ENHANCING ANTIVIRAL EFFICACY: THE SYNERGY OF DEEP EUTECTIC SOLVENTS AND NUCLEOSIDE ANALOGS

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by

ROOPAL GARG **(2K22/MSCCHE/51)**

RAVEEN KUMAR **(2K22/MSCCHE/29)**

Under the supervision of
DR. RICHA SRIVASTAVA
(DTU)

Department of Applied Chemistry

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-110042. India

May, 2024

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-42

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ROOPAL GARG

RAVEEN KUMAR

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-42

DECLARATION

We, **ROOPAL GARG & RAVEEN KUMAR**, hereby declare that the work which is being submitted in this dissertation entitled "**Enhancing Antiviral Efficacy: The Synergy of Deep Eutectic Solvents and Nucleoside Analogs**" in the partial fulfillment for the award of the degree of **Master of Science** in the **Department of Applied Chemistry**, Delhi Technological University is an authentic record of our own work carried out during the period from **August, 2023 to April, 2024** under the supervision of **Dr. Richa Srivastava (Assistant Professor, Department of Applied Chemistry, Delhi Technological University)**.

We, further declare that the dissertation has not been submitted by us for the award of any other degree of this or any other Institute.

ROOPAL GARG — 2K22/MSCCHE/51

RAVEEN KUMAR — 2K22/MSCCHE/29

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)
Shahbad Daulatpur, Main Bawana Road, Delhi-42

CERTIFICATE

This is to certify that the Project report entitled **"Enhancing Antiviral Efficacy: The Synergy of Deep Eutectic Solvents and Nucleoside Analogs"** which is submitted by **Roopal Garg (2K22/MSCCHE/51)** and **Raveen Kumar (2K22/MSCCHE/29)**, Department of Applied Chemistry, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of **Master of Science**, is a record of the project work carried out by the students under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Dr. Richa Srivastava
SUPERVISOR
Assistant Professor
Department of Applied Chemistry
Delhi Technological University
New Delhi - 110042

ABSTRACT

The recent COVID-19 pandemic has revealed weaknesses in our readiness to address emerging viral threats, stressing the urgent need for sustainable strategies to combat such outbreaks. Nucleoside analogs, an important class of antiviral drugs, have shown their effectiveness, but their synthesis often relies on hazardous organic solvents, presenting significant sustainability challenges and environmental concerns. This study aims to tackle these challenges by exploring the potential of deep eutectic solvents (DESs) as eco-friendly alternatives for the greener manufacturing of these life-saving therapies.

The research begins by providing a comprehensive overview of the SARS-CoV-2 virus lifecycle, identifying key targets where nucleoside analogs can effectively intervene to disrupt viral replication. This fundamental understanding serves as a crucial foundation for the development of potent antiviral candidates against COVID-19 and future coronavirus threats, ensuring their targeted and efficient action.

DESs, an emerging class of solvents derived from renewable bioresources such as sugars and organic acids, offer unique solvation properties while being non-toxic and biodegradable. The focus of this work is on synthesizing and optimizing DESs composed of glucose combined with tartaric acid or citric acid as sustainable media for the production of nucleoside analogs.

The research focuses on using optimized deep eutectic solvents (DES) as more environmentally friendly alternatives to traditional solvents typically used in the synthesis, purification, and formulation of nucleoside analogs.

This comprehensive analysis aims to demonstrate that DES are a practical solution. By combining bio-based DES with potent nucleoside analogs, this work presents an environmentally responsible approach to sustainably producing important therapies against viral threats such as COVID-19. This approach enables us to safeguard public health by providing these essential pharmaceuticals while minimizing their environmental impact.

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INTRODUCTION

1.1 Overview and Significance:

In late December 2019, an outbreak of an unidentified respiratory illness occurred in Wuhan, Hubei, China. Patients exhibited symptoms such as fever, cough, shortness of breath, and myalgia. Subsequently, independent laboratories identified the causative agent as a novel coronavirus, SARS-CoV-2 [1]. Coronaviruses, first characterized in 1960, constitute a diverse family of enveloped viruses with single-stranded RNA genomes [2]. They are classified into four genera: Alpha, Beta, Delta, and Gamma. SARS-CoV-2 is a betacoronavirus closely related to the virus responsible for the 2003 SARS outbreak. The virus predominantly targets the respiratory system by binding to ACE2 receptors in the lungs. Complications may include acute respiratory distress syndrome (ARDS) and multi-organ dysfunction. This virus has an incubation period ranging from 2 to 14 days and primarily spreads via respiratory droplets generated during coughing and sneezing. On March 11, 2020, the World Health Organization declared COVID-19 as a pandemic due to the significant number of global cases and fatalities. As of April 2024, over 775 million confirmed cases and 7 million deaths have been recorded [3]. The pandemic has resulted in profound social and economic consequences, leading to widespread implementation of lockdown measures. Nucleoside analogs have emerged as a promising area of focus for therapeutic interventions against COVID-19 owing to their broad-spectrum antiviral activity and capacity to overcome resistance [4].

1.1.1 Nucleoside analogs

Nucleoside analogs are artificially synthesized compounds designed to mimic the structure of natural nucleosides. They find widespread application in medical contexts, primarily as antimicrobial [5], antineoplastic [6], and antiviral agents [7]. These analogs are often derivatives of natural nucleosides and their synthesis involves modifying the molecular structure through three specific methods which are given below [8]:

1. *Heterocyclic base modification:*

The first method involves changing nucleobases by adding different moieties or substituting the base with another nitrogen-containing heterocycle. These changes lead to synthesizing nucleoside analogs like AL-335, Azvudine, Ribavirin, Molnupiravir, and Favipiravir. Altering the nucleobase gives unique properties to the analogs, making them effective against specific viruses or cellular processes.

2. Sugar Modification:

The alternative approach involves altering the D-ribofuranosyl component of nucleosides. This can be accomplished through the addition of various substituents, the removal of hydroxy groups at the C-2' and C-3' positions, the elimination of hydrogen atoms at these positions, the introduction of oxygen &/or sulphur atoms, or the replacement of the oxygen atom with sulphur. Another common variation is the substitution of the D-ribofuranosyl moiety with a cyclopentane or cyclopentene ring. This modification leads to the synthesis of nucleoside analogs such as Abacavir, Remdesivir, & Acyclovir, giving specific properties to the analogs and impacting their effectiveness and target specificity.

3. Addition of the Phosphate group to the hydroxyl group at the C-5' position:

The third method involves adding a phosphate group to the hydroxy group at the C-5' position of the nucleoside. This addition improves the medicinal properties of the nucleoside analog by adding one or more phosphate groups. Phosphorylation is an important change observed in AZT, a nucleotide inhibitor used against viruses like HIV. It ensures that the nucleoside analog is activated and included in cellular processes, making it a crucial strategy in developing antiviral and therapeutic drugs.

1.1.2 Rationale for using Nucleoside analogs against SARS-CoV-2

The global COVID-19 pandemic has emphasized the critical requirement for effective antiviral therapeutics. Nucleoside analogs, a category of antiviral medications, have surfaced as a promising treatment in our battle against this ailment. These agents have demonstrated efficacy in combating viral infections such as HIV, herpes, and hepatitis C by selectively targeting the viral RNA-dependent RNA polymerase (RdRp), an important enzyme in viral replication [9]. Remdesivir, a nucleoside analog initially designed for Ebola, has exhibited effectiveness against SARS-CoV-2 & has obtained emergency use authorization, confirming the potential of this drug class.

Ongoing research is concentrating on structurally modifying nucleoside analogs to improve their antiviral strength and effectiveness, particularly against COVID-19. Furthermore, these medications can be employed in combination with other antiviral agents, potentially resulting in synergistic effects and enhanced treatment outcomes. Initiatives to develop nucleoside analogs for oral administration are also underway, which would improve accessibility and facilitate outpatient treatment, a critical consideration for managing COVID-19 cases.

Nucleoside analogs have well-established safety profiles in the treatment of various viral infections. This existing safety data could accelerate their utilization against COVID-19 following detailed studies. With their ability to inhibit viral replication through targeted mechanisms, coupled with promising clinical data and ongoing optimization efforts, nucleoside analogs represent a highly compelling therapeutic approach deserving of strong support in the battle against the COVID-19.

1.2 Viral Life Cycle and Drug Targets

The SARS-CoV-2 virus, responsible for the COVID-19 pandemic, goes through several important phases during its life cycle that allow it to reproduce and propagate within host cells [Fig 1].

The SARS-CoV-2 virus initiates infection by entering host cells through viral-cell membrane fusion. This process is dependent on the binding of the viral spike (S) protein to the host cell receptor angiotensin-converting enzyme 2 (ACE2) [10]. The host protease TMPRSS2 primes the S protein for fusion by cleaving it into the S1 and S2 subunits [11]. S1 facilitates ACE2 binding, while S2 mediates the fusion of viral and host cell membranes, enabling the viral genome to enter the cell cytoplasm.

Upon entry, SARS-CoV-2 takes over the host machinery to replicate its genome and synthesize viral proteins. The viral genome is a single-stranded positive-sense RNA of approximately 30 kilobases, encoding structural proteins such as spike (S), membrane (M), envelope (E), and nucleocapsid (N), as well as non-structural proteins (NSPs). Two large polyproteins, pp1a and pp1ab, are translated from the viral genome. These are cleaved by viral proteases (3CLpro and Mpro) into 16 NSPs that constitute the replication-transcription complex (RTC) [12]. Key enzymes in the RTC include the RNA-dependent RNA polymerase (RdRp, NSP12), which initiates viral replication by generating negative-sense RNA copies from the positive-sense genome to act as templates for new genome synthesis [13]. It operates in combination with cofactors NSP7 and NSP8. The helicase (NSP13) unwinds RNA duplexes, while the proofreading exonuclease (NSP14) enhances replication accuracy by eliminating mismatched nucleotides. NSP14 and NSP16 methylate the 5' cap of viral mRNAs, safeguarding them from degradation by host cells. NSP16 functions with the cofactor NSP10 [14]. Several NSPs participate in capping the viral mRNAs, including NSP13 (helicase/triphosphatase), NSP14 (N7-methyltransferase), and NSP16 (2'O-methyltransferase). This cap allows viral mRNAs to escape detection by the host's innate immune system [15].

Following the synthesis of viral proteins and genomic RNA, the subsequent stages involve virion assembly and release from the host cell. At the endoplasmic reticulum and Golgi complex, the viral structural proteins M, E, and N drive the assembly of new virions incorporating the viral genome and spike protein [16]. The assembled virus particles are released from the host cell through one of three mechanisms: 1) budding through the plasma membrane, 2) secretion via exocytosis, or 3) cell lysis and rupture. Upon release, the new virions can infect other cells and spread the viral infection [17].

The viral RdRp and methyltransferases are crucial targets for antiviral drugs such as nucleoside analogs (e.g., remdesivir, molnupiravir, favipiravir, ribavirin). By inhibiting these enzymes through mechanisms like chain termination & mutagenesis,

the drugs can effectively disrupt viral replication and hold promise as COVID-19 therapeutics.

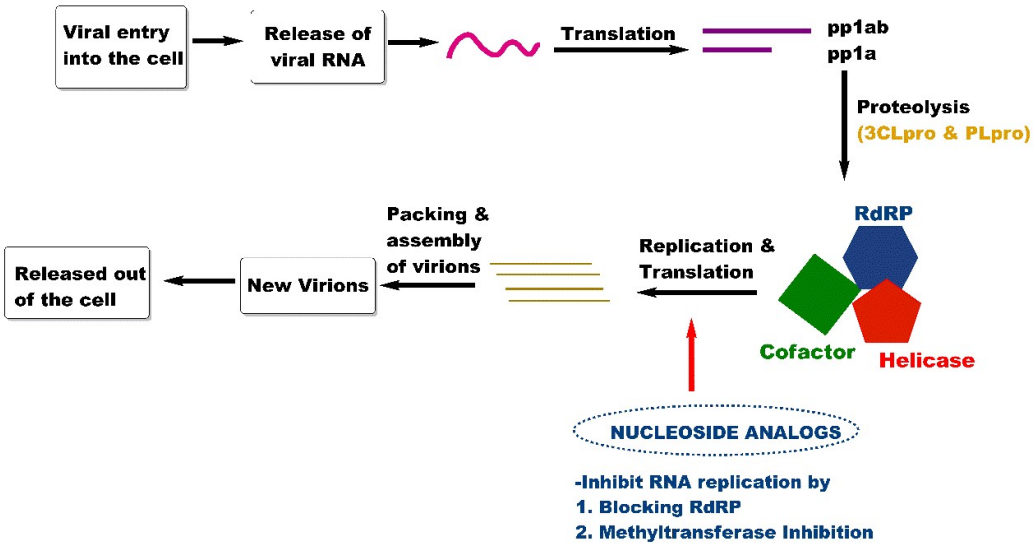


Fig 1 Life cycle of SARS-CoV-2

The exploration of the SARS-CoV-2 life cycle highlights the significance of the viral RNA-dependent RNA polymerase (RdRp) & methyltransferase as crucial targets. Inhibiting these targets can effectively hinder viral transmission. Nucleoside analogs demonstrate potent inhibition of these enzymes, suggesting their potential as promising therapeutics for COVID-19. The next section will present comprehensive explanation of the mechanisms of action employed by nucleoside analog drugs in targeting the methyltransferase & RdRp activities of the virus.

1.3 Mechanism of Action of Nucleoside Analogs as Antivirals

1.3.1 Nucleoside Analogs as RdRP inhibitor

In order for nucleoside analogs to exhibit efficacy against viral infections, they require enzymatic modification by cellular kinases. These kinases catalyze the conversion of nucleoside analogs into their active triphosphate form. Upon reaching their active state, the nucleoside analogs act through competitive inhibition of viral polymerases. This process can be accomplished via following pathways:

Chain Termination

It mainly occurs as a result of these methods.:

Obligate Chain Termination: In antiviral therapy, it is a process that stops the replication of viral DNA or RNA chains by incorporating a nucleoside analog. This

mechanism is specific to nucleosides lacking the 3'-hydroxyl group, which is essential for linking the next incoming nucleotide [18]. Antiviral agents like Tenofovir, Acyclovir, & Abacavir follow this mechanism to effectively combat DNA viruses and retroviruses. [Fig. 2].

