Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Approaches and advances in the development of potential therapeutic targets and antiviral agents for the management of SARS-CoV-2 infection

Gayathri Krishna [1], Vinod Soman Pillai [1], Mohanan Valiya Veettil [*]

Virology Laboratory, Department of Biotechnology, Cochin University of Science and Technology, Cochin, 682022, Kerala, India

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ABSTRACT

Virus onslaughts continue to spread fear and cause rampage across the world every now and then. The twenty first century is yet again witnessing a gross global pandemic, Coronavirus Disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Globally no vaccines or drug specific to COVID-19 is available. Corona viruses have been in mutual relationship with humans and other hosts over many decades though aggressive zoonotic strains have caused havoc. Zoonotic emergent corona viruses prior to SARS-COV-2 included severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), with the former leading to aggressive infectious spread and the later with high mortality rate. Although they emerged in the early period of the twenty first century, resilient biomedical and expertise in pharmaceutical domain could not appropriate any proprietary therapeutics. Studies envisaged towards curtailing their spread employed different stages of the virus life cycle with all zoonotic coronaviruses (CoVs) sharing genomic and structural similarities. Hence the strategies against SARS-CoV and MERS-CoV could prove effective against the recent outbreak of SAR-CoV-2. The review unravels key events involved in the life-cycle of SARS-CoV-2 while highlighting the possible avenues of therapy. The review also holds the scope in better understanding a broad-spectrum antivirals, monoclonal antibodies and small molecule inhibitors against viral glycoproteins, host cell receptor, viral mRNA synthesis, RNA-dependent RNA polymerase (RdRp) and viral proteases in order to design and develop antiviral drugs for SARS-CoV-2.

1. Introduction

Since the beginning of the twenty-first century, corona viruses have succeeded in their adaptive potential by traversing through host barrier to cause deadly diseases in humans. So far, they have evolved into three emerging viruses of zoonotic origin, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). SARS-CoV, which was identified as the cause of severe acute respiratory syndrome (SARS) outbreak, was reported in the year 2002 in China, and within a short time it infected 8089 people from five continents to claim 774 lives (de Wit et al., 2016). Effective control measures facilitated in containing SARS-CoV by 2004 with no new reported cases till date. Saudi Arabia witnessed the emergence of Middle East respiratory syndrome (MERS), a viral respiratory syndrome caused by MERS-CoV, in 2012 spreading across 27 countries and killing 858 out of 2500 affected persons (de Wit et al., 2016). Though both SARS-CoV and MERS-CoV caused severe respiratory disease in humans with similar disease progression, the mortality rate of MERS-CoV was 35%, which was much greater than a 10% mortality rate with SARS-CoV (Petrosillo et al., 2020).

The emergence of a novel corona virus which was later named SARS-CoV-2 was first reported in the city of Wuhan, China during December 2019. SARS-CoV-2 infection causes coronavirus disease 2019 (COVID-19) which is predominantly characterized by respiratory distress of varying severity with a fatality rate of about 3%. By January 2020, the World Health Organization (WHO) declared that it is a Public Health Emergency of International Concern. Remarkably, SARS-CoV-2 and SARS-CoV is associated with milder infection and rapid transmission in the community, compared to MERS-CoV (Munster et al., 2020).

Precise vaccination or treatment is currently unavailable for CoVs though several therapeutics have been identified successfully in cell lines and animal models. Moreover, symptomatic treatments and control measures alone cannot dampen the severity of diseases or future virus

* Corresponding author.
E-mail addresses: mohanwiwi@gmail.com, mohanan.veettil@cusat.ac.in (M.V. Veettil).
[1] Both authors contributed equally.

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spill overs. The lessons learnt from previous SARS-CoV and MERS-CoV outbreaks reveal a realm of research emphasizing the requirement to develop effective therapeutic measures against SARS-CoV-2. In this review, we describe various treatment modalities for SARS-CoV and MERS-CoV and the scientific avenues that could be employed for the development of drugs towards prevention and control of SARS-CoV-2 infection and COVID-19. This review also draws attention to prospects and challenges likely to be confronted by the scientific and biomedical community during the development of therapeutics for SARS-CoV-2.

2. Origin of coronaviruses

Bats are often considered to be the primary reservoir of all zoonotic CoVs (Cui et al., 2019; Lau et al., 2005; Li et al., 2005b). Civets and camels serve as intermediate hosts for SARS-CoV and MERS-CoV respectively (Gong and Bao, 2018). SARS-CoV-2's similarity to SARS-like bat CoVs potentiates a possible transmission route of SARS-CoV-2 from bats (Zhou et al., 2020). As SARS-like bat CoVs cannot directly infect humans, mutational changes in an intermediate host is inevitable. Reports on the similarity between S1 protein of Pangolin-CoV and SARS-CoV-2 has suggested a possibility of pangolins as intermediate host for SARS-CoV-2, though further evidences are required to prove this concept (Zhang et al., 2020) (Fig. 1).

From a phylogenetic point of view, SARS-CoV-2 shares 96.2% genomic similarity with SARS-like bat CoV while SARS-CoV and MERS-CoV are 79% and 50% identical to SARS-CoV-2, respectively (Ren et al., 2020). This suggests an unequivocal origin of SARS-CoV-2 from bats, as in the outbreaks of SARS-CoV and MERS-CoV. However, similar to SARS-CoV, the possibility of human transmission in SARS-CoV-2 is assumed to be from an animal market in China, though the probability of an intermediate host remain unsubstantiated (Walls et al., 2020b). Though studies report similar genomic structures among these CoVs, they differ in exploiting receptors to enter host cell. MERS-CoV uses dipeptidyl peptidase 4 (DPP4) while SARS-CoV and SARS-CoV-2 shares the same receptor angiotensin-converting enzyme 2 (ACE2) for cellular entry with latter having higher affinity to receptor (Wan et al., 2020).

3. SARS-CoV-2

3.1. Structure and replication

SARS-CoV-2 is a spherical virus possessing a positive sense RNA genome of 29.9 kb in size (Wu et al., 2020c). CoVs possess 15 non-structural proteins which are essential for viral replication and four structural proteins, spike (S) glycoprotein, small envelope (E) protein, matrix (M) protein and nucleocapsid (N) proteins (Cui et al., 2019). The RNA genome along with N proteins form the nucleocapsid which is surrounded by an envelope. The M proteins provide shape to the virus whereas the heavily glycosylated S proteins used for cellular entry are embedded on the surface of the virus like a crown, hence the name coronavirus (Siu et al., 2008).

