Targeting SARS-CoV-2: An evaluation of the efficacy, mode of action and synthesis of remdesivir

Student Dissertation

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Targeting SARS-CoV-2: An evaluation of the efficacy, mode of action and synthesis of remdesivir.

A Report submitted as the examined component of the Project Module SXM390.

Mark Duffy
Drug Design and Synthesis
26 August 2021

4936 words
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Boundless thanks go to my wife Fiona for her support and my children Eilidh and Rory in bearing with me all these years whilst I completed my chemistry degree.

My final thoughts and thanks go to Father Lamb, my chemistry teacher at St Aloysius College from 1974-1979, who ignited my passion for chemistry and who couldn’t disguise his disappointment when I told him I was going to university to study accountancy. I hope I didn’t disappoint in the end.
ABSTRACT

The outbreak of a novel coronavirus in Wuhan Province, China in 2019 has had a devastating global impact with over 4.4 million deaths recorded by 26 August 2021. There has been no pipeline of available drugs to tackle SARS-CoV-2 and the disease it causes, COVID-19. The aim of this review is to evaluate Gilead Science’s remdesivir (RDV), originally aimed at Ebola Virus Disease, and now proposed as a treatment for COVID-19.

This report is based upon a review of the current scientific literature on RDV comprising primary research, review papers and other sources. These have been identified using major scientific databases, search engines and other sources including Web of Science, Scopus, the Royal Society of Chemistry and Google Scholar. Key words used for searches included remdesivir, mode of action or mechanism, synthesis, SARS-CoV-2 and COVID-19.

SARS-CoV-2 possesses a highly conserved replication polymerase, RNA-dependent RNA polymerase (RdRp). This is targeted by RDV whose metabolite, remdesivir triphosphate, acts as a substrate for synthesis of viral RNA by RdRp, replacing adenosine triphosphate. This leads to RNA chain termination and cessation of replication. Research conducted in-vitro has demonstrated that treatment of SARS-CoV-2 with RDV can lead to a powerful reduction of replication in human epithelial airway cells with an EC50 (half-maximal effective concentration) of 0.01µM.

The synthesis of RDV however is challenging and a number of improvements have recently been made. These include the use of flow chemistry and alternative methods for precursor synthesis.

RDV can be developed further for tackling SARS-CoV-2. This report sets out suggested improvements to its structure, reviews recent improvements in its synthesis and signposts areas for further research.

To date RDV has been given either full or temporary approval for treating SARS-CoV-2 in around 50 countries. It remains the only currently approved antiviral drug for COVID-19.
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACTT-1</td>
<td>Adaptive COVID-19 Treatment Trial</td>
</tr>
<tr>
<td>AMP</td>
<td>Adenosine monophosphate</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BOC</td>
<td>Tert-butyloxycarbonyl</td>
</tr>
<tr>
<td>COVID-19</td>
<td>Coronavirus disease 2019</td>
</tr>
<tr>
<td>CoVs</td>
<td>Coronaviruses</td>
</tr>
<tr>
<td>Cryo-EM</td>
<td>Cryogenic electron microscopy</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DyKAT</td>
<td>Dynamic kinetic asymmetric transformation</td>
</tr>
<tr>
<td>ExoN</td>
<td>Exonuclease</td>
</tr>
<tr>
<td>EVD</td>
<td>Ebola virus disease</td>
</tr>
<tr>
<td>Gilead</td>
<td>Gilead Sciences Inc.</td>
</tr>
<tr>
<td>MERS-CoV</td>
<td>Middle East respiratory syndrome coronavirus</td>
</tr>
<tr>
<td>NA</td>
<td>Nucleoside analogue</td>
</tr>
<tr>
<td>NMP</td>
<td>N-Methyl-2-pyrrolidone</td>
</tr>
<tr>
<td>NSP</td>
<td>Non-structural protein</td>
</tr>
<tr>
<td>HAE</td>
<td>Human airway epithelial</td>
</tr>
<tr>
<td>RdRp</td>
<td>RNA dependent RNA polymerase</td>
</tr>
<tr>
<td>RDV</td>
<td>Remdesivir</td>
</tr>
<tr>
<td>RMP</td>
<td>Remdesivir monophosphate</td>
</tr>
<tr>
<td>RTP</td>
<td>Remdesivir triphosphate</td>
</tr>
<tr>
<td>rt</td>
<td>Room temperature</td>
</tr>
<tr>
<td>SARS-CoV</td>
<td>Severe acute respiratory syndrome</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>Severe acute respiratory syndrome-2</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>SAGE</td>
<td>Scientific Advisory Group for Emergencies</td>
</tr>
<tr>
<td>TfOH</td>
<td>Trifluoromethanesulfonic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TMSOTf</td>
<td>Trimethylsilyl trifluoromethanesulfonate</td>
</tr>
<tr>
<td>TMSCI</td>
<td>Trimethylsilyl chloride</td>
</tr>
<tr>
<td>TMSCN</td>
<td>Trimethylsilyl cyanide</td>
</tr>
<tr>
<td>UTP</td>
<td>Uridine trisphosphate</td>
</tr>
</tbody>
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CHAPTER 1
Introduction