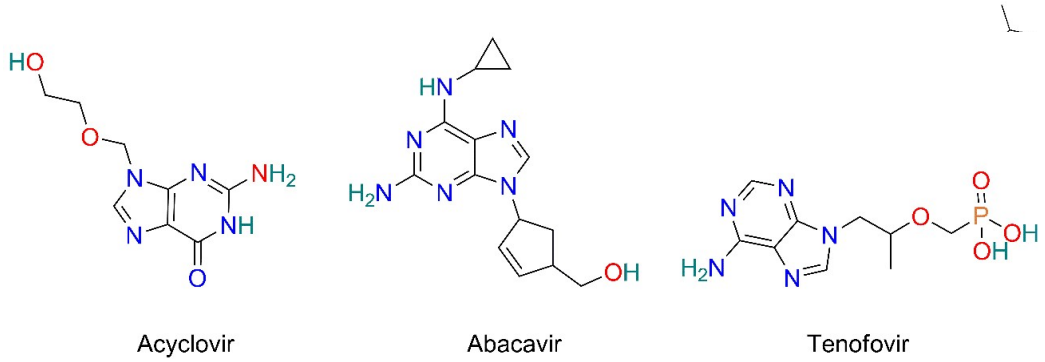


Fig 2 Nucleoside Analogs That Work by Obligate Chain Termination

Non-Obligate Termination: Nucleosides that induce non-obligate chain termination possess a 3'-hydroxyl group capable of incorporating incoming nucleotides. Islatravir, Azvudine, Balapiravir, & Adafosbuvir are examples of nucleoside analogs that operate via this mechanism. [Fig. 3].

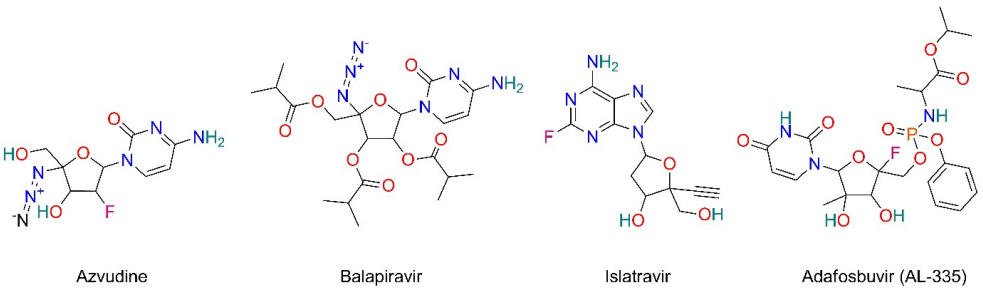


Fig 3 Nucleoside Analogs That Work by Non-Obligate Chain Termination

Delayed Termination: In this mechanism of action, termination does not occur immediately but instead takes place after the addition of a few more nucleotides. This process results in a delay but ultimately leads to the termination of the chain. Nucleosides that function through delayed chain termination possess a 3'-hydroxyl group, enabling the continued integration of incoming nucleotides. However, due to steric hindrances at neighbouring positions, each subsequent addition occurs at a slow pace. Eventually, after 2-4 additions, further incorporation terminates. Compounds such as Entecavir, Remdesivir, & others exhibit this type of termination mechanism [Fig. 4] [19].

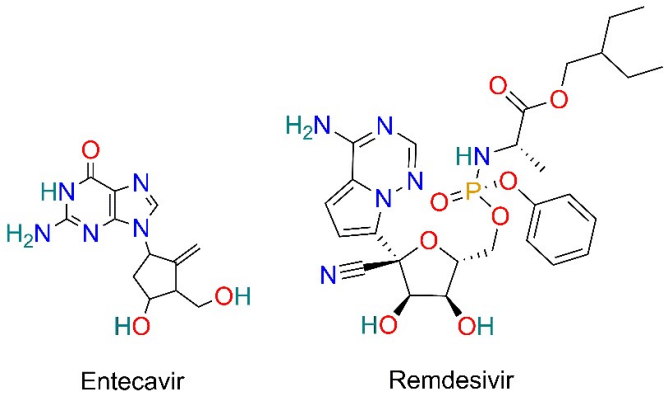


Fig 4 Nucleoside Analogs That Work by Delayed Chain Termination

Lethal Mutagenesis

This approach used against RNA viruses involves increasing the error rate during the virus's replication. This higher mutation rate can weaken the virus's essential genes and eventually lead to its death. Nucleoside analogs can cause mutations in RNA by changing the base pairing patterns. When these nucleoside analogs are introduced into the RNA synthesis process, they add to the accumulation of mutations in the virus, weakening it and making it harder for it to replicate. Nucleoside analogs like ribavirin, favipiravir, & molnupiravir have shown that this approach works [Fig 5-6] [20]. However, there are risks, such as the virus becoming resistant and potential adverse effects like cancer and birth defects. So, careful consideration and ongoing research are needed before using this approach.

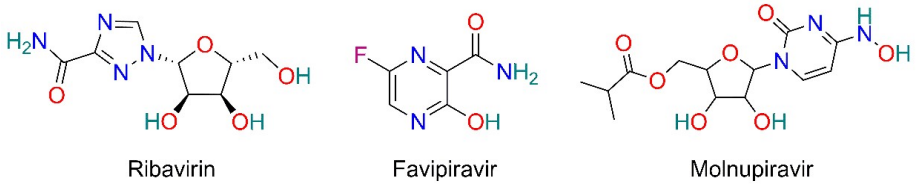


Fig 5 Nucleoside Analogs That Work by Lethal Mutagenesis

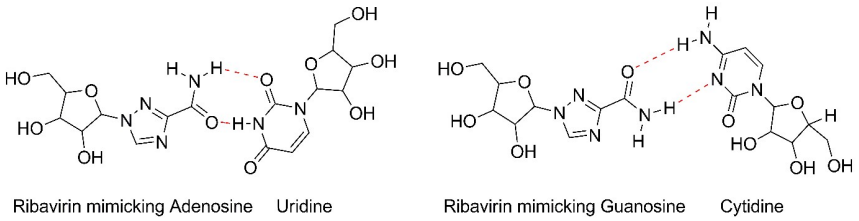


Fig 6 Ribavirin Mismatch Base Pairing

1.3.2 Nucleoside analogs as Methyltransferase inhibitor

Methyl Transferases inhibition: The genomes of RNA viruses contain methyltransferase enzymes that are crucial for capping viral RNA, allowing it to mimic host mRNA. This enables viruses to evade the host cell's immune response, improve mRNA translation, and promote stability. Due to their high conservation, viral methyltransferases are a promising target for antiviral drug development. Guanosine analogs like RBV disrupt the GTP binding site of viral enzymes & endogenous eIF4E, reducing viral RNA capping efficiency. Additionally, newly developed Flex analogs of acyclovir have shown greater inhibition of flavivirus methyltransferase compared to the endogenous enzyme [21].

1.4 Prominent Nucleoside Drugs for COVID-19

1.4.1 Remdesivir

Remdesivir, also known as GS-5734, received approval from the US FDA on October 22, 2020, as a primary treatment for SARS-CoV-2 in adults. It has demonstrated significant antiviral potential in laboratory experiments against various viruses, including MERS-CoV, EBOV, SARS-CoV, and SARS-CoV-2 [22]. Remdesivir, a 10-cyano-substituted adenosine nucleotide analog, acts as a monophosphorylated prodrug metabolized by the body into an active nucleoside triphosphate, known as GS-443902 (a natural counterpart of ATP), through the action of intracellular kinase. This active form inhibits viral RNA-dependent RNA polymerases early in viral infection, competing with endogenous nucleotides for viral RNA incorporation via RNA-dependent RNA polymerase. Once integrated into the RNA chain, remdesivir does not immediately terminate the chain, as the presence of 3’OH allows the addition of three more nucleotides until RNA chain termination (delayed chain termination). Additionally, remdesivir may induce lethal mutagenesis, as remdesivir triphosphate mimics ATP. The delayed chain termination mechanism may shield remdesivir from removal from the RNA chain by viral proofreading proteins due to the presence of 3–5 additional natural nucleotides.

1.4.2 Favipiravir

The orally bioavailable prodrug favipiravir, also referred to as T-705, received initial approval in Japan in 2014 for treating influenza virus infections. Favipiravir has gained significant interest due to its antiviral potential as a candidate therapy for both the treatment and prevention of COVID-19. In vitro studies by Wang et al. demonstrate that favipiravir markedly inhibits SARS-CoV-2 with an EC50 of 61.88 μM and moderate selectivity (SI = 6.46). In SARS-CoV-2-infected hamsters, favipiravir exhibits potent dose-dependent effects, leading to notable improvements in lung histology [23].

Favipiravir undergoes a series of enzymatic phosphorylation steps to convert to its active form, favipiravir-ribofuranyl-triphosphate (Favipiravir-RTP). Of note, the binding mechanism of Favipiravir-RTP to the replicating SARS-CoV-2 RNA strand differs from that of remdesivir-RTP. Specifically, it inhibits the RNA-dependent RNA

polymerase (RdRp) of the influenza virus. Acting as a purine analog, it is incorporated in place of guanine & adenine. Post-incorporation, favipiravir-RTP acts as a mutagen, hindering the coronavirus's self-repair capabilities. Given the low cytosine content in the SARS-CoV-2 genome, favipiravir-RTP increases pressure on the CoV nucleotide content. By disrupting the virus's cytopathic effects, reducing viral RNA levels, and preventing the spread of infectious particles, favipiravir-RTP exerts a beneficial impact on SARS-CoV-2.

1.4.3 Molnupiravir

Molnupiravir, also known as EIDD-2801 or MK-4482, is an oral antiviral drug developed by Plemper's group for treating COVID-19. It is a novel prodrug of β-D-N4-hydroxycytidine (NHC, EIDD-1931) and targets the RdRp, making it effective against various viruses including influenza A, HCV, EBOV, Venezuelan equine encephalitis virus, SARS-CoV, and SARS-CoV-2 [24]. Molnupiravir received approval for use in the UK in November 2021 and emergency use authorization from the FDA in December 2021 for treating mild to moderate COVID-19 in high-risk adults with no alternative treatment options. When used with favipiravir (FVP), molnupiravir shows a significant cooperative effect in the SARS-CoV-2 hamster infection model. Molnupiravir quickly converts to its active form, molnupiravir-TP or MTP, through cellular kinases. The MTP acts as a substrate for the viral RdRp and triggers lethal mutagenesis.

1.4.4 Ribavirin

Ribavirin, a nucleoside antiviral medication discovered in the 1970s, exhibits broad-spectrum antiviral activity against DNA and RNA viruses [25]. It is authorized for treating hepatitis C, often in combination with other drugs. Inside cells, ribavirin undergoes phosphorylation to form its monophosphate and triphosphate derivatives.

The antiviral mechanism of ribavirin involves multiple actions, including host-targeted mechanisms such as inhibition of inosine monophosphate dehydrogenase (IMPDH) and modulation of the host immune response. It also demonstrates virus-targeted effects by interacting with RNA capping enzymes, inhibiting viral RNA-dependent RNA polymerases (RdRp), and inducing viral lethal mutagenesis. The inhibition of IMPDH, critical for DNA and RNA synthesis, accounts for ribavirin's efficacy against a broad spectrum of viruses. Its immunomodulatory effect alters host T-cell responses, and its interference with RNA capping triggers the activation of the host immune response against foreign viral RNA. Ribavirin's interaction with RdRp inhibits viral RNA synthesis. Lethal mutagenesis is another mechanism by which ribavirin increases the mutation rate of RNA viruses to non-viable levels, making them incapable of maintaining genetic information.

1.4.5 Sofosbuvir

Sofosbuvir, a nucleotide analog approved for the treatment of hepatitis C virus (HCV) infection, also known as GS-7977 or SOF, was developed by Pharmasset Ltd in 2010 and later acquired by Gilead Sciences. The drug underwent various preclinical and clinical trials to advance its development. Upon entering the host cell (hepatocyte),

sofosbuvir is converted into its active form of nucleoside triphosphate by a cellular enzyme. Its primary mode of action involves inhibiting the NS5B RNA-dependent RNA polymerase of HCV, leading to chain termination and inhibiting the virus's replication through the disruption of the hydrogen bonding network. Despite its efficacy against HCV, sofosbuvir has shown potential as an antiviral candidate against SARS-CoV-2 due to the similarity in their positive-stranded RNA structures. In a study involving hospitalized COVID-19 patients receiving sofosbuvir treatment, no significant reduction in viral load was observed compared to a control group [26]. Therefore, larger clinical trials are necessary to determine the efficacy of this treatment approach.

1.5 Synthesis of Nucleoside Analogs

Focusing specifically on the solvents used in the synthesis of nucleoside analogs like remdesivir, molnupiravir, and favipiravir, we can identify several hazardous and environmentally concerning solvents that demand replacement with greener alternatives.

For the synthesis of remdesivir [Fig 7], solvents such as Tetrahydrofuran (THF) and Dichloromethane (CH_2Cl_2) are employed. THF, widely used in organic synthesis, is highly volatile, flammable, and contributes to air pollution and environmental degradation. Exposure to THF vapors can cause respiratory irritation and adverse health effects [27]. Similarly, CH_2Cl_2, also known as methylene chloride, is classified as a potential carcinogen and can contribute to environmental pollution due to its volatile nature, potentially causing adverse health effects with prolonged exposure.

In the synthesis of molnupiravir [Fig 8], solvents like Isopropyl alcohol (IPA) and Methyl tert-butyl ether (MTBE) are used. IPA is a flammable solvent that can contribute to air pollution and poses fire hazards, with exposure to its vapors causing respiratory irritation and other health effects [28]. MTBE, a volatile organic compound (VOC) and flammable solvent, can also contribute to air pollution and is associated with potential health risks upon exposure.

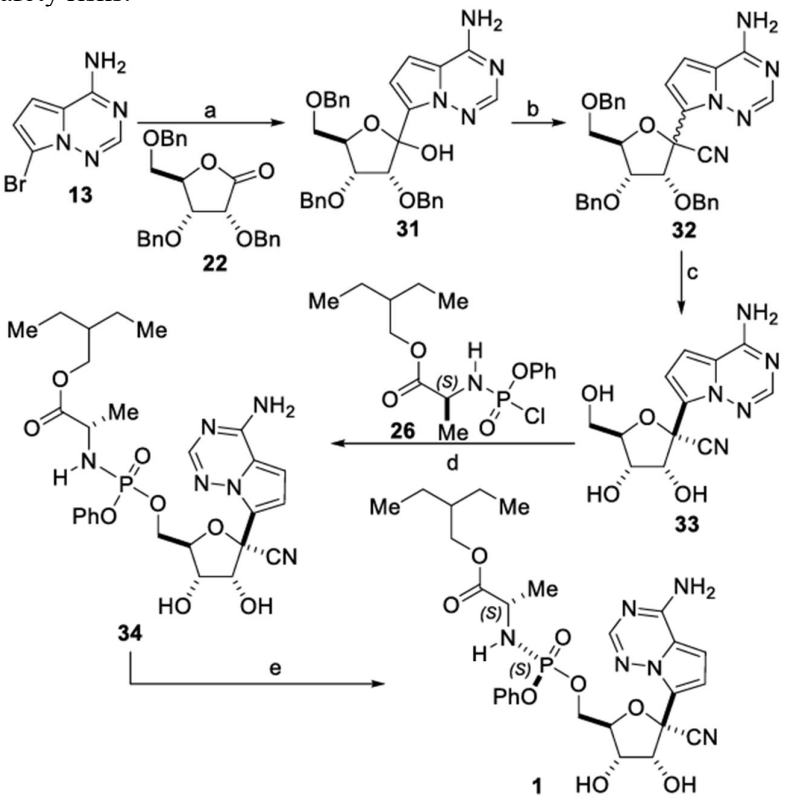
For the synthesis of favipiravir [Fig 9], ethanol is used as a solvent. Ethanol is a volatile organic solvent that can contribute to air pollution and greenhouse gas emissions, in addition to being flammable and posing fire hazards [29].

