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REVIEW ARTICLE

# Current understanding on molecular drug targets and emerging treatment strategy for novel coronavirus-19

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## Abstract

SARS-CoV-2 is an enveloped positive-sense RNA virus, contain crown-like spikes on its surface, exceptional of large RNA genome, and a special replication machinery. Common symptoms of SARS-CoV-2 include cough, common cold, fever, sore throat, and a variety of severe acute respiratory disease (SARD) such as pneumonia. SARS-CoV-2 infects epithelial cells, T-cells, macrophages, and dendritic cells and also influences the production and implantation of pro-inflammatory cytokines and chemokines. Repurposing of various drugs during this emergency condition can reduce the rate of mortality as well as time and cost. Two druggable protein and enzyme targets have been selected in this review article due to their crucial role in the viral life cycle. The eukaryotic translation initiation factor (eIF4A), cyclophilin, nucleocapsid protein, spike protein, Angiotensin-converting enzyme 2 (ACE2), 3-chymotrypsin-like cysteine protease (3CLpro), and RNA-dependent RNA polymerase (RdRp) play significant role in early and late phase of SARS-CoV-2 replication and translation. This review paper is based on the rationale of inhibiting of various SARS-CoV-2 proteins and enzymes as novel therapeutic approaches for the management and treatment of patients with SARS-CoV-2 infection. We also discussed the structural and functional relationship of different proteins and enzymes to develop therapeutic approaches for novel coronavirus SARS-CoV-2.

**Keywords** SARS-CoV-2 · Epidemiology · Pathogenesis · eIF4A · Cyclophilin · Nucleocapsid protein · Spike protein · ACE2 · 3CLpro · RNA-dependent RNA polymerase

## Abbreviations

| | |
|---|---|
| SARS-CoV-2 | Severe acute respiratory syndrome coronavirus-2 |
| SARD | Severe acute respiratory disease |
| BCoV | Bovine CoVs infectious |
| IBV | Bronchitis virus |
| TGEV | Transmissible Gastric Enteritis Virus |
| RBD | Receptor Binding Domain |
| DPP4 | Dipeptidyl-peptidase 4 |
| eIF4A | Eukaryotic translation initiation factor 4 A |
| Cyps | Cyclophilins |
| ALV | Alisporivir |
| HCV | Hepatitis C virus |
| NTD | N terminal domain |
| CTD | C-terminal domain |
| IRF-3 | Interferon regulatory factor-3 |
| ACE2 | Angiotensin-converting enzyme |
| RdRp | RNA-dependent RNA polymerase |

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## Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a highly transmissible and pathogenic coronavirus that mainly affects the human respiratory system. SARS-CoV-2 is responsible for two distinct endemics like Middle East respiratory syndrome (MERS) and acute respiratory syndrome (SARS), which have significant affected on public health (Raoult et al. 2020). SARS-CoV-2 is named due to the presence of crown-like spikes on their surface and consisted of four sub-groups, called as alpha, beta, gamma, and delta (Fehr and Perlman, 2015). It is a positive sense-stranded RNA virus with 29,891 bases; among these, 96% bases are identical to a bat coronavirus (CoVs), at the full level of genome stage, and share 79.6% of gene similarity with SARS-CoV (Denison et al. 2011). SARS-CoV-2 encodes spike (S) protein consisting of a receptor-binding

domain (RBD) that binds to the angiotensin-converting enzyme-2 (ACE-2) of humans and facilitates membrane fusion as well as virus uptake into human lungs (Fig. 1) (Hofmann and Pöhlmann, 2004). SARS-CoV-2 enter into human cells and capture the protein synthesis machinery to synthesize the viral proteins for replication and proliferation (Hofmann and Pöhlmann, 2004). SARS-CoV-2 contains the largest genomic structure (26.4–31.7 kb) among all known RNA viruses. Large numbers of small open reading frames (ORFs) are present between the various conserved genes [ORF1ab, spike (S), envelope (E), membrane (M), nucleocapsid (N)] and the nucleocapsid genes of various CoVs lineages (Mousavizadeh and Ghasemi, 2020). The viral genomes consist of distinctive characteristics, including a unique N-terminal fragment within the spike protein. Genes for main structural proteins in all SARS-CoV-2 occur in 5′–3′ order, such as S, E, M, and N. A typical SARS-CoV-2 contains at least six ORFs in their genome. ORF1a and ORF1b provide a frameshift between two polypeptides that are pp1a and pp1ab (Prajapat et al. 2020). These polypeptides are converted into 16 nsps (nsp1-16) by virally encoded chymotrypsin-like protease (3CLpro) or main protease (Mpro) and one or two papain-like proteases. ORFs 10,11 encode four specific structural proteins containing S, E, M, N proteins on one-third of the genome near to the 3′-terminus (van Boheemen et al. 2012). In addition to these four main structural proteins, such as HE protein, 3a/b protein and 4a/b protein are encoded by various CoVs (Fig. 2) (Chen et al. 2020). Such mature proteins are responsible for maintaining genomic structural integrity maintenance and virus replication roles.

The genome gets transcribed after the virus enters into host cell. The reproduction and transcription of the CoVs genome occur on cytoplasmic membrane and regulate by the viral replicate (Shulla et al. 2011). It is assumed that the replicase complex has consisted of approximately 16 subunits and a various cellular protein. In addition to RNA-dependent RNA polymerase (RdRp), RNA helicase, and activities of proteases which are common in many RNA viruses, CoVs replicase is known to use a variety of RNA-dependent processing enzymes which are not present in other RNA viruses, including a putative specific sequence of endoribonuclease, 3′- to 5′-exoribonuclease, 2′-O-ribose methyltransferase, ADP ribose 1′-phosphatase, and cyclic phosphodiesterase behaviors in a subset of group 2 CoVs (Sola et al. 2015; Ziebuhr, 2005). The proteins are packaged on the cellular membranes and genomic RNA is introduced by budding from the internal cell membrane as the mature particles emerge (Almazán et al. 2006). SARS-CoV-2 N-proteins have 3 distinct and highly conserved domains include 2 structural and independently folded structural regions, known as N terminal domain (NTD/domain 1) and C-terminal domain (CTD/domain 3), separated by intrinsic disordered central region (RNA-binding domain/domain 2) (Fig. 3) (Huang et al. 2004).

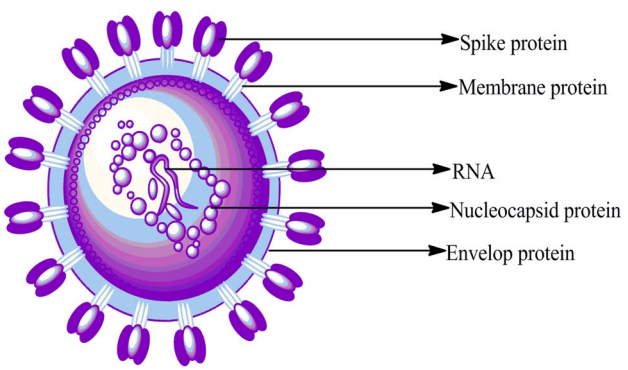


**Fig. 1** Structure of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)

Number of patients were hospitalized with initial diagnosis of unknown pneumonia in December 2019. Available studies have indicated that bat may be the potential reservoir of SARS-CoV, which cause serious illness in humans and agricultural animals. However, there is no confirmation to date that SARS-CoV-2 was originated from the seafood market but bats are the ideal repository for a variety of SARS-CoV-2, including MERS-CoV and SARS-CoV (Guo et al., 2020). The genome sequencing of COVID-19 was analyzed and found 96.2% similar to Bat CoV RaTG13 because both types of viruses might be shared the same ancestor (Zhang et al. 2020a, b).

## Drug repurposing against SARS-CoV-2

Repurposing various drugs during this emergency condition can control the rate of mortality and reduces both time- and cost-effective product development (Singh et al. 2020). Repurposing is scientific research currently underway to develop safe and effective treatments for COVID-19. Drug repurposing strategy is favorable options and considered to be gold standard for development new drugs. In addition, drug repurposing lies basically on structure-based design prediction of efficacy and off-drug target toxicity (Farha and Brown, 2019). During COVID-19 pandemic, some antiviral medications, previously used as treatments for HIV/AIDS, Malaria, MERS, and SARS, have investigated for COVID-19 and some of them undergo to clinical trials investigations (Senanayake, 2020).

