REVIEW ARTICLE

SARS-CoV-2: Pathophysiology, Prophylaxis and Treatment Opportunities – A Current Review

M. Mohan[1*], V.Aroulmoji[1], M.Manohar[2], P.Ganesh[3], P. Rekha[4], Shreyas Saride[5], Riaz Khan[6]

[1]Centre for Research & Development, Mahendra Engineering College (Autonomous), Mallasamudram, Namakkal (Dt.) - 637 503, Tamil Nadu, India.
[2]Department of Microbiology, Sadakathullah Appa College (Autonomous),Tamil Nadu, India.
[3]Department of Microbiology, Faculty of Science, Annamalai University, Chithamparam,Tamil Nadu, India.
[4]Department of Physical Sciences, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Chennai, 602105 Tamil Nadu, India.
[5]School of Behavioral and Brain Sciences, The University of Texas at Dallas, Richardson, TX, United States (USA)
[6]Rumsey, Old Bath Road, Sonning, Berkshire, RG4 6TA, England, United Kingdom.

ABSTRACT: Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) was emerged in 2003, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) got reported in the year 2012. Following, SARS-CoV-2 was observed as pandemic around the globe in the end of 2019. COVID-19 has produced significant rate of morbidity and mortality rate causing severe loss of life. An immediate attention is required to find out active therapeutic methods and appropriate vaccines to control this epidemic condition. This is the crucial time to find out the lead compounds for the inhibition of SARS-CoV-2 by targeting its enzymes. The present review will make it possible to better understand the pathogenesis caused by the virus through their various proteins and finding path for the development of effective new drug targets and vaccines for this newly raised pathogen. In order to achieve these objectives, the current review will focus on the biology of the virus and describe the chemistry of a cellular targeted drug delivery pattern of two novel drug conjugates: HA-2-Deoxy-D-Glucose and HA-Hydroxychloroquine for treatment of COVID-19.

KEYWORDS: SARS-CoV-2, phylogeny, pathogenesis, Drug Conjugation, Structural Proteins, drug targets, cellular targeted drug target pattern, Hyaluronic acid.

https://doi.org/10.29294/IJASE.9.1.2022.2637-2657

INTRODUCTION

The COVID-19, a viral disease, was initiated in February 2020 and accelerated in the beginning of March 2020. Globally, COVID-19 has caused serious health, social, trade, and economic setback. In response to this severe condition, it is the vital challenge for the scientists to invent appropriate drugs and vaccines to keep the pandemic under control. They have produced a number of COVID-19 drugs and vaccines but as yet, there is no definite prescription of drugs for the disease. This important issue must be solved immediately to avoid any more social and economic encumbrance.

Coronaviruses (CoVs) are a large group of viruses, enveloped with positive-sense, single-stranded RNA genomes. SARS-CoV-2 is the seventh type of coronavirus [1]. Coronaviruses have gained regency due to the global pandemic, as its rapid infection rate has caused worldwide disruptions [2]. The most common symptoms reported like fever (83%), cough (82%), and shortness of breath (31%) [3], other symptoms includes [4] fatigue, muscle/body aches, the new loss of taste or smell, and nausea. Unfortunately, the pandemic has had an impact beyond patients and their families. Social distancing and self-isolation have contributed too many travel restrictions and reduced workforce leading to many short-term and long-term economic issues throughout the world [5]. Also, the pandemic was a major threat to health care systems around the globe. Many areas had resources and staff diverted to focus on adequate testing and

**Corresponding Author: mohanm@mahendra.info*
Received: 18.06.2022 **Accepted:** 15.07.2022 **Published on**: 01.08.2022

treatment for COVID-19. This disruption has led to decreased access to other forms of care, surgeries, and hospital services [6].

The coronaviruses are divided into four genera like alpha, beta, gamma, and delta [7,8]. The alpha and beta coronaviruses are causing only common cold like illness. But in contrast SARS-CoV and MERS-CoV are posing life-threatening disease and potentially pandemic [9,10]. World Health Organization (WHO) have reported the SARS-CoV emerged during 2002-2003 in China and rapidly extended around the world caused more than 8000 infections and nearly 800 deaths [11]. MERS-CoV was reported in 2012 around the Middle East and noticed in other countries [12]. Initially the virus infection is started with the binding of viral particles to the cellular receptors of the host surface. The recognition of the receptor is the determinant of the viral tropism on the cell. The receptor of the human coronaviruses is proteinaceous peptidases. Specifically, human aminopeptidase N (hAPN) is the receptor for HCoV – 229E and human dipeptidyl peptidase 4 (hDPP4) for MERS CoV [13,14]. The SARS-CoV and SARS-CoV-2 enter into the cell by the interaction of human angiotensin-converting enzyme 2 (hACE2) [15, 16]. The present review will attempt to examine the current status of the pandemic *vis a vis* the biology of this viral disease and what has been achieved in terms of efficacious medicines without any toxicity and side effects in order to cure SARS-CoV-2 (Figure 1).

Phylogeny of the virus

Phylogenetic analysis had revealed that the bats might be the source for SARS-CoV-2, there is a another intermediate host is involved in between these zoonotic pathway. CoVs are belonging to the order Nidovirales and family Coronaviridae. It can be divided into four genera: the alpha, beta, gamma, and delta coronaviruses. Zhou et al., [17] showed that the bat coronavirus sequence is 96.2% similar with the human coranavirus. In the genomes of SARS-Cov-2, [18] found that there are three variants by distinguished amino acid changes in which two types are significant at outside East Asia and one another type is common type in East Asia. The researchers were used the data for the phylogenetic study was obtained from the Global Initiative on Sharing All Influenza Data (GISAID) database (https://www.gisaid.org/) possessing 253 compilations of genes related to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

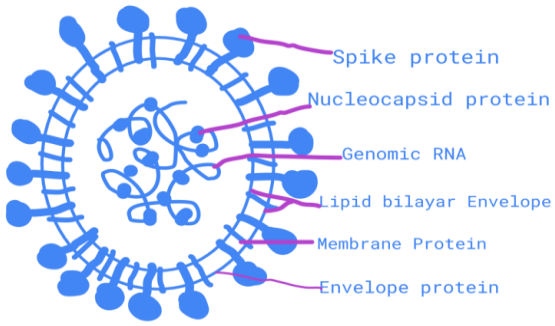


Figure 1 Structure of SARS CoV- 2

Alpha variant or B.1.1.7 was found in the United Kingdom and it comprises seventeen mutations in which the maximum number of mutations occurred in spike protein [19]. Beta variant or B.1.351 with multiple mutations in the spike protein was responsible for the second wave of the pandemic situation observed in South Africa. This variety expanded the transmission and decreased the neutralization effect of monoclonal antibodies and post vaccination [20]. The next variety is P.1 or gamma variant noted in Brazil that also have spike protein mutations. Delta variant or B.1.617.2 was detected in India and found to be the reason for deadly second wave of the pandemic during April, 2021 in India. This variant is drastically spread around the globe and considered to be a most dominant variety of SARS-CoV-2. The variant B.1.1.529 or omicron initially recorded in South Africa. Spike protein of omicron has more than thirty changes and it increases the spreading rate [21].

PATHOGENESIS

Antigenic and Genomic variance in Coronavirus

The genome structure of the Coronaviruses is well known among the RNA viruses. Two thirds of the viral genome is having ORF1ab encoded with replicase polyproteins and the remaining is having genes encoded with structural proteins like spike (S), envelope (E) membrane (M), and nucleocapsid (N) proteins. ORF1ab has enzymes like papain-like protease (PLpro), 3-chymotrypsin-like protease (3CLpro), RNA-dependent RNA polymerase (RdRp) and helicase (Hel). There is a huge variation observed in the genome by four nonstructural proteins present in ORF1a/ORF1b [22].

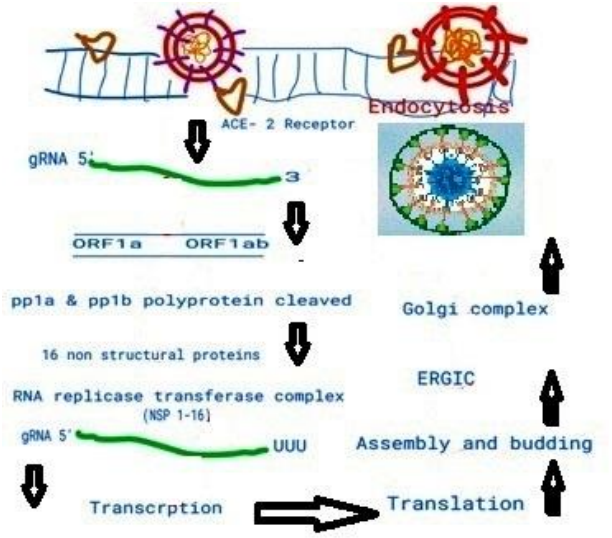


Figure 2 SARS-CoV- 2 Virus life cycle

ERGIC - Endoplasmic reticulum – Golgi complex intermediate compartment; gRNA-Genomic RNA; pp-polyprotein; NSP - Nonstructural proteins; ORF- open reading frame

Mode of Infection

Initially the viral infection is caused by the interaction of the spike protein of the virus with the human cells. The spike protein of the Coronavirus is a type I glycoprotein which formed the peplomers on the viral particles. A stalk-like structure present in the spikes of the virus is attached on the cell membrane. The attachment of coronavirus produces the conformational change in the spike protein that activates the fusion of the cell membrane by the viral particle. Encoding and expression of genes happen after the entry of the viral particles which enable the adaptation of CoVs to the human host [23]. Genome changes were observed to be more frequent amount CoV by the way of recombination, exchange of the genes, insertion and deletion ofgenes. This is the reason for the outbreaks by the CoVs during past epidemics and current pandemic in recent periods.

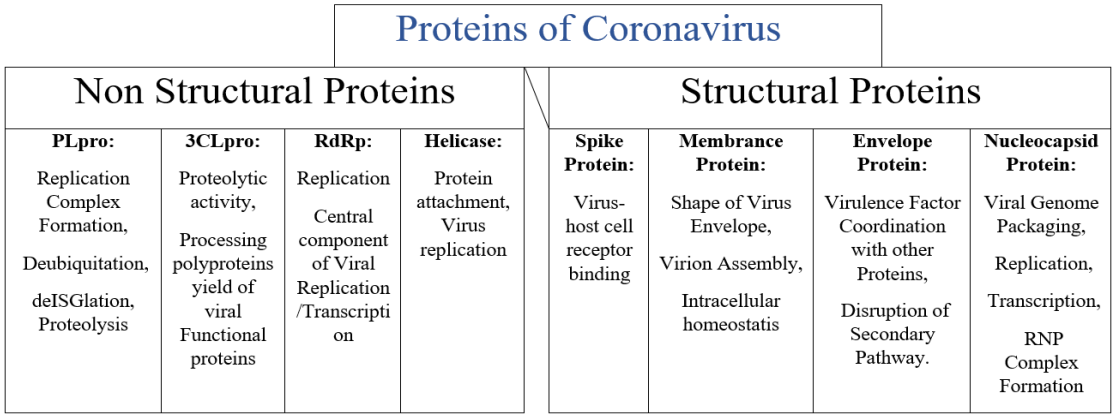
The encapsulated, polyadenylated viral genomic RNAs are encoded with structural and nonstructural proteins exhibiting chymotrypsin-like activity, resulting in the production of RNA through replication and transcription. Replication of the genome had resulted in the full length RNA genome generation by using RNA copies as a template. The sub genomic RNAs and all structural proteins are synthesized by discontinuous transcription. In the cytoplasm of the host cell, the viruses are actively inducing the viral protein synthesis in large amounts. The larger production of viral protein resulted in misfolded proteins observed in the endoplasmic reticulum. This leads to the limited amount of chaperone in the endoplasmic reticulum and thereby the process of unfolded protein response. It can result in either the production of more chaperones or the host cell to apoptosis. The virions are assembled and budded into the lumen of the endoplasmic reticulum. The new virions from the virus infected cells are released by exocytosis (Figure-2). The new viruses released are capable of infecting kidney cells, hepatic cells, intestinal cells, respiratory tract and T lymphocytes. After the infection of these cells, the symptoms and signs are formed [24].