While these solvents offer favorable properties for organic synthesis, their hazardous nature and potential for environmental pollution highlight the need for greener alternatives. The use of volatile organic solvents like THF, CH_2Cl_2, IPA, MTBE, and ethanol raises concerns about air pollution, greenhouse gas emissions, and potential health risks to workers through inhalation or skin contact. They can contribute to environmental degradation, climate change, and pose safety hazards due to their flammability. Moreover, the generation of solvent waste streams creates challenges in terms of proper disposal and treatment, with improper handling and disposal leading

to environmental pollution, water source contamination, and potential harm to ecosystems.

Addressing these solvent-related concerns in the synthesis of these crucial antiviral compounds is a significant step toward more sustainable and environmentally responsible manufacturing practices within the pharmaceutical industry, aligning with the principles of green chemistry and promoting a safer and healthier environment for workers and the general public.

To mitigate these issues, it is essential to explore greener and more sustainable solvent alternatives. Potential replacements could include bio-based solvents derived from renewable sources, ionic liquids, supercritical fluids, or deep eutectic solvents (DES). These alternative solvents often exhibit favourable properties, such as low volatility, non-flammability, and biodegradability, while minimizing environmental impact and reducing safety risks.



[a]Reagents and conditions: (a) *n*-BuLi, TMSCl, THF, −78 °C (26%) or NaH, *n*-BuLi, $ClSi(Me)_2CH_2CH_2(Me)_2SiCl$, THF, −78 °C (60%); (b) TMSCN, TMSOTf, CH_2Cl_2, −78 °C, 5 h (65%; β:α = 89:11); (c) BCl_3, CH_2Cl_2, −78 °C, 1 h (74%); (d) NMI, $(MeO)_3P{=}O$, THF, 0 °C (21%); (e) chiral HPLC.

Fig 7 Synthesis of Remdesivir [30]

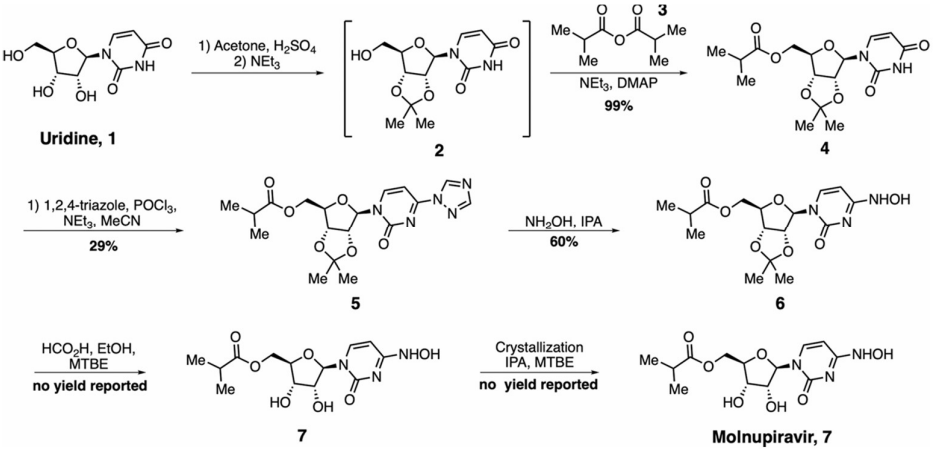


Fig 8 Synthesis of Molnupiravir [31]

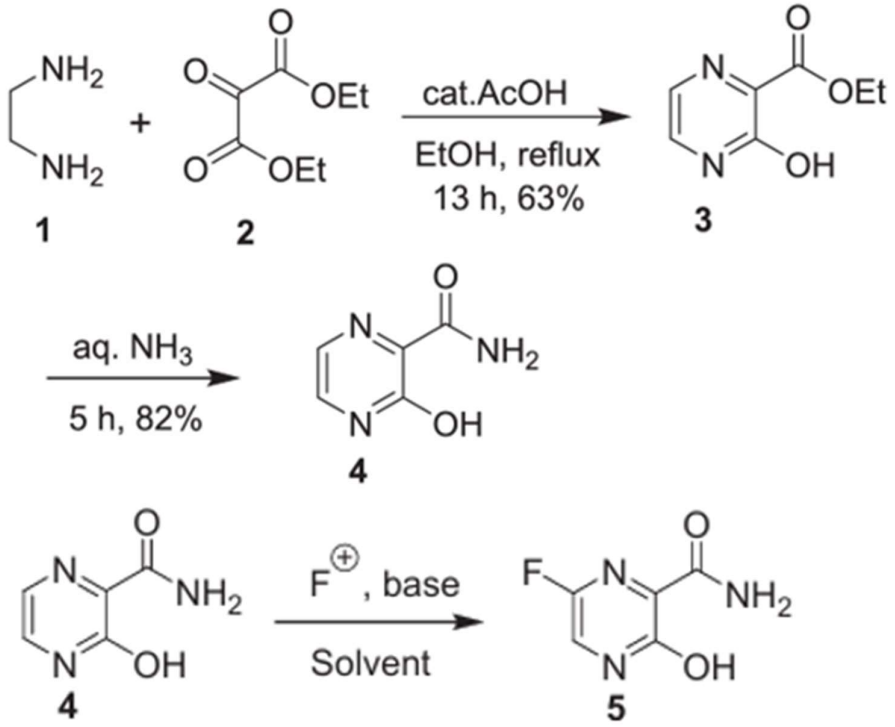


Fig 9 Synthesis of Favipiravir [32]

1.6 Research Gap

The synthesis of nucleoside analogs has gained significant interest due to their wide-ranging applications in pharmaceutical and biomedical fields. However, traditional synthetic routes often involve the use of hazardous organic solvents, raising environmental and safety concerns. Additionally, some nucleoside analogs with

complex structures or specific functional groups are challenging to synthesize efficiently, resulting in low yields and high production costs.

In recent years, deep eutectic solvents (DESs) have emerged as promising alternatives to traditional organic solvents. These unconventional solvents possess unique properties, such as adjustable polarity, low volatility, and good solubilizing ability, making them attractive candidates for various chemical processes. Despite their potential advantages, the application of DESs in nucleoside analog synthesis remains largely unexplored.

This gap in the literature presents an opportunity to explore the feasibility and benefits of using DESs as solvents for nucleoside analog synthesis. By utilizing the unique properties of DESs, such as their tunability, hydrogen-bonding capabilities, and potential for improved reaction kinetics, it may be possible to develop more sustainable and efficient synthetic routes for targeted nucleoside analogs.

Exploring DESs in this context could lead to several advantages, including:

- Improved reaction yields and selectivity, especially for challenging nucleoside analog syntheses.
- Reduced environmental impact and enhanced safety compared to traditional organic solvents.
- Potential cost savings through the use of renewable or waste-derived DES components.
- Opportunities for discovering new reaction pathways or mechanisms facilitated by the unique solvent properties of DESs.

This study aims to connect nucleoside analog synthesis with deep eutectic solvents, potentially creating a more sustainable and effective method for producing important pharmaceutical compounds. Additionally, it could contribute to a better understanding and wider application of DESs in organic synthesis.

1.7 Proposed Objectives:

To address these gaps, the following objectives are proposed in the present work:

1. To explore the potential of deep eutectic solvents as alternative solvents for organic synthesis reactions.
2. To synthesize selected deep eutectic solvents as potential alternative solvents for the synthesis of nucleoside analogs.
3. To optimize the reaction conditions in the synthesis of deep eutectic solvents.
4. To potentially use the synthesized deep eutectic solvents in the synthesis of nucleoside analogs.

1.8 Deep Eutectic Solvents

In the search for sustainable & environmentally friendly alternatives to traditional organic solvents, deep eutectic solvents (DESs) have emerged as a promising class of innovative solvents. These unique solvents, typically composed of binary or ternary

mixtures of compounds that primarily interact through hydrogen bonds, have attracted significant attention in the field of green chemistry because of their distinct properties, versatility, & potential applications across various domains.

When these compounds are combined at a specific molar ratio, they form a eutectic mixture. The term "eutectic" comes from the Ancient Greek εὔτηκτος or eútēktos, which means easily melted [33]. A eutectic point signifies the chemical composition & temperature at which a mixture of two solids becomes fully molten at the lowest melting temperature in comparison to either compound.

The definition of a deep eutectic solvent is a topic of debate. Various reported definitions fail to distinguish them from other mixtures, as all mixtures of immiscible solid compounds have a eutectic point, & many compounds can form hydrogen bonds when combined. To address this, Martins et al. recently defined a deep eutectic solvent as "a mixture of two or more pure compounds for which the eutectic point temperature is below that of an ideal liquid mixture, presenting significant negative deviations from ideality ($\Delta T2>0$)," where $\Delta T2$ represents the temperature depression, the difference between the ideal & real eutectic points [34].

It is important that a decrease in temperature results in a liquid mixture at the required operating temperature, regardless of the mixture's composition. The absence of a fixed composition allows for even more adjustability in these systems. Deep eutectic solvents (DESs) usually form when two or more compounds are mixed, with at least one serving as a hydrogen bond donor (HBD) & the other as a hydrogen bond acceptor (HBA). This interaction, primarily through hydrogen bonding but also involving van der Waals forces, ionic interactions, & entropy contributions, leads to a significant reduction in the lattice energy of the system, causing the observed depression in melting point. The exact nature of these interactions & their contributions to the behavior of DESs are still under research & discussion [35].

DESs offer economic, environmental, & performance benefits, which could revolutionize various industries. One advantage of DESs over conventional solvents is their versatility & adjustability. By selecting the HBD & HBA components & adjusting their molar ratios, it is possible to fine-tune the physicochemical properties of the resulting DES. This adjustability allows for the optimization of DESs for specific applications, making them potential "designer solvents." Tailoring the properties of DESs to meet the requirements of various processes & reactions presents an opportunity for improving efficiency, selectivity, & sustainability in various industries [36].

1.8.1 Comparison with Ionic liquids:

DESs possess several desirable properties similar to ionic liquids (ILs), including negligible vapor pressure, high thermal stability, non-flammability, & a wide liquid range. However, DESs have additional benefits such as ease of preparation, low cost, & generally lower toxicity. In contrast to ILs, which are often synthesized through complex & energy-intensive processes, DESs can be easily prepared by mixing the HBD & HBA components without the need for further purification steps. This

straightforward preparation, combined with the affordability of the starting materials, makes DESs an appealing option for large-scale industrial applications [37].

The constituents of DESs are often obtained from renewable sources, such as choline chloride, urea, glycerol, lactic acid, carbohydrates, polyalcohols, amino acids, & vitamins. This not only contributes to their low cost & biodegradability but also reduces their environmental impact & toxicity, making DESs an attractive alternative to traditional organic solvents & ionic liquids. The use of renewable & naturally occurring components aligns with the principles of green chemistry & sustainable development, further enhancing the attractiveness of DESs as environmental friendly solvents.

The unique properties & potential applications of DESs have led to extensive research efforts aimed at understanding their practical applications. Various experimental techniques, such as nuclear magnetic resonance (NMR) spectroscopy, X-ray diffraction, infrared (IR) spectroscopy, & differential scanning calorimetry (DSC), have been employed to characterize the structure & dynamics of DESs. Additionally, computational methods, including molecular dynamics (MD) simulations & quantum chemical calculations, have proven to be powerful tools for gaining insights into the microscopic interactions & dynamics of these solvents.

1.8.2 Classification of Deep Eutectic Solvents:

The understanding & systematic study of DESs have been facilitated by several classification systems based on the nature of their constituents & the interactions involved in their formation. The most widely adopted classification system was introduced by Abbott et al., which categorizes DESs into four main types based on the general formula Cat^+X^-zY, where Cat^+ represents a cation (typically an ammonium, phosphonium, or sulfonium cation), X^- is a Lewis base (usually a halide anion), Y is a Lewis or Brønsted acid, & z is the number of Y molecules interacting with the corresponding anion [Fig. 10].

Type I DESs: These DESs are formed by mixing a quaternary ammonium or phosphonium salt with a metal chloride, such as zinc chloride or tin chloride. They are similar to the well-studied metal halide/imidazolium salt systems & are often considered as a subclass of ionic liquids. Examples of Type I eutectics include the chloroaluminate/imidazolium salt melts & ionic liquids formed with imidazolium salts & various metal halides, including iron (II) chloride, silver chloride, copper(I) chloride, lithium chloride, cadmium chloride, copper (II) chloride, tin (II) chloride, zinc chloride, lanthanum chloride, yttrium chloride, & tin (IV) chloride.

Type II DESs: This type of DESs involves the use of hydrated metal halides instead of non-hydrated metal halides as in Type I. The hydrated metal halides are combined with quaternary ammonium salts, such as choline chloride, to form the eutectic mixture. The cost-effectiveness of many hydrated metal salts, along with their natural resistance to air & moisture, makes them suitable for large-scale industrial processes.

Type III DESs: This class of deep eutectic solvents (DESs) has been extensively researched & widely investigated. These solvents are formed by combining a quaternary ammonium or phosphonium salt with a hydrogen bond donor (HBD) such as amides, carboxylic acids, alcohols, or carbohydrates. The most common example is the mixture of choline chloride & urea, often known as "reline." Other widely studied HBDs include glycerol, ethylene glycol, malonic acid, succinic acid, & carbohydrates like fructose & glucose. The wide variety of available HBDs makes this class of DESs highly adaptable, allowing for easy customization of physical properties for specific applications.

