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## Drug targets for corona virus: A systematic review

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### Abstract

The 2019-novel coronavirus (nCoV) is a major source of disaster in the 21<sup>th</sup> century. However, the lack of specific drugs to prevent/treat an attack is a major need at this current point of time. In this regard, we conducted a systematic review to identify major druggable targets in coronavirus (CoV). We searched PubMed and RCSB database with keywords HCoV, NCoV, corona virus, SERS-CoV, MERS-CoV, 2019-nCoV, crystal structure, X-ray crystallography structure, NMR structure, target, and drug target till Feb 3, 2020. The search identified seven major targets (spike protein, envelop protein, membrane protein, protease, nucleocapsid protein, hemagglutinin esterase, and helicase) for which drug design can be considered. There are other 16 nonstructural proteins (NSPs), which can also be considered from the drug design perspective. The major structural proteins and NSPs may serve an important role from drug design perspectives. However, the occurrence of frequent recombination events is a major deterrent factor toward the development of CoV-specific vaccines/drugs.

**Keywords:** Coronavirus, drug targets, Middle East respiratory syndrome, severe acute respiratory syndrome

### Introduction

Coronaviruses (CoVs) have a single-stranded RNA genome (size range between 26.2 and 31.7 kb, positive sense), covered by an enveloped structure.[1] The shape is either pleomorphic or spherical, and it is characterized by bears club-shaped projections of glycoproteins on its surface (diameter 80–120 nm).[1] Among all the RNA viruses, the RNA genome of CoV is one among the largest.[2] The number of open reading frames (ORFs) in the CoV genome ranges from six to ten.[2] CoV genetic material is susceptible for frequent recombination process, which can give rise to new strains with alteration in virulence.[3] There are seven strains of human CoVs, which include 229E, NL63, OC43, HKU1, Middle East respiratory syndrome (MERS)-CoV, severe acute respiratory syndrome (SARS)-CoV, and 2019-novel coronavirus (nCoV), responsible for the infection with special reference to the involvement of the

respiratory tract (both lower and upper respiratory tract), e.g., common cold, pneumonia, bronchiolitis, rhinitis, pharyngitis, sinusitis, and other system symptoms such as occasional watery and diarrhea.[4,5] Among these seven strains, three strains proved to be highly pathogenic (SARS-CoV, MERS-CoV, and 2019-nCoV), which caused endemic of severe CoV disease.[5] The reservoir of SARS-CoV is unknown, but bats and subsequent spread to Himalayan palm civets are hypothesized.[6] MERS-CoV also has a zoonotic origin in the Middle East, and the transmission is through camels.[7] Among these, the SARS-CoV outbreak started in 2003 in Guangdong province of China and the second outbreak of the MERS-CoV outbreak in 2012 in Saudi Arabia.[1,4,6] Previous to these two attacks, CoV was known to cause milder disease, and these two outbreaks highlighted their adaptive potential to the changing environmental conditions and they are classified under “emerging viruses.” Knowledge about the structure, metabolic pathways of CoV, and pathophysiology of CoV-associated diseases is important to identify possible drug targets.[8]

The most important structural proteins of CoV are spike (S) protein (trimeric), membrane (M) protein, envelop (E) protein, and the nucleocapsid (N) protein. Some of the viruses such as beta-CoVs also have hemagglutinin esterase (HE) glycoprotein.[3] The RNA genome of CoV has seven genes that are conserved in the order: ORF1a, ORF1b, S, ORF3, E, M, N in 5' to 3' direction. The two-third part of the RNA genome is covered by the ORF1a/b, which produces the two viral replicase proteins that are polyproteins (PP1a and PP1ab).[9] Sixteen mature nonstructural proteins (NSPs) arise from further processing of these two PPs. These NSPs take part in different viral functions including the formation of the replicase transcriptase complex. The remaining genome part of the virus encodes the mRNA which produces the structural proteins, i.e., spike, envelope, membrane, and nucleocapsid, and other accessory proteins.[9] Another important envelop-associated protein which is expressed by only some strains of CoV is the HE protein.[10] The RNA genome of CoV is packed in the nucleocapsid protein and further covered with envelope.[11]

## Molecular Basics of Transmission of Coronavirus

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In case of SARS-CoV, transmission is through droplet infection (respiratory secretions) and close person-to-person contact.[11,12] It can also spread through sweat, stool, urine, and respiratory secretions.[13] When virus enters into the body, it binds to the primary target cells such as enterocytes and pneumocytes, [11,12] thereby establishing a cycle of infection and replication. Other target cells of CoV are epithelial renal tubules, tubular epithelial cells of kidney, immune cells, and cerebral neuronal cells.[11,12]

