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Elucidation of the Pharmacological Development and Action Mechanism of Remdesivir and Paxlovid in Combatting COVID-19

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Abstract:

COVID-19, the disease identified in 2019 as coronavirus disease, stems from an infection by severe acute respiratory syndrome coronavirus 2, or SARS-CoV-2, which possesses a single-stranded RNA genome. Since the onset of the pandemic attributed to COVID-19, a variety of vaccines and antiviral medications have received approval from international regulatory bodies. Two notable antiviral agents, remdesivir and paxlovid, serve as treatments for viral infections. Initially developed to combat Ebola, remdesivir has also demonstrated efficacy against a broad spectrum of coronaviruses (CoV). It is a nucleoside analogue and functions by being incorporated into the RNA chain of the virus and thus induce the early termination of RNA synthesis. Paxlovid comprises two pharmacological agents, nirmatrelvir and ritonavir. The mechanism of nirmatrelvir involves the specific targeting and inhibition of the viral protease's active site, thereby impeding the viral replication process. By inhibiting the cytochrome CYP3A4 enzyme which metabolites nirmatrelvir, ritonavir acts as a pharmacokinetic enhancer in this combination. The initial section of this review provides a comprehensive analysis of SARS-CoV-2, detailing the mechanisms of its pathogenesis and the subsequent immune responses elicited by the body. Then this article generalizes the history of development, the structure, and the mode of action of remdesivir and paxlovid.

Keywords: COVID-19, SARS-CoV-2, remdesivir, nirmatrelvir / ritonavir, polymerase inhibitor, nucleoside analogue, protease inhibitor, main protease (Mpro)

1. Introduction

1.1 COVID-19: symptoms, mortalities, and significance

In December 2019, Wuhan, China, reported the first incidents of infections linked to the novel coronavirus, SARS-CoV-2. Those affected exhibited symptoms such as elevated body temperature, persistent cough, significant fatigue, shortness of breath, and production of sputum [1]. As of April 13, 2024, the global pandemic of COVID-19 has resulted in a cumulative death toll of 7,010,681 [2].

1.2 SARS-CoV-19: structure and pathogenesis

SARS-CoV-19 is a member of the β-coronaviruses which is one of the genera of coronaviruses (CoV). CoVs possess an enveloping structure enclosing a single-stranded RNA of positive sense. The SARS-CoV-2 genome encodes 29 proteins, which are classified into three categories: 16 nonstructural proteins, 4 structural proteins, and 9 accessory proteins. The structural proteins of this virus inclued the spike (S), membrane (M), envelope (E), and nucleocapsid (N). The pathogenic phenotype and viral tropism are significantly influenced by the S glycoprotein. Research has shown that the S protein predominantly aids in the attachment of SARS-CoV-2 to the ACE2 receptor found on host cells, which is important for the virus membrane fusion and entry. Additionally, the M protein is crucial in maintaining the structural integrity of the virus, facilitating the development of the viral envelope, and enhancing virus release and budding. This protein also plays a key role in stablizing the N protein to the viral RNA, which is essential for the replication process of SARS-CoV-2. Study shows that the E protein is a virulence domain that causes SARS-CoV infection-related immunopathology [3]. The coronavirus genome includes 16 nonstructural proteins (NSPs), which contain 14 functional open reading frames (ORFs) and are flanked by two noncoding regions, one at each end of the genome. The 16 NSPs (nsp1-nsp16) that ORF1a and ORF1b encode are necessary for viral RNA synthesis [4].

1.3 Vaccines

At the moment, 104 potential vaccines are in the clinical phases of development and 184 are in the preclinical stages. Recent data indicate that 18 COVID-19 vaccines have received authorization for use worldwide. There are four primary categories of COVID-19 immunizations, distinguished by their technological foundations: 1) vaccines based on the entire virus, 2) those that use protein subunits, 3) immunizations employing viral vectors, and 4) accines developed using nucleic acid methodologies.