Numbers of antiviral agents have been tested in early phase of clinical trials which showed beneficial results with minimum adverse effects. These molecules inhibit viral replication by targeting viral enzymes or their functions

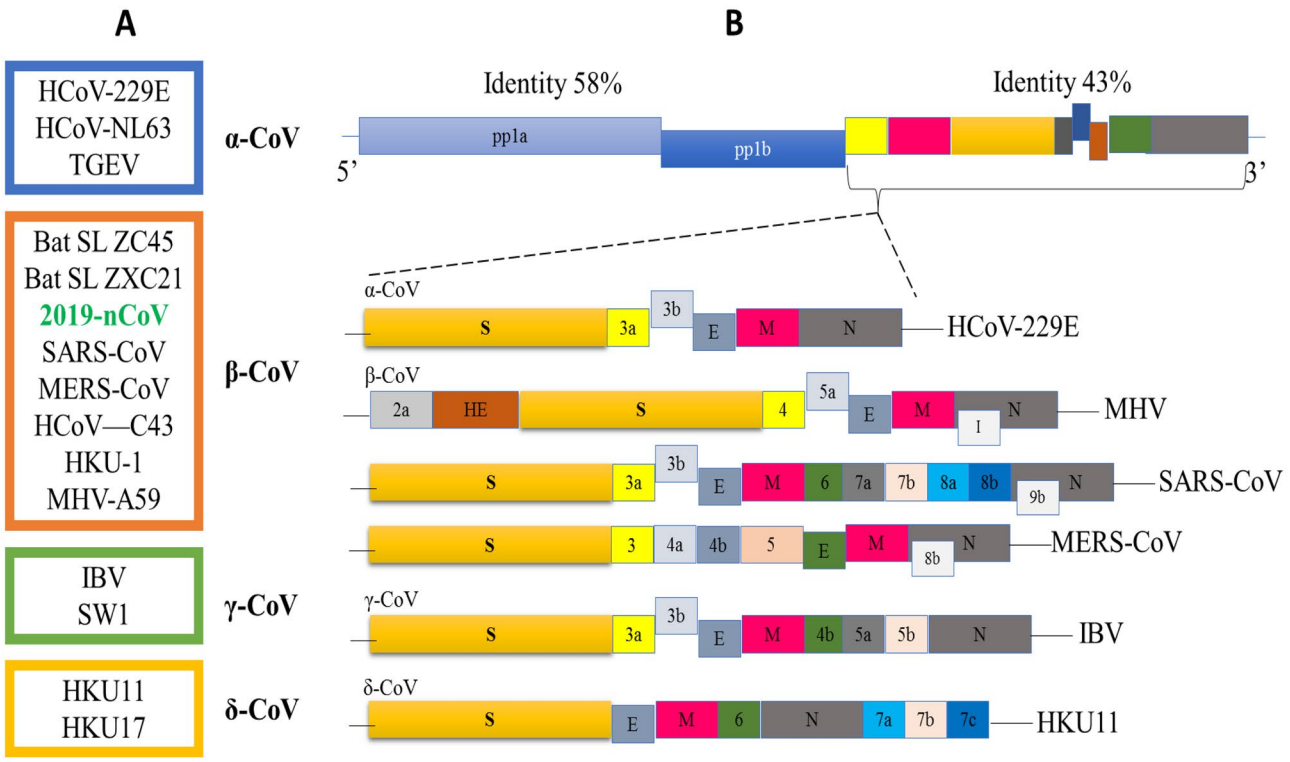


**Fig. 2** The genomic structure and phylogenetic of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2): **a** The phylogenetic tree of coronavirus with the new COVID-19 shown in green color. **b** The genome structure of four genera of coronaviruses (CoVs): two long polypeptides with 16 nonstructural proteins initiated from Pp1a to pp1b represent. E, S, M, and N are consisted of the four structural proteins envelope, spike, membrane, and nucleocapsid. Abbreviations: CoVs, coronavirus; HE, hemagglutinin-esterase. HCoV, human coronavirus; HKU, coronaviruses identified by Hong Kong University; MHV, murine hepatitis virus; IBV, infectious bronchitis virus; TGEV, transmissible gastroenteritis virus; HCoV-229E, human coronavirus OC43; MERS-CoV, Middle East respiratory syndrome coronavirus

and used to treat SARS-CoV-2 patients (Abd El-Aziz and Stockand, 2020). Umifenovir is a membrane fusion inhibitor that inhibits the viral entry and ritonavir/lopinavir is the combination of drugs that target viral protease, which is well approved for influenza and HIV indications (Andersen et al. 2020). These molecules are currently under phase II clinical trial (75 patients) for COVID-19-related pneumonia in various combinations. The treatment course included 75 mg oseltamivir oral administration, 500 mg ritonavir, 500 mg lopinavir, and 250 mg ganciclovir intravenous administration for 3–14 days (Wu et al. 2020). These antiviral molecules were used with a safety track record in human patients. Remdesivir is a viral RNA-dependent polymerase inhibitor for mild and moderate COVID-19 under investigation at phase III level (Harrison, 2020). Chloroquine was found to have antiviral activity at the entry and post-entry stages of COVID-19 infection, in addition to its immune-modulating actions (Cao et al. 2020). The viral RNA polymerase inhibitor favipiravir is also under a phase II clinical trial for COVID-19-related pneumonia. So, these therapeutic drugs could be considered for treatment of CoVs infection after found beneficial effects in clinical trials (Li et al. 2020). Additionally, there is a large number of compounds that are under developmental phases (Fig. 3). These compounds include EIDD-2801 as a clinical molecule which has shown high therapeutic potential activity against SARS-CoV-2 infection (Zhang et al. 2020a, b).

Modern drug discovery, propelled by computational modeling and bioinformatics, has enabled virtual screening of biologically active compounds for hit identification and lead optimization. There are two types of simulation methods perform, like structure-based and ligand-based, to discover a new drug (Lionta et al. 2014). Therefore, these techniques are useful for development of drugs to inhibit SARS-CoV-2-associated infection. Several experiments have used molecular docking technology for virtual screening and repurposing of existing medications and natural products as a solution for the COVID-19 pandemic (Lionta et al. 2014). However, the discovery of multi-targeted, receptor selective, and low toxicity compounds is also equally important to overcome SARS-CoV-2 infection. According to the new

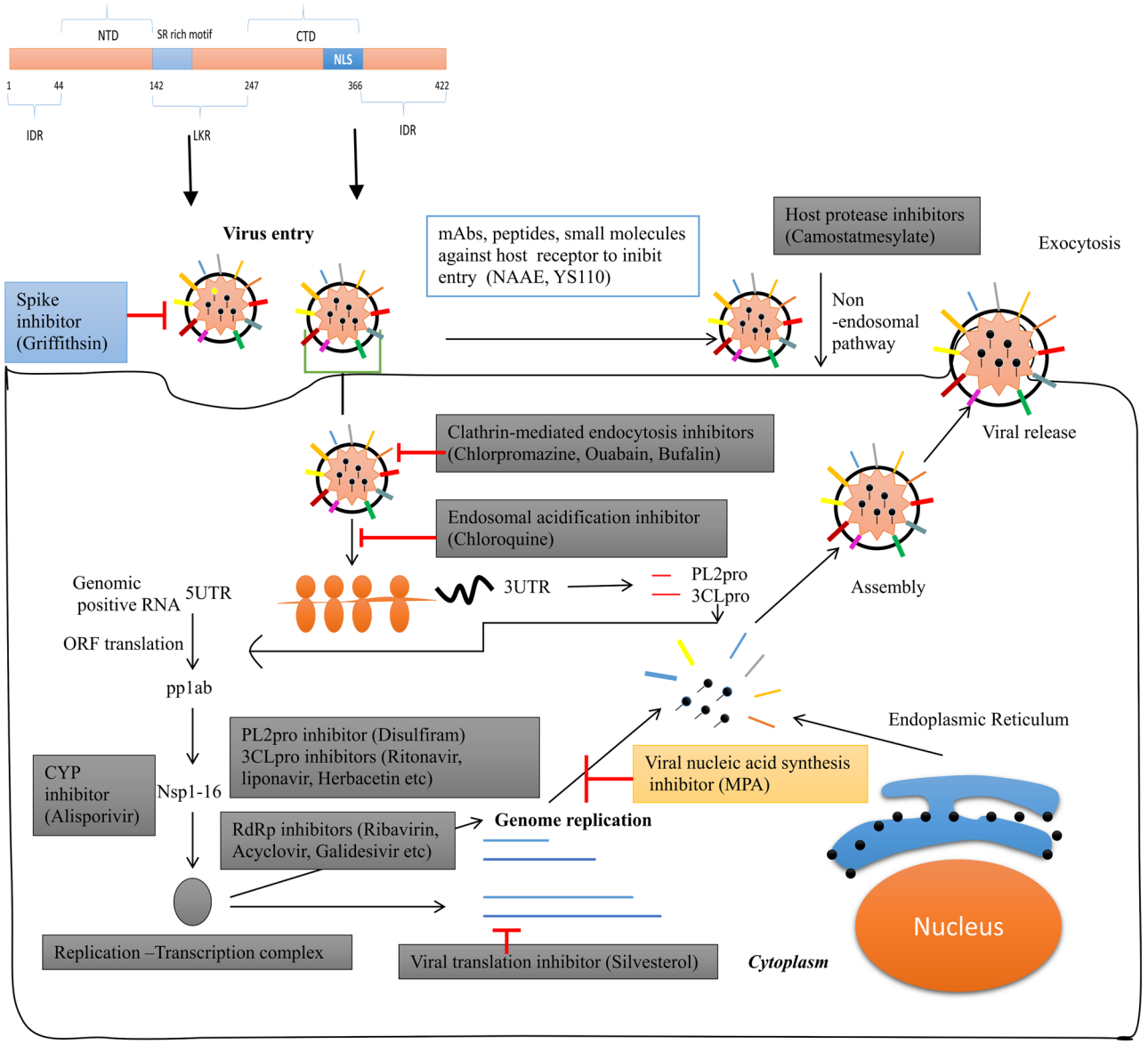


**Fig. 3** Structure of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) nucleocapsid protein and target sites of potential antiviral agents. The virion enters by endocytosis or direct fusion of cell through viral membranes. The viral genome is translated into two polyproteins, which are cleaved by two viral proteases (3CLpro PLpro) to generate a large replication and transcription complex orchestrating genome replication and synthesis of mRNAs. New viral genomes recruit viral structural proteins to generate new virions released by exocytosis process. Red arrow indicates the potential inhibitors used to inhibit various targets. Abbreviations: 3CLpro, chymotrypsin-like protease; PLpro, papain-like protease; 3UTR, 3 untranslated region; 5UTR, 5 untranslated region; pp 1 ab, polypeptide 1ab; CYP, cyclophilin; RdRP, RNA-dependent RNA polymerase