1.1 – Background

The outbreaks of a new severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 led to scientists warning of a future zoonotic viral pandemic (Menachery et al., 2015). These fears were realised with the detection of a new and deadly respiratory virus in Wuhan Province, China in December 2019. This was named as a novel coronavirus by Chinese authorities in January 2020 and subsequently designated SARS-CoV-2 and the resulting disease as COVID-19. As of 26 August 2021 COVID-19 has led to over 4.4 million deaths globally (World Health Organisation, 2021).

No antiviral had been developed or approved for coronaviruses. The outbreak of Ebola Virus Disease (EVD) in Africa in 2013-2016 however led to the development of a new antiviral drug targeting the replication machinery of EVD, namely RNA-dependent RNA polymerase (RdRp) (Warren et al., 2016). RdRp is a highly conserved enzyme common to many viruses, including EVD and coronaviruses. Scientists at Gilead Sciences Inc. (Gilead) had been working on antivirals for many years and the most promising candidate developed to target RdRp was remdesivir (RDV). This had shown much promise during in-vitro testing and was the subject of clinical trials for EVD.

RDV was proposed as a potential drug to target SARS-CoV-2 and a number of clinical trials commenced worldwide. A peer reviewed Adaptive COVID-19 Treatment Trial (ACTT-1) run by the National Institute of Allergy and Infectious Diseases demonstrated a five day reduction in hospital stay in patients administered RDV compared with a placebo (Beigel et al., 2020).

1.2 – Objectives

This project will:

 assess the potential of RDV in targeting SARS-CoV-2 and review its efficacy;
 review the mode of action of RDV;
 review existing synthetic routes for RDV and suggest alternatives where possible;
 propose any suitable modifications to the structure of RDV;
 consider any further work in developing nucleoside analogues for the treatment of SARS-CoV-2 and possible future variants.

1.3 – Scope

The project comprises a review and critical evaluation of the recent scientific literature regarding RDV. It investigates cutting-edge research into the mode of action of RDV in tackling SARS-CoV-2 and synthetic routes in scaling up production of RDV for clinical use. Identification of potential improvements in either area is an aim of this review. A brief review of efficacy is included as a backdrop. Pharmacological aspects of RDV however are not covered.
1.4 – Methodology

Primary research papers largely form the basis of this project together with a limited number of review papers and other sources. Papers have been identified using major scientific databases, search engines and other sources including Web of Science, Scopus, the Royal Society of Chemistry and Google Scholar. Key words used for searches included remdesivir, mode of action or mechanism, synthesis, SARS-CoV-2 and COVID-19 or combinations thereof.

RDV has been given either full or temporary approval for the treatment of SARS-CoV-2 in around 50 countries (Gilead, 2021). It remains the only currently approved antiviral drug for COVID-19.
CHAPTER 2

Efficacy

2.1 – Background to development of remdesivir

Although RDV was developed for use in treating EVD, a study conducted by Sheahan et al., (2017) assessed its broad spectrum potential in treating MERS-CoV, SARS-CoV and other coronaviruses (CoVs).

Key findings were:

- replication of the coronaviruses MERS-CoV and SARS-CoV in human airway epithelial (HAE) cells was virtually halted by GS-5734 (later named remdesivir);
- administering GS-5734 as a prophylactic led to less severe disease arising from SARS-CoV in mice models;
- treatment with GS-5734 after exposure to SARS-CoV reduced disease levels in mice; and
- GS-5734 could inhibit other coronaviruses including zoonotic strains with the potential to cause a pandemic and circulating in bats.

2.2 – SARS-CoV-2 In-vitro results

The RdRp which RDV targets is highly conserved and this should lead to similar efficacy results against SARS-CoV and SARS-CoV-2. This was confirmed by Pruijssers et al., (2020) who found that treatment with RDV led to a powerful reduction of replication of SARS-CoV-2 in HAE cells with an EC$_{50}$ (half-maximal effective concentration) of 0.01µM.

