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Grau de Química

Treball Final de Grau

Remdesivir, Favipiravir and Sinefungin, three nucleosidic products with potential antiviral properties against SARS-CoV-2

Remdesivir, Favipiravir i Sinefungina, tres nucleòsids amb potencial activitat antiviral contra SARS-CoV-2

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Res a la vida s'ha de témer; només s'ha d'entendre. Ara és el moment d'entendre més, de manera que puguem témer menys.

Marie Curie

Vull dedicar el treball especialment a la meva família, Montse, Josep i Mireia pel suport, el recolzament i els ànims del dia a dia.

Dedicar-lo també al meu tutor, Jordi Robles, per fer que el treball a distància fos molt més senzill davant la situació extraordinària actual, per la seva preocupació i dedicació en tot moment.

REPORT

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1. SUMMARY

In December 2019, an outbreak of pneumonia of unknown origin was reported in Wuhan, China. The causing agent was promptly recognised as a new coronavirus (CoV) titled as SARS-CoV-2. The accelerated set of scientific findings on SARS-CoV-2 provided in a record time a meaningful amount of potential drug targets. Current promising strategies to pharmacologically target SARS-CoV-2 are vaccines and repurposing drugs. Repurposing antivirals have the advantage of accelerated market approval because of the previously broad knowledge of the drug's behaviour in humans and the feasibility of combining the repurposed drugs with other ones to reach much more effective treatments. Nonetheless, more therapeutic trials are required to establish whether these drugs are indeed efficacious and safe against SARS-CoV-2.

In this TFG report, through a previous analysis of the biology and activity of the virus, we will review three current potential antivirals against SARS-CoV-2: Remdesivir, Favipiravir and Sinefungin. Concretely, the chemical methodologies adopted for either development and synthesis, mode of biological action and therapeutic and pharmacological properties will be specifically treated for each antiviral.

Keywords: Covid-19, SARS-CoV-2, antivirals, Remdesivir, Favipiravir, Sinefungin, drug repurposing.

2. RESUM

Al desembre de 2019 es va donar notícia d'un brot de pneumònia d'origen desconegut a Wuhan (Xina). L'agent causant va ser reconegut ràpidament com un nou coronavirus (CoV) denominat SARS-CoV-2. El progressiu abast de les troballes científiques sobre el SARS-CoV-2 ha proporcionat en un temps rècord una quantitat significativa de possibles dianes terapèutiques. Actualment, les estratègies prometedores per actuar farmacològicament contra la SARS-CoV-2 són les vacunes i la reutilització de fàrmacs. El reposicionament d'antivirals té com a avantatges una aprovació de comercialització més ràpida donat que es coneix àmpliament el comportament del fàrmac en els éssers humans i la possibilitat de combinar-los amb d'altres per aconseguir tractaments molt més eficaços. No obstant això, es segueixen requerint més assaigs clínics per establir si aquests fàrmacs són realment eficaços i segurs contra el SARS-CoV-2.

En aquesta memòria de TFG, partint d'una anàlisi prèvia de la biologia i l'activitat del virus, es revisaran tres potencials antivirals actuals contra la SARS-CoV-2: el Remdesivir, el Favipiravir i la Sinefungina. Concretament, de forma específica per a cada antiviral, es tractaran les metodologies químiques tant de desenvolupament com de síntesi, el mode d'acció biològica i les propietats terapèutiques i farmacològiques.

Paraules clau: Covid-19, SARS-CoV-2, antivirals, Remdesivir, Favipiravir, Sinefungina, antivirals, reposicionament de fàrmacs.

3. INTRODUCTION

3.1. WORLD'S CURRENT AND CRITICAL SITUATION

Due to the unpredictable Covid-19 pandemic, the world is currently in a state of uncertainty as Covid-19 has changed and stopped the world in just half a year. SARS-CoV-2, also known as 2019-nCoV or Wuhan coronavirus, is the virus responsible for the outbreak and the respiratory disease caused by it is COVID-19. The symptoms of the SARS-CoV-2 infection are quite varied as it ranges from asymptomatic, to mild or severe with the possibility of death.[1] Although the mortality rate is currently quite low, the virus has spread rapidly around the world due to the ease connection between countries. In the last six months, a large number of people died due to virus-induced pneumonia and as a consequence of the global paralysis, the economy is in an unknown and prolonged situation with highly unfavourable consequences in both short and long term.[2]

In December 2019, in Wuhan, China, an outbreak of pneumonia of unknown origin was reported. It was speculated that the first infected human caught the infection in a seafood market where wild animals were also traded in Wuhan City. After that happened, it was quickly identified as a new coronavirus (CoV). Since the virus started infecting in December, an unprecedented fast-spreading worldwide pandemic was stabilized in the globe. As we could observe since the virus arrived, the level of person-to-person transmission of CoV increased exponentially due to the modern world's traffic highways.[3]

Coronavirus family members have been responsible for several outbreaks and deadly epidemics over the past decade (SARS 2002, MERS 2012).[3] For this reason, and based on previous experience with other coronaviruses, China took strict measures while facing the new situation and several cities were isolated and confined in an attempt to prevent the spread of the pandemic.

The origin of the virus is not completely clear but the scientific community says that the possible zoonotic transfer came from bats or pangolins.[2] The spread of the virus that began in 2019 is growing at an unprecedented rate. SARS-CoV-2 has travelled around the world and as a

consequence of that, the current situation is the following one with more than 80 million cases, more than one and a half million deaths and more than 60 million people recover.

As we can prove in the last decade, the CoV family has attained to stay, whether one mutation or another. COVID-19 could either disappear or settle in the human population and reappear seasonally in future mutations through animal zoonosis. However, outbreaks of CoV and other viruses are likely to occur in the coming years, so scientific communities need to be prepared to combat an unpredictable situation like the current one. That is the reason why research groups recently started to study emerging variants of coronavirus because the more we learn about novel viruses, the better we can respond.[1]

On the one hand, a short-term solution is needed to find a drug to treat the high number of seriously ill patients as an emergency solution due to the unstable situation. On the other hand, a long-term solution must be required for developing drugs to prevent and cure future coronavirus outbreaks. [1]

Since the beginning of the global pandemic, the scientific community has been fully involved in research within a wide range of antivirals to combat coronavirus.[4] The current exceptional situation is testing scientists due to the urgent need for coronavirus treatment or prevention.[5] Thanks to technology's progression, the scientific community could react more straightforward to the situation as information about the biology and spread of the virus was quickly shared and available for scientists. As a result of the global pandemic, research is additionally being done to combat outbreaks of possible future mutations in CoV or other viruses.

Moreover, more and more companies are getting involved in the global pandemic because of the demand for new medicines to treat COVID-19. There are several potential options to pharmacologically fight COVID-19 as vaccines, natural medicines, small-molecule drugs, monoclonal antibodies and drug repurposing.[1] The timelines for the development of a vaccine could be faster rather than developing a *de novo* small-molecule drug but due to the challenging situation, it is required a quick solution in a short period of time. Drug repurposing[6] existing antiviral agents seems to be one of the best ways to fight COVID-19 because of the already immense knowledge of the drug's behaviour in humans. That is an overwhelming advantage as those antivirals were previously clinically evaluated and due to the demand for a short-term solution, it seems a potential way of success.[4]

3.2. SARS-CoV-2

SARS-CoV-2 is a type of coronavirus that causes COVID-19 disease and can infect both humans and animals. The coronavirus family can be classified into alpha, beta, gamma, and delta classes. Specifically, SARS-CoV-2 is a β-coronavirus and is believed to be derived from bats that are asymptomatic because they don't show signs of disease.[7] The β-coronavirus had already caused previous outbreaks of severe acute respiratory syndromes, SARS, such as SARS-CoV in 2002 in China and MERS-CoV in 2012.[3]

Similar to earlier SARS-CoV and MERS-CoV, SARS-CoV-2 first infects the alveolar epithelial cells of the lungs and could lead to severe pneumonia with a mortality rate of 2% to 5%. Moreover, the virus can affect different organs such as the heart, liver, central nervous system, kidney and gastrointestinal tract, originating organ failure.[3]

The scientific community has demonstrated that there are some common clinical manifestations in patients. The main symptoms of infected people are fever, followed by cough and a loss or change to your sense of smell or taste. Other symptoms related to Covid-19 are dyspnoea, myalgia, headache and diarrhoea but not as common as the three main ones.[2]

3.2.1. Life cycle of SARS-CoV-2

The principal tracts that SARS-CoV-2 infects are the respiratory and the gastrointestinal ones. The virus enters the cell within the interaction of the viral protein S and the ACE2 cell receptor. Following entry, viral RNA is released from the endosome and translated into viral replicase polyproteins, which are cleaved into functional proteins, such as RdRp, N protein or helicase, thanks to viral proteases. After the viral protease cleavage, the viral genome replication is set up by the viral replication complex that includes RdRp, helicase and protein N. Once the replication finishes, viral nucleocapsids from the packaged viral genomes and translated viral structures are freed from the cell through exocytosis.[8,9]

Potential targets and mechanisms of action against SARS-CoV-2 have been postulated such as blocking the interaction between the virus and the membrane receptors of host cells through the use of antibodies, inhibiting the processing of polyproteins into viral proteins by protease, and inhibiting viral RNA replication via nucleoside/nucleotide analogues like Remdesivir.[8,10]

3.2.2. Molecular biology and viral structure

The β-coronaviruses are pathogenic to humans and contain a single-stranded RNA genome that is encapsulated by a membrane envelope (see Figure 1).[3] Although the genome of the virus is quite large in size, it contains approximately 30,000 bases, encoding few proteins. On the one hand, there are structural proteins: the Spike protein (S), Membrane protein (M), Envelope protein (E) and Nucleocapsid protein (N) which are essential for the structure of a viral particle. The N protein holds the RNA genome, whereas the M, S and E proteins create the viral envelope. On the other hand, the viral genome also encodes 16-17 non-structural proteins (ns1 to ns17) such as 3-chymotrypsin-like protease (3CLpro), papain-like protease (PLpro), helicase (H) and RNA-dependent RNA polymerase (RdRp).[1,7]