CoV attaches to the target cells with the help of spike protein–host cell protein interaction (angiotensin converting enzyme-2 [ACE-2] interaction in SARS-CoV[14] and dipeptidyl peptidase-4 [DPP-4] in MERS-CoV[15]). After the receptor recognition, the virus genome with its nucleocapsid is released into the cytoplasm of the host cells. The viral genome contains ORF1a and ORF1b genes, which produce two PPs that are pp1a and pp1b,[16] which help to take command over host ribosomes for their own translation process.[17] Both pp1a and pp1b take part in the formation of the replication transcription complex.[16] After processing of PP by protease, it produces 16 NSPs. All NSPs have their own specific functions such as suppression of host gene expression by NSP1 and NSP2, formation of a multidomain complex by NSP3, NSP5 which is a M protease which has role in replication,[17] NSP4 and NSP6 which are transmembrane (TM) proteins,[18] NSP7 and NSP8 which act as a primase,[16] NSP9 – a RNA-binding protein, the dimeric form of which is important for viral infection. Induction of disturbance to the dimerization of NSP9[19] can be a way to overcome CoV infection.[20] NSP10 acts as a cofactor for the activation of the replicative enzyme.[21] NSP12 shows RNA-dependent RNA polymerase activity, NSP13 shows helicase activity, NSP14 shows exoribonuclease activity, NSP15 shows endoribonuclease activity, and NSP16 has methyltransferase activity.[18] All NSPs have an important role in replication and transcription.[18]

Synthesized proteins such as M, E, and S are entered into the endoplasmic reticulum (ER)-Golgi intermediate compartment (ERGIC) complex and make the structure of viral envelope.[22] On the other hand, the replicated genome binds to N protein and forms the ribonucleoprotein (RNP) complex. The outer cover is formed by the M, E, and S proteins.[22] Finally, the virus particle comes out of the ERGIC by making a bud-like structure.[23] These mature virions form a vesicle, which fuses with the plasma membrane and releases the virus particles into the extracellular region.[23,24] The detailed structure of

CoV and its life cycle is depicted in Figures [1](#) and [2](#). On infection, the SARS-CoV and MERS-CoV cause a surge of pro-inflammatory cytokines and chemokines, which cause damage to lung tissue,[\[13\]](#) deterioration of lung function, and then finally lung failure in some cases.[\[25\]](#)

Currently, there is no specific antiviral drug for the treatment of CoV-associated pathologies. Most treatment strategies focus on symptomatic management and supportive therapy only.[\[26,27\]](#) Some therapeutic agents that are under development or being used off-label are ribavirin, interferon (IFN)- $\alpha$ , and mycophenolic acid.[\[7\]](#) There are many newspaper articles citing effectiveness of anti-HIV drugs: ritonavir,[\[28,29\]](#) lopinavir,[\[29\]](#) either alone or in combination with oseltamivir,[\[29\]](#) remdesivir, and chloroquine;[\[28\]](#) and among these, ritonavir, remdesivir, and chloroquine showed efficacy at cellular level[\[28\]](#) which further need experimental support and validation.

As there is no well-defined therapy available, which specifically targets CoV, in this article, we have reviewed the possible protein structures, which could be potential targets for the development of a therapeutic approach for the treatment of CoV.

## Materials and Methods

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### Database screen

We screened PubMed and RCSB database with the keywords HCoV, NCoV, corona virus, SERS-CoV, MERS-CoV, 2019-nCoV, crystal structure, X-ray crystallography structure, NMR structure, target, and drug target till Feb 3, 2020. The database files were extracted using endnote, and title and abstract screening was done using Rayyan QCRI. Full texts of these screened articles were further screened for possible inclusion in the systematic review. Articles that evaluated different druggable targets of CoV and evaluated different therapeutic measures against some identifiable target were included for further review.

## Results and Discussion

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A total of 392 articles were found after preliminary screening of the databases. After title and abstract screening, a total of 230 articles were excluded. Full-text screening of the remaining 154 articles was done. Among these studies, after full-text screening, a total of 122 articles were included in the final review. The PRISMA flowchart of the study is shown in [Figure 3](#). Thirty-two articles were excluded after full-text screen (review articles = 7, articles not specifying drug targets against CoV = 22, articles in other language other than English = 3). Details of studies with important structural and functional target proteins are summarized in [Table 1](#).

### Spike protein

The spike protein is a clove-shaped, type I-TM protein.[\[2\]](#) The spike protein has three segments that are ectodomain (ED) region, TM region, and intracellular domain, which comprises the intracellular short tail part.[\[2\]](#) The receptor-binding S1 domain (three S1 heads) and the membrane fusion subunit S2 (trimeric stalk) on C-terminal together comprise the ED. Spike proteins gather in the trimeric form on the outer surface of the virion, giving it the appearance of a crown, due to which it is called CoV.[\[2\]](#) The spike protein plays an important role in virus entry into the host.[\[10\]](#) Initial interactions between the S1 domain and its host receptor (ACE2 in case of SARS-CoV and PP 4 In case of MERS-CoV) and subsequent S2 segment mediated fusion of the host and viral membranes allow the CoV- RNA genome to enter inside the host cells and thus, these proteins represent as important targets from drug discovery side.[\[10\]](#) The spike protein also activates the immune response of the host cell toward CoV.[\[10\]](#)