Structural and functional target proteins of SARS - CoV

The virus genes encoded with structural proteins like spike (S), envelope (E) membrane (M), and nucleocapsid (N) proteins (Table.1). The size of each protein is displayed in Table 1. Initially the viral infection is caused by the interaction of human cells with the spike glycoprotein. The S protein is cleaved by proteases of the host into S1 and S2 subunits. The S1 subunit is involved in receptor recognition and S2 in membrane fusion. S1 subunit is again divided into two receptor binding units called N-terminal domain (NTD)

and C-terminal domain (CTD) [25]. Receptor binding domain (RBD) is found on S1 subunit which acts as a key antigen observed on the surface of the virus and targeted by ample drug designing experimental studies [26,27]. The S2 subunit comprises two heptad-repeat regions (HR1 and HR2) along with hydrophobic fusion peptide [28].

Table.1 SARS-CoV-2 Proteins and its functions



Spike Protein

The development of vaccine by the researchers is focusing on the coronavirus spike glycoprotein which is responsible for the entry to the host cells. Angiotensin-converting enzyme 2 (hACE2) is a type I transmembrane metallo carboxypeptidase related to ACE and which is responsible for the infection of the host cell by SARS-CoV-2. S-glycoprotein and ACE-2 receptors are glycosylated which contain covalently linked complex oligosaccharides known as glycans. It is reported that the spike glycoprotein consists of 66 glycosylation sites [28]. Ten different glycoforms are occupied in glycosylation sites of S glycoproteins which are site specific N-linked glycosylation of MERS and SARS. The glycans are extending the epitope diversity [29]. When compared to SARS-CoV, the cells infected with SARS-CoV-2 were established to produce typical syncytium, which might use the plasma membrane fusion pathway for the entry and replication in the host cells [30]. Studies supported the host recognition by SARS-CoV-2 by spike protein [31]. It is reported that S-protein O-glycosylated on threonine near to the cleavage site of furin. LC-MS analysis also provided the spike protein structural motifs like LacdiNAc and PolyLacNacs [32]. Spike protein processed by human transmembrane protease serine 2 (TMPRSS2) and fusion peptide was observed in the subunit S2 exposed to ACE-2, a host receptor [33]. Primary and processing of S-protein by TMPRSS2 plays an important role for the infection of SARS-CoV- 2 [34].

Table 2 Coronavirus virion-associated proteins

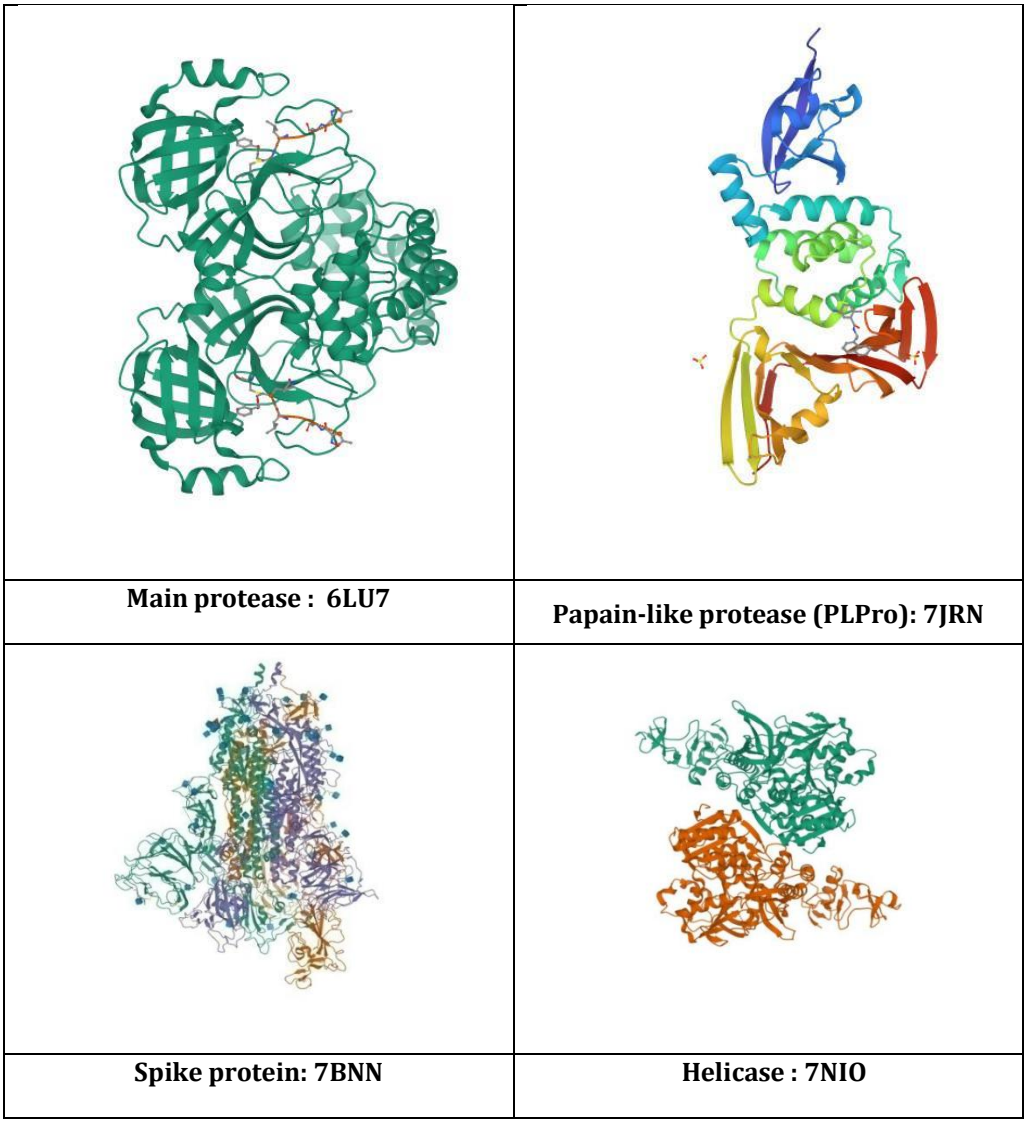
Name of the Protein	Size (kDa)*
Spike glycoprotein (S)	180–220
Membrane protein (M)	23–35
Nucleocapsid protein (N)	50–60
Small envelope protein (E)	9–12
Hemagglutinin-esterase protein (HE)	65

*= Molecular weight given by [35] using SDS-PAGE estimation

Envelope Protein

Envelop protein (E) is a tiny protein consisting of two domains like hydrophobic domain and charged cytoplasmic tail. It is involved in the viral morphogenesis having variable structure compared with the different groups of coronaviruses. Several studies reported that the E protein is acting as a virulence factor, showing immature, inefficient progenies and

coordinating with other intracellular proteins. Ion channels of E proteins formed by oligomerization and the significance of these channels are involved to disrupt the secondary pathway during viral infection. In cell culture, the E protein shows lower virus titers, but it plays an important role in assembly and budding formation in coronaviruses [36-39].



Main protease : 6LU7	**Papain-like protease (PLPro): 7JRN**
Spike protein: 7BNN	**Helicase : 7NIO**

Figure 3 Molecular target proteins observed in SARS COV- 2: Structure obtained from Protein Data Bank (PDB)

Membrane protein

Membrane protein (M) is a structural protein, as a central organizer interacting with other structural proteins. These proteins found abundantly in the coronaviruses which are responsible for the shape of the viral envelope [40,41]. Virion envelope formation driven homotypic interactions by the M protein, but this is not alone adequate for virion formation. Retention of S protein in the Endoplasmic reticulum–Golgi complex intermediate compartment (ERGIC) is mediated by the interaction between S with M protein. The M protein binds to the nucleocapsid and leads to the virion assembly [42]. The M and E proteins interact together to form an envelope of the virus. Their interaction is adequate for the production and discharge of virus-like particles (VLPs) [43]. M protein is acting as an important role in intracellular homeostasis of viruses by multiple protein-protein interaction and these proteins sensitize the host cells by the virus. The

M and N proteins define the shape of the virus. They are the envelope proteins of the virus [44].

Nucleocapsid protein

The size of the nucleocapsid protein (N) is 50-60 KDa (see Table 2) which comprises three regions known as intrinsically disordered regions viz., N-arm, Central Linker (CL) and C-Tail and similar characters found in the nucleocapsid proteins of all coronaviruses. These proteins are involved in the replication, transcription of virus and viral genome packaging. N proteins are participated in the active phase of viral infection and highly conserved. The N protein is proportionately high immunogenic in nature and having conserved amino acid sequences giving rise pathway for making diagnostic assay and vaccine and therapeutic formulations [45-48]. The N protein is involved in the vital function known as the formation of ribonucleoprotein (RNP) complexes consisting of nucleocapsids by the binding of viral RNA genome. The nucleocapsid protects the viral genome by maintaining the replication process of the virus on time and consistent transmission. The RNA binding and oligomerization are caused by the presence of nucleocapsid protein terminals known as N Terminal Domain (NTD) and C Terminal Domain (CTD) respectively [49]. The N protein causes the translational suppressive effect through the CTD with the interaction of elongation factor 1α (EF1α) which are the major translation factor of mammalian cells. SARS-CoV- N protein inhibits the translation of the protein by direct binding of EF1α. Recent research reported that the N protein epitope triggered T cell response revealed prolonged protection up to 11 years and can be used to design prophylactic trials [50]. A motif rich in serine/arginine was reported to be important for oligomerization of N protein [51]. Folding of nucleic acid in proper mode assists RNA chaperone. This chaperone activity suggests a common activity of all coronaviruses N proteins and it has been validated for N proteins of the SARS-CoV [52]. The N protein of SARS-CoV also regulates the host cell cycle by controlling cyclin-dependent kinase (CDK) activity. Similar to other viruses, Coronaviruses deliberately encourage host translational shut off during the steady synthesis of their own viral gene products through affecting the defense mechanism of the host cells. The N protein of the SARS-CoV, ORF3b and ORF6 are the three β interferon (IFβ) antagonists and N protein inhibits the synthesis of IFβ. Synthesis of type-1 interferon (1FN) was inhibited by N protein [53].