Receptor recognition plays a significant role in cell tropism and forms the primary step of any viral infection. During entry of the virus to human cells, the S glycoprotein of SARS-CoV-2 attaches to the cellular receptor human angiotensin-converting enzyme 2 (ACE2), which is a single pass transmembrane protein found to be expressed in lung, heart,

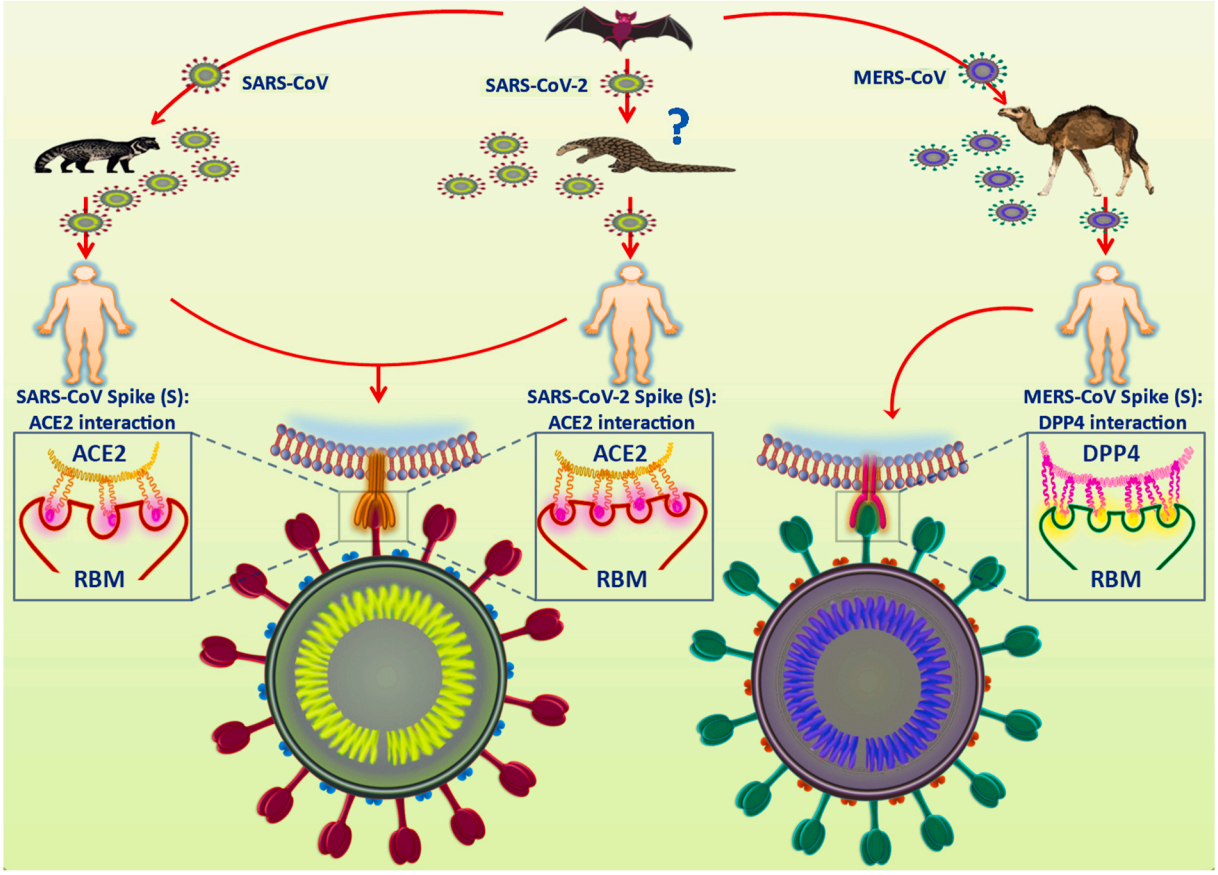

Fig. 1. Zoonosis of corona viruses. Bats are the primary reservoirs for the three zoonotic corona viruses, SARS-CoV, SARS-CoV-2 and MERS-CoV. Human transmissions often occur through intermediate hosts. Civets and dromedary camels serve as intermediate hosts for SARS-CoV and MERS-CoV, respectively while intermediate host for SARS-CoV-2 is yet to be confirmed but are anticipated to be pangolins. For cellular entry, the receptor binding domain (RBM) of SARS-CoV S protein interacts with the host ACE2 receptor. SARS-CoV-2 also utilizes the same RBM-ACE2 receptor interaction but possesses a greater binding affinity compared to SARS-CoV. The entry of MERS-CoV is mediated by the interaction of RBM with the host receptor DPP4.

kidney, intestine and testis (Tipnis et al., 2000). The S protein comprises of 2 functional domains, S1 and S2, responsible for receptor binding and membrane fusion respectively (Zhang et al., 2014). Receptor binding domain (RBD) on the S1 domain binds to ACE2 receptor, a major factor influencing the tropism of virus (Kuo et al., 2000). Studies have reported six RBD amino acids to be critical for binding in SARS-CoV, of which none has been reported to be substituted in SARS-CoV-2, however few other amino acids in the RBD have been modified in SARS-CoV-2 (Wu et al., 2020a). Structural analysis confirmed the binding of SARS-CoV-2 with ACE2 at 10 folds greater affinity compared to SARS-CoV which clarifies the efficient transmission of SARS-CoV-2 in humans (Wrapp et al., 2020). Immediately after binding, a conformational change is triggered in S2 domain exposing the fusion peptide mediating the virus-cell membrane fusion eventually delivering the capsid into the cytoplasm (Masters, 2006) (Fig. 2).

Once inside the cytoplasm, the viral RNA uncoats and translates into polyproteins pp1a and pp1ab that encodes non-structural proteins (Fang et al., 2008). Subsequently, polyproteins pp1a is cleaved into 11 non-structural proteins (nsp1-nsp11) while 15 non-structural proteins (nsp1-nsp10 and nsp12-nsp16) are produced due to the cleavage of pp1ab. nsp3 and nsp5 regulate the autoproteolytic cleavage while RNA-dependent RNA polymerase (RdRp) is positioned within nsp12. Mutations in nsp2 and nsp3 may affect the infectious ability and differentiation mechanism of SARS-CoV-2 (Fung and Liu, 2014). In addition to translating polyproteins, the viral RNA also acts as a template to synthesize negative sense genomic RNA which is further used as template to produce positive sense RNA genomes. On the other hand, subgenomic RNAs are produced as a result of non-contiguous transcription of the genome which are later translated into structural proteins (Sawicki et al., 2007). During non-contiguous transcription, RdRp is expected to jump from one template to another resulting in CoV recombination which can play a significant role in virus evolution (Wu and Brian, 2010). The viral assembly occurs in the endoplasmic reticulum-Golgi compartment of the host cell, and the virions are transported to the plasma membrane via vesicles to ultimately release the mature viruses (Masters, 2006; Siu et al., 2008).

