

A review on Drug discovery and development in the last year 2020-21 for the corona virus

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ABBREVIATIONS

Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2)
 RNA-dependent RNA polymerase (RdRp)
 Angiotensin-converting enzyme 2 (ACE2).
 Middle East respiratory virus corona virus (MERS-CoV)
 Guanosine monophosphate (GMP)
 Toll-like receptor (TLR)
 Centers for Disease Control (CDC)
 3C-like proteases (3CLpro)
 Glucocorticoid receptor (GR)
 2-deoxy-D-glucose (2-DG)

INDEX

Sr. No.	Content
1.	Abstract
2.	Introduction
3.	Drug Design Targets
4.	Approaches for drug design targeting SARS COV-2
5.	Approaches for Drug Repurposing
6.	Potential treatment and drug design strategies
	6.1. Inhibiting the RNA-Dependent RNA polymerase
	6.2. Viral Protease Inhibitors
	6.3. Viral Entry Inhibitor
	6.4. Immune Modulators
	6.5. Monoclonal antibodies
	6.6. Janus kinase Inhibitors
7.	Drug targets at different stages of covid-19 in human cell
8.	Development of Ramdesivir
9.	Repurposing strategy and use of Lopinavir / Ritonavir
10.	Designing strategy used for Tocilizumab
11.	Corticosteroids / Dexamethasone
12.	Development of 2DG for covid treatment
13.	Conclusion
14.	References

Abstract: The COVID-19 pandemic caused by SARS-CoV-2 infection is spreading at an alarming rate and has created an unprecedented health emergency around the globe. The development of antiviral agents was an urgent priority. Biochemical events critical to the corona virus replication cycle provided a number of attractive targets for drug development. These include, spike protein for binding to host cell-surface receptors, proteolytic enzymes that are essential for processing poly proteins into mature viruses, and RNA-dependent RNA polymerase for RNA replication. There has been a lot of ground work for drug discovery and development against these targets. Also, high throughput screening efforts have led to the identification of diverse lead structures, including natural product-derived molecules. This review highlights past and present drug discovery and medicinal-chemistry approaches against SARS CoV and COVID-19 targets. The review hopes to stimulate further research and will be a useful guide to the development of effective therapies against COVID-19 and other pathogenic corona viruses. [1]

Keywords: antiviral agents, COVID-19, drug discovery.

1. Introduction

Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) originated in Wuhan in central China's Hubei Province, in December 2019. The most common symptoms include fever, headache, non-productive cough, and fatigue. Patients with severe disease develop viral pneumonia, acute respiratory distress, and hypoxia, requiring intubation and mechanical ventilation.

They are a family of positive-sense, single-stranded RNA viruses diversely prevalent in humans and wildlife. There are now seven known corona viruses that cause disease in humans that include, HCoV-229E, HCoV OC43, HCoV-NL63, HCoV-HKU1, severe acute respiratory syndrome corona virus (SARS-CoV) and Middle East respiratory virus corona virus (MERS-CoV), and now SARS-CoV-2. The first four CoVs cause mild self-limiting disease. However, the last three corona viruses are highly pathogenic, leading to communicable outbreak causing fatal respiratory diseases.

The SARS-CoV-2 outbreak leading to COVID-19 has grown to become the most serious public health emergency. Attempts to repurpose historic anti-malarial drugs or existing antivirals have yet to show efficacy. Therefore, development of new and effective broad-spectrum antivirals against current SARS-CoV-2 and future outbreaks of pathogenic corona viruses is an urgent priority. It is imperative that multiple drugs will likely be essential to tackle this pandemic. In this review, I highlight the potential drug development targets, protein X ray structure-based design, lead generation and recent medicinal chemistry efforts toward the evolution of drug like small molecules. [1]

In this review, the potential drug targets for drug candidates against SARS-CoV-2 are discussed and an overview of the current status of drug development against SARS-CoV-2 infection is provided. Also their development, different approaches and strategies used are mentioned.

2. Drug Design Targets

The availability of the virus RNA genome sequence (GenBank ID: MN908947.3) represents a valuable starting point for the identification of effective treatments. Most importantly, SARS-CoV-2 features 82% similarity with SARS-CoV (GenBank ID: NC_004718.3) with a 90% resemblance in various essential enzymes.

SARS-CoV-2 features a spike protein in charge of binding its host cell-surface receptor, namely the angiotensin-converting enzyme 2 (ACE2). Upon cell entry, viral RNA attaches to the host ribosome in order to produce two polyproteins that are essential for the production of new mature virions. The proteolytic cleavage of these two polyproteins is carried out by the corona virus main proteinase (3CLpro) and the papain-like protease (PLpro). Moreover, all CoVs feature an RNA-dependent RNA polymerase (RdRp), responsible for replicating the RNA genome. All those proteins can represent potential targets in order to tackle SARS-CoV-2. In this context, the medicinal chemistry efforts performed towards novel therapeutic options for both SARSCoV and MERS-CoV could be of great help to identify potential treatments for SARSCoV-2. Among them, the development of broad-spectrum antivirals targeting the major viral proteases, shared by all coronaviruses, could represent a very promising strategy in order to generate powerful and versatile therapeutic options against these potentially fatal respiratory illnesses.

3. Approaches for drug discovery targeting SARSCoV-2

Antiviral drugs targeting the SARS-CoV-2 can be classified into two major classes, with the first group targeting virus-host interactions or inhibiting viral assembly. The other approach would include drugs that modulate broad-spectrum host innate immune responses or interfere with signaling pathways involved in viral replication. These drugs may be capable of engaging host receptors or proteases utilized for viral entry or may impact the endocytosis pathway.

Essentially, three general approaches can be utilized for screening of antiviral compounds capable of inhibiting the COVID-19 infection:

4.1. Repurposing of antiviral compounds

The first approach is to check existing antiviral compounds and molecules and estimate their effect on viral replication and packaging. Molecules like interferon alpha, beta and gamma, ribavirin and chemical inhibitors of cyclophilin 8 can be evaluated for their antiviral activities. These known antivirals have a strategic advantage since they are in active clinical use and their pharmacokinetic and pharmacodynamics properties are well studied. On the flip side, such drugs might lack specificity against SARS-CoV-2, and thus may have severe adverse effects.

3.2. High-throughput screening of compounds

The second approach involves screening of chemical libraries that constitute compounds targeting transcriptional machinery of various cell lines. High-throughput screening technology has the potential to screen large libraries of 'drug-likely' chemical

compounds for chemical entities having antiviral effects. Even libraries of existing drugs can be screened to support drug repurposing efforts, thereby leading to the identification of new functions of many known drug molecules. Marketed drugs like Lopinavir/ritonavir which was earlier intended to be used in anti-HIV therapy and was subsequently used to treat SARS emerged as a result of the successful execution of such screening programs. However, a serious disadvantage of this approach is that the 'hits' obtained from such screenings may have immunosuppressive or cytotoxic effects at higher concentrations. Another disadvantage is that the half-maximal effective concentration (EC₅₀) of drugs required to be effective against the SARS-CoV-2 infection might exceed the highest serum concentration (C_{max}) levels that can be achieved by pharmacological dosing.

3.3. Inhibition of SARS-CoV-2 replication

The third approach could involve the development of specific novel agents resulting from strong basic research around understanding of the SARS-CoV-2 life cycle. siRNA molecules or inhibitors that have the capability to inhibit specific viral enzymes involved in viral replication cycle, or monoclonal antibodies targeting the host receptor ACE-2 could be the result of such an endeavor. Such an approach has the potential to return a large number of virus-specific promising therapies against the SARS-CoV-2 virus. One of the major hurdles in such therapies is the specific drug delivery of these molecules and a lack of understanding of siRNA based therapy.

4. Approaches for drug repurposing

Due to the immense financial implications, resource implications and time implications involved in novel drug discovery process, pharmaceutical companies and researchers in the field are inclining towards and relying on 'Drug Repurposing' efforts. As the name suggests, using this approach, a known drug or an investigational drug candidate drug is studied for new uses that are beyond their scope of original intended medical indication. Some researchers and institutions also term 'Drug repurposing' as Drug Repositioning, Drug re-profiling or Drug re-tasking depending on the final outcome of studies. This strategy can considerably lower the risk of failure of investigational drugs since the toxicity profile of the drug is already well evaluated and in most cases its adverse effects are well documented. More importantly, this strategy can help save time involved in Drug development since the preclinical testing, safety assessment and even formulation development has already been completed for repurposed drugs. Also, since the drugs have undergone clinical trials earlier, repurposed drugs can potentially skip phases 1 and 2 trials, and based on therapeutic indication and adverse effect profile, repurposed drugs can be considered directly for large scale phase 3 trials.

5.1. Computational approach:

Computational approaches for Drug repurposing are largely data-driven and involve a systematic analysis of gene expression, chemical structure, proteomic data or electronic healthcare records.

5.1.1. 'Signature' matching:

Every drug or investigational drug candidate possesses some unique characteristics or 'signature' like its transcriptomic effect profile, structural or adverse effect profile and by matching these characteristics/signatures with another disease or drug, repurposing can be achieved.

5.1.2. Computational molecular docking:

Computational molecular docking is an indispensable tool for Drug repurposing activities. Here, by using structure based computational strategy, binding efficiency is predicted between the drug and the target molecule. This method large- and small-scale screens can be conducted with known drugs against a disease target. However, this technique has its own limitations, such as, for many targets 3D structure is not elucidated or there is a lack of available screen-able macromolecular database that can provide structural information for a varied molecular class of drug.

5.1.3. Network mapping:

Molecular Pathway or Network mapping is one of the most commonly used methods for drug repurposing. Many identified drug targets are not directly druggable as their direct inhibition might lead to severe adverse effects and thus network mapping can inform about the upstream or downstream druggable targets thereby enabling drug repurposing. Based on Gene expression pattern and disease pathology, drug and disease networks can be created using network mapping tools. Such maps and networks can open enormous possibilities for drug repurposing.

5.1.4. Artificial intelligence and drug repurposing:

Advances in Information technology with Artificial Intelligence (AI) and 'Big-data analysis' are revolutionizing drug repurposing efforts and studies. With the help of machine learning tools, computational algorithms can be developed that can predict new drug target engagement with far greater accuracy than earlier used methods. Huge data generated by High-throughput Next Gen Sequencing (NGS) from numerous patients when combined with disease characteristics and treatment options can lead to the identification of new disease biomarkers and drug targets. AI-driven supervised machine learning algorithms can implement multiomics and multitask learning to facilitate drug response elicited by engagement of multiple drug targets.