### S1 domain

The main components of the S1 domain are the N-terminal domain (NTD) and the C-terminal domain (CTD). The S1 domain acts as a major antigen on the surface of the virus[\[40\]](#) and has a receptor-binding domain (RBD).[\[25\]](#) The 18 residues of ACE-2 interact with the RBD (contain 14 amino acids) of SARS-CoV spike protein,[\[45\]](#) and for this contact, K341 of ACE-2 and R453 residue of RBD play the most important role. If point mutated on the D454 or R441 of RBD, it disturbs the binding activity with ACE-2.[\[25\]](#) The S1 domain interacts with the ACE-2 or DPP-4 receptors of the host. Anti-ACE-2 antibody

blocked viral entry and replication in Vero E6 cells.[14,45] One another mechanism of virus for binding to host cell is using dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN receptor) or L-SIGN in lymph nodes or in liver.[46,47] S protein has seven (109, 118, 119, 158, 227, 589, and 699) glycosylation asparagine-linked sites, which is pivotal for both L-SIGN- or DC-SIGN-based virus entry into the host.[48]

## S2 subunit

The S2 subunit has two heptad repeat regions (HR 1 and 2) and hydrophobic fusion peptide.[25]

## Drug designing strategies targeting S protein and its interactions

The RBD is targeted in many drug designing studies.[25] A peptide sequence with sequence similarity to the RBD of S protein hampered S1-RBD: ACE-2 interaction and prevented entry of SARS-CoV into Vero cells (IC<sub>50</sub> around 40 μM).[25,49,50]

A SARS-CoV RBD-specific antibody (FM6) failed to inhibit the occurrence of infection.[39]

OC43-HR2P, a peptide derived from heptad repeat 2 regions of S2 domain of HCoV-OC43 and its optimized form EK1, showed pan-CoV fusion inhibition property.[39] The structure (protein data bank [PDB] ID 5ZUV and 5ZVM) shows a stable 6-helix bundle structure with α-HCoV and long β-HCoV-HR1 domain.[39]

Chloroquine, an antimalarial agent, inhibits SARS-CoV by elevation of endosomal pH and alters the terminal glycosylation of ACE-2, which ultimately interferes with the virus receptor binding.[51]

Other inhibitors SSAA09E2 block the S-ACE2 interaction, SSAA09E1 inhibits the host protease cathepsin L (which is important for viral entry), and SSAA09E3 prevents fusion of host and viral cell membrane. [52]

Kao *et al.* identified 18 small molecules that targeted the S-ACE-2-mediated entry of virus into human cell.[53] In 293T cells expressing ACE-2, one of these agents (VE607) showed a significant inhibition of SARS-pseudovirus entry.[53] In Vero E6 cells, two other molecules tetra-O-galloyl beta-D-glucose and luteolin also inhibited SARS-pseudovirus and SARS-CoV infection.[53] In virus-infected Vero E6 cells, a siRNA against the S sequences of SARS-CoV inhibited SARS-CoV replication.[25,54]

The S230 antibody (origin: memory B-cells of SARS-CoV-infected persons) neutralizes wide spectrum of isolates of SARS-CoV.[55] S230 antibody Fab fragment binds to the SARS-CoV complex to neutralize it, and their structures are also available (PDB IDs: 6NB6, 6NB7, and 6NB8.[55] The monoclonal antibody, m396, has a competitive role for RBD binding (PDB ID: 2DD8).[56]

Monoclonal antibody can be generated by immunizing the spike protein of SARS-CoV (transgenic mice) or from the B-cells of CoV-infected persons.[25] Spike-specific monoclonal antibodies 80R and CR301 block the S-ACE-2 interactions and thus neutralize infection by human SARS-CoV (HKu39849 and Tor2) and palm civet strain (SZ3).[25]

Mice vaccinated with SARS-n DNA showed T-cell immune response (both induction and proliferation), [57] and cytotoxic T-cell response was seen against SARS-DNA-transfected alveolar epithelial cells.

## Envelop protein (E)

The E protein is the smallest (8.4–12 kDa size) TM structural protein of CoV.[58,59] Two distinct domains comprise the E protein: the hydrophobic domain and the charged cytoplasmic tail. However, the structure is highly variable among different members of the CoV family.[59]

The E protein has a special role in viral morphogenesis, especially during assembly and egress.[59] CoVs lacking E protein show lower viral titer, immature, and inefficient progenies.[58,60] Oligomerization of E proteins leads to the formation of ion channels.[61] However, the importance of these ion channels is still not clear. Many other studies infer that the E protein acts in coordination with other intracellular proteins and modulates the activity of those proteins.[59] E protein also acts as a virulence factor.[59] E protein has

an important role in CoV assembly and budding formation.[24] Apart from this, E protein found around the ER and Golgi body regions.[60] Hexamethylene amiloride blocks this E protein-associated ion channel activity in the mammalian cells expressing SERS-CoV envelop protein.[62]

### Membrane protein

Maintenance of the shape of the viral envelope is the most important function of the M protein,[60] and the M protein performs this job by interacting with other CoV proteins,[63] incorporation of Golgi complex into new virions,[60] and stabilization of nucleocapsid protein.[60]