2.3 – SARS-CoV-2 clinical trials

A number of clinical trials have been undertaken to assess the efficacy of RDV in a human population. A peer reviewed ACTT-1 COVID-19 trial run by the National Institute of Allergy and Infectious Diseases demonstrated a five day reduction in hospital stay in patients administered RDV compared with a placebo (Beigel et al., 2020). However, this was contradicted by data from the COVID-19 Solidarity trial which found no significant improvement in patients treated with RDV (Pan et al., 2020). More recently a retrospective study found that, for a cohort of largely non-white patients, time to clinical improvement was reduced on average by two days following treatment with RDV (Garibaldi et al., 2021).

Despite these conflicting results RDV has been given either full or temporary approval for the treatment of SARS-CoV-2 in around 50 countries. It remains the only currently approved antiviral drug for COVID-19.
CHAPTER 3

Mode of action

3.1 – Chain termination

The biological target for RDV is viral RdRp which synthesises RNA. RdRp comprises non-structural protein 12 (nsp12) in complex with cofactors nsp7 and nsp8. These heterodimers are thought to help stabilise those regions of nsp12 interacting with RNA (Kirchdoerfer and Ward, 2019). The overall complex is crucial to the importance of nsp12 and significantly increases binding to RNA (Yin et al., 2020).

RDV is a nucleotide analogue (NA) prodrug which is converted in the cell by hydrolysis to remdesivir monophosphate (RMP) (Figure 3.1). Delivery and conversion of RDV into RMP in this way avoids an otherwise rate limiting first phosphorylation step in-vivo. RMP in turn is doubly phosphorylated by the action of cellular kinases into remdesivir triphosphate (RTP) (Siegel et al., 2017). This is the pharmacologically active form of the drug which mimics its natural counterpart adenosine triphosphate (ATP).

Figure 3.1 – Conversion of RDV to RMP to RTP (Adapted from Eastman et al. (2020).
RTP competes favourably with ATP as a substrate for RNA replication in a number of different viruses, acting as a delayed chain terminator. The elongation of the RNA chain is catalysed by the RdRp, releasing RMP and pyrophosphate from RTP. RMP is incorporated into the growing RNA chain instead of adenosine monophosphate (AMP), however after inclusion of three nucleobases chain synthesis is halted and viral replication ceases.

A schematic for the incorporation of RMP opposite uridine into a growing RNA chain is set out below in Figure 3.2 (Yin et al., 2020, fig 4, p4).

Figure 3.2 – Positioning of RMP opposite uridine into elongating RNA primer strand (Yin et al., 2020). Also shown are interactions between primer and template strands and adjoining residues.

The movement of the synthesised RNA strand through the RdRp is known as translocation and a barrier to this arises from a steric clash between the 1′-CN (cyano) group of RMP and a serine-861 residue (Figure 3.3). This steric clash is understood to result from the short distance of 1.7 Å between the oxygen atom of the serine-861 residue and the nitrogen atom of the 1′-CN in RMP (Gordon et al., 2020). This mechanism was confirmed in a very recent cryo-EM study by Bravo et al., (2021) who additionally demonstrated that inhibition of chain synthesis is not absolute but can be overcome by increased concentrations of nucleotides. However, it should be noted that Shannon et al., (2020) report that Arg858 is responsible for the steric clash and further work is therefore required to clarify the mode of action.

Figure 3.3 – steric clash arising from RMP incorporation (adapted from Kokic et al., 2021, fig 3, p4).
Recent research (Zhang et al., 2020) had found additional roles for the cyano modification of RDV. Firstly, electrostatic interactions with a salt bridge comprised of Asp865 and Lys593 induce instability. Secondly, a steric clash between the cyano group and Asn104 prevents cleavage of RMP by an exonuclease (ExoN).

3.2 – Alternative modes of action

A recent paper by scientists at Gilead has claimed a further mechanism of action of RDV (Tchesnokov et al., 2020). They note that RDV, via RMP, can become incorporated into an RNA strand which is later used as a template. The efficiency with which uridine trisphosphate (UTP) is incorporated into the new daughter strand is then affected. This results in RMP in the template being improperly positioned for base pairing with UTP due to a clash with an alanine-558 residue.

An additional mode of action has recently been suggested by Koulgi et al. (2020) whose in-silico study looked at interactions of RdRp with surrounding residues both when bound to RDV and in the apo (unbound) state. The authors determined that when bound to RDV a conformational change took place which blocked entry to the template site. This prevents entry of additional nucleotides to the site and leads to cessation of replication of the virus.