5.1.5. Drug repurposing in antiviral drug discovery:

The approaches for drug repurposing can also be utilized to scout for drugs that can be effective antivirals. By screening the database of small molecules against viral drug targets using computational methods, drugs or molecules can be identified that may possess antiviral activity.

Essentially two different scenarios can be discussed to facilitate antiviral drug repurposing:

- (1) Known target/new virus: In this scenario, an established antiviral drug targeting a specific protein/pathway is found to possess antiviral activity against other viruses. Known viral RNA polymerase Favipiravir and sofosbuvir were initially developed for the treatment of Influenza virus and Hepatitis C virus (HCV) infection and were repurposed for treatment of Ebola virus.

(2) Known target/new indication: In this scenario, the pharmacological target is implicated to be affected in a new pathogenic infection. In such cases, drugs targeting these proteins can be repurposed as effective antiviral agents.

5.1.6. Lessons from SARS: pharmacological interventions

Lessons from SARS and MERS epidemic can be used to develop some therapies for SARS-CoV-2 infection. Previously used antiviral drugs like oseltamivir, peramivir, zanamivir, ganciclovir, acyclovir and ribavirin are not recommended for COVID-19 treatment.

In such a scenario, given the similarity of SARS-CoV and MERS virus along with the SARS CoV-2 virus, an insight into the treatment options available for SARS and MERS could provide valuable inspirations for Drug discovery and repurposing.

5.1.7. Key CoV targets for drug development and available therapies

As of date, no specific and definite antiviral drug is available for the treatment of CoV-associated pathologies. However, some therapeutic agents based on the biology of the virus and some potential drug targets have been identified. Since the onset of previous global coronavirus pandemics like MERS and SARS, considerable research has gone into the search for suitable drug targets and subsequent drug candidates. Based on this and life cycle stages of SARS-CoV virus, the therapies that have the potential to act on corona virus can be divided into five broad categories/approaches:

- (1) Inhibition of virus binding to the host receptor by either chemical compounds or monoclonal antibodies. These agents can block or effectively engage the host's cell surface receptor thereby preventing virus binding and subsequent.
- (2) Target the viral endocytosis. This process enables the virus to enter the host cell and release its genetic material for further replication and therefore blocking virus-mediated endocytosis is a logical target for antiviral therapy.
- (3) Neutralize the virus particle. This can be accomplished by the compounds and antibodies acting on enzymes or functional proteins critical to virus replication and multiplication.
- (4) Targeting the viral structural proteins like the membrane, envelope and Nucleocapsid protein thereby blocking virus repackaging.
- (5) Restoration of host's innate immunity by the agents capable of producing virulent factors.

5. Potential Treatment and Drug Designing Strategies

As of today, there is no clinically proven and specific antiviral drug available for treating the SARS-CoV-2 infection. Most treatment trials are based on molecular mechanisms and genomic organization of SARS-CoV2. There are several existing antiviral agents that could potentially be repurposed or developed into effective interventions for this novel coronavirus. Some of these approaches are detailed below. [8]

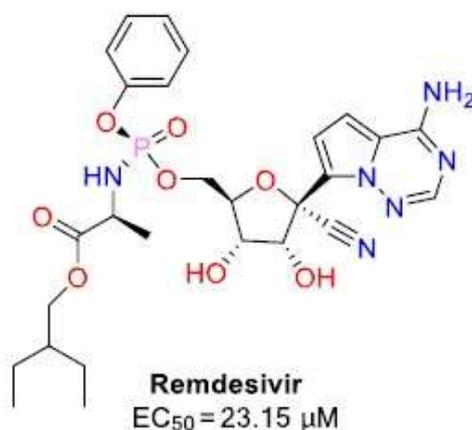
6.1. Inhibiting the RNA-Dependent RNA polymerase

6.1.1. Remdesivir

Remdesivir or GS-5734, is a broad-spectrum, antiviral, phosphoramidate pro-drug. In the body it is converted to GS-441524, a ribonucleotide analog. It is currently in clinical trials for the treatment of the Ebola viral infection. Remdesivir is an adenosine analog that interferes with the RNA-dependent RNA polymerase enzyme, which incorporates ribonucleotides into nascent viral RNA chains. Due to this mechanism, Remdesivir confuses viral RNA-dependent RNA polymerase and delays, or prematurely terminates, RNA chains which in turn inhibits viral RNA production and replication of EBOV Viruses with mutated RNA polymerase often develop partial resistance against Remdesivir. Recent research has demonstrated that Remdesivir is a potential therapeutic for the treatment of SARS-CoV-2. According to the New England Journal of Medicine. The first case in the United States (reported in Washington State) was treated with an intravenous administration of Remdesivir which mitigated symptoms, and did not cause any side-effects. However, prior clinical trials in

Ebola reported side effects such as liver inflammation, nausea, and sweating, shivering, and low blood pressure.

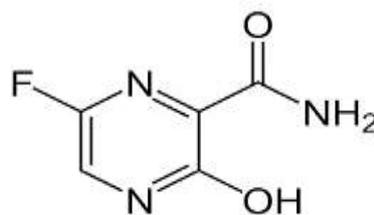
Remdesivir is currently in clinical trial evaluation for the treatment of COVID-19 in various countries including France (NCT04365725), USA (NCT04431453), Canada (NCT04330690), and Egypt (NCT04345419). Remdesivir was in Phase 3 clinical trials in the USA, but on May 1st, 2020, the FDA issued an emergency use authorization for Remdesivir to treat COVID-19 patients. [8]



6.1.2. Favipiravir

Favipiravir, also known as Avigan or T-705, is a potential antiviral, and anti-influenza drug approved in Japan for the treatment for influenza A, B, and C viruses, including

Oseltamivir-resistant strains. Favipiravir is under clinical trials for the treatment of the Ebola virus infection and has been reported to be effective against low to moderate levels of Ebola infections, but not for high-risk groups. Favipiravir is a pro-drug that Cells undergoes intracellular metabolism by the human hypoxanthine guanine phosphoribosyltransferase enzyme which then yields the active favipiravir-ribofuranosyl-5-triphosphate. Favipiravir is a nucleoside analog that inhibits the viral RNA-dependent-RNA-polymerase enzyme and prevents the viral RNA replication process. Previous studies have shown that Favipiravir not only reduced the viral load in the upper respiratory tract, but also reduced the viral load in the lungs Favipiravir is currently under clinical trials for the treatment of novel SARS-CoV-2.



Favipiravir

6.1.3. Galidesivir

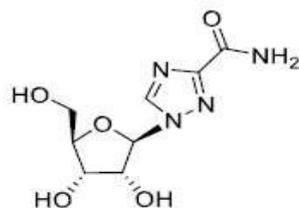
Galidesivir, also known as BCX4430 or Immucillin-A, is a potential antiviral drug, and was originally developed to treat hepatitis C Galidesivir prevents the replication and transcription of the viral genome. Galidesivir binds to the viral enzyme's active sites and incorporates itself into viral RNA strands, leading to chain termination It is currently under clinical trials for the treatment of the Ebola virus infection, Filovirus Infections, Marburg Virus Disease, and for novel SARS-CoV-2.[8]



6.1.4. Ribavirin

Ribavirin, also known as Tribavirin, is a broad-spectrum antiviral drug Ribavirin is a guanosine analog and inhibits guanosine monophosphate (GMP) synthesis as a competitive inhibitor of the inosine monophosphate dehydrogenase enzyme. Inosine monophosphate dehydrogenase catalyzes the rate-limiting step where inosine 5'-monophosphate is converted to xanthine monophosphate during GMP synthesis. GMP is then further converted into guanosine triphosphate. By inhibiting this pathway, Ribavirin inhibits the viral mRNA synthesis, viral protein synthesis, as well as replication of RNA and DNA viruses. Ribavirin is approved for the treatment of hepatitis C infections and also for viral hemorrhagic fevers. Ribavirin also causes mutations in the targeted viral RNA, premature termination of nascent RNA, and increases mutagenesis by producing defective virions. Due to these properties, Ribavirin is currently in clinical trials for the treatment of SARS-CoV-2.[8]

6.1.5. Sofosbuvir



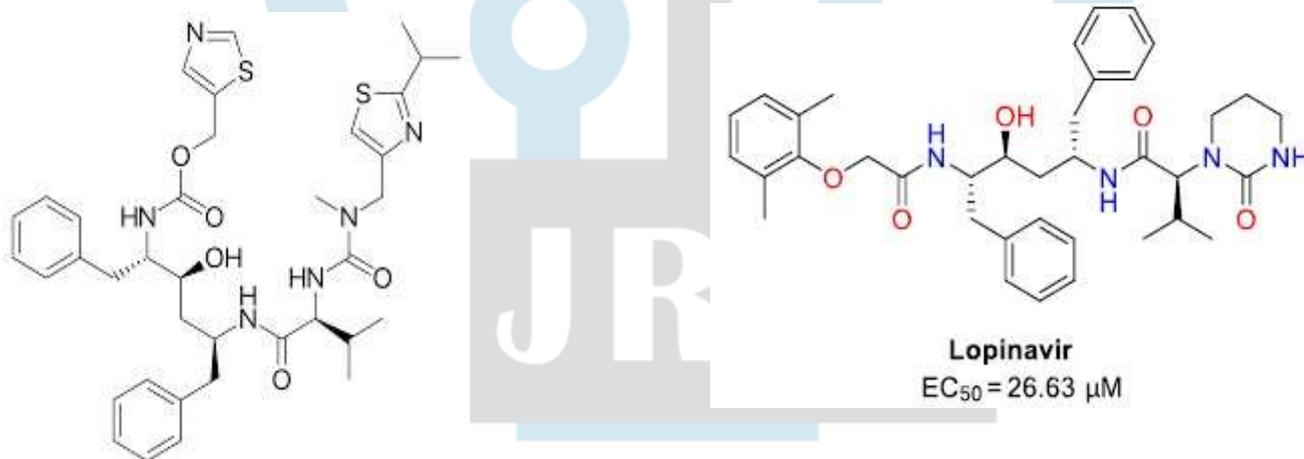
Ribavirin
EC₅₀ = 109.50 μM

Sofosbuvir, also known as Sovaldi, is a direct-acting antiviral drug used to treat hepatitis C in combination with other antiviral drugs such as Ribavirin, Velpatasvir, and Elbasvir. Sofosbuvir is a pro-drug that undergoes hepatic metabolism to form the active antiviral compound 20-deoxy-20_-fluoro_-C-methyluridine-50-triphosphate. This compound acts as a nucleotide analog inhibitor for the RNA-dependent RNA polymerase enzyme, which is vital for hepatitis C viral RNA synthesis and replication. This drug is also reported to inhibit the synthesis of the Zika virus by inhibiting pTBK1 localization and mitosis in human neuroepithelial stem cells. Sofosbuvir is currently in Phase 2/3/4 clinical trials in combination with other antiviral drugs for the treatment of SARS-CoV-2.[8]

