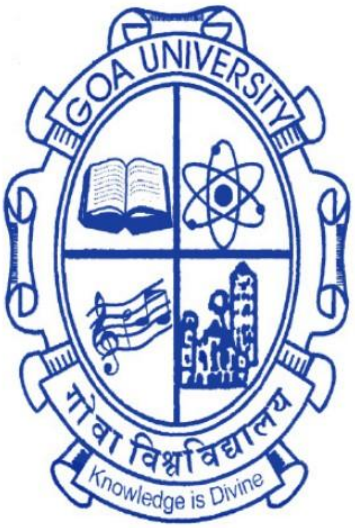
Antiviral Therapeutic Molecules For Covid-19

A MSc Dissertation report by:

PRAJWAL P. NAIK



School Of Chemical Sciences
Goa university
Goa 403206

April 2022

ANTIVIRAL THERAPEUTIC MOLECULES FOR COVID-19

A DISESSERTION REPORT

Submitted in Partial Fulfillment

Of

The degree M.Sc. (Analytical Chemistry)

By

Mast. Prajwal P. Naik

To the

School of Chemical Sciences

Goa University

Goa 403206

April 2022

CERTIFICATE

This is to certify that **Prajwal Naik** of M.Sc.(Analytical Chemistry) has successfully completed dissertation work on the topic ***"Antiviral Therapeutic Molecules for Covid-19"*** in the year 2021-2022, it is further certified that this project is the individual work of the candidate.

Dr. Vidhyadatta Vernekar
Dean of SCS,
Goa University

Dr. Rupesh Patre
Guiding Teacher
SCS, Goa University

INDEX

I. INTRODUCTION :

Pandemics and epidemics of respiratory viruses continue to be recognized as being among the most common causes of morbidity and mortality around the world. In 2019, some pneumonia cases with indefinite reasons have been reported. Through the epidemiological investigation, virus isolation and nucleic acid sequencing, it was confirmed that the pathogenic factor was a novel coronavirus named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses. And this disease is known as COVID-19 which standing for coronavirus disease 2019. With the continuous spread of the epidemic, the WHO announced on March 11, 2020 that COVID-19 is a global pandemic[1].

The current coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a major threat to human civilization and leaves many challenges ahead. As of July 11, 2020, there were more than 12 million confirmed cases of COVID-19, with more than 500,000 deaths. The most common symptoms of COVID-19 include fever, dry cough, dyspnea, chest pain, fatigue and myalgia, whereas headache, dizziness, abdominal pain, diarrhea, nausea, and vomiting are less commonly observed[2]. Although most of the SARS-CoV-2 infections are asymptomatic or have mild clinical symptoms, 20.3% of the hospitalized patients require intensive care unit (ICU) admission, resulting in a significant burden on health care facilities[3].

SARS-CoV-2 belongs to the family *Coronaviridae*, subfamily *Coronavirinae* and genus *Betacoronavirus*, along with SARS-CoV and the Middle East respiratory syndrome coronavirus (MERS-CoV). SARS-CoV-2 has a spherical enveloped particle-containing positive-stranded RNA that binds to the nucleocapsid (N) inside the membrane protein (M) and the envelope (E) comprises of glycoprotein spikes(S). S protein is a primary receptor-binding domain (RBD) and is critical for viral entry into the host cells through cellular receptor angiotensin-converting enzyme 2 (ACE2). Similar to other viruses, SARS-CoV-2 hijacks the host cell machinery and multiplies via viral attachment, fusion, penetration, uncoating, transcription, translation, and virion release[3].

The statistics have considered till the end of July 2021, SARS-CoV-2 has spread to 220 countries or areas, with a total of 198 million confirmed cases and 4.2 million deaths. Although the mortality rate of SARS-CoV-2 is about 2.0%, lower than that of SARS CoV (8422 confirmed cases, 916 deaths, mortality rate 10.9%) and MERS-CoV (2468 confirmed cases, 851 deaths, case fatality rate 34.5%), SARS-CoV-2 is more contagious and now is spreading very quickly around the globe. The high number of infections has demonstrated the ability of SARS-CoV-2 to spread quickly and sustainably in the community[1].

Unfortunately, there are no satisfactory anti-SARS-CoV-2 drugs so far. Although the FDA has approved remdesivir for the treatment of mild and moderate patients with COVID-19, WHO suggests no remdesivir for patients with COVID-19 at any severity. Besides, the clinical trial results of lopinavir-ritonavir and hydroxychloroquine did not show effectiveness with statistical significance. Therefore, there is an unmet medical need to develop novel antiviral drugs and better therapeutic options to combat this deadly disease. Taking these facts into consideration , an in-depth study of the life cycle and pathogenic mechanism of SARS-CoV-2 is urgently needed to pave the way for the development of SARS-CoV-2 inhibitors[1].

II. STRUCTURE OF SARS-CoV-2:

SARS-CoV-2 is an enveloped positive-sense RNA virus and belongs to the lineage B betacoronavirus that also includes the highly pathogenic coronaviruses MERS-CoV and SARS-CoV. Based on the phylogenetic analysis, SARS-CoV-2 shares high sequence identity with that of SARS-like coronaviruses (89.1% nucleotide similarity) and SARS-CoV (79% nucleotide similarity). Like other coronaviruses, the structure of SARS-CoV-2 is composed of 16 nonstructural proteins (Nsps) (Nsp1–16), 5–8 accessory proteins, and 4 structural proteins including the spike (S), membrane (M), envelope (E) and nucleocapsid (N) proteins. The S protein is a heavily glycosylated type I membrane protein and uniformly arranged as trimers anchored in the viral membrane. The trimeric S protein consists of two fragments: the receptor-binding fragment S1 and the fusion fragment S2 , and is crucial for viral fusion, entry, and transmission[4].

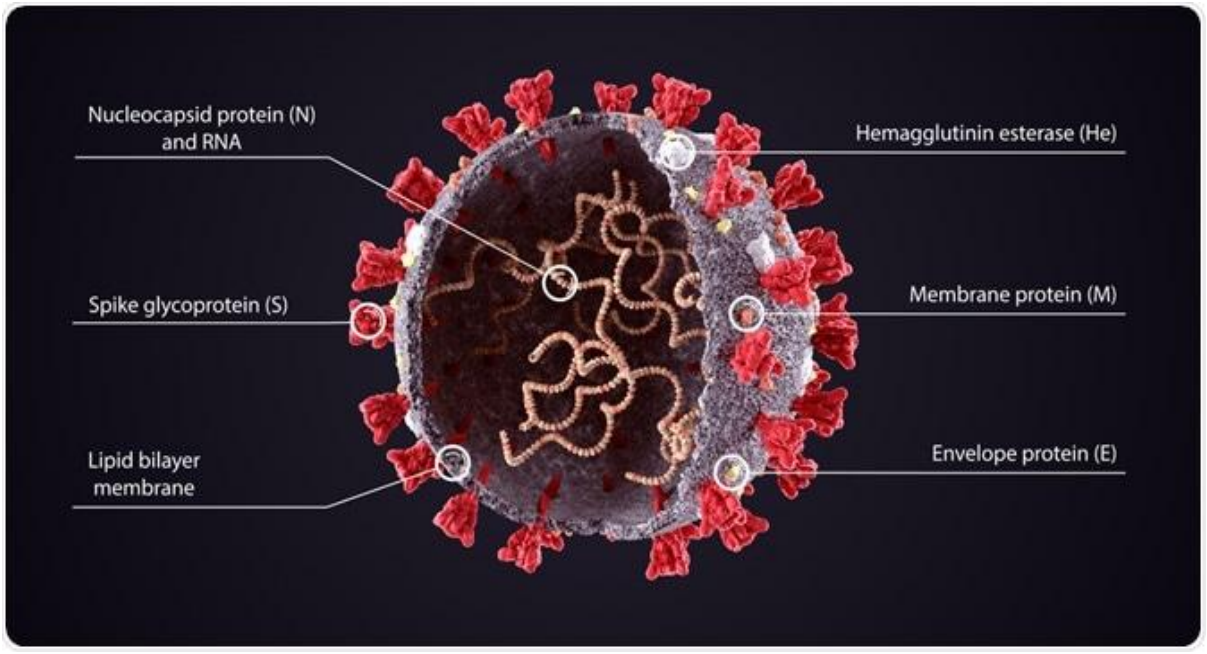


Fig.1. Structure of SARS-CoV-2.