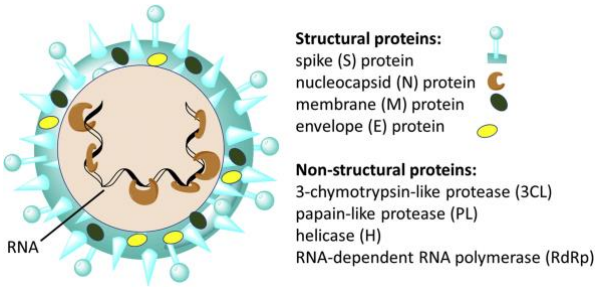


Figure 1. Structure of SARS-CoV-2 and some of its molecular protein targets *(image taken from Dömling, A. et al, ref. 1).*

3.2.2.1. Structural proteins

The morphology of the virus is due to transmembrane S glycoproteins which form homotrimers that protrude from the viral surface creating the crown-shaped structure of the virus. The S protein is anchored to the membrane and acts as an intermediary for virus entry into host cells by first binding to a host receptor and then fusing the membranes of the host cell and the viral one.[3]

The affinity between the viral receptor-binding domain (RBD) and the host receptor in the first binding is what mainly determines which host is susceptible to SARS-CoV-2 infection. SARS-CoV-2 uses the receptor angiotensin-converting enzyme 2 (ACE2), an exopeptidase expressed on epithelial cells of the respiratory system, and the transmembrane serine protease 2 (TMPRSS2) to facilitate cells entry.[3]

After host cell entry, the viral RNA is released for viral RNA replication and translation of virus polyproteins that finally shift into mature effector proteins via virus proteases. The S protein interaction with ACE2 on host cells membrane launches viral infection.

Earlier researches employed to prevent binding SARS-CoV to its host cell receptor ACE2 might be relevant since SARS-CoV-2 also uses ACE2 as a way to get into the cell. The information about the viral structure and biology is remarkably useful to determine the antivirals that can interact with the virus and inhibit it. There are multiple mechanisms identified that could be useful for the pharmacological intervention in the infection and replication processes of the virus due to the ability to block viral host cell entry. For instance, inhibiting ACE2 may constitute a pharmacological target to avoid SARS-CoV-2 entry into the host cell.

The scientific community discovered some capable strategies for interrupting protein S binding with receptor ACE2 which could have significant therapeutic value. The affinity of ACE2-binding has been demonstrated to be one of the most important determinants of SARS-CoV infectivity. It is known that the binding affinity of the protein S of SARS-CoV-2 to ACE2 is 10 or 20 times greater than protein S of earlier SARS-CoV, which may contribute to higher contagiousness of SARS-CoV-2 compared to the elder SARS-CoV. The affinity of ACE2-binding has been demonstrated to be one of the most important determinants of SARS-CoV infectivity. Pharmacologists proved that a single N501T mutation in the SARS-CoV-2 Spike protein may significantly intensify the binding affinity between 2019-nCoV RBD and human ACE2.[9] Considering that Spike protein of SARS mutated over the pandemic of 2002-2004 to better bind human cell-surface receptors, this is a situation that can be probably repeated in the future.[11]

Mutations in the S protein are the ones causing zoonosis, and as a result, not all of them will lead to their union to ACE2. Hence, antiviral drugs focused on the current interaction between protein S and ACE2 might not be useful for future CoVs.[1]

3.2.2.2. Non-structural proteins

Although SARS-CoV and SARS-CoV-2 are not identical, there are some conserved proteins associated with both of them, including 3CLpro, also named MPro, and RdRp which share over 95% of similarity between the two viruses. 3CLpro and PLpro are viral proteases which decompose viral polyproteins into functional units within host cells that are finally assembled into new viruses. RdRp is essential for replicating the viral genome within the host cell.

The 3CLpro is a cysteine protease that cleaves and processes viral polyproteins. The majority of 3CLpro inhibitors are covalent in nature, binding to the active-site cysteine. Their selectivity towards other potential objectives in the human body has not been proved yet. Even if antivirals cannot be quickly developed for the current situation, their development is highly supported to be prepared for probable future CoV outbreaks. In fact, computational approaches have been published to offer approved drugs potentially binding to 3CLpro. Viral protease inhibitors such as Ritonavir/Lopinavir that were used to inhibit diseases like Ebola virus or HIV, were also considered candidate drugs against SARS-CoV-2.

Another attractive antiviral drug target that is essential for CoV replication is PLpro. The structure, function and inhibition of SARS-CoV-2 PLpro have been widely analysed. Although the main function of PLpro and 3CLpro is to process the viral polyprotein in a coordinated way, PLpro has an additional function of removing ubiquitin and ISG15 from host-cell proteins to help CoV to evade natural immune responses of the host cell. Accordingly, it was newly discussed that targeting PLpro with antiviral drugs could be an improvement not only for inhibiting viral replication but also inhibiting the dysregulation of signalling cascades in infected cells that might end to cell death of uninfected cells that are surrounding.[1]

The RdRp enzyme enables the viral genome to be transcribed into new RNA copies employing the host cell's engine. Inhibitors of RdRp are being analysed as a new strategy to fight against viral infections, that's why viral biology and chemistry have been recently reviewed. Due to the similarity of sequence identity between RdRp proteins of SARS-CoV-2 and SARS-CoV, inhibitors of RNA polymerase are likely agents to combat Covid-19. Both of them share 96% of sequence identity. Promising RdRp inhibitors of SARS-CoV-2 are Remdesivir and Favipiravir which were used previously for treatments of Ebola and influenza pandemic respectively.[1]

3.3. DISCOVERY AND DEVELOPMENT OF ANTIVIRALS AGAINST SARS-COV-2

Antiviral drugs are a class of medicine used for treating viral infections. The majority of antivirals target particular viruses, while a broad-spectrum antiviral is efficacious against a large-scale variety of viruses. Before creating the antiviral, it is required to identify the viral proteins, or parts of proteins, that can be disabled. The targets generally need to be as unlike any proteins or parts of proteins in humans as possible, to reduce the possibility of side effects. Moreover, the targets need to be common over many viral strains within the same family, so then a single drug will have more extended effectiveness.

Once targets are distinguished, candidate drugs can be elected, either from known drugs that have suitable effects or by creating the candidate one from the beginning at a molecular level with a computer-aided design program. Then, the target proteins can be developed in laboratories for testing with diverse treatments by injecting the gene that synthesizes the target protein within bacteria cells. Following that, cells are cultivated for massive production of the protein, so then those proteins can be exposed to different treatment candidates and finally be evaluated. Utilizing rapid screening technologies, drugs can be analysed, and possible side effects could be identified.

Potential target drugs are easier to discover and develop if the viral structure and biology is deeply reviewed. For the arrangement of the replication and transcription processes, there is an enormous network described between the non-structural proteins of SARS-CoV-2. Likewise, viral particle assembly requires coordinated interaction between structural S, N, M and E proteins.[1]

All these interactions could be interesting targets for future CoV because nowadays more structural information is required. As soon as structural targets are determined, leading structural inhibitors will be provided.[1]

Developing a novel drug is a lengthy process that requires many competencies from the scientific community, such as biologists, pharmacologists and chemists. In fact, it is a highly priced development and most of the compound candidates fail along the way (one of every ten candidates).

The drug development process incorporates five different steps. The first step consists of the discovery and research for a new drug in the laboratory. Next step is the preclinical research where drugs undergo laboratory and animal testing to solve basic questions about safety. Following that, drugs are tested on people to make sure they are safe and effective during clinical research.

Four-phase studies are needed for drug approval and they aim to perform clinical research and study drug's safety on humans:

· In phase 1, the study involves between 20 and 100 healthy volunteers or people with the disease to analyse the drug's safety and dosage. In phase 1, approximately 70% of drugs move to phase 2.

· In phase 2, the study includes up to several hundred people with the disease to define the efficacy and side effects. In the second phase, around 33% of drugs move to the next phase.

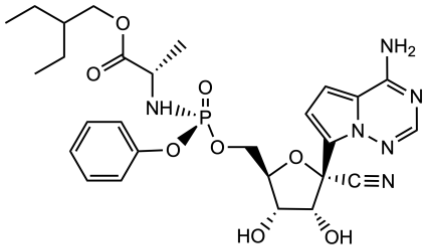
· The longest phase is the third one due to the study's length around 1 to 4 years. In phase 3, the number of participants goes from 300 to 3000 volunteers who have the disease, and the objective is to determine the efficacy and monitoring of adverse reactions. In that phase, nearly 25-30% of drugs move to the last phase.

· In the last phase, several thousand volunteers who have the disease participate to define the safety and efficacy of the drug.

Later, regulatory authorities (European Medicines Agency - EMA, Agencia Española de Medicamentos y Productos Sanitarios – AEMP, USA Food and Drugs Agency Administration – FDA) wholly examine all of the submitted data associated with the drug and decide to approve it or not.

3.3.1. RNA-dependent RNA polymerase (RdRp) inhibitors

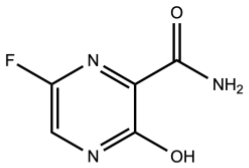
Remdesivir (**1**) and Favipiravir (**2**) are inhibitors related to replication, membrane fusion and assembly of SARS-CoV-2. Remdesivir, formally known as GS-5734, is a monophosphate prodrug that metabolizes to an active C-adenosine nucleoside triphosphate analogue.[5] Remdesivir (see Figure 2) was discovered while screening process against RNA viruses. That small-molecule adenine nucleotide analogue antiviral drug has demonstrated earlier effectiveness against Ebola virus in rhesus monkeys.[12] Also, Remdesivir has proved antiviral activity against other single-stranded RNA viruses such as MERS-CoV and earlier SARS-CoV. Remdesivir is a prodrug that is metabolized into its active triphosphate form and interferes with the activity of viral RNA-dependent RNA polymerase (RdRp), pointing to inhibition of viral RNA synthesis. That prodrug, which has to be administrated intravenously, acts directly in the first steps of infection and diminishes the viral RNA levels in a dose-dependent way. In recent studies, some mechanisms of action of that prodrug have been proved in vitro for Ebola virus, MERS- CoV and earlier SARS-CoV. Experiments on SARS-CoV-2 infecting cells such as simian Vero E6 cells or human liver cancer cells, which are sensitive to SARS-CoV-2 infection, were efficiently inhibited by Remdesivir.[13] Besides, the prophylactic and therapeutic effectiveness of Remdesivir has been proved lately in rhesus monkeys against MERS-CoV infection.[3]



1

Figure 2. Chemical structure of Remdesivir (**1**).