6.2. Viral Protease Inhibitors

6.2.1. Lopinavir/Ritonavir

Lopinavir and Ritonavir are anti-retroviral protease inhibitors which are used alone or in combination with other anti-retroviral agents to treat the human immunodeficiency virus (HIV) infection. The aspartyl protease enzyme, which is encoded by the pol gene of HIV, cleaves the precursor Polypeptides in HIV and plays an essential role in viral replication. Lopinavir and Ritonavir both inhibit the HIV protease enzyme by blocking its active site. Although coronaviruses encode a different enzymatic class of protease, the cysteine protease, but research studies have shown that Lopinavir and Ritonavir are effective against the SARS and MERS viruses. Clinical trials using both Lopinavir and Ritonavir on SARS-CoV-2 infected patients have exhibited little to moderate benefits for improving the clinical outcome.



Lopinavir
EC₅₀ = 26.63 μM

6.2.2. Nelfinavir

Nelfinavir, also known as Viracept, is an antiviral drug used to treat HIV infections as a first-line therapy in combination with other HIV medications. Nelfinavir inhibits HIV-1 and HIV-2 retroviral proteases, which are vital for both viral replication within the cell and also for the release of mature viral particles from an infected cell. The underlying mechanism for how Nelfinavir inhibits SARS-CoV-2 replication remains unknown. The main proteases of SARS-CoV-2 play a vital role both during and after infection as well as in viral replication, therefore, the effect of Nelfinavir on the main protease activity of SARS-CoV-2 should be investigated. In vitro studies have shown that Nelfinavir inhibits the replication of SARS-CoV-2 and is a promising drug for treating the corona virus infection. [8]

6.2.3. Atazanavir

Atazanavir, also known as Reyataz, is an anti-retroviral drug that belongs to the protease inhibitor class and is used to treat HIV infections. Atazanavir binds to the active sites of the HIV-1 protease enzyme in infected cells, and selectively inhibits the processing of viral Gag and Gag-Pol polyproteins. Atazanavir inhibits the SARS-CoV-2 proteases enzyme and prevents the formation of mature viral particles in combination with other inhibitors. Atazanavir is currently in Phase 2 clinical trials for the treatment of SARS-CoV-2 in combination with Nitazoxanide, Ritonavir, Dexamethasone, and Daclatasvir. [8]

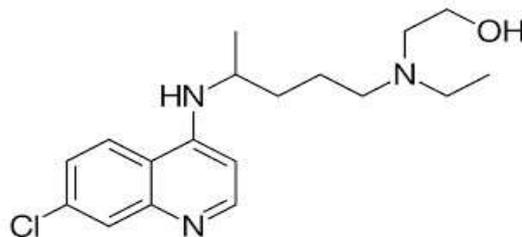
6.2.4. Darunavir

Darunavir, also known as Prezista, is an anti-retroviral protease inhibitor and is used to treat HIV infections. Darunavir has a similar mechanism of action as Atazanavir, where it inhibits the cleavage of viral Gag and Gag-Pol polyproteins, as well as prevents enzymatic binding, dimerization, and catalytic activity of viral proteases this drug is currently in Phase 3 and Phase 4 clinical trials for the treatment of SARS-CoV-2 in combination with other drugs in multiple countries worldwide [8]

6.3. Viral Entry Inhibitor

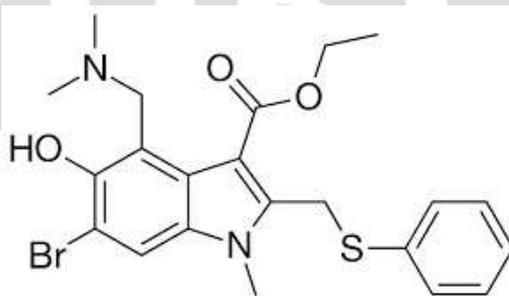
6.3.1. Hydroxychloroquine

Hydroxychloroquine is an immunosuppressive agent used to treat various autoimmune disorders, such as rheumatoid arthritis, Sjogren's syndrome, and systemic lupus erythematosus. Hydroxychloroquine is also used as a potent antiparasitic drug and has been FDA-approved to treat malaria since 1955. The proposed antimalarial activity results from the elevation of intravesicular pH to a level that inhibits the liposomal activity of antigen-presenting cells, thereby preventing antigen processing and MHC class II-mediated auto antigen presentation to T cells and ultimately inhibiting autophagy. By preventing antigen processing, hydroxychloroquine reduces T cell activation and differentiation. Hydroxychloroquine also reduces the release of certain cytokines like IL-1 and TNF α that help to reduce inflammation. The alteration of endosomal pH by hydroxychloroquine also suppresses toll-like receptor (TLR) signaling by interrupting the binding between TLR7 and TLR9 as well as to their respective RNA or DNA ligands. Hydroxychloroquine is also shown to inhibit the terminal glycosylation of the ACE2 receptor, which in turn inhibits SARS-CoV-2 entry, infection, and disease progression. Hydroxychloroquine has also been associated with reducing the risk of thrombosis, one of the major risk-factors in SARS-CoV-2 patients. Although hydroxychloroquine is a major drug used both prophylactically and for the direct treatment of COVID-19 patients, but recently some adverse side-effects were reported, including cardiac arrest and ventricular arrhythmias. Careful clinical examinations are still underway to validate the effects of hydroxychloroquine on COVID-19 patients. Due to the severity of its progression and the non-availability of other effective drugs, the FDA gave an accelerated approval for hydroxychloroquine for the treatment of COVID-19 on March 28th, 2020.



6.3.2. Arbidol

Arbidol, also known as Umifenovir, is an indole-based antiviral drug approved for the treatment of the influenza virus in China and Russia, but not currently approved by the FDA in the USA. Arbidol also exerts its antiviral activity against the Zika virus, hepatitis virus, respiratory syncytial virus, coronaviruses MERS-Co-V, and SARS-Co-V. Arbidol predominately inhibits the membrane fusion of the viruses while also decreasing the interaction between the viruses and the host during both endocytosis and exocytosis processes. Additionally, Arbidol interrupts multiple phases of viral cycle replication as a host-targeting agent; from entry, attachment to internalization, and membrane fusion. Arbidol is currently under clinical trials for various disease conditions including SARS-CoV-2

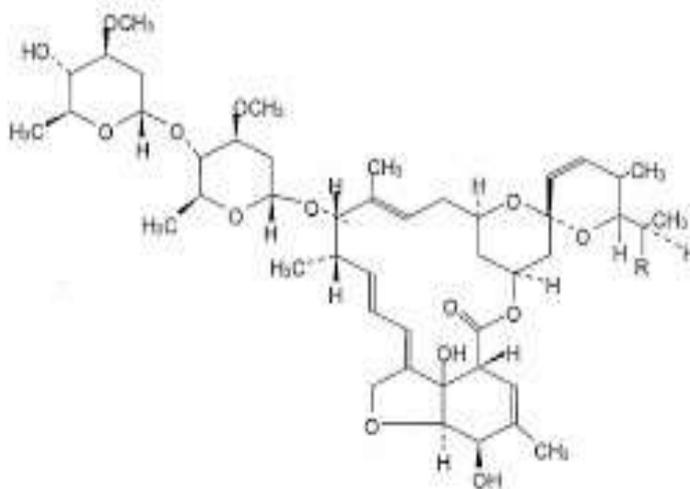


6.3.3. APN01

APN01 is a human recombinant ACE2 developed for the treatment of pulmonary arterial hypertension, acute lung injury, and acute respiratory distress. As discussed earlier, SARS-CoV-2 enters human cells through the ACE2 receptor. APN01 prevents this ACE2-mediated SARS-CoV-2 interaction, restores the physiological signaling of the ACE2 receptor, and may minimize lung injury and multiple organ dysfunction. APN01 is currently under clinical trials in multiple countries for the treatment of novel SARS-CoV-

6.3.4. Ivermectin

Ivermectin, also known as Soolantra, Sklice, or Stromectol, is an FDA approved, broad-spectrum, antiviral/antiparasitic drug. Ivermectin selectively binds to glutamatergated chloride ion channels and increases the permeability of the cell membrane to chloride ions, which in turn causes hyperpolarization of the cell leading to paralysis and parasite death. Ivermectin exhibited antiviral properties against SARS-CoV-2 in vitro through the inhibition of IMP_/_1-mediated nuclear import of viral proteins. Ivermectin is currently under clinical trials for the treatment of SARS-CoV-2.