The SARS-CoV-2 virus is known to infect the host cell via binding to the angiotensin-converting enzyme (ACE2) with the help of its S proteins. The S protein has two subunits, named as S1 and S2. The S1 subunit is known to attach to the receptor on the cell i.e. ACE2 with the help of its receptor-binding domain (RBD). The S2 subunit is involved in the membrane fusion of the virus and host cell. The E protein also has two domains, a hydrophobic domain and a charged cytoplasmic tail. It is known to be functional in viral assembly by the formation of ion channels which help in coordinating with other viral proteins. The M protein has three transmembrane domains with long C-terminals and short N-terminals. Its major function is to maintain the viral envelope by interaction with other viral proteins by stabilizing the N protein. The N protein plays an important role in RNA binding in the viral replication cycle. It forms a ribonucleoprotein complex with the help of its N-terminal domain (NTD) and performs dimerization using the C-terminal domain (CTD). This protein has three

domains, known as N-arm, C-tail, and the central linker region. It also performs roles like inhibition of host cell protein translation, alteration of host cell metabolism, and cell apoptosis[5].

III.ACTIVE BINDING SITES ON SARS-CoV-2:

There are many binding sites present on the SARS-CoV-2 that might be the potential drug targets.

1. Chymotrypsin-like protease (3CLpro)

3CLpro, also known as main protease (Mpro) or Nsp5 is an important enzyme found in SARS-CoV that is responsible for proteolytic function in the maturation stage of the virus. It is found to cleave at least 11 sites on polyprotein-1a and polyprotein-1ab found in the viral genome to cleave Nsp4-Nsp16. These non-structural proteins namely RNA dependant RNA polymerase (RdRp), helicase, endonuclease, exonuclease, and 2′ -O-methyltransferase (2′ -O-MTase) are very important proteins in the viral genome. Hence, 3CLpro is the most important target for drugs against coronaviruses . Small molecules and peptide inhibitors are expected to inhibit 3CLpro activity[5].

2. RNA dependant RNA polymerase (RdRp)

RdRp (Nsp12) is the crucial enzyme that has an important role in the replication and transcription of the virus. With the help of its other cofactors Nsp7 and Nsp8, the interaction between Nsp12 and the RNA is strengthened, thereby enhancing the RdRp activity. Hence, the Nsp12-RdRp is an important drug target for treatment against COVID-19. The RdRp domain of the protein is at the C-terminus with a Ser-Asp-Asp motif[5].

3. Hemagglutinin esterase (HE)

HE is a structural protein present specifically in β-coronaviruses and is recognized as one of its markers. It acts both as a receptor-destroying enzyme and a lectin that attaches to the O-acetylated-sialic-acids . HE-sialic acid complex (PDB ID: 3CL5) is thus another drug target in COVID-19[5].

4. Papain like protease (PLpro)

PLpro cleaves the N-terminus of the polyprotein to generate Nsps; Nsp1, Nsp2, and Nsp3 by cleaving them at their boundaries with the help of 3CLpro. PLpro is also crucial in alienating the innate immunity of the host cell. Due to its important function in the viral replication cycle, PLpro is another important drug target[5].

5. 2′ -O-methyltransferase (2′ -O-MTase)

2′ -O-MTase (Nsp16) and S-adenosylmethionine (SAM) dependent nucleoside perform the role of methylating the ribose 2′ -O position of the first two nucleosides of mRNA of the virus, thereby camouflaging and protecting the virus from the immunity system of the host cell. 2′ -O-MTase gets activated on binding with the Nsp10. Hence, Nsp10-Nsp16 complex (PDB: 3R24) is a drug target owing to its indispensable role in viral replication[5].

6. Helicase

Helicase (Nsp13) has the function of unwinding the double-stranded oligonucleotides in an NTP-dependant manner in the 5′ − 3′ direction. It has a metal-binding domain at N-terminal and a helicase domain. Nsp 13 is another important target to develop a treatment method against coronaviruses[5].

7. Spike RBD

RBD is the major binding domain of the S protein located on the S1 subunit. It complexes with the ACE2 receptor on the host cells. A total of 18 residues of ACE2 attach with 14 amino acids of RBD, the most important being K341 of ACE2 with the R453 of the RBD. The binding activity of the RBD can be altered if there is a mutation on D454 or R441 residues of the RBD (Du et al., 2009). Any alteration in the structural proteins of RBD could be an effective prophylactic measure and inhibition of RBD-ACE2 complex can be another promising treatment strategy[5].

8. ACE2 receptor

ACE2 receptor is the primary binding site of the S protein which facilitates viral entry. The affinity of SARS-CoV-2 S protein to the ACE2 receptor is higher than that of SARS-CoV. The ACE2 receptor and the ACE2-RBD complex are thus the two important sites for prophylaxis of COVID-19[5].

9. Glucose-regulated proteins

GRP78 also known as Binding immunoglobulin protein (BiP) or heat shock 70 kDa protein 5 (HSPA5), is a chaperone protein found in the lumen of the endoplasmic reticulum (ER). Its function is to prevent the unfolding of proteins that get translocated into the ER. In conditions where the unfolded proteins get accumulated above a threshold, GRP78 releases enzymes like activating transcription factor 6, inositol-requiring enzyme 1, and protein kinase RNA-like endoplasmic reticulum kinase, which enhance the protein folding and inhibit protein synthesis. GRP78 is overexpressed under cell stress and translocates from the ER to the plasma membrane, where it can be recognized as a receptor to mediate viral infection with the help Pep42 protein of the virus-cell. The interactions of the S protein of SARS-CoV-2 with the GRP78 and concluded that the S protein GRP78 binding site is a potential druggable target for treatment of COVID-19[5].

IV. MECHANISM OF COVID INFECTION AND REPLICATION :

In both SARS-CoV and SARS-CoV-2, the S protein binds to ACE2 as its receptor on host cells . Cryo-EM structure analysis has revealed that the binding affinity of S protein to ACE2 in SARS-CoV-2 is about 10–20 times higher than that of the S protein from SARS-CoV. Since the process of viral infection begins with the interaction of S protein and ACE2 on the host cell surface, this step is of particular importance[6].

Initially, the S1 domain of spike protein and surface receptor ACE2 mediates coronavirus binding to the host cell. The S1 protein is then cleaved by transmembrane serine protease 2 (TMPRSS2) or by cathepsin L, and fusion peptide of S2 domain facilitates the fusion of E protein with cellular membranes. After the virus enters the cell, it is uncoated and genomic RNA of the virus is then released into the cytoplasm and translated into two polyproteins , namely, PP1A and PP1AB. The viral genome is then converted into a negative-sense viral RNA genome, used as a template to

synthesize positive sense genomic and sub-genomic viral RNA. Genomic RNA and nucleocapsid (N) protein are replicated or transcribed in the host cytoplasm. However, other viral structural proteins, such as spike (S), envelope (E), and membrane (M), are transcribed and translated into the endoplasmicreticulum, which is then inserted into the Golgi body. The viral genomic RNA and proteins such as N, S, E, and M are further assembled in the ER–Golgi intermediate compartment (ERGIC). Finally, the newly generated positive-sense RNA genomes are released through the plasma membrane.