3.2. Diagnosis

With the continuous spread of COVID-19 in a tremendous rate, the most important challenge faced by the global health community is rapid and early detection of SARS-CoV-2 positive cases. Various diagnostic methods employed to detect the presence of SARS-CoV-2 are nucleic acid detection tests, immunological tests and imaging techniques. Of which, detection of viral RNA using real time reverse transcriptase PCR (RT-PCR) is considered the most accurate and reliable diagnostic test often conducted using nasopharyngeal or upper respiratory tract swabs (Sethuraman et al., 2020). S, E, N, RdRp/ORF1ab genes of SARS-CoV-2 are the different targets used for conducting real-time PCR (Corman et al., 2020; Tang et al., 2020). For the primary screening of positive

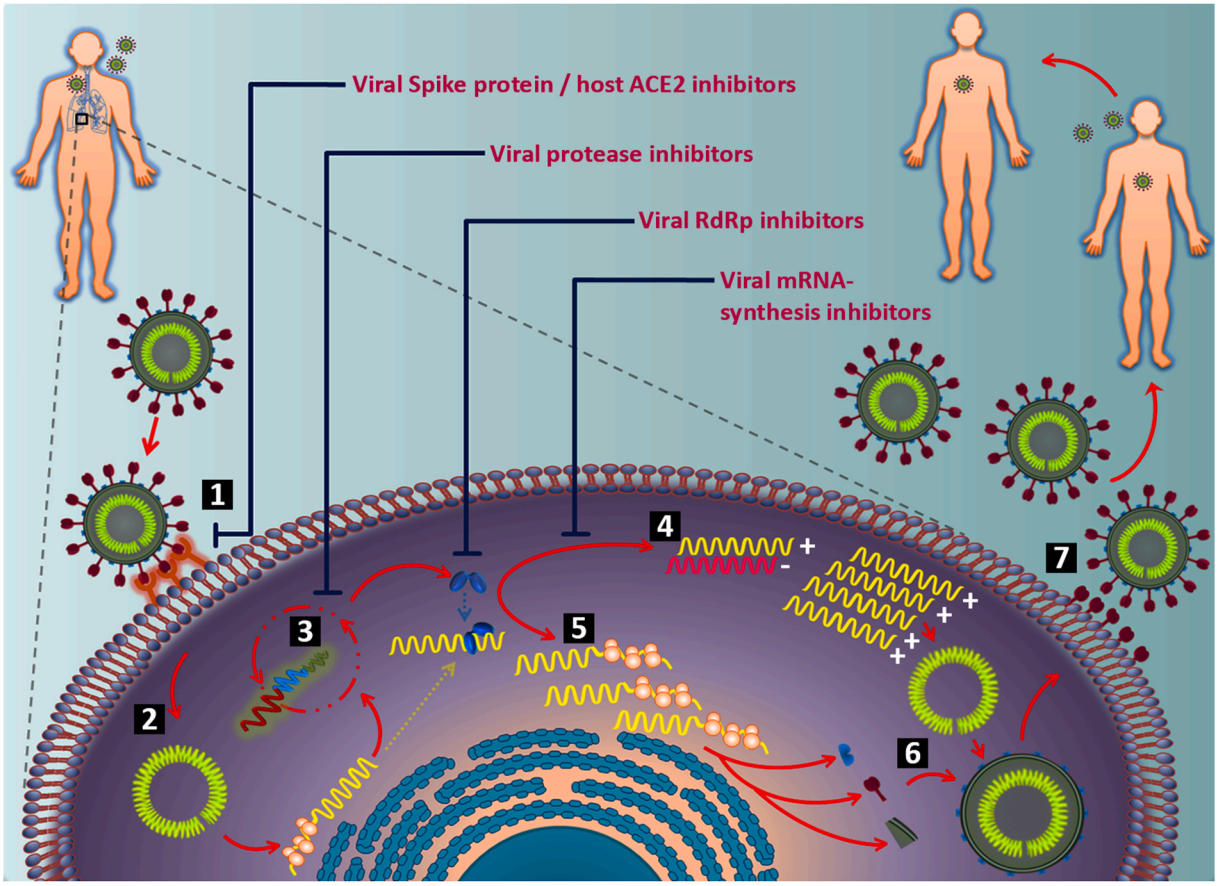

Fig. 2. Schematic diagram of SARS-CoV-2 life cycle and potential antiviral drug targets based on life cycle. SARS-CoV-2 life cycle comprises several stages. 1) Attachment of the viral S glycoprotein RBM with cellular receptor ACE2 to facilitate entry into the host cell. 2) Disassembly of SARS-CoV-2 to release the RNA into the cytoplasm of the host cell. 3) The viral RNA is translated into replicase polyproteins, which are further cleaved by the viral proteases, papain like protease (PL^{pro}) and 3C like protease ($3CL^{pro}$) to produce non-structural proteins (nsps). 4) The viral RNA also acts as a template for synthesis of negative sense RNA which subsequently converts to positive sense genomic RNAs. 5) RdRp, one of the nsps, get involved in non-contiguous transcription to produce subgenomic RNAs which are consequently translated into the viral structural proteins. 6) The RNA genome and structural proteins assemble to form new virions. 7) Subsequently mature virions are released from the host cell. Bar-headed lines and red fonts indicate potential antiviral drug targets-viral spike protein/host cellular receptors, protease inhibitors, RdRp inhibitors, and mRNA synthesis inhibitors-that can block different stages of viral life cycle.

cases, WHO recommends to test the target E with subsequent confirmation with RdRp primers as two target assays are most preferred for efficient diagnosis (Corman et al., 2020). Though considered the gold standard in COVID-19 tests, false negatives may occur due to sampling errors or untimely sample collection during a real-time PCR assay (Sethuraman et al., 2020).

