The emergence of a novel coronavirus (SARS-CoV-2), their biology and therapeutic options

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Abstract

The new decade of the 21st century (2020) started with the emergence of novel coronavirus known as SARS-CoV-2 that caused an epidemic of coronavirus disease (COVID-19) in Wuhan, China. It is the third highly pathogenic and transmissible coronavirus after severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in humans. The source of origin, transmission to humans and mechanisms associated with the pathogenicity of SARS-CoV-2 are not clear yet, however, its resemblance with SARS-CoV and several other bat coronaviruses was recently confirmed through genome sequencing related studies. The development of therapeutic strategies is necessary in order to prevent further epidemics and cure infected people. In this Review, we summarize current information about the emergence, origin, diversity, and epidemiology of three pathogenic coronaviruses with a specific focus on the current outbreak in Wuhan, China. Furthermore, we discuss the clinical features and potential therapeutic options that may be effective against SARS-CoV-2.

KEY WORS: Novel coronavirus; Outbreak; Therapeutics

Introduction

Coronaviruses are enveloped, positive-sense single-stranded RNA viruses with a nucleocapsid of helical symmetry (1). Coronaviruses have widely been known to cause respiratory and intestinal infections in humans after the outbreak of "severe acute respiratory syndrome (SARS)" in Guangdong, China (2, 3). SARS was caused by SARS-CoV during 2002 and 2003, emerged in a market where civets were sold out (2, 3). Only a decade later, the world witnessed another outbreak of "Middle East respiratory syndrome (MERS)" caused by MERS-CoV in the Middle East (4, 5). While the researchers were still investigating the underlying mechanisms of pathogenicity and developing effective therapeutic strategies against MERS, the world witnessed the deadliest outbreak of COVID-19 (6). The causative coronavirus of this outbreak was named SARS-CoV-2 due to its resemblance to SARS-CoV (7–9). The SARS-CoV infects ciliated bronchial epithelial cells and type-II pneumocytes through angiotensin-converting enzyme 2 (ACE2) as receptor (2, 10). MERS infects unciliated bronchial epithelial cells and type-II pneumocytes by using dipeptidyl peptidase 4 (DPP4) also known as CD26, as a receptor (2, 11). The mechanisms associated with the infectiousness of SARS-CoV-2 is not clear, however, structural analysis suggests it is likely entering human cells through the ACE2 receptor (12). This newly emerged virus has much more similarity with SARS-CoV than MERS-CoV, thus both SARS-CoV and SARS-CoV-2 may cause pathogenesis through similar mechanisms. The transmission of SARS-CoV to humans was reported from market civets, while that of MERS- CoV was from dromedary camels (13, 14). Similarly, the newly emerged SARS-CoV-2 also transmitted to humans form the markets where wild animals were sold out (8). However, the zoonotic source of its transmission is not clear yet. According to the previous reports, the aforementioned three coronaviruses are thought to have originated in bats (2, 11, 15, 16).

Since the first epidemic of SARS, the pathogenic coronaviruses have harmed thousands of people worldwide (1, 17). Considering the adverse outcomes of the current COVID-19 epidemic, developing effective therapeutic strategies is necessary to cope with the lack of effective drugs, high mortality rate and the potential of the virus to cause further epidemics. In this Review, we focus on the origin, evolution, and pathogenicity of SARS- CoV, MERS- CoV, and SARS-CoV-2. We also discuss the therapeutic options for SARS-CoV-2, due to its importance in the current scenario of COVID-19 outbreak in Wuhan, China. This review will be

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useful in terms of preparation against future spillover and pathogenic infections with novel coronaviruses in humans.