Favipiravir (see Figure 3), chemically known as 6-fluoro-3-hydroxy-2-pyrazinecarboxamide, is an oral guanine analogue developed in Japan by Toyama Chemical. The antiviral was discovered throughout phenotypic screening against the influenza virus. Favipiravir selectively and potently inhibits RdRp of RNA viruses and causes lethal RNA transversion mutations, whereby forming a nonviable virus phenotype.[14] This drug undergoes a phosphoribosylation in the cells to be transformed into an active form, known as Favipiravir-RTP, which is identified as the substrate by RdRp that inhibits the enzyme activity. As the RdRp catalytic domain is conserved in various sorts of RNA viruses, this mechanism allows a wide antiviral spectrum for Favipiravir.[15] That drug was reported to inhibit the replication of multiple types of RNA viruses, including influenza virus and Ebola virus.[3]



2

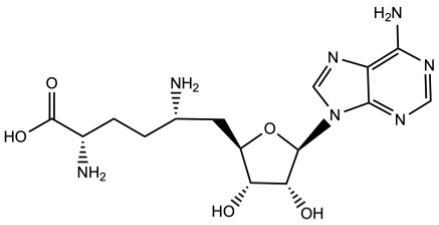
Figure 3. Chemical structure of Favipiravir (**2**).

3.3.2. 2'-O-RNA methyltransferases (MTase) inhibitors

Nucleoside derivatives that combine a nucleoside, mostly uridine or adenosine, with amino acids, lipids, peptides or glycosides, are popular secondary metabolites of wide structural variety. Many of them, isolated from bacteria, show diverse biological activities, hence they have been repeatedly used in the design and development of various antitumors, antibiotics and antivirals.[16]

MTase is a likely target for antiviral therapy because is crucial for the RNA cap formation, a fundamental process for viral RNA stability. This MTase function is associated with the nsp16 protein, which requires nsp10 as a cofactor for its satisfactory activity.[17]

Sinefungin (**3**) (see Figure 4), also known as adenosine-ornithine, which was discovered in 1971 from *Streptomyces griseolus* was initially identified as an antibiotic but later on, it was recognised as an antimalarial and antifungal.[18] Sinefungin was first ruled out due to its low cell penetrability and toxicity in humans. Most likely due to its structural resemblance to *S*-adenosylmethionine (SAM), its activity is due to the inhibition of 2'-O-RNA methyltransferases (MTase). In fact, that is the main reason why it has stirred interest as an antiviral, as MTase is a vital enzyme for the replication of certain RNA viruses included SARS-CoV-2.[19]



3

Figure 4. Chemical structure of Sinefugin (**3**).

4. OBJECTIVES

The aim of this project was to review the recent bibliography of drugs that could interact with SARS-CoV-2 and inhibit the virus. Remdesivir, Favipiravir and Sinefungin were chosen as potential drugs of SARS-CoV-2 which have revealed likely antiviral properties against coronaviruses.

The bibliographic research focused on the present state of knowledge of the properties, structure-activity studies, synthetic routes and the antiviral potential of these three drugs.

Furthermore, a secondary purpose of this project was also to analyse the current chemistry's role in the current global pandemic.

5. METHODS

The bibliographic research has been possible by using various scientific databases such as SciFinder, Reaxys, Web of Science, Mendeley, Science Direct, PubMed and PubChem to contrast papers and information.

Searching keywords like Covid-19, SARS-CoV-2, Remdesivir, Favipiravir or Sinefungin, it has been achievable to obtain satisfactory results for the research.

Since the beginning of the research, SciFinder and Web of Science were the most helpful database by searching keywords like Covid-19, SARS-CoV-2 and antivirals to get global information of the theme. The compilation of general information included approximately twenty papers and was mostly related to the world's current situation and the biology, life cycle and viral activity of SARS-CoV-2. Once general research was fulfilled, specific research of antivirals and drug repurposing was done by using databases like SciFinder, Reaxys, Science Direct, PubMed

and PubChem. The compilation of approximately 30 papers for specific research was essential to contrast information.

6. BIBLIOGRAPHIC ABSTRACT AND DISCUSSION

The discovery and development of new drugs require a long period of research and that is the reason why it is not a short-term solution for the current global pandemic. However, the reuse of drugs (drug repurposing)[6] previously used in other diseases seems to be one of the possible short-term solutions to fight the coronavirus as vaccination is a solution that requires clinical approval and takes normally a longer time to obtain.[20]

The extraordinary challenge for the scientific community during the current global public health crisis has made them rush because of the necessity of a vaccine. After conducting the final efficacy analysis in the phase 3 study, the Pfizer vaccine met all of the study's primary efficacy endpoints and seems to be safe and effective. Since the FDA's approval, the USA and UK started in early December the massive vaccination with the Pfizer vaccine, which primary efficacy analysis demonstrates to be 95% effective against SARS-CoV-2.[21] EMA have recently approved Pfizer's and Moderna's vaccines to be administered in Europe, and it is expected that other (Oxford-Astra Zeneca, Sinopharm) will be also validated soon.

Although other vaccines and antibodies targeting SARS-CoV-2 are under study, these still need a rigorous evaluation of efficacy and safety. Hence, drug therapy is possibly the unique practicable approach for a hurried response to the pandemic.[22]

Despite their high species variety, coronaviruses share key genomic components that are fundamental for the design of therapeutic targets. Due to the similarity of different coronaviruses, some drugs such as Remdesivir and Ritonavir/Lopinavir that were used to treat diseases like Ebola virus or HIV, constitute candidate drugs against SARS-CoV-2 and are now investigated for their therapeutic efficacy in COVID-19 patients.[3]

Drugs that have broad-spectrum activity against an ample range of coronaviruses and other viruses are the ones targeting either nucleosides or nucleotides and/or viral nucleic acids.[23] This present work was devoted to summarize the information on three compounds that have been proposed to address this issue, Remdesivir (**1**), Favipiravir (**2**) and Sinefungin (**3**), depicted below in Figure 5.

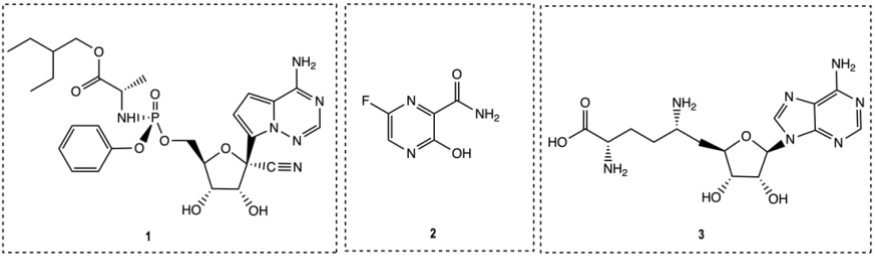


Figure 5. Chemical structures of Remdesivir (**1**), Favipiravir (**2**) and Sinefungin (**3**).

6.1. REMDESIVIR

Remdesivir (**1**, see Figure 5) is a prodrug of an adenosine analogue that can be incorporated in the nascent viral RNA chains and inhibit the RNA-dependent RNA polymerase, stopping the replication of the viral genome and result in premature termination.[22]

Remdesivir is a wide-ranging antiviral medication developed by the pharmaceutical company Gilead Sciences and came out from a collaboration between Gilead, the U.S. Centers for Disease Control and Prevention and the U.S. Army Medical Research Institute of Infectious Diseases, intending to identify therapeutic agents for treating RNA-based viruses with global pandemic potential such as Ebola and other rising viruses.[24] Later on, different clinical studies analysed its mechanism of action and its efficacy versus these viruses and they proved the broad-spectrum antiviral activity against RNA viruses. The unfavourable effect of Remdesivir is possible kidney damage due to the increment in transaminases.

6.1.1. Prodrug development

Structural modification of natural *N*-nucleosides (*N*-glycosides of pyrimidines and purines) on either the sugar or the base has driven to the development of diverse therapeutic agents, which involve antiviral and anticancer agents. This strategy has been often applied to identify additional agents with improved efficacy and safety over existing drugs and expand into novel therapeutic areas.

Amongst all the likely alterations of the sugar moiety, the incorporation of a substituent at 1'-position of the *N*-nucleosides has been unusually employed in drug design. This is slightly due to the chemical instability of the *N*-glycosidic bond provoked by namely dissociation of the base and the sugar at lower pH. Nonetheless, *C*-nucleoside, in which the sugar and the base are connected by the C-C bond, should be hydrolytically stable even with a 1'-substituent. As a result

of that, *C*-nucleoside could be an exemplary structure to investigate diverse 1'-substituted nucleosides for their therapeutic potential. A range of 1'-substituted *C*-nucleosides analogues of 4-aza-7,9-dideazaadenosine (see Figure 6) were evaluated for their antiviral potential against various RNA viruses. These nucleoside inhibitors should be intracellularly transformed by kinases to the triphosphorylated nucleosides, which then operate as competitors of the natural nucleoside triphosphates in RNA synthesis.[25]

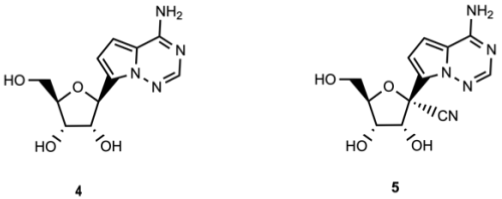


Figure 6. Structure of 4-aza-7,9-dideazaadenosine (**4**) and 1'-cyano-substituted adenine C-nucleoside ribose analogue (**5**).