Immunological test is an indirect detection method by measuring the antibodies generated due to the host immune response against SARS-CoV-2 infection. Studies have reported the presence of viral specific Immunoglobulin G (IgG) and Immunoglobulin M (IgM) in high levels during the second and third week after infection (Sethuraman et al., 2020). Furthermore, serological diagnosis based on IgG and IgM enzyme-linked immunosorbent assay (ELISA) are reported to have high specificity for detection of COVID-19 (Xiang et al., 2020). However these test are qualitative and can only show the presence or absence of virus specific antibodies but is often considered an important tool to study the prevalence of COVID-19 and spread rate in a community. Even though real-time PCR is the most reliable method of detection, radiological evaluation like the chest computed tomography (CT) scan is also considered a standard diagnosis method of COVID-19 for pneumonia detection (Li and Xia, 2020). However, CT scan is limited to detect any specific viral disease but has proved necessary to ensure early detection and control of transmission during a pandemic situation.

3.3. Current treatment modalities for SARS-CoV-2

No specific vaccines or anti-viral drugs are discovered for the control of SARS-CoV-2 as yet. Therefore, symptomatic treatment is the mainstay of clinical handling at present. Though a number of anti-viral drugs such as remdesivir, arbidol, lopinavir/ritonavir and many more are available for the treatment of SARS-CoV-2, none of them are considered the most potential therapeutics till date (Jin et al., 2020; Wang et al., 2020a).

Remdesivir, an adenosine analogue, has shown anti-viral activity against many RNA viruses including Ebola, SARS-CoV and MERS-CoV (Lo et al., 2019; Sheahan et al., 2017; Warren et al., 2016). It interferes with the viral RdRp and integrates into the nascent viral RNA resulting in premature termination. In addition, it was reported to effectively restrain SARS-CoV-2 *in vitro* (Wang et al., 2020a). However, its efficacy and side effects in patients need to be substantiated by clinical trials. Arbidol, an indole derivative broad spectrum anti-viral, affects various stages of viral life cycle, particularity targeting virus associated cellular host molecules or viral proteins (Blaising et al., 2014). Arbidol blocks the viral fusion process in influenza virus whereas it inhibits viral attachment and vesicle trafficking in hepatitis C virus (Blaising et al., 2013; Kadam and Wilson, 2017). Similarly, *in vitro* studies have also reported arbidol's activity to interfere with attachment and vesicular trafficking in SARS-CoV-2 potentiating its candidature for the treatment of COVID-19 though *in vivo* studies and clinical trials are yet to be accomplished (Wang et al., 2020b). An additional candidate used for the treatment of COVID-19 is a combination of HIV protease inhibitors, lopinavir and ritonavir. They have reported to bind on the target site of M protease (M^{Pro}) to supress its activity in SARS-CoV. Treatment with lopinavir and ritonavir could also improve the condition of marmosets infected with MERS-CoV (Chan et al., 2015; Yao et al., 2020). Moreover, they were found to be effective on COVID-19 patients, validating them as potential drug candidates though their potency need to be validated by clinical trials (Lim et al., 2020). Chloroquine, an anti-malarial drug that increases endosomal pH is used as a treatment option against COVID-19. It is reported to increase the endosomal pH required for virus-cell membrane fusion and also interrupts with the glycosylation of host cell receptors of SARS-CoV (Savarino et al., 2003). Moreover, chloroquine also holds promise as an autophagy inhibitor along with its reported anti-tumor properties (Golden et al., 2015). In Vero-E6 cells, chloroquine functioned at both entry, and at post-entry stages of the SARS-CoV-2 infection categorizing its role as a potent COVID-19 drug (Wang et al., 2020a).

4. Research scope

In an era of emerging novel viruses, the process of developing anti-viral drugs is complex yet is of paramount importance for sustenance of mankind. Adversely, the complexity worsens as viruses with lower mortality or comorbidities evolve and re-emerge to elude current therapeutic strategies as observed in the case of SARS-COV-2. Since the discovery of first antiviral drug, a few novel drugs were established to be therapeutically effective and safe but none reckoned for the treatment of CoVs (De Clercq and Li, 2016). Developing antiviral drugs include strategies like screening of existing therapeutic molecule databases, prevailing broad-spectrum antivirals or even *de novo* synthesis of drugs by harnessing the viral genomic characteristics (Zumla et al., 2016). Systematic analysis have identified significant and potential antiviral targets against SARS-COV-2 like viral spike protein (S), host cellular ACE2 receptor, viral genomic RNA, moieties included in viral mRNA synthesis like the RdRp, replication complex and viral proteases (Wu et al., 2020). Furthermore, many antiviral drugs and small molecules have been proven to block SARS-CoV and MERS-CoV in preclinical studies, while their treatment potency are argued due to meagre results from human clinical trials. Considering the structural and genomic similarity of SARS-CoV-2 to SARS-CoV and MERS-CoV, repurposing the existing drugs may imply a practical solution to ramp down the recent pandemic outbreak. Numerous novel possibilities can be envisaged to prevent and treat COVID-19, such as viral glycoprotein and viral receptor targeted drugs and antibodies, small molecule inhibitors, siRNAs, viral mRNA and replicase targeted drugs, viral protease targeted drugs, and vaccines. However, novel therapeutic interventions entail a prolonged period of time for screening and development.

4.1. Anti-viral drugs targeting viral glycoproteins and viral receptors

A promising target for the treatment of COVID-19 is the viral S glycoprotein. The most variable part of S protein is the RBD domain which is essential for interaction with ACE2 (Wu et al., 2020c; Zhou et al., 2020). Peptide drugs targeting SARS-CoV-2 specific RBD domains could possibly block the RBD-ACE2 interaction during SARS-CoV-2 infection. Consistently, a polypeptide containing two RBD-binding motifs of ACE2 displayed strong inhibitory effects on SARS pseudo virus entry into HeLa cells expressing ACE2 with an IC50 of ~100 μM (Han et al., 2006).

The polybasic cleavage site at the junction of S1 and S2 domains of S protein constitutes another notable target for anti-viral agents as effective cleavage at this site determines the infectivity of SARS-CoV-2 (Walls et al., 2020a). Reports on synthetic peptides derived from cleavage site sequences exhibited inhibitory action against the GZ50 strain of SARS-CoV infection in fetal rhesus kidney cells (Zheng et al., 2005). Therefore, synthesis of such peptides specific to the SARS-CoV-2 polybasic cleavage site can impede the production of functional S1 and S2 domain eventually blocking the fusion of virus-host membranes.