Diversity and origin of highly pathogenic coronaviruses

Coronaviruses are members of the subfamily "Coronavirinae" (family; Coronaviridae, order; Nidovirales) that contains four genera alpha-coronavirus, beta-coronavirus, gamma-coronavirus and delta-coronavirus (2). Gamma and delta coronaviruses generally infect birds, although some of them can cause infection in mammals. Whereas, alpha and beta coronaviruses are known to harm humans and animals. The SARS-CoV (beta coronavirus), 229E (alpha coronavirus), HKU1 (beta coronavirus), NL63 (alpha coronavirus), OC43 (beta coronavirus) and MERS-CoV (beta coronavirus) can cause infectiousness in humans (2). However, beta-coronaviruses are the most important group as this group contains the highly pathogenic viruses in humans including SARS-CoV-2, MERS-CoV and SARS- CoV (2, 18, 19). The highly pathogenic MERS and SARS coronaviruses originated form bats (2, 18, 19), however, the origin of the newly emerged SARS-CoV-2 is debatable. Investigations revealed that the detected SARS-CoV strains in market civets (20, 21), were transmitted from horseshoe bats (22). These viruses were found phylogenetically related to SARS-CoV in bats from China, Europe, Southeast Asia and Africa (2, 22, 23). In addition, the genome sequences of SARS-CoV isolated from humans were very much similar to those in bats (21). However, some variations were found among the *s* gene and *orf* gene, which encode the binding and fusion proteins, and dispensable protein for replication respectively (2, 23). Nevertheless, clade2 of *s* region (22, 24), *orf8* (23) and *orf3b* in SARS-CoV from bats contain major variations if compared with SARS-CoV from humans (23).

Different strains of MERS-CoV obtained from camels were found similar to those isolated from humans (14, 25, 26), except, genomic variations among S, ORF4b and ORF3 regions (26). Furthermore, genome sequencing-based studies revealed that MERS-CoVs from humans are phylogenetically related to those form bats. They have identical genomic and protein structures except for the S proteins (27). In addition recombinations analysis of genes encoding orf1ab and S revealed that MERS-CoV originated from the exchange of genetic elements between coronaviruses in camels and bats (26, 28).

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Although the zoonotic source of SARS-CoV-2 is not confirmed, its genome sequence exhibited close relatedness (88% identity) with two bat-derived SARS-like coronaviruses (bat-SL-CoVZC45 and bat-SL-CoVZXC21). Phylogenetic analysis revealed that SARS-CoV-2 was genetically distinct from SARS-CoV and MERS-CoV. However, homology modeling revealed that both SARS-CoV and SARS-CoV-2 had similar receptor-binding domain structures, despite amino acid variation at some key residues. Such as the absence of 8a protein and fluctuation in the number of amino acids in 8b and 3c protein in SARS-CoV2 (29). In contrast, the main protease is highly conserved between SARS-CoV-2 and SARS-CoV with a 96% overall identity (30). These observations suggest that bats are the source of origin, while an animal sold at the Wuhan seafood market might represent an intermediate host facilitating the emergence of the virus in humans (12, 31).

Epidemiology and clinical features of human coronaviruses

After the emergence of SARS-CoV in the Guangdong province of China, it rapidly spread around the globe (2, 3). During November 2002, an epidemic of pneumonia with a high rate of transmission to the people, occurred in Guangdong, China (32), followed by subsequent outbreaks in HongKong. In HongKong, a total of 138 people contracted the infection within 2 weeks after the exposure to an infected patient in the general ward of a hospital (1, 32). Overall, SARS-CoV infected 8098 people and caused 774 fatalities in 29 different countries by the end of the epidemic (1). Later on, during June 2012 a patient infected by MERS-CoV developed severe pneumonia and died in Jeddah, Saudi Arabia (1, 33). Analysis of cluster of nosocomial cases in Jordan during April 2012, confirmed that MERS-CoV caused the outbreak (34). The spread of MERS-CoV continued beyond the Middle East, causing further reports of infected individuals (1, 4). Until 2020, 2468 cases and 851 fatalities have been reported globally (35, 36). Again, during December 2019, clusters of patients with atypical pneumonia were reported by local health facilities, in Wuhan, China. On December 31, 2019, a rapid response team was dispatched by the Chinese Center for Disease Control and Prevention (China CDC) to conduct an epidemiologic and etiologic investigation (37). The patients were found epidemiologically linked to the wet animal wholesale market and seafood in Wuhan, China. Later on, the infectious agent responsible for this atypical pneumonia was confirmed reported a coronavirus SARS-CoV-2, which caused the first fatality during the start of January 2020 (15). During the first two 6 weeks of the outbreak, several cases were reported in more than 37 countries including the USA, Japan, Iran and South Korea (38). The infection rapidly spread all over the globe