Whole virus vaccines employ either inactivated or attenuated versions of SARS-CoV-2 to stimulate an immune response. In the case of live attenuated vaccines, the employed virus retains the ability to replicate and disseminate but is incapable of causing human infection due to its significantly reduced virulence. Inactivated vaccines utilize viruses that have undergone genetic modification through exposure to radiation, heat, or chemicals. These viruses are incapable of replication and infection due to the destruction of their genetic integrity. Nevertheless, they remain effective in provoking an immune response. The foundation of many current vaccines is built on the principle of using whole virus particles.

Protein-based vaccines are classified into two main types: subunit vaccines and virus-like particle (VLP) vaccines. The former involves the utilization of viral antigens synthesized through recombinant protein techniques. On the other hand, VLP vaccines consist of non-infectious hollow structures that mimic the morphology of CoV without containing viral genetic material.

To propagate and thrive, viruses must penetrate host cells and commandeer their protein synthesis machinery. This hijacking enables transcription and replication of the viral genetic code, leading to the production of new viral entities. Antigens, integral components of viral particles, can trigger immune responses. Viral vector vaccines employ a similar principle but are engineered to deliver only the genetic instructions necessary to produce specific antigens, without producing new viruses. The viral vector acts as a delivery system, introducing the genetic blueprint of the SARS-CoV-2 antigen into the host cells to facilitate cellular entry.

Nucleic acid vaccines targeting SARS-CoV-2 utilize sequences encoding proteins that are capable of provoking an immune response. These sequences are embedded within either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) frameworks specific to the vaccine design [5].

2. Remdesivir

2.1 History of development

A collaborative effort involving Gilead Science, U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), and the U.S. Centers for Disease Control and Prevention (CDC) resulted in the development of the antiviral agent Remdesivir, designated GS-5734. They looked for treatment options for RNA-based viruses that still had the potential to spread globally. A compound library of around 1000 small molecules, mainly centered on analogues of nucleosides, was assembled as an initial resource for antiviral drug discovery against RNA viruses. Given that nucleosides generally exhibit low permeability across cell membranes, which may result in a decreased hit rate during cell-based antiviral screening, a substantial portion of the library was composed of chemically modified nucleosides. These modifications included monophosphates, esters, and phosphonamidite prodrugs to potentially enhance cell permeability.

The compiled library was used to find and rank chemicals that were effective against Ebola when it broke out in 2014. The research conducted by Madelain et al. identified that remdesivir (GS-5734) effectively sustained its antiviral activity in in vivo models of EBOV infection using nonhuman primates. Additionally, the compound was found to inhibit EBOV replication, achieving an IC50 = 100 nM.

Evaluations both in vitro and in vivo were performed by researchers to assess the antiviral capabilities of GS-5734. This study follows on from earlier studies demonstrating the efficacy of this compound against various RNA viruses. The compound's antiviral efficacy against zoonotic CoVs, including MERS and SARS, has also been demonstrated.

The antiviral effects of remdesivir against SARS-CoV-2 has been confirmed by *in vitro* research. Further investigations by Sheahan *et al*. and de Wit *et al*. have confirmed its efficacy against related coronavirus by showing its function in stopping the viral replication and mitigating the damage caused by the virus in live organisms. Such evidence supports that remdesivir could serve as a viable therapeutic option for combating the SARS-CoV-2 pandemic [6].

2.2 Mechanism

RNA-dependent RNA polymerase (RdRp) is needed by CoVs for both the transcription and replication of their RNA genome. The process of the metabolism of remdesivir produces the active metabolite remdesivir triphosphate (RTP). This compound acts as an analogue of natural nucleoside triphosphate (NTP). Studies show

that RTP is incorporated by RdRp into the growing RNA strand. This process leads to the inclusion of remdesivir monophosphate (RMP). Following the insertion of this analogue, RdRp is only able to add three more nucleotides to the RNA chain before the process is halted [7].

After remdesivir is incorporated into the growing RNA chain, the RdRp undergoes a translocation hindrance. This process leads to the incorporation of three additional nucleotides followed by a stalling of the polymerase. The C1'-cyano motif of the rameltegravir ribose molecule is responsible for the translocation barrier [7].