Proteases

The genome of SARS-CoVs have two open reading frames ORF1a and ORF1b joined by a ribosomal frameshift and that encode two overlapping viral replicase polyproteins PP1a and PP1ab by host ribosomes. These are the functional proteins produced by the proteolytic process. The polyprotein pp1a of 5′-terminal open reading frame ORF1a cleaved by two viral proteases called as a papain-like protease (PLpro) and 3- chymotrypsin-like protease (3CLpro). The PLpro is employed to cut the first three cleavage sites of the polyprotein, whereas 3CLpro is engaged for the cleavage of the rest of the 11 locations to release 16 nonstructural proteins (Nsp). The Nsp included RNA-dependent RNA polymerase (RdRp) and helicase and participated in the transcription and replication process of the virus [54]. Replication of SARS-CoV-2 happened in the cytoplasm of the host cells to produce a capping mechanism. 2'O-Methyl transferase dependent to S-adenosyl – L-Methionine (SAM) catalyzes viral mRNA structure in nsp-16 which prevents the response of cellular immune system [55, 56].

Main Protease (Mpro)

3CLpro capable to cleave 11 sites available in p1 position of pp1a and pp1ab by the way it produces mature proteins which attached on the replication/transcription complex and finally releases matured nonstructural proteins [57]. The other name of 3CLpro enzyme is main protease (M^{pro}) actively involved in the replication and viral infection processes and it is considered as an ideal target for antiviral therapeutics. 3CLpro is a cysteine protease consists of domains I to III engaged in the maturation cleavage events occurred in the polyprotein precursor. Highly conserved active sites observed in 3CLpro of all coronaviruses consist of four sites namely S1′, S1, S2 and S4. Among which S1 sites have thiol of a cysteine residue responsible for attaching inhibitors. The covalent linkage by the inhibitors is considered to be important for sustaining antiviral activity [58, 59]. Mpro consist of catalytic dyad His41 and Cys145 [60]. His41 received a proton from Cys145 and nucleophilic attack by suphur atom

observed in Cys145 on carbonyl carbon atom in the peptide bond resulted thiohemiketal intermediate formation. This transformation produces intermediate of acyl-enzyme complex supports the breakage of peptide bonds [61]. The enzyme protease acts as a prominent role in viral protein synthesis machinery transformation into the cellular cytosol found in the host cells. The protease is the opt target to control the viral multiplication. The SARS-CoV-2 has conserved main protease and the virus lethally affected by mutation and the progeny of the virus is affected the mutation. Targeting this protease greatly reduces the impact of mutated viruses and its resistance to antiviral drugs [62]. It was reported that ebselen, an organo selenium drug strongly inhibit the Mpro and recommended for the usage of therapeutic purpose [63].

Papain-like protease (PLpro)

The N terminal region of polyprotein splits by the PLpro to produce three nonstructural proteins viz., Nsp 1, 2 and 3 [64]. PLpro is the major multi domain viral protein important for replicase transcriptase complex [65] and it has proteolytic and other related functions [66]. PLpro has 3.5 % of cystein and its structure has Cys111 and zinc ions. It is more reactive and consists of active cystein like Mpro [67]. The multi-domain non-structural protein (Nsp3) of SARS-CoV protein consist of 1,922 amino acids and it plays important role during the viral replication complex formation through their insertion in to the membranes of the host and several interaction with Nsp and Nsp6 [68]. PLpro of SARS-CoV is the peptidase enzyme having catalytic triad consisting of Cys112, His273 and Asp287 [67] involved in the key role including viral replication and pathogenesis [68]. It is reported that PLpro of SARS-CoV function is mediated by cysteine protease catalytic activity in which Cys112 functions as nucleophile, His273 acts as a general acid-base, and Asp287 is combined with histidine assisting for the alignment process and encourage the deprotonation of Cys112 [69]. The enzymatic activities of the PLpro are established that the viral polyprotein processing, deubiquitination and DeISGylation from the proteins of the host cells. The deubiquitination is the process of removing ubiquitin and DeISGylation is the removal of Interleukin Stimulating Genes (ISG). The different functions mediated by PLpro of SARS-CoV to escape from the anticoronaviral innate immune response of the host.

Helicase

Helicases(HE) are motor proteins that couple the released energy from nucleoside triphosphate hydrolysis with double-stranded (ds) nucleic acid unwinding into single stranded nucleic acids [70]. The helicase of SARS-CoV is the part of the Nsp13 and the residues of the protein sequences from 5302 to 5902 mediating ATPase and DNA unwinding process [71]. Hemagglutinin esterase (HE) is a homodimeric class I protein present in the envelope of betacoronavirus associated with the enzyme activity. The membrane fusion activity is lacking in this virion and attached to the spike protein which is considered as a receptor binding protein. HE of coronavirus is binding to O-acetylated sialic acids and involved in the virion attachment [72,73]. HE is considered to function as a second attachment protein in addition to the S protein which initiates the infection [74].

Prophylactic options for COVID-19

Vaccines are an important tool in the medical field to prevent infection, decrease the prevalence of disease and finally global eradication of the disease [75]. Inactivated and attenuated pathogenic strains, subunits of the pathogens are used in the vaccine production to propel the immune response. Milestone of vaccine comprises use of virus like particle, recombinant viral vectors, and conjugates of proteins, polysaccharides and toxoids. Nearly six million lives can be saved by vaccines and it increases the lifespan of human beings [76].

Vector vaccines

Viral vector constructed by using the genetic material of SARS-CoV-2 virus is placed in a modified version of a different kind of virus. The genetic information is delivered to host cells, where the initiation of the immune defense system creates antibodies.

Protein subunit vaccines

Subunit vaccines implement on the parts of the virus that activate the immune system. In the case of SARS-CoV-2, these happen to be harmless spike proteins. This provides the body

with a better immune response in the case of an infection. Antigenic proteins from the subunit of proteins have more immune response. Protein subunit vaccines or acellular vaccines stimulate immunity of the host and these fragments protein molecules do not cause disease. These vaccines have peculiar protein molecules developed against the COVID–19 virus. The protein subunit has adjuvants which support the immune system.

mRNA & DNA Vaccines

History of mRNA and DNA vaccines

First discovered in 1990 when scientists experimented by injecting mice with mRNA and DNA. This resulted in the mouse cells creating proteins that lasted for a few weeks. mRNA vaccines potentially used in the year 1990. The *in vivo* expression of proteins observed when mRNA code is injected into the skeletal cells of mice [77]. After the successful expression noted in the experiments, many studies were carried out to give rise to the immune response by using mRNA in mammalian cell culture. mRNA vaccines drastically work due to its expression as a specific antigenic response [78]. Humoral and cell mediated immune response happens by using mRNA vaccine and increases the innate immune response [79].

In 1992, mRNA coding for vasopressin was injected in mice, resulting in resolved diabetes insipidus symptoms. The only issue was the instability of mRNA outside of the cell. During the SARS-CoV-2 coronavirus pandemic, some institutions found ways to deliver the mRNA vaccine with relatively stable delivery methods. Large-scale funding and public urgency allowed for human trials.

A piece of messenger RNA (mRNA) found in the outer membrane of the virus introduced to produce mRNA vaccine. mRNA is responsible for viral protein synthesis. When administered, the mRNA vaccine is used as a blueprint for making viral proteins, spike proteins in the case of SARS-CoV-2 coronavirus, and then the mRNA may break down. The immune system of the host recognizes the protein as a foreign particle and synthesizes antibodies specific to the virus. The function of antibodies is to find the pathway surrounding them and mark them for phagocytosis. These antibodies remain in the body and allow for quicker response times if the body is infected by a specific pathogen.

DNA vaccines work very similarly to mRNA vaccines, except for the fact that they involve DNA genetic information instead of mRNA. DNA-based vaccines are also believed to be less potent, requiring higher doses in order to activate an immune response. DNA vaccines in India require a device that pushes a stream of vaccines into the skin.

Liposome encapsulated RNA developed for the effective immune response without the limiting degradation in the host cells [80]. It can induce antigen specific antibody and T-cell multifunctional response during cancer trials [80].

Benefits and limitations of mRNA vaccines

Benefits of mRNA vaccines

In the case of SARS-CoV-2 vaccines, mRNA vaccines have an efficacy rate of more than 90%. Large-scale administration of the vaccines could promote the needed levels of herd-immunity in order to return to the status quo pre-pandemic, allowing for the global economy to recover. mRNA vaccines are independent of developing cellular processes, making them extremely adaptable, inexpensive, and able to manage the scale of the pandemic. The adaptability factor is best expressed when considering the mutation ability of the SARS-CoV-2 virus. If a new variant were to become more prevalent, it would be relatively easy to code the nucleotide sequences. This also facilitates its production, therefore contributing to the inexpensiveness and ability to produce vaccines on a larger scale at a faster rate. mRNA vaccines are produced void of the infectious pathogen, relieving the responsibility of pathogen cultivation and purification. mRNA/DNA vaccines can be produced in comparatively low-resource settings in comparison to handling highly contagious pathogens like SARS.

Limitations of mRNA vaccines

The Pfizer SARS-CoV-2 vaccine was the fastest formulated, in 11 months, and the fastest approved. About a week later, the Moderna COVID-19 vaccine was released. This is frankly not enough time to acquire data on the long-term safety of these vaccines. Low dosage of single vaccine or combined vaccines might possess the capacity to induce neurological, articular, and autoimmune disorders in the system. There is no risk for genomic integration with an mRNA vaccine but could lead to damaged immune response due to T-cell exhaustion as the mRNA and DNA vaccines serve

as long-term antigen production. Duration of protection is another limitation as it can only last for about a couple of months.

Virus mediated drug targets

The virus life cycle mediated by many proteins discussed previously provides highly potential opening to inventors for the drug discovery and therapeutic targets. The viral replication is mediated by the prominent enzymes and proteins present in the coronaviruses. The capable targets are including nonstructural proteins like 3-chymotrypsin-like protease, papain like protease, RNA-dependent RNA polymerase which resembled with other coronaviruses (Figure. 4). The 3-chymotrypsin like protease (3CLpro) of CoV is the key protein also known as main protease (Mpro) that is involved in the cleavage of replicase polyproteins in the time of viral replication. The *invitro* studies were carried out for finding the potent antagonist against CoV found that the drugs lopinavir–ritonavir combination basically developed for HIV-1 protease was showing protease inhibiting activity on 3CLpro [81]. Lopinavir–ritonavir combination is used to inhibit the protease activity to treat the SARS-CoV-2 [82]. Lopinavir/ritonavir targeted to 3CL protease potentially inhibit the main protease especially in the His/Cys catalytic dyad [83] and arrest the viral entry. Lopinavir developed by Abbott Laboratories for the inhibition of human immunodeficiency virus (HIV) protease. They are peptodomimetic drug containing hydroxyl ethylene inhibits HIV protease. Earlier stage of SARS-CoV-2 has been controlled by the combination of lopinavir, ritonavir and ribavirin [84]. Overdose of these drugs cause risk to the infants, it may pose cardiac effect, hepatotoxicity, hypersensitivity and other side effects (drug bank). Based on the report released by National Institute of health (NIH) showed that the drug Lopinavir/ritonavir was not effective to treat COVID- 19 patients [85].