Type IV DESs: In this category, transition metal halides, such as zinc chloride or aluminium chloride, are combined with HBDs like urea, ethylene glycol, or acetamide to form the eutectic mixture. These DESs have been studied for applications involving the processing of metal oxides & the synthesis of various materials.

In addition to these four types, a new class of DESs, referred to as **Type V**, has been introduced by Coutinho & coworkers. These DESs consist solely of non-ionic, molecular HBAs & HBDs, such as the mixture of thymol & menthol. The depression of the melting point is caused by strong hydrogen bonding interactions between the non-ionic components. This type of DES broadens the range of potential components & offers more opportunities to adjust the properties of these solvents by combining different non-ionic hydrogen bond donors & acceptors.

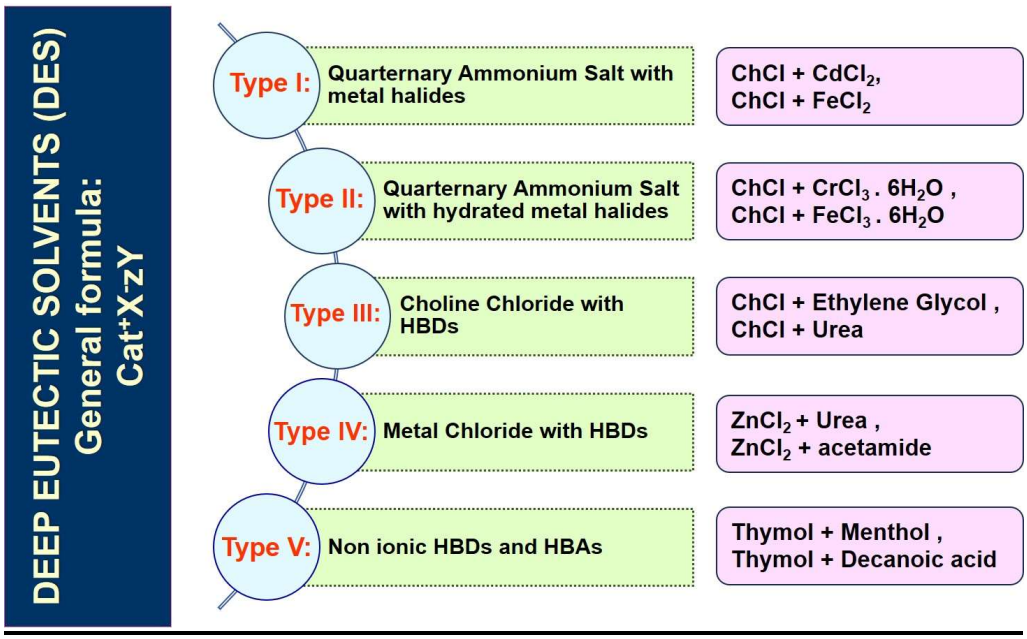


Fig 10 Classification of Deep Eutectic Solvents

The concept of natural deep eutectic solvents (NADESs) has emerged, involving the use of naturally occurring compounds such as sugars, sugar alcohols, amino acids, &

organic acids as hydrogen bond donors (HBDs) & hydrogen bond acceptors (HBAs). NADESs can be classified into five main groups [38]:

1. *Ionic liquids:* Made of an acid & a base, such as choline chloride & lactic acid.

2. *Neutral:* Made of only sugars or sugars & other polyalcohols, such as glucose & glycerol.

3. *Neutral with acids:* Made of sugar/polyalcohols & organic acids, such as glucose & oxalic acid.

4. *Neutral with bases:* Made of sugar/polyalcohols & organic bases, such as glucose & choline chloride.

5. *Amino acids-containing NADESs:* Made of amino acids & sugars/organic acids, such as proline & lactic acid.

Recently, cyclodextrins, non-toxic cyclic oligosaccharides, are being used as HBAs to form liquid supramolecular mixtures at room temperature, expanding the scope of DESs.

The reported DESs may not necessarily fit into one of these categories, given the versatility & the wide range of potential starting compounds. As the field continues to evolve, new classification systems may emerge to accommodate the diverse range of DESs being explored.

In addition to the classification based on constituents, DESs can also be categorized based on their hydrophobicity. While the majority of DESs are hydrophilic, a new class of hydrophobic DESs has been introduced, which are based on the use of hydrophobic compounds such as tetrabutylammonium bromide, menthol, thymol, & fatty acids as HBAs, along with long alkyl chain alcohols & carboxylic acids as HBDs. These hydrophobic DESs exhibit unique properties & can be used in extraction processes & as alternatives to organic solvents in various chemical reactions.

Furthermore, deep eutectic solvents can be synthesized using active pharmaceutical ingredients (APIs) like ibuprofen, lidocaine, & phenylacetic acid. These solvents are termed therapeutic deep eutectic solvents (TDESs) & exhibit potential applications in pharmaceutical formulations & drug delivery systems.

The diverse range of DESs & their classification systems are indicators of the complexity & versatility of these solvents. As ongoing research in this field continues to progress, it is expected that novel classes & subcategories of DESs will emerge, broadening the scope of potential applications & facilitating the systematic design of these innovative solvents for specific purposes.

1.8.3 Applications of Deep Eutectic Solvents

The exceptional properties of deep eutectic solvents have led to their use in a wide variety of areas, transforming numerous industries and processes [Fig. 11] [39].

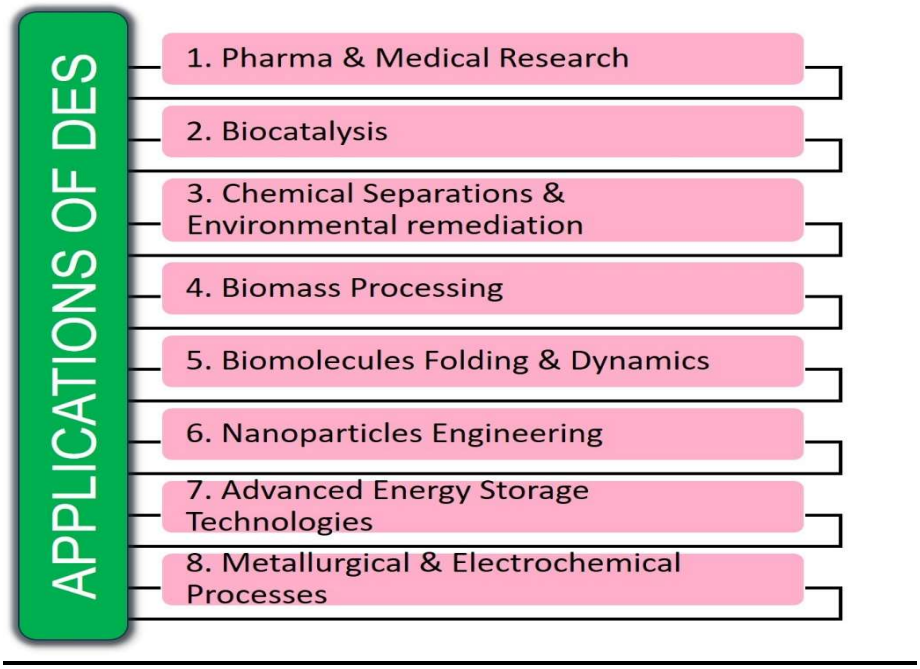


Fig 11 Application of Deep Eutectic Solvents

i. **Pharma & Medical Research:** Deep eutectic solvents (DESs) are being recognized for their potential in pharmaceutical & medical research as they can significantly enhance the solubility of poorly soluble drugs. Research has shown that DESs can increase solubilities up to 22,000 times higher than water for certain drug compounds. This improved solubility has the potential to lead to the development of more effective drug delivery systems. Furthermore, DESs have been studied as co-solvents to improve drug solubility & as carriers for time-release antibacterial dental composites, demonstrating their versatility in biomedical applications.

ii. **Biocatalysis:** DESs offer several attractive properties for biocatalytic reactions involving enzymes. They have the ability to accommodate a wide variety of substrates, enzymes, & other bioactive solutes. Additionally, many DESs are composed of naturally occurring compounds like choline chloride, sugars, amino acids, & alcohols, making them environmentally friendly & potentially biocompatible. Researchers have studied the use of DESs as reaction media, co-solvents, or suspensions in enzymatic synthesis, particularly in the production of biofuels & other biochemicals. Studies have shown that enzymes can maintain high activity & stability in DES systems, which makes them promising alternatives for traditional organic solvents.

iii. **Chemical Separations & Environmental remediation:** DESs have shown promising applications in various chemical separation processes & environmental remediation. Their tunable properties, low volatility, & environmentally friendly nature make them attractive alternatives to conventional volatile organic solvents. DESs have been investigated for liquid-liquid extraction of azeotropic mixtures, CO2 capture & sequestration, & desulfurization of fuels. Their capacity to selectively dissolve compounds &

extract specific components from mixtures has resulted in their utilization in these separation processes. Additionally, DESs have shown potential for the extraction & recovery of valuable compounds from dilute aqueous solutions, showing their versatility in environmental remediation applications.

iv. **Biomass Processing:** The utilization of DESs in biomass processing has attracted significant attention due to their ability to dissolve, extract, & facilitate the production of value-added products from lignocellulosic biomass. DESs have been used as pretreatment solvents to break down & extract lignin & other biopolymers from plant-based materials. They have also been used for extraction of natural chemicals, such as flavonoids & saponins, from plants. Additionally, DESs have demonstrated the ability to convert carbon dioxide into calcite nanoparticles, providing new opportunities for carbon capture & utilization. The eco-friendly characteristics & tunability of DESs make them promising alternatives to conventional harsh solvents used in biomass processing.

v. **Biomolecules Folding & Dynamics:** DESs have become a valuable tool for investigating the characteristics of biomolecules such as proteins, enzymes, and nucleic acids. Researchers have explored how DESs impact the conformation, activity, and thermal stability of these biomolecules, providing valuable insights into their behaviour in these unique solvent systems. Various techniques, including circular dichroism, fluorescence spectroscopy, differential scanning calorimetry, and computational methods such as molecular dynamics simulations, have been utilized to examine biomolecular interactions and dynamics in DESs. These studies have shown that DESs have the potential to either stabilize or destabilize biomolecular structures, influence folding pathways, and modify the kinetics of unfolding processes, depending on the specific composition and conditions of the DES.

vi. **Nanoparticles Engineering:** DESs have various applications in nanotechnology, particularly in synthesizing and dispersing nanoparticles, nanocomposites, and nanomaterials. Their ability to dissolve metals and metalloids, along with their adjustable properties, makes DESs a promising medium for nanomanufacturing processes. Researchers have employed DESs for electrochemically depositing nanostructured metal films, self-assembling nanoparticles, and dispersing carbon nanotubes, graphene, and other nanomaterials. The unique characteristics of DESs, such as high ionic conductivity and low toxicity, have facilitated the development of innovative nanomaterials with enhanced properties and reduced environmental impact.

vii. **Advanced Energy Storage Technologies:** DESs have generated significant interest for use in advanced energy storage technologies, specifically as electrolytes for lithium-ion batteries and redox flow batteries. Their reduced flammability, wide temperature range, and high ionic conductivity make them promising alternatives to traditional electrolytes. Research indicates that DESs can achieve broad electrochemical stability and favorable characteristics for lithium-ion battery applications. Furthermore, DESs have been investigated as electrolytes for redox flow batteries, which are utilized in large-scale energy storage systems. The non-toxic and environmentally friendly properties of

DESs, combined with their adjustable features, present opportunities for the development of safer and more sustainable energy storage technologies.

viii. **Metallurgical & Electrochemical Processes:** DESs have found significant applications in the fields of metallurgy and electrochemical processes since their early days. They have shown impressive solubility for metals and metal salts, as well as high electrical conductivities, making them suitable solvents for a wide range of metallurgical applications. These applications include metal extraction and recycling, ore refining, electroplating, and electrodeposition processes. The efficient and precise ability of DESs to dissolve and deposit metals has led to their investigation in processes such as metal dissolution, deposition, and processing. Furthermore, DESs have been studied for use in electrochemical applications like electropolishing and electrochemical machining, indicating their potential in the metallurgical and electrochemical industries.

CHAPTER 2

EXPERIMENTAL WORK: GREEN SYNTHESIS OF NUCLEOSIDE ANALOGS USING DEEP EUTECTIC SOLVENTS (DES)

2.1 Materials and Methods

D-Glucose (LR grade), Tartaric acid (LR grade) & citric acid (LR grade)

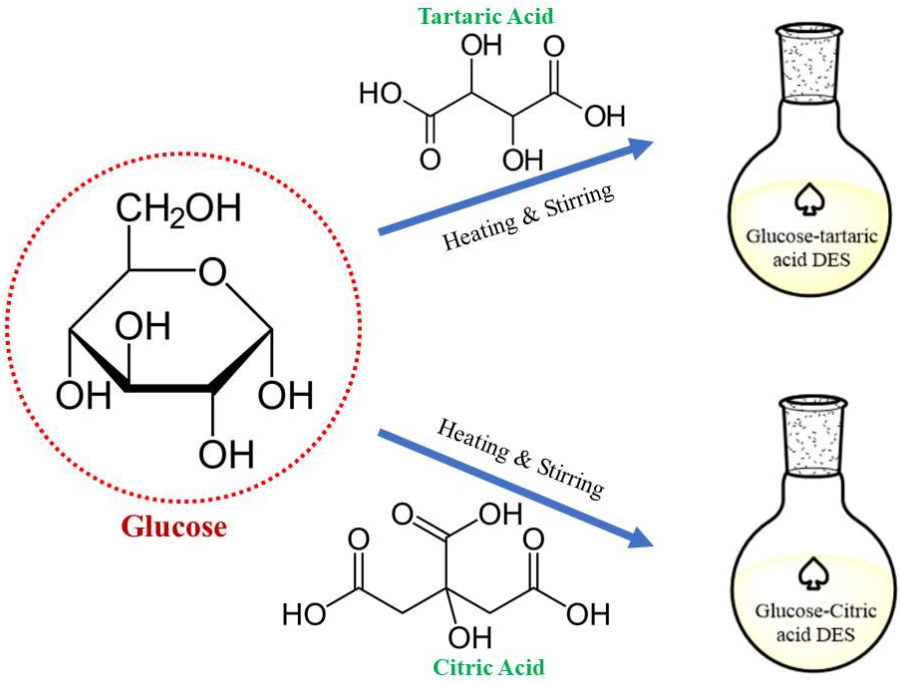


Fig 12 Synthesis of Deep Eutectic Solvents

2.1.1 Synthesis of Glucose-Tartaric Acid Deep Eutectic Solvent (DES)

The glucose-tartaric acid DES was synthesized by carefully weighing out D-(+)-glucose and L-(+)-tartaric acid according to molar ratios of 1:1, 1:2, and 2:1. The weighed solid components were quantitatively transferred into separate clean, dry round-bottom flasks.