It has been shown that entry of SARS-CoV-2 is not possible in cells without ACE2 expression. Other receptors e.g. dipeptidyl peptidase 4 (DPP4) or aminopeptidase N do not mediate SARS-CoV-2 entry, indicating that ACE2 is essential for SARS-CoV-2 entry into the host cell. The studies have shown that the S protein of SARS-CoV-2 has a much higher binding affinity towards ACE2, which is 10–20 fold greater than that of SARS-CoV, making SARS-CoV-2 more contagious than other coronaviruses[7].

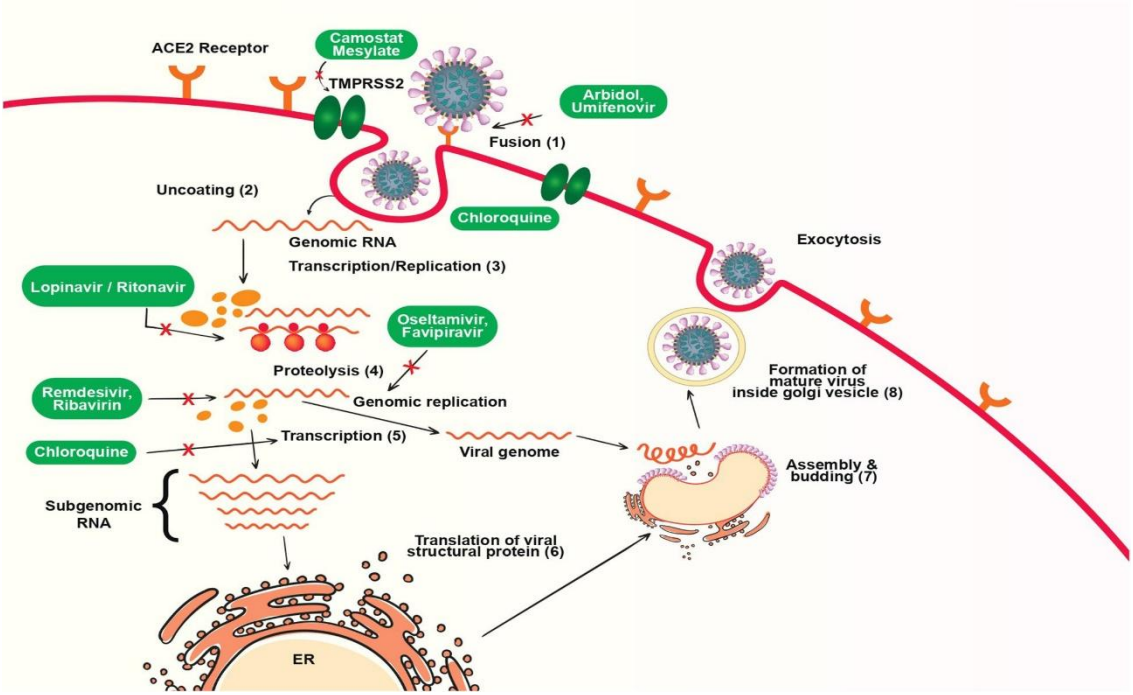


Figure 2: Mechanism of covid -19 infection

V. DEVELOPMENT OF ANTIVIRAL DRUG:

Any steps or any proteins that are essential in the SARS-CoV-2 replication cycle can be targeted to develop antiviral drug[8].

From the medicinal chemistry point of view, many general approaches might be helpful to identify new therapies to combat SARS-CoV-2 infections. Drug repurposing is an efficient approach where the screening of chemical libraries containing drugs or investigational new drugs (INDs) occurs to find effective SARS-CoV-2 inhibitors. Those drugs or INDs with appropriate pharmaceutical properties possess approved safety profile. They can be massively produced in the pharmaceutical industry which can save a lot of time in this urgent situation. One additional possibility involves the large-scale screening in silico or in vitro. Through the methods of virtual screening and high-

throughput screening (HTS), it is possible to identify promising lead compounds in a short time from millions of candidates. Another is the rational drug design based on target structures such as the functional proteins of SARS-CoV-2. However, this process may not respond quickly to such a emergency situation under pandemic, but if we take the great genetic diversity of bat SARS related-CoVs into consideration, it is necessary to enrich our arsenal in case of the emergence of new coronavirus mutants in the future. To be noted, these three approaches are often used in combination to facilitate drug development. Besides, natural products derived from herbal plants has also been an important source of lead molecules for SARS-CoV-2 treatment[1,9].

VI. DRUG REPURPOSING:

Drug repurposing, also called repositioning or rediscovering, refer to the process of developing a known drug for a novel use that is different from its original clinical indication[10].

New drug development is generally acknowledged as time-consuming and expensive. However, drug repositioning is a shortcut to the discovery of effective coronaviral inhibitors in this pandemic situation. There are generally some classical methods that can be used to develop or screen for novel broad-spectrum coronaviral inhibitors. The first method is the repurposing of nucleoside analogues as the inhibitors of SARS-CoV-2 RdRp which is conserved across different coronavirus family and plays an important part in coronavirus life cycle. It makes RdRp an ideal target for broad-spectrum coronaviral drugs. Nucleoside analogues are converted into its active triphosphate metabolite by intracellular kinase and incorporated into the growing RNA chain leading to the termination of the replicative cycle, which accounts for their antivirus activity[11] .

Some of the broad-spectrum antiviral drugs that are repurposed in this COVID-19 pendemic includes Remdesivir, Favipiravir, Azvudine, beta-D-N4-Hydroxycytidine, Terrflunomide,Ivermectin[12].

VII. THE DRUG CANDIDATE FOR THE TREATMENT OF COVID-19:

With a better understanding of the structure, the infection mechanisms of SARS-CoV-2 and the clinical symptoms of COVID19 patients, many drugs had been used for the clinical practice of COVID-19.

1. *Remdesivir*

Remdesivir (GS-5734) is a novel prodrug of an adenosine analogue and effective against a broad spectrum of human and preepidemic zoonotic CoVs . Remdesivir can be triphosphorylated in cells and act as a substrate of virus RNA-dependent RNA polymerase(RdRp) and penetrate into the virus newly synthesized RNA strand, thus interrupting the synthesis of the virus genome[13].

The first case of COVID-19 in Washington, USA, was compassionately treated with intravenous (IV) administration of remdesivir to stop the progression of pneumonia on day 7 of hospitalization . Studies have shown that remdesivir is a potential therapy for COVID-19 due to its in vitro potent and broad-spectrum, activity against several SARS-CoV-2 strains with EC50 and EC90 values of 0.77 μM and 1.76 μM, respectively.

Despite the side effects, remdesivir has yielded acceptable results for treating patients with COVID-19. However, further studies are needed to prescribe remdesivir with minimal side effects on patients with COVID-19. An in vivo study in mice with MERS-CoV infection showed that remdesivir is an effective drug in the prevention of pulmonary hemorrhage and is capable of reducing the viral titer [90]. Albeit, it should not be neglected that remdesivir was not originally designed to target COVID-19[14].