6.4. Immune Modulators

6.4.1 Interferon-alpha (IFN_{-2b})

IFN_{-2b} is a recombinant interferon alpha-2 protein used as an antiviral and/or antineoplastic drug. IFN_{-2b} binds to type-1 interferon receptors, leading to the dimerization of JAK1 and JAK2 receptors, that leads to JAK trans-phosphorylation, and phosphorylation of STAT1 and STAT2. Dimerized STAT activates multiple antiviral proteins and immunomodulators. IFN_{-2b} also inhibits viral replication, viral proteases, increases immunomodulating activities such as phagocytic activity of the macrophages, and augmentation of the specific cytotoxicity of lymphocytes for target cells. IFN_{-2b} is approved by the FDA for the treatment of malignant melanomas, hairy cell leukemia, follicular lymphoma, condylomata acuminata, AIDS-related Kaposi's sarcoma, as well as chronic hepatitis B and C. Based on its antiviral properties, IFN_{-2b} could be a potential therapeutic compound to treat novel SARS-CoV-2 and is currently under clinical trials. . [8]

6.5. Monoclonal Antibodies

6.5.1 Sarilumab

also known as Kevzara, is a human monoclonal antibody that blocks the IL-6 receptor. Sarilumab is approved by the FDA for the treatment of rheumatoid arthritis and is currently under clinical trials for the treatment of critically ill COVID-19 patients with pneumonia, either alone or in combination with hydroxychloroquine, azithromycin, and/or corticosteroids. [8]

6.5.2. Tocilizumab

Tocilizumab, also known as Actemra, is an immunosuppressive drug mainly used for the treatment of rheumatoid arthritis and juvenile idiopathic arthritis. Tocilizumab is a humanized monoclonal antibody that binds both soluble and membrane bound IL-6 receptors, and inhibits the IL-6 signaling pathway. The SARS-CoV-2 virus binds to alveolar epithelial cells and activates both the innate and adaptive immune system, which leads to the release of a vast number of cytokines including IL-6, IL-10, and IL-23. Among these cytokines, IL-6 acts as both a proinflammatory and anti-inflammatory cytokine, while also being present at high-levels for specific autoimmune diseases such as rheumatoid arthritis. Additionally, IL-6 is found to be one of the most important cytokines involved in COVID-19 disease-mediated inflammatory conditions. IL-6 increases vascular permeability which then allows a large number of bodily fluids and blood cells to enter into lung alveoli, ultimately leading to dyspnea and respiratory failure. Tocilizumab has exhibited promising results for critically ill COVID-19 patients with pneumonia and is currently under clinical trial evaluation either alone or in combination with hydroxychloroquine, methylprednisone, or azithromycin for the treatment of SARS-CoV-2 patients. . [8]

6.6. Janus Kinase Inhibitors

6.6.1. Fedratinib

Fedratinib is a selective JAK2 inhibitor approved by FDA for the treatment of myelofibrosis. Several symptoms of COVID-19, such as pulmonary edema and lung failure, liver, heart, and kidney damage, are associated with cytokine storm, manifesting elevated serum levels of IL-17. Both IL-6 and IL-23 activate STAT3 through JAK2, which then promotes IL-17 expression. Fedratinib inhibits JAK2 and in turn reduces expression of the inflammatory cytokine IL-17, ultimately reducing cytokine storm-mediated symptoms in critically ill COVID-19 patients. Fedratinib can be used in combination with other antiviral drugs and supportive treatments but cannot be used alone because JAK2 inhibition is reversible. [8]

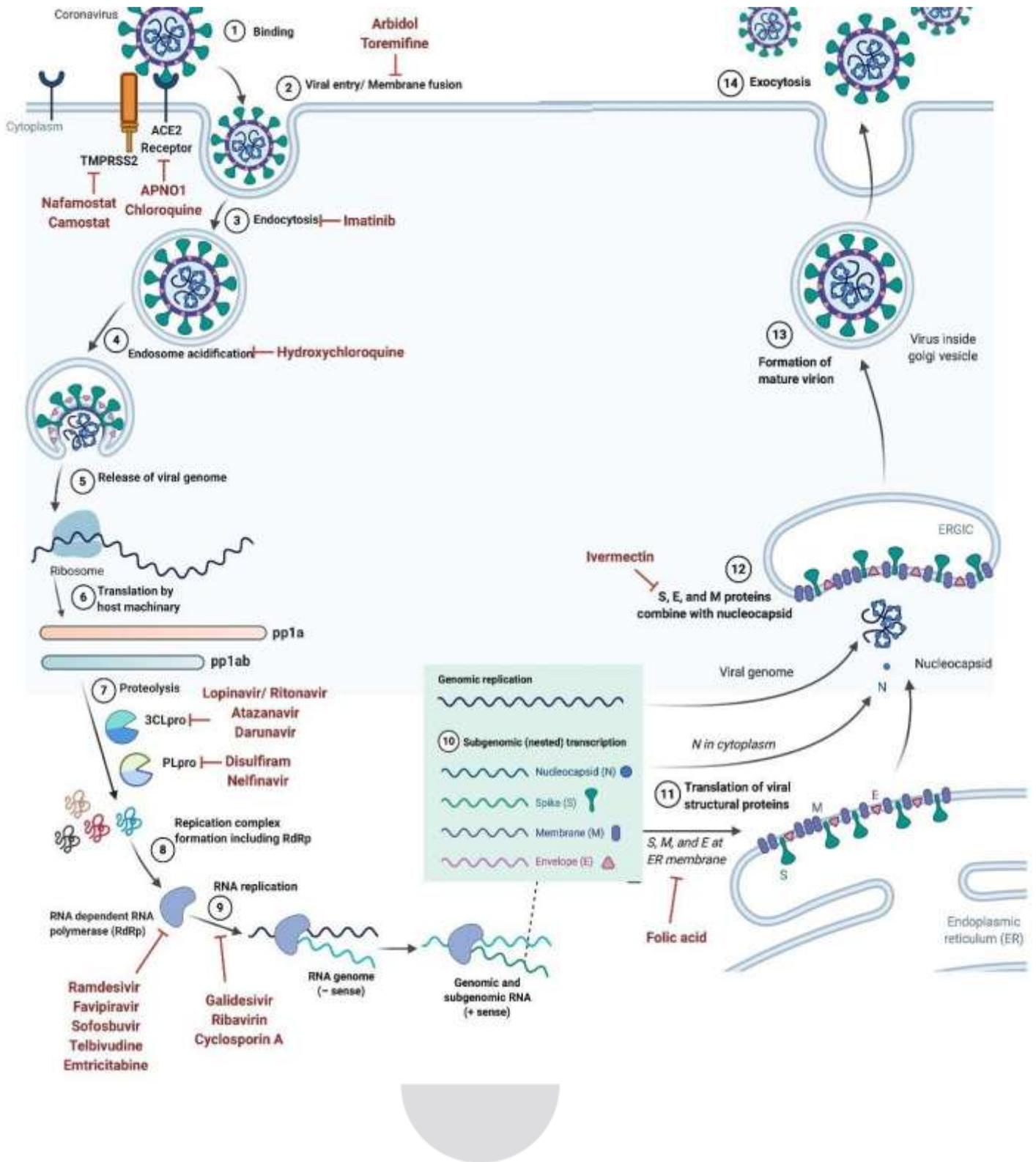
6.6.2. Baricitinib

Baricitinib is a selective and reversible inhibitor of both JAK1 and JAK2 which is approved for the treatment of rheumatoid arthritis. Baricitinib has the same mechanism of action as Fedratinib, as it prevents the release of pro inflammatory cytokines. Baricitinib can be used in combination with other antiviral drugs as well as with supportive treatment. Baricitinib is currently in clinical trials for the treatment of SARSCoV-2. [8]

6.
[1]

DRUG TARGETS AT DIFFERENT STAGES OF COVID 19 IN HUMAN CELL.





Current efforts from top pharmaceutical companies

Many pharmaceutical giants have now jumped into the race to find a drug for SARS-CoV-2 infected patients. Given the high number of fatalities across the globe, even regulatory authorities are giving rapid approvals to conduct clinical trials for promising candidate drugs. [2]

Table 1. List of probable drug targets against SARS-CoV-2 and compounds/agents effective against these targets

Targeted viral components	Examples	Mechanism of action	Status	Pros	Cons
<i>Inhibition of SARS-CoV-2 fusion/entry</i>					
RBD of the S1 subunit of S	REGN3051 and REGN3048 mAbs	Antibodies target the RBD domain of the S1 subunit	Preclinical	Efficacy demonstrated <i>in vitro</i>	Narrow spectrum
S2 subunit of S	HR2P and P1 peptides	Antiviral peptides that inhibit fusion of S with host cell receptor	Preclinical	anti-HIV peptidol has been marketed	Narrow spectrum
TMPRSS2	Camostat Mesylate	TMPRSS2 inhibitor that blocks the TMPRSS2-entry pathway	Marketed	Promising results <i>in vitro</i> . Effect on patients need to be tested	Broad spectrum. Developed for therapy against SARS
<i>Inhibition of endocytosis</i>					
Endosomal acidification	chloroquine	An antimalarial that sequesters protons in lysosomes to increase the intracellular pH	Marketed	Broad spectrum; many SARS-CoV-2 affected patients show good recovery	No concrete clinical data to suggest efficacy
Clathrin-mediated endocytosis	Oubain	ATP1A1- binding steroids; inhibits clathrin-mediated endocytosis	Marketed	Active against MERS-CoV	May have risk of cardiac toxicity
<i>Inhibition of Viral Enzymes</i>					
3CLpro	Lopinavir	Inhibits 3CLpro activity	Marketed	Broad spectrum	Toxicity Adverse impact on immune system
PLpro	GRL0617	Inhibits PLpro activity	Preclinical	Narrow spectrum	No animal or clinical data available
RdRp	Remdesivir	Nucleotide analogue; Broad spectrum: many viral infections, inhibits viral RNA synthesis	Marketed	Active against SARS-CoV and MERS-CoV at high doses <i>in vitro</i>	Side effects are common and may be severe with high dose regimens
<i>Inhibition of Viral envelope (E), membrane (M), Nucleocapsid (N) and accessory proteins</i>					
E and M Protein	siRNA	Short chains of dsRNA that interfere with the expression of SARS-CoV proteins	Preclinical	Promising <i>in vitro</i> studies.	Optimal delivery method in humans uncertain
N Protein	Pj34	Impairs viral replication	Preclinical	Narrow spectrum Effective <i>in vitro</i> and in animal studies	Optimal delivery method in humans is uncertain
Membrane and Accessory proteins	Lj001 and JL103	Induces membrane damage	Preclinical	Broad spectrum	Anti-CoV activity yet to be demonstrated Unstable physiologically and photo dependent

Also, the present development stage as well as the pros and cons of therapies are listed

Table shows a list of companies that are conducting clinical trials or are seeking approval from regulatory authorities to conduct trials.

Since the onset of the SARS epidemic, much knowledge was generated about drug targets and some candidate molecules were developed as well. However, these molecules could not be taken to clinical trials since there were not enough patients suffering from SARS virus by the time these drugs were developed. Nevertheless, this information is of tremendous use since SARS-CoV and SARS-CoV-2 share striking similarities in the genome, replication cycle and even symptoms experienced by patients.