The M protein is characterized by three TM domains[64] with C-terminal inside (long) and N-terminal (short) outside.[63] The details of the protein structure is available in UniProt.[65] Through multiple protein–protein interactions, the M protein plays a crucial role in viral intracellular homeostasis.[60] Interaction between M–M, M–S, and M–N proteins takes a special part in viral assembly.[60] The M–S interactions are necessary for the interaction of spike protein in the ERGIC complex, also known as the Golgi complex, which is later incorporated into new viral progenies.[60] The M–N interactions are crucial for the stabilization of the RNP complex (nucleocapsid–RNA complex), which forms the viral core.[60] The M protein and the N protein are the major viral envelope proteins, defining viral shape, but it also takes part in the formation and release of virus-like particles.[60]

M protein also takes part in the sensitization of the host by the virus.[66] The M protein of SARS-CoV activates the nuclear factor kappa pathway and IFN-beta pathway, through a Toll-like receptor-dependent mechanism. Again, a mutated M protein (V-68) failed to illicit an IFN-beta response.[66]

Mice vaccinated with SARS-M DNA showed T-cell immune response (both induction and proliferation), [57] and cytotoxic T-cell response was seen against SARS-DNA-transfected alveolar epithelial cells.

### Nucleocapsid protein (N)

The structure of nucleocapsid protein (N protein) is conserved across different members of the CoV family. The three characteristic intrinsically disordered regions (IDRs) of the nucleocapsid (N) protein are the N-arm, central linker (CL), and the C-tail.[4] The NTD and the CTD are the major structural and functional domain of the nucleocapsid protein. The most important function of the N protein NTD is RNA binding, while the primary job of the CTD is dimerization.[4,9] As the CL region is rich in arginine and serine residue content, it also contains a large number of phosphorylation sites.[26] The C-terminal IDRs take an important part in nucleocapsid protein oligomerization and N–M protein interactions.[67]

Formation and maintenance of the RNP complex are the most important functions of the N protein.[9] It also regulates the replication and transcription of viral RNA, and in the host, it inhibits protein translation through EF1 $\alpha$ -mediated action,[9] alteration of host cell metabolism, host cell cycle (N proteins are reported to inhibit CDK4), and apoptosis.[3,9] In human peripheral blood, N protein inhibits cell proliferation through the inhibition of cytokinesis.[68]

The NTD contains sites for RNA binding. The RNA-binding sites on the NTD of N protein were identified by observing its interactions with ribonucleoside 5'-monophosphates (AMP, UMP, CMP, and GMP).[26] Using the information about interaction between AMP and UMP binding to the NTD of nucleocapsid protein, inhibitors of RNA binding were designed. Three-dimensional structure with all complex can see from PDB that is 4LMC, 4LM9, 4LM7, and 4LI4, respectively.[26] One such molecule which was designed with this strategy is N-(6-oxo-5,6-dihydrophenanthridine-2-yl) (N, N dimethyl amino) (PJ34), which was designed using the HCoV-OC43 model.[26] Binding of PJ34 on NTD affects the genome binding and replication process of CoV.[26] The crystal structure of COV-OC43 N-NTD with inhibitor PJ34 complex is given in PDB ID: 4KXJ.[26] On the basis of interactions between PJ34 and NTD of nucleocapsid protein, another inhibitor was designed that is H3 (6-chloro-7-(2-morpholin-4-yl-ethylamino) quinoxaline-5,8-dione), which also inhibits RNA binding.[26,69] This highlights the importance of NTD in RNA binding. Some of the herbal products, such as catechin gallate and gallic acid (both are polyphenolic compounds), have shown the inhibitory action against SARS-CoV.[70]

The CTD of N protein has a primary role in oligomerization, especially the C-terminal end. A C-terminal tail peptide sequence N377–389 competes with the oligomerization process and significant inhibition of viral titer was seen at 300  $\mu\text{M}$  concentration.[71]

N220, which is a nucleocapsid protein peptide, showed a high binding affinity to human MHC-1 in T2 cells, and the peptide sequence was successful in activating T-cells (cytotoxic). In transgenic animals, the peptide further showed potential to selective killing of nucleocapsid protein expressing cells and is a potential candidate for DNA vaccine.[72] Other N protein-targeted peptides of importance are NP111, NP331, and NP351.[72,73]

## Proteases

The SERS-CoV genome encodes a number of proteins. The replicase gene, which is a major component of the CoV genome encoded for 16 NSPs in the form of two large PPs (PP1a and PP1ab).[74] Two types of cysteine proteases act on these PPs to release the NSPs. The C-terminal end of these PPs is cleaved by chymotrypsin-like cysteine protease (main protease [ $\text{M}^{\text{pro}}$ ] or 3C-like protease [3CLpro]) and the N-terminal end is processed by the  $\text{M}^{\text{pro}}$  (also known as papain-like protease [ $\text{PL}^{\text{pro}}$ ]).[74] The first three cleavage sites of the PPs is cut by  $\text{PL}^{\text{pro}}$  while the rest 11 sites are cleaved by CLpro, and this cleavage results in release of 16 NSPs.[75]

### 3C-like protease

The 3CLpro is present in homodimer form and has cys-his dyad on active site which shows protease activity.[27] If mutated on the Ser139 and phe140 positions, it abolishes the dimerization of 3CLPro (PDB ID: 3F9G).[76] This protease can cleave 11 sites in the p1 position of PP1a and PP1ab and can produce a mature protein that anchors the replication/transcription complex[3,77] and also releases the mature NSPs.[78]