To evaluate the safety and pharmacokinetics of remdesivir, the drug was evaluated in single- and multiple-doses associated to phase I clinical trial. The results revealed that IV infusion of 3–225 mg remedsivir posed no adverse risk to the kidneys or liver. Remdesivir was approved by FDA on May 01, by Japan on May 07, 2020, and subsequently by various European countries and Canada. Further studies are needed on its safe and efficient prescription in children and pregnant women[15].

Since April 19, 2020, several studies have been conducted in the United States to investigate the effect of remdesivir on the treatment of patients with COVID-19. In a recent study by, the efficacy of remdesivir was evaluated in 61 patients with COVID-19. Several moderate side effects such as renal impairment, rash, diarrhea, increment of hepatic enzymes, and hypotension have been reported in 32 patients. Also, 12 patients experienced severe side effects, including septic shock, multiple-organ-dysfunction syndrome, hypotension, and acute kidney injury. In addition, the treatment was stopped in four patients due to deteriorating preexisting renal failure and multiple organ failure as well as increased transaminases in two patients, including one patient with a maculopapular rash[13] .

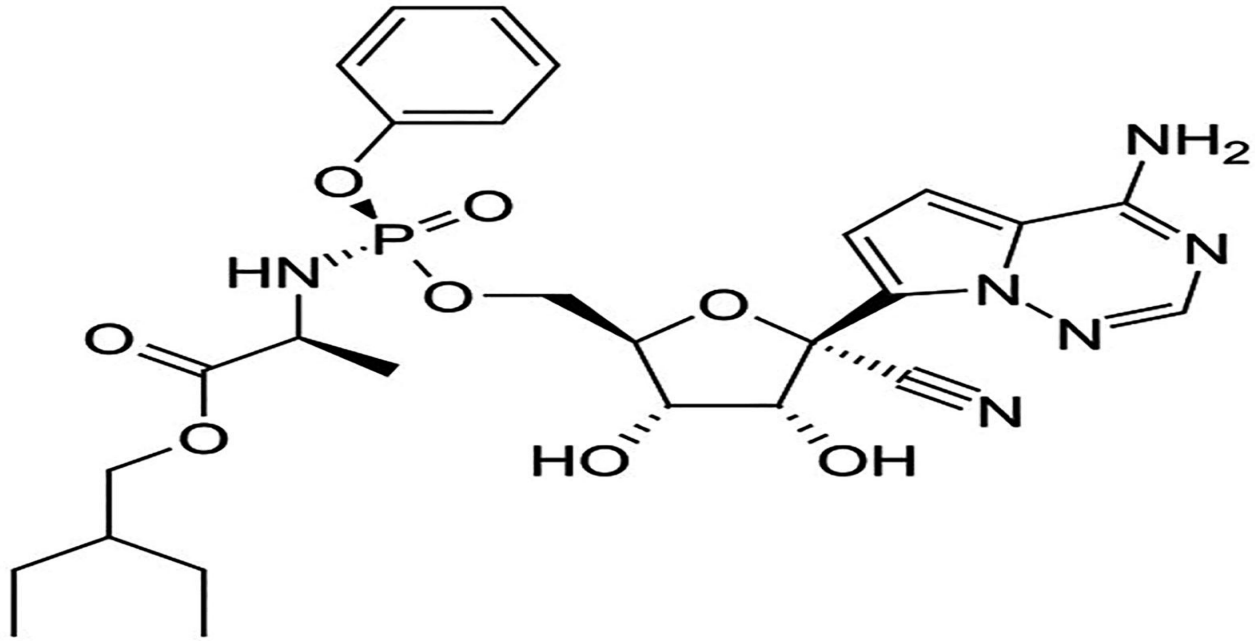


Figure 3: Remdesiver structure

2.Ribavirin

Ribavirin is a guanosine analogue with broad-spectrum antiviral activity against RNA viruses. The antiviral mechanisms of Ribavirin includes fatal mutagenesis, specific or non-specific chain termination and inhibition of nucleotide biosynthesis etc[13].

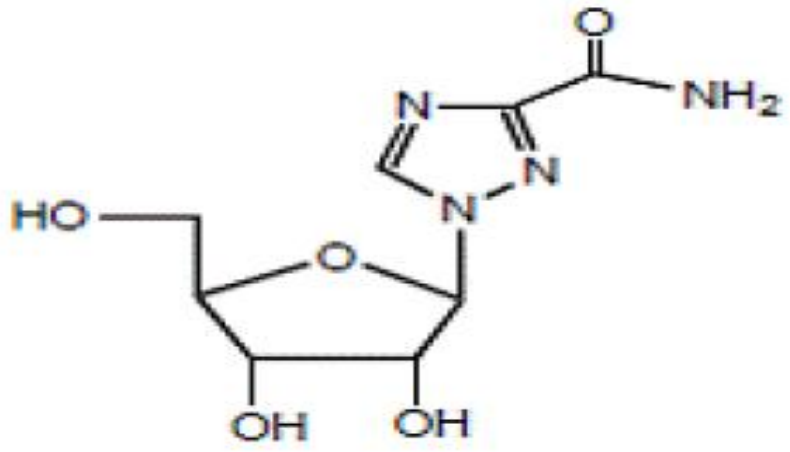


Figure 4: Ribavirin structure

3.Favipiravir

Favipiravir is a nucleoside analogue that can be triphosphorylated in cells and act as a substrate of virus RNA-dependent RNA polymerase (RdRp)[13]. Favipiravir offers broad-spectrum anti-RNA virus activity, and its effectiveness in both animal and human trials has been shown in the literature[16,17].

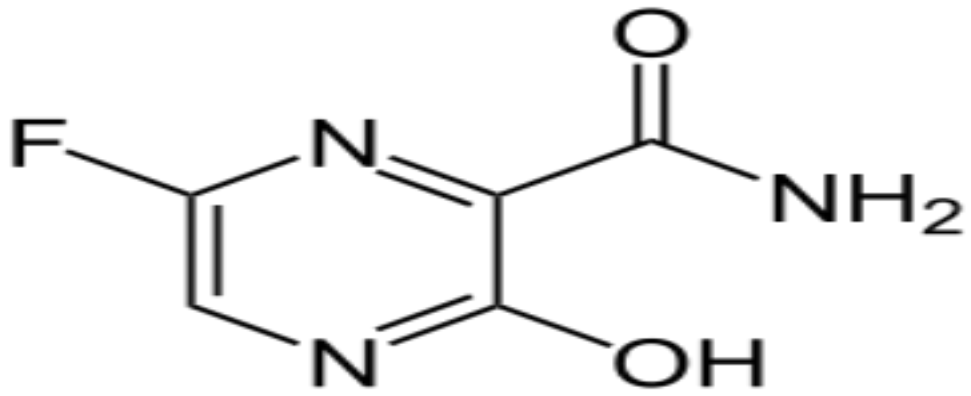


Figure 5: Favipiravir structure

4. Kaletra (Lopinavir/Ritonavir)

Kaletra is a compound preparation with two specifications (Lopinavir/Ritonavir). Lopinavir is an HIV protease inhibitor, which can influence the formation of matured virus particles and weaken the infectivity of the virus [39]. Lopinavir has poor bioavailability and short half-life time. Ritonavir is a cytochrome CYP3A4 enzyme inhibitor, which can inhibit the metabolism of Lopinavir. The combined treatment of Lopinavir and Ritonavir can significantly enhance the bioavailability of Lopinavir and improve its antiviral effect in vivo[13].