A library of approximately 1000 different nucleoside and nucleoside phosphonate analogues was collected from over two decades of investigation across various antiviral programs and assayed in the research project. The 1'-methyl analogue was less active against Ebola virus and additionally presented a higher level of toxicity compared to the 1'-CN analogue which showed how small variations in the polarity and size of the 1'-substituent can impact the overall molecule.[26] The 1'-cyano-substituted adenine *C*-nucleoside ribose analogue (Nuc) presented antiviral activity against many RNA viruses. The Nuc requires intracellular anabolism to generate the active triphosphate metabolite (NTP) that interferes with the viral RdRp activity. Structurally, the 1'-cyano group granted potency and selectivity towards viral RNA polymerases.[12]

Remdesivir (GS-5734) is the single *Sp* isomer of the 2-ethylbutyl L-alaninate phosphoramidate prodrug that efficiently avoided the rate-limiting first phosphorylation step of the Nuc. Remdesivir was shown to be more active than the parent non-phosphorylated nucleoside.[12]

The initial phosphorylation step to synthesize the nucleoside monophosphate is frequently rate-limiting, that is the reason why the application of monophosphate prodrugs, principally phosphoramidates (ProTides), has been widely investigated in nucleoside analogues to avoid this initial phosphorylation step.[26]

The interest in the application of the ProTide technology in drug discovery has been growing due to the effectiveness in the in vivo intracellular delivery of nucleoside analogue monophosphates. In the 1980s the initial work on the ProTides had the aim of masking the oxygen atoms of phosphate groups in nucleoside monophosphate analogues so they are neutral at physiological pH and therefore have a better absorption into cells. The process took at least two decades to be developed and could be divided into six phases that are summarized in Table 1.

· The first stage consisted of using alkyl and haloalkyl phosphate esters as masking groups. These showed great activity due to more favourable membrane-crossing ability because the biological activity relates to lipophilicity, rather than increased intracellular levels of the phosphate species. Such conclusion moved towards the upcoming strategy.

· The second stage of nucleoside phosphate prodrugs' design was focused on alkyloxy and haloalkyl phosphoramidates. As a result of a non-clear structure-activity relationship from the haloalkyl phosphoramidates, they did not proceed any further. Nevertheless, the improvement of activity with nucleoside phosphoramidate prodrugs, principally with L-alanine, confirmed to be important for the evolution of this strategy.

· Once proved that the use of amino acids to generate phosphoramidates led to improved anti-HIV activity, it was probable that the following step would be to mask both oxygen groups of the phosphate group by amino acid esters to obtain phoshorodiamidates.

· In stage four, the masking groups studied in the design of a novel phosphate prodrug strategy were lactyl-derived systems. These were not further developed as they showed poor anti-HIV activity.

· In the fifth stage, the next masking groups studied in the design of the new prodrug strategy were diaryl motifs but after some studies, it was concluded that the development of diaryl monophosphate prodrugs would not progress any further and the focus turned back to phosphoramidates because of their good biological activity.

· The last stage is commonly referred to as ProTides and was inspired by the improvement of the biological activities of the initial examples of nucleoside monophosphate prodrugs, especially the aryls and the amino acid esters. McGuigan and co-workers took the next step by combining these two masking groups for the novel monophosphate prodrug. These prodrugs showed improved anti-HIV activity as compared to the parent nucleoside and the ProTide technology was widely pursued in the future discovery of novel antiviral and anticancer nucleotide therapeutics.[27]

Stages of development	Phosphate masking group(s)
1. Alkyl and haloalkyl phosphate triesters	 **R**$_1$ and **R**$_2$ = Me, Et, Pr, -CH_2CCl_3 and -CH_2CF_3
2. Alkyloxy phosphoramidates	 **R** = H, Me, -$CH(CH_3)_2$, -$CH_2CH(CH_3)_2$, -$CH(CH_3)$Et or Bn **R**$_1$ = Me, Et, Pr, Bu or Hex  n = 1-6  **R** = H, Me or iPr **X** = H, F or Cl

Stages of development	Phosphate masking group(s)
3.Phosphorodiamidates	 **R** = H, Me, iPr, -CH_2Ph or -$CH_2CO_2CH_3$.
4. Lactyl-derived systems	 **R** = Me or Et, **R**$_1$ = H or Me, **R**$_2$ = Me, nPr or $nC_{12}H_{25}$.
5. Diaryl phosphates	 **Ar** = Ph or p-X-Ph (x = various)
6. Aryloxy phosphoramidates	 **Ar** = Ph or substituted Ph **R** = H, Me, -CH_2iPr, -CH_2Ph…etc. **R**$_1$ = Me, Et, iPr, tBu, Bn…etc.

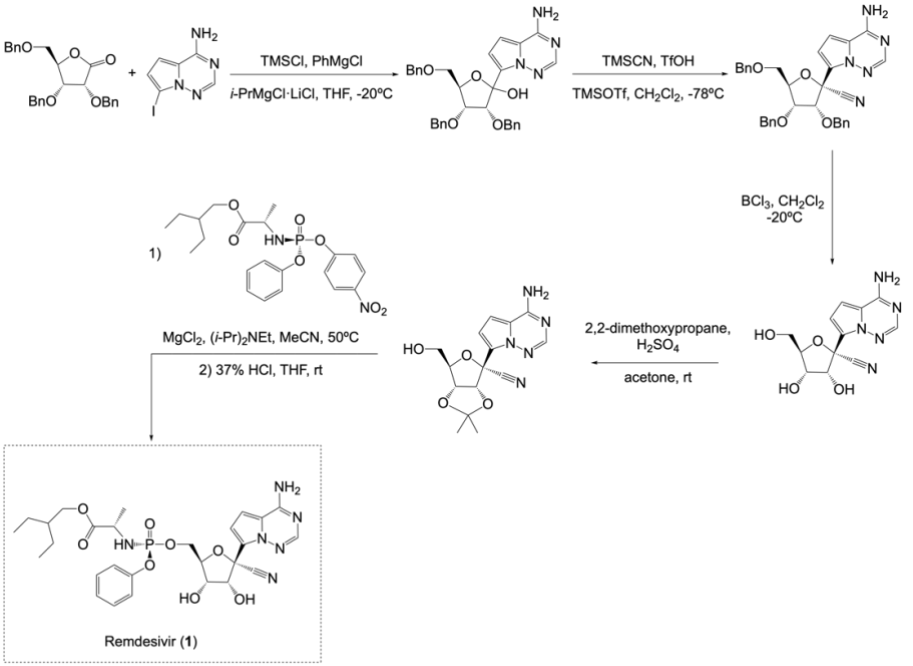
Table 1. Stages of development of ProTide prodrugs *(image taken from Mehellou, Y. et al, ref. 27).*

Remdesivir is a prodrug based on the ProTide technology, designed to increase the efficiency of intracellular delivery of antiviral nucleoside analogues as monophosphoramidates. Protides are comprised of a nucleoside phosphoramidate of an aryl group and an amino acid ester. The monophosphate is masked by alkyl groups so the in vivo stability increments and the transport into the cells is improved. Once inside the cell, these groups are metabolically transformed into the active nucleoside triphosphate (NTP).[27]

6.1.2. Synthesis of Remdesivir

The route that enabled the diastereoselective synthesis of the single *S*p isomer of the phosphoramidate prodrug is depicted in Scheme 1. The first step consisted of glycosylation and used the iodo-base instead of the bromo-base due to an easier metal-halogen exchange compatible with the isopropylmagnesium chloride lithium chloride complex (*i*-PrMgCl·LiCl). The treatment with phenylmagnesium chloride (PhMgCl) and trimethylsilyl chloride (TMSCl) granted the protection of the amino group before the addition of *i*-PrMgCl·LiCl and the ribolactone at -20ºC to obtain the glycosylated product. The next step consisted of cyanation and the inclusion of trifluoromethanesulfonic acid (TfOH) was essential to promote either high yield and selectivity favouring the desired β-anomer. The following step was the benzyl deprotection through

treatment with boron trichloride. The penultimate step was acetonide protection of the 2',3'-hydroxyl moieties with 2,2-dimethoxypropane in the presence of sulphuric acid. Some studies demonstrated that the 2',3'-acetonide protection was optimal as the yield of coupling reaction with the *p*-nitrophenyl 2-ethylbutyl-L-alaninate phosphoramidate was highly improved compared to straight coupling reaction to the unprotected diol nucleoside. The last step consisted of two reactions, the phosphorylation and deprotection of the ribose: the prodrug was produced in 70% yield as a single *Sp* isomer. [26,28]



Scheme 1. Synthesis of Remdesivir (**1**).

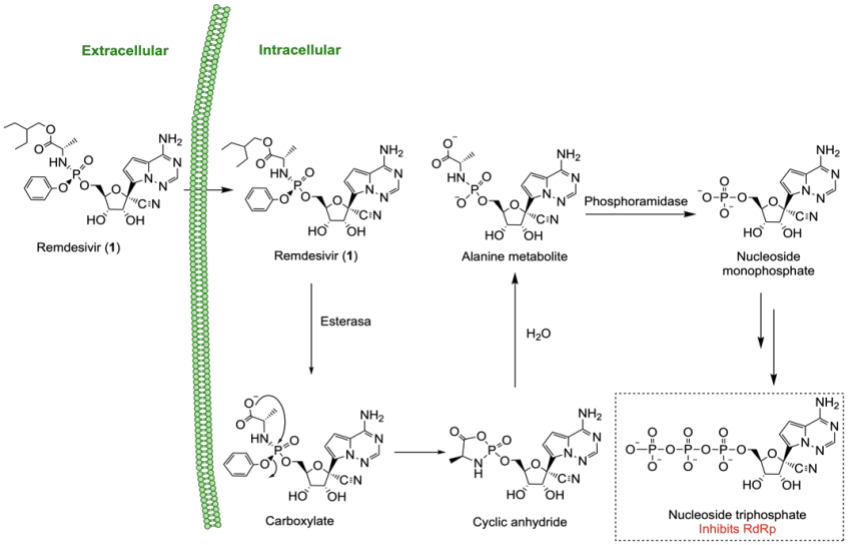
6.1.3. Mode of action of Remdesivir

Remdesivir (GS-5734) is a prodrug metabolized within cells into an alanine metabolite (GS-704277), which it is further processed into the monophosphate and finally into the active nucleoside triphosphate derivative (see Scheme 2).