3.3 – RDV interactions with the RdRp

A number of studies have examined the binding of RDV to adjacent residues of the RdRp. Wu et al., (2020, fig 7C, p785) have reported that RDV is involved in hydrogen bonding with Asn497, Arg569 and Asp684 residues (Figure 3.4). They also suggest there may be a number of hydrophilic interactions which direct a preferred conformation of RDV (residues Leu576, Ala685 and Tyr687).

![Figure 3.4 – RDV interactions with RdRp residues](Wu et al., 2020). Dotted lines show hydrogen bonds.
However, there are considerable differences noted in the scientific literature in terms of which amino acid residues are involved in binding to RDV. For example, Koulgi et al., (2020) performed molecular simulations between RDV and adjacent residues and reported a number of hydrogen bonds not noted by other authors. A summary of interactions noted in eight different recent papers and highlighting such differences is set out in Table 3.1 below.

<table>
<thead>
<tr>
<th>Amino acid residue</th>
<th>Authors</th>
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<tbody>
<tr>
<td>Ala550</td>
<td>Wu et al., (2020)</td>
</tr>
<tr>
<td>Ala685</td>
<td>Wang &amp; Yang (2020)</td>
</tr>
<tr>
<td>Ala688</td>
<td>Pruijssers et al. (2020)</td>
</tr>
<tr>
<td>Arg553</td>
<td>Shannon et al. (2020)</td>
</tr>
<tr>
<td>Arg555</td>
<td>Yin et al. (2020)</td>
</tr>
<tr>
<td>Arg569</td>
<td>Zhang et al. (2020)</td>
</tr>
<tr>
<td>Arg858</td>
<td>Kouli et al. (2020)</td>
</tr>
<tr>
<td>Asn104</td>
<td>(Gao et al., 2020)</td>
</tr>
<tr>
<td>Asn497</td>
<td></td>
</tr>
<tr>
<td>Asn691</td>
<td></td>
</tr>
<tr>
<td>Asp618</td>
<td></td>
</tr>
<tr>
<td>Asp623</td>
<td></td>
</tr>
<tr>
<td>Asp684</td>
<td></td>
</tr>
<tr>
<td>Asp760</td>
<td></td>
</tr>
<tr>
<td>Asp761</td>
<td></td>
</tr>
<tr>
<td>Asp865</td>
<td></td>
</tr>
<tr>
<td>Cys813</td>
<td></td>
</tr>
<tr>
<td>Leu576</td>
<td></td>
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<tr>
<td>Lys545</td>
<td></td>
</tr>
<tr>
<td>Lys551</td>
<td></td>
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<tr>
<td>Lys593</td>
<td></td>
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<tr>
<td>Gln805</td>
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<tr>
<td>Ser549</td>
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<tr>
<td>Ser759</td>
<td></td>
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<tr>
<td>Ser814</td>
<td></td>
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<tr>
<td>Thr687</td>
<td></td>
</tr>
<tr>
<td>Thr680</td>
<td></td>
</tr>
<tr>
<td>Val557</td>
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Table 3.1 demonstrates a wide variety of recorded interactions with RDV in the recent literature with 28 amino acid residues noted across eight different studies. Despite such differences, however, a number of similarities can be noted. In particular interactions of four or more residues are noted across multiple papers-Arg555, Asp760, Asp761 and Thr687. Of these residues there appears agreement amongst authors that Asp760 and Asp761 are involved in binding to magnesium (Mg$^{2+}$) ions. These magnesium ions are involved in catalysis at the active site of the RdRp enzyme. Yin et al., (2020) report that RMP is covalently bound to the primer RNA strand with the magnesium ions and the pyrophosphate group from RTP located nearby. They also note that a sidechain of Arg555 helps to stabilise
inbound nucleotides at the catalytic site. This is supported by Pruijssers et al., (2020) who state that Arg553 and Arg555 help to coordinate RTP. The latter authors also found that the OH group of the ribose 2′C is involved in hydrogen bonding to Thr680 and Asn691 and the 1′-CN group lies in a pocket formed by Thr687 and A688.

The differences noted in Table 3.1 perhaps demonstrate the infancy of the current research. A greater understanding of the interactions between RDV (via RMP and RTP) and RdRp will be necessary to further develop RDV and other NA’s and offers the potential for considerable further research.