An appropriate amount of distilled water was added to each flask, and a magnetic stir bar was introduced. Each round-bottom flask containing the solid mixture was securely

positioned on a heating plate equipped with a temperature controller and a magnetic stirrer set to 300 rpm.

For the 1:1 molar ratio, two separate syntheses were carried out - one with the solid mixture heated and stirred at 60°C for 40 minutes, and the other at 80°C for 40 minutes.

For the 1:2 molar ratio, two syntheses were performed - one with heating and stirring at 80°C for 60 minutes, and the other at 90°C for 60 minutes.

The 2:1 molar ratio mixture was heated and stirred at three different temperatures: 60°C, 80°C, and 120°C, each for 60 minutes.

After each heating cycle, the molten mixture was allowed to cool undisturbed to room temperature, forming a homogeneous liquid DES. The resulting cooled liquids were carefully evaluated for yield [Fig. 12] [40].

2.1.2 Synthesis of Glucose-Citric Acid Deep Eutectic Solvent (DES)

The synthesis of the glucose-citric acid DES was carried out using molar ratios of 1:1, 1:2, and 2:1. Precisely weighed amounts of D-(+)-glucose and citric acid were quantitatively transferred into separate clean, dry round-bottom flasks. An appropriate amount of distilled water was added to each flask, and a magnetic stir bar was introduced. The flasks were then immersed in a water bath, and the mixtures were continuously stirred at 300 rpm using a magnetic stirrer.

For the 1:1 molar ratio, three separate syntheses were carried out - one with heating and stirring at 60°C for 40 minutes, another at 80°C for 60 minutes, and the third at 90°C for 60 minutes.

For the 1:2 molar ratio, the mixture was heated and stirred at 60°C for 60 minutes, and another synthesis was performed at 80°C for 60 minutes.

The 2:1 molar ratio mixture was3 heated and stirred at two different temperatures: 60°C and 80°C, each for 60 minutes.

The heating and stirring process continued until clear, homogeneous, and viscous liquids were obtained, indicating the successful formation of the glucose-citric acid DESs. The formed DESs were carefully evaluated for yield [Fig. 12] [40].

2.2 Optimization of DES

The synthesis conditions, including the molar ratio of glucose to tartaric acid, reaction temperature, and time, were optimized. The investigated parameters are summarized in Table 1.

Table 1 Optimization studies for Glucose-Tartartic Acid DES

S. No.	Glucose-Tartaric Acid	Reaction time (min)	Magnetic rotation speed (rpm)	Temperature (°C)	Yield (%)
1.	1:1	40	300	60	72
2.	1:1	40	300	80	77
3.	1:2	60	300	60	80
4.	1:2	60	300	80	88
5.	1:2	60	300	90	85
6.	2:1	60	300	60	74
7.	2:1	60	300	80	78

The optimization of molar ratio, reaction temperature, and time was carried out for the synthesis of the glucose-citric acid DES system, as shown in Table 2.

Table 2 Optimization studies for Glucose-Citric Acid DES

S. No.	Glucose-Citric acid	Reaction time (min)	Magnetic rotation speed (rpm)	Temperature (°C)	Yield (%)
1.	1:1	40	300	60	87
2.	1:1	60	300	80	92
3.	1:1	60	300	90	89
4.	1:2	60	300	60	86
5.	1:2	60	300	80	88
6.	2:1	60	300	60	82
7.	2:1	60	300	80	85

The optimal synthesis conditions for the glucose-tartaric acid & glucose-citric acid DES systems were determined based on the observations from these optimization experiments.

CHAPTER 3

RESULTS & DISCUSSION

The optimization table provides valuable insights into the synthesis conditions for two distinct deep eutectic solvents (DESs): glucose-tartaric acid DES and glucose-citric acid DES. The data presented in the table helps in the identification of the optimum conditions for achieving the highest yield of each DES.

3.1 Glucose-Tartaric Acid Deep Eutectic Solvent

According to the data collected, the glucose-tartaric acid deep eutectic solvent (DES) achieved its highest yield (88%) when synthesized at a molar ratio of 1:2 (glucose:tartaric acid), a reaction time of 60 minutes, & a temperature of 80°C (Entry 4). These specific reaction conditions seem to be the most optimal for producing the glucose-tartaric acid DES with the maximum yield.

The observation of a slight decrease in yield to 85% at a temperature of 90°C (Entry 5) suggests that elevated temperatures may have an adverse impact on the formation or stability of the deep eutectic solvent (DES). Furthermore, at temperatures of 60°C and 80°C, molar ratios of 2:1 (glucose:tartaric acid) resulted in reduced yields of 74% and 78%, respectively (Entries 6 and 7). This implies that a higher proportion of tartaric acid relative to glucose is advantageous for optimizing the yield of this specific DES.

3.2 Glucose-Citric Acid Deep Eutectic Solvent

Upon analysis, it was determined that the most favorable parameters for the synthesis of the glucose-citric acid deep eutectic solvent (DES) were a molar ratio of 1:1 for glucose to citric acid, a reaction duration of 60 minutes, & a temperature of 80°C. Under these conditions, the highest yield of 92% was achieved, indicating the optimal synthesis of the glucose-citric acid DES.

At higher temperatures, such as 90°C (Entry 3), the yield of the system remained relatively high at 89%, indicating potential thermal stability compared to the glucose-tartaric acid deep eutectic system (DES). However, deviations from the 1:1 molar ratio led to decreased yields. For instance, a 1:2 molar ratio at 60°C and 80°C resulted in yields of 86% and 88% (Entries 4 and 5), while a 2:1 molar ratio at the same temperatures led to yields of 82% and 85% (Entries 6 and 7)

So, it can be said that the most favourable parameters for the production of glucose-tartaric acid deep eutectic solvent (DES) were determined to be a 1:2 molar ratio of glucose to tartaric acid, a 60-minute reaction time, a magnetic rotation speed of 300 rpm, and a temperature of 80°C, resulting in an 88% yield of the target product. For the synthesis of glucose-citric acid DES, the optimal conditions involved a 1:1 molar ratio of glucose to citric acid, a 60-minute reaction time, & a temperature of 80°C, yielding a 92% product yield.

The results offer significant understanding into the synthesis conditions that can be utilized to optimize the production of these two deep eutectic solvents (DESs), which hold promise for diverse applications including as solvents, catalysts, or additives in industrial processes.

CHAPTER 4

FUTURE PROSPECTS & CONCLUSION

4.1 FUTURE PROSPECTS

Nucleoside analogs have become a crucial category of antiviral medications, with substances such as remdesivir playing a significant role in the global fight against the COVID-19 pandemic. Nevertheless, the production of these complex compounds often depends on conventional organic solvents, which can be harmful to health, dangerous, & environmentally disturbing. Optimization investigations carried out with glucose/tartaric acid & glucose/citric acid deep eutectic solvents (DESs) have revealed a promising substitute, indicating a potential shift towards more sustainable and environmentally friendly production of nucleoside analogs.

In the field of nucleoside analog synthesis, using traditional organic solvents presents major challenges. Solvents such as dichloromethane, tetrahydrofuran, and N,N-dimethylformamide are frequently used, but they are derived from non-renewable sources, are highly toxic, and contribute to environmental pollution. Moreover, these solvents often require thorough purification and disposal procedures, further increasing the overall environmental impact and operational expenses.

Deep eutectic solvents, on the other hand, represent a significant advancement towards greener chemistry. These solvents are composed of readily available, bioderived components like sugars and organic acids. They are inherently biodegradable, non-toxic, & can be synthesized using simple, energy-efficient processes.

Future efforts should focus on utilizing the unique properties of these DESs to develop more sustainable & efficient synthetic routes for a broad spectrum of nucleoside analogs. By expanding the range of substrates beyond the initial model reactions, researchers can unlock the true potential of these DES systems for a wide range of antiviral drug candidates targeting not only COVID-19 but also other viral threats like HIV, hepatitis, and influenza.

The systematic exploration will be important in comprehending how these bio-derived deep eutectic solvents (DESs) enable effective synthesis of nucleoside analogs. Utilizing computational simulations and empirical methodologies may explain the complex hydrogen-bonding networks & solvation environments within the DES environment, offering detailed molecular-level insights into reaction pathways and transition states. This understanding could guide the strategic development of next-

generation DESs for specific nucleoside analog targets, ultimately improving reaction kinetics, selectivity, and yields.

Moreover, the unique properties of these DESs, such as their high thermal stability and tunable polarity, present opportunities for process intensification strategies. Combining with advanced technologies like continuous-flow reactors, microwave-assisted synthesis, or advanced manufacturing platforms could lead to more efficient and scalable production of nucleoside analogs. By taking the advantage of these intensified processes, pharmaceutical companies may be able to reduce manufacturing costs, minimize waste generation, and achieve higher output. This could ultimately result in more affordable and accessible antiviral therapies.

Addressing the challenges of waste minimization and solvent recycling will be cruicial in realizing the full environmental and economic benefits of DES-based nucleoside analog synthesis. Collaborative efforts should focus on developing effective strategies for recovering and reusing these bioderived solvents after synthesis, potentially through membrane separations, advanced distillation techniques, or other innovative solvent recovery methods. By implementing closed-loop systems, the environmental footprint of nucleoside analog production could be significantly reduced, & thereby aligning with the principles of green chemistry and circular economy.

While the optimization studies focused on glucose/tartaric acid and glucose/citric acid DESs, the large chemical space of deep eutectic solvents remains largely unexplored for this application. Systematic screening and evaluation of other bioderived DES compositions, which incorporate different sugar, acid, and salt components, could help uncover superior or complementary solvent systems for nucleoside analog synthesis. This expanded DES toolkit has the potential to offer tailored solutions for specific nucleoside targets, effectively addressing challenges related to solubility, reactivity, and selectivity.

Moreover, the concepts illustrated in these DES (Deep Eutectic Solvent) systems may have the potential to be expanded beyond nucleoside analogs to other categories of medications and high-quality chemicals. The distinctive solvation characteristics and adjustable properties of deep eutectic solvents offer possibilities for environmentally friendly synthesis across a diverse set of molecular objectives, thereby contributing to the objectives of eco-friendly chemistry and environmental sustainability within the chemical sector.

4.2 CONCLUSION

The increasing environmental concerns and the necessity for cost-effective chemical processes have led to a significant focus on developing sustainable alternatives to traditional organic solvents. This research concentrated on the synthesis and optimization of two deep eutectic solvent (DES) systems, namely glucose-tartaric acid & glucose-citric acid, with potential applications in the synthesis of nucleoside

analogs, a class of compounds known for their potent antiviral properties against emerging viral pathogens such as coronaviruses.

A comprehensive literature review highlighted the crucial role of nucleoside analogs in inhibiting viral replication processes. Compounds like remdesivir, favipiravir, ribavirin, sofosbuvir & molnupiravir have shown promising antiviral activities by using their structural similarities to natural nucleosides and disrupting vital stages of the viral life cycle. However, the conventional synthesis of these compounds often involves the use of hazardous organic solvents, necessitating the exploration of more sustainable synthetic methodologies.

The synthesis of the glucose-tartaric acid and glucose-citric acid DESs was achieved through the optimization of various parameters, including molar ratios, reaction temperatures, and times. Extensive optimization studies were conducted to identify the ideal combination of these factors, ensuring the formation of homogeneous, clear, and viscous DES formulations with high yields.

While the actual synthesis of nucleoside analogs using the prepared DESs was not conducted in this study, the identification of a research gap and the proposal of future research prospects were key components. The lack of exploration of DESs as alternative solvents in nucleoside analog synthesis was emphasized, presenting an opportunity to investigate the feasibility and potential benefits of these innovative solvents in this domain.

The utilization of DESs in the synthesis of nucleoside analogs could potentially offer several advantages, including improved reaction yields and selectivity, reduced environmental impact, enhanced safety, and potential cost savings. By exploiting the unique properties of DESs, such as their tunability, hydrogen-bonding capabilities, & potential for improved reaction kinetics, it may be possible to develop more sustainable and efficient synthetic routes for targeted nucleoside analogs.

Furthermore, exploring DESs in this context could lead to the discovery of new reaction pathways or mechanisms facilitated by the unique solvent properties of these systems, potentially unlocking novel synthetic routes or enabling the synthesis of previously inaccessible nucleoside analogs. This could contribute to the broader understanding and application of DESs in organic synthesis and pharmaceutical chemistry, paving the way for their adoption in other chemical research and industry areas.

While this dissertation has laid the groundwork for future investigations, several challenges and limitations must be addressed. Thorough optimization of the DES systems for specific nucleoside analog syntheses, comprehensive evaluation of reaction kinetics and mechanisms, and rigorous economic viability and scalability assessment will be crucial. Additionally, the toxicity profiles & environmental impact

of the DESs should be thoroughly evaluated to ensure their true sustainability and safety.

In conclusion, this study has underscored the potential of deep eutectic solvents as promising alternatives to conventional organic solvents in the synthesis of nucleoside analogs. By bridging these two research areas, this work has opened avenues for further exploration and collaboration, contributing to the development of sustainable and efficient synthetic methodologies for these vital pharmaceutical compounds. The findings and future prospects outlined in this work facilitate continued research efforts, encouraging innovation, and addressing the global need for effective and environmentally responsible chemical processes.

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MEDICINAL CHEMISTRY RESEARCH

REVIEW ARTICLE

Exploring nucleoside analogs: key targets in the viral life cycle - advancing strategies against SARS-CoV-2

Roopal Garg[1] · Raveen Kumar[1] · Ritika Srivastava[2] · Richa Srivastava[1]

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Abstract

The COVID-19 pandemic has been a major reason behind the increased research aimed at the identification of effective antiviral agents. Among these, Nucleoside analogs have shown a promising effect on the inhibition of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus replication, the pathogen of COVID-19. Nucleoside analogs are synthetic compounds designed to mimic natural nucleosides, the building blocks of RNA and DNA. This review provides a comprehensive examination of the pivotal role nucleoside analogs play in combating SARS-CoV-2 infections. These analogs function by incorporating into the viral RNA during replication, disrupting the synthesis process and preventing the virus from proliferating. Researchers have identified multiple nucleoside analogs exhibiting robust antiviral efficacy against SARS-CoV-2, including remdesivir, favipiravir, and molnupiravir. This review explores the mechanisms of action, pharmacokinetics, and safety profiles of these nucleoside analogs. Furthermore, it discusses the challenges and limitations associated with their use, including the emergence of resistant viral strains and potential side effects. Additionally, the review delves into ongoing research efforts to optimize nucleoside analogs for enhanced efficacy and reduced adverse effects. In summary, the article aims to enhance our overall understanding of nucleoside-based treatments by combining information about their chemistry, mechanisms of action, and activation pathways. The goal is to contribute to advancements in addressing emerging viral threats in the future.