2.3 Mode of action

Remdesivir is a nucleoside analogue. There are several restrictions to the use of nucleoside analogue medication. Nucleoside analogues are hydrophilic, they have low membrane permeability, which makes it difficult for them to efficiently enter cells. To enable the molecule to enter cells efficiently, researchers used the ProTide strategy to it. ProTides are altered nucleotides. These nucleotides include a nucleoside monophosphate which is attached to an aromatic moiety and an amino acid ester. The hydrophobic segment of the aryl group enables remdesivir to pass through cell membranes. The ester bond enables the molecule to pass through the cell membrane by having additional hydrophobic properties. Once the molecule enters the cell, these additional groups (aryl group and amino acid ester) are cleaved by intracellular enzymes including phosphoramidases and esterase and the active RMP is released. Cellular kinases then quickly phosphorylate the active RMP and forms the active triphosphate that can prevent viral RNA polymerase from acting [6] [8].

Remdesivir contains a nucleoside monophosphate. This structure is modified with an acrylic moiety and an amino acid ester. Once the compound enters the cell, metabolic transformations occur and finally lead to the formation of the nucleoside monophosphate form. Transformations are then initiated by hydrolytic enzymes. These enzymes are primarily esterases and function by cleaving amino acid esters to release carboxylate groups. These groups then cyclically bond to the phosphorus atom, consequently ejecting the phenoxide group. This reaction forms an unstable cyclic anhydride. This cyclic anhydride is rapidly hydrolyzed by water, forming the alanine metabolite GS-704277. Subsequently, enzymes known as phosphonamidites facilitate the cleavage of the phosphorus-nitrogen bond, resulting in the liberation of the nucleoside monophosphate. The active NTP analogue is formed through additional phosphorylation events and is subsequently utilized by the viral RdRp. Then chain termination is caused, which prevents viral replication [6].

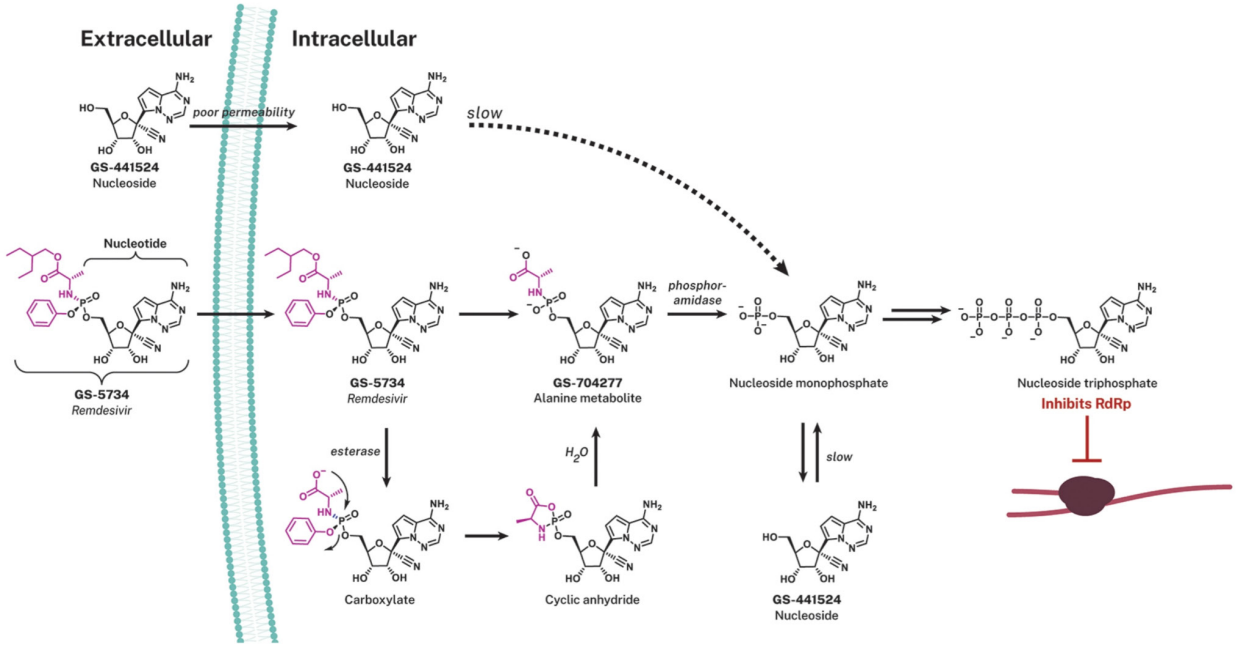

Figure 2. The conversion of remdesivir prodrug to nucleoside monophosphate. Reproduced from [6], copyright: 2020, ACS Central Science