N finger residues available in the SARS 3CLpro are engaged in the prominent role in enzyme dimerization. N-terminal octapeptide known as N8 is the inhibitor actively targeting the dimeric interface noted in the SARS 3CLpro explicitly making a way to find out the drug designing pathway [86]. The promising and efficient inhibitors for 3CLpro are mercury containing compounds like thimerosal, phenylmercuric acetate and hexachlorophene [87], compounds with metal-conjugates with mercury and zinc [88], stable benzotriazole esters based inactivators [89], the keto-methylene isosteres and tripeptide a,b-unsaturated esters [90] series of trifluoro methyl ketones [91], C2-symmetric diol [92] and anilide derived from 2-chloro-4-nitroaniline, l-phenylalanine and 4 (dimethylamino) benzoic acid [93].

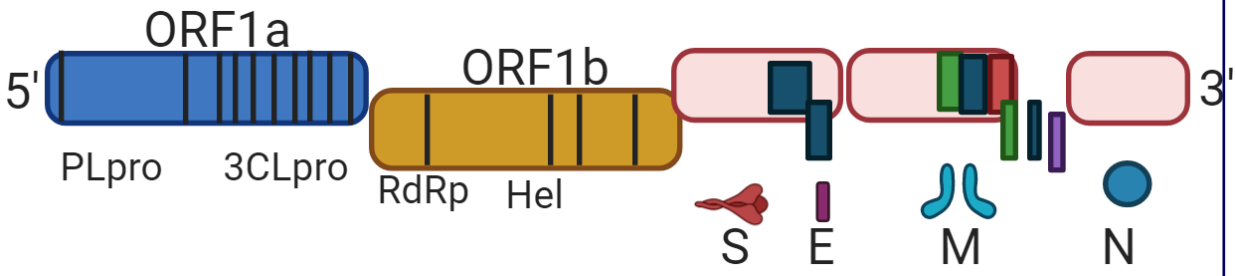


Figure 4 Coronavirus gene Structure

PLpro enzymes of SARS-CoVare important proteolytic enzymes involved in the specific activities including deubiquitylating and deISGylating and the characterization of these enzymes are performed by using crystallography [94]. Many classes of PLpro inhibitors are reported which includes zinc ion and conjugates of zinc molecule, natural products, thiopurine, naphthalene and small molecule inhibitors [95]. Further studies are required to find out the vibrant drug to inhibit the enzymatic activities of PLpro and to prevent the viral replication process facilitated by the enzyme. Hydroxychloroquine is used to inhibit the PLpro during the post entry stage of viral infection and formoterol is used as a bronchodilator to treat the patients and inhibit the PLpro enzyme activity. SARS PLpro can be inhibited by zinc and conjugates of zinc with high dose [96]. Benzodioxol is also inhibiting the enzyme activity [97]. All the coronaviruses are structurally different and it shows narrow spectrum activity by the PLpro enzyme inhibitors. The structural difference between

SARS-CoV and MERS Cov is due to the flexible blocking loop 2 (BL2) domains observed in the PLpro enzymes [98].

Broad spectrum antiviral activity is noted with nucleoside ribavirin for the treatment of severe respiratory syncytial infection caused by virus [99, 100]. Remdesivir is an adenosine analog experimentally demonstrated monophosphoramidate prodrug against some RNA viruses comprising Filoviridae, Pneumoviridae, and Coronaviridae. RdRp is the main target of remdesivir [101] and it is extremely conserved within the gene groups that are beta 2b with the amino acid similarity among the groups showing variation between 70 to 90% [102]. A research reported that the RdRp binding efficiency of approved drugs like Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenofovir which are highly potent against SARS-CoV-2 [103].

Sheahan et al., [104] reported many small molecules which are actively inhibiting replication of SARS-CoV. Further they indicated that SSYA10-001, a 1,2,4 triazole which block SARS-CoV replication and inhibits the helicase activity of SARS-CoV Nsp13. Helicases comprise vastly variable glycoproteins observed in the surface and this SSYA10-001 is showing broad spectrum inhibition from different coronaviruses. Triazoles are well known heterocyclic moieties showing broad spectrum antiviral activities which are inhibiting helicase activity [105].

Griffithsin is a lectin derived from red algae which binds to the glycans of spike protein it is utilized for the potential antiviral agent inhibiting the entry of the virus observed *in-vitro* and in *vivo*. [106, 107]. HR1(Heptad Repeat 1) domain of HCoV spike protein is the vital target and the structures of protein data bank (PDB) id 5zvm, 5zvk, and 5zuv are showing stable six helix bundle structure with long HCoV HR1 region [108]. HR2P is a hopeful antiviral agent which is inhibiting spike protein mediated cell fusion and can be used to treat and prevent the infection by HCoV [109]. Studies show that panel of monoclonal antibodies isolated by using immunized mice exhibit an efficient viral neutralizing effect on alpha, beta, delta and omicron variety of SARS-CoV-2 [110].

Viroporin decreases the ion channel activity of envelope protein of SARS-CoV-2 and shows narrow spectrum activity. Lipophilic thiazolidine derivatives are photo sensitizers which generate singlet oxygen molecule making changes in the lipid membrane properties by the way it is preventing the fusion of virus and target cell membranes. They are showing broad spectrum against various enveloped viruses [111-113].

Host mediated drug targets

The viral infection resulted in the replication of virus cells inside the host cells controlling the innate immune response through the production of interferon. Approved recombinant interferons including pegylated alpha 2a (IFN-α2a) and beta 2b (IFN- β1a, 1b) are used to treat metastatic renal carcinoma and melanoma and these are used to inhibit the replication of SARS-CoV and MERS-CoV observed during *in vitro* and animal model based studies [114-116].Trial testing was approved for the pegylated interferon combined with ribavirin for coronavirus treatment [117].

Development of the Anti-COVID Drugs

There are many drugs and vaccines are observed in and around the world to race the SARS-CoV-2 prophylaxis, treatment and eradication of this current pandemic development. The familiar antiviral drug Chloroquine and hydroxychloroquine are reported and recommended by many researchers by showing their maximum publication of articles recently. These compounds are exhibiting its potential through their active inhibition of SARS-CoV-2 in the *in vitro* studies [118]. These drugs are modifying the ACE 2 receptor profiles and maximizing the pH of the intra cellular organelles. Virus entry to the host cells and their subsequent replication is interrupted by the action of the drug candidate. The top ten drugs ranked by IBM based on the total publication of articles and clinical trial undertaken were Remdesivir, Chloroquine, Hydroxychloroquine, Hidroxicloroquina, Angiotensin II, Plaquenil, Lopinavir, Ribavirin, Ritonavir and Serine [119].

The N protein of the SARS-CoV encoded with DNA vaccines creates a robust humoral and T cell mediated immune response specific to the N protein extensively decreasing the viral titer during the vaccination studies using mice [120].

Few months after the virulence and fatality of the SARS-CoV-2, there are nearly hundred vaccines for preventing viral infection which are at various stages to meet the present threat faced by millions of people globally. There are different types of vaccines under trial basis prepared by using virus and viral parts including viral derived, virus vector used, nucleic acid and protein based vaccines. WHO's immunization head Katherine O'Brien told that under emergency situations no vaccine has even been deployed and in the path of finding new vaccine extra reassurance for the safety of the virus by the regulators is required.

A number of antiviral drugs have been considered and some have been used for the treatment of different viral diseases such as Hydroxychloroquine, originally used for malaria, rheumatoid arthritis, Remdesivir (GS.5734-RDV), developed for the Ebola virus disease, MERS and SARS Viruses (SARS-CoV-2) has been proposed for COVID-19 and so has the Favipiravir (Avigan), an antiviral drug with activity against RNA diseases, is approved in Japan and China for COVID-19; Cyclodextrins, a sugar based polymers, show some promise in the treatment of COVID-19; Lopinavir and Ritonavir for HIV/AIDS and other antiretroviral diseases has been proposed for COVID-19.

It is of interest to note that with all the current scientific research activities on this disease, yet no definitive prescription drug has emerged. We are facing a new and a very difficult and challenging problem but provided the considerable scientific research and financial resources it demands, a drug for COVID-19 will be found.

Research on the development of organic molecules with anticancer drugs activity have highlighted the importance of the use of hyaluronic acid (HA), a natural biopolymer present in our body, as a conjugate in improving the efficiency of the physiological properties of the drugs[121-124]. Hyaluronic acid (HA), also known as hyaluronan, is a naturally occurring polysaccharide of a linear repeating disaccharide unit consisting of β-(1→4)-linked D-glucopyranuronic acid and β-(1→3)-linked 2-acetamido-2-deoxy-D-glucopyranose, which is present in extracellular matrices, the synovial fluid of joints, and scaffolding that comprises cartilage. Despite the simplistic structure of hyaluronan, it behaves quite differently from the glycosaminoglycans in its mechanism of synthesis, its size, its biochemical properties such as the network-forming, viscoelasticity, and its charge characteristics, important to many biochemical properties of living tissues. It mediates its biological functions through specific protein receptors present on the different cell surfaces, which include CD44 [125], HARE [126], RHAMM [127], and LYVE [128].

The Conjugation Technology of Hyaluronic acid with drugs

Hyaluronic acid has been covalently conjugated with a number of pharmacologically active compounds, for example anticancer drug Taxol, a poorly soluble antimitotic chemotherapeutic agent, has been conjugated to HA by way of their intermediate Taxol 2'-hydroxyl succinate and the adipicdihydrazide-modified HA to afford HA–Taxol conjugates [129]**.** Akima et al., [130] have described a process to conjugate Mitomycin C at the C-5' position of the glucuronic acid residue of the HA by way of an amide bonding of hyaluronic acid and the Mitomycin C; the conjugate under metabolism is completely decomposed in the cell by metabolism in the action region, whereby the medicinal ingredient is gradually and quantitatively released.

Camptothecin 20-(S), (CPT), a naturally occurring alkaloid, with a significant antitumor activity against a variety of human solid tumors**;** however because of its limited solubility in water and severe toxicity its trial as an anticancer drug was stopped. In order to enhance its water solubility and impart targeting ability it was conjugated with the hyaluronic acid at the C-6 position of the *N*-acetyl-**D**-glucosamine moiety involving the following chemical steps: (a) replacement of the C-6 hydroxyl group by the amino group and then treating with CPT-20-*O*-hemisuccinate with HA-6-ammino TBA salt in DMSO to afford HA-CPT conjugate [131,132].