from Wuhan, China. Therefore, the Chinese authorities implemented several strategies including massive lockdown in Wuhan and suspension of transport to and from Wuhan to control the spread (17). According to situation report 35, published on the WHO website, SARS-CoV-2 caused 79331 confirmed cases and 2618 deaths around the globe. However, COVID-19 caused 77262 confirmed cases and 2595 deaths only inside Mainland China (38). Until February 24, WHO has reported 8 deaths in Iran. It is now the second country after Chian, bearing the highest fatalities due to SARS-CoV-2 infection. (38). The spread of SARS-CoV-2 in Iran can pose a higher risk of pandemics in the Middle East and South Asian countries. The epidemic growth rate on the basis of data analyzed between December 10 and January 4, was estimated and the basic reproductive number (R_0) calculated, which was 2.2. It means that each patient has been spreading the infection to 2.2 other individuals (39). The estimated R_0 value for SARS was around 3, however, SARS was successfully controlled by isolation and of patients (39). Moreover, The R_0 for MERS ranged from 0.45 in Saudi Arabia to 8.1 in South Korea (36). Considering the lower R_0 value, the rapid increase in suspected as well confirmed cases with COVID-19 may be inferred with viral transmission through the fecal-oral route and aerosol formation. Moreover, the asymptomatic persons are thought to be potential sources of SARS-CoV-2 infection (40), which may have caused the rapid spread of SARS-CoV-2. This asymptomatic spread may be one of the reasons that the control strategy based on the isolation of patients was not fully successful. To overcome these problems a complete quarantine for the general public is necessary. So that all of the infected individuals could develop symptoms without spreading the virus randomly. Thus the direct and indirect contacts of infected individuals can be easily identified and isolated.

Clinical features associated with patients infected with SARS-CoV, MERS-CoV and SARS-CoV-2 range from mild respiratory illness to severe acute respiratory disease (1, 17). Both MERS and SARS patients in later stages develop respiratory distress and renal failure (1, 17). Pneumonia appears to be the most frequent manifestation of SARS-CoV-2 infection, characterized primarily by fever, cough, dyspnea, and bilateral infiltrates on chest imaging (17). The period from infection to appearance of symptoms varies. Generally, it is thought to be 14 days, however, a research group at Guangzhou Medical University reported the incubation period to be 24 days. In a family cluster of infections, the onset of fever and respiratory symptoms occurred approximately three to six days after presumptive exposure (41).

Diagnostic testing

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Diagnostic testing for the SARS-CoV-2 is primarily done in public health laboratories. Delays in testing result from the need for administrative oversight of testing at the national or regional level, as well as the time needed to transport specimens and the high volume of testing needed in some regions. More rapid testing should widely available in days of epidemics. High-level testing facilities at the regional hospital and commercial laboratories are needed, in addition to the commercially available tests that have undergone regulatory approval. Several tests have been validated by public health authorities, including those in China, Germany, Thailand, Japan and the United States (WHO, COVID-19, technical guidance, Feb 12, 2020). These tests are reverse-transcriptase PCR tests that use primers and probes designed to detect a variety of targets in the SARS-CoV-2. Although these have been designed and validated, there is currently very limited information available related to the performance of these tests. The sensitivity and specificity of the tests are not widely known, and some of them might detect other coronaviruses such as SARS-CoV. In addition, the utility of different specimen types for detection of the viruses is not known. As a result, testing of multiple specimen types is recommended by some agencies, including the CDC (CDC, guidelines for samples for COVID-19, Feb 11, 2020). The availability of serological tests is unclear, and presumably, such tests are in development. Moreover, the collection and submission of sera from potentially infected patients is recommended by some public health laboratories.

The CDC and WHO have both issued recommendations for laboratory safety when testing specimens from patients suspected of being infected with SARS-CoV-2 (WHO, document Laboratory biorisk management for laboratories handling human specimens suspected or confirmed to contain novel coronavirus 2012, Interim recommendations and CDC, guidelines for samples for COVID-19, Feb 11, 2020). Both guidelines recommend that manipulation of potentially infectious specimens should be done in a biosafety cabinet if there is potential for splashes or generation of droplets or aerosols. Viral isolation (culture) should be done only in BL-3 laboratories. Testing in chemistry and hematology laboratories can be done following routine laboratory precautions recommended for such work.