2.4 Mode of delivery

Remdesivir is given intravenously (IV) over a maximum of 10 days. On the first day, an adult loading dose of 200 mg is delivered, tailored according to the body weights of pediatric patients. Subsequently, adults receive a daily maintenance dose of 100 mg. Administered at 10 mg/kg

daily, Remdesivir exhibits a brief prodrug plasma half-life (t1/2= 0.39 h) in nonhuman primates but maintains effective intracellular levels of the active triphosphate derivative [6].

3.Paxlovid

3.1 Ingredient

Paxlovid is a combination of ritonavir and nirmatrelvir. Nirmatrelvir acts as a reversible covalent inhibitor, structurally designed as a peptidomimetic, which specifically targets the main protease (Mpro) of SARS-CoV-2. This protease is vital for the replication cycle of the virus. Concurrently, ritonavir serves to enhance the pharmacokinetic profile of nirmatrelvir by inhibiting CYP3A4 irreversibly, which is the enzyme mainly responsible for nirmatrelvir's rapid metabolism. This inhibition significantly increases the plasma half-life and improves the systemic availability of nirmatrelvir [9].

3.2 Nirmatrelvir: Development of chemical structure

PF-00835231, developed by Pfizer, was initially engineered to inhibit the primary protease of the SARS-CoV-1 virus and subsequently served as a precursor to nirmatrelvir (PF-07321332). The effectiveness of this compound in targeting the Mpro of SARS-CoV-2 stems from its structural congruence with the proteases of both SARS-CoV-1 and SARS-CoV-2.

PF-00835231's extremely low oral absorption is the issue. Pfizer initiated a development strategy to address the issue (Figure 3). The reduction in hydrogen bond donor groups within a molecule can enhance its oral bioavailability. Accordingly, the α-hydroxymethyl ketone warhead was substituted with a benzothiazol-2-yl ketone group. Additionally, the synthesis of compound 1 involved replacing the P2 unit with a pyrrolidine ring, thereby eliminating the N–H group, a known hydrogen bond donor. These modifications, however, impeded the capacity of the amino acid Gln-189 in Mpro to form a hydrogen bond, diminishing the molecule's interaction with the target enzyme and decreasing its inhibitory effectiveness. Additionally, the indole moiety at P3 was substituted with a smaller, non-cyclic sulfonamide group to better fit the S3 pocket of Mpro. This increased the enzyme's binding affinity and, as a result, the inhibitory effect. Compound 2 outperformed Compound PF-00835231 in terms of antiviral activity and had a higher oral bioavailability. The trifluoroacetamide group (highlighted in green in figure 3) was subsequently added to the P3 cap, significantly enhancing its antiviral activity and improving oral pharmacokinetics and metabolic stability. Incorporating a nitrile warhead at the P1′position, highlighted in green within the nirmatrelvir molecule structure, has substantially improved both its oral bioavailability and its effectiveness against viruses. This enhancement can be attributed to the increased solubility and reduced propensity for epimerization of the nitrile-containing compound, which, in turn, simplified the manufacturing process [9].