Hydroxychloroquine (HCQ) and 2-Deoxy-D-glucose have both exhibited anti-COVID-19 but they also revealed some toxicity as side effects. On the basis of the *insilico* molecular docking studies HA-2-Deoxy-D-Glucose (HA-2DG) conjugate has been proposed as novel drug for the treatment of COVID-19 (Figure 5). *In silico* molecular docking studies of HA-2DG and 2-Deoxy-D-Glucose (2DG) against four different SARS-CoV-2 viral proteins (Mpro, RdRp, PLpro and S protein) revealed that HA-2DG conjugate

showed better binding affinity (-6.2, -7.2, -7.0 and 6.4 Kcal/mol) with all four screened SASR-CoV-2 viral targets than the antimetabolite drug 2DG alone (-4.8, -4.9, -4.6 and 4.7 Kcal/mol) respectively [133]. Similarly, *In-Silico* anti-viral activity of Hydroxychloroquine (HCQ) and its Hyaluronic Acid-derivative (HA-HCQ) towards different SARS-CoV-2 protein molecular targets were reported (Figure 6). Four different SARS-CoV-2 proteins molecular target i.e., three different main proteases and one helicase were chosen for *in-silico* anti-viral analysis. The HA-HCQ conjugates exhibited superior binding affinity and interactions with all the screened SAR-CoV-2 molecular target proteins with the exception of a few targets [134].

The Potential Mode of Action of the HA-HCQ Conjugate Drug against SARS-CoV-2

The role of hyaluronic acid-binding receptors, CD44 and receptor for hyaluronan-mediated motility RHAMM have attracted much attention because they are believed to be involved in cancer metastasis and it also possesses therapeutic potential in the treatment of arthritis and wound healing. The following diagram reveals the potential process of the HA-HCQ drug conjugate internalization in the cell and its mode of action against SARS-CoV-2.

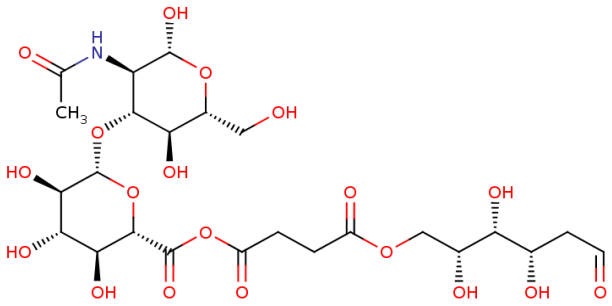


Figure 5 Structure of Hyaluronic Acid- 2-Deoxyglucose conjugate (HA-2DG)

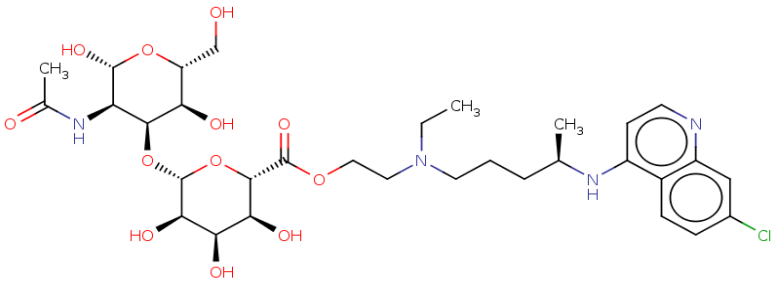


Figure 6 Hyaluronic acid - Hydroxychloroquine Conjugate (HA-HCQ)

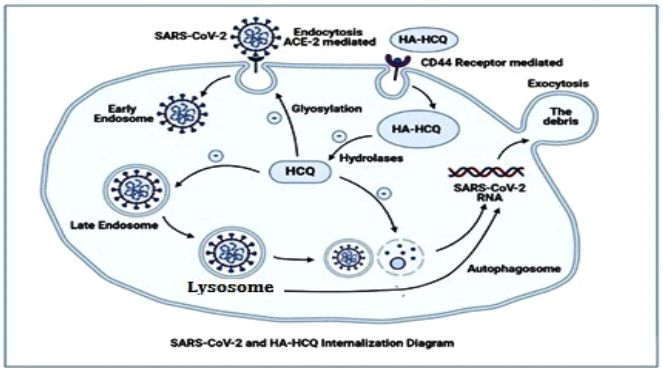


Figure 7 SARS-CoV-2 and HA-HCQ internalization Diagram

The Diagram (SARS-CoV-2 and HA-HCQ internalization) shows the key biological steps indicating how the HA- HCQ drug conjugate would function in the cell to deactivate and eliminate the SARS-CoV-2 from the cell (Figure 7).

The SARS-CoV-2 virus enters the cell *via* Angiotensin Converting Enzyme 2, ACE-2, receptor mediated endocytic pathway, which is composed of a series of highly dynamic membrane-enclosed tubule-vesicular structures with different biological functions such as early endosomes, late endosomes, recycling endosomes and lysosomes.

The following key steps are involved in the metabolism of the drug conjugate:

a) The HA-HCQ drug enters into the cell mediated by the CD44 receptor.
b) The HA-HCQ drug then under the influence of hydrolytic enzymes, released from the lysosome, splits the HA-HCQ ester covalent bond and releases the HCQ drug into the cell.
c) Glycosylation is an important and highly regulated mechanism in the cell that determines protein structure, function, recruitment, interaction, and activates their signaling properties.
d) Autophagosome initiates the important catabolic process for the virus that delivers to the cytoplasmic material to the lysosome for degradation of viral protein aggregates by facilitating bioenergetic homeostasis; it delivers the cytoplasmic degradation components to lysosomes for digestion allowing them to either excrete out of the cell using the exocytosis process or the cell to recycle them to produce new cells.

CONCLUSION

Finding the appropriate drug target to capture viral or host mediated targets needs further research. The lining of the nose readily accepts the viral pathogen when someone inhales virus containing droplet. The cell surface receptors ACE-2 is suppressed by virus assembly machinery or the cell and making innumerable copies of cells. The drugs reported in this review are very limited and appropriate and many more vibrant medications are constantly reported by researchers every day. Many drugs and vaccines are under trial and preclinical are proving the hectic efforts made by the research community constantly. The review also highlights a new cellular targeted drug target pattern employing hyaluronic acid conjugates of not only anti-COVID drugs but other pharmacological active compounds.

Acknowledgement: The authors are thankful to Dr. R. Mothi, Associate Professor, Electronics and Communication Engineering, Mahendra Engineering College for his support during the writing of this article. One of the author (V.A) wish to thank DST-FIST-137 / 2013 and UGC/SERO-2192, Government of India for providing financial support to carry out this work.

REFERENCES

[1] Lu.G., Liu.D. 2012. SARS-like virus in the Middle East: a truly bat-related coronavirus causing human diseases. Protein Cell, 3, 803-805.
[2] Li Yang, Shasha Liu, Jinyan Liu, Zhixin Zhang, Xiaochun Wan, Bo Huang, Youhai Chen, Yi Zhang. 2020. COVID-19: immunopathogenesis and Immuno therapeutics. Sig Transduct Target Ther, 5, 128.
[3] Marco Ciotti, Massimo Ciccozzi, Alessandro Terrinoni, Wen-Can Jiang, Cheng-Bin Wang., Sergio Bernardini (2020) The COVID-19 pandemic, Critical Reviews in Clinical Laboratory Sciences, 57:6, 365-388,
[4] https://www.cdc.gov/coronavirus /2019-ncov/symptoms testing/symptoms.html.
[5] Nicola M, Alsafi Z, Sohrabi C, Kerwan A, Al-Jabir A, Iosifidis C, Agha M, Agha R. The socio-economic implications of the coronavirus pandemic (COVID-19): A review. Int J Surg. 2020 Jun; 78:185-193. Epub 2020 Apr 17.
[6] Núñez A, Sreeganga SD, Ramaprasad A. Access to Healthcare during COVID-19. Int J Environ Res Public Health. 2021 Mar 14;18(6):2980.
[7] De Groot, R.J., Baker, S.C., Baric, R., Enjuanes, L., Gorbalenya, A.E., Holmes, K.V., Perlman, S., Poon, L., Rottier, P.J.M., Talbot, P.J.2012. Family Coronaviridae; Academic Press: San Diego, CA, USA, 19.
[8] Woo, P.C.; Lau, S.K.; Lam, C.S.; Lau, C.C.; Tsang, A.K.; Lau, J.H.; Bai, R.; Teng, J.L.; Tsang, C.C.; Wang, M.; et al. Discovery of seven novel mammalian and avian coronaviruses in the genus delta coronavirus supports bat coronaviruses

as the gene source of alpha coronavirus and beta coronavirus and avian coronaviruses as the gene source of gamma coronavirus and delta coronavirus. J. Virol. 2012, 86, 3995–4008.

[9] G.J. Gorse, T.Z. O'Connor, S.L. Hall, J.N. Vit ale, K.L. Nichol. 2009. Human coronavirus and acute respiratory illness in older adults with chronic obstructive pulmonary disease, J. Infect. Dis., 199, 847-857

[10] A.Jean, C. Quach, A. Yung, M. Semret.201 3. Severity and outcome associated with human coronavirus OC43 infections among children Pediatr. Infect. Dis. J., 32, 325-329

[11] WHO. 2004. Cumulative number of reported probable cases of severe acute respiratory syndrome (SARS). https://www.who.int/csr/sars/country /en/

[12] Ali M Zaki , Sander Van Boheemen, Theo M Bestebroer, Albert D M E Osterhaus, Ron A M Fouchier.2012. Fouchier Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia, N. Engl. J. Med., 367, 1814-1820.

[13] Lu, G., Liu. D. 2012. SARS-like virus in the Middle East: a truly bat-related coronavirus causing human diseases. Protein Cell, 3, 803-805

[14] Song, W., Wang, Y., Wang, N., Wang, D., Guo, J., Fu, L., Shi, X. 2014. Identification of residues on human receptor DPP4 critical for MERS-CoV binding and entry. Virology. 471-473:49-53.

[15] Wu, K., Li, W., Peng, G., and Li, F. 2009. Crystal structure of NL63 respiratory coronavirus receptor-binding domain complexed with its human receptor. Proc. Natl. Acad. Sci. USA 106, 19970–19974

[16] Zhou, P., Yang, X.-L., Wang, X.-G., Hu, B., Zhang, L., Zhang, W., Si, H.-R., Zhu, Y., Li, B., Huang, C.-L., et al. 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270–273.

[17] Zhou P., Fan H., Lan T., Yang X.L., Shi W.F., Zhang W., Zhu Y., Zhang Y.W., Xie Q.M., Mani S., et al. 2018. Fatal swine acute diarrhoea syndrome caused by an HKU2-related coronavirus of bat origin. *Nature.* 556:255–258.

[18] Forster. P, Forster L, Renfrew. C and Forster. M. 2020. Phylogenetic network analysis of SARS-CoV-2 genomes. PNAS Latest Articles.1-3.

[19] Wu K, Werner AP, Koch M, Choi A, Narayanan E, Stewart-Jones GBE, Colpitts T, Bennett H, Boyoglu-Barnum S, Shi W, Moliva JI, Sullivan NJ, Graham BS, Carfi A, Corbett KS, Seder RA, Edwards DK. 2021. Serum Neutralizing Activity Elicited by mRNA-1273 Vaccine. N Engl J Med. 15;384(15):1468-1470.

[20] Wang P, Casner RG, Nair MS, Wang M, Yu J, Cerutti G, Liu L, Kwong PD, Huang Y, Shapiro L, Ho DD. Increased Resistance of SARS-CoV-2 Variant P.1 to Antibody Neutralization. bioRxiv. 2021 Apr 09.