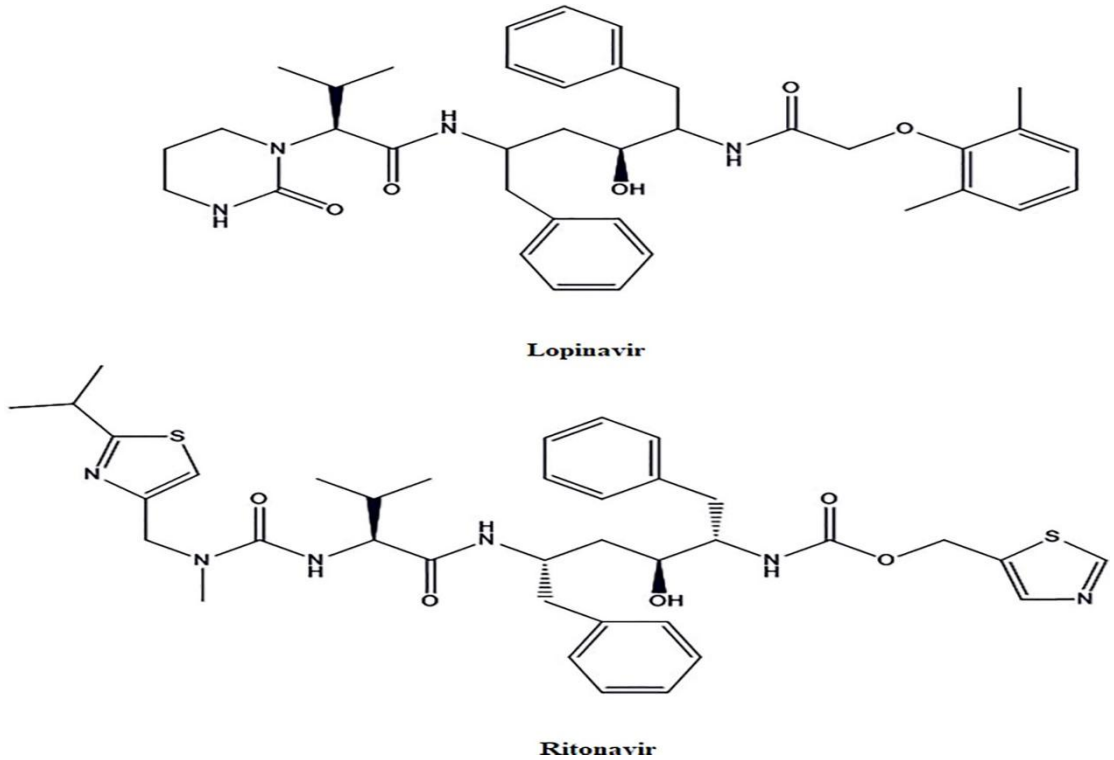


Figure 6: structure of Lopinavir and Ritonavir

5.Chloroquine/Hydroxychloroquine

Chloroquine (CQ)is a weak base, which can enter cells and accumulate in lysosome,trans Golgi network and other acidic organelles through protonation, so as to increase the pH value and destroy the structure and function of the organelles Some viruses can enter the lysosome in cells through endocytosis. The acidity of lysosomes and the function of related enzymes can destroy virus particles and make virus release reproducible nucleic acid, thereby infecting host cells.After entering cells, Chloroquine can block virus infection by increasing lysosomal pH via protonation. Therefore, it has a broad-spectrum antiviral effect. Hydroxychloroquine(HCQ) is a less toxic derivative of CQ which shows better clinical efficacy than CQ[13].

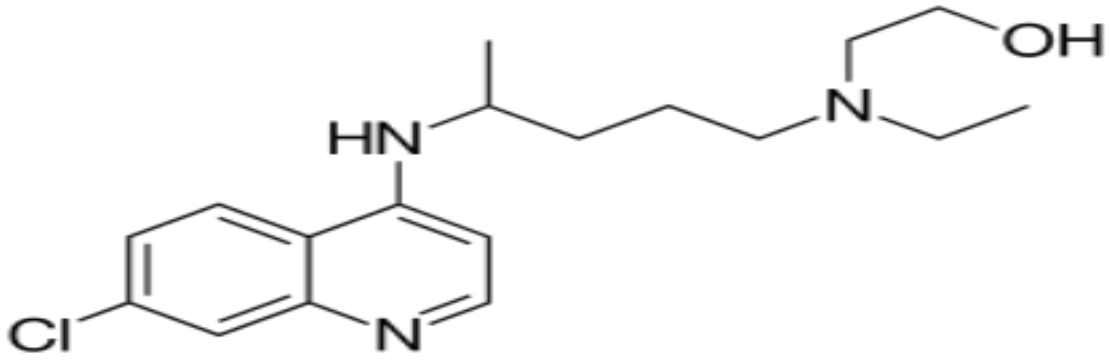


Figure 7: structure of Hydroxychloroquine

6. Arbidol

Arbidol hydrochloride is a non-nucleoside antiviral drug. Arbidol can specifically inhibit the contact, adhesion and fusion of virus lipid envelope with host cell membrane by activating the antiviral protein in the host, thereby inhibiting the replication of virus in host cells. Arbidol has dual pharmacological activity on the one hand, as a broad-spectrum antiviral drug, it can inhibit a variety of viruses, including influenza virus, respiratory syncytial virus (RSV), adenovirus (ADV), hepatitis C virus (HCV) and hepatitis B virus (HBV), on the other hand, it can activate the immune induce the body to produce IFN (interferon), and regulate the immunologic function of the body[13].

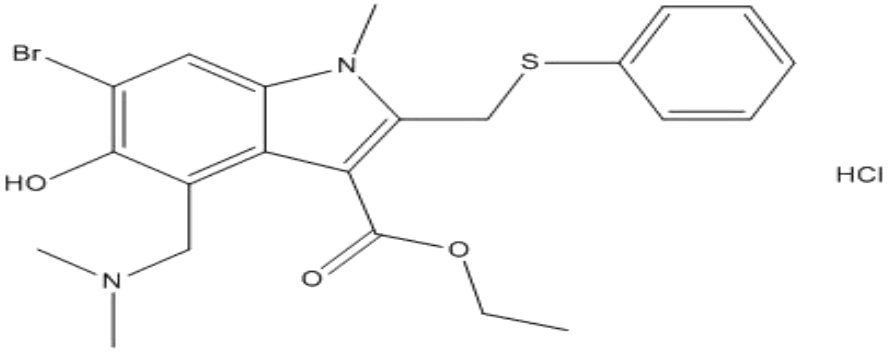


Figure 8: structure of Arbidol hydrochloride

7.Ruxolitinib

Ruxolitinib is commonly used for treating patients with intermediate or high-risk myelofibrosis . Ruxolitinib as a Janus kinase (JAK) inhibitor was prescribed in a phase III clinical trial of patients with COVID-19 associated with cytokine storm. However, due to the broad immunosuppressive effects of JAK kinase inhibitors, the US National Institute of Health (NIH) did not recommend the application of ruxolitinib for control of cytokine storm in patients with COVID-19[6].