Keywords Nucleosides · Analogs · SARS-CoV-2 · Viral replication · Multi-Drug Resistant strains

Introduction

During the final days of December 2019, a breakout of a respiratory disease originating from an unknown source took place in Wuhan, situated in Hubei, China [1, 2]. The prevalent symptoms observed in the patients included fever, cough, shortness of breath, and muscular fatigue [3, 4]. In a matter of days after the COVID outbreak, several independent laboratories successfully identified the responsible agent behind this pneumonia as a newfound coronavirus, SARS-CoV-2 [5]. SARS-CoV-2 has an incubation period ranging from 2 to 14 days and it primarily spreads through the respiratory tract via respiratory droplets released during coughing and sneezing by people infected with this disease [6, 7].

On 11 March 2020, more than 118 thousand cases and 4291 deaths were reported from 114 countries, which made WHO declare COVID-19 a pandemic [8, 9]. As of August 2023, over 769 million confirmed cases including over 6.9 million deaths have been reported to WHO [10]. Along with these deaths, the world has faced the social and economic effects of the pandemic as well. It significantly disrupted normalcy worldwide as the governments had to lock down the cities/countries [7, 11, 12].

Coronaviruses (CoVs), first discovered in 1960 [13, 14] form a varied family of enveloped viruses with positive-sense single-stranded RNA genome [15] and helically symmetrical nucleocapsid, can be classified into four genera: Alpha coronavirus, Beta coronavirus, Delta coronavirus, and Gamma coronavirus. The first two genera mainly infect mammals, Gamma coronaviruses mainly

These authors contributed equally: Roopal Garg, Raveen Kumar

✉ Richa Srivastava
richasrivastava@dtu.ac.in

[1] Department of Applied Chemistry, Delhi Technological University, Delhi 110042, India

[2] Department of Chemical Sciences, Indian Institute of Science Education and Research Berhampur, Berhampur, Odisha 760010, India

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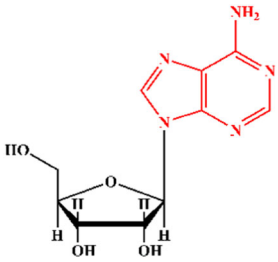
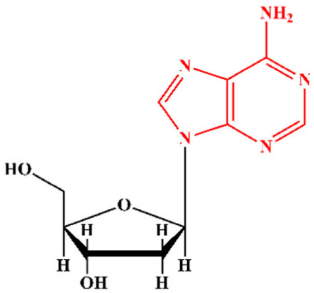
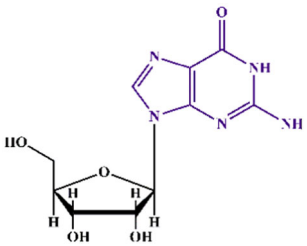
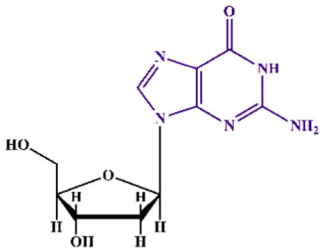
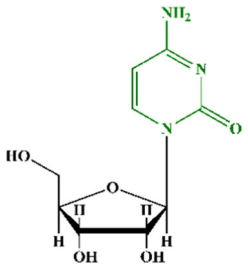
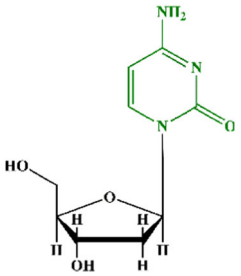
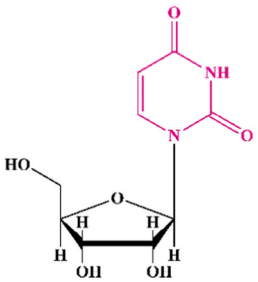
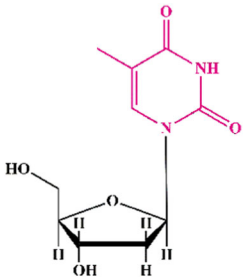
Table 1 Natural nucleosides found in the polynucleotides RNA and DNA (known as Ribonucleosides and Deoxyribonucleosides respectively) have been given

N-Base	Ribonucleoside	Structure	N-Base	Deoxyribonucleoside	Structure
Adenine	Adenosine		Adenine	Deoxyadenosine	
Guanine	Guanosine		Guanine	Deoxyguanosine	
Cytosine	Cytidine		Cytosine	Deoxycytidine	
Uracil	Uridine		Thymine	Deoxythymidine	

target birds, and Delta coronaviruses are distinctive in their capacity to infect both birds and mammals [16, 17].

Amidst the challenges posed by the pandemic, the quest for effective treatments against SARS-CoV-2 has intensified, prompting a surge in research focusing on nucleoside analogs which emerged as a ray of hope. These compounds mimic the structure of nucleosides essential for viral replication, making them promising candidates for antiviral therapies. These compounds, known for their broad-spectrum applicability against various viral strains and their ability to overcome resistance, have become a focal point in the search for therapeutic solutions against COVID-19 [18, 19]. The present article provides a comprehensive overview of the mechanisms by which nucleoside analogs target key stages of viral replication, including viral entry, genome replication, and protein synthesis. It delves into the structural and functional properties of these analogs, elucidating how they interfere with viral processes and inhibit viral propagation (Table 1).

Chemistry of nucleosides

The term "nucleoside(s)," introduced by Levene and Jacobs in 1909, originally pertains to nucleic acids. They were initially isolated by breaking down nucleic acids and are the building blocks of nucleic acids DNA & RNA. Natural nucleosides are composed of either a purine base (like adenine and guanine) or a pyrimidine base (such as cytosine, uracil, and thymine) coupled with a pentose sugar residue (either β -D-ribofuranose or β-D-deoxyribofuranose) [20].

Nucleoside analogs

Nucleoside analogs are synthetic compounds that mimic the structure of natural nucleosides. They are commonly used in medical applications, particularly as antibacterial [21–23], anticancer [24–26] and antiviral agents [27–29]. Nucleoside analogs interfere with normal cellular processes such as DNA replication and transcription by being incorporated into growing DNA or RNA chains. This interference inhibits the growth of cancer cells or the replication of viruses making them valuable in pharmaceutical treatments. Presently, over 30 nucleoside analogs have been approved for public use [30].

These analogs can be modified versions of natural nucleosides. The process of synthesis of nucleoside analogs involves changing the structure of natural nucleosides through three distinct methods [31]:

Heterocyclic base modification

The first method involves the modification of nucleobases by adding various substituents or substituting the base with a different nitrogen-containing heterocycle. By manipulating the nucleobase, the analogs gain unique structural features that confer specific advantages. For example Molnupiravir's mutagenic activity can be attributed to the substitution of the nucleobase with a modified isoxazole ring. This mutagenic activity results in its broad-spectrum antiviral effects [32]. Ribavirin, on the other hand, inhibits viral RNA synthesis by adding an amide group to the guanine base, which enhances its ability to do so. This mechanism contributes to its effectiveness against various RNA viruses [33]. Finally, Favipiravir's inhibitory effects on the viral RNA-dependent RNA polymerase are due to the pyrazine ring replacement of the purine base. This inhibition of the viral polymerase is key to its broad-spectrum antiviral activity [34].

Sugar modification

The second method is the modification of the D-ribofuranosyl component of nucleosides. This is achieved by adding different substituents, removing hydroxy groups at the C-2' and C-3' positions, eliminating hydrogen atoms at these positions, introducing oxygen and/or sulphur atoms, or replacing the oxygen atom with sulphur. Remdesivir, a nucleotide prodrug with a cyclopentane sugar mimic, is a potent antiviral drug against SARS-CoV-2 [35]. The substitution of the D-ribofuranosyl moiety with a cyclopentane ring contributes to its potent antiviral activity. Abacavir lacks the 3'-hydroxyl group present in the natural nucleoside, which enhances its potency against HIV reverse transcriptase [36]. The replacement of the D-ribofuranosyl moiety with an acyclic linker confers selectivity for viral over cellular enzymes, making Acyclovir a selective inhibitor of viral DNA polymerases over cellular DNA polymerases [37].

Addition of Phosphate group to the hydroxyl group at C-5′ position

The third method involves phosphorylation of the hydroxy group at the C-5' position of the nucleoside. This phosphorylation step enhances the pharmacological properties of the nucleoside analog by adding one or more phosphate groups. Phosphorylation is a critical modification observed in AZT, a nucleotide inhibitor used against viruses such as HIV. It ensures the activation and incorporation of the nucleoside analog into cellular processes, making it an essential strategy in the design of antiviral and therapeutic drugs.

Rationale for using nucleoside analogs against SARS-CoV-2

Nucleoside analogs have emerged as a potential therapeutic strategy in the fight against COVID-19 owing to their ability to impede viral replication and their broad-spectrum antiviral activity against various viruses. Their successful application in HIV, Herpes, and Hepatitis C has demonstrated their efficacy and safety [38]. These analogs target the viral RNA-dependent RNA polymerase (RdRp), which is essential for viral replication, by acting as competitive inhibitors or alternative substrates, disrupting the RdRp's functioning and preventing viral RNA synthesis and replication. Remdesivir, initially developed for Ebola, has showcased the potential of this class of drugs against SARS-CoV-2, exhibiting potent inhibitory effects on SARS-CoV-2 replication in vitro and showing clinical benefits in COVID-19 patients, leading to its emergency use authorization [39]. Structural modifications to the nucleoside scaffold can enhance binding affinity to the viral RdRp, increase intracellular stability, and improve bioavailability, thus optimizing their antiviral potency, selectivity, and drug-like properties [40]. Nucleoside analogs can be used in combination with other antiviral agents or therapeutic

approaches, potentially enhancing their efficacy and reducing the risk of resistance development [41]. Some analogs, like molnupiravir and AT-527, are being developed for oral administration, improving accessibility and convenience for outpatient treatment. Although the development and evaluation of nucleoside analogs require rigorous preclinical and clinical studies, their broad-spectrum antiviral activity, established safety profiles, and potential for prophylactic use make them promising candidates in the ongoing global effort to manage and treat COVID-19.

Viral life cycle and drug targets

The SARS-CoV-2 virus, responsible for the COVID-19 pandemic, undergoes various critical stages in its life cycle that allow it to replicate and propagate inside host cells. These key steps, which have been extensively studied, are as follows:

Viral fusion

SARS-CoV-2 is a type of beta coronavirus that has five structures, namely spike (S), membrane (M), envelope (E), nucleocapsid (N), and hemagglutinin-esterase dimer (HE) glycoproteins [42, 43]. The virus enters host cells through either endosomes or plasma membrane fusion. In both mechanisms, the virus uses the S protein to bind to the host cell membrane and the angiotensin-converting enzyme 2 (ACE2) as the entry receptor [44–47]. ACE2 shows high specificity for SARS-CoV-2 and binds strongly with it, which may explain its easy transmission from human to human.

Recent studies have shown that the attachment between the S protein and ACE2 is activated by a host protease called transmembrane serine protease 2 (TMPRSS2) [48, 49]. TMPRSS2 also plays a role in the proteolytic cleavage of the S protein into S1 (globular domain) and S2 (biomembrane-anchored stalk domain) subunits [50–55]. The S1 segment allows the CoV to attach to ACE2, while the S2 assists the fusion of the virus inside the host cell [56, 57]. ACE2 plays a vital function in facilitating viral infection, thereby limiting its defensive influence in the lungs and heart [58].

ACE2 and TMPRSS2 play a crucial role in allowing the virus to enter the host cells by facilitating the fusion process. These proteins are the primary targets for drug development for a certain class of drugs known as Fusion inhibitors [48, 59]. Fusion inhibitors are a type of antiviral medication that can hinder the fusion process and prevent viruses from infecting the host cells. There are several drugs available, including umifenovir and camostat mesylate besides monoclonal antibodies that have demonstrated antiviral fusion inhibitory activity against SARS-CoV-2 [60, 61].

Replication and transcription

Once the fusion process occurs, the protein envelope is removed, and the genetic material of SARS-CoV-2, along with its nucleocapsid, enters the host cell cytoplasm [48, 62, 63]. Coronaviruses have the largest genomes among all RNA viruses, ranging from 26.4 to 31.7 kilobases, with a high guanine-cytosine content [64]. The SARS-CoV-2 genome is a single-stranded RNA of 30 kilobases with 14 open reading frames (ORFs) encoding different conserved genes, including ORF1a, ORF1b, S, E, M, and N protein regions. ORF1a and ORF1b produce two critical polypeptides, pp1a and pp1ab. The pp1a encodes for spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins (located at the 3' end) and is important for the integrity of virus protein. In contrast, the pp1ab encodes integral non-structural proteins (NSPs) pivotal for the development of the intricate replicase machinery, NSPs 1-16 [65, 66]. These polypeptides are also processed by viral enzymes, chymotrypsin-like protease (3CLpro) and main protease (Mpro), to yield non-structural proteins (NSPs 1-16), which encode for endoribonuclease activity and play a vital role in viral replication and transcription [67]. Protease inhibitor drugs, such as Lopinavir/Ritonavir, have been shown to inhibit viral proteases [68].

Replication pathway

The process of replicating SARS-CoV-2 involves three main steps: RNA synthesis, template proofreading, and capping [69]. To achieve these steps, several NSP complexes are critical for the virus, including NSP12 (RNA-dependent RNA polymerase; RdRp), NSP13 (zinc-binding helicase; HEL), NSP14-16 complex (mRNA capping), NSPs14 (RNA proofreading), NSP15 (uridylate-specific endoribonuclease activity (NendoU)), and NSP7-NSP10 (non-structural proteins) [69–76]. All of these components are present in the host cell endoplasmic reticulum complexed with the transcription enzyme of SARS-CoV-2, leading to the production of new genome molecules, including sub-genomic (sg) messenger RNAs [77, 78].

RNA polymerization and proofreading

RNA polymerization is a process that involves the catalytic activity of NSP12 coordinated with NSPs7-8 as cofactors [58]. The conserved N-terminal domain of NSP12 (NiRAN) is found to be showing nucleotidylation activity across most coronaviruses. The bifunctional protein NSP 14 plays a crucial role in RNA proofreading and mRNA capping. NSP 14 consists of two domains: the N-terminal domain and the C-terminal domain. Former shows exoribonuclease activity (DEDD guanine)-methyl exo-nucleases family) for proofreading, whereas the latter has N7 transferase activity for

viral mRNA cap synthesis [58]. The cap structure has an N7-methylated GTP molecule connected through a 5′–5′ triphosphate bond, transcribed by the foremost nucleotide [71, 75].

