# Structure and Inhibitor Development of Coronavirus Main Protease NSP5

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Abstract: The global health crisis triggered by COVID-19, a SARS-CoV-2 virus, has presented unparalleled challenges on the global health system, resulting in nearly 770 million infections and more than 7 million fatalities by the end of 2024. Despite significant advancements in vaccines and therapeutic interventions, the continuous emergence of viral variants underscores the urgent need for effective antiviral therapies. The non-structural protein (NSP) 5, a key enzyme for viral replication, has emerged as promising target for drug development due to its critical role in viral polyprotein cleavage and high specificity, lacking homologs in human cells. This article provides a methodical and comprehensive overview of NSP5 structural and functional characteristics, along with recent development of NSP5 inhibitor discovery. Clinically approved inhibitors, such as Paxlovid (Nirmatrelvir) and Xocova (Ensitrelvir), have demonstrated significant efficacy in reducing severe disease outcomes and mortality rates. However, challenges such as viral mutations, drug resistance, and pharmacokinetic limitations remain obstacles to long-term therapeutic success. The integration of advanced computational strategies, including structure-based drug design (SBDD), ligand-based drug design (LBDD), and artificial intelligence (AI)-driven approaches, has accelerated the discovery and optimization of novel NSP5 inhibitors. Additionally, multi-target synergistic therapies and innovative drug design strategies offer promising avenues to enhance antiviral efficacy and overcome resistance. This review also highlights the importance of rigorous efficacy evaluations to ensure the safety, pharmacokinetic stability and clinical viability of lead compounds. By consolidating existing knowledge and exploring future directions, this work aims to contribute to the ongoing development of next-generation antiviral therapies, ultimately strengthening global management of COVID-19 and preparedness for future coronavirus pandemics.

## 1 INTRODUCTION

The widespread COVID-19 pandemic has created extraordinary damage to global society, causing severe disruptions in several aspects, such as healthcare, economic welfare, and social well-being (Lew et al., 2020). The pandemic, triggered by the rapidly spreading respiratory disease, emerged in late 2019 and quickly became the most profound global health challenges in recent history (Lew et al., 2020). By the end of 2024, nearly 770 million people around the world have been infected by SARS-CoV-2, with over 7 million reported deaths (WHO, 2024). The mortality rate, although varying significantly across regions