Systemic genomic comparisons have revealed a striking 79% similarity at the nucleotide level between SARS-CoV-2 and SARS-CoV. However, only 72% nucleotide similarity was observed in the spike (S) protein of both the viruses. At the biochemical level both the virus display preferential binding to the ACE-2 receptor. Even at the clinical level, the chest X-rays of patients infected

with either SARS-CoV or SARS-CoV-2 display multi lobar ground glass like opacities. Similarly the CT scan of patients infected with either virus display lobar consolidations.

However, since the SARS epidemic, the virus has mutated considerably, and as a result, it now has the Spike protein which is quite different from the previous version. This fact makes efforts to find drugs that inhibit virus entry to host cells quite difficult. Takeda Polyclonal antibody therapy Preclinical Collaboration with several health and regulatory agencies and health care partners across the globe on polyclonal antibody TAK-888 Program initiated in March 2020. Lilly Antibody drug Preclinical Eli Lilly developing antibody treatments for coronavirus infection. Using a blood sample from a coronavirus survivor Partner company AbCellera identified more than 500 antibodies that might protect against the virus CTs in humans to be started in the next four months of 2020 the information was collected from the company web sites or press release. [2]

7. Development of remdesivir

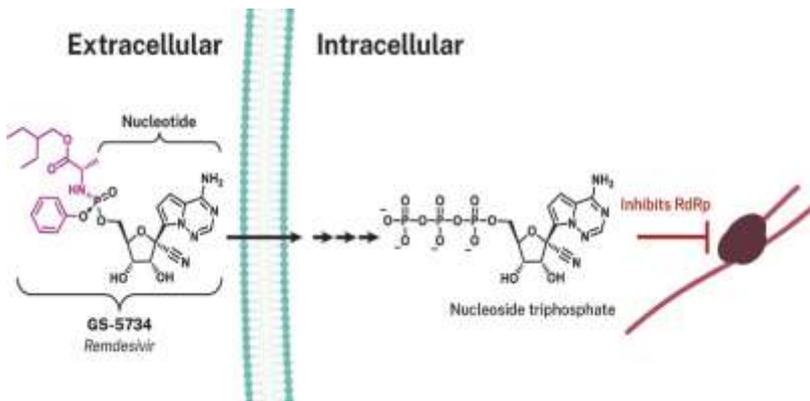
Remdesivir (GS-5734) was developed by Gilead Sciences and emerged from a collaboration between Gilead, the U.S. Centers for Disease Control and Prevention (CDC) and the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID). They sought to identify therapeutic agents for treating RNA-based viruses that maintained global pandemic potential, such as those that indeed emerged following the initiation of the program, including EBOV and the Coronaviridae family viruses exemplified by Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS).

As a starting point for discovery, a library of 1000 small molecules focused around nucleoside analogues was compiled, based on prior knowledge of effective antiviral compounds targeting RNA viruses. Nucleosides are poorly cell-permeable (and therefore can have a low hit rate in cell-based screens such as antiviral screens), so modified nucleosides such as monophosphate, ester, and phosphoramidate prodrugs composed a significant portion of the library. Such prodrugs are typically more permeable and metabolized to liberate the nucleoside or phosphorylated nucleoside within cells. While the data from the original full screen does not appear to have been disclosed, a 1'-CN modified adenosine C-nucleoside hit (GS-441524), along with a prodrug form of the monophosphate of GS-441524 (GS-5734, later renamed as remdesivir), was found to be highly potent. GS-441524 and its S-acyl-2-thioethyl monophosphate prodrug had previously been reported in 2012 as potent leads from a series of 10- substituted 4-aza-7,9-dideazaadenosine C-nucleosides, with broad activity against a panel of RNA viruses: yellow fever virus (YFV), Dengue virus type 2 (DENV-2), influenza A, parainfluenza 3, and SARS. The primary assay used was the cytoprotection effect (CPE) assay, in which live virus is incubated with a target cell line and the antiviral activity is inferred by the ability of a test agent to rescue cell death, measured using a standard cell viability reagent. In a 2012 study, GS-5734 showed CPE activity against SARS strain Toronto 2 (IC₅₀ = 2.2 μM) without causing cytotoxicity toward the host Vero African green monkey kidney epithelial cells used in the CPE assay (note that different target cells were utilized in viral CPE assays).

When the Ebola outbreak occurred in 2014, the assembled library was utilized to identify and prioritize compounds with efficacy against EBOV. The study by Madelain et al. found that GS-5734 reduced EBOV replication in HeLa cells with an IC₅₀ ≈ 100 nM, and it retained potency in in vivo nonhuman primate EBOV infection models, while GS-441524 was inactive. In addition to demonstrating activity against EBOV, Warren et al. showed that remdesivir also had antiviral activity against several other viruses, including the coronavirus MERS, with an IC₅₀ of 340 nM in vitro. With the demonstration that GS-5734 (remdesivir) possessed broad activity against RNA viruses, multiple groups assessed antiviral activity both in vitro and in vivo, validating its activity against corona viruses. Antiviral activity was confirmed against SARS, MERS zoonotic coronaviruses, as well as the circulating human corona viruses HCoV-OC43 and HCoV-229E, causative agents of the common cold [4]

Furthermore, de Wit et al. demonstrated that remdesivir had both prophylactic and therapeutic activity against MERS in a nonhuman primate in vivo model. The pharmacokinetics of remdesivir have been summarized in compassionate use documentation published by the European Medicines Agency (EMA, 2020). Remdesivir is administered via an intravenous injection (IV) with a loading dose on day 1 (200 mg in adults, adjusted for body weight in pediatric patients) followed by a daily maintenance dose (100 mg in adults) for up to 10 days. In nonhuman primates, daily administration of 10 mg/kg of remdesivir yielded a short plasma half-life of the prodrug (t_{1/2} = 0.39 h), but sustained intracellular levels of the triphosphate form. In vitro and preclinical in vivo animal models supported the effectiveness of remdesivir against SARS-CoV-2 and related corona viruses. These include a recent in vitro study of remdesivir assessing antiviral activity against SARS-CoV-2 (previously known as 2019-nCoV, strain nCoV-2019BetaCoV/Wuhan/WIV04/2019) using qRT-PCR quantification of viral copy number in infected Vero E6 cells. This study demonstrated an IC₅₀ of 770 nM and an IC₉₀ equal to 1,760 nM (with cytotoxic concentration >100 nM). In addition, works by Sheahan et al. and de Wit et al. demonstrated in vivo efficacy of remdesivir at inhibiting viral replication and reducing viral related pathology against related coronaviruses.

These findings, along with the safety profile of remdesivir in the clinical trial assessment against EBOV, support the evaluation of remdesivir as a potential therapeutic drug for repurposing against the SARS-CoV-2 pandemic. Driven by the EBOV outbreak in 2014 and based on in vitro and animal model in vivo efficacy against EBOV, Gilead Sciences initiated clinical evaluation of remdesivir for EBOV. Gilead pursued FDA evaluation under the FDA's Animal Rule, permitting the reliance on efficacy findings from animal studies for drugs in which it is not feasible or ethical to conduct human trials. As such, remdesivir was included in a randomized, controlled trial of Ebola virus therapeutics in patients within the Democratic Republic of the Congo (NCT02818582); however, midstudy primary analyses found remdesivir inferior to the antibody based therapeutics MAb114 and REGN-EB3, with respect to mortality, and the remdesivir intervention arm was terminated. Mulangu et al. reported one serious adverse event related to remdesivir, an instance of hypotension, along with elevated creatinine and aspartate aminotransferase plasma levels (a suggestive marker for impaired kidney or liver function, respectively) in remdesivir treated patients compared to either antibody based therapeutic arms. Although remdesivir was inferior against EBOV based on efficacy compared to antibody therapy, the study arm did provide an initial insight into the safety profile in patients. [4]

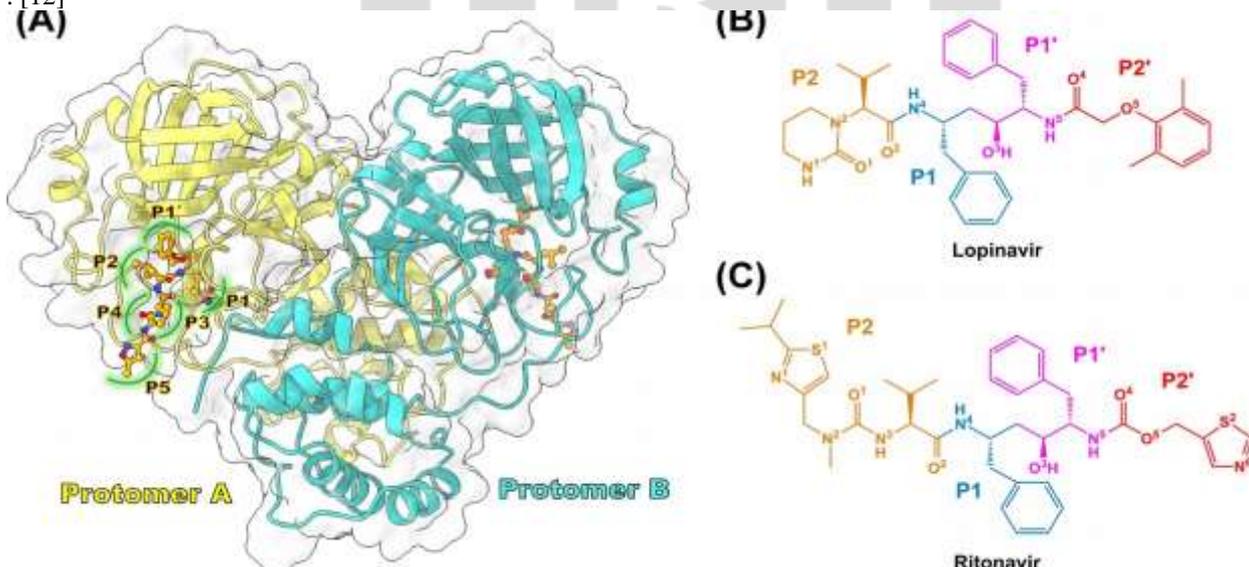


8. Repurposing strategy and use of Lopinavir and Ritonavir

To date, many companies and academic research groups around the world have focused on searching for and developing a specific vaccine or antiviral drug to prevent or control emerging SARS-CoV-2 infections (e.g., vaccine, monoclonal antibodies, and small-molecule drugs). However, these options need several months to years for their development. Because of the urgent need to alleviate the COVID-19 pandemic, the use of repurposed existing antiviral drugs approved for treatment of other viral infections such as human immunodeficiency virus (HIV), hepatitis B virus, hepatitis C virus, and influenza is somewhat promising, based on previous successes of the therapeutic treatment with two relevant human coronaviruses, SARS-CoV and MERS-CoV. According to numerous previous studies, the nonstructural protein of coronavirus, in particular, main proteases or 3C-like proteases (3CLpro), is considered an attractive drug target for the treatment of coronavirus infection. The role of this protease involves the proteolytic processing of the replicase polyprotein and is crucial for viral replication and maturation. Moreover, 3CLpro has a similar common cleavage site among coronaviruses. The sequence alignment of SARS-CoV-2 3CLpro shows that the SARS-CoV-2 proteinase is highly conserved compared to that of SARS-CoV with a 96.1% sequence identity. A combination of the two approved drugs for HIV infection, lopinavir and ritonavir (KALETRA), has been reported to be active toward SARS and MERS. Both anti-HIV drugs were initially purposed to inhibit 3CLpro of SARS-CoV and MERS CoV, and they appeared to be related to clinical benefits of patients with SARS in a nonrandomized open-label trial.