The heptad repeat (HR) domains, HR1 and HR2 in the S2 domain form a six helix bundle fusion core which supports membrane fusion by bringing the cellular and viral membranes into close vicinity. (Gao et al., 2013). During the fusion process, three conformational stages are believed to occur in the CoV HR motifs: pre-fusion native state, a pre-hairpin intermediate state, and a stable post-fusion hairpin state (Eckert and Kim, 2001). The formation of six helix bundle fusion core represents the post-fusion hairpin state. However, prior to this state, both HR1 and HR2 should probably be exposed in an intermediate state where they could function as target for anti-viral therapeutics (Eckert and Kim, 2001). Based on this theory, a number of inhibitory peptides have been designed to stop viral infections of HIV, Ebola and SARS-CoV (Bosch et al., 2004; Shi et al., 2016; Watanabe et al., 2000). One of such peptides that overlapped HR2 could bind and interact with HR1 to form a stable six helix fusion core thereby inhibiting SARS-CoV infection in Vero E6 cells (Liu et al., 2004). A peptide derived from HR2 domain of

human coronavirus OC43 (HCoV-OC43) demonstrated fusion inhibition property against many HCoVs (Xia et al., 2019). Nevertheless, these peptides showed poor anti-viral activity compared to the anti-HIV peptides, the reason was attributed to the capability of SARS-CoV S protein HR2 region to form the trimeric coiled-coil which was absent in other class I viral fusion proteins (Du et al., 2009). However, increasing the binding affinity of HR2 peptide with HR1 to form six helix bundle fusion core while reducing the formation of trimer could escalate the antiviral efficiency. Additionally, SARS-CoV-2 exhibits much higher capacity of membrane fusion than SARS-CoV, suggesting the fusion machinery of SARS-CoV-2 as potential target (Xia et al., 2020). In a recent study, a lipopeptide EK1C4, derived from EK1 targeting the HR1 domain was reported to be highly effective against SARS-CoV-2 membrane fusion and pseudovirus infection (Xia et al., 2020). Another group of carbohydrate binding drugs that inhibits SARS-CoV *in vitro* and *in vivo* by binding to the viral surface glycoprotein are Griffithsin isolated from the red alga *Griffithsia* (Barton et al., 2014; O'Keefe et al., 2010). Soluble forms of ACE2 were also demonstrated to block the infection of SARS-CoV with comparable affinities to monoclonal antibodies (Li et al., 2003; Sui et al., 2004). However, to increase the lifespan of such circulating molecule, it is recommended to attach an immunoglobulin Fc domain which alters soluble ACE2 into an immunoadhesin format (Kruse, 2020). Though all the above mentioned peptide drugs are effective in *in vitro*, the *in vivo* anti-viral efficacy should be estimated in animal models for further therapeutic development. In addition to the efficacy, the optimum delivery methods and safety profiles of the above mentioned drugs should also be evaluated.

Another potential approach to curtail COVID-19 would be to target the host receptor, ACE2. A few added advantages of targeting ACE2 are that the host protein cannot alter thereby chances of escaping from therapeutic agent are abated. Furthermore, the capability of SARS-CoV-2 to mutate and bind to a different host receptor during an ongoing outbreak is beyond scope. Peptides drugs corresponding to SARS-CoV-2 RBD domain that can successfully bind to ACE2 is an important therapeutic realm to be studied. It has been shown that SARS-CoV infection in Vero cells were inhibited by an RBD overlapping peptide sequence by hindering RBD-ACE2 interaction at an IC50 of ~40 μM (Hu et al., 2005). Similarly a 193 residue fragment of S protein was reported to block the S protein mediated interaction of SARS-CoV by binding on to ACE2 receptor in cell culture (Wong et al., 2004). Enhanced tissue penetration and effective receptor binding could be accomplished by the production of nanobodies as a therapeutic agent (Arbabi-Ghahroudi, 2017). An rRBD protein attached to Fc fragment was demonstrated to effectively block MERS-CoV infection in MERS-CoV specific receptor expressing cells (Du et al., 2013).

4.2. Antibodies targeting viral glycoproteins and viral receptors

Convalescent plasma (CP) therapy is an adaptive immunotherapy that has been used to prevent and treat many contagious diseases. CP therapy is considered to be a promising treatment strategy for COVOD-19 due to the resemblance of viral and clinical characteristics among SARS-CoV, MERS-CoV, and SARS-CoV-2. The donor source of CP therapy are those patients who have recovered from COVID-19 with high neutralizing antibody titers. CP therapy involves transfer of sera containing anti-SARS-CoV-2 antibodies that can block the virus infection and will aid in viral clearance (Rojas et al., 2020). A study of CP therapy in few severe COVID-19 patients in China, showed a decrease in viremia within seven days of treatment with no adverse effects (Duan et al., 2020). Though the therapy was successful and well tolerated by patients, further investigations regarding optimum dose and time points need to be conducted. Contrastingly, some *in vitro* and *in vivo* studies have demonstrated disease worsening due to treatment conducted with low titre blood products (Weingartl et al., 2004; Yang et al., 2005b).

To tackle these issues, neutralizing antibodies like monoclonal antibodies (mAbs), their functional antigen-binding fragment (Fab), the single-chain variable region fragment (scFv), or single-domain antibodies (nanobodies), targeting various regions of CoVs have been produced by cloning scFv or Fab from convalescent patients, immortalization of B cells from convalescent patients or immunizing human immunoglobulin into transgenic mice (Coughlin and Prabhakar, 2012; Traggiai et al., 2004). mAbs are a dominant approach of therapeutic intervention which is predominantly significant for viruses in which the neutralizing antibody reaction is vital for protection (Berry, 2005). Majority of these mAbs target the S1 subunit to block the RBD-ACE2 binding while some inhibit the membrane fusion by binding to the S2 subunits. Apart from the common method of using mice and other animals for selecting lead molecules, the faster phage or yeast display methods could be employed in the current pandemic situation (Keck et al., 2019; Shin et al., 2019). However, laborious *in vitro* and *in vivo* methods should be performed to confirm the therapeutic efficiency.