N-(benzo[1,2,3]triazol-1-yl)-N-(benzyl) acetamido phenyl) carboxamides are also found to be important inhibitors of CLPro. The structure of CLPro inhibitor is with ML188 ( $\text{IC}_{50}$  1.5  $\mu\text{M}$ ) is reported (CID: 46897844, PDB ID: 3V3M).[79,80] Another structure with CLPro inhibitor ML300 (PDB ID: 4MDS,  $\text{IC}_{50}$ : 6.2  $\mu\text{M}$ ) is reported.[79] Some metal-conjugated and peptidomimetic compounds showed inhibitory activity against 3CLpro.[77] Some of the small molecules also act as an inhibitor that is arylboronic acids, quinolinecarboxylate derivatives, thiophenecarboxylate, and phthalhydrazide-substituted ketoglutamine analogs.[77] Some flavonoids are also reported to inhibit  $\text{M}^{\text{pro}}$ . [75] GC376 also has protease inhibitor activity.[81] A crystal structure of  $\text{M}^{\text{pro}}$  with small molecule inhibitor N3 is also reported (PDB ID: 2AMQ).[82] Lopinavir and ritonavir, which are the inhibitors of HIV protease, also inhibit  $\text{M}^{\text{pro}}$ . [83] *In silico* studies directed that among commercially available drugs, colistin, valrubicin, icatibant, bepotastine, epirubicin, epoprostenol, vapreotide, aprepitant, caspofungin, and perphenazine also bind to the lopinavir/ritonavir-binding site on CoV.[83]

### Papain-like protease

The  $\text{PL}^{\text{pro}}$  cleaves the N-terminal region of the PP to generate three NSPs (NSP 1, 2, and 3).[3,74]  $\text{PL}^{\text{pro}}$  has a catalytic core domain that contains 316 amino acid, which is responsible for cleaving replicase substrates, and a consensus sequence LXGG was required for cleavage.[78] Higher doses of zinc and zinc conjugates were found to inhibit both types of SARS protease (CLpro and PLpro).[84] Benzodioxole can inhibit the  $\text{PL}^{\text{pro}}$  enzyme. The crystal structure of interaction is shown in PDB ID: 4OVZ, 4OWZ.[31] Through *in silico* approach, another new lead was identified (6577871) which was further optimized, and compound 15h (S configuration, enzyme  $\text{IC}_{50}$  =0.56  $\mu\text{M}$ , antiviral  $\text{EC}_{50}$  =9.1  $\mu\text{M}$ ) and 15g (R configuration, enzyme  $\text{IC}_{50}$  =0.32  $\mu\text{M}$ ; antiviral  $\text{EC}_{50}$  =9.1  $\mu\text{M}$ ) were found to be the most important inhibitors.[32] The crystallized structural details of these interactions can be visualized in the PDB database (PDB ID: 2FE8 and 3E9S).[32]

Many of the protease inhibitors are being used in the treatment of COVID-19, e.g., lopinavir–ritonavir combinations.[85]

## Hemagglutinin esterase

This HE enzyme is present in the envelope of CoV, more specifically among beta-coronaviridae.[86] The HE is a marker of CoV and influenza virus evolution.[86] HE mediates reversible attachment to O-acetylated-sialic-acids by acting both as lectins and as receptor-destroying enzymes.[86] Interactions between HE in complex with sialic acid can be visualized in PDB ID: 3CL5.[86]

## NTPase/helicase

NTPase/helicase plays an important role in the central dogma of the virus.[87] SARS-CoV helicase enzyme is a member of the SF1. This enzyme prefers ATP, dATP, and dCTP as substrates; it also hydrolyzed all NTPs.[88] Toxicity issues are main obstacles in the development of inhibitors of helicase, and nonspecificity of inhibitors may cause serious toxicity.[87] However, despite theoretical limitations, helicase is being increasingly recognized as a druggable target for different disease conditions.[89]

## Other Strategies to Counter Coronavirus: Endosomal pH

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Once entered into the host cell, the subsequent life cycle of SERS-CoV requires low pH.[90] Inhibitors of pH-sensitive endosomal protease block CoV infection.[90,91] Several different small compounds and molecules have been reported against virus infection. Amiodarone gets accumulated in the acidic organelles. Vacuoles on exposure to amiodarone shows alteration in intracellular organelles especially enlargement of late endosomes. In *in-vitro* environment, amiodarone inhibited coronavirus infection in Vero cells.[92] At priori trypsin, cleavage of S protein is essential for a successful viral entry. However, trypsin cleavage also does not affect the efficacy of amiodarone.[92]

## 2019-Novel Coronavirus: Challenges

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In the RCSB database, only one PDB (PDB ID: 6LU7) is there on the 2019-nCoV which is in complex with N3 (inhibitor). The complete sequence of the 2019-nCoV is available.[93] However, it is only 95% similar to bat-SL-CoVZC45 and 88% to SIRS CoV-ZSc (nucleotide blast, NCBI). This highlights the amount of recombination processes or changes that occurred in the 2019-nCoV and changes in protein structural and functional levels.