Nucleotide analogues are not very cell-permeable, and once within the cell, they require to phosphorylate twice to obtain the active nucleoside triphosphate (NTP) that can be used for the

viral RNA-dependent RNA polymerases (RdRp) for genome replication. To avoid the pharmacokinetic limitations of nucleosidic drugs, phosphate and phosphonate derived nucleosides are usually used as prodrugs. As noted above, ProTides nucleotides are an optimized antiviral prodrug design in the form of phosphoramidates.

Nucleosides are internalized into the cell through an intracellular diffusion process. The prodrug Remdesivir gets within the cell where it is metabolized into the nucleoside monophosphate via sequential hydrolytic steps that are undertaken by esterase-mediated hydrolysis of the amino acid ester to release a carboxylate that then cyclizes intramolecularly to form a cyclic anhydride ejecting the phenoxide as leaving group. Due to the instability of this cyclic anhydride, it is hydrolysed by water to the alanine metabolite (GS-704277) whose P-N bond is ultimately hydrolysed by phosphoramidases to release the nucleoside monophosphate. This monophosphate is highly polar and does not diffuse back through the cell membrane which means that it is basically confined within the cell. Then, the nucleoside monophosphate undertakes further phosphorylation processes by host cell kinases yielding the active NTP analogue form which being recognized as a substrate by the viral RNA-dependent RNA polymerase enzyme leads to the inhibition of this activity.[8,29]



Scheme 2. Remdesivir (**1**) and its intracellular conversion.

Enzymatic experiments determined that Remdesivir-TP was a substrate for the purified EBOV RdRp complex and competes with ATP for incorporation as an adenosine competitor. However, it does not act as a chain terminator, but rather as a delayed chain terminator.[30] The inhibition of RNA synthesis is seen predominantly at position i+5 and RNA synthesis is generally terminated at this point. Even if the concentration of the next nucleotide is increased, the effect does not overcome.[31]

6.1.4. Therapeutic and pharmacological properties

In 2014, Gilead Sciences started a clinical evaluation of Remdesivir for the Ebola virus (EBOV) outbreak. Nowadays, the FDA approved in May 2020 the emergency use of Remdesivir for the treatment of COVID-19 in adults and children hospitalized with suspected or laboratory-confirmed Covid-19.[31] Shortly after, EMA granted conditional marketing approval. In August 2020, Gilead Sciences has petitioned the FDA to approve its drug Remdesivir to treat COVID-19 patients with its brand name of Veklury. The company Pfizer has reached an agreement with Gilead to fabricate Remdesivir.

An article was recently published in October 2020 by Beigel, J.H. *et al.*[28] about a randomized, placebo-controlled trial of intravenous Remdesivir in adults who were hospitalized with Covid-19 and had proof of lower respiratory tract infection.

A total of 1062 patients were randomly selected to receive either Remdesivir (200 mg loading dose on the first day, followed by 100 mg daily for up to 9 additional days) or placebo for up to 10 days. Those who received Remdesivir, 541 patients in total, had a recovery time of approximately 10 days among those who received placebo, 521 patients in total. In an analysis, they demonstrated that those who received Remdesivir were more likely to have clinical improvement at day 15 rather than the ones who received placebo. It was estimated that the percentage of mortality was 6.7% with Remdesivir and 11.9% with placebo by day 15 and 11.4% with Remdesivir and 15.2% with placebo by day 29. Also, the research group reported that serious adverse effects were observed in 24.6% of patients who received Remdesivir in comparison with 31.6% of patients who received placebo.

That study concluded that Remdesivir was better to placebo in making shorter the recovery time in adults who were hospitalized with Covid-19 and had proof of lower respiratory tract infection.

However, despite the use of Remdesivir, it is obvious that treatment with just an antiviral drug is not likely to be enough for all patients. Recent studies evaluated Remdesivir in combination with modifiers of the immune response, for instance, the inhibitor baricitinib. A diversity of therapeutic approaches such as novel antivirals, modifiers of the immune response or other intrinsic pathways, and mixed approaches are essential for improving outcomes in patients with Covid-19.[32]

6.2. FAVIPIRAVIR

Favipiravir (**2**, see Figure 5) was developed in 2000 and was approved for medical use in Japan in 2014. The drug has been proved to be strongly and selectively inhibitory against Influenza viruses. Additionally, Favipiravir is recognised as a novel viral RNA polymerase inhibitor. Thus, alike to Remdesivir, Favipiravir acts as an RNA-dependent RNA polymerase inhibitor.[22]

The regularly good tolerance of human patients to Favipiravir indicate that this drug holds large promise for clinical use around the world. The Zhejiang Hisun Pharmaceutical company from China has published that Favipiravir could be a viable medication against SARS-CoV-2.[14]

Appropriate doses of Favipiravir against coronaviruses are still under research. Although it is commonly well tolerated, the safety's knowledge of the effects with higher doses regimens is limited. Favipiravir can cause an increase in transaminases or diarrhoea.[22]

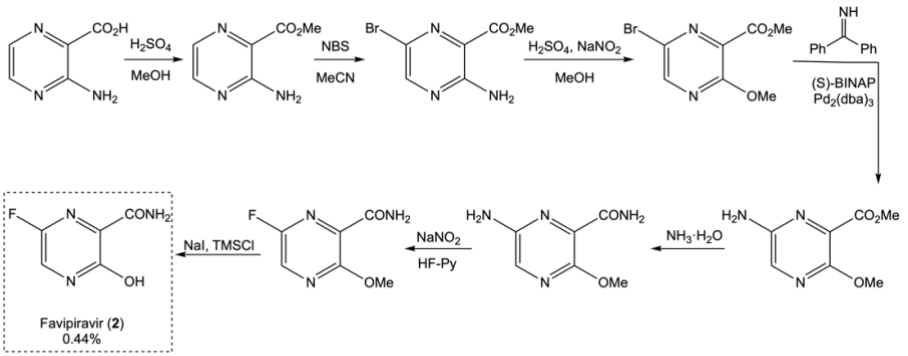
6.2.1. Prodrug development

Favipiravir is a pyrazine carboxamide derivative (6-fluoro-3-hydroxy-2-pyrazinecarboxamide) and a wide range antiviral drug approved in Japan for the treatment of Influenza viruses.[24] During the Ebola virus outbreak initiated in West Africa during 2014, a trial with Favipiravir was carried out in Guinea and patients treated with the prodrug showed an improved survival trend.

Favipiravir was first synthesized by the Japanese Chemical Company Toyama in 2000. However, in 2009 when Beldar *et al.*[33] reported an optimization of the first synthetic procedure. Afterwards, further modifications to the synthetic route were published by different research groups in the following years. In 2013, Favipiravir was successfully developed by Zhang's group[33] from 3-amino-2-pyrazinecarboxylic acid with an overall yield of 22%.

6.2.2. Synthesis of Favipiravir

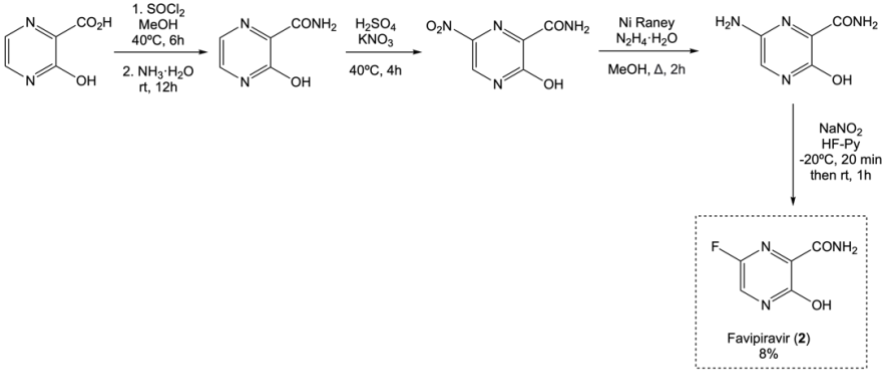
Favipiravir was first synthesized in 2000 by a chemical route consisting of seven different steps[14] (see Scheme 3). Starting with 3-aminopyrazine-2-carboxylic acid, the first compound was transformed into an ester by an esterification step using sulfuric acid and methanol. The second step consists of a bromination via aromatic electrophylic substitution reaction employing *N*-bromosuccinimide in acetonitrile. The third step consisted of the conversion of 2-aminopyridine into 2-methoxypyridine via diazotization in methanol. Later, the 5-amination step was carried out by reaction with benzophenone imine was catalysed by Pd(0) in the presence of (*S*)-(-)-2,2'-bis(diphenyl-phosphino)-1,1'-binaphthyl or (*S*)-BINAP. The following step consisted of the conversion of 3-ester into amide by using aqueous ammonia. The penultimate step was fluorination via diazotisation in the presence of the highly corrosive Olah reagent (HF-Py). Finally, an ether *O*-demethylation was performed by using sodium iodide and trimethylsilyl chloride. After these seven steps, the Favipiravir drug was created in an overall reaction yield of approximately 0.44%.



Scheme 3. First synthetic route of Favipiravir (**2**).

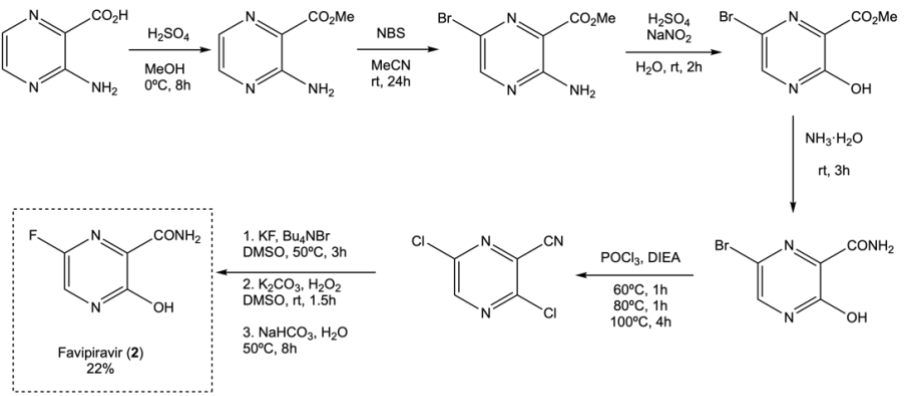
In recent years, refined methodologies for the synthesis of Favipiravir have been described. In particular, it was reported a four-step route[14] for the synthesis of Favipiravir (see Scheme 4) with a higher yield compared to Scheme 3. The overall yield of Favipiravir was 8% and the route consisted of four steps. The starting product was the commercially available 3-hydroxypyrazine-2-carboxylic acid. The initial compound was subjected to esterification and followed by amidation in the first step. The second step consisted of the nitration of the pyrazine

ring by using sulphuric acid and potassium nitrate. Once the nitration took place, the following step was the reduction of the nitro group by treatment with hydrazine in the presence of Raney nickel, which minimized the number of by-products. The last step was fluorination via diazotisation in the presence of HF·pyr.