Although ritonavir is a protease inhibitor, it is generally used to inhibit cytochrome P450 3A4 and markedly increases the plasma concentrations of other protease inhibitors. Nevertheless, whether HIV protease inhibitors could effectively target SARS-CoV-2 3CLpro is under debate. This is based on the fact that HIV protease is from the aspartic protease family, whereas SARS-CoV-2 3CLpro belongs to the cysteine protease family. Previously, a theoretical study of the molecular interaction of lopinavir and ritonavir with 3CLpro of SARS-CoV suggested that these two drugs could bind well at the substrate-binding pocket of SARS-CoV 3CLpro. To date, the three-dimensional structure of SARS-CoV-2 3CLpro in a complex with lopinavir and ritonavir has not been reported. Thus, in our study, we aimed to investigate the binding interactions of lopinavir and ritonavir with the SARS-CoV-2 proteinase using both molecular modeling and quantum chemical methods. It is our hope that this information can be useful for the future design or development of more specific inhibitors for the treatment of human coronaviruses. [12]

[12]



(A) Three-dimensional structure of the peptide like inhibitor binding to the active site of the SARS-CoV-2 3CLpro homodimer (PDB entry 6LU7) in one asymmetric unit (A, yellow; B, cyan). Protomers are shown as ribbons, and the inhibitor is shown as an orange ball and stick model. Chemical structures of (B) lopinavir and (C) ritonavir, where the atomic labels are also given.

In this work, the binding pattern and susceptibility of the two HIV-1 protease inhibitors lopinavir and ritonavir in complex with SARS-CoV-2 3CLpro were fully revealed by all-atom MD simulations, binding free energy estimation, and PIEDA based on the

MM/PB(GB)SA and FMO-MP2/PCM/6-31G* calculations, respectively. According to ΔG_{bind} prediction, the susceptibility against SARS-CoV-2 3CL_{pro} of ritonavir was somewhat higher than that of lopinavir, supported by energy stabilization from individual residues that resulted from both methods: (i) M49, M165, P168, and Q189 from MM/GBSA for lopinavir and L27, H41, M49, F140,

N142, G143, H164, M165, and E166 from MM/GBSA for ritonavir and (ii) H41, A46, M49, E166, L167, L187, Q189, A191, and A193 from FMO-MP2/PCM/6-31G* for lopinavir and N142, G143, S144, C145, M165, E166, D187, and Q189 from FMO-MP2/PCM/6-31G* for ritonavir. In addition, the oxyanion whole residues N142 and G143 were found to interact with ritonavir via hydrogen bonds.

From the FMO-MP2/PCM/6-31G* data, the electrostatics, dispersion, and charge transfer were considered as the important interactions for drug binding. The obtained results demonstrated how repurposed anti-HIV drugs could be used to combat COVID-19 and how fundamental knowledge at the atomic level could also be helpful

for the further design or development of more specific inhibitors in treating human corona virus.[12]

9. Designing strategy used for Tocilizumab for targeting to calm inflammatory storm.

Inflammatory storm refers to an excessive inflammatory response flaring out of control and the immune system gone awry. To identify which kind of immune cells are involved in and which inflammatory cytokine is the critical target in these severe COVID-19 patients, analyzed peripheral blood samples from patients with severe or critical COVID-19 from The First Affiliated Hospital of University of Science and Technology of China and observed monocytes and T cells from severe or critical COVID-19 patients decreased significantly compared to normal controls. These aberrant pathogenic T cells from critical ICU care COVID-19 patients showed activated characteristic accompanied with co-expressing IFN- γ and GM-CSF. This phenomenon aroused our alarm, for GM-CSF has the capability to control diverse pathogenic capabilities of inflammatory myeloid cells, especially monocytes. As expected, inflammatory monocyte with CD14⁺ CD16⁺ phenotype exists in peripheral blood of COVID-19 patients and has larger population in critical COVID-19 patients from ICU. Note that without any re-stimulation with PMA or incubation with monensin, large amount of IL-6 could be tested from these inflammatory monocytes especially in ICU patients. Therefore, these pathogenic Th1 cells (GM-CSF+IFN- γ +) and inflammatory monocytes (CD14⁺CD16⁺ with high expression of IL-6) exist especially in critical ICU COVID-19 patients. Given that large amount of mononuclear inflammatory lymphocytes have been observed in the biopsy samples at autopsy from COVID-19 patients, we believe that these pathogenic T cells and inflammatory monocytes may enter the pulmonary circulation in large numbers and incite inflammatory storm in severe or critical COVID-19 patients.

Tocilizumab treatment is effective to reduce the mortality of severe COVID-19

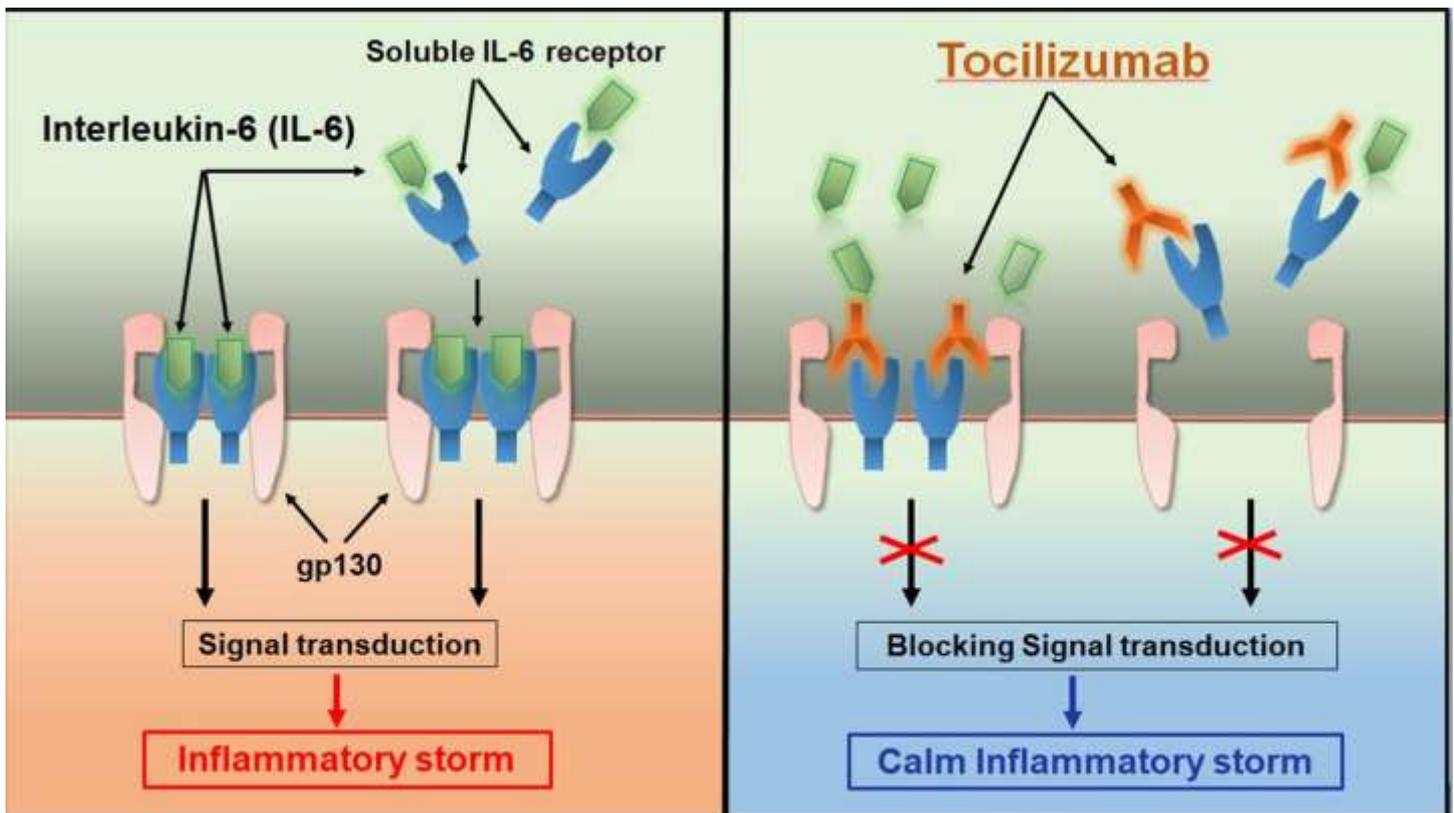
Tocilizumab is the first marketed IL-6 blocking antibody through targeting IL-6 receptors and has proved its safety and effectiveness in therapy for rheumatoid arthritis. In order to verify whether targeted

IL-6, may potentially be the effective and safe way to reduce mortality of COVID-19, 21 patients diagnosed as severe or critical COVID-19 from The First Affiliated Hospital of University of Science and Technology of China and Anhui Fuyang Second People's Hospital were recruited and given tocilizumab therapy. Patients received standard treatment according to the Diagnosis and Treatment Protocol for COVID-19 (7th edition), including lopinavir, methylprednisolone, other symptom relievers and oxygen therapy. The results of Tocilizumab treatment are inspiring. The temperature of all the patients returned to normal very quickly. The respiratory function and all other symptoms improved remarkably. Among these 21 patients, 20 patients have been recovered and discharged within 2 weeks after the Tocilizumab therapy. One left patient is recovering and out of ICU care. No adverse drug reactions were reported during the treatment with Tocilizumab. With these promising preliminary clinical results, we further launched the multicenter, large-scale clinical trials (ChiCTR2000029765) and have already about 500 severe or critical patients treated this way. [6]

The immunotherapy strategy about Tocilizumab treatment has been formally included in the diagnosis and treatment program of COVID-19 (7th edition) of the national health commission of China since

3th March 2020 as following: Tocilizumab can be used in patients with extensive bilateral lung lesions opacity or in severe or critical patients, who have elevated laboratory detected IL-6 levels. The first dose is 4–8 mg/kg (the recommended dose is 400 mg, diluted to 100 ml with 0.9% normal saline, and the infusion time is more than 1 h). For patients with poor initial efficacy, an additional application can be made after 12 h (the dose is the same as before). The maximum number of times of administration is two, and the maximum dose of a single dose should not exceed 800 mg. Note that patients with allergic reactions, such as tuberculosis and other active infection are contraindicated. We suggest that IL-6 concentrations can be detected if fever persists for more than

3 days. By chemiluminescence detection, if serum IL-6 content is over 20 pg/ml, Tocilizumab can be used. The IL-6 will be temporarily increased in serum in the next few days, for its receptors have been blocked by Tocilizumab. Together, Tocilizumab treatment is recommended to reduce the mortality of severe COVID-19[6].