## Clinical Trial Update on 2019-nCoV

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A total of 233 trials are registered till date in the Chinese Clinical Trial Registry[94] (dated Feb 24, 2020, keywords 2019-nCov and COVID-19). Among the pharmacotherapeutic agents evaluated, some of the highlighted agents, which are being evaluated, are high-dose Vitamin C, favipiravir, adalimumab, dihydro-artemisinin piperazine, leflunomide, dipyridamole, chloroquine or hydroxychloroquine, suramin sodium, lopinavir/ritonavir and arbidol (umifenovir) tablets, and IFN-alpha 2b. Other important agents being evaluated are Huo-Shen particles, Xiyanping injection, Shen-Fu injection, etc., many of which are from traditional Chinese medicines background. Use of stem cells is also evaluated frequently.[94]

## Conclusion

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Drug discovery against the CoV is a challenging job owing to frequent recombination events. The development of a vaccine is another important aspect. We need more structural biology details and details of the life cycle of the CoV, which can speed up the drug/vaccine development process against CoV. Again, as a preventive measure, strict vigilance of viral changes in different hosts for prediction of an event is important.

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## Conflicts of interest

There are no conflicts of interest.

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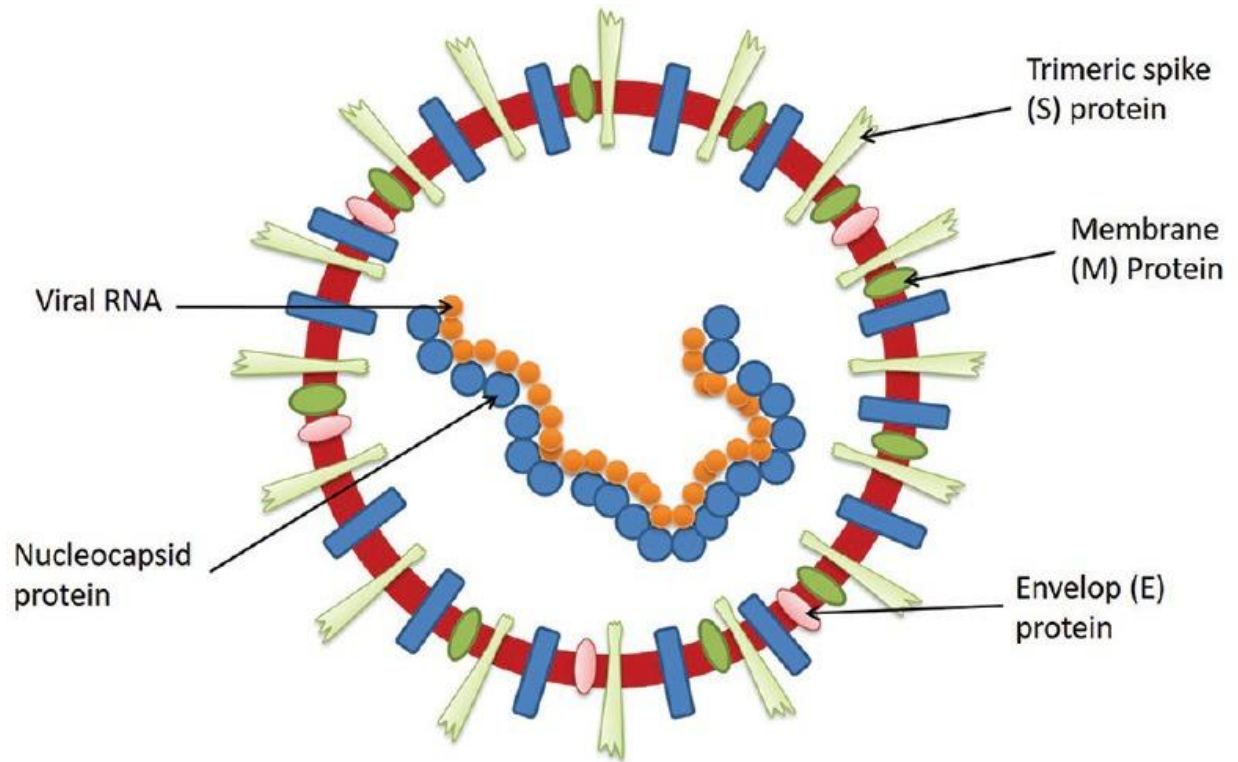
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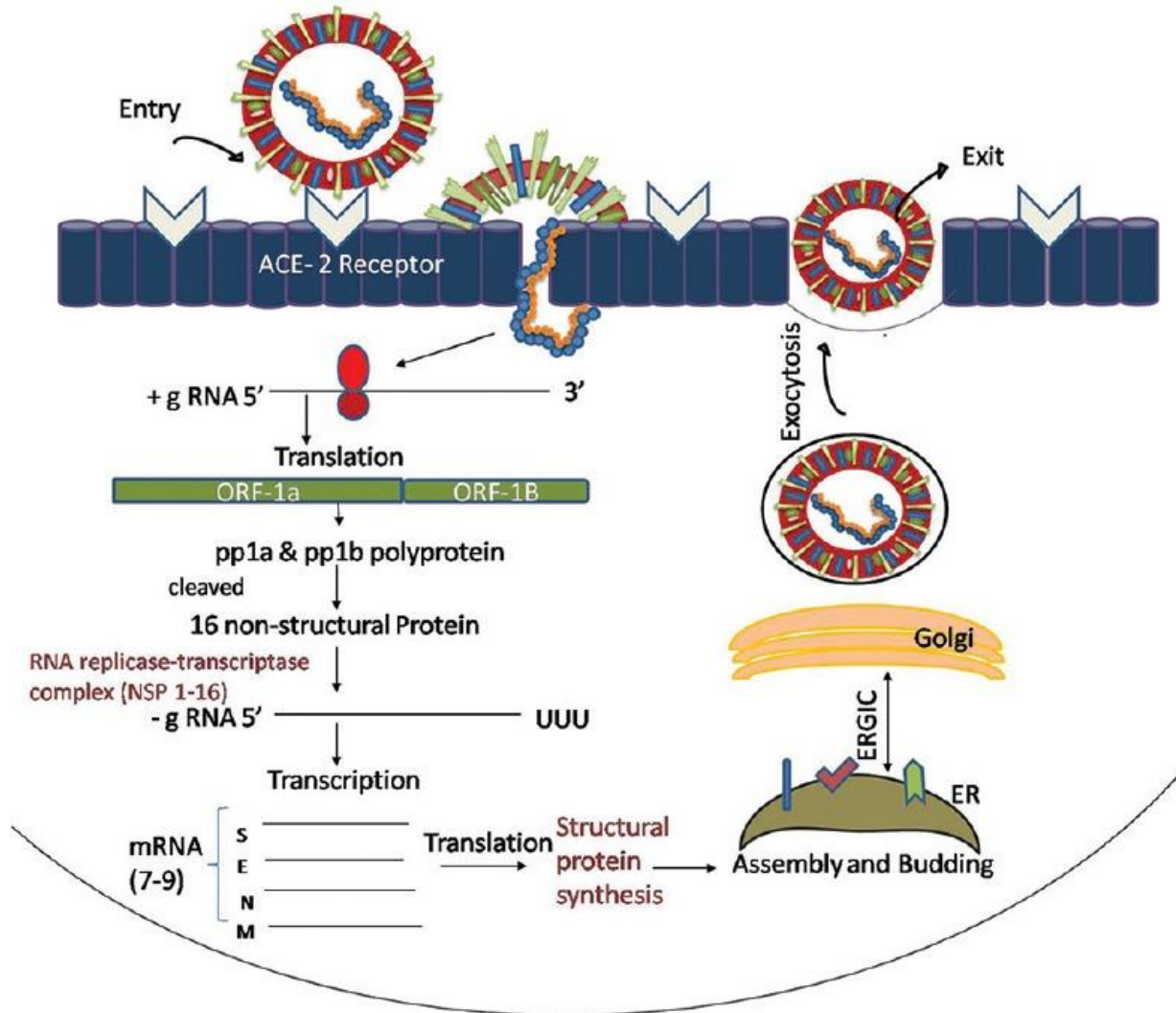
## Figures and Tables