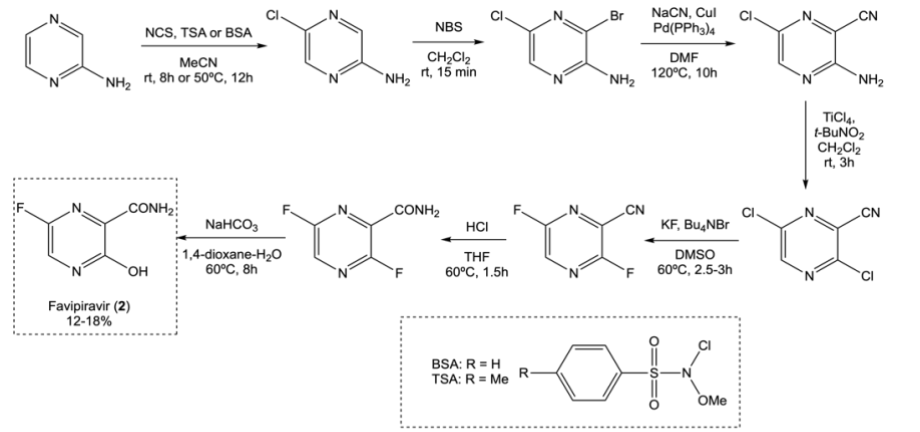
Scheme 4. Second synthetic route of Favipiravir (**2**).

Furthermore, a different route for the synthesis of Favipiravir (see Scheme 5) was developed by a research group from China[14] through a key intermediate, the methyl 3-amino-6-bromopyrazine-2-carboxylate. According to the research group, the purity of this compound was determinant for the successful preparation of 3,6-dichloropyrazine-2-carbonitrile. In that synthesis, the starting material was 3-aminopyrazine-2-carboxylic acid and following a similar procedure such as in Scheme 3, it was possible to obtain the 6-bromo-3-hydroxypirazine-2-carboxamide. After that, by using phosphoryl chloride acting both as a dehydrating and chlorinating agent it was possible to produce the double substitution of the bromine and the hydroxyl with chlorine and convert also the primary amide to nitrile. The last step comprised the replacement of the chlorine atom with fluorine, followed by hydrolysis of the nitrile group obtaining the amide of Favipiravir's molecule. The overall yield was 22%.



Scheme 5. Third synthetic route of Favipiravir (**2**).

A fourth procedure[14] (see Scheme 6) permitted the preparation of Favipiravir from pyrazin-2-amine as the starting material. First, regioselective chlorination of pyrazine ring, followed by bromination produced 2-amino-5-chloropyrazine. After that, the next two steps were a regioselective 5-bromination followed by a palladium-catalyzed cyanide substitution. The fourth step consisted of one-pot diazotisation followed by chloride substitution to render 2,5-dichloropyrazine. The synthesis of the drug was completed by double nucleophilic fluorination, followed by nitrile hydrolysis into carboxamide and final replacement of fluorine to hydroxyl. In that synthesis, the overall yield of Favipiravir was 12-18%, depending on the reagents that were used for the first step of the route.



Scheme 6. Fourth synthetic route of Favipiravir (**2**).

6.2.3. Mode of action of Favipiravir

Although Favipiravir can be considered a nucleoside aglycone and is administered unphosphorylated, the mechanism of action[34] against the virus is similar to the one for Remdesivir (see Figure 7).[35] Favipiravir is incorporated into cells and once inside the cells, the prodrug is ribosylated intracellularly to form favipiravir-RMP and after two phosphorylations it is converted to favipiravir ribofuranosyl-5'-triphosphate, known as Favipiravir-RTP, by host cells. The nucleoside triphosphate form, Favipiravir-RTP, is the active metabolite which competes with purine nucleosides and interferes with viral replication by inclusion into the RNA virus and hence, potentially inhibits the activity of viral RNA dependent RNA-polymerase (RdRp).[36]

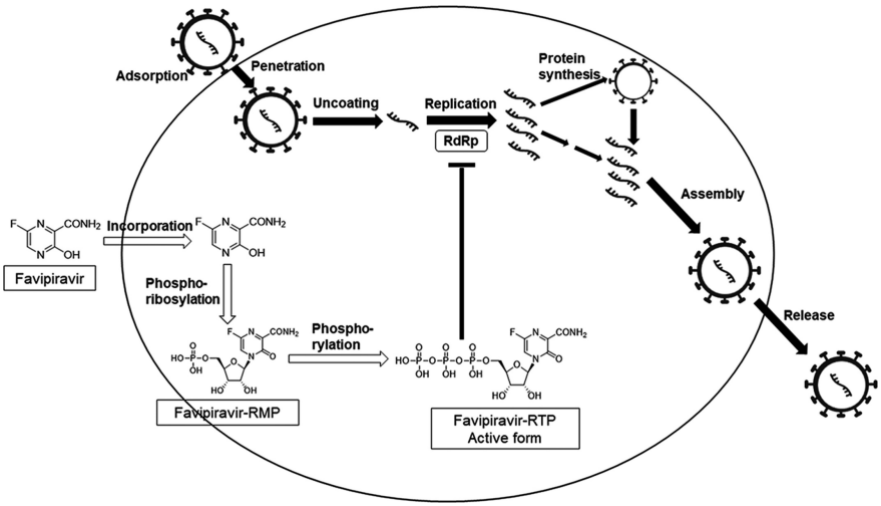


Figure 7. Mechanism of action of Favipiravir against the virus *(image taken from Du, Y.X., Chen, X.P., ref. 36)*.

6.2.4. Therapeutic and pharmacological properties

A clinical trial to evaluate the efficacy and safety of Favipiravir was conducted in Shenzhen with the recruitment of 80 patients in a non-randomized trial. The results demonstrated a significant reduction in the time of viral clearance in patients treated with Favipiravir compared to patients treated with Lopinavir/Ritonavir, a combined antiretroviral used to treat AIDS. Thirty-five patients received Favipiravir 1600mg orally twice daily on day 1 followed by 600mg orally twice daily on days 2 to 14 and forty-five patients received the corresponding doses of Lopinavir/Ritonavir. The results showed a meaningful decrease in the median time to viral

clearance with the inhibitor Favipiravir compared to Lopinavir/Ritonavir. X-ray examinations demonstrated a higher rate of improvement in chest imaging in the Favipiravir arm of 91.43% versus 62% in the Lopinavir/Ritonavir arm. Also, there was a significantly decreased rate of adverse effects in patients who received Favipiravir (11.4%) compared to Lopinavir/Ritonavir (55.6%).[37,38]

Verified in vitro, Favipiravir is recognized as one likely candidate for the treatment of Covid-19 and preclinical animal studies on-going.[39,40] The results of some ongoing randomized and controlled trials that study the efficacy of Favipiravir for Covid-19 will be fundamental for further investigation of the prodrug's role in the current coronavirus pandemic.[38,41]

6.3. SINEFUNGIN

Sinefungin (**3**, see Figure 5) is a natural nucleoside isolated from cultures of *Streptomyces Griseolus* and experimentally used as antibiotic. The nucleoside is structurally similar to *S*-adenosylmethionine (SAM) and it has proved to inhibit various fungi and viruses, but its potential antiparasitic activity is the main current attraction.[18] Sinefungin is an antibiotic that has important characteristics such as antiprotozoal, antifungal and antiviral properties.[42,43] The natural nucleoside inhibits transmethylation reactions related to RNA, proteins, DNA and other molecules.

Very recently, Sinefungin was also found to be an effective nsp16 MTase inhibitor against MERS-CoV and SARS-CoV. However, the inhibitory activity of SFG towards SARS-CoV-2 nsp16/nsp10 MTase need further investigation for their potential for treating Covid-19.[44,45]

6.3.1. Drug development

The isolation of the antibiotic was reported from cultures of *Streptomyces griseolus* and *Streptomyces incarnatus* at Lilly Research Laboratories in 1971. The inhibitory effect of the drug was studied on some *S*-adenosylmethionine mediated transmethylation reactions in cell culture and *in vivo* due to structural similarity to analogues *S*-adenosylmethionine (SAM) and *S*-adenosylhomocysteine (SAH).[46]

6.3.2. Synthesis of Sinefungin

Sinefungin was first synthesized by Mock and co-workers[47] in 1982 and the C6' stereocenter was the main synthetic challenge. The product was obtained as a mixture of C6'-epimers that

could be separable by chromatographic methods or through stereoselective transformations such as the alkylation of a chiral imide auxiliary by Ghosh team or the diastereoselective reduction of a ketone at C6' by Rapoport and co-workers.[47] In every case, it was necessary to interconvert multiple functional groups to elaborate the alkyl chain of the natural nucleoside molecule, limiting the tractability of these approaches for the effective synthesis of Sinefungin and its potential analogues.[47]

The promising use of Sinefungin and the structurally related analogues to target SAM-dependent methyltransferases inspired for the synthesis of C9'-*epi*-sinefungin[47] (see Scheme 7). A study provided a satisfactory solution to the obtention of the key C6' amine of the drug scaffold, which can be applied to the synthesis of new analogues of Sinefungin in order to investigate new MT inhibitors.