Therapeutic options for human coronaviruses

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Currently no promising antiviral treatment available, however, numerous compounds have been proven effective against SARS-CoV and MERS-CoV but have not been tested widely for newly emerged SARS-CoV-2. Remdesivir and chloroquine were found highly effective in vitro for the control of 2019-nCoV infection (42). Treatment with remdesivir alone or in combination with chloroquine or interferon beta was found effective against COVID-19 infection. This strategy has not caused any obvious side effects yet (35, 42, 43). However, more investigations are necessary to confirm the impacts of remdesivir. As coronaviruses share key genomic elements thus, common therapeutic targets can be of greater importance. Therapeutic agents targeting nucleosides, nucleotides, viral nucleic acids and enzymes/proteins involved in the replication and transcription of coronaviruses can be promising strategies to treat coronavirus diseases (1). The surface spike glycoprotein (S) is an important potential target for anti-viral agents, due to its vital role in the interaction between the virus and the cell receptor. S consists of two subunits, S1, the amino-terminal receptor binding subunit, and S2, the carboxy-terminal membrane fusion subunit (44). In addition, Activation of membrane fusion and virus entry requires the cleavage at the junction of S1–S2 (44). Hence, the S1 subunit targeting monoclonal antibodies and S2 subunit targeting fusion inhibitors may be effective therapeutics to target coronaviruses (1). Furin (a serine endoprotease) cleaves off S1/S2 (45), thus, could be a suitable antiviral agent. Further, the helical nucleocapsid interacts with S protein, envelope proteins, and membrane proteins to form the assembled virion (1). Therefore targeting the structural genes using small interfering RNAs could be an effective therapeutic strategy against coronaviruses (1). The host receptors are also associated with the viral entry into host cells, thus agents targeting these receptors also inhibit coronaviruses (44). Inhibitors of endosomal cysteine protease and transmembrane protease serine 2 can partially block viral entry into the cell (46). K22 targets membrane-bound RNA synthesis in coronaviruses and inhibit double-membrane vesicles formation (47) thus could be effective against SARS-CoV-2.

Broad-spectrum antivirals, for instance, dsRNA-activated caspase oligomerizer (DRACO) selectively induces apoptosis in virus-containing host cells, thus can be evaluated for its effectiveness against SARS-CoV-2 (48). However, it may not be a very promising strategy alone, as it cannot block the virus from entry or disrupt the viral nucleic acid. On the other hand thiopurine compounds, naphthalene inhibitors, protease inhibitors, zinc, and mercury conjugates target 3CLpro (3C-like protease) and PLpro (papain-like protease) enzymes in coronaviruses and

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can block the pathogenicity of coronaviruses (49, 50). Therefore, combinational therapy of these antiviral agents with DRACO may enhance the overall impact on the recovery of patients. Despite the higher rate of infectiousness, coronaviruses are thought to have the ability to suppress counteracting response from host innate interferons. This response can be augmented by the utilization of interferon inducers or recombinant interferons (1). The previously tested recombinant interferon against SARS-CoV, such as interferon alfa and beta (1) can be utilized either alone or in combination with other potential antiviral drugs including remdesivir. Both interferon-alpha and beta inhibit viral replication (1). The use of interferon inducers in combination effective antiviral agents may be evaluated for their synergistic effects against SARS-CoV-2. In addition, calcineurin inhibitors such as cyclosporine (51) could also be evaluated for SARS-CoV-2 in combination with antibiotics and traditional Chinese medicines.

