the efficacy of these drugs for COVID-19.[58]

Glycyrrhizic Acid and Its Derivatives. Glycyrrhizic acid, a triterpene glycoside, and its aglycone 18β-glycyrrhetinic acid are bioactive compounds derived from the licorice root (*Glycyrrhiza radix*).[104] Both compounds have been reported to have antitumor, anti-inflammatory, and antiviral activity against a wide variety of viruses, including flaviviruses, herpes viruses, and influenza viruses.[105−107] Against CoVs, glycyrrhizic acid was has been shown to inhibit SARS-CoV *in vitro* in Vero (IC_{50} = 365 μM)[108] as well as in Vero E6 cells (IC_{50} = 100 μM),[56] and chemical modifications to the glycyrrhizin backbone further enhanced the potency of its anti-SARS-CoV activity.[109] These antiviral effects of glycyrrhizic acid could potentially be mediated via the upregulation of nitric oxide production.[110] While the exact antiviral mechanism of glycyrrhizic acid still remains undefined, it is tempting to speculate that this NO production is responsible for its efficacy as the latter has been reported to exhibit antiviral activity against SARS-CoV.[108] There are currently eight clinical trials assessing the efficacy of NO in COVID-19, and if these prove effective, then further development of glycyrrhizic acid and its derivatives would be warranted.

Concluding Remarks. The current COVID-19 pandemic is one that will require intense and sustained effort to control. Availability and implementation of antiviral strategies are key for the successful resolution of this pandemic. Despite the fact that the antiviral strategies and drugs discussed in this review are still undergoing efficacy testing, it is heartening to know that there are many novel compounds that are being fed into the drug pipeline at a preclinical stage as well as a number of already approved drugs, including drugs used for other conditions being repurposed for use against SARS-CoV-2. This has of course been made possible because of all the research efforts that were previously made in understanding the basic biology and pathogenesis of SARS-CoV as well as MERS-CoV. With the commitment from all drug discovery sectors, including academia and industry as well as buy in from all governments and international organizations, we are confident that among the over 400 new antiviral strategies being tested, at least one of these will be successful for the treatment of mild, moderate, and severe COVID-19 disease.

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