[21] Vaughan A. Omicron emerges. New Sci. 2021 Dec 04;252(3363):7.

[22] Luk HK, Li X, Fung J, Lau SK, Woo PC. 2019. Molecular epidemiology, evolution and phylogeny of SARS coronavirus. Infection, Genetics and Evolution 71:21–3.

[23] Weiss. S.R. and Navas-Martin. S. 2005. Coronavirus Pathogenesis and the Emerging Pathogen Severe Acute Respiratory Syndrome Coronavirus. Microbiology and Molecular Biology Reviews. 69.4.635–664.

[24] Ding, YQ, Wang, HJ, Shen, H, Li, ZG, Geng, J, Han, HX, Cai, JJ, Li, X, Kang, W, Weng, DS, Lu, YD, Wu, DH, He, L, Yao, KT. 2003. The clinical pathology of severe acute respiratory syndrome (SARS): a report from China. J Pathol, 200:282–289.

[25] Lu G, Wang Q, Gao GF.2015. Bat-to-human: spike features determining 'host jump' of coronaviruses SARS-CoV, MERS-CoV, and beyond. Trends Microbiol. Aug; 23(8):468-78.

[26] Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S.2009. The spike protein of SARS-CoV--a target for vaccine and therapeutic development. Nat Rev Microbiol. Mar;7(3):226-36.

[27] Yuan, Y., Cao, D., Zhang, Y. Jun Ma, Jianxun Qi, Qihui Wang, Guangwen Lu, Ying Wu, *et al.* 2017.Cryo-EM structures of MERS-CoV and SARS-CoV spike glycoproteins reveal the dynamic receptor binding domains. Nat Commun **8,** 15092

[28] Daniel Wrapp , Nianshuang Wang , Kizzmekia S Corbett , Jory A Goldsmith , Ching-Lin Hsieh , Olubukola

Abiona , Barney S Graham , Jason S McLellan. 2020. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science (80). 367, 1260–1263.

[29] Yasunori Watanabe, Zachary T. Berndsen, Jayna Raghwani, Gemma E. Seabright, Joel D. Allen, Jason S. McLellan, Ian A. Wilson, Thomas A. Bowden. 2020. Vulnerabilities in coronavirus glycan shields despite extensive glycosylation. bioRxiv 2020.02.20.957472.

[30] Xia. S, Liu. M., Wang. C., Xu. W., Lan. Q., Feng. S., Qi.F., Bao. F., Du.L., Liu.L., Qin. C., Sun.F., Shi .Z., Zhu.Y. , Jiang.S., Lu.L.2020. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell Research,30:343 – 355.

[31] Ibrahim, I.M., Abdelmalek, D.H., Elshahat, M.E.2020. COVID-19 spike-host cell receptor GRP78 binding site prediction. J. Infect, 80(5):554–562.

[32] Miloslav Sanda., Lindsay Morrison., Radoslav Goldman. 2021. N- and O-Glycosylation of the SARS-CoV-2 Spike Protein, Anal. Chem. 93, 4, 2003–2009

[33] Qi, F., Qian, S., Zhang, S., Zhang, Z. 2020.Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses. Biochem. Biophys. Res. Commun. 526, 135–140.

[34] Limburg H, Harbig A, Bestle D, Stein DA, Moulton HM, Jaeger J, et al. 2019. TMPRSS2 is the major activating protease of influenza A virus in primary human airway cells and influenza B virus in human type II pneumocytes. J Virol. 93:e00649–19.

[35] C.M. Fauquet, M.A. Mayo, J. Maniloff, U. Desselberger, L.A.2005. Ball Virus taxonomy: eighth report of the international committee for virus taxonomy, Elsevier Academic Press, San Diego, CA .

[36] Arbely E, Khattari Z, Brotons G, Akkawi M, Salditt T, Arkin IT. 2004. A highly unusual palindromic transmembrane helical hairpin formed by SARS coronavirus E protein. J Mol Biol, 341:76979

[37] Verdiá-Báguena C , Nieto-Torres JL, Alcaraz A, DeDiego ML, Torres J, Aguilella VM, Enjuanes L.2012. Coronavirus E protein forms ion channels with functionally and structurally-involved membrane lipids. Virology. 432(2):485-94.

[38] Venkatagopalan P, Daskalova SM, Lopez LA, Dolezal KA, Hogue BG.2015. Coronavirus envelope (E) protein remains at the site of assembly. Virology 478:7585.

[39] Schoeman D, Fielding BC. 2019. Coronavirus envelope protein: Current knowledge. Virol J, 16:69.

[40] Masters PS. 2006. The molecular biology of coronaviruses. Adv Virus Res. 66: 193–292

[41] Neuman BW, Kiss G, Kunding AH, Bhella D, Baksh MF, Connelly S. 2011. A structural analysis of M protein in coronavirus assembly and morphology. J Struct Biol. 174(1):11–22.

[42] Fehr AR, Perlman S.2015. Coronaviruses: An overview of their replication and pathogenesis. Coronaviruses: Springer; 1–23.

[43] Ruch.T.R. and Machamer. C.E. 2012. The Coronavirus E Protein: Assembly and Beyond. Viruses 2012, 4, 363-382;

[44] Wang Y, Liu L.2016. The membrane protein of severe acute respiratory syndrome coronavirus functions as a novel cytosolic pathogenassociated molecular pattern to promote beta interferon induction via a toll like receptorrelated TRAF3 independent mechanism. mBio, 7:e0187215.

[45] Mau-Sun Chang , Yen-Ta Lu, Shin-Tsung Ho, Chao-Chih Wu, Tsai-Yin Wei, Chia-Ju Chen, Yun-Ting Hsu, Po-Chen Chu, Ching-Hsin Chen, Jien-Ming Chu, Ya-Lin Jan, Chia-Chien Hung, Chi-Chen Fan, Yuh-Cheng Yang. 2004. Antibody detection of SARS-CoV spike and nucleocapsid protein. BiochemBiophys Res Commun 314:931-6.

[46] Li Guo, Lili Ren , Siyuan Yang , Meng Xiao, De Chang , Fan Yang , Charles S Dela Cruz , Yingying Wang , Chao Wu , Yan Xiao , Lulu Zhang, Lianlian Han , Shengyuan Dang , Yan Xu , Qi-Wen Yang, Sheng-Yong Xu , Hua-Dong Zhu , Ying-Chun Xu , Qi Jin , Lokesh Sharma , Linghang Wang, Jianwei Wang. 2020.

Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). Clin Infect Dis, 71(15):778-785.

[47] Monique H. Verheije, Marne C. Hagemeijer, Mustafa Ulasli, FulvioReggiori, Peter J. M. Rottier, Paul S. Masters, and Cornelis A. M. de Haan. 2010.The Coronavirus Nucleocapsid Protein Is Dynamically Associated with the Replication - Transcription Complexes. Journal of Virology, Nov. 84-21: 11575–11579.

[48] Zhang T, Wu Q, Zhang Z.2020. Probable pangolin origin of SARS-CoV-2 associated with the COVID-19 outbreak. CurrBiol, 30:1-6.

[49] Jayaram, H., Fan, H., Bowman, B.R., Ooi, A., Jayaram, J., Collisson, E.W., Lescar, J., Prasad, B.V., 2006. X-ray structures of the N- and C-terminal domains of a coronavirus nucleocapsid protein: implications for nucleocapsid formation. J. Virol. 80, 6612–6620.

[50] Ahmed SF, Quadeer AA, McKay MR.2020. Preliminary identification of potential vaccine targets for the COVID-19 coronavirus (SARS-CoV-2) based on SARSCoV immunological studies. Viruses,12:254.

[51] Nelson, G.W.; Stohlman, S.A.; Tahara, S.M. High affinity interaction between nucleocapsid protein and leader/intergenic sequence of mouse hepatitis virus RNA. *J.* Gen. Virol.**2000**, *81*, 181–188.

[52] Zuniga, S., Sola, I., Moreno, J.L., Sabella, P., Plana-Duran, J., Enjuanes, L.2007. Coronavirus nucleocapsid protein is an RNA chaperone. Virology*, 357*, 215–227

[53] Lu, X.; Pan, J.; Tao, J.; Guo, D. 2011.SARS-CoV nucleocapsid protein antagonizes IFN-beta response by targeting initial step of IFN-beta induction pathway, and its C-terminal region is critical for the antagonism. Virus Genes*, 42*, 37–45

[54] Chuck CP, Chow HF, Wan DC, Wong KB.2011. Profiling of substrate specificities of 3C-like proteases from group 1, 2a, 2b and 3 coronaviruses. PLoS One, 6:e27228

[55] Hyde, J.L.; Diamond, M.S. Innate immune restriction and antagonism of viral RNA lacking 20-O-methylation. Virology 2015, 479–480, 66–74.

[56] Paula Morales, Natalie L. Curtis, Sandra G. Zárate, Agatha Bastida and Victor M. Bolanos-Garcia.2020. Interfering with mRNA Methylation by the 2 0O-Methyltransferase (NSP16) from SARS-CoV-2 to Tackle the COVID-19 Disease. Catalysts, 10, 1023;

[57] Min-Feng Hsu , Chih-Jung Kuo, Kai-Ti Chang, Hui-Chuan Chang, Chia-Cheng Chou, Tzu-Ping Ko, Hui-Lin Shr, Gu-Gang Chang, Andrew H-J Wang, Po-Huang Liang.2005. Mechanism of the maturation process of SARSCoV 3CL protease. J Biol Chem. 280:3125766.

[58] H. Yang, W. Xie, X. Xue, K. Yang, J. Ma, W. Liang, Q. Zhao, Z. Zhou, D. Pei, J. Ziebuhr, R. Hilgenfeld, K. Y. Yuen, L. Wong, G. Gao, S. Chen, Z. Chen, D. Ma, M. Bartlam, Z. Rao,2005. Design of wide-spectrum inhibitors targeting coronavirus main proteases. PLOS Biol.3, e324.

[59] L. Zhang, D. Lin, Y. Kusov, Y. Nian, Q. Ma, J. Wang, A. von Brunn, P. Leyssen, K. Lanko, J. Neyts, A. de Wilde, E. J. Snijder, H. Liu, R. Hilgenfeld. 2020. α-Ketoamides as Broad-Spectrum Inhibitors of Coronavirus and Enterovirus Replication: Structure-Based Design, Synthesis, and Activity Assessment. J. Med. Chem. acs.jmedchem.9b01828.

[60] Kneller, D.W, Gwyndalyn Phillips, Hugh M O'Neill, Robert Jedrzejczak , Lucy Stols , Paul Langan, Andrzej Joachimiak , Leighton Coates , Andrey Kovalevsky ,2020.Structural plasticity of SARS-CoV-2 3CL M(pro) active site cavity revealed by room temperature X-ray crystallography. Nat. Commun. 11(1), 3202.

[61] Mengist, HM, Dilnessa, T., Jin T. 2021. Structural Basis of Potential Inhibitors Targeting SARS-CoV-2 Main Protease. Front. Chem. 9:622898.