The synthesis of C9'-*epi*-sinefungin had as a starting material acetonide **6**, which is commercially available or easily accessible from D-ribose. Triflation of acetonide, followed by alkynylation upon treatment with lithium acetylide generated the silyl-protected propargyl alcohol **7**. Next step consisted of the cleavage of the TBS protecting group and subsequent reduction to the (*E*)- allylic alcohol **8** with lithium aluminium hydride. The following step converted the alcohol **8** to trichloroacetimidate **9** in the presence of trichloroacetonitrile obtaining the required precursor for the envisioned Overman rearrangement.

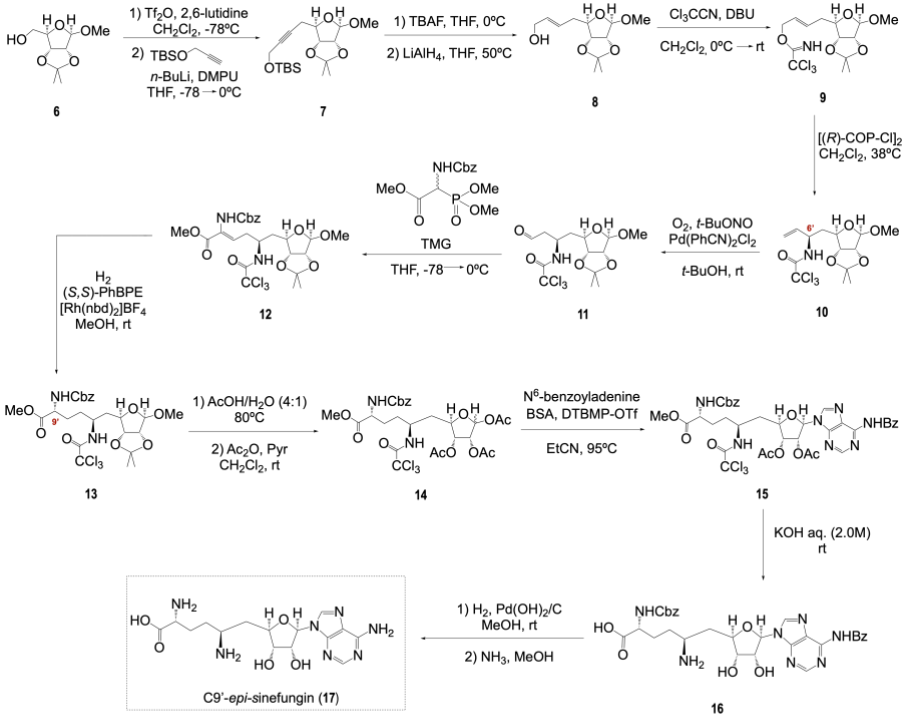
The treatment of (*E*)-allylic trichloroacetimidate **9** in dichloromethane with Overman's catalyst resulted with the desired [3,3]-sigmatropic rearrangement producing allylic trichloroacetamide **10** as the major diastereomer which could be isolated by chromatography. The catalytic diastereoselective rearrangement provided an efficient solution to the installation of the stereocenter at C6'.

Once the stereocenter at C6' was build, the next steps consisted of the elaboration of the amino acid moiety. The terminal alkene **10** was oxidated to aldehyde **11** and followed a Horner-Wadsworth-Emmons olefination that produced the enamide **12**. The C9' amino stereocenter was set by asymmetric hydrogenation with (*S,S*)-Ph-BPE-Rh catalyst, that provided (*R*)-carbamate **13** as a single diastereoisomer.

Once the C5'-C10' amino acid chain was elaborated, the addition of the adenine moiety and succeeding global deprotection were undertaken. The acetonide **13** was converted in two steps

into triacetate **14** and after that, Vorbrüggen glycosylation supported by 2,6-di-*tert*-butyl-4-methylpyridinium triflate synthesized the trichloroacetamide nucleoside **15** as a single diastereomer.

The final two steps involve the removal of various protecting groups. The importance of adding certain conditions which would selectively deprotect the C6' amine over the C9' one, was of potential interest because of its practical way for the synthesis of Sinefungin analogues at this position. The penultimate step was the treatment of the nucleoside **15** with aqueous potassium hydroxide that delivered the amine **16** in good yield, leaving intact the C9'-Cbz-protected amine. The final step was the cleavage of the other protecting group, Cbz, by hydrogenolysis and removal of the Bz protecting group with methanolic ammonia that ended in the obtention of C9'-*epi*-sinefungin **17**. That route synthetic comprised a total of 11 steps with an overall yield of 7.6%.[47,48]



Scheme 7. Synthetic route of C9'-*epi*-sinefungin (**17**).

6.3.3. Mode of action of Sinefungin

Structural similarity between analogues with the natural nsp16 co-substrate SAM revealed that analogues that operate as competitive binders of SAM might be effective inhibitors of 2'-O-MTase catalytic activity.[49] The nsp16 protein from SARS-CoV-2 is a SAM-dependent (nucleoside-2'-O)-methyltransferase (2'-O-MTase) that its activity is regulated by nsp10 allosteric activator. The nsp16 protein catalyzes the methyl transfer from SAM to Cap-0, generating the products SAH and Cap-1. This transmethylation can be inhibited by Sinefungin, an analogue of SAH used as a pan-inhibitor of MTases.[50]

Based on early research, recent crystallographic studies showed that MTase inhibitors like Sinefungin adhered to SAM binding site and could suppress coronaviral MTase activity of nsp16.[51,52]

6.3.4. Therapeutic and pharmacological properties

Some studies showed that Sinefungin exhibited a variety of biological effects including the inhibition of diverse fungi's growth, virus replication and antiparasitic effects *in vivo* and *in vitro*. Despite these meaningful biological activities for antiviral research, Sinefungin has limited use as an antibiotic because of its nephrotoxicity that induces bone marrow depression in laboratory animals.[53]

Further studies will be required to review and analyse if Sinefungin could be a potential inhibitor of nsp16 MTase of earlier MERS-CoV and SARS-CoV.

7. CONCLUSIONS

The rapid spread of the novel beta coronavirus SARS-CoV-2, first reported in Wuhan in December 2019 due to high human-to-human transmission, along with the inability to stop the pandemic, is causing a high mortality and the paralysation of world's economy.

The scientific community from all over the world has been working together to fight against SARS-CoV-2. Although some vaccines have been recently approved and some countries like the UK or Spain started a massive vaccination, the repositioning of available drugs could be also an essential strategy in the development of novel drugs. Within this aim, Remdesivir, Favipiravir or Sinefungin are currently studied at different stages to determine their antiviral potential against SARS-CoV-2 although more studies will be required to comprehensively understand their antiviral potential.

Remdesivir and Favipiravir are prodrugs and potent inhibitors of viral RNA-dependent RNA polymerase (RdRp). Both prodrugs have been studied for other outbreaks because of their multiple RNA viruses' inhibitions. Remdesivir is currently under evaluation for COVID-19 and it is considered the most promising antiviral drug. That is the main reason why a large-scale study investigating the clinical efficacy of Remdesivir is on-going. Favipiravir, known as an anti-influenza RdRp, also started being clinically investigated for its effectiveness in COVID-19 patients.

Sinefungin, a bacterial metabolite, is a novel drug that inhibits the activity of viral MTases. Due to nephrotoxicity, it will require further investigation and therapeutic trials to establish whether the drug is indeed effective against SARS-CoV-2.

Drug repurposing is possibly a crucial and global strategy for novel drugs' development for the treatment of emerging diseases such as COVID-19 because of its key benefits such as limited clinical trials, especially concerning phases I and II, and the opportunity for improving and discovering new types of medicines from the early drugs with higher efficacy and safety profiles.

Unfortunately, outbreaks of CoV or other viruses are likely to happen in the coming years, that is the principal reason why we, as a society, need to adapt our lives to the new normality. As

a future projection, further clinical and therapeutic investigations will be essential to prevent and treat future viruses to avoid pandemics like the current one.

8. REFERENCES AND NOTES

1. Dömling, A. & Gao, L. Chemistry and Biology of SARS-CoV-2. *Chem* **6**, 1283–1295 (2020).
2. Lai, C. C., Shih, T. P., Ko, W. C., Tang, H. J. & Hsueh, P. R. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. *Int. J. Antimicrob. Agents* **55**, 1-9 (2020).
3. McKee, D. L., Sternberg, A., Stange, U., Laufer, S. & Naujokat, C. Candidate drugs against SARS-CoV-2 and COVID-19. *Pharmacol. Res.* **157**, 1-9 (2020).
4. Jeon, S. *et al.* Identification of antiviral drug candidates against SARS-CoV-2 from FDA-approved drugs. *Antimicrob. Agents Chemother.* **64**, 1-9 (2020).
5. Sanders, J. M., Monogue, M. L., Jodlowski, T. Z. & Cutrell, J. B. Pharmacologic Treatments for Coronavirus Disease 2019 (COVID-19): A Review. *JAMA - J. Am. Med. Assoc.* **323**, 1824–1836 (2020).
6. Riva, L. *et al.* Discovery of SARS-CoV-2 antiviral drugs through large-scale compound repurposing. *Nature* **586**, 113–119 (2020).
7. V'kovski, P., Kratzel, A., Steiner, S., Stalder, H. & Thiel, V. Coronavirus biology and replication: implications for SARS-CoV-2. *Nat. Rev. Microbiol.* 1-16 (2020).
8. Eastman, R. T. *et al.* Remdesivir: A Review of Its Discovery and Development Leading to Emergency Use Authorization for Treatment of COVID-19. *ACS Cent. Sci.* **6**, 672–683 (2020).
9. Wan, Y., Shang, J., Graham, R., Baric, R. S. & Li, F. Receptor Recognition by the Novel Coronavirus from Wuhan: an Analysis Based on Decade-Long Structural Studies of SARS Coronavirus. *J. Virol.* **94**, 1–9 (2020).
10. De Clercq, E. Potential antivirals and antiviral strategies against SARS coronavirus infections. *Expert Rev. Anti. Infect. Ther.* **4**, 291–302 (2006).
11. Jean, S. S., Lee, P. I. & Hsueh, P. R. Treatment options for COVID-19: The reality and challenges. *J. Microbiol. Immunol. Infect.* **53**, 436–443 (2020).
12. Warren, T. K. *et al.* Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. *Nature* **531**, 381–385 (2016).
13. Wang, M. *et al.* Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res.* **30**, 269–271 (2020).
14. Titova, Y. A. & Fedorova, O. V. Favipiravir – a Modern Antiviral Drug: Synthesis and Modifications. *Chem. Heterocycl. Compd.* **56**, 659–662 (2020).