Tocilizumab calms the inflammatory storm through blocking IL-6 receptors[6]

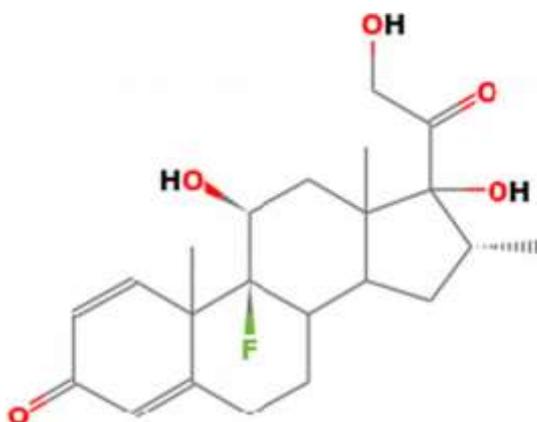
All three coronaviruses, including SARS-CoV, MERS CoV and SARS-CoV-2, induce aberrant non-effective host immune responses that are associated with severe lung pathology. The new SARS-CoV-2 additionally causes serious alveolar mucus infiltration and multiple organ failure. As the SARS-CoV-2 continues to spread, the numbers of fatal cases rise exponentially in many countries, advancing novel therapeutic development becomes crucial to minimize the number of deaths from COVID-19. In the absence of specific antiviral drugs, existing host-directed therapies could potentially be repurposed to treat COVID-19. China's plan of Tocilizumab treatment has shown its remarkable effectiveness and safety in clinical practice over the past 2 months, hoping it will benefit other countries fighting the pandemic and reduce the mortality of severe COVID19 [6]

10. CORTICOSTEROIDS / DEXAMETHASONE

Corticosteroid drugs are a class of synthetic steroid hormones that are produced in the adrenal cortex in healthy individuals. Corticosteroids include glucocorticoids and mineralocorticoids; they are used to treat a wide range of diseases and symptoms. Dexamethasone is a steroid compound, belonging to the corticosteroid class (more precisely a glucocorticoid). It is used in the treatment of numerous conditions, including chronic obstructive lung disease, severe allergies, rheumatic problems, asthma, several skin conditions, brain swelling and alongside antibiotics in tuberculosis [10]

Chemical and Physical Properties of Dexamethasone

Dexamethasone is a white, odorless crystalline powder. It is stable when exposed to air. It is practically insoluble in water (≤ 0.1 mg/mL). The molecular formula is C₂₂H₂₉FO₅. The molecular weight is 392.47 Da and is also chemically known as 1-dehydro-9 α -fluoro-16 α -methyl hydrocortisone.



Designing strategy for Dexamethasone

Dexamethasone exerts a good inhibitory effect on inflammatory factors and is predominantly used as an auxiliary treatment for viral pneumonia. The action of dexamethasone mimics the action of the compounds the body produces to quell inflammation, naturally. It is about 25 times more active than other corticosteroid compounds and this higher potency might be one of the reasons as to why dexamethasone has been shown to be effective in treating SARS-CoV-2 patients. [10]

I - Genomic Mechanisms Being small, lipophilic substances, dexamethasone can easily pass through the cell membrane by diffusion and enter the cytoplasm of the target cells and proceed by binding to glucocorticoid receptors in the cytoplasm. Dexamethasone binds to the glucocorticoid receptor (GR) on the cell membrane and the formation of this complex leads to translocation of the corticosteroid into the cell, where it travels to the nucleus. Here, it reversibly binds to several specific DNA sites resulting in stimulation (transactivation) and suppression (transrepression) of a large variety of gene transcription. It can inhibit the production of proinflammatory cytokines such as interleukin IL-1, IL-2, IL-6, IL-8, TNF, IFN-gamma, VEGF and prostaglandins. Importantly, five of these are linked to SARS-CoV-2 severity. At the same time, it can also induce the synthesis of glucocorticoid response element resulting in the activation of anti-inflammatory cytokine synthesis, notably IL-10 and lipocortin-1. [10]

II - Non-Genomic Mechanisms At high doses of the medication, dexamethasone binds to the membrane-associated GR on cells, such as T lymphocytes, resulting in the impairment of receptor signalling and a T lymphocyte-mediated immune response. The glucocorticoid receptor combines to integrins, leading to the activation of FAK (focal adhesion kinase). As well as that, a high dose of dexamethasone also interacts with the movement of Ca²⁺ and Na⁺ across the cell membrane, resulting in a rapid decrease in inflammation.

Due to their quick anti-inflammatory and immunosuppressive impact, corticosteroid medications are broadly used to treat hyper-inflammatory conditions, including the previous coronavirus diseases such as Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS). There are, however, only a few clinical studies where corticosteroids have been used to treat patients with SARS-CoV-2 and most of these studies have a high heterogeneity regarding the dose, the type of corticosteroid drug, and the course for which the medication was given and which patients are suitable for the drug. [10]

One of the main roles of glucocorticoids, to consider, is the fact that they cause immunosuppression and are anti-inflammatory. As they suppress the adaptive immune response, glucocorticoids play an important role in the modulation of several biological functions in immune cells and in different organs and tissues in the human body. The latest research suggests that glucocorticoids could have both stimulatory and inhibitory impacts on the immune response depending on their concentration in the blood and how long it is taken. Clinically, the primary reason for the use of glucocorticoids is that it might be beneficial in preventing damage of structures, like pulmonary in the case of SARS-CoV-2, by inhibiting cytokine production. A number of studies have been conducted on the use of this drug for treating hyper-inflammatory states secondary to viral infections caused by respiratory syncytial virus (RSV); MERS; influenza; and, now, SARS-CoV-2.

High doses of corticosteroids may cause more harm than good, especially if the treatment is given at a time when there is uncontrolled viral replication but with a low level of inflammation. Although there are potential hazards associated with high doses of corticosteroids when treating patients with SARS-CoV-2 pneumonia, including secondary infections and prolonged virus shedding, but in severely ill patients, if the hyper-inflammatory state is not controlled, cytokine-related lung injury could cause rapidly progressive pneumonia, the outcomes of which can be long-term and irreversible.

According to these latest findings, the WHO has welcomed the preliminary results regarding the use of dexamethasone in the treatment of SARS-CoV-2 patients, as this drug treatment was proven to save lives.

Side Effects

The common side effects of dexamethasone include an increase in appetite, mood changes, agitation and headache. Sometimes, it causes blurred vision with dizziness, and in the long term (more than a week), it could lead to arrhythmias. Therefore, in people with chronic diseases like heart disease and diabetes, high doses of corticosteroid should be used with caution. [10]

11. DEVELOPMENT OF 2-DEOXY-D-GLUCOSE FOR COVID-19 TREATMENT

The first step of attachment and entry of Corona viruses is dependent on the binding of SARS-CoV-2 spike glycoprotein (S2) to cellular receptors (Angiotensin converting enzyme 2, ACE2) of the host. Secondly, after entry into the host cell, the virus starts replicating with the aid of viral nucleases (NSP15 endoribonuclease) and protease (Main Protease 3CLpro). All these said viral virulence factors are vital for the viral life cycle hence, unraveling the pathogenesis of these virulence factors might provide insights into the etiology of COVID-19 and reveal therapeutic targets.

WHO has recently announced restricted use permission for repurposed anti-HIV, anti-malarial, anti-flu and anti-Ebola drugs. Considering such a considerable emergency of this outbreak, the current in silico study is aimed at investigating the possibilities of a glucose anti-metabolite, 2-deoxy-D-glucose (2-DG) as a repurposed drug for the treatment of novel SARS-CoV-2 virus. Post entry of virus, the host cells have been observed to undergo metabolic reprogramming to meet the increased demand of nutrients and energy for replication of the virus, wherein 2-DG might serve as a probable drug candidate as it acts as a dual inhibitor of glycolysis as well as glycosylation. 2-DG has already been granted permission for clinical trials, as evidenced from previously published results.

In the present study, the drug-like potential of 2-DG will be studied by targeting SARS-CoV-2 spike glycoprotein (S2), viral nucleases (NSP15 endoribonuclease) and protease (Main Protease 3CLpro). The binding mechanism of 2-DG with the said viral virulence factors will be assessed by means of in silico molecular docking as well as pharmacophore modeling. Moreover, another tetra-acetate glucopyranose derivative of 2-DG (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) has also been assessed for studying its binding affinities with the said viral virulence factors. The rationale for selecting this tetra-acetate glucopyranose derivative as a probable antiviral drug is dependent on its activity of impairing glycolysis and glycosylation. Hence, this derivative can possibly be used as a prodrug for 2-DG [11].