study, virtual screening of new antiviral compounds against SARS-CoV-2 would also be useful to elucidate other vaccines like antibody and protein preparation (Chowdhury, 2020). Beclabuvir and Saquinavir were identified as the good candidates for SARS-CoV-2 therapy based on virtual high throughput screening (HTS) of clinically approved drugs and the structure of SARS-CoV-2 Mpro determined by X-ray diffraction technology (Quimque et al. 2020). HTS is an automated process used in drug discovery for identification of hits from library compounds, which are pharmacologically active like proteins, antibodies, peptides, and inhibitors. HTS can be used for screening most promising drug candidates for efficacy analysis and development of new antivirus drugs (Talluri, 2020). HTS of large compound libraries (approved drugs by FDA, proteins, peptides, antibodies, and inhibitors) have identified effective antiviral candidates against SARS-CoV-2 infection (Touret et al. 2020).

## Targeting antiviral protein

### eIF4A protein

Eukaryotic translation initiation factor 4 A (eIF4A) is a member of the DEAD-box protein helices family. It consists of two recA-like domains that are separated by flexible hinge region in the center, lined by conserved motifs. This conserved motif is called DEAD box which contains amino acids like aspartic acid, glutamic acid, and alanine (Andreou and Klostermeier, 2013). The motif of eIF4A interacts with nucleic acid, involved in ATP binding and ATPase activity. As a consequence, eIF4A has been demonstrated to have RNA-dependent ATPase activity, ATP-dependent duplex RNA unwinding activity, and also involved in initiation of translation shown in Fig. 4. The activity of eIF4A is synchronized with complementary initiation factors of translation, which propagate its all activities as well as interaction with RNA for protein synthesis (Andreou and Klostermeier, 2013; Andreou et al. 2017). Furthermore, major functions of eIF4A are to remove secondary multifaceted structures within the 5′-untranslated region and to displace proteins attached to mRNA during protein synthesis (Hilbert et al. 2011). The eIF4A protein is a key factor involved in translation during viral protein formation and mediating infection. A study demonstrated that viral mRNA uses eIF4A for synthesis of its protein (Montero et al. 2019). Genomic mRNAs of SARS-CoV-2 have a 5-cap structure and go through cap-dependent translation via eIF4F. The eIF4A is a part of eIF4F protein complex which is associated with other two translation initiation factors such as eIF4E and eIF4G, in turn connected with eIF4A which is further connected with eIF4E (Nakagawa et al. 2016). In the cap-dependent mechanism of translation, the viral mRNA is engaged with eIF4F protein complex, consisted of three functional proteins: eIF4E, eIF4A, and eIF4G. The eIF4A and eIF4F are essential for recruitment of ribosomes for protein synthesis during SARS-CoV-2 infection. Consequently, eIF4A is important for controlling translation and regulating gene expression at the translational level (Montero et al. 2019).

Recently, research has revealed that specific inhibition of eIF4A can block viral replication and thus help the immune system for establishing an effective antiviral response. Inhibition of eIF4A with synthetic or natural antiviral drugs shows similar inhibition of replication and translation in SARS-CoV-2. Similarly, natural compounds like silvestrol and rocaglamide have been reported as a precise inhibitor of eIF4A in viral translation using virus-infected primary cells (Fig. 5). It is also revealed to retain an inhibitory activity toward Ebola virus in viral-infected human macrophages (Nebigil et al. 2020). Additionally, another study conducted using human embryonic lung fibroblast (MRC-5) cells infected with CoVs has demonstrated that inhibits eIF4A by silvestrol leads to separation of cap-dependent viral mRNA translation. Silvestrol has been shown protection against MERS-CoV and HCoV-229E with EC50 = 1.3 nM and 3 nM respectively (Song et al., 2019). Morever, Zotatifin is another inhibitor of eIF4A recently comes under clinical trials for treatment of SARV-CoV-2 (Biedenkopf et al. 2017), which inhibit an enzyme responsible for unwinding of messenger RNA structures initiate their translation into proteins (Prabhu et al. 2020). The Zotatifin has shown potent anti-proliferative activity through inhibition of eIF4 against a group of B-cell lymphoma cell lines (Müller et al. 2018). Furthermore, in vivo study is separated in which influenza virus–infected cells were treated with Pateamine A and Silvestrol. They found that inhibition of viral protein synthesis and prevention of viral genome replication through inhibition of eIF4A binding with mRNA can overcome infection. Pateamine A irreversibly binds to eIF4A and produces long-term inhibition of IAV replication with least cellular toxicity (Slaine et al. 2017). In addition, pateamine A disrupts interaction with eIF4G and decreases the levels of eIF4A present in the eIF4F complex. Flavaglines are cyclopenta [b] benzofurans found in Aglaia and use as a traditional Chinese medicine. These compounds work by targeting the eIF4A translation initiation factor and the

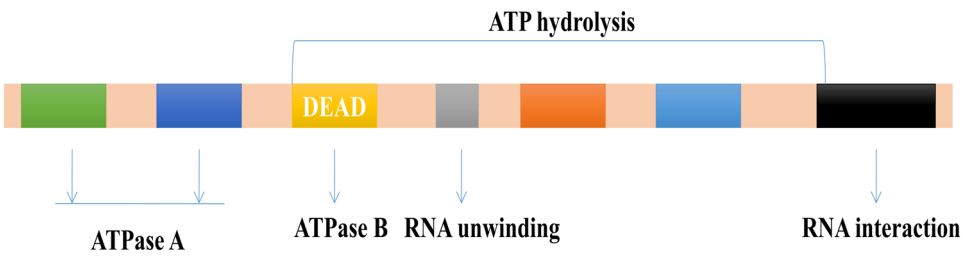


**Fig. 4** Structure of eukaryotic translation initiation factor 4 A (eIF4A). DEAD box proteins are one of the conserved motifs, consisted of amino acid sequence of proteins containing aspartic acid-glutamic acid-alanine-aspartic acid. Abbreviations: ATP, adenosine triphosphate; RNA, ribonucleic acid