[62] Goyal B, Goyal D. Targeting the Dimerization of the Main Protease of Coronaviruses: A Potential Broad-Spectrum Therapeutic Strategy. ACS Comb Sci. 2020 Jun 8;22(6):297-305.

[63] Amporndanai Kangsa, Xiaoli Meng ,Weijuan Shang, ZhenmigJin, Michael Rogers, Yao Zhao, Zihe Rao, Zhi-Jie Liu , Haitao Yang, Leike Zhang, Paul M. O'Neill & S. Samar Hasnain (2021). Inhibition mechanism of SARS-CoV-2 main protease by ebselen and its derivatives. Nature Communications, 12:3061.

[64] Lindner HA, FotouhiArdakani N, Lytvyn V, Lachance P, Sulea T, Ménard R. The papain like protease from the severe acute respiratory syndrome coronavirus is a deubiquitinating enzyme. JVirol 2005;79:15199208

[65] Lei, J., Kusov, Y. &Hilgenfeld, R. Nsp3 of coronaviruses: structures and functions of a large multi-domain protein. Antivir. Res. 149, 58–74 (2018).

[66] Donghyuk Shin, Rukmini Mukherjee, Diana Grewe, Denisa Bojkova, Kheewoong Baek, Anshu Bhattacharya, Laura Schulz, Marek Widera, Ahmad Reza Mehdipour, Georg Tascher, Paul P. Geurink, Alexander Wilhelm, Gerbrand J. van der Heden van Noort, Huib Ovaa, Stefan Müller, Klaus-Peter Knobeloch, Krishnaraj Rajalingam, Brenda A. Schulman, Jindrich Cinatl, Gerhard Hummer, Sandra Ciesek ,Ivan Dikic. 2020. Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity, *Nature* 587, 657–662

[67] Jerzy Osipiuk, Saara-Anne Azizi , Steve Dvorkin, Michael Endres, Robert Jedrzejczak, Krysten A. Jones , Soowon Kang, Rahul S. Kathayat , Youngchang Kim, Vladislav G. Lisnyak , Samantha L. Maki , Vlad Nicolaescu, Cooper A. Taylor, Christine Tesar, Yu-An Zhang, Zhiyao Zhou , Glenn Randall, Karolina Michalska, Scott A. Snyder, Bryan C. Dickinson.,Andrzej Joachimiak. 2021. Structure of papain-like protease from SARS-CoV-2 and its complexes with non-covalent inhibitors. Nature Communications, 12:743.

[68] Angelini MM, Akhlaghpour M, Neuman BW, Buchmeier MJ. 2013. Severe acute respiratory syndrome coronavirus nonstructural proteins 3, 4, and 6 induce double-membrane vesicles. mBio. Aug 13; 4(4):e00524-13.

[69] Baez-Santos, Y. M., St John, S. E. &Mesecar, A. D. 2015. The SARS-coronavirus papain-like protease: structure, function and inhibition by designed antiviral compounds. Antivir. Res. 115, 21–38.

[70] Ratia, K., Saikatendu, K.S., Santarsiero, B.D., Barretto, N., Baker, S.C., Stevens, R.C., Mesecar, A.D., 2006. Severe acute respiratory syndrome coronavirus papain-like protease: structure of a viral deubiquitinating enzyme. Proc. Natl. Acad. Sci. U.S.A. 103, 5717–5722

[71] Thiel, V., K. A. Ivanov, A´. Putics, T. Hertzig, B. Schelle, S. Bayer, B. Weibrich, E. J. Snijder, H. Rabenau, H. W. Doerr, A. E. Gorbalenya, and J. Ziebuhr. 2003. Mechanisms and enzymes involved in SARS coronavirus genome expression. J. Gen. Virol. 84:2305–2315.

[72] de Groot RJ. 2006. Structure, function and evolution of the hemagglutinin-esterase proteins of corona- and toroviruses. Glycoconj J23:59–72.

[73] Zeng Q, Langereis MA, van Vliet AL, Huizinga EG, de Groot RJ. Structure of coronavirus hemagglutininesterase offers insight into corona and influenza virus evolution. Proc Natl AcadSci USA 2008; 105: 90659.

[74] Parker, M. D., Cox, G. J., Deregt, D., Fitzpatrick, D. R., and Babiuk, 1. A., 1989, Cloning and in vitra expression of the gene for the E3 hemagglutinin glycoprotein of bovine coronavirus, 1-Gen. Viral. 70:155.

[75] Andre, FE, Booy, R, Bock, HL, Clemens J, Datta SK, John TJ, et al. 2008. Vaccination greatly reduces disease, disability, death and inequity worldwide. Bull World Health Organ, 86(2):140–6.

[76] Rappuoli R.2020. Timeline: Vaccines. Cell, 183:552.

[77] Wolff J, Malone R, Williams P, Chong W, Acsadi G, Jani A, et al. 1990. Direct gene transfer into mouse muscle in vivo. Science, 247(4949):1465–8.

[78] Hoerr I, Obst R, Rammensee H-G, Jung G.2000. In vivo application of RNA leads to induction of specific cytotoxic T lymphocytes and antibodies. Eur J Immunol, 30:1–7.

[79] Pollard C, De Koker S, Saelens X, Vanham G, Grooten J. 2013. Challenges and advances towards the rational design of mRNA vaccines. Trends Mol Med,19(12):705–13.

[80] Ulmer JB, Mason PW, Geall A, Mandl CW. 2012. RNA-based vaccines. Vaccine, 30(30):4414- 8.

[81] Wu C-Y, Jan J-T, Ma S-H, et al.2004. Small molecules targeting severe acute respiratory syndrome human coronavirus. Proc Natl AcadSci USA. 101(27):10012–10017.

[82] Lim J, Jeon S, Shin HY, Kim MJ, Seong YM, Lee WJ, *et al*. 2020. Case of the index patient who caused tertiary transmission of COVID19 infection in Korea: The application of lopinavir/ritonavir for the treatment of COVID19 infected pneumonia monitored by quantitative RTPCR. J Korean Med Sci 35:e79.

[83] Muthuraj. S., Manohar, M., Mohan, M. 2021. Potential binding efficiency of antiviral drug lopinavir targeted to the catalytic dyad,His41-Cys145 of SARS CoV-2 Main protease. International Journal of life sciences and research. 3(1) 22-25.

[84] Yu-San Han, Gu-Gang Chang, Chiun-Gung Juo, Hong-Jen Lee, Shiou-HweiYeh, John Tsu-An Hsu, Xin Chen. 2005. Papainlike protease 2 (PLP2) from severe acute respiratory syndrome coronavirus (SARSCoV): Expression, purification, characterization, and inhibition. Biochemistry, 44:1034959.

[85] Báez-Santos. Y.M., St. John.S.E and Mesecar. A.D. 2015. The SARS-coronavirus papain-like protease: Structure, function and inhibition by designed antiviral compounds. Antiviral Research 115, 21–38.

[86] Wei, P.; Fan, K.; Chen, H.; Ma, L.; Huang, C.; Tan, L.; Xi, D.; Li, C.; Liu, Y.; Cao, A.; Lai, L. 2006. The N-terminal octapeptide acts as a dimerization inhibitor of SARS coronavirus 3C-like proteinase. Biochem. Biophys. Res. Commun. 339, 865-872.

[87] Hsu. J.T.A., Chih-Jung Kuo ,Hsing-Pang Hsieh, Yeau-Ching Wang, Kuo-Kuei Huang, Coney P.-C. Lin, Ping-Fang Huang, Xin Chen, Po-Huang Liang.2014. Evaluation of metal conjugated compounds as inhibitors of 3CL protease of SARS-CoV. FEBS Letters 574, 116–120.

[88] Lee. C.C, Chih-Jung Kuo, Min-Feng Hsu, Po-Huang Liang, Jim-Min Fang, Jiun-JieShie, Andrew H.-J. Wang.2007. Structural basis of mercury- and zinc-conjugated complexes as SARS-CoV 3C-like protease inhibitors. FEBS Letters 581,5454–5458.

[89] Wu,C.Y, Ke-Yung King, Chih-Jung Kuo, Jim-Min Fang, Ying-Ta Wu, Ming-Yi Ho, Chung-Lin Liao, Jiun-JieShie, Po-Huang Liang and Chi-Huey Wong. 2006. Stable Benzotriazole Esters as Mechanism-Based In activators of the Severe Acute Respiratory Syndrome 3CL Protease. Chemistry & Biology 13, 261–268, March.

[90] Shie,J.J., Jim-Min Fang, Tun-HsunKuo, Chih-Jung Kuo, Po-Huang Liang, Hung-Jyun Huang, Yin-Ta Wu, Jia-Tsrong Jan, Yih-Shyun E. Cheng and Chi-Huey Wong.2005. Inhibition of the severe acute respiratory syndrome 3CL protease by peptidomimetica,b-unsaturated esters. Bioorganic & Medicinal Chemistry 13.5240–5252.

[91] Shao,Y.M., Wen-Bin Yang, Tun-HsunKuo, Keng-Chang Tsai, Chun-Hung Lin, An-Suei Yang, Po-Huang Liang., Chi-Huey Wong.2008. Design, synthesis, and evaluation of trifluoromethyl ketones as inhibitors of SARS-CoV 3CL protease. Bioorganic & Medicinal Chemistry 16,4652–4660.

[92] Shao, Y.M., Wen-Bin Yang, Hung-Pin Peng, Min-Feng Hsu, Keng-Chang Tsai, Tun-HsunKuo, Andrew H.-J. Wang, Po-Huang Liang, Chun-Hung Lin, An-Suei Yang, Chi-Huey Wong.2007. Structure-Based Design and Synthesis of Highly Potent SARSCoV 3CL Protease Inhibitors. ChemBioChem, 8, 1654 – 1657.

[93] Shie, JJ, Jim-Min Fang, Chih-Jung Kuo, Tun-HsunKuo, Po-Huang Liang, Hung - Jyun Huang, Wen - Bin Yang, Chun-Hung Lin, Jiun-Ling Chen, Yin-Ta Wu, Chi-Huey Wong. 2005. Discovery of Potent Anilide Inhibitors against the Severe Acute Respiratory Syndrome 3CL Protease. Journal of Medicinal Chemistry,*48* (13), 4469-4473.

[94] Patel SS, Donmez I. 2006. Mechanisms of helicases. J. Biol.Chem. 281: 18265-18268.

[95] Tsai,K.C., Shih-Yuan Chen, Po-Huang Liang, I-Lin Lu, NeerajMahindroo, Hsing-Pang Hsieh, Yu-Sheng Chao, Lincoln Liu, Donald Liu, Wei Lien, Thy-Hou Lin., Su-Ying Wu. 2006. Discovery of a Novel Family of SARS-CoV Protease Inhibitors by Virtual Screening and 3D QSAR Studies. Journal of Medicinal Chemistry, *49* (12), 3485-3495.

[96] Hilgenfeld, R.2014. From SARS to MERS: crystallographic studies on coronaviral

proteases enable antiviral drug design. FEBS J.**281**, 4085–4096.

[97] Baez-Santos, Y. M., St John, S. E., Mesecar, A. D.2015. The SARS-coronavirus papain-like protease: structure, function and inhibition by designed antiviral compounds. Antiviral Res. **115**, 21–38.