Spike protein specific neutralizing human mAbs, 80R and CR3014, inhibited S protein-ACE2 interaction thereby neutralizing the infection by SARS-CoV strains Tor2 and HKU39849 (Sui et al., 2005). Similarly, infection caused by human SARS-CoV strains Urbani, Tor2 and GD03 could also be neutralized by mAbs, m396 and S230.15 (Zhu et al., 2007). Interestingly, CR3014 and m396 were unsuccessful in neutralizing SARS-CoV-2 infections as they failed to bind to the S protein indicating that the cross reactivity seen in mAbs could be due to the difference in the RBD domains of SARS-CoV and SARS-CoV-2 (Tian et al., 2020). Therefore development of mAbs specific for SARS-CoV-2 is crucial in controlling the disease. Moreover, CR3022, a SARS-CoV specific mAb isolated from blood of a convalescent SARS patient was found to interact with the RBD domain of SARS-CoV-2 (Tian et al., 2020). Mouse mAbs are also used for initial and urgent treatment of CoV infections. However repeated use of mouse mAbs can cause human-anti mouse antibody response which subsequently clears the mAb from blood to lessen the therapeutic effect. Mouse mAbs generated using rRBD and inactivated SARS-CoV could inhibit RBD binding and also neutralized pseudoviruses of human SARS-CoV strains Tor2 and GD03T13 (He et al., 2005, 2006). Several MERS-CoV neutralizing antibodies like MERS-27, MERS-GD27, hMS-1, Mersmab1 were reported to recognize RBD epitopes to block infection while SAB-301, isolated from transchromosomic cattle has been evaluated in Phase I clinical trials (Zhou et al., 2019). Apart from isolating mAbs from mice few researchers have used large animals like rhesus macaques, llama and camel for isolating antibodies. Rhesus macaques immunized with combined DNA and protein vaccines isolated a group of mAbs with inhibitory effects that targeted both MERS-CoV RBD and non-RBD S1 region of the S glycoprotein (Wang et al., 2018). Nanobodies targeting RBD isolated from camels immunized with MERS-CoV S protein had potent neutralization capabilities (Stalin Raj et al., 2018). Till now, no SARS-CoV-2 specific antibodies have been reported, though once produced they will have to undergo *in vitro* evaluation for neutralizing capabilities and *in vivo* testing for efficacy and safety prior to the approval for clinical trials.

Another approach to prevent COVID-19 would be to deliver antibodies against ACE2 receptors. ACE2 could be effectively blocked by scFv or nanobodies, however, without Fc domain these molecules show shorter half-life. Furthermore, ACE2 binding antibody would eliminate the concern of viral escape which is an advantage over S protein specific antibodies (Kruse, 2020).

4.3. Small molecule inhibitors and siRNAs targeting viral glycoproteins

Studies on several small molecules targeting S protein have been reported, emphasizing the fact that small molecules and other compounds can be effective in inhibiting SARS-CoV-2. Previously, two small molecules tetra-o-galloyl-beta-d-glucose (TGG) and luteolin were identified to block the entry of SARS-CoV into Vero cells while 18 small molecules were also reported to target the S protein- ACE2 mediated viral entry (Kao et al., 2004; Yi et al., 2004). A strong repressive activity of VE607 was observed on SARS-CoV pseudovirus entry into

ACE2-expressing 293T cells (Kao et al., 2004). Even though these small molecule inhibitors are effective *in vitro*, further *in vivo* studies on efficacy and optimal concentration for drug delivery should be evaluated. Spike specific small interfering RNA (siRNA) repressed SARS-CoV replication in virus-infected Vero E6 cells by RNA interference, enlightens with another potent target for the development of SARS-CoV-2 drugs (Wu et al., 2005).

4.4. Anti-viral drugs targeting viral mRNA synthesis and replicase

The viral RdRp is another promising therapeutic target of SARS-CoV-2 that does not cause any significant side effects in the host. Repurposing the existing therapeutic drugs can also be considered as an accomplishable strategy. Remdesivir, HIV reverse transcriptase inhibitor and one of the approved drugs for treatment during the COVID-19 outbreak was shown effective in animal models against SARS-CoV and MERS-CoV and is also under phase III clinical trials for the treatment of Ebola virus (de Wit et al., 2020; Sheahan et al., 2017). Favipiravir, a broad spectrum anti-viral drug has gained its attention as a potentially promising drug for specifically untreatable RNA viral infections due to its unique profile. This purine nucleotide analogue undergoes intracellular phosphoribosylation to an active favipiravir-ribofuranosyl-5′-triphosphate (RTP) form, thus acting as a substrate and inhibits RdRp activity and is already under clinical trials for COVID-19 (Furuta et al., 2017; Mifsud et al., 2019). Since various types of RNA viruses share similar catalytic domain, favipiravir can be considered as a therapeutic strategy to prevent COVID-19. A fleximer nucleoside analogue designed based on the acyclic sugar scaffold of acyclovir were capable of inhibiting Human coronavirus NL63 (HCoV-NL63) and MERS-CoV replication in cell culture by disrupting viral replication through an imprecise mechanism of action (Peters et al., 2015). The RdRp inhibitor guanosine analogue, ribavirin, is also a potent drug candidate for COVID-19 as ribavirin treatment in combination with interferons has shown to be effective during MERS-CoV and SARS-CoV infections (Omrani et al., 2014; Saijo et al., 2005). However the therapeutic efficiency of all these RdRp inhibitor drugs in COVID-19 patients must be estimated by clinical trials. K22, a compound targeting the CoV replication complex, inhibited a broad range of CoV RNA synthesis *in vitro* (Lundin et al., 2014). A chimeric RNA-DNA ribozyme targeting the loop region of SARS-CoV could considerably minimize the expression of recombinant SARS-CoV RNA in cell culture. Conversely, delivery of ribozymes in humans has to be optimized for productive clinical procedure as they were rapidly degraded *in vivo* (Fukushima et al., 2009). In addition to ribozymes, a chimeric protein, dsRNA-activated caspase oligomerizer (DRACO) possessing viral dsRNA binding domain that selectively kill cells harboring viral dsRNA was found to be effective against many RNA viruses (Rider et al., 2011). Such broad spectrum antivirals that potentially target the viral RNA sequences could also be employed as an effective therapeutic strategy against COVID-19. Production of mAbs against RdRp or nsp12, a fundamental component that plays a central role in the replication and transcription of SARS-CoV-2, along with the support of nsp7 and nsp8 has to be studied in detail.