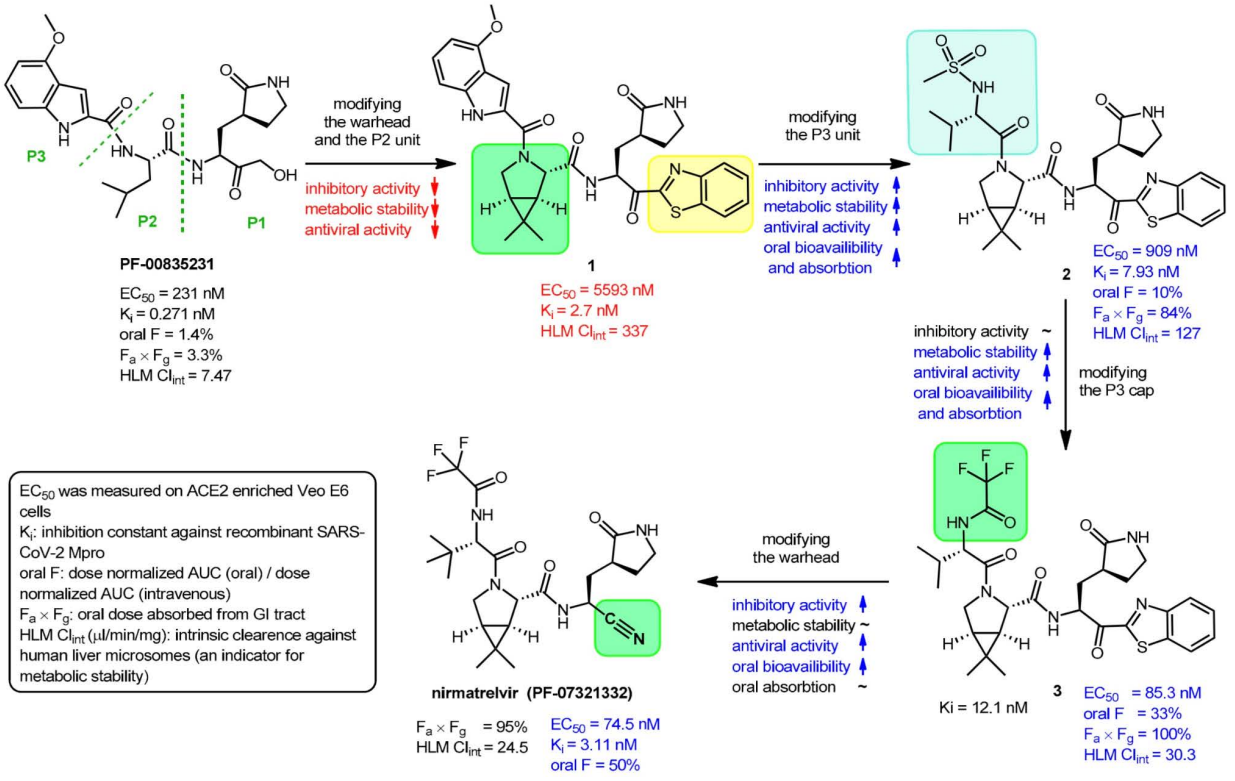

Figure 3. Development of nirmatrelvir. Reproduced from [9], copyright: 2024, MDPI

3.3 Nirmatrelvir: Mechanism

The SARS-CoV-2 genome is responsible for the coding of four structural proteins along with two polyproteins, designated as pp1a and pp1ab. The main protease, Mpro, executes cleavages at 11 distinct sites on these polyproteins, facilitating the generation of NSPs vital for the virus replication cycle. Inhibiting these cleavage actions would effectively halt viral propagation. Characterized as a cysteine protease, Mpro demonstrates a preference for glutamine at its P1 position. Across the Coronaviridae family, the conservation of Mpro is notable; the protease active sites in both SARS-CoV-1 and SARS-CoV-2 are highly similar, sharing approximately 96% amino acid sequence identity [10].

Figure 4 shows the main structural parts of nirmatrelvir. In this molecular configuration, the warhead positioned at the P1′ site incorporates a nitrile group, which actively participates in reactions within the enzyme. At the P1 position, introduction of a γ-lactam ring as a cyclic side chain enhances stability and hydrogen bonding potential compared to the original Gln amide group, which could adversely interact with specific warhead types and reduce molecular efficacy. This modification not only bolsters molecule binding affinity but also simplifies its synthesis. Furthermore, the P2 position features dimethyl cyclopropyl proline (DMCP), a leucine derivative, facilitating lipophilic interactions at the enzyme S2 site. The residue at position P3, which mirrors the properties of valine, is structurally identified as tertiary leucine, enhancing its efficacy in interacting with the target site. The molecule also has a trifluoroacetyl group at N-termina [9].