3.4 – Exonuclease

Coronaviruses (CoVs) possess an exonuclease, also known as nsp14, which is capable of removing a mis-incorporated nucleotide. Such a mechanism has been demonstrated for another antiviral drug, ribavirin (Ferron et al., 2017). However Kokic et al., (2021) note that RTP is able to partially evade excision by the ExoN. Agostini et al., (2018) suggest that drugs effective against CoVs must be able to either directly inhibit the ExoN, be incorporated in the growing RNA strand more quickly than copying errors can be corrected or not be recognised for excision by the ExoN. The ability of the ExoN to recognise and correct modifications made to the ribose or the nucleobase in NA’s could be a productive area in future development of this class of drugs (Shannon et al., 2020). As an example they show that RMP is positioned in nsp14 in such a way that its base becomes distorted forcing the ribose to move close to the catalytic Mg\(^{2+}\) ions which prevents efficient catalysis taking place. The same authors suggest that coupling drugs such as RDV with others which have the ability to counteract the ability of the ExoN to excise nucleotides may be another option to explore. Such an approach has been taken further in a recent pre-print study (Khater, Dasgupta and Das, 2020) where the authors have identified through molecular docking studies a number of promising drug candidates for co-administering with RDV.

3.5 – Where next

3.5.1 – GS-441524

The core active component of RDV is the adenosine analogue GS-441524 (Figure 3.5) which has been suggested as an alternative to RDV (Yan and Muller, 2020). The authors argue that the prodrug moiety of RDV is not designed for delivery to the lungs to combat a respiratory virus such as SARS-CoV-2 and that the predominant metabolite is GS-441524. Compared to RDV this is a less complex molecule with a less complex synthetic route.

These conclusions were borne out recently by another research group which found that GS-441524 was highly effective against SARS-CoV-2 using in-vitro mouse models (Li et al., 2021). The authors claim that GS-441524 can be efficiently triply phosphorylated to the active drug, RTP which would avoid the need to use the synthetically complex prodrug. However, the authors acknowledge in the same paper that studies have shown that RDV is more effective than GS-441524 in SARS-CoV-2 infected HAE cells (Pruijssers et al., 2020). It is clear that more research is needed in this area however the ability to deliver the active ingredient of RDV orally rather than intravenously as is the case for the current prodrug could be a major step forward.
3.5.2 – Alternative nucleoside analogues

An alternative drug candidate proposed to treat COVID-19 is β-D-N4-hydroxycytidine (EIDD-1931) and its related prodrug EIDD-2801 (also known as molnupiravir—Figure 3.6) These drugs have a similar structure to RDV but they achieve efficacy by the process of lethal mutagenesis whereby accumulated copying affects the secondary structure of RNA (Robson et al., 2020). According to Yin et al., (2020), EIDD-2801 forms an additional two hydrogen bonds with the RNA template compared to RMP. These are between the hydroxyl group adjacent to the cytidine ring and the side chain of Lys545 together with a further bond between the cytidine and guanine bases. The authors note that EIDD-2801 has been shown to be up to 10 times more effective than RDV in stopping replication of SARS-CoV-2 (Sheahan et al., 2020). These results are supported by a very recent study which used mice implanted with human lung tissue and which demonstrated a more than 25,000 fold reduction in SARS-CoV-2 virus levels when treated with EIDD-2801 24 hours after exposure (Wahl et al., 2021).

Other NA’s which have a similar structure to RDV and could form the basis for incremental changes to improve the efficacy of RDV include:

- Galidesivir;
- Sofosbuvir; and
- Carbovir

The structure of all these molecules is shown in Figure 3.6 below:
3.5.3 – Proposed modifications to RDV

By comparing the core structural component of RDV, GS-441524, with the other NA’s in Figure 3.6 it is possible to envisage structural modifications to RDV which may enable RMP to bond more efficiently to the RdRp and possibly increase its efficacy. Any modifications to RDV however would have to take into account the 2′-OH on ribose as this appears to be required for the proofreading function of the ExoN (Jockusch et al., 2020).

Some suggested improvements (Duffy, 2021) are set out in Figure 3.7 below:

![Figure 3.7 – Proposed modifications to GS-441524 (highlighted in yellow), (Duffy, 2021).](attachment:image.png)

Galidesivir (Figure 3.6) has a similar structure to GS-441524 however the oxygen atom in the ribose is replaced with an amine. This could be a modification worth exploring further and a proposed structure is shown as 1 in Figure 3.7.

Both sofosbuvir and carbovir possess a carbonyl group on the nucleobase. This may lead to the formation of additional hydrogen bonds and increase the overall binding of the drug to its target. It is proposed that a RDV could be modified with addition of a carbonyl group as shown in 2 in Figure 3.7.