Vaccines

Effective vaccines are important to prevent and control sporadic viral attacks and emerging virus-mediated epidemics, such as the recent outbreak caused by SARS-CoV-2. Although SARS-CoV was fully controlled during 2003, and MERS-CoV has been controlled from causing high mortalities, yet the newly emerged SARS-CoV-2 is spreading efficiently with a significant increase in the number of cases and fatalities each passing day. Vaccines are required to prevent SARS-CoV-2 from causing COVID-19. Live-attenuated vaccines, designed for SARS (1), may be evaluated for SARS-CoV-2 infected patients. In addition rhesus θ-defensin 1 and protein cage nanoparticles are innate immunomodulators with high anti-SARS-CoV efficiency (52, 53). Based on the higher similarities and phylogenetic relatedness between SARS-CoV and SARS-CoV-2, protein cage nanoparticles designed for SARS-CoV can be evaluated for SARS-CoV-2. Meanwhile, following the similar strategies utilized for SARS-CoV, novel protein cage nanoparticles specified for novel coronavirus can be designed on an urgent basis. Based on the urgency in the current scenario of COVID-19 outbreak, vaccination strategies based on viral vectors, recombinant protein, and viral-like particles, which have been developed or being developed for SARS and/or MERS can be modified for utilization against SARS-CoV-2 (54). Despite the current progress, further work is needed to develop safe and effective vaccines, available for individuals at high risk of SARS-CoV-2 endemics, to control the ongoing and risk of future epidemics. An interesting feature of plasma from recovered patients is

the presence of active antibodies, thus transferring plasma from people recovered from COVID-19 into infected individuals could enhance immunity against SARS-CoV-2. Monoclonal antibodies that could inhibit virus-cell receptor binding, and interrupt virus-cell fusion have been developed (1). Combining two or more monoclonal antibodies may be suitable for the quicker recovery of patients. Lastly, antiviral peptides that target different regions of S such as, HP2P-M2 peptide (effective against viral infections) (1), should also be considered against COVID-19.

Although some strategies against SARS-CoV are being developed including RBD based vaccines, they need further evaluation (2). Given the importance of the current outbreak in Wuhan, further studies are necessary to provide deep understating of replication, pathogenesis, and biological properties using reverse genetics and related molecular techniques. These investigations will help the control and prevention of SARS-CoV-2 mediated pneumonia disease and novel emerging diseases in the future.

Conclusions and Perspective

SARS-CoV and MERS-CoV were reportedly originated in recombination from bats viruses, before their introduction into Guangdong province through civets, and the middle east through camels respectively. Some of the SARS-CoV strains became epidemic after several independent spillovers to humans during the outbreak of 2002 in Guangdong, China (3). Similarly, MERS-CoV became endemic after a series of infections to humans during 2012 in middle eastern countries (33). Both viruses further transferred to several countries other than countries of origin. However, unlike the continuous propagation of MERS-CoV epidemics, the SARS-CoV outbreak was successfully controlled in 2003. Based on the origin of other coronaviruses, SARS-CoV-2 is likely originated in bats and introduced to Wuhan, China through an unknown intermediate. Until now, no effective clinical treatments or prevention strategies are available to be used against human coronaviruses.

Testing the drugs for coronaviruses requires suitable animal models prior to their use in humans. The currently established models are not very promising for the studies of pathogenesis and treatment of highly pathogenic coronaviruses. For instance, non-human primates were unable to reproduce the characteristics of the severe human disease and even mortality was not observed (55). However, some of the small animals developed the clinically apparent disease

(55), such as transgenic mice expressing human ACE2 and mouse-adapted SARS-CoV strains are one of the most suitable models (1). Additionally, transgenic mice expressing human DPP4 are a suitable small animal model for MERS (1). Like the animal models for SARS-CoV, transgenic animal models may also be standardized for SARS-CoV-2. The development of clinical drugs for coronaviruses is challenging because of the repeated emergence of novel coronaviruses with diverse features, thus, each newly emerged virus requires specific drugs. Moreover, only a limited number of animal models are available and most of them can only be used in highly demanding biosafety level 3 labs (1). From the perspective of the current outbreak, designing effective therapeutics for SARS-CoV-2 is yet another challenge for scientists. Although a large number of antiviral treatment options for SARS and MERS have been reported with potent in vitro activities, a very limited number from them may have the potential in the clinical setting.

Now moving forward treatment options are available that could be utilized clinically during the ongoing SARS-CoV-2 epidemics. Some of the broad-spectrum antiviral drugs may be effective for SARS-CoV-2, and it is a congenial opportunity to test them in the current scenario of pneumonia in Wuhan, China. Broad range combinational therapies including, lopinavir and interferon antiviral peptides can also be evaluated and examined as these agents have shown significant effects against MERS in non-human primates (1). The designing and development of novel broad-spectrum antiviral drugs that can potentially target all coronaviruses, in general, maybe the only treatment option against reemerging and circulating coronaviruses.