Nirmatrelvir's protease inhibitory action heavily relies on the nitrile warhead. It is important to remember that the nitrile group targets serine and cysteine peptidases in a variety of medications and therapeutic candidates. Despite its lower reactivity compared to other functional groups like aldehydes, the nitrile group maintains exceptional metabolic stability and enhanced selectivity.

Figure 5 illustrates the covalent interaction between nirmatrelvir and the primary protease of SARS-CoV-2. The action mechanism involves the P1′ nitrile moiety of nirmatrelvir establishing a thioimidate bond with the thiol group located on Cys-145 within the target enzyme, a process similar to that observed in the Pinner reaction. This bond is essential as it impairs the function of the enzyme by targeting the critical thiol group at Cys-145, which is indispensable for the hydrolysis of peptide bonds [9].

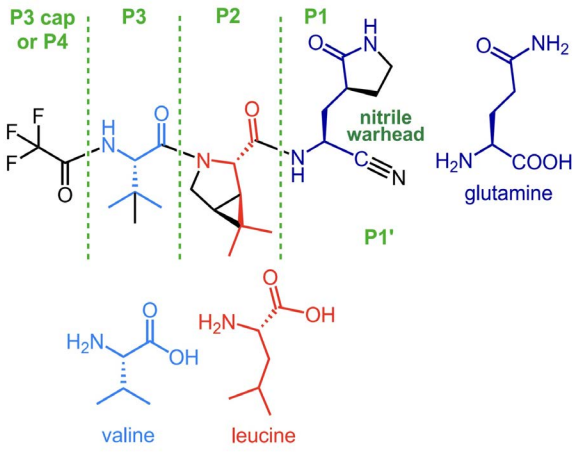

Figure 4. Main structural elements of nirmatrelvir. Reproduced from [9], copyright: 2024, MDPI

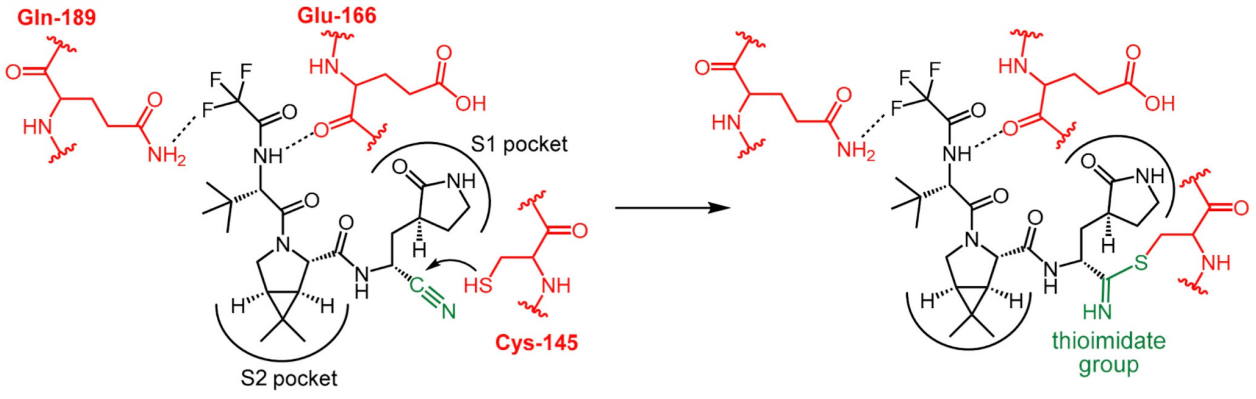

Figure 5. Mechanism of action of nirmatrelvir Reproduced from [9], copyright: 2024, MDPI

3.4 Ritonavir: Development

In Paxlovid, ritonavir functions as a CYP3A4 inhibitor, amplifying the effects of nirmatrelvir. CYP3A4 belongs to the cytochrome P450 family of enzymes, predominantly synthesized in the liver. It plays a critical role in the metabolic breakdown of a myriad of both intrinsic and foreign substances, encompassing various pharmaceuticals [9].