4.5. Anti-viral drugs targeting viral proteases

SARS-CoV-2 genome encodes two types of proteases 3C-like protease ($3CL^{pro}$) also called main protease (M^{pro}) and papain-like protease (PL^{pro}) which are necessary for the cleavage of viral polyproteins. They are considered perfect drug targets as they are distinct from host cellular proteases. Protein sequence similarity of 96% has been identified between SARS-CoV M^{pro} and SARS-CoV-2 M^{pro} (Chen et al., 2020). HIV protease inhibitors, lopinavir and ritonavir have been reported to effectively inhibit SARS-CoV and MERS-CoV though their efficacy should be evaluated through clinical trials (Chan et al., 2015; Yao et al., 2020). Therefore, substantiated clinical trial studies can establish lopinavir and ritonavir as an effective treatment against COVID-19. Additionally, few peptidomimetic chymotrypsin-like protease inhibitors were found to inhibit SARS-COV at high concentrations (Ghosh et al., 2005). Two PL^{pro} targeting compounds, 6-mercaptopurine and 6-thioguanine, were reported to inhibit SARS-CoV and MERS-CoV while mycophenolic acid effectively blocked replication of MERS-CoV *in vitro* (Cheng et al., 2015; Hart et al., 2014). Benzodioxole was found to inhibit PL^{pro}, whereas zinc and its conjugates was reported to inhibit the activity of both PL^{pro} and $3CL^{pro}$ of SARS-CoV (Baez-Santos et al., 2014; Han et al., 2005). Small molecule inhibitors of $3CL^{pro}$ like ML188, ML300 and N3 have also reported inhibitory effects (Jacobs et al., 2013; Turlington et al., 2013; Yang et al., 2005a). Additionally, mycophenolic acid did not exhibit any effect on marmosets, whereas the efficiency of other molecules are yet to be tested in animal models (Chan et al., 2015) (Table 1). However, high levels of functional complexities mark SARS-CoV-2 proteases a significant target for therapeutic interventions.

4.6. Bioactive compounds against coronaviruses

Numerous bioactive natural products have also been reported to show anti-viral activities against SARS-CoV, MERS-CoV and SARS-CoV-2. Bioactive compounds ginsenoside-Rb1 isolated from *Panax ginseng* and aescin isolated from *Aesculus hippocastanum* were reported to possess anti-SARS-CoV properties (Wu et al., 2004). Similarly, pharmacologically active compounds reserpine, leptodactylone and lycorine shown to be effective against SARS-CoV were extracted from *Rauwolfia serpentina*, *Boenninghausenia sessilicarpa* and *Lycoris radiate* respectively (Li et al., 2005a; Wu et al., 2004; Yang et al., 2007). MERS-CoV infection was inhibited by resveratrol and dihydrotanshinone, a lipophilic compound isolated from *Salvia miltiorrhiza* (Kim et al., 2018; Lin et al., 2017). Few other natural compounds like celastrol, tingenone, pristimererin, iguesterin isolated from *Triterygium regelii* inhibited the activity of SARS-CoV $3CL^{pro}$ while PL^{pro} activity was blocked by tanshinones I–VII and hirsutenone (Park et al., 2012a, 2012b; Ryu et al., 2010). In addition, a bioactive compound emodin extracted from *Rheum palmatum* blocked the interaction of SARS-CoV S protein with ACE2 receptor (Ho et al., 2007). However, a recent study has reported an ACE2 inhibitory activity of cepharanthine extracted from *Stephania japonica* on SARS-CoV-2 related pangolin CoV infection (Fan et al., 2020). Furthermore, molecular docking studies have identified stilbene-based natural compounds and a potent M^{pro} inhibitor, oolonghomobisflavan-A from tea plant as promising candidates against SARS-CoV-2 (Bhardwaj et al., 2020; Wahedi et al., 2020) (Table 2).

4.7. Drugs in clinical trials

Sudden onset of COVID-19 pandemic along with the challenge of discovering specific anti-viral drug against SARS CoV-2 has lead researchers to repurpose potent antiviral and non-antiviral drugs or their combinations to slow down the spread. Drugs repurposed for COVID-19 are still undergoing intensive preclinical and clinical trials and may prove effective in limiting the infection.

The first and foremost clinical trial study on repurposed drug for COVID-19 was with remdesivir on 61 patients with critical condition on compassionate use, although the study was criticized due to absence of control cases (Grein et al., 2020). This potent nucleotide analogue RdRp enzyme inhibitor prodrug has been found to support severe pulmonary cases with little advantage of shorter viral clearance period over placebo control group in a randomized double blind study on 237 infected patients (Wang et al., 2020c). Although the drug had adverse effects, a bigger cohort study of 1063 advanced stage critical patients, remdesivir aided advantage over placebo while had a marginal advantage on reducing mortality (Beigel et al., 2020). Being a non-specific drug, smaller advantage over other therapies could be accounted as a life saver, better combination of drugs are also suggested.

Favipiravir, another nucleoside analogue, had similar effects to that of remdesivir when administered in higher drug doses and supported

Table 1
Potential antivirals against corona viruses and their therapeutic targets and mechanisms of action.