As already noted above, molnupiravir forms two additional hydrogen bonds with RNA compared to RDV. One of these is located at the hydroxyl group adjacent to the cytidine ring and this together with addition of a carbonyl group may be productive modifications to RDV as shown in 3 especially in the light of very promising therapeutic results for molnupiravir noted above. Addition of these groups may increase the number of hydrogen bond donor-acceptor interactions between RMP and proximate amino acid residues. This may promote stability of RMP in the synthesised RNA strand and help to resist the action of the ExoN thereby improving efficacy.

It is recognised that the proposed modifications may give rise to unnatural base pairings e.g. the additional carbonyl on 3 could induce pairing with cytosine. However a recent paper argues that such pairings induce mutations in the extension of RNA causing chain termination.
to occur (Jena, 2020). The nature of the unique RNA base pairings between adenine and its natural partner uracil is an ongoing area of research (Ding et al., 2018).

Initial development of GS-441524 had involved screening a large number of candidate drug compounds. Potential shortlisted drugs included modified versions of GS-441524 shown in Figure 3.8 below:

![Figure 3.8 – Previous modifications to GS-441524 (Siegel et al., 2017).](image)

Siegel et al. (2017) had been investigating potential treatments for EVD. Results had indicated that compounds 4 and 5 had little efficacy against EVD however they had demonstrated considerable activity against another virus, Hepatitis C. Although the RdRp of SARS-CoV-2 is highly conserved and likely to be similar to the RdRp of EVD, it could be worthwhile modifying the structure of RDV with either of the additional features shown in 4 or 5 above and testing these for efficacy against the virus. A review of the literature does not reveal this as having been done to date.
CHAPTER 4

Synthesis

4.1 – Existing synthetic routes

RDV is an example of a C-nucleoside drug which has a carbon (1′C) of the ribose sugar ring bonded to another carbon in the nucleobase. Although many C-nucleosides have been developed since the late 1950’s, only N-nucleosides (1′C bonded to nitrogen in the base) had been approved by the US Food and Drug Administration (Wang and Yang, 2020). An example of an N-nucleoside is ribavirin (Figure 4.1).

Researchers at Gilead had pursued development of new antiviral drugs leading to synthesis of a novel C-nucleoside drug candidate, GS-441524. In addition to this new molecule being a C-nucleoside, a key modification was the cyanation at the 1′C position (Siegel et al., 2017). This is shown in Figure 4.1.

The newly developed GS-441524 was subsequently developed into its current prodrug form, RDV (Figure 4.2).
Prodrugs like RDV possess an additional moiety, which in RDV’s case masks a negative charge which would otherwise prevent efficient diffusion into the cell. Key building blocks in the synthesis of RDV are therefore:

- prodrug moiety;
- ribose sugar; and
- nucleobase.

The prodrug moiety consists of a 2-ethylbutyl-2-aminopropanoate molecule bonded to anisole via a phosphoramidate group (Figure 4.3).

Siegel et al., (2017) noted that Gilead had published two routes to the synthesis of RDV. The initial synthetic route was carried out at very low temperature of -78°C giving a low yield of 5.4%. This initial synthesis of RDV produced a roughly equal mixture of stereoisomers, known as Sp and Rp, however Siegel et al., (2017) determined the Sp version to be more effective. Stereoisomers were separated by chiral HPLC in this synthetic route. The requirement for chiral HPLC together with a combination of very low temperatures and yields led to development of a second-generation synthesis which was carried out at -20°C with a yield of 12.7%.

The second-generation synthesis of RDV took advantage of the favourable properties of the turbo Grignard reagent i-PrMgCl-LiCl (Scheme 1). This offers greater reactivity and selectivity compared with conventional Grignard reagents whilst enabling functional groups to tolerate milder conditions (Li-Yuan Bao, Zhao and Shi, 2015). This second-generation synthesis produced a diastereoselective route for the Sp isomer which generated greater amounts of RDV for preclinical testing.
Key steps in the synthesis of RDV are:

- protection of the reactive hydroxyl groups in the ribose sugar (an initial step not shown above in Scheme 1 and step D);
- attachment of the ribose to the nucleobase (C-Glycosylation- step A);
- cyanation of the 1'C of the ribose (step B)
- deprotection of the ribose benzyl and hydroxyl groups (steps C, F); and
- attachment of the prodrug moiety (step E).