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499 Table 1. Therapeutic options for COVID-19

Therapeutic Name	Activity	Effectiveness	Reference
K22	It targets membrane-bound replication complex of virus in host cell to inhibit RNA synthesis	It has been found effective against SARS and MERS, thus could be effective against SARS-CoV-2.	(56)
Draco	It targets viral dsRNA to induce apoptosis in cells containing virus	It has been found effective against a large group of viruses, therefore may have potential to target SARS-CoV-2.	(49, 57)
Mycophenolic Acid	It targets Nucleosides and/or nucleotides to inhibit synthesis of guanine monophosphate	Effective against wide range of viruses, however combinatorial therapy with interferon beta-1b may be useful for SARS-CoV-2.	(54)
Lopinavir	It targets 3CLpro enzyme to inhibit its activity	Effective against wide range of viruses including SARS-CoV and MERS-CoV, thus could be suitable choice for treatment of SARS-CoV-2 infection.	(58)
Remdesivir	It terminates transcription of the viral RNA transcription at premature level	It has been found effective against broad spectrum viruses including MERS-CoV and SARS-CoV. The efficacy is	(35)

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		very promising when combined with IFNb, hence could be a suitable therapeutic strategy for SARS-CoV-2.	
Ribavirin	It targets RdRp (RNA-dependent RNA polymerase) enzyme to inhibit synthesis of viral RNA synthesis and capping of mRNA	Effective against wide range of viruses including MERS-CoV and SARS-CoV but high doses are required which may have severe side effects. It may be reevaluated for SARS-CoV-2 and recommended if low doses are found effective.	(35, 58)
Bcx4430	It targets RdRp (RNA-dependent RNA polymerase) enzyme to inhibit synthesis of viral RNA synthesis and capping of mRNA	It is broad-spectrum and effective against SARS-CoV and MERS-CoV, thus may be effective against SARS-CoV-2 however, evaluation using animals' models is required.	(59)
Bananins	It targets helicase to Inhibit its unwinding and activities of ATPase	It can affect broad-spectrum viruses and can be evaluated for SARS-CoV-2	(1)
Aryl Diketoacids (Adks)	Targets helicase to inhibit its unwinding	Effective for broad range viruses and including SARS/MERS-CoV and may be a suitable choice for SARS-CoV-2	(1)

Griffithsin	It targets Oligosaccharides on S to block viral binding with host cell	It has been found effective against SARS/MERS-CoV and other high pathogenic viruses, thus can be used against SARS-CoV-2	(60)
Hexamethylene Amiloride	It targets viral envelope to inhibit ion channel activity	It is effective against different coronaviruses thus a one of the most suitable treatment options for SARS-CoV-2	(61)
Jl103	It targets lipid membrane and causes modification of phospholipids	It has shown effects against different viruses and may be promising anti-SARS-CoV-2 agent.	(1, 58)
Recombinant Interferons	These induce the innate interferon responses against viral pathogens	Inducing immune responses through recombinant interferons has been found effective against a wide range of viruses and can be the most suitable option in case of SARS-CoV-2.	(1, 58)
Nitazoxanide	These induce the innate interferon responses against viral pathogens	Inducing immune responses through recombinant interferons has been found effective against a wide range of viruses and may be promising to use against SARS-CoV-2.	(62)

Cyclosporine, Alisporivir	It inhibits cyclophilin to affect calcineurin–NFAT pathway	These agents can inhibit broad-spectrum viruses specifically coronaviruses and thus could be suitable option to treat people infected with SARS-CoV-2.	(1)
Rapamycin	It inhibits kinase signaling associated pathways to block viral entry	It is effective against SARS/MERS-CoV and possibly be effective against SARS-CoV-2.	(1, 63)
Imatinib	It inhibits kinase signaling associated pathways to block viral entry	It is effective against SARS/MERS-CoV and possibly be effective against SARS-CoV-2.	(1)
Dasatinib	It inhibits kinase signaling associated pathways to block viral entry	It is effective against SARS/MERS-CoV and possibly be effective against SARS-CoV-2.	(1)

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