	Anti-virals	Anti-viral targets	Mechanism of action	References
Viral S glycoprotein	P6 peptide	RBD of S protein	Binds to S protein preventing the entry of virus	Han et al. (2006)
	20-mer synthetic peptides	Cleavage site of S protein	Block the production of functional S1 and S2 domain	Zheng et al. (2005)
	EK1	HR1	Inhibits fusion of S protein with host receptor	Xia et al. (2019)
	EK1C4 peptide	HR1	Inhibits fusion of S protein with host receptor	Xia et al. (2020)
	CP-1	HR2	Interacts with HR1 inhibiting viral infection	Liu et al. (2004)
	Griffithsin	S protein	Binds to S protein blocking virus –host interaction	(Barton et al., 2014; O'Keefe et al., 2010)
	Soluble ACE2	S1 subunit of S protein	Blocks S1 subunit of S protein	(Li et al., 2003; Sui et al., 2004)
	80R, CR3014, m396, S230.15, CR3022, MERS-27, MERS-GD27, hMS-1, Mersmab1	RBD of S protein	Blocks interaction with the host receptor	(Sui et al., 2005; Tian et al., 2020; Zhou et al., 2019; Zhu et al., 2007)
	TGG and luteolin	S protein	Binds to S protein and blocks entry into the host cell	Yi et al. (2004)
	VE605	S protein	Blocks entry into the host cell	Kao et al. (2004)
	siRNA	S protein	Inhibits the expression of S protein	Wu et al. (2005)
Host cell receptor	$S_{471\text{-}503}$ peptide	ACE2	Blocks binding between RBD and host receptor	Hu et al. (2005)
	193aa fragment (318–510)	ACE2	Blocks S protein mediated viral infection	Wong et al. (2004)
	Recombinant S377-588-Fc	DPP4	Blocks binding of viral RBD to host receptor	Du et al. (2013)
Viral mRNA synthesis and replicase	Remdesivir	RdRp	Inhibits viral RNA synthesis	Sheahan et al. (2017)
	Favipiravir	RdRp	Inhibits viral RNA synthesis	(Furuta et al., 2017; Mifsud et al., 2019)
	Fleximer nucleoside analogue	RdRp	Inhibits viral replication	Peters et al. (2015)
	Ribavirin	RdRp	Inhibits viral RNA synthesis	(Omrani et al., 2014; Saijo et al., 2005)
	K22	mRNA	Inhibits viral RNA synthesis	Lundin et al. (2014)
	Ribozymes	mRNA	Inhibits viral RNA synthesis	Fukushima et al. (2009)
	DRACO	Viral dsRNA	Kills cells containing dsRNA	Rider et al. (2011)
Viral proteases	Lopinavir and ritonavir	$3CL^{pro}$	Inhibits the activity of $3CL^{pro}$	(Chan et al., 2015; Yao et al., 2020)
	6-mercaptopurine and 6-thioguanine	PL^{pro}	Inhibits the activity of PL^{pro}	Cheng et al. (2015)
	Mycophenolic acid	PL^{pro}	Blocks replication of virus	Hart et al. (2014)
	Benzodioxole	PL^{pro}	Inhibits the activity of PL^{pro}	Baez-Santos et al. (2014)
	ML188, ML300 and N3	$3CL^{pro}$	Inhibits the activity of $3CL^{pro}$	(Jacobs et al., 2013; Turlington et al., 2013; Yang et al., 2005a)

with IFN-α. A non-randomized open label clinical trial with 80 patients on favipiravir demonstrated that COVID-19 patients could recover rapidly in contrast to Ritonavir/Lopinavir treated patients. Betterment of lung CT opacity was evidenced on favipiravir treatment (Cai et al., 2020). Similarly, broad spectrum purine analogue drug ribavirin in combination with lopinavir/ritonavir along with IFN-α supportive therapy could efficiently clear SARS-CoV-2 virus in phase 2 clinical trial with 127 patients, but the drug ribavirin alone had adverse effects like hypocalcemia, hemolytic anemia and hypomagnesemia (Hung et al., 2020). Ritonavir/Lopinavir both potent protease inhibitors known in use for HIV treatment were known to reduce SARS-CoV and MERS-CoV infection and thus had been a potent candidate of study on humans. Ritonavir/Lopinavir combination treatment in an open label trial of 199 patients improved recovery of critically infected case while failed in reducing mortality rates (Cao et al., 2020; Young et al., 2020).

Apart from the repurposed anti-viral drugs various monoclonal antibody, steroids and bioactive compound are being administered for immediate recovery of critically ill patients. Similarly, hydroxy-chloroquine and chloroquine although non-anti-viral drug has been used extensively as a first line treatment modality and various clinical trials are under progress evaluating the efficiency of such compounds and drugs towards effectively reducing the disease severity and recovery.

5. Conclusion

World economy and global industries plunged under the rampant spread of SARS-CoV-2 among populations. Unavailability of COVID-19 specific vaccines or drugs forced the authorities to repurpose drugs, however the current calamitous public health and unstable socio-economy, indispensably demands a scientific investigation for COVID-19 therapeutics. Considering genomic similarity of SARS-CoV-2 with SARS-CoV and MERS-CoV and their structural protein homology holds the avenues of therapeutics. Though clinical evaluation of drug candidates on young and naturally non-susceptible model animals often falsifies the outcome and in availing protection to the elderly population. Hence future testing should be mandated in proper and susceptible animal models thereby reducing escape mutants in mAbs as well as clinical trial failures. Additionally, combinatorial antiviral drugs or novel host acting broad spectrum antivirals could overcome impediments of dosage toxicities and immune evasion. Establishing more biosafety level containment facilities accompanied by meticulous research can benefit development of novel therapeutics and also limit future pandemics to greater extend. Nevertheless, ensuring critical research data exchange with data transparency along with proper preparedness and alertness among the global regulatory authorities and public awareness can effectively contain future viral outbreaks.

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Table 2
Potential bioactive compounds against corona viruses and their mechanisms of action.

Bioactive compound	Plant	Mechanism of action	References
Celastrol	*Triterygium regelii*	Inhibits $3CL^{pro}$	Ryu et al. (2010)
Tingenone	*Triterygium regelii*	Inhibits $3CL^{pro}$	Ryu et al. (2010)
Pristimererin	*Triterygium regelii*	Inhibits $3CL^{pro}$	Ryu et al. (2010)
Iguesterin	*Triterygium regelii*	Inhibits $3CL^{pro}$	Ryu et al. (2010)
Tanshinones I–VII	*Salvia miltiorrhiz*	Inhibits PL^{pro}	Park et al. (2012b)
Hirsutenone	*Alnus japonica*	Inhibits PL^{pro}	(Park et al., 2012a, 2012b)
Emodin	*Rheum palmatum*	Blocks the interaction of S protein and ACE2	Ho et al. (2007)
Ginsenoside-Rb1	*Panax ginseng*	Blocks glycoprotein	Wu et al. (2004)
Aescin	*Aesculus hippocastanum*	unknown	Wu et al. (2004)
Leptodactylone	*Boenninghausenia sessilica*	unknown	Yang et al. (2007)
Lycorine	*Lycoris radiate*	unknown	Li et al. (2005a)
Reserpine	*Rauvolfia serpentina*	unknown	Wu et al. (2004)
Dihydrotanshinone	*Salvia miltiorrhiza*	unknown	Kim et al. (2018)
Resveratrol	*Polygonum cuspidatum*	unknown	Lin et al. (2017)
Oolonghomobisflavan-A	Tea plant	Inhibits M^{pro}	Bhardwaj et al. (2020)
Cepharanthine	*Stephania japonica*	Blocks ACE2	Fan et al. (2020)

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Declaration of competing interest

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