The third-generation synthesis patented by Gilead used a variation in the method of protection for the hydroxyl groups on the ribose (Kokic et al., 2021).
4.2 – Continuous flow routes

Alternative synthetic approaches to RDV include a continuous flow method for the C-Glycosylation step (Von Keutz, Williams and Kappe, 2020). This could simplify the existing batch process which uses three temperature ranges from -20°C to +20°C and takes more than 11 hours. By comparison the continuous flow method can achieve complete synthesis in one minute at a temperature of +20°C. Both these processes use the Grignard reagents PhMgCl and i-PrMgCl·LiCl for formation of a new carbon to carbon bond between the ribose and nucleobase. The same authors have also proposed in a follow-up study a variation of the continuous flow process using flash chemistry. Flash chemistry flow processes are very fast and in the order of seconds permitting reactions which would otherwise be very difficult or impossible (Von Keutz, Williams and Kappe, 2021). This process resulted in the formation of the product in just 8 seconds at a temperature of -30°C. This flash process uses organolithium reagents for the formation of the carbon to carbon bond.

A continuous flow route has also been proposed for the cyanation of the 1′C of the ribose constituent of RDV (Vieira et al., 2020). This process essentially uses the same chemical precursors as the existing route developed by Gilead however it is performed at a higher temperature of -30°C (compared with -78°C). Yield is comparable at 78% (versus 85%) with 99.9% purity. A scaled up plant produced around 500kg of product enabling provision of RDV at quantities for clinical trials.

4.3 – Other syntheses

The potential need for the manufacturing and supply of large quantities of RDV has seen attention focussed on synthetic routes to its precursors. In particular, two recent approaches have been described towards the synthesis of the nucleobase fragment, pyrrolotriazine. The first of these (Paymode et al., 2020 – Scheme 2) used pyrrole as a starting point synthesising pyrrolotriazine in two steps compared with the published four step route. This increased the yield of pyrrolotriazine from 31% to 59% and the authors claim that their process uses only readily available starting materials.

Scheme 2 – Alternative synthesis of pyrrolotriazine. Adapted from Paymode et al.(2020). (A) POCl3, DMF then NH₂OH. (B) NH₂Cl then HN=CHNH₂ · CH₃COOH.
An alternative approach to the synthesis of pyrrolotriazine is described by Knapp et al., (2020). The starting material for this route is 2,5-dimethoxyfuran with a two-step reaction producing formamide. Next a condensation reaction with cyanamide produces cyanoamidine as an intermediate with the final product delivered via a Lewis acid cyclisation (Scheme 3). The authors suggest this method could in theory just produce water as a by-product and that it would provide an economical route to the nucleobase in RDV and other similar small-molecule drugs.

Scheme 3 – Formation of nucleobase via a cyanoamidine cyclization. Adapted from Knapp et al., (2020). (A) Step 1: H₂N-NHBoc, HCl, NMP, 90°C; Step 2: Ac₂O, 23°C, formic acid. (B) NC-NH₂, NaOMe, MeOH, 23°C. (C) SnCl₄, 1,2-dichloroethane, 90°C, 16 hours.

An important recent development has been in the coupling of the prodrug moiety to GS-441524 (Wang et al., 2020). In this paper the authors describe the use of a novel catalyst to significantly increase the yield of RDV to around 65%. This has been achieved using a dynamic kinetic asymmetric transformation (DyKAT) with a bicyclic imidazole catalyst which achieved excellent stereoselectivity towards the $S_p$ isomer. This is shown in Scheme 4 below:
Scheme 4 – coupling of the prodrug moiety to nucleoside GS-441524 using a bicyclic imidazole catalyst. Adapted from Wang et al., (2020).

4.4 – Where next

The multi-step nature of the above syntheses demonstrate the challenges faced in the manufacture of RDV. Hardy et al., (2020) note that further improvements in synthesis are required in the following areas:

- C-Glycosylation;
- Use of protecting groups; and
- Coupling of the Phosphoramidate moiety.
Further improvements in the yield of RDV using imidazole carbamates catalysts have been shown in a recent study by Gannedi et al., (2021). In a 10g scale experiment this group demonstrated synthesis of RDV with a yield of 70% using this catalyst with 83% of the catalyst being recovered for re-use. The catalyst is shown in Figure 4.4.