15. Furuta, Y., Komeno, T., & Nakamuba, T. Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. *Proc Jpn Acad Ser B Phys Biol Sci.* **93**, 449–463 (2017).

16. Shiraishi, T. & Kuzuyama, T. Recent advances in the biosynthesis of nucleoside antibiotics. *J. Antibiot. (Tokyo).* **72**, 913–923 (2019).

17. Krafcikova, P., Silhan, J., Nencka, R. & Boura, E. Structural analysis of the SARS-CoV-2 methyltransferase complex involved in RNA cap creation bound to sinefungin. *Nat. Commun.* **11**, 1–8 (2020).

18. Nolan, L. L. Molecular target of the antileishmanial action of sinefungin. *Antimicrob. Agents Chemother.* **31**, 1542–1548 (1987).

19. Decroly, E. *et al.* Crystal structure and functional analysis of the SARS-coronavirus RNA cap 2'-o-methyltransferase nsp10/nsp16 complex. *PLoS Pathog.* **7**, 1-14 (2011).

20. Kiplin Guy, R., DiPaola, R. S., Romanelli, F. & Dutch, R. E. Rapid repurposing of drugs for COVID-19. *Science.* **368**, 829–830 (2020).

21. Vaccines and Related Biological Products Advisory Committee Meeting FDA Briefing Document Pfizer-BioNTech COVID-19 Vaccine Sponsor : Pfizer and BioNTech. (2020).

22. Nittari, G., Pallotta, G., Amenta, F. & Tayebati, S. K. Current pharmacological treatments for SARS-COV-2: A narrative review. *Eur. J. Pharmacol.* **882**, 1-11 (2020).

23. Zumla, A., Chan, J. F. W., Azhar, E. I., Hui, D. S. C. & Yuen, K. Y. Coronaviruses-drug discovery and therapeutic options. *Nat. Rev. Drug Discov.* **15**, 327–347 (2016).

24. Martinez, M. A. Clinical trials of repurposed antivirals for SARS-CoV-2. *Antimicrob. Agents Chemother.* **64**, 1–6 (2020).

25. Cho, A.; Saunders, O. L. . et al. Synthesis and antiviral activity of a series of 1'-substituted 4-aza-7,9-dideazaadenosine C-nucleosides. *Bioorganic Med. Chem. Lett.* **22**, 2705–2707 (2012).

26. Siegel, D. *et al.* Discovery and Synthesis of a Phosphoramidate Prodrug of a Pyrrolo[2,1-f][triazin-4-amino] Adenine C-Nucleoside (GS-5734) for the Treatment of Ebola and Emerging Viruses. *J. Med. Chem.* **60**, 1648–1661 (2017).

27. Mehellou, Y., Rattan, H. S. & Balzarini, J. The ProTide Prodrug Technology: From the Concept to the Clinic. *J. Med. Chem.* **61**, 2211–2226 (2018).

28. Vieira, T. *et al.* Development of a Large-Scale Cyanation Process Using Continuous Flow Chemistry en Route to the Synthesis of Remdesivir. *Org. Process Res. Dev.* **24**, 2113–2121 (2020).

29. Yan, V. C. & Muller, F. L. Advantages of the Parent Nucleoside GS-441524 over Remdesivir for Covid-19 Treatment. *ACS Med. Chem. Lett.* **11**, 1361–1366 (2020).

30. Gordon, C. J. *et al.* Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. *J. Biol. Chem.* **295**, 6785–6797 (2020).

31. Tchesnokov, E. P., Feng, J. Y., Porter, D. P. & Götte, M. Mechanism of inhibition of ebola virus RNA-dependent RNA polymerase by remdesivir. *Viruses* **11**, 1–16 (2019).

32. Beigel, J. H. *et al.* Remdesivir for the Treatment of Covid-19 — Final Report. *N. Engl. J. Med.* **383**, 1813–1826 (2020).
33. Liu, F. L., Li, C. Q., Xiang, H. Y. & Feng, S. A practical and step-economic route to Favipiravir. *Chem. Pap.* **71**, 2153–2158 (2017).
34. Furuta, Y. *et al.* Mechanism of action of T-705 against influenza virus. *Antimicrob. Agents Chemother.* **49**, 981–986 (2005).
35. Peng, Q. *et al.* Structural basis of SARS-CoV-2 polymerase inhibition by Favipiravir. *bioRxiv* 2020.10.19.345470 (2020).
36. Du, Y. X. & Chen, X. P. Favipiravir: Pharmacokinetics and Concerns About Clinical Trials for 2019-nCoV Infection. *Clin. Pharmacol. Ther.* **108**, 242–247 (2020).
37. Coomes, E. A. & Haghbayan, H. Favipiravir, an antiviral for COVID-19? *J. Antimicrob. Chemother.* **75**, 2013–2014 (2020).
38. Cai, Q. *et al.* Experimental Treatment with Favipiravir for COVID-19: An Open-Label Control Study. *Engineering* **6**, 1192–1198 (2020).
39. Chen, C. *et al.* Favipiravir versus Arbidol for COVID-19: A Randomized Clinical Trial. *medRxiv,* 1-30 (2020).
40. Kaptein, S. J. F. *et al.* Favipiravir at high doses has potent antiviral activity in SARS-CoV-2−infected hamsters, whereas hydroxychloroquine lacks activity. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 26955–26965 (2020).
41. Boretti, A. Favipiravir use for SARS CoV-2 infection. *Pharmacol. Reports* **72**, 1542–1552 (2020).
42. Yadav, M. K., Park, S. W., Chae, S. W. & Song, J. J. Sinefungin, a natural nucleoside analogue of S-adenosylmethionine, inhibits streptococcus pneumoniae biofilm growth. *Biomed Res. Int.* **2014**, 1-10 (2014).
43. Hamill, R. L. & Hoehn, M. M. A9145, a new adenine-containing antifungal antibiotic: I. discovery and isolation. *J. Antibiot. (Tokyo).* **26**, 463–465 (1973).
44. Mahalapbutr, P., Kongtaworn, N. & Rungrotmongkol, T. Structural insight into the recognition of S-adenosyl-L-homocysteine and sinefungin in SARS-CoV-2 Nsp16/Nsp10 RNA cap 2'-O-Methyltransferase. *Comput. Struct. Biotechnol. J.* **18**, 2757–2765 (2020).
45. Perveen, S. *et al.* A high throughput RNA displacement assay for screening SARS-CoV-2 nsp10-nsp16 complex towards developing therapeutics for COVID-19. *bioRxiv,* 416–432 (2020).
46. Geze, M., Blanchard, P., Fourrey, J. L. & Gero, M. R. Synthesis of Sinefungin and Its C-6' Epimer. *J. Am. Chem. Soc.* **105**, 7638–7640 (1983).
47. Decultot, L., Policarpo, R. L., Wright, B. A., Huang, D. & Shair, M. D. Asymmetric Total Synthesis of C9'- epi-Sinefungin. *Org. Lett.* **22**, 5594–5599 (2020).
48. Ghosh, A. K. & Liu, W. Total synthesis of (+)-sinefungin. *J. Org. Chem.* **61**, 6175–6182 (1996).

49. Morales, P., Curtis, N. L., Sandra, G. Z., Bastida, A. & Bolanos-Garcia, V. M. Tackle the COVID-19 Disease. *Catalysts* **10**, 1–13 (2020).

50. Rosas-Lemus, M. *et al.* High-resolution structures of the SARS-CoV-2 2'-O-methyltransferase reveal strategies for structure-based inhibitor design. *Sci. Signal.* **13**, 1–12 (2020).

51. Hijikata, A. *et al.* Knowledge-based structural models of SARS-CoV-2 proteins and their complexes with potential drugs. *FEBS Lett.* **594**, 1960–1973 (2020).

52. Tazikeh-Lemeski, E. *et al.* Targeting SARS-COV-2 non-structural protein 16: a virtual drug repurposing study. *J. Biomol. Struct. Dyn.* **1**, 1–14 (2020).

53. Maguire, M. P., Feldman, P. L. & Rapoport, H. Stereoselective Synthesis and Absolute Stereochemistry of Sinefungin. *J. Org. Chem.* **55**, 948–955 (1990).

9. ACRONYMS AND ABBREVIATIONS

ACE2 – Angiotensin-converting enzyme 2

AEMP – Agencia Española de Medicamentos y Productos Sanitarios

BPE – bis(phospholano)-ethane

BSA (Favipiravir) – *N*-chloro-*N*-methoxybenzenesulfonamide

BSA – N,O-bis(trimethylsilyl)acetamide

COP – Cobalt Oxazoline Palladacycle

CoV – Coronavirus

COVID-19 – Coronavirus Disease 2019

DIEA – Diisopropylethylamine (Hunig's Base)

DTBMP-OTf – 2,6-di-tert-butyl-4-methylpyridinium triflate

EMA – European Medicines Agency

FDA – American Food and Drug Administration

GS-5734 – Gilead Sciences 5734 (Remdesivir's investigational name)

H – Helicase

MERS – Middle East Respiratory Syndrome

MERS-CoV – Middle East Respiratory Syndrome Coronavirus

MTase – 2'-O-RNA Methyl Transferase

nbd - norbornadiene

NTP – Nucleoside Triphosphate

Plpro – Papain-like protease

RBD – Receptor-binding domain

RdRp – RNA-dependent RNA polymerase

SAM – *S*-adenosylmethionine

SARS – Severe Acute Respiratoy Syndrome

SARS-CoV – Severe Acute Respiratoy Syndrome Coronavirus

SARS-CoV-2 – Severe Acute Respiratory Syndrome Coronavirus 2

TMG – 1,1,3,3-tetramethylguanidine

TMPRSS2 – Transmembrane protease, serine 2

TSA - *N*-chloro-*N*-methoxy-4-methylbenzenesulfonamide

3CLpro – 3-chymotrypsin-like protease