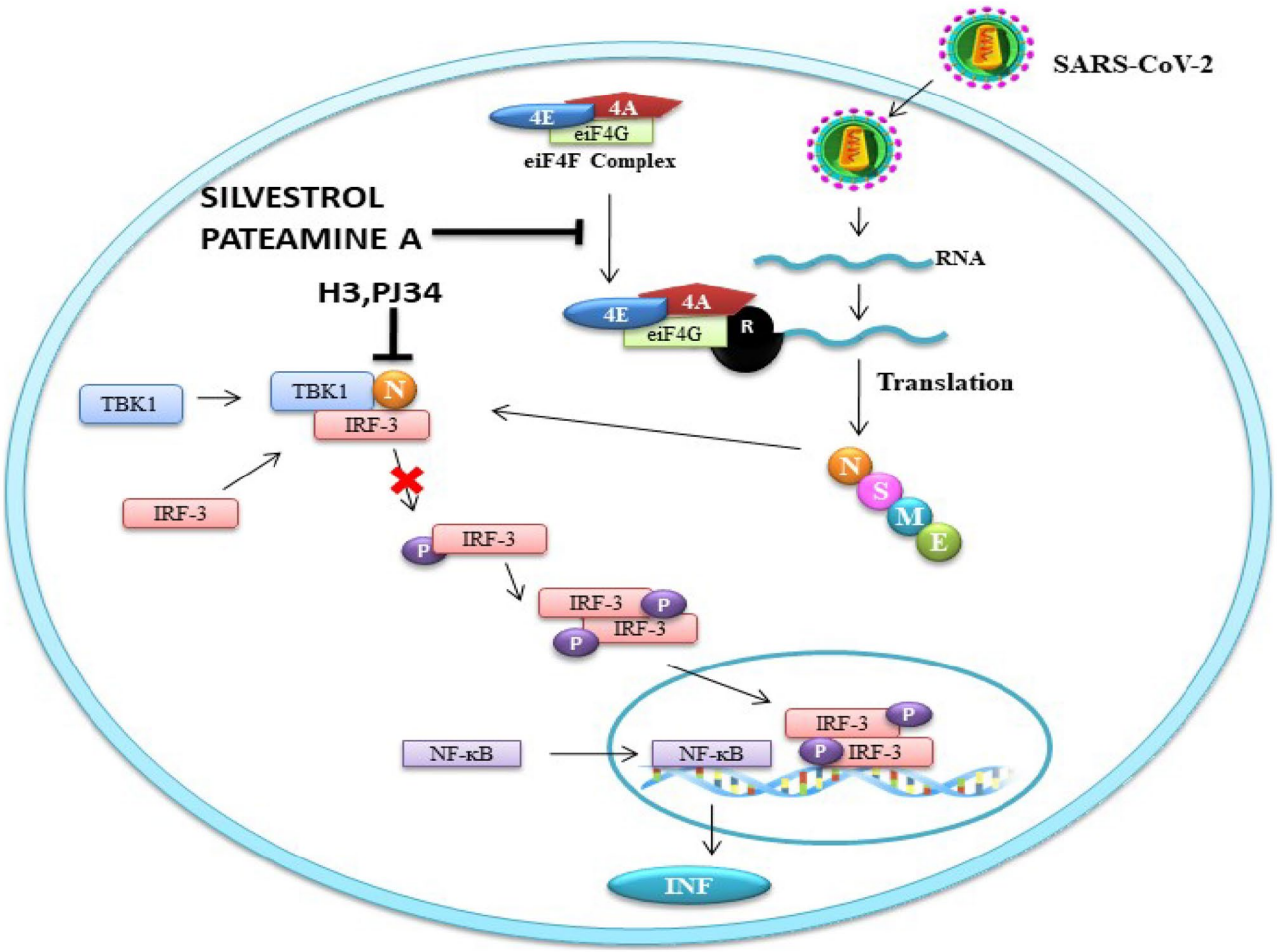


**Fig. 5** Mechanism of nucleocapsid inhibitor and eiF4A inhibitor. Inhibition of eIF4A with synthetic or natural antiviral drugs prevents replication and translation in SARS-CoV-2. Pateamine A and silvestrol irreversibly bind with eiF4A and inhibit the binding of eiF4A with mRNA. On the other hand, nucleocapsid block the phosphorylation of IRF3 which in turn cause transcription of INF, H3, and PJ34 which are inhibitors of nucleocapsid. These inhibitors reduced the N protein's binding affinity with IRF3, which leads to the activation of INF and hindered viral replication. Abbreviations: IRF-3, interferon regulatory factor-3; eiF4A, eukaryotic translation initiation factor 4 A; NF-kB, nuclear factor kappa B; INF, interferon; TBK1, TANK-binding kinase

scaffold proteins prohibitins-1 and 2 (PHB1/2) to perform antiviral activity against different types of viruses, including SARS-CoV-2 (Nebigil et al. 2020). Flavaglines stabilize the eIF4A and 5′UTR interaction by altering the conformation of both mRNA and eIF4A. As a result, eIF4A recycling is blocked, which leads to an inhibition of cap-dependent translation. 40S, small ribosome subunit, m7G, and 7-methylguanosine found at the 5′ end of the mRNA to which eIF4E binds (Dmitriev et al. 2020).

Hippuristanol is a polyhydroxysteroid obtained from the golden fan coral Isis hippuris. It interacts with the C-terminal domain of eIF4A via motifs V and prevents the binding of RNA. Hippuristanol is a selective inhibitor of eIF4A because of the high sequence variance of motifs V and VI through DEAD-box helicases (Karthik et al. 2014). Antiviral activity of hippuristanol has been reported against several viruses such as the norovirus and encephalomyocarditis virus (EMCV) and the two positive-stranded RNA viruses, and human T cell leukemia virus type 1 (HTLV-1) (Tsumuraya et al. 2011; Taroncher-Oldenburg et al., 2021).

Plitidepsin was clinically approved for the treatment of multiple myeloma with a well-established pharmacokinetics and safety profile (White et al. 2021). Plitidepsin inhibits the activity of eEF1A and is predicted to interact with the same binding site as didemnin B, which is structurally linked to plitidepsin. Plitidepsin has showed better results in a phase I/II clinical trial for the treatment of COVID-19 and is moving forward into a phase II/III COVID-19 (Amanat et al. 2020). Hence, eIF4A could be utilized as a therapeutic intervention target in COVID-19 infections and may obtain promising results in future.

## Cyclophilin

Cyclophilins (Cyps) are sub-group of immunophilins belong to enzyme peptidyl-prolyl *cis/trans* isomerases family. Cyps are present in the cells of prokaryote and eukaryotes organisms, and regulate intracellular protein synthesis, folding, and transportation, and replication of RNA viruses, such as influenza A virus, HIV, and HCV (Liu and Zhu, 2020). Totally 80 iso-forms of different molecular masses have been illustrated in human tissues. Out of these isoforms, seven are major Cyps present in humans such as Cyclophilin A, Cyclophilin B, Cyclophilin C, Cyclophilin D, Cyclophilin E, Cyclophilin 40, and Cyclophilin NK. Cyps are present in both extracellular and intracellular space of the cell and secreted in response to a variety of stimuli having different natures and intensity (O'Meara et al. 2020). The extracellular cyps like Cyclophilin A and Cyclophilin B are concerned with cell to cell communication. Cyps are also involved in various signaling pathways such as mitochondrial apoptosis, inflammation, RNA splicing, and adaptive immunity (Thompson et al. 2019). Cyps bind to the CD147 cell membrane receptor as well as heparins and then initiate arrays of signaling pathways in the cell which are concerned with inflammatory outcomes. In addition, CypA is also competent to control human IFN-I reaction to viral infections (Rajiv and Davis, 2018).

Moreover, Cyclophilin A and Cyclophilin B play important role in replication of many viruses including CoVs, human immunodeficiency virus (HIV), hepatitis C virus (HCV), measles virus, and influenza A virus (Zhou et al. 2012). A study demonstrated that Cyclophilin A is an essential cyps that acts as binding factors for SARS-CoV-2 proteins and required for SARS-CoV-2 proliferation (von Hahn and Ciesek, 2015). Another study conducted using plasmon resonance biosensor technology reported the interaction of Cyclophilin A with nucleocapsid (N) protein of SARS-CoV. This statement gets confirmed by another technique in which they observed Cyclophilin A as one of the cellular proteins integrated into purified SARS-CoV-2 particles by using spectrometric pro-filing (Luo et al. 2004; Tanaka et al. 2017). Furthermore, research using nucleocapsid protein (NP) of SARS-CoV showed that segment of Val235-Pro369 of SARS-NP interact with human Cyclophilin A (hCypA) more accurately and SARS-NP loop Trp302-Pro310 lock into the catalytic-site of hCypA with the help of hydrogen bonding indicate hCypA binds NP of SARS-CoV with high affinity, resulting in Cyclophilin A play important role in the replication and growth of SARS-CoV-2 (Carbajo-Lozoya et al. 2012).

Collectively, this information revealed the significant functions of Cyclophilin A in intervening SARS-CoV-2 infections and inhibition of Cyclophilin A can be a target for the advancement of anti-viral therapy. Similarly, Cyp inhibitor Alisporivir (ALV) has been demonstrated to inhibit viral replication in SARS-CoV, MERS-CoV, MHV, and HCoV-229E infected in different culture cells (Dawar et al. 2017). Cyclophilin inhibitors can inhibit the replication and infection of SARS-CoV-2 into host cells via interacting with CD147 (Liu and Zhu, 2020). ALV with ribavirin has been revealed to enhance the antiviral response during chronic HCV infection treatment in phase III clinical trials. Although more than a 100-fold higher concentration of ALV required for SARS-CoV inhibition in cell culture than that required for inhibition of HCV replication. However, ALV has been showed to lack of antiviral activity against SARS-CoV mouse model recommending that the drug might not be well matched for CoVs infection treatment (De Wilde et al. 2017). Various non-immunosuppressive cyclophilin inhibitors are developed, such as NIM811, SCY-635, sangliferins, CRV431, and STG175. Available studies have reported that many of these inhibitors can effectively inhibit the replication of hCoV-229E, and indicated its potential for human SARS-CoVs infection (Liu and Zhu, 2020). On the other hand, Cyp is still an attention-grabbing target and inhibition of Cyclophilin A is valuable for overwhelming viral infections leading to the advancement of host-directed anti-CoVs therapy.

## Nucleocapsid protein

The nucleocapsid protein (N) is a fundamental RNA-binding protein fixed in the 3′ end portion of the viral genome, which plays an imperative function in viral infection through their structural and functional activities. The N proteins from different types of SARS-CoV-2 have difference in length and primary sequence (Surjit and Lal, 2008). However, some motifs of N protein with functional application are conserved and have a three-discrete and extremely conserved domain association according to sequence similarity. Out of these three, two domains, i.e., N terminal domain (NTD) and C-terminal domain (CTD) are independently folded structural regions. The former domain is also known as domain 1 and later as domain 3. These two domains are separated by central region RNA-binding domain/domain 2 (Li, 2016).