![Imidazole carbamate catalyst](image)

**Figure 4.4 — Imidazole carbamate catalyst** — Adapted from Gannedi et al., (2021).

The use of imidazole catalysts by Wang et al., (2020) described earlier follows on from previous work by DiRocco et al., (2017) which addressed the challenges of stereoselectivity at the phosphorus centre of prodrugs. This same group has very recently published an important paper describing significant improvements in the synthesis of uprifosbuvir, a nucleoside prodrug related to RDV (Klapars et al., 2021). The authors describe in this paper a new synthetic route to uprifosbuvir in 5 steps compared with 11 in the existing synthesis. This has led to a 50-fold improvement in yield from 1% to 50% and features a number of synthetic improvements including the use of imidazole carbamate catalysts. The authors believe their work should help in improving the efficient synthesis of other nucleoside antivirals. Indeed a leading researcher in HIV antivirals has recently stated that this new synthetic approach could be useful in the preparation of C-nucleoside drugs such as RDV (De Clercq, 2021).
CHAPTER 5

Conclusions and recommendations

5.1 – Conclusions

The current pandemic caused by SARS-CoV-2 demonstrates the urgent need for effective antiviral drugs which can be widely administered to deal with future variants of the virus, entirely new coronavirus strains or other novel viruses. NA’s can be further developed to meet this need. In particular RDV and similar NA’s such as molnupiravir have shown great promise in tackling coronaviruses and incremental changes to the structure of RDV may have a significant effect on its efficacy.

The objectives of this paper were to review the efficacy, mode of action and synthesis of RDV and other similar NA’s and to make suitable suggestions for improvements where possible. These objectives have been achieved. Efficacy has been briefly reviewed as a backdrop to mode of action and synthesis. Suggestions for improvements in mode of action have been made and are summarised further below. Developments in the synthesis of RDV are fast moving and whilst it has not been possible to make any additional suggestions for improving these, the latest research has been reviewed and set out.

5.2 – Recommendations

This paper has reviewed the only antiviral drug approved to date by the US Food and Drug Administration to treat COVID-19 (Li et al., 2021) and has suggested potential improvements to its structure and reviewed improvements in its synthesis. It is recommended that the modifications to the structure set out in Figure 3.7 are carried out and that these are tested for safety and efficacy. Compound 3 in particular could offer great potential with the addition of carbonyl and hydroxyl groups. Furthermore compounds already developed by Gilead scientists for EVD as shown in Figure 3.8 should be tested against current and future strains of SARS-CoV-2.

There are considerable differences across the studies reviewed in this paper relating to the mode of action of RDV and its interactions with RdRp. It is recommended therefore that further research is carried out to clarify the mode of action as this will inform further drug development.

In relation to the synthesis of RDV, this project has identified the key synthetic steps and highlighted the latest research. It is recommended that research continues into improvements in these key steps and recent papers have shown that novel catalysts in particular could be a fruitful area of research.

The United Kingdom’s Scientific Advisory Group for Emergencies (SAGE) has recently reported that the future emergence of a SARS-CoV-2 variant with mortality rates between 10% and 35% is a realistic possibility (SAGE, 2021). To prepare for such a possibility they have advocated continued development of prophylactic and therapeutic drugs to treat future outbreaks. It is vital therefore that development of new antivirals drugs continues apace and this paper has signposted a number of potential areas of research.
REFERENCES:


<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>C-Glycosylation</td>
<td>Attachment of a sugar group via a new carbon-carbon bond.</td>
</tr>
<tr>
<td>C-nucleoside</td>
<td>Attachment of a nucleoside to a sugar group via a carbon-carbon bond.</td>
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<tr>
<td>Chain termination</td>
<td>Cessation of synthesis of a new RNA primer strand from a template due to a stalling mechanism.</td>
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<tr>
<td>Cyano group</td>
<td>A chemical group comprising a carbon atom triple bonded to a nitrogen atom.</td>
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<tr>
<td>DyKAT</td>
<td>Dynamic kinetic asymmetric transformation technique used in organic chemistry reactions.</td>
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<tr>
<td>EC₅₀</td>
<td>That concentration of a drug which results in a half-maximal response.</td>
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<tr>
<td>Exonuclease</td>
<td>An enzyme which can remove a nucleotide from a polynucleotide chain.</td>
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<tr>
<td>HAE cells</td>
<td>Culture of cells derived from epithelium tissue found in human airways.</td>
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<tr>
<td>Lethal mutagenesis</td>
<td>Accumulation of mutations in a virus leading to permanent loss of viability.</td>
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<tr>
<td>Lewis acid cyclisation</td>
<td>Formation of a ring compound via the use of a Lewis acid.</td>
</tr>
<tr>
<td>N-nucleoside</td>
<td>Attachment of a nucleoside to a sugar group via a carbon-nitrogen bond.</td>
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<tr>
<td>Prodrug</td>
<td>A drug which becomes pharmacologically active after being subject to further chemical reactions in-vivo.</td>
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<tr>
<td>Translocation barrier</td>
<td>A barrier to the movement of the RNA strand through the RdRp.</td>
</tr>
<tr>
<td>Zoonotic</td>
<td>Description of a virus capable of being transmitted from animals to humans or vice-versa.</td>
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