[98] Lee. C.C, Chih-Jung Kuo, Min-Feng Hsu, Po-Huang Liang, Jim-Min Fang, Jiun-JieShie, Andrew H.-J. Wang. Structural basis of mercury- and zinc-conjugated complexes as SARS-CoV 3C-like protease inhibitors. FEBS Letters 581 (2007) 5454–5458.

[99] Markland, T. J. Mcquaid, j. Jain and A. D. Kwong.2000. Broad-Spectrum Antiviral Activity of the IMP Dehydrogenase Inhibitor VX-497: a Comparison with Ribavirin and Demonstration of Antiviral Additivity with Alpha Interferon. Antimicrobial Agents and Chemotherapy. 2000, p. 859–866.

[100] Noel J C Snell. 2001. Ribavirin - current status of a broad spectrum antiviral agent, Expert Opinion on Pharmacotherapy, 2:8, 1317-1324,.

[101] Hyun Lee , Hao Lei , Bernard D Santarsiero , Joseph L Gatuz , Shuyi Cao , Amy J Rice , Kavankumar Patel , Michael Z Szypulinski, Isabel Ojeda, Arun K Ghosh , Michael E Johnson. 2015.Inhibitor recognition specificity of MERSCoV papain-like protease may differ from that of SARS-CoV. ACS Chem. Biol. **10**, 1456–1465.

[102] Báez-Santos. Y.M., Scott J. Barraza, Michael W. Wilson, Michael P. Agius, Anna M. Mielech, Nicole M. Davis, Susan C. Baker, Scott D. Larsen., Andrew D Mesecar. 2014. X-ray Structural and Biological Evaluation of a Series of Potent and Highly Selective Inhibitors of Human Coronavirus Papain-like Proteases. J Med Chem. Mar 27; 57(6): 2393–2412.

[103] Agostini, M.L., Andres, E.L., Sims, A.C., Graham, R.L., Sheahan, T.P., Lu, X., Smith, E.C., Case, J.B., Feng, J.Y., Jordan, R., Ray, A.S., Cihlar, T., Siegel, D., Mackman, R.L., Clarke, M.O., Baric, R.S., Denison, M.R., 2018. Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. mBio 9.

[104] Sheahan,T.P., A. C. Sims, R. L. Graham, V. D. Menachery, L. E. Gralinski, J. B. Case, S. R. Leist, K. Pyrc, J. Y. Feng, I. Trantcheva, R. Bannister, Y. Park, D. Babusis, M. O. Clarke, R. L. Mackman, J. E. Spahn, C. A. Palmiotti, D. Siegel, A. S. Ray, T. Cihlar, R. Jordan, M. R. Denison, R. S. Baric. 2017. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci. Transl. Med. 9, eaal3653.

[105] Abdo A Elfiky. 2020. Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): A molecular docking study, Jul 15;253:117592

[106] O'Keefe BR, Giomarelli B, Barnard DL, et al. Broad-spectrum in vitro activity and in vivo efficacy of the antiviral protein griffithsin against emerging viruses of the family Coronaviridae. J Virol. 2010 Mar;84(5):2511-21.

[107] Barton C, Kouokam JC, Lasnik AB, et al. Activity of and effect of subcutaneous treatment with the broad-spectrum antiviral lectin griffithsin in two laboratory rodent models. Antimicrob Agents Chemother. 2014;58(1):120-7.

[108] Zhao H, Zhou J, Zhang K, et al. 2016.A novel peptide with potent and broad-spectrum antiviral activities against multiple respiratory viruses. Sci Rep. Feb 25, 6:22008.

[109] Adeyemi O Adedeji , Kamalendra Singh , Ademola Kassim , Christopher M Coleman , Ruth Elliott , Susan R Weiss, Matthew B Frieman , Stefan G Sarafianos, 2014. Evaluation of SSYA10-001 as a replication inhibitor of severe acute respiratory syndrome, mouse hepatitis, and Middle East respiratory syndrome coronaviruses, Antimicrob Agents Chemother Aug;58(8):4894-8

[110] Yan Fang, Pengcheng Sun, XupingXie, Mingjian Du, FengheDu ,Jianfeng Ye, Birte K. Kalveram, Jessica A. Plante, Kenneth S. Plante, Bo Li, Xiao-chen Bai, Pei-Yong Shi, Zhijian J. Chen. An antibody that neutralizes SARS-CoV-1 and SARS-CoV-2 by binding to a conserved spike epitope outside the receptor binding motif. 2022. Sci. Immunol.10.1126/sciimmunol.abp9962.

[111] Zaher NH, Mostafa MI, Altaher AY.2020. Design, synthesis and molecular docking

of novel triazole derivatives as potential CoV helicase inhibitors. Acta Pharm, Jun 1;70(2):145-159.

[112] Xia S, Yan L, Xu W, Agrawal AS, Algaissi A, Tseng CK, *et al*.2019. A pancoronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike. SciAdv, 5:eaav4580.

[113] Xia S, Xu W, Wang Q, et al.2018. Peptide-based membrane fusion inhibitors targeting HCoV-229E Spike Protein HR1 and HR2 Domains. Int J Mol Sci. Feb 6;19(2).

[114] Nieto-Torres. J.L., Carmina Verdiá-Báguena., Carlos Castaño-Rodriguez., Vicente M. Aguilella., Luis Enjuanes. 2015. Relevance of Viroporin Ion Channel Activity on Viral Replication and Pathogenesis. Viruses, 7, 3552-3573

[115] Pillaiyar.T ,Sangeetha Meenakshi sundaram and ManojManickam. 2020. Recent discovery and development of inhibitors targeting coronaviruses. Drug Discovery Today, Apr;25(4):668-688

[116] Vigant,F, Nuno C. Santos, Benhur Lee. Broad-spectrum antivirals against viral fusion. Nat Rev Microbiol. 2015; 13(7): 426–437. 2015.

[117] JindrichCinatl Jr, Martin Michaelis, Martin Scholz & Hans Wilhelm Doerr (2004) Role of interferons in the treatment of severe acute respiratory syndrome, Expert Opinion on Biological Therapy, 4:6, 827-836.

[118] Martin J Vincent, Eric Bergeron, Suzanne Benjannet, Bobbie R Erickson, Pierre E Rollin, Thomas G Ksiazek, Nabil G Seidah,Stuart T Nichol, 2005. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread, Virol J, 2:69.

[119] https://www.ibm.com/in-en/cloud/databases. accessed on 27.04.2020.

[120] Kim, T.W.; Lee, J.H.; Hung, C.F.; Peng, S.; Roden, R.; Wang, M.C.; Viscidi, R.; Tsai, Y.C.; He, L.;Chen, P.J.; et al. 2004. Generation and characterization of DNA vaccines targeting the nucleocapsid protein of severe acute respiratory syndrome coronavirus. J. Virol. 78, 4638–4645.

[121] Norbedo, S., Dinon, F., Bergamin, M., Bosi, S., Aroulmoji, V., Khan, R., Murano, E. 2009. Synthesis of 6-amino-6-deoxyhyaluronan as an intermediate for conjugation with carboxylate-containing compounds: application to hyaluronan - camptothecin conjugates. Carbohydrate Research, 344, 98-104.

[122] Sorbi, C., Bergamin, M., Bosi, S., Dinon, F., Aroulmoji, V., Khan, R., Murano E., Norbedo. S. 2009. Synthesis of 6-O methotrexylhyaluronan as a drug delivery system. Carbohydrate Research, 344, 91-97.

[123] Luo Y, Prestwich GD.1999. Synthesis and selective cytotoxicity of a hyaluronic acid-antitumor bio-conjugate, Bioconjugate Chem., Sep-Oct;10(5)755-63.

[124] Glen Prestwich, Pre blocking with non-HA gags increases effectiveness of HA conjugated anticancer agents; US20050169883 (2003).

[125] Underhill C,1992. CD44: the hyaluronan receptor, J Cell Sci., Oct 103 (Pt 2):293-288.

[126] Pandey, MS., Weigel, PH. 2014. Hyaluronic Acid Receptor for Endocytosis (HARE)-mediated Endocytosis of Hyaluronan, Heparin, Dermatan Sulphate, and Acetylated Low Density Lipoprotein (AcLDL), but Not Chondroitin Sulphate Types A, C, D, or E, Activates NF-κB-regulated Gene Expression ,J Biol. Chem., Jan 17, 289(3): 1756–1767.

[127] Park, D., Youngmi Kim., Hyunah Kim., Kyungjong Kim., Yun-Sil Lee., JongseonChoe., Jang-Hee Hahn., Hansoo Lee., Jongwook Jeon., Chulhee Choi Young-Myeong Kim., Dooil Jeoung,2012. Hyaluronic Acid Promotes Angiogenesis by Inducing RHAMM-TGFβ Receptor Interaction via CD44-PKCδ, Molecules and Cells, 33, 563–574.

[128] Lawrance, W., Banerji S, Day AJ., Bhattacharjee S., Jackson DG. 2016. Binding of Hyaluronan to the Native Lymphatic Vessel Endothelial Receptor LYVE-1 Is Critically Dependent on Receptor Clustering and Hyaluronan Organization. J Biol. Chem. Apr 8, 291(15), 8014-30.

[129] Luo, Y., Prestwich, G. D.1999. Synthesis and selective cytotoxicity of a hyaluronic acid - antitumor bio-conjugate. Bioconjugate Chem. 10: 755-763.

[130] Akima, K., Iwata, Y., Matsuo, K., Watar, N., US Patent, 5,733,891, March 311998..

[131] Khan, R., Mahendhiran, B., Aroulmoji, V. 2013. Chemistry of hyaluronic acid and its significance in drug delivery strategies: A review. International Journal of Pharmaceutical Sciences and Research, 4, 3699-3710.

[132] Norbedo, S., Dinon, F., Bergamin, M., Bosi, S., Aroulmoji, V., Khan R., Murano, E. 2009. Synthesis of 6-amino-6-deoxyhyaluronan as an intermediate for conjugation with carboxylate-containing compounds: application to hyaluronan-camptothecin conjugates. Carbohydrate Research, 344, 98-104.

[133] Thirumalaisamy, R., Aroulmoji, V., Riaz Khan., Sivasankar C., Deepa, M. 2021. Hyaluronic Acid - 2-Deoxy-D-Glucose Conjugate Act as a Promising Targeted Drug Delivery Option for the Treatment of COVID-19, Int. J. Adv. Sci. Eng. 7(4) 1995-2005.

[134] Riaz Khan, Aroulmoji, V. 2021. Hyaluronic Acid - Hydroxychloroquine Conjugate Proposed for Treatment of COVID-19, Int. J. Adv. Sci. Eng. 6(4) 1469-1471.

[135] Thirumalaisamy, R., Aroulmoji, V. Muhammad Nasir Iqbal., Deepa, M., Sivasanka, C., Riaz Khan, Selvankumar, T.2021. Molecular insights of hyaluronic acid-hydroxychloroquine conjugate as a promising drug in targeting SARS-CoV-2 viral proteins, J of Molecular Structure, 1238, 15 August, 130457.