Functionally N protein of SARS-CoV-2 has been informed to be valuable for the packaging of viral genome via interacting with genome RNA and leads to formation of elongated, stretchy, helical ribonucleoprotein (RNP) complexes known as viral nucleocapsid. N protein also interrelates with the membrane protein of virus during participating in viral assembly (Chang et al. 2014). Moreover, several studies have verified that N protein is essential for RNA replication of SARS-CoV-2. The involvement of N protein in the synthesis of RNA is carried out through only two steps: firstly, intracellularly co-localization of SARS-CoV N protein with elements of replicase during the commencement of

infection and secondly, depends on translocation of N protein responsible for initiation of gRNA infection (McBride et al. 2014).

SARS-CoV-infected cells inhibit the production of interferon with the help of SARS-CoV N protein (Shah et al. 2020). Thus, N protein acts as a β interferon (IF-β) antagonist. The mechanism behind the inhibition of IF-β synthesis by N protein might be due to blockage of interferon regulatory factor-3 (IRF-3) and nuclear factor kappa B (NF-kB) (Frieman and Baric, 2008). Both IRF-3 and NF-kB are important transcription factors, essential for interferon gene expression. So, inhibition of the interferon response is liable to contribute to the SARS-CoV pathogenesis (DeDiego et al. 2014).

Therefore, N protein of SARS-CoV-2 is involved during viral infection and inhibition of N protein may be useful to combat viral infection. The new molecules synthesized such as N protein inhibitors prevent the interaction between RNA and N protein, resulting in inhibition of viral replication during infection (Prajapat et al. 2020). Likewise, in silico virtual study developed compound H3 as a blocker for SARS-CoV-2 NPs which has been further verified by X-ray crystallography (Zhou et al. 2020). Moreover, N-(6-oxo-5, 6-dihydro phenanthridin-2-yl) (N, N-dimethyl amino) acetamide hydro-chloride (PJ34) is another N protein inhibitor which has been developed using virtual screening. This inhibitor decreased the binding capacity of N proteins with RNA and precluded replication of virus (Wang et al. 2016). Consequently, the discovery of novel NP-targeting agents is very beneficial for the treatment of COVID-19 infections.

## Envelope protein

The envelope protein of SARS-CoV-2 is a short, chief viral structural protein containing 76 to 109 amino acids (Kuo et al. 2007). Moreover, the primary and secondary structure confirms that E protein, having a short hydrophilic amino terminus, exposed in the membrane toward the cytoplasmic side which consisted of 7–12 amino acids along with large hydrophobic transmembrane cytoplasmic domain consisted of 25 amino acids (Li et al. 2014). The hydrophobic region of the transmembrane domain contains at least one predicted amphipathic α-helix which upon oligomerizes form an ion-conductive pore in membrane (Torres et al. 2007). Studies revealed that E protein contains a binding motif known as the postsynaptic density protein 95 (PSD95)/*Drosophila* disk large tumor suppressor (Dlg1)/(PDZ)-binding motif (PBM), which are located at the last four amino acids of carboxyl terminus (Teoh et al. 2010). The PDZ domain is a protein–protein interaction unit that binds with carboxyl terminus of target proteins, involved in the viral infection (Hung and Sheng, 2002). Some interaction partners are capable to binding with PBM of E protein and are thought to be involved in the pathogenesis of COVID-19 (Jimenez-Guardeño et al., 2014).

Despite its enigmatic nature, several studies are conducted to date to demonstrate the function of E protein. The interaction between the cytoplasmic units of the E and M protein drives VLP production suggesting that E protein participates in viral assembly, release of virions, and crucial to the pathogenesis of the virus (Hogue and Machamer, 2007; Ye and Hogue, 2007). The E protein is also involved in maintaining the morphogenesis and phenotype of virus. This phenotype suggests that E protein is essential for creating the membrane curvature, which is necessary to acquire the rounded and stable virions. Similar to other viruses, the E protein of SARS-CoV-2 was shown to form membrane channels with selectivity for monovalent cations along with enhanced the membrane permeability of bacterial and mammalian cells (Madan et al. 2005). This channel-forming activity of SARS-CoV-2 E protein was recently comprehensive to the human coronavirus 229E (HCoV-229E), MHV, and IBV (Wilson et al. 2004). More interestingly, the channels formed by E proteins show greater preference for sodium ions ($Na^{+2}$) over potassium ions ($K^{+2}$), but in contrast, the ion channels formed by the E protein of coronavirus HCoV-229E exhibit greater preference for potassium ions ($K^{+2}$) over sodium ions ($Na^{+2}$) (Wilson et al. 2006).

Hexamethylene amiloride (HMA) is an amiloride analog which blocks the ion channel activity of HIV, HCV, and dengue virus (Ewart et al. 2002). This molecule could also inhibit the ion channel activity of the HCoV-229E, suggesting a more divergent structure of coronavirus E protein. Furthermore, HMA is also able to inhibit the replication of HCoV-229E along with MHV, but not the replication of a recombinant MHV with deletion of the entire E gene (Wilson et al. 2006). These results indicate that the ion channel activity of coronavirus E protein is important for virus replication.

## Spike protein and ACE2

After immense research work, the researchers now revealed that COVID-19 is an enveloped virus. This envelope contains a number of unique spike-like proteins known as S-glycoproteins, which is a clove-shaped type I-transmembrane protein that allow the entry of viral into target cells (Mittal et al. 2020). The S-glycoprotein is made of two smaller protein subunits S1 and S2 and shares 76% amino acid identity (Coutard et al. 2020).

The S1 part is consisted of receptor-binding domain (RBD) that interacts with the peptidase domain (PD) of ACE 2 while the S2 subunit is cleaved by the host proteases in post-interaction and causes membrane fusion (Shang et al. 2020). Entry depends on the binding of the S1 surface unit to a cellular receptor, which promotes viral attachment to

the target cell surface. SARS-S engages ACE2 as the entry receptor and uses the TMPRSS2 cell serine protease for the priming of S proteins (Hoffmann et al. 2020). SARS-CoV-2 protein association with ACE2 (cellular receptor) is the central determinant of the COVID-19 host system. The central domain of COVID-19 spike in other beta-CoVs spike is homologous to a related region, which is a specific contract ACE2. Evidence indicates that human alpha-CoVs, such as NL-63, also uses ACE2 receptor (Ortega et al. 2020) and might have provided this linking loop. The spike replacement with one or two amino acids may have significant effects on COVID-19 spike activity and human ACE2 receptor. The S-protein binds with ACE2 by fusing with plasma membrane and releases RNA genome. This leads to replication and initiates exocytosis thereby releases number of virus species inside the host alveolar cells (Fig. 6).

Increased prevalence of COVID-19 is also implicated for viral entry and modulation of the rennin angiotensin mechanism, which is propagated by the downregulation of ACE2 expression on the plasma membrane arising from infection with SARS-CoV-2 (Robson, 2020). In many models of lung injury, ACE2 has been publicized to be pneumoprotective because of its impact on angiotensin II degradation (Sparks et al. 2011). During the infection with SARS-CoV-2, the production of ACE2, downregulation the SARS-CoV-2 receptors, on the surface of cells. The cause of this downregulation seems to be attributed due to internalization of ACE2 after the initiation of SARS-CoV-2 (Perrotta et al. 2020) and the activation of TNFα or metalloproteases of Adams family. Because they cleave the extracellular ACE2 domain from the trans-membranous domain sheds into the media (Gheblawi et al. 2020). ACE2 shows pneumoprotective impact on acute lung damage triggered by acid damage (Kuba et al. 2005) and addition of a recombinant fusion protein comprising of SARS S protein (Hamming et al. 2004). These findings concluded that SARS-CoV-2 S protein binds to receptor of the host cell and activates the membrane fusion process of virus that take part in virus invasion process. The SARS-CoV-2 is replicated in myocardium whereas pulmonary inflammation is correlated with ACE2 (Hamming et al. 2004). Several proteases, including cathepsin L, have been reported to affect SARS-CoV-2 entry through cleavage of the S-protein and activation of its membrane fusion activity (Simmons et al. 2013).

Several types of vaccinations and antiviral drugs, based on S protein, have been evaluated. A study has shown that vaccines can be grounded on the S proteins consisted of full-length S protein, viral vector, DNA, recombinant S protein, and recombinant RBD protein (Kaur and Gupta, 2020). In vitro analysis of S-based antiviral treatments is comprised of RBD-ACE2 blockers, S cleavage inhibitors, fusion center inhibitors, neutralizing antibodies, protease inhibitors, S-protein inhibitors, and minor interfering RNAs (Cannalire et al. 2020). There are some recombinant complexes such as IFN with ribavirin known to partially reduce COVID-19 infection.

Monoclonal antibodies mainly target the S1 subunit and fusion inhibitors bind to S2 subunit, which could be effective therapeutic target for the treatment of COVID-19 infections (Millet and Whittaker, 2014). A serine endoprotease, furin, cleaves off S1–S2 could be a suitable anti-COVID-19 agent (Gioia et al. 2020). Griffithsin is a lectin derived from red algae, which binds to spike glycoprotein of SARS-COV and HIV glycoprotein 120. However, delivery mechanisms and the efficacy of S inhibitors are generally re-evaluated for the prevention or treatment of COVID-19 (O'KEEFE et al. 2010; Wondmkun and Mohammed, 2020).