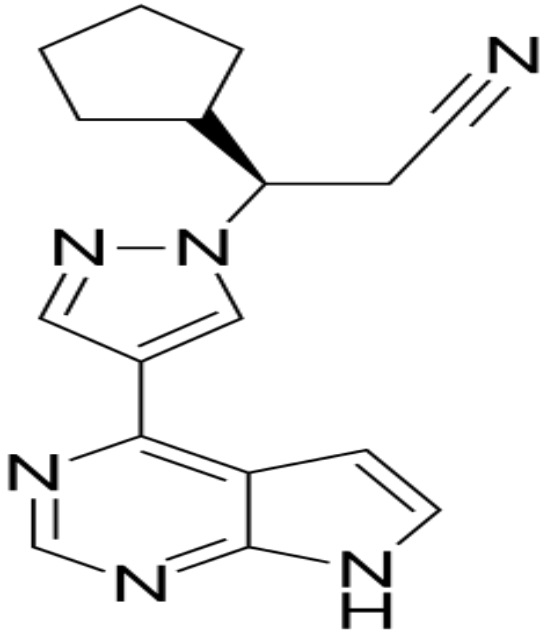


Figure 9: Structure of ruxolitinib

8. Azithromycin

Although the preponderance of evidence indicates that there is no benefit of HCQ for treating COVID-19, fewer studies have evaluated azithromycin along with HCQ, a broad-spectrum antibiotic with anti-inflammatory properties. Azithromycin is an antibacterial compound, which exhibits significant anti-inflammatory properties against bacterial lipopolysaccharide (LPS)-induced inflammation in pneumonia. In the case of COVID-19, this antibiotic had no direct effect on patients' recovery process, while some scientists have suggested that the antibacterial properties of azithromycin have been clinically effective in the experimental treatment of severe acute respiratory syndrome, which occurs in patients with COVID-19.

Based on duality, it is required that the clinical pharmacology and characteristics of azithromycin could be considered in clinical trials alone or combined with other agents to ensure its efficacy for treating patients with COVID-19 and to increase the probability of achievement to a definitive treatment protocol[6].

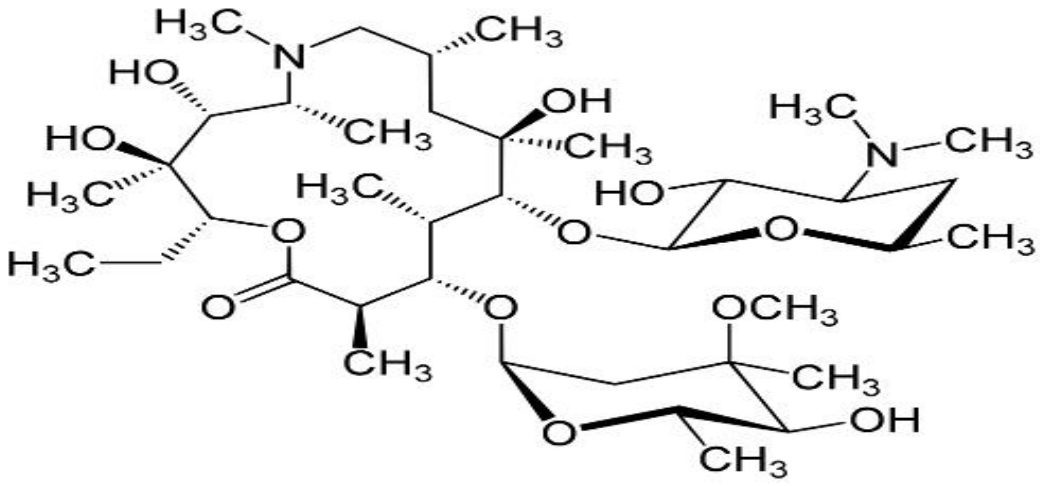


Figure 10. Structure of Azithromycin.

9. Ivermectin

Ivermectin is an antifungal drug, which is prescribed as a treatment for cutaneous larva migrans. Ivermectin boosts the immune system by increasing the production of IL-1 and other cytokines, as well as activation of superoxide anion production and augmentation of lymphocyte response to mitogens. Ivermectin has been reported to be effective in treating infections caused by RNA viruses such as respiratory syncytial virus, dengue, influenza, rabies, and Zika viruses.

The 2015 Nobel Prize in Physiology and Medicine was awarded to William C. Campbell and Satoshi Omura ¨ for their discoveries leading to ivermectin . In addition to its extraordinary efficacy against

parasitic diseases, ivermectin continues to offer new clinical applications due to its ability to be repurposed to treat new classes of diseases. Beyond its invaluable therapeutic role in onchocerciasis and strongyloidiasis, an increasing body of evidence points to the potential of ivermectin as an antiviral agent[18].

In an in vitro study by Caly et al., ivermectin was used as a potential replication inhibitor of SARS-CoV-2. They showed that the addition of 5 μM ivermectin to virus-infected Vero/hSLAM cells was capable of reducing SARS-CoV-2 RNA level about 5000-fold, compared to controls within 48 h[6].

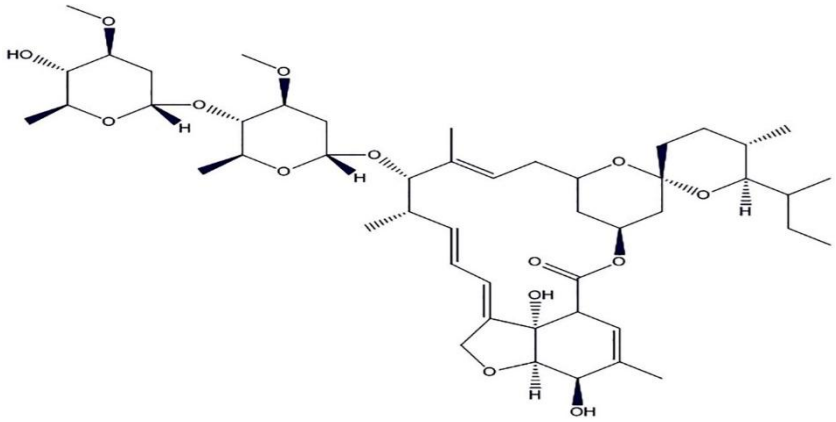


Figure 11.Structure of Ivermectin

10. Baricitinib

Similar to other viruses, SARS-CoV-2 enters the host cells through receptor-mediated endocytosis. The process of endocytosis is regulated by AP2-associated protein kinase 1 (AAK1). Therefore, the disruption of AAK1 will not only block the viral entry but also the intracellular viral assembly. Baricitinib is a Janus kinase (JAK) inhibitor with high potential to bind to and inhibit AAK1.53 Hence baricitinib can be used to inhibit both viral entry as well as the inflammatory response associated with SARS-CoV-2 infection. JAK inhibitors such as ruxolitinib and fedratinib that are closely related to baricitinib inhibited clathrin-mediated endocytosis at higher doses and hence these may not be effective in reducing the viral infectivity at tolerable doses. Therapeutic use of baricitinib is associated with the occurrence of neutropenia, lymphocytopenia, and viral reactivation. Since SARS-CoV-2 infected patients have a lower absolute lymphocyte count, use of baricitinib may increase the incidence of co-infection.Further studies are required to analyze the risk-benefit ratio as well as the clinical utility of baricitinib therapy.