Materials and Methods

The RCSB Protein Data Bank is a global archive of three-dimensional structural data of biomolecules, per say viral receptors in this study (Rose et al., 2015). Proteins Plus server is a common online server for computational drug modeling, wherein one of its counterparts, namely, Pose View is used to visualize receptor structures and create pose depictions of ligand-receptor binding. Moreover, another counterpart of Proteins Plus server, namely, DoG Site Scorer is used to predict the active binding sites and drug ability of binding pockets of receptors. [11]

Preparation of 3D structure of viral virulence factors as receptors

The crystal structures of SARS-CoV-2 spike glycoprotein (S2; PDB code: 6VSB), viral nuclease (NSP15 endoribonuclease; PDB code: 6VWW) and protease (Main Protease 3CLpro; PDB code: 1Q2W) were obtained from RCSB Protein Data Bank (<https://www.rcsb.org/>). Hydrogen atoms were introduced in all these 3D structures using Argus Lab (4.0.1), so as to customize the viral receptors for rigid docking. [11]

Preparation of 3D structure of 2-DG and 2-DG derivative as ligands

The structure of 2-deoxy-D-glucose and 2-DG derivative (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) were downloaded in xml format from PubChem database and structures were validated (Butkiewicz et al., 2013). Hydrogen atoms were introduced into the ligands structure using Argus lab (4.0.1), so as to customize them for rigid docking. The hydrogenated ligand molecules were then converted into pdb format using Open Babel (2.4) interface (openbabel.org/docs/dev/OpenBabel.pdf), as required for rigid docking. Similarly, 3D structures of standard chemotherapeutic agents (lopinavir, favipiravir, hydroxychloroquine) were also customized for docking. [11]

Active site analysis of viral virulence factors

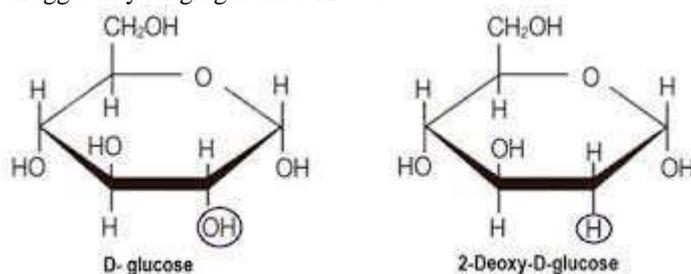
DoG Site Scorer, a web based tool (<https://proteins.plus/>), was used to predict the possible binding sites in the 3D structure of spike glycoprotein, viral nuclease and viral main protease. Predictions with DoG Site Scorer were based on the difference of gaussian filter to detect potential pockets on the protein surfaces and thereby splitting them into various sub-pockets. Subsequently, global properties, describing the size, shape and chemical features of the predicted pockets were calculated so as to estimate simple score for each pocket, based on a linear combination of three descriptors, i.e. volume, hydrophobicity and enclosure. For each queried input structure, a druggability score between 0-to-1 was obtained. Higher the druggability score, higher the physiological relevance of the pocket as potential target [11]

Molecular Docking and Ligand Receptor Binding analysis

The docking analysis of pdb structures of 2-deoxyglucose and its analogue (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) with viral receptors (spike glycoprotein, viral nuclease and viral main protease) was carried by Hex Cuda 8.0.0 software. Receptor and Ligand files were imported in the software (Harika et al., 2017). The grid dimension of docking was defined according to the binding site analysis of DoG Site Scorer (Volkamer et al., 2012). Graphic settings and Docking parameters were customized so as to calculate the binding energies (E values) of ligand receptor docking. The parameters used for the docking process were set as (i) Correlation type: Shape + Electro + DARS, (ii) FFT mode: 3D fast lite, (iii) Grid Dimension: 0.6, (iv) Receptor range: 180°, (v) Ligand range: 180°, (vi) Twist range: 360°. The best docked conformations with lowest docking energy were selected for further MD simulations using Pose View for creating pose depictions of selected ligand-receptor binding (Ezat et al., 2014). Molecular Docking and MD simulations for the standard chemotherapeutic agents (lopinavir, favipiravir, hydroxychloroquine) were also conducted. The MM-PBSA method was used to compute the binding free energy of receptor-ligand docking during simulation. In this study, the binding free energy of the receptors to ligands was calculated using the GROMACS tool, wherein the binding free energy of the receptor and ligand was defined as $DG_{\text{binding}} = DG_{\text{complex}} - (DG_{\text{receptor}} + DG_{\text{ligand}})$. For each subunit, the free energy, G, can be presented as summation of mechanical potential energy (Electrostatic and Vander Waals interaction) and solvation free energy (G_{polar} + G_{nonpolar}), wherein the total entropy is excluded from the total value [11]

Active site analysis

Active site analysis of SARS-CoV-2 spike glycoprotein (S2), viral nuclease (NSP15 endoribonuclease) and protease (Main Protease 3CLpro) as conducted by DoG Site Scorer indicated that there are various active pockets within the studied viral virulence factors with druggability ranging from 0.12 to 0.86.



Ligand Receptor binding pose depictions

The best docking pose of 2-DG, and its derivatives with SARS-CoV-2 viral receptors was also identified using Pose View tool so as to visualize the interactions of the ligands with that of the residues present in the active sites of the viral receptors. Both 2-deoxy-D-glucose and its derivative were found to form salt bridges with the amino acid residues of the viral receptors, namely, main protease 3CLpro and viral spike glycoprotein, respectively. The orientational binding of the ligands and the viral receptors showing the pose view and residue interactions have been depicted in Fig. 3. It was observed that the hydroxyl group of 2-deoxy-

D-glucose formed a hydrogen bond with the carbonyl residue of Proline amino acid (108th position) found in the viral main protease. In earlier studies it has been found that the proline amino acid residues are found in the conserved domains of HIV viral infectivity factor (Vif) and these proline-rich motifs are therapeutic targets for neutralizing the human immunodeficiency virus. Chemical bridging of 2-deoxy-D-glucose and proline residues of viral main protease 3CLpro present a similar case where proline residues were invariably bound and neutralized, thereby possibly neutralizing the COVID-19 virus. Similarly, the 2-DG derivative (1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose) formed a hydrogen bond with the amide group of Glutamine amino acid (804th position) found in the viral spike glycoprotein. Reynard and Volchkov have also previously highlighted that mutation or any change in the glutamine residues of Ebola virus spike glycoprotein causes viral. In conclusion, the binding interactions of 2-deoxy-D-glucose with viral main protease and 1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose with viral spike glycoprotein is now evident, as analysed by using Pose View tool. [11]

Molecular property analysis

After analyzing the binding energies and ligand-receptor binding pose depictions, it was requisite to evaluate the drug likeliness of the ligands. Analysis of molecular descriptors is necessary in elucidating the pharmacokinetic parameters of the drugs such as absorption, distribution, metabolism, and excretion. Molinspiration software was used to analyze the Lipinski Rule of Five, including the Log P value (partition coefficient), molecular weight, polar surface area, number of hydrogen bond donor and number of hydrogen bond acceptor.

According to the Lipinski's rule, a drug like moiety should have a low molecular weight (500 D), log P value ≤ 5 , number of hydrogen bond acceptors ≤ 10 , and number of hydrogen bond donors ≤ 5 . A bioactive drug like molecule should ensue to at least 4 of the 5 Lipinski rules. In the present study, it was found that 2-deoxy-D-glucose and 1, 3, 4, 6-Tetra-O-acetyl-2-deoxy-D-glucopyranose befalls within the said permissible limits of Lipinski rules and hence, both these drugs can be said to possess satisfactory oral bioavailability. [11]

13. CONCLUSIONS

The Covid-19 pandemic caused by highly transmissible SARS-CoV-2 has become a major public health crisis in the world, today. The pandemic is spreading at an alarming rate overwhelming existing healthcare system, and causing inordinate fatalities, particularly to elderly and immunocompromised patients around the globe. In our current pandemic situation, effective antiviral treatment could have made a significant impact on reducing morbidity and mortality. Antivirals could also be used as cheap prophylaxis. Thus, it is essential to proceed with very serious efforts for the development of effective, broad-spectrum antivirals against Covid-19. Past and present research efforts on the coronavirus replication cycle provided a number of significant biochemical targets for drug development. As the SARS-CoV-2 genome has over 80% similarity to SARS CoV, previous work on the development of antivirals against SARS is very beneficial and timely. Repurposing or repositioning an effective small-molecule therapeutic promises the fastest therapeutic means to stem the tide of the pandemic.

1. Among the candidate therapies, remdesivir has demonstrated efficacy in both in vitro and in vivo models against corona viruses. Based on these initial findings, the U.S. Food and Drug Administration has issued an Emergency Use Authorization for the emergency use of remdesivir for the treatment of hospitalized COVID-19 patients.

2. Patients treated with LPV/RTV had a numerically lower in-hospital mortality rate compared to patients treated with HCQ. Early treatment initiation may be crucial to improve patient outcome, which might explain the lower mortality rate in our LPV/RTV. Higher doses of LPV/RTV might be necessary to inhibit SARS-CoV-2 replication more efficiently.

3. In the absence of specific antiviral drugs, existing host-directed therapies could potentially be repurposed to treat COVID-19. China's plan of Tocilizumab treatment has shown its remarkable effectiveness and safety in clinical practice over the past 2 months, hoping it will benefit other countries fighting the pandemic and reduce the mortality of severe COVID-19 as well.

4. The outcome data regarding the use of corticosteroids, especially dexamethasone, for SARS-CoV-2 so far, although not conclusive, are promising with some findings suggesting that low-to-moderate doses of corticosteroids (dexamethasone and methylprednisolone) could lower the mortality rate in patients with a severe form of the condition. It is, however, not recommended for patients with mild symptoms.

To further improve our understanding of the parameters and the effect of glucocorticoids on patients with SARS-CoV-2 infection, more randomized clinical trials on this treatment are necessary.

5. It is noteworthy that 2-deoxy-D-glucose have shown significant activity towards inactivating the SARS-CoV2 viral receptors and is significantly better than that of the standard drug lopinavir and favipiravir. Present results also indicate that both 2-DG and 2-DG derivative possess adequate oral bioavailability without any major signs of toxicity or side effects.

Much ground work has been laid in terms of small-molecule lead generation, identification and limited medicinal chemistry optimization of lead structures. This review outlines various important protein targets for drug development and highlights principles and strategies for drug design along with a host of small-molecule lead structures. I hope that this review will stimulate drug design and discovery efforts toward the development of broad-spectrum antivirals against COVID-19 and future pathogenic coronaviruses.

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