Capping machinery

The capping machinery responsible for the stability of mRNA and dodging host immune response consists of NSPs 13, 14 &16, and cofactor NSP10 [79, 80]. NSP13 plays a vital role in initiating mRNA capping by acting as a multifunctional helicase and hydrolyzing NTPs. It also unwinds RNA duplexes in the 5'-3' direction and possesses RNA 5'-triphosphatase action [73]. Ultimately, NSP16, in complex with NSP10, terminates the mRNA capping process. NSP16 belongs to the O-methyl Transferase category and features a reversed β hairpin at its carboxyl end.

The NSP13 helicase, NSP14 GTPase, NSP15 N7-Methyl Transferase, and NSP16 2'-O-Methyl Transferase synthesize a nascent RNA strand with a cap1 structure at its 5' end [75, 81].

The various non-structural proteins (NSPs) involved in the process of viral RNA replication are crucial for the formation of new viral particles. Nucleoside drugs, such as Remdesivir, Favipiravir, Ribavirin, Sofosbuvir, Molnupiravir, etc. have shown potential as antiviral candidates against SARS-CoV-2 by inhibiting these NSPs [82]. These drugs can inhibit the RdRp (NSP12) through mechanisms like chain termination and lethal mutagenesis [19]. Remdesivir also acts by escaping the proofreading of the ExoN (NSP15). Nucleoside analogs, like Ribavirin, also demonstrate their activity against methyltransferases [40].

Assembly of virions

The host's endoplasmic reticulum and Golgi apparatus play a vital role in assembling viral RNAs and associated proteins into virions for SARS-CoV-2 [83]. The M and E proteins combine to form virus-like particles, which are further enhanced by the N protein in the endoplasmic reticulum and Golgi apparatus. Additionally, the new virions also contain the S protein [84].

Virion release

An infected cell releases virus particles in one of three ways: budding, exocytosis, or cell death. Budding is the process by which undeveloped virus particles are released. The N protein interacts with the virus's E protein (which is glycosylated) at the host cell membrane, allowing the proper orientation for budding to occur. This process can occur in the plasma membrane, endosomal, nuclear, or perinuclear membranes [58]. The release of the virions which are matured in ER or Golgi complex takes place via Exocytosis. Sometimes the virus can damage the host cell machinery, causing the release of lysosomes. This can lead to the death of the cell and the release of virus particles. The new virus particles are then released from the host cell in vesicles through exocytosis [83, 85]. These particles can either infect other healthy cells or be shed into the environment through the mouth or breathing, possibly infecting other individuals.

The SARS-CoV-2 life cycle study highlights the viral RNA-dependent RNA polymerase (RdRp) and methyltransferase as critical targets, inhibition of which can impede viral propagation. Nucleoside analogs potently inhibit these enzymes, positioning them as promising COVID-19 therapeutics. The following section will elaborate on the mechanisms of action employed by these nucleoside analog drugs in targeting the RdRp and methyltransferase activities of SARS-CoV-2.

Nucleoside analogs as RdRP inhibitor

For nucleoside analogs to be effective against viral infections, they require modification by cellular enzymes called kinases. These enzymes convert the nucleoside analogs into their active form, which is a triphosphate. Once the nucleoside analogs are in their active form, they function by competitively inhibiting the viral polymerases [29]. This process can be achieved through the following pathways:

Chain termination

It majorly occurs following these methods:

Obligate chain termination

It is a process in antiviral treatment that involves stopping the growth of a viral DNA or RNA chain once a nucleoside analog is incorporated into it. This mechanism only occurs in nucleosides that lack the 3'-hydroxyl group, which is required for attaching the next incoming nucleotide [86]. Antivirals such as Acyclovir, Tenofovir, and Abacavir use this mechanism to combat DNA viruses and retroviruses effectively (Fig. 1) [87]. RNA viruses are less affected by

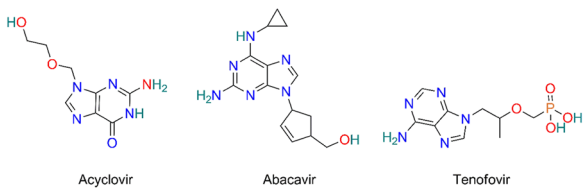


Fig. 1 Nucleoside Analogs that work by obligate chain termination

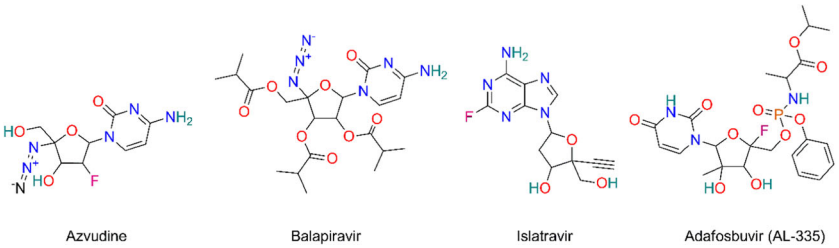


Fig. 2 Nucleoside Analogs which work by non-obligate chain termination

antiviral drugs as RNA-dependent RNA polymerases choose substrates based on the presence of a 2′-hydroxyl and exhibit selectivity for the existence of a 3′-hydroxyl [88, 89]. Additionally, the nucleic acids incorporated can be eliminated efficiently through pyrophosphorolysis [90].

Non-obligate termination

Nucleosides that cause non-obligate chain termination have a 3'-hydroxyl group that can add incoming nucleotides (Fig. 2). It is further divided into these classes

i. Translocation Inhibition: Nucleosides that operate through this mechanism possess a 3'-hydroxyl. However, they are unable to continue adding to the growing chain due to substituents on adjacent carbons that cause steric hindrance [87]. These nucleosides are known as pseudo-obligate terminators and include 4'-modified nucleosides such as azvudine (also known as FNC), islatravir, balapiravir, and AL-335 [91–94].
ii. Inhibition of Chain Elongation via Disruption of Hydrogen Bonding Network:

    Nucleoside Analogs (NAs) that are 2'-methylated, such as sofosbuvir and bemnifosbuvir, have strong antiviral properties. This is because they disrupt the hydrogen-bonding network during ribonucleotide incorporation into viral RdRp [95]. Sofosbuvir, which has a high resistance barrier against HCV, is a non-obligate chain terminator that demonstrates this mechanism [96]. Bemnifosbuvir is also effective against coronavirus RdRp, which further emphasizes the potential of this class of drugs [97].
iii. Polymerase Backtracking: T1106 is a nucleoside analog that mimics purine bases. It has been found to disrupt the movement of viral polymerase. Based on the Cryo-EM data, when influenza virus polymerases use two T1106 molecules in the RNA chain, it causes the polymerase to backtrack. This results in removing the recently added nucleotides, including both the molecules of T1106, from the nucleotide entry channel. This release of nucleotides leads to the termination of RNA synthesis [98].

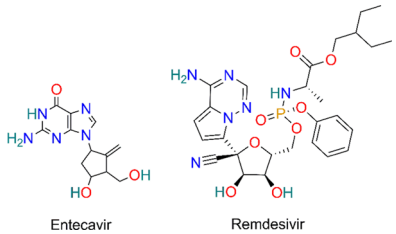


Fig. 3 Nucleoside Analogs that work by delayed chain termination

Delayed termination

In this case, the termination is not immediate but occurs after adding a few more nucleotides. It delays the process but eventually leads to chain termination. Nucleosides that operate through delayed chain termination have a 3'-hydroxyl group, allowing for further integration of incoming nucleotides. However, due to steric hindrances at adjacent positions, each subsequent addition takes place slowly. Eventually, after two to four additions, further incorporation stops. Drugs like Entecavir, Remdesivir, etc. show this type of termination (Fig. 3) [99, 100].

Lethal mutagenesis

It is a strategy used against RNA viruses & works by increasing the error rate during the virus's replication process. This elevated mutation rate can weaken the virus's essential genes and eventually lead to its death. Nucleoside analogs can induce mutagenesis in RNA. They introduce base modifications, which create different base pairing patterns. When these mutagenic nucleoside analogs are introduced into the primer strand, they become part of the template strand during RNA synthesis. This process further contributes to the accumulation of mutations in the viral genome, which ultimately weakens the virus and compromises its ability to replicate successfully [101]. Nucleoside analogs such as ribavirin, favipiravir, and molnupiravir have demonstrated the effectiveness of this strategy (Figs. 4 and 5) [102]. As these nucleoside analogs do not work as chain terminators, these are less likely to be removed from the viral RNA by the action of ExoN [103]. This strategy is effective, but it also carries risks, such as the

Fig. 4 Nucleoside Analogs that work by lethal mutagenesis

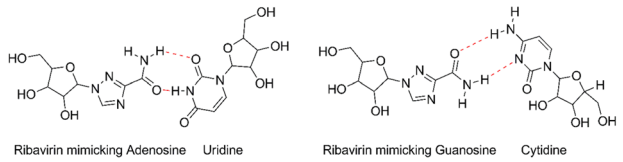


Fig. 5 Ribavirin mismatch base pairing

emergence of resistant strains and potential adverse effects like carcinogenesis and teratogenicity. Therefore, careful consideration and ongoing research validation are necessary before its application [104].

Nucleoside analogs as Methyltransferase inhibitor

Methyl Transferases inhibition: Viral RNA genomes encode methyltransferase enzymes which are essential for the capping of viral RNA. It ensures a resemblance between the viral RNA and the host intrinsic mRNA. As a result, viruses can avoid the host cell's immune response, enhance the translation efficiency of mRNA, and ensure stability. These viral methyltransferases being highly conserved, become a promising target for the development of antiviral agents. Guanosine analogs such as RBV occupy the GTP binding site of both viral enzymes and endogenous eIF4E, reducing the efficiency of viral RNA capping and inhibiting viral RNA capping [105–108]. Flex analogs of acyclovir have recently been developed, which have demonstrated even greater inhibition of flavivirus methyltransferase compared to the endogenous enzyme [109]. These compounds exhibit submicromolar activity but also have micromolar toxicity [19].

Prominent nucleoside drugs for COVID-19

Remdesivir

Remdesivir, created by Gilead Sciences Inc. and also known as GS-5734, was approved by the US FDA on October 22, 2020, for use as the main treatment for SARS-CoV-2 in adults [110]. Remdesivir has shown considerable antiviral promise in laboratory settings against a variety of viruses, including SARS-CoV, MERS-CoV, EBOV, and SARS-CoV-2 [111, 112]. The drug's EC_{50} values, which are 0.069, 0.090, 0.012, and 0.77 μM against these respective viruses, serve as a measure of its potency [113–115].

Remdesivir is a 10-cyano-substituted monophosphoramidate prodrug of adenosine analog, that requires metabolic activation within target cells to exert its antiviral activity. The activation process begins with the uptake of RDV into the cells. Once inside, the enzyme carboxylesterase 1 (CES1) hydrolyzes RDV, removing one of the masking groups. The resulting metabolite then undergoes further hydrolysis catalyzed by the enzyme cathepsin A (CatA), forming the alanine intermediate metabolite called MetX. MetX is then acted upon by the enzyme histidine triad nucleotide-binding protein 1 (HINT1), which hydrolyzes MetX to form the monophosphate nucleoside GS-441524. The monophosphate nucleoside GS-441524 is subsequently subject to consecutive phosphorylation reactions mediated by cellular phosphotransferases. First, it is phosphorylated to form the diphosphate nucleotide, which is then further phosphorylated to generate the active triphosphate form, GS-443902 (Fig. 6). This active triphosphate metabolite, GS-443902, acts as a potent and selective inhibitor of multiple viral RNA polymerases, including the RNA polymerase of SARS-CoV-2, the virus responsible for COVID-19. By inhibiting the viral RNA polymerase, GS-443902 effectively blocks the replication of the virus, thereby exerting its antiviral activity [116–122].

This active form inhibits viral RNA-dependent RNA polymerases during the early stages of a viral infection competing with endogenous nucleotides for viral RNA incorporation via RNA-dependent RNA polymerase. Being incorporated into the RNA chain, remdesivir does not immediately terminate the chain [123]. The presence of 3'-OH allows the addition of 3 more nucleotides until the RNA chain termination (delayed chain termination) [119, 124]. In addition to this primary mechanism, Remdesivir may also impact the virus through lethal mutagenesis (Remdesivir triphosphate mimics ATP) [125]. The delayed chain termination mechanism might protect Remdesivir from removal from the RNA chain by viral proofreading proteins due to the 3–5 additional natural nucleotides [126]. Remdesivir is poorly bioavailable in oral form and has a short life so it must be administered intravenously [125, 127–129].

Favipiravir

The orally accessible prodrug favipiravir, also known as 6-fluoro-3-hydroxy-2-pyrazine-carboxamide (T-705), was initially authorized for use in Japan in 2014 for the treatment of infection caused by influenza virus. It exhibits a wide-ranging antiviral impact on various viruses, including influenza A (H1N1) [130], H5N1 [131], Rift Valley fever virus [132], yellow fever virus [133], West Nile virus [134],

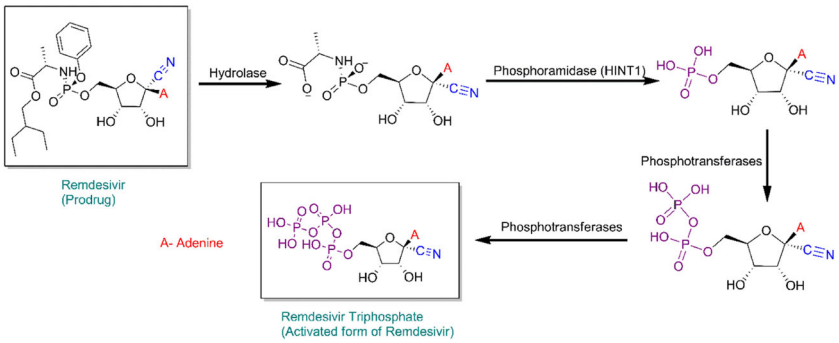


Fig. 6 Activation of Remdesivir prodrug [121]

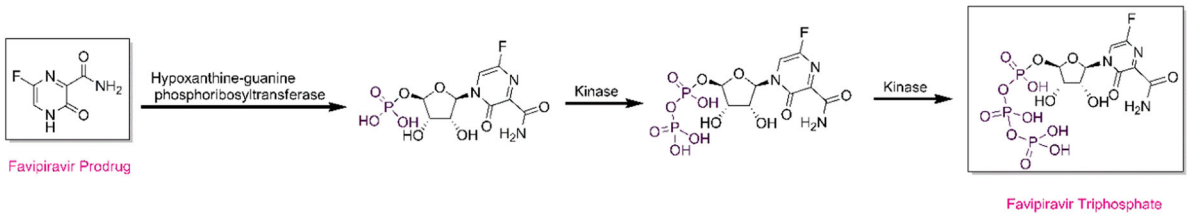


Fig. 7 Activation of Favipiravir prodrug [139]

foot-and-mouth disease virus [135], and Punta Toro virus [136] in animal models. Favipiravir has drawn a lot of attention because of its antiviral potential as a potential treatment and prevention for developing COVID-19 [137]. Favipiravir significantly suppresses SARS-CoV-2 in vitro according to Wang et al., with an EC_{50} of 61.88 µM and moderate selectivity (SI = 6.46) [123]. Favipiravir exhibits a potent dose-dependent impact on SARS-CoV-2-infected hamsters, resulting in appreciable improvements in lung histology [138].