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**Figure 1**

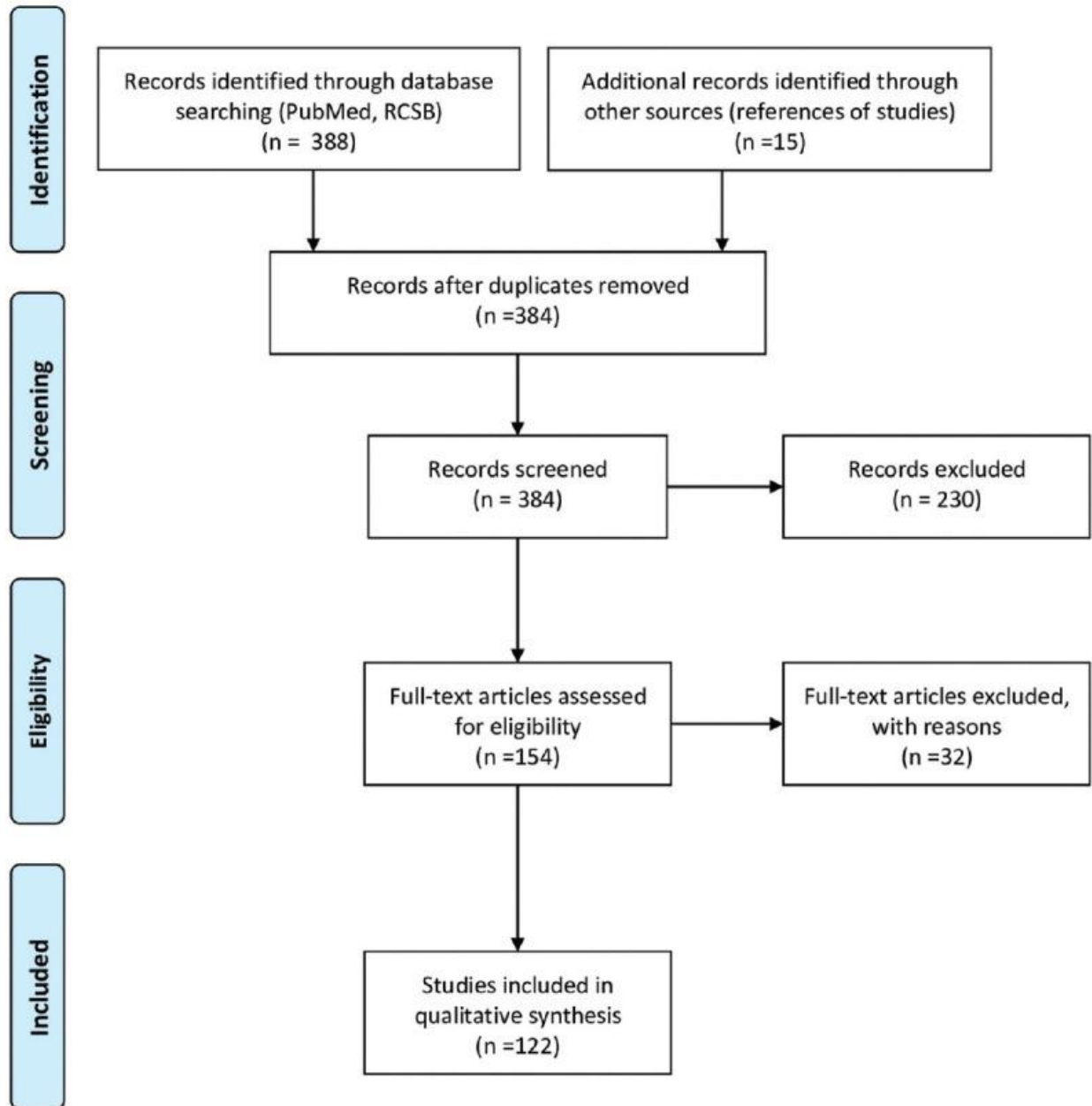
Structural details of Coronavirus

Figure 2



The life cycle of CoV in host cells. The S proteins of CoV binds to cellular receptor angiotensin-converting enzyme 2 (ACE2) which is followed by entry of the viral RNA genome into the host cell and translation of structural and non structural proteins (NSP) follows. ORF1a and ORF1ab are translated to produce pp1a and pp1ab polyproteins, which are cleaved by the proteases that are encoded by ORF1a to yield 16 non-structural proteins. This is followed by assembly and budding into the lumen of the ERGIC (Endoplasmic Reticulum Golgi Intermediate Compartment). Virions are then released from the infected cell through exocytosis. S: spike, E: envelope, M: membrane, N: nucleocapsid. PP: polyproteins, ORF: Open reading frame, CoV: coronavirus

Figure 3



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Flowchart



**Table 1**

Details of studies representing protein database structures of major targets in coronavirus and their structures

PDB ID	Details	Inhibitor	IC <sub>50</sub>	Reference
<b>N protein</b>				
4KXJ	Interaction between PJ34 and NTD of N protein of HCoV-OC43	PJ34	-	[26]
3V3P	Structure not released			[30]
4LM7	Interactions of NTD of N protein of HCoV-OC43 with UMP	-		[26]
4LI4	Interactions of NTD of N protein of HCoV-OC43 with AMP	-		[26]
<b>Protease</b>				
4TWY	3CLPro of SARS-CoV with an inhibitor	3BL		[27]
4TWW	3CLPro of SARS-CoV with an inhibitor	41	63 μM	[27]
4WY3	3CLPro of SARS-CoV with an inhibitor	3X5	240 μM	[27]
4OVZ	CoV PLPro complexed with inhibitor	P85	490 nM	[31]
3MJ5	SARS-CoV PL <sup>Pro</sup> complexed with inhibitor	GRM	320 nM	[32]
2FE8	SARS-CoV PL <sup>Pro</sup>	-	-	[33]
1UK4	SARS-CoV 3CL <sup>Pro</sup> and its interactions with an inhibitor	Substrate analog hexapeptidyl CMK inhibitor	IC <sub>50</sub> ca. 2 mM	[34]
1UJ1, 1UK3, 1UK2	SARS-CoV M-pro, apo-enzyme at different pH	-	-	[34]
3VB6	SARS-CoV 3CLPro in complex with C6Z	C6Z	39 μM	[35]
3VB5	SARS-CoV 3CLPro with C4Z	C4Z	1.3-4.6 μM	[35]
3TLO	HCoV-NL63 3CLPro	-	-	[36,37]
6LU7	Main protease of 2019-nCoV and its complex with N3 (inhibitor)	-	-	[38]
<b>Spike protein</b>				

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NTD=N-terminal domain, CoV=Coronavirus, 3CLPro=3C-like protease, PL<sup>Pro</sup>=Papain-like protease, MERS=Middle East respiratory syndrome, SARS=Severe acute respiratory syndrome, ACE-2=Angiotensin converting enzyme-2, RBD=Receptor-binding domain, nCoV=Novel coronavirus, S protein=Spike protein

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