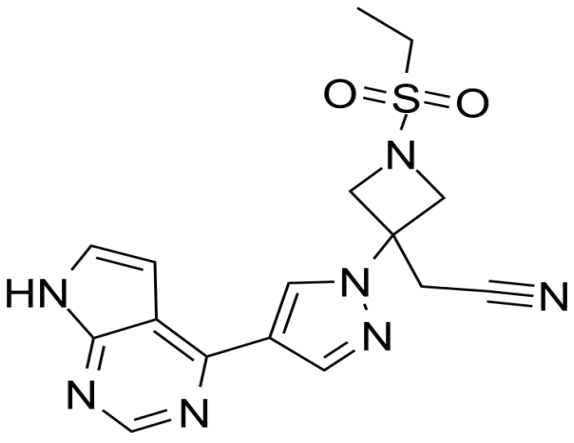


Figure. 12 : Structure of Baricitinib

11. Camostat mesylate

Camostat mesylate – a serine protease inhibitor – is another candidate drug that targets the fusion step in viruses. SARS-CoV-2 gains entry within the target host cells either through ACE-2 receptor and/or TMPRSS2 receptors, and camostat mesylate acts as a TMPRSS2 inhibitor. It downregulates expression of SARS-CoV-2 spike (S) protein to prevent surface fusion and thereby blocks the cellular entry of the virus. A previous study found that camostat mesylate prevented SARS-CoV entry into human bronchial epithelial cells. Another in vitro study showed that camostat mesylate and E-64d (a cysteine protease inhibitor) could efficiently block TMPRSS2 binding of SARS-CoV-2. Clinical trials are ongoing to assess the effectiveness of a combination therapy of hydroxychloroquine and camostat mesylate vis-a-vis hydroxychloroquine alone in Denmark and Germany[3].

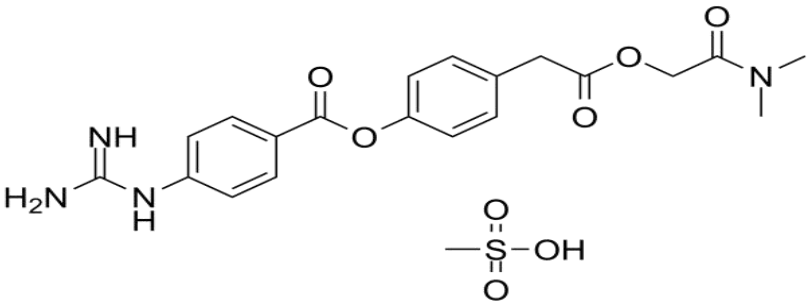


Figure.13: structure of Camostat mesylate

12. Atazanavir

An in silico study showed that atazanavir bound more strongly to the active site of SARS-CoV-2 MPro as compared to lopinavir and an in vitro study found that atazanavir inhibited SARS-CoV-2 replication. A previous study on HIV infected patients showed that a combination of atazanavir with ritonavir improved glucose uptake and lipid parameters and decreased fasting glucose more effectively as compared to lopinavir-ritonavir combination. This suggests that atazanavir might be an alternative for lopinavir when combined with ritonavir for COVID-19 treatment; however, further study is warranted. Currently, this antiviral drug is as an option for COVID-19 treatment[3].

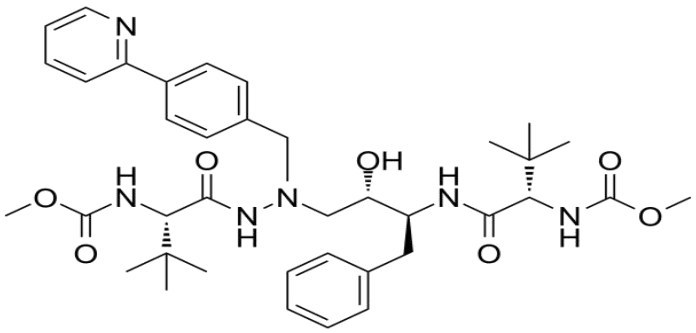


Figure.14: Structure of Atazanavir.

13. Ebselen

Ebselen is an organoselenium compound which has been investigated for the treatment of multiple diseases such as bipolar disorders and hearing loss, and its safety in humans has been evaluated in a number of clinical trials. Ebselen displayed strong inhibition of Mpro activity against SARS-CoV-2 with a half maximal inhibitory concentration (IC50) of 0.67μM and a half maximal effective concentration (EC50) of 4.67μM, respectively. It also showed the capacity to penetrate cellular membrane to access their targets and extremely low cytotoxicity, with half maximal lethal dose (LD50) in rats > 4,600 mg/kg[19].

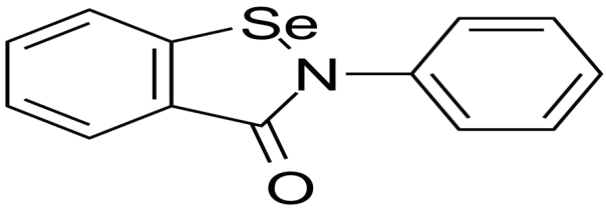


Figure.15:Structure of Ebselen

14. Disulfiram

It is an FDA-approved drug for the treatment of alcohol dependence (Garbutt et al., 1999). It was also investigated as a repurposed drug for the treatment of cancers during the past few years. It exhibited strong inhibition of Mpro activity against SARS-CoV-2 with an IC50 of 9.35μM. Remarkably, both ebselen and disulfiram are currently under evaluation in phase II clinical trials for the treatment of COVID-19 (NCT04484025 and NCT04485130). Taken together, these data strongly support the clinical potential of these drugs to treat COVID-19[19].

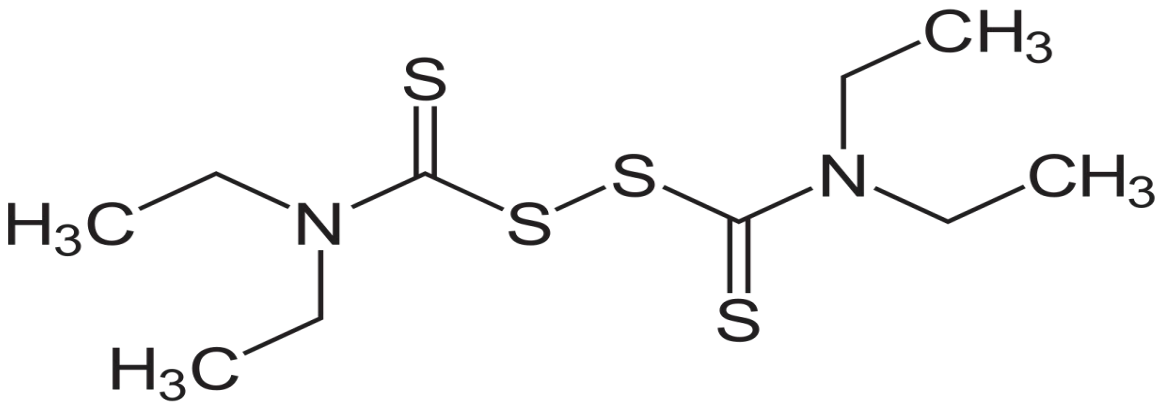


Figure.16: Structure of Disulfiram

15. Carmofur

It is an approved antineoplastic drug, derived from 5-fluorouracil (5-FU), and has been investigated in curing breast, gastric, bladder, and colorectal cancers . It was also reported as a novel therapeutic agent for acute lung injury. Carmofur displayed inhibition of Mpro activity and against SARS-CoV-2 with an IC50 of 1.82μM and an EC50 values of 24.87μM. Complex structure of SARS-CoV-2 Mpro with Carmofur showed that Carmofur covalently binds to the catalytic cysteine via an electrophilic carbonyl reactive group. Its fatty acid tail occupies the hydrophobic S2 subsite of Mpro whilst its 5-fluorouracil head is cleaved off. These findings make carmofur a novel and promising lead compound for the development of antivirals to target COVID-19[19].