Favipiravir undergoes a sequence of enzymatic phosphorylation steps to convert into its active state, favipiravir-ribofuranyl-triphosphate (Favipiravir-RTP). The initial rate-limiting step in the activation of favipiravir involves the conversion of the drug to its monophosphate form, favipiravir-ribofuranosyl-5'-monophosphate (favipiravir-RMP), by the host enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) [139]. Subsequently, the monophosphate favipiravir-RMP is further metabolized by host cell enzymes to generate the pharmacologically active triphosphate form, favipiravir-RTP (Fig. 7) [140]. It specifically blocks the RNA-dependent RNA polymerase (RdRp) of the influenza virus [141]. It functions as a purine analog and is incorporated instead of guanine and adenine [142, 143]. Following incorporation, favipiravir-RTP acts as a mutagen and can inhibit the coronavirus's ability to repair itself. The SARS-CoV-2 genome has a low cytosine content, and favipiravir-RTP increases the pressure on the CoV nucleotide content. By inhibiting the virus's cytopathic effect, reducing the amount of viral RNA, and preventing the spread of infectious particles, favipiravir-RTP has a beneficial effect on SARS-CoV-2 [143].

Molnupiravir

Molnupiravir, also known as EIDD-2801 or MK-4482, is a promising oral drug developed by Plemper's group for the treatment of COVID-19. It is a novel isobutyryl ester prodrug of β-D-N^4-hydroxycytidine (NHC, EIDD-1931) that targets the RdRp, making it a broad-spectrum therapeutic against several viruses including EBOV, influenza A, HCV, Venezuelan equine encephalitis virus, SARS-CoV, and SARS-CoV-2 [144, 145]. Molnupiravir has been approved for use since November 2021 in the UK and FDA approved this drug in December 2021 for its emergency use in the treatment of mild to moderate COVID-19 in adults who are at high risk of disease progression to severe, and for whom alternative permitted treatment options are unavailable or clinically unsuitable [118, 146]. When used in combination with favipiravir (FVP), molnupiravir displays a notable cooperative effect in the SARS-CoV-2 hamster infection model [145].

Molnupiravir undergoes a swift conversion into its triphosphate form, known as molnupiravir-TP or MTP, by the action of cellular kinases [118]. The initial step in this process involves the hydrolysis of the isopropyl ester moiety by the human carboxylesterase enzyme CES2. This generates the metabolite N-hydroxycytidine (NHC). After its formation, NHC undergoes three sequential phosphorylation steps catalyzed by cellular kinases that are yet to be

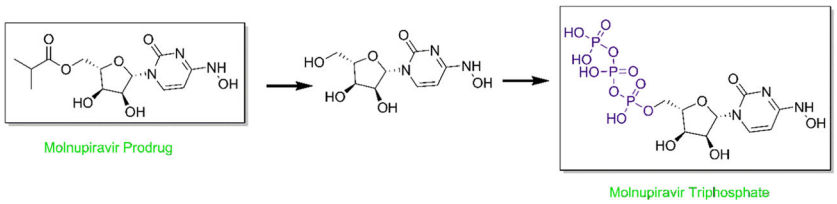


Fig. 8 Activation of Molnupiravir prodrug [147]

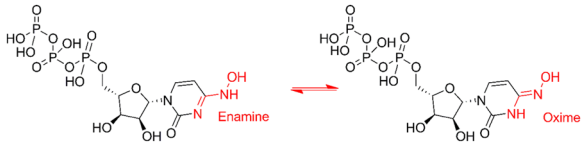


Fig. 9 Tautomers of the activated form of molnupiravir [150]

identified. These steps lead to the production of the NHC monophosphate, diphosphate, and ultimately the active NHC triphosphate metabolite (Fig. 8) [147, 148]. The triphosphate can then be used as a substrate by the viral RdRp and induces lethal mutagenesis or error catastrophe [149]. The tautomerisation of the oxime molecule to the enamine form provides the analog with two base-pairing opportunities. These two isomers mimic uridine & cytidine respectively (Fig. 9). Different isomeric forms of molnupiravir have the potential to increase G-to-A and C-to-U transition mutations in the course of viral replication, which can significantly increase the drug's effectiveness in combating SARS-CoV-2 [150].

Nevertheless, it's crucial to emphasize that molnupiravir is approved for brief usage (up to 5 consecutive days) and is discouraged for pregnant women or young patients (under the age of 18 years) due to the risk of fatal toxicity as well as bone & cartilage toxicity. [151].

Ribavirin

Ribavirin is a nucleoside antiviral drug discovered in the 1970s, which is known for its wide-ranging antiviral activity against both DNA and RNA viruses [152]. It is approved for the treatment of hepatitis C, often in combination with other drugs [153]. Structurally, ribavirin consists of a typical ribose connected to a triazole aromatic ring with a rotatable amido group. Ribavirin undergoes intracellular phosphorylation by adenosine kinase to form ribavirin monophosphate (RMP), which is then further phosphorylated by nucleoside mono- and diphosphate kinases to generate ribavirin triphosphate (RTP), the primary intracellular metabolite of ribavirin (Fig. 10) [33, 154, 155].

Ribavirin's antiviral mechanism comprises multiple actions, including host-targeted mechanisms, such as inhibition of inosine monophosphate dehydrogenase (IMPDH) and regulation of the host immune response [33]. It also exhibits virus-targeted effects, interacting with RNA capping enzymes, inhibiting viral RNA-dependent RNA polymerases (RdRp), and inducing viral lethal mutagenesis [152].

The inhibition of IMPDH (crucial for DNA and RNA synthesis) explains ribavirin's effectiveness against a wide range of viruses. Its immunomodulatory effect alters host T-cell responses, and its interference with RNA capping leads to the activation of the host immune response against foreign viral RNA. Ribavirin's interaction with RdRp inhibits viral RNA synthesis [152].

Ribavirin also causes lethal mutagenesis which increases the mutation rate of RNA viruses to non-viable levels, rendering them unable to maintain genetic information [156].

The docking analysis demonstrated that ribavirin can bind to the SARS-CoV-2 replication enzyme (RdRp) with comparable binding energy to native nucleotides, indicating its potential to hinder the virus's replication process [157]. However, ribavirin's similarity to adenosine and guanosine presents challenges in terms of selectivity and toxicity, often leading to side effects like severe anaemia [158]. In conclusion, ribavirin's capability to emulate both adenosine and guanosine allows it to interact with various critical enzymes and mechanisms in virus replication. While it demonstrates broad-spectrum antiviral activity, efforts to develop compounds with similar mutagenic properties but improved selectivity are desirable to reduce side effects.

Sofosbuvir

Sofosbuvir, a nucleotide analog that has been primarily approved for the treatment of hepatitis C virus (HCV) infection also known as GS-7977 or SOF (specifically known as PSI-7977), was developed by Pharmasset Ltd in 2010, later acquired by Gilead sciences which took care of the advancement of the drug in various preclinical and clinical trials [159, 160].

Upon entering the host cell (hepatocyte), sofosbuvir is converted by a cellular enzyme into an active form of the nucleoside triphosphate. The initial stage of activation involves stereospecific hydrolysis of the carboxyl ester prodrug by the enzymes cathepsin A (CatA) and/or carboxylesterase 1 (CES1), producing the alaninyl phosphate metabolite PSI-352707. The amino acid moiety is then

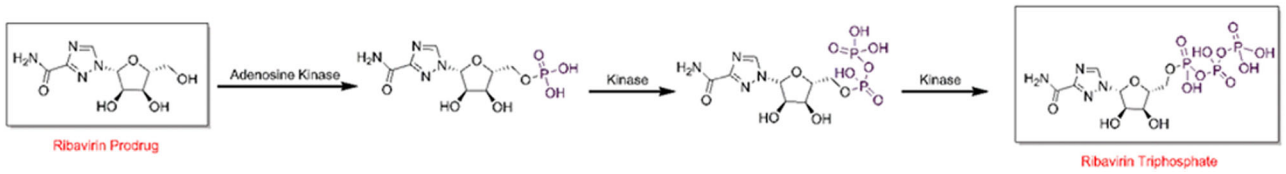


Fig. 10 Activation of Ribavirin prodrug [154]

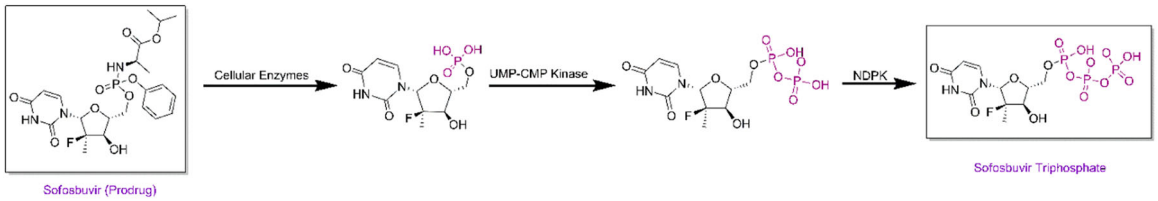


Fig. 11 Activation of Sofosbuvir prodrug [161]

removed from PSI-352707 by the enzyme histidine triad nucleotide-binding protein 1 (HINT1), forming the monophosphate PSI-7411. HINT1 is confirmed to be responsible for this conversion. The monophosphate PSI-7411 subsequently undergoes two sequential phosphorylation steps, catalyzed by the enzymes UMP-CMP kinase and nucleoside diphosphate kinase, to produce the diphosphate PSI-7410 and finally the active triphosphate form PSI-7409 (Fig. 11). The active triphosphate primarily functions by inhibiting the NS5B RNA-dependent RNA polymerase of HCV (following a mechanism that involves the inhibition of the hydrogen bonding network) which plays a crucial role in virus replication [161–165].

However, Sofosbuvir, effective against HCV, becomes a potential antiviral candidate against SARS-CoV-2, which shares a similarity in having a positive-stranded RNA. In a study involving COVID-19 patients who were hospitalized and received this treatment, there was no significant reduction in the viral load compared to a control group [16]. Therefore, larger clinical trials are needed to determine the effectiveness of this treatment plan.

Future prospects

As we look ahead to the future of SARS-CoV-2 treatment, nucleoside analogs are showing promise in the fight against the virus. However, there are both potential advancements and obstacles to consider. Nucleoside analogs, which use lethal mutagenesis to disrupt viral replication, have proven effective, but concerns about potential side effects require researchers to evaluate and refine these compounds for a safer profile. Resistance to antiviral drugs remains a challenge, and to address this, the future of nucleoside analogs involves strategic approaches such as combination therapies or developing novel analogs with different mechanisms of action.

Researchers are exploring various strategies to develop more effective treatments against COVID-19. One of these strategies involves developing dual-acting nucleoside analogs that can simultaneously target multiple viral components or processes. By designing molecules that inhibit the viral RNA-dependent RNA polymerase (RdRp) while also disrupting other essential viral functions, such as the SARS-CoV-2 helicase or protease, researchers aim to achieve potent antiviral effects and potentially create a higher barrier to resistance development. Another promising approach is to target host factors required for viral replication. This dual-targeting approach could lead to potent antiviral activity while reducing the risk of resistance. Furthermore, the combination of nucleoside analogs with other therapeutic modalities, such as host-directed therapies, may unlock synergistic antiviral effects and help prevent the emergence of drug-resistant viral variants. Exploring these combination approaches in preclinical and clinical studies will be a priority for future research.

Improving the pharmacokinetic properties of nucleoside analogs, such as enhanced tissue distribution and prolonged half-life, will be crucial to optimize their therapeutic efficacy. Strategies like the development of lipophilic prodrug formulations could enhance the delivery of these compounds to the primary sites of SARS-CoV-2 infection and maintain sustained antiviral concentrations.

Personalized medicine is becoming increasingly important, as patient response to treatment can vary. Research is underway to identify individual genetic factors influencing responses to nucleoside analogs, paving the way for tailored treatment approaches that maximize therapeutic benefits.

Global accessibility and affordability remain imperative, and efforts are needed to overcome logistical and financial

barriers to ensure that nucleoside analogs reach all corners of the world, especially in resource-limited settings. Collaborations between pharmaceutical companies, governments, and international organizations are crucial to achieving equitable access and affordability for all.

By leveraging these innovative design strategies, researchers aim to develop the next generation of nucleoside analogs that can offer improved potency, selectivity, pharmacokinetic properties, and the ability to overcome resistance challenges, ultimately enhancing the arsenal of effective treatments against COVID-19.

Conclusion

This review highlights the crucial role of nucleoside analogs in combating COVID-19. Looking ahead, there is promise in the development of new analogs with better antiviral properties, which offers hope for more effective treatments. However, there are also challenges that must be addressed. Antiviral resistance is a persistent threat, which requires vigilant surveillance and adaptable strategies. Safety concerns are also important to consider in drug development to ensure patient well-being. Additionally, navigating the complex landscape of regulatory frameworks presents a hurdle to the widespread adoption of these promising agents. Despite these challenges, there is a call to action. Researchers must continue exploring nucleoside analogs to unravel new therapeutic dimensions. Interdisciplinary collaboration is crucial to bring together the realms of virology, pharmacology, and regulatory science. Sustained research efforts will be the cornerstone in overcoming hurdles and realizing the full potential of nucleoside analogs in the fight against COVID-19. In conclusion, this review not only captures the current state of knowledge but also serves as a guide for future endeavours. The journey toward effective antiviral strategies is ongoing, and the importance of nucleoside analogs in this narrative cannot be denied. Through collective efforts and unwavering dedication, we move forward with the shared vision of a healthier, more resilient global community.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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