The RBD of SARS-CoV-2 has a higher ability to bind with ACE2 than CoVs and acts as binding receptors for COVID-19. Gurwitz recommended the use of accessible angiotensin receptor 1 (AT1R) antagonists, such as losartan, as a therapy to minimize COVID-19 infection intensity (Matsuyama et al. 2010). Treatment is focused on the detection and production of unique and efficient monoclonal antibodies to treat COVID-19 infection such as Bevacizumab (NCT04305106), Meplazumab (NCT04275245), and Tocilizumab (NCT04317092).

SSAA09E2 inhibits the S-ACE2 interaction, SSAA09E1 inhibits the host protease cathepsin L, and SSAA09E1 prevents the fusion of the host and viral cell membranes (Adedeji et al. 2013). Kao et al. identified 18 small molecules, targeted the virus entry into human cells through S-ACE-2 (Kao et al. 2004). VE607 showed a strong inhibition of SARS-pseudovirus entry in 293 T cells. Other two molecules luteolin and tetra-O-galloyl beta-D-glucose showed significant inhibition of SARS-CoV and SARS-pseudovirus infection (Kao et al. 2004; Wu et al. 2005). Monoclonal antibodies generated by immunizing spike protein of SERS-CoV or B-cells of CoV-infected person. M396 is a monoclonal antibody that competes with RBD binding (PDB ID: 2DD8) (Prabakaran et al. 2006). Spike-specific monoclonal antibodies 80R and CR301 block S-ACE-2 interactions and neutralize the human SARS-CoV (HKu39849 and Tor2) and palm civet strain infections (Du et al. 2009; Prajapat et al. 2020). However, further work is required to confirm the mechanism of inhibiting SARS-CoV-2 and reducing associated infection.

### 3CLpro and PLpro

The non-structural proteins 3CLpro and PLpro are the major component of SARS-CoV-2 and play an important role in viral replication by translating polyproteins from viral RNA-genome to active functional proteins (Astuti, 2020). Genomes of SARS-CoV-2 are comprised of two open reading frames ORF1a and ORF1b, encoded by host

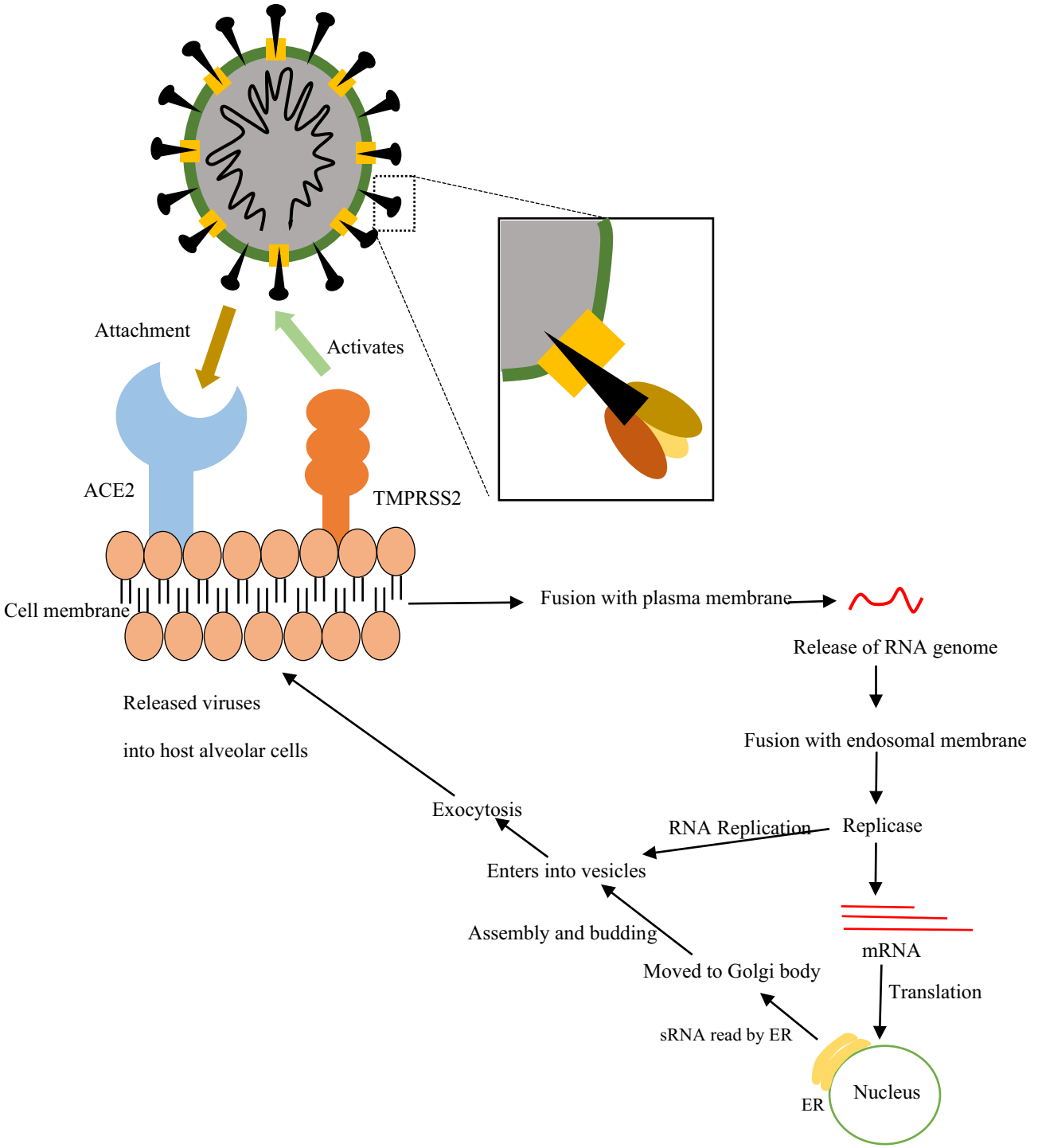


**Fig. 6** Schematic representation of SARS-CoV-2 spike attachment protein using cellular attachment factor ACE2 for its pathogenesis. The S-protein binds with ACE2 by fusing with plasma membrane and releases RNA genome. This leads to replication and initiate exocytosis of virus species inside the host alveolar cells. Green arrow signifies activation/enhancement and the red arrow signifies inhibition/negative impact; blue arrow shows permeability. Abbreviations: ACE-2, Angiotensin-converting enzyme-2; TMPRSS2, Transmembrane protease serine 2; RNA, ribonucleic acid; mRNA, messenger ribonucleic acid; ER, endoplasmic reticulum

ribosomes into two respective viral polyproteins pp1a and pp1ab. ORF1a contains two cysteine proteases, a protease specific to papain (PLpro) and a protease specific to 3CLpro (Othman et al. 2020). Although PLpro cuts the polyprotein's first three cleavage sites, and 3CLpro is accountable for cleavage of subsequent 11 positions

culminating in a sum of 16 non-structural proteins (nsp) released into SARS-CoV-2. The 3CLpro controlled the activities of SARS-CoV-2 replication complex, represents as an attractive target for SARA-CoV-2 therapy. Both 3CLpro crystal structures revealed that each monomer contains structural domains like domains I and II construct of chymotrypsin-like framework with a catalytic cysteine and are linked via an extended loop toward a third C-terminal domain (Al-Tawfiq et al. 2020). 3CLpro monomer domain contains further domain I (residues 8–101), domain II (residues 102–184), and domain III (residues 201–303). The large loop binds to domain II and III (residues 185–200). The effective zone of 3CLpro seems to have a Cys-His catalytic dyad (Cys145 and His41) found at a distinct length between domains I and II (Ulferts et al. 2010). At the proteolytic stage, both 3CLpro carry glutamine at positions P1 and leucine (low hydrophobic residues) at positions P2, P3, and P4 respectively. Limited residues are expected at positions P1′ and P2′; however, position P3′ shows no clear preference. Recently, it has been reported that the structure of 3CLpro from SARS-CoV2 (PDB code 6LU7) and the accessible assembly of 3CLpro from CoV (PDB code 1UK4) contain two main proteases differentiated by only 12 amino acids, with α carbon atoms all present at a distance 1 nm away from the 3CLpro active site (ul Qamar et al. 2020). The substrate-binding pockets of COVID-19 are main proteases that exhibit an amazingly high level of some residues participated in substrate binding, including the CYS145-HIS41 dyad, and HIS163/HIS172/GLU166. The latter residues are supposed to deliver the introductory gateway for the substrate in the active state of the protomer (ul Qamar et al. 2020). Two viral proteases, PLpro and 3CLpro, process ORFs and construct 16 non-structural proteins that are essential for the membrane-associated duplication complex. PLpro has been observed to be multipurpose enzymes with deISGylating (deletion of ISG15 conjugates from host cell factors) and deubiquitinating (cleavage of ubiquitin from host cell factors) properties (Chuck et al. 2010). In addition, the PLpro C-terminus of nsp3 contains transmembrane domains that anchor the dsDNA, unwinding/RNA binding domain, which are essential for replications (Neuman, 2016). PLpro is a most drug targeting area due to their involvement in the viral polyproteins into mature nsp3 and assisting the coronavirus into host immune response by competing interaction with ubiquitin and ISG15 on host-cell proteins (Kouznetsova et al. 2020). Although there is no any protease inhibitor available for treatment of MERS, SARS, and COVID-19 but various studies showed that MERS, SARS-CoV, and SARS-CoV-2 PLpro are underway and evidenced that such protease inhibitors can prevent SARS-CoV-2 replication in cultured cells.