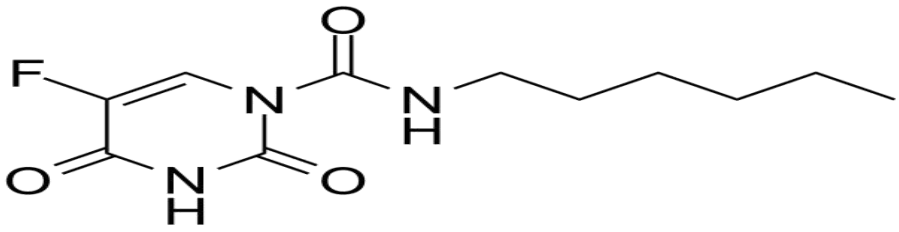


Figure.17: Structure of Carmofur

VIII. CONCLUSION

- The current COVID-19 pandemic have brought major challenge to public health. Since then, significant efforts have been made by scientists around the world and impressive progress has been gained in the research of structural biology, epidemiology and antiviral interventions of the SARS-CoV-2. Unfortunately, there are no effective drugs to combat SARS-CoV-2 until now. It is an enormous challenge for the development of effective drugs, especially for halting the suddenly out broken SARS-CoV-2.
- Even though specific antiviral drugs for COVID-19 have not been discovered or approved by the FDA, the use of some available antiviral drugs that target specific steps within the life cycle of SARS-CoV-2 could be an alternative therapeutic strategy for dealing with this pandemic.
- It's worth noting that SARS-CoV-2 has a highly mutative genetic sequence, resulting in the generation of drug-resistant mutant strains (alpha, beta, gamma, delta), Among them, delta mutant has stronger transmission ability and greater virus load. The pandemic changed the strategies and methods of researchers, and it showed them what works and what doesn't during a global emergency.
- We need to increase the investment in the basic research of antiviral drugs, to build the systematic platform for de novo drug design, lead compound discovery and optimization, drug-likeness evaluation.
- A series of drug candidates have been reported to date, Some of these agents were repurposed drugs, which previously designed for other applications with approved druggability. They showed good performance in in vitro antiSARS-CoV-2 study and could rapidly enter further clinical trials.
- A COVID-19 vaccine may be the best defense against SARS-CoV-2, but there is no guarantee that vaccines will protect a hundred percent of the people. Moreover, the virus has a high mutation rate; therefore, it is uncertain that the vaccine prepared against one strain will protect against other mutated strains of SARS-CoV-2.

ACKNOWLDGEMENT:

It is indeed a great sense of joy achieved to see this project completed. It has been very faithful and joyable learning experience for me. A project on which I have worked on, named "*Antiviral Therapeutic Molecules for Covid-19"* could have not been possible without significance guidance and a lot of hard work. The fruit of this hard work comes from good guidance, so I thank my project guide Dr. RUPESH PATRE Sir who have helped me during the project and without his help this project would not have been possible.

X.REFRENCES:

1. Gao S, Huang T, Song L, et al. Medicinal chemistry strategies towards the development of effective SARS-CoV-2 inhibitors. *Acta Pharmaceutica Sinica B*. 2022;12(2):581-599. doi:10.1016/j.apsb.2021.08.027

2. Sharma V, Singh AP, Singh AP. Therapeutic approaches in COVID-19 followed before arrival of any vaccine. In: *Materials Today: Proceedings*. Vol 48. Elsevier Ltd; 2021:1258-1264. doi:10.1016/j.matpr.2021.08.265

3. Frediansyah A, Tiwari R, Sharun K, Dhama K, Harapan H. Antivirals for COVID-19: A critical review. *Clinical Epidemiology and Global Health*. 2021;9:90-98. doi:10.1016/j.cegh.2020.07.006

4. Tian X, Li C, Wu Y, Ying T. Insights into biological therapeutic strategies for COVID-19. *Fundamental Research*. 2021;1(2):166-178. doi:10.1016/j.fmre.2021.02.001

5. Mhatre S, Srivastava T, Naik S, Patravale V. Antiviral activity of green tea and black tea polyphenols in prophylaxis and treatment of COVID-19: A review. *Phytomedicine*. 2021;85. doi:10.1016/j.phymed.2020.153286

6. Mirtaleb MS, Mirtaleb AH, Nosrati H, Heshmatnia J, Falak R, Zolfaghari Emameh R. Potential therapeutic agents to COVID-19: An update review on antiviral therapy, immunotherapy, and cell therapy. *Biomedicine and Pharmacotherapy*. 2021;138. doi:10.1016/j.biopha.2021.111518

7. Dowarah J, Marak BN, Yadav UCS, Singh VP. Potential drug development and therapeutic approaches for clinical intervention in COVID-19. *Bioorganic Chemistry*. 2021;114. doi:10.1016/j.bioorg.2021.105016

8. Gil C, Ginex T, Maestro I, et al. COVID-19: Drug Targets and Potential Treatments. *Journal of Medicinal Chemistry*. 2020;63(21):12359-12386. doi:10.1021/acs.jmedchem.0c00606

9. Gil C, Ginex T, Maestro I, et al. COVID-19: Drug Targets and Potential Treatments. *Journal of Medicinal Chemistry*. 2020;63(21):12359-12386. doi:10.1021/acs.jmedchem.0c00606

10. Alonso DF, Farina HG. Repurposing of host-based therapeutic agents for the treatment of coronavirus disease 2019 (COVID-19): a link between antiviral and anticancer mechanisms? *International Journal of Antimicrobial Agents*. 2020;56(3). doi:10.1016/j.ijantimicag.2020.106125

11. Martinez MA. Efficacy of repurposed antiviral drugs: Lessons from COVID-19. *Drug Discovery Today*. Published online 2022. doi:10.1016/j.drudis.2022.02.012

12. Gao S, Huang T, Song L, et al. Medicinal chemistry strategies towards the development of effective SARS-CoV-2 inhibitors. *Acta Pharmaceutica Sinica B*. 2022;12(2):581-599. doi:10.1016/j.apsb.2021.08.027

13. Wang D, Li Z, Liu Y. An overview of the safety, clinical application and antiviral research of the COVID-19 therapeutics. *Journal of Infection and Public Health*. 2020;13(10):1405-1414. doi:10.1016/j.jiph.2020.07.004

14. Li Z, Wang X, Cao D, Sun R, Li C, Li G. Rapid review for the anti-coronavirus effect of remdesivir. *Drug Discoveries & Therapeutics*. 2020;14(2):73-76. doi:10.5582/ddt.2020.01015

15. Li Z, Wang X, Cao D, Sun R, Li C, Li G. Rapid review for the anti-coronavirus effect of remdesivir. *Drug Discoveries & Therapeutics*. 2020;14(2):73-76. doi:10.5582/ddt.2020.01015

16. Shiraki K, Sato N, Sakai K, Matsumoto S, Kaszynski RH, Takemoto M. Antiviral therapy for COVID-19: Derivation of optimal strategy based on past antiviral and favipiravir experiences. *Pharmacology and Therapeutics*. 2022;235. doi:10.1016/j.pharmthera.2022.108121

17. Shiraki K, Sato N, Sakai K, Matsumoto S, Kaszynski RH, Takemoto M. Antiviral therapy for COVID-19: Derivation of optimal strategy based on past antiviral and favipiravir experiences. *Pharmacology and Therapeutics*. 2022;235. doi:10.1016/j.pharmthera.2022.108121

18. Formiga FR, Leblanc R, de Souza Rebouças J, Farias LP, de Oliveira RN, Pena L. Ivermectin: an award-winning drug with expected antiviral activity against COVID-19. *Journal of Controlled Release*. 2021;329:758-761. doi:10.1016/j.jconrel.2020.10.009

19. Cui W, Yang K, Yang H. Recent Progress in the Drug Development Targeting SARS-CoV-2 Main Protease as Treatment for COVID-19. *Frontiers in Molecular Biosciences*. 2020;7. doi:10.3389/fmolb.2020.616341