3.5 Ritonavir: Mechanism

The mechanisms by which ritonavir inhibits the CYP3A4 enzyme remain not fully elucidated, with various theories proposed in scholarly discussions. One theory suggests that ritonavir may bind directly to the heme iron of the enzyme. Another hypothesis posits that a yet unidentified metabolic byproduct of ritonavir could coordinate with the heme component of the enzyme. Additionally, it is speculated that a reactive derivative of ritonavir might form a covalent bond with the CYP3A4 apoprotein. For the last mechanism, two specific pathways have been proposed (Figure 6). One hypothesis is that an elimination reaction occurs after CYP3A4 oxidizes ritonavir, leading to the formation of an isocyanate derivative (route B).Isocyanate groups in ritonavir react with nucleophilic groups in proteins, leading to the formation of covalent bonds with CYP3A4, which inactivates the enzyme. As a result, new synthesis of CYP3A4 molecules is required to recover enzymatic function, accounting for the gradual decline in the inhibitory effect of ritonavir after discontinuation. An alternate pathway involves the oxidation of the thiazole ring in ritonavir, resulting in epoxide formation. Subsequently, this epoxide is attacked and opened by the lysine residue at position 254, enabling a covalent attachment of ritonavir to the apoprotein, as delineated in route A. [9].

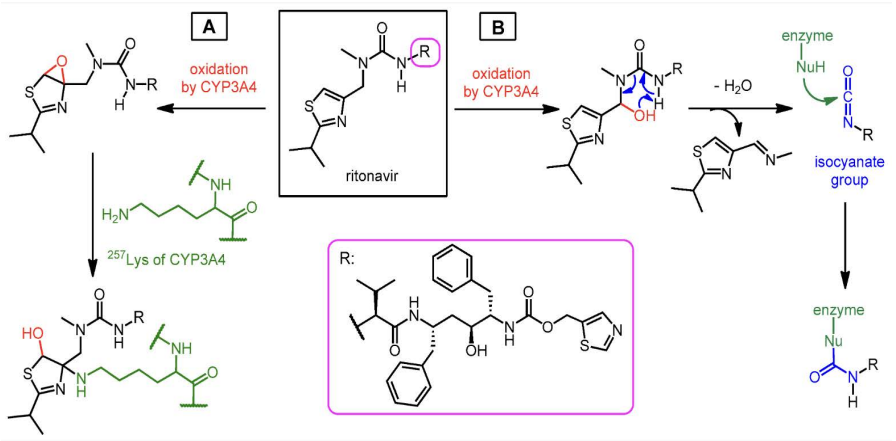

Figure 6. Possible mechanism of the irreversible inactivation of CYP3A4 by ritonavir. Reproduced from [9], copyright: 2024, MDPI

3.6 Ritonavir: limitations

One of the drawbacks of ritonavir is its potential to cause drug-drug interactions (DDI). Since ritonavir inhibits CYP3A4, any medications a patient takes that are metabolized by this enzyme are likely to interact. This interaction can result in ritonavir increasing the concentration of the other medication to potentially toxic levels. Given the proximity of effective and toxic doses in drugs with a narrow therapeutic index, it is imperative to manage interactions cautiously. For ritonavir, known to alter metabolic processes, clinicians advise suspending or replacing drugs that are mainly metabolized by CYP3A4 during and for three days following Paxlovid therapy. Nonetheless, this recommendation might not hold for drugs with prolonged half-lives, as their plasma levels may remain elevated well beyond the cessation period [9].

4. Conclusion

SARS-CoV-2, a single-stranded RNA virus, causes COVID-19. Researchers have formulated multiple vaccines and antiviral agents targeting this virus. Specifically, Remdesivir disrupts the RdRp, while Paxlovid blocks the Mpro protease, both essential for viral replication. Remdesivir is a prodrug which consists of a nucleoside analogue and a protide compound. Once enters the cell, it needs to be metabolized and turns into nucleoside monophosphate, then being incorporated into the RNA chain. Paxlovid comprises the antiviral agents nirmatrelvir and ritonavir. The former functions as the protease inhibitor, the later inhibits the CYP3A4 enzyme to prevent the nirmetralvir from being metabolized.

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