## RNA-dependent RNA polymerase

The RNA-dependent RNA polymerase (RdRp) or nsp12 is a core component of the virus replication and transcription complex. All RNA viruses and some DNA viruses encode RdRp that is required for SARS-CoV-2 transcription, replication, and are involved in synthesis of genomic and subgenomic RNAs (Wang et al. 2021). The RdRp complex of SARS-CoV-2 is consisted of a nsp 12 core catalytic unit, nsp7-nsp8 (nsp8-1) heterodimer, additional nsp8 subunit (nsp8-2), and nsp12 for virus RNA replication (Peng et al. 2020). The polymerase RdRp domain is located on the C-terminus and a retained amino acid sequence of Ser-Asp-Asp (Báez-Santos et al., 2015). RdRp also acts as therapeutic target due to important role in replication of the RNA genome. Furthermore, there is absence of counterpart to RdRp in mammalian cells, and inhibition of this does not cause target-related side effects (Tian et al. 2021). Pharmaceutical companies are still looking to develop effective RdRp inhibitors and block viral replication. There are two known classes of RdRp inhibitors: nucleoside analog inhibitors (NIs) and non-nucleoside analog inhibitors (NNIs) are used for treatments of virus infections (Tian et al. 2021). The well-known RdRp inhibitors are nucleoside analogs such as favipiravir, ribavirin, penciclovir, remdesivir, Sofosbuvir, EIDD-2801, and galidesivir which are under investigation for the treatment of SARS-CoV-2 infection. Remdesivir is a prodrug of an adenosine nucleotide analog, which is under the clinical trial phase III for COVID-19 treatments. Based on clinical trial data, remdesivir got emergency use permit in the United States (US) on May 1, 2020, and a special approval for emergency use in Japan on May 7, 2020 (Lamb, 2020) and in Taiwan in late May 2020 with safety ensure.

Favipiravir is an antiviral drug that selectively and potently inhibits the RdRp of RNA viruses. It undergoes intracellular phosphoribosylation into favipiravir ribofuranosyl-5′-triphosphate (favipiravir-RTP) (Furuta et al. 2017). Active favipiravir-RTP acts as a nascent RNA strand elongation terminator by competing with purine nucleosides for RdRp binding (Sangawa et al. 2013). Some comparative study has found that favipiravir exerts more powerful antiviral activity against COVID-19 due to faster viral clearance and a higher improvement rate in chest imaging than lopinavir/ritonavir-treated patients (Furuta et al. 2017).

Ribavirin show antiviral activity against a wide range of DNA and RNA viruses. Due to broad-spectrum antiviral efficacy of ribavirin, used as an antiviral therapy during the outbreaks of extreme SARS in 2003 and MERS in 2012 (Stockman et al. 2006; Momattin et al. 2013). The National Health Commission of China recommended intravenous infusion of ribavirin (500 mg) in combination with lopinavir/ritonavir or interferon in the most recent COVID-19 diagnosis and treatment plan (Wang et al. 2020).

**Table 1** List of potential therapeutic drugs for COVID-19. Abbreviations: *SARS-CoV-2*, severe acute respiratory syndrome coronavirus-2; *IFNs*, interferons; *IL-6*; interleukin 6; *mAb*, monoclonal antibody; *ACE2*, angiotensin-converting enzyme 2; *RdRP*, RNA-dependent RNA polymerase; *mTOR*, mechanistic target of rapamycin; *i.v.*, intravenous; *p.o.*, per oral; *PaO2*, arterial oxygen partial pressure; $FiO_2$, fractional inspired oxygen

| S. No | Drug name | Other names | Target | Mechanism of action | Tests type and clinical trial ID | Current status and no. of participants enrolled | Dose |
|---|---|---|---|---|---|---|---|
| Drugs inhibit viral replication | | | | | | | |
| 1 | Remdesivir | Veklury | RdRp | Remdesivir specifically targets key viral RNA polymerase proteins that involved in making new copies of the virus and prevents them from working by halting genome replication | In silico, in vitro, humans (237 participants) | Phase III NCT04257656 | 200 mg loading dose on day 1, followed by 100 mg i.v. once daily for 9 days |
| 2 | Molnupiravir | EIDD-2801 | RNA synthesis | Inhibiting viral replication | Humans (80 participants) | Phase II NCT04405739 | Oral capsule twice a day for 5 days |
| 3 | Favipiravir | Favir 200 | RdRp | Inhibits viral replication | In silico, in vitro, humans (676 participants) | Phase III NCT04694612 | 1800 mg/p.o. on day first and followed by 800 mg for 2 days |
| 4 | Ribavirin | DuACT | RdRp | Inhibits viral RNA synthesis and mRNA capping | In silico, in vitro, humans (40 participants) | Phase II NCT04563208 | 400 mg BID for 5 days |
| 5 | Penciclovir | - | RdRp | Inhibits viral replication | - | - | - |
| 6 | Galidesivir | - | RdRp | Inhibits viral RNA polymerase function by terminating non-obligate RNA chain | In silico, in vitro, humans (132 participants) | Phase I NCT03891420 | - |
| 7 | Elbasvir | - | RdRp | Blocks viral replication | In silico | None | - |
| 8 | Cepharanthine | - | Viral RNA | Blocks viral entry and replication | In silico, in vitro | - | - |
| 9 | Sofosbuvir | Mpiviropack Sovaldy | RdRP | Blocks viral replication | In silico, humans (100 participants) | Phase II NCT04497649 | - |
| 10 | Daclatasvir | Daklinza, Daklanork | RdRP | Inhibits 3CLpro | In silico, humans (100 participants) | Phase III NCT04497649 | - |
| 11 | Acyclovir fleximer analogs | - | RdRp | Inhibiting the viral DNA polymerase | In silico | Pre-clinical | - |
| 12 | Sirolimus | Rapamune | mTORC1 | Inhibition of mTORC1 and viral replication | Humans (40 participants) | Phase II NCT04461340 | 6 mg/p.o. on day 1 followed by 2 mg/day for 9 days |
| 13 | Budesonide dry powder inhaler | Pulmicort | Replications | Inhibits viral replications | Humans (146 participants) | Phase II NCT04416399 | 400 µg BID by inhalation route |
| 14 | Clofazimine | - | Replications | Inhibits the replications of SARS-CoV-2 | Humans (81 participants) | Phase II NCT04465695 | 100 mg BID for first day followed by 100 mg OD for 2 days |

**Table 1** (continued)

| S. No | Drug name | Other names | Target | Mechanism of action | Tests type and clinical trial ID | Current status and no. of participants enrolled | Dose |
|---|---|---|---|---|---|---|---|
| Protease inhibitors/drugs inhibit viral entry | | | | | | | |
| 1 | Darunavir and cobicistat | - | Protease | Binds to the site of HIV-1 protease activity and inhibits cleavage of viral Gag-Pol polyprotein precursors into individual proteins | In silico, in vitro, humans (200 participants) | Phase III NCT04425382 | 800 mg/150 mg p.o. OD |
| 2 | Arbidol | - | Spike glycoprotein | Inhibits viral entry | In silico, in vitro, humans (380 participants) | Phase IV NCT04260594 | 2 tablets TID for 14-20 days |
| 3 | Prulifloxacin | - | Proteases | Blocks the active sites or interrupt the dimer formation of viral protein | In silico | None | - |
| 4 | Tegobuvir | - | Proteases | Blocks the active sites or interrupt the dimer formation of viral protein | In silico | - | - |
| 5 | Nelfinavir | - | Proteases | Blocks the active sites or interrupt the dimer formation of viral protein | In silico | - | - |
| 6. | Lopinavir- ritonavir | Kaletra | Protease | Lopinavir/ritonavir are protease inhibitors, which block viral replication. Ritonavir is a CYP3A inhibitor | In silico, in vitro, humans (75 participants) | Phase II NCT04455958 | Lopinavir/ritonavir p.o. BID for 14 days |
| Drugs inhibit cytokine release | | | | | | | |
| 1 | Azithromycin | - | Inhibits viral replication and IL-6 | Azithromycin inhibits translation of mRNA and takes place in protein synthesis action | In silico, in vitro, humans (2271 participants) | Phase III NCT04332107 | 1.2 gm/p.o. OD |
| 2 | Doxycycline | | Cytokines | Inhibitsviral replication and IL-6 production | Humans (400 participants) | Phase III NCT04523631 | 100 mg/p.o. BID for 5 days |
| 3 | Tocilizumab | EMPACTA | IL-6 receptor | Inhibits IL-6 release | Humans (379 participants) | Phase III NCT04372186 | 8 mg/kg; i.v. infusion |
| 4 | Auranofin | - | Viral RNA | Inhibits viral RNA and Cytokines | In vitro | - | - |
| 5 | Ruxolitinib | jakafir | Janus-kinase 1/2 | Inhibits cytokine storm | In silico, humans (80 participants) | Phase II/III NCT04348071 | 10 mg/p.o. BID for 14 days |
| 6 | Baricitinib | LY3009104 | Janus-kinase 1/2 | Inhibits cytokine | In silico, humans (1400 participants) | Phase III NCT04421027 | 4 mg/p.o |

**Table 1** (continued)

| S. No | Drug name | Other names | Target | Mechanism of action | Tests type and clinical trial ID | Current status and no. of participants enrolled | Dose |
|---|---|---|---|---|---|---|---|
| 7 | Dexamethasone | - | Inflammatory cells | Inhibits release of cytokines | In silico, humans (300 participants) | Phase IV NCT04707534 | 20 mg/day for 5 days |
| 8 | Cholecalciferol (Vitamin D) | D-Cure | B and T cells | Inhibits cytokine storm | Humans (100 participants) | Phase IV NCT04636086 | 25,000 IU/ml/day by i.v. route |
| 9 | Zinc | - | T lymphocytes | Boost immune system and show anti-viral activities | Humans (700 participants) | Phase III NCT04641195 | 40 mg/p.o. OD |
| 10 | Vitamin C | - | T cells | Inhibits cytokine release | Humans (600 participants) | Phase II NCT04335084 | 12 gm/i.v. BID for 7 days |
| 11 | Iloprost | Ilomedin | Cytokines | Suppression TNF and IL-6 production | Humans (80 participants) | Phase II NCT04420741 | 1 ng/kg/min. i.v. infusion at 3 ml/hour continuously for 72 h |
| 12 | Sarilumab | REGN88 | IL-6 receptor | Inhibits IL-6 release | Humans (420 participants) | Phase III NCT04327388 | 1st dose by i.v. infusion OD |
| 13 | Siltuximab | Sylvant | IL-6 receptor | Inhibits IL-6 release | Humans (200 participants) | Phase II NCT04329650 | 11 mg/kg i.v. infusion within 1 h |
| 14 | Tocilizumab | Actemra | IL-6 receptor | Inhibits IL-6 release | Humans (402 participants) | Phase II NCT04317092 | 8 mg/kg by i.v. route |
| 15 | Meplazumab | - | CD147 | Inhibition of proinflammatory factors | Humans (456 participants) | Phase II NCT04586153 | 0.2 mg/kg i.v. route |
| Supporting therapy/miscellaneous agents | | | | | | | |
| 1 | Famotidine | Famotac 20 mg | $H_2$ receptor | Inhibits histamine release from activated mast cells | Humans (200 participants) | Phase III NCT04504240 | 20 mg/p.o./day |
| 2 | Nebulized unfractionatedheparin | - | $PaO_2/FiO_2$ ratio | Heparin can reverse the hypercoagulability in severe cases of COVID 19 | Humans (712 participants | Phase III NCT04635241 | 25,000 Units in 5 ml/6 h by the Aerogen Solo vibrating mesh nebulizer |
| 3 | Atorvastatin | Atrovastatin calcium | ACE 2 | Improve endothelial dysfunction | Humans (300 participants) | Phase II NCT04380402 | 40 mg/p.o |
| 4 | 6′Fluorinated-aristeromycin analogs | - | - | Inhibits the activity of RdRp and host cell S-adenosyl-L- homocysteine hydrolase | In silico | Pre-clinical | - |
| 5 | Convalescent plasma | - | Immunity system | Convalescent plasma from cured patients provides protective antibody against SARS-CoV-2 | Humans (80 participants) | Phase III NCT04373979 | 200–230 ml over 2 h for 2 consecutive days |

**Table 1** (continued)

| S. No | Drug name | Other names | Target | Mechanism of action | Tests type and clinical trial ID | Current status and no. of participants enrolled | Dose |
|---|---|---|---|---|---|---|---|
| 6 | Cholchicines | colcorona | NLRP3 inflammasome | Inhibitions of NLRP3 and disruption of cytoskeletal functions by inhibitions of microtubule polymerization | Humans (4506 participants) | Phase III NCT04322682 | 0.5 mg/p.o. BID for 3 days |
| 7 | Epoprostinol | Ventaprost | $PaO_2/FiO_2$ ratio | Improved oxygenation via vasodilating process | Humans (20 participants) | Phase II NCT04452669 | 50 ng/kg/min via mechanical ventilations |
| 8 | Rifampicin | - | DNA dependent RNA polymerase | Inhibition of late stage viral protein synthesis, virion assembly and also suppresses de novo synthesized viral polymerase | In silico, humans | Phase I | 600 mg per day |
| 9 | IMU-838 + Oseltamivir | - | - | Neuraminidase inhibitors | Humans (120 participants) | Phase II NCT04516915 | IMU-838 22.5 mg BID + Oseltamivir 75 mg BID for 14 days |
| 10 | Nafamostat | | Prevents membrane fusion | Inhibits spike-mediated membrane fusion | In vitro, humans (84 participants) | Phase III NCT04418128 | 0.1–0.2 mg/kg/h i.v. infusion |
| 11 | Losartan | Cozaar | Angiotensin II receptor | Block the activity of angiotensin II receptor | Humans (580 participants) | Phase II NCT04311177 | 25 mg/p.o./day |

Daclatasvir and sofosbuvir are well-effective and tolerated antiviral drugs against HCV. Sofosbuvir has a broad antiviral activity against various viruses, including Dengue and Zika virus. Based on experimental in silico and in vitro report that sofosbuvir/daclatasvir and ribavirin binds to RdRp of SARS-CoV-2 (Eslami et al. 2020). The clinivaltrials.gov and Chinese Clinical Trail Registry (ChiCTR) websites show several ongoing randomized controlled trials of RdRp inhibitors, which are mention in Table 1. Some studies has suggested that theaflavin is a natural product, which can be used as a lead compound for developing a SARS-CoV-2 inhibitor via targeting RdRp (Raj et al., 2020). The exact in vivo effect of these drugs is yet unclear, however, and further finding may confirm the mechanism of inhibiting SARS-CoV-2 and reducing associated infections.

### Neuraminidase and M2 ion-channel protein

Neuraminidase plays an important role in cleavage of terminal sialic acid residues from glycoconjugates and is essential for virus replication and infectivity (Akhtar, 2020). Neuraminidase inhibitors (oseltamivir, zanamivir, and peramivir) are not expected to be effective against COVID-19 due to absence of this enzyme in SARS-CoV-2. Moreover, oseltamivir with ganciclovir and lopinavir/ritonavir was found beneficial to treat COVID-19 infections in Wuhan city (Chu et al. 2020; Huang et al. 2020). In silico study also found that combination of oseltamivir-lopinavir-ritonavir c had synergistic effects against SARS-CoV-2 (Muralidharan et al. 2020). In Indonesia and Singapore, oseltamivir is currently being used as a recommended COVID-19 treatment option.

The M2 channel protein is essential viral envelope protein for maintaining pH across the viral envelope, and plays an important role during entry and movement across the trans-Golgi host cell membrane during viral maturation (Skehel et al. 1978). Previous studies have shown that amantadine could block the p7 protein of HCV, which is crucial to form ion channels in host cell membranes (Griffin et al. 2003). In 1973, amantadine was found to have a potent antiviral effect against coronavirus 229E in vitro, and later, it was able to block SARS-CoV's protein-membrane channel activity. Furthermore, amantadine showed good antiviral activity against SARS-CoV-2 (Frediansyah et al. 2020) but more molecular analysis determines its specificity toward particular statin.

## Conclusion and future perspective

SARS-CoV-2 a is single-stranded positive RNA virus and uses several host viral proteins and cellular components to complete its replication cycle, including the steps of viral entry, replication. Development of drug and vaccine against the SARS-CoV-2 is a challenging job due to lack of predictive in vitro and animal model, insufficient knowledge regarding underlying mechanism of action of disease, lack of targets and biomarkers, and a high rate of failed clinical trials. We need to know more structural biology, life cycle details, which can speed up the drug/vaccine development process against SARS-CoV-2. Again, to avoid these types of pandemic insult, strict vigilance of viral infection and understanding of viral protein and enzyme structure are necessary. Several series of small-molecule SARS-CoV-2 inhibitors targeting these protein and enzymes (eIF4A, cyclophilin, nucleocapsid protein, spike protein, ACE2, 3CLpro, and RdRp) have discussed in our article. However, most of them were tested in vitro, while only a small percentage of these compounds have been evaluated in animal study, and few have advanced into clinical trial study. Therefore, further studies should be focused on exploring novel strategies to identify new anti-CoVs compounds, elaborated their mechanism of action, improving the efficacy of anti-CoVs compounds, and evaluating the in vivo efficacy and safety of these compounds in different preclinical and clinical studies. Furthermore, development of small-molecule CoVs inhibitors with high efficacy and low toxicity will be brought for treatment of SARS-CoV-2 infection and related disease in the future.

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**Data availability** Not applicable.

## Declarations

**Ethical approval** This article is a review article, so it does not contain any studies with human participants performed by any of the authors.

**Consent to participate** Not applicable.

**Consent to publish** Not applicable.

**Competing of interest** The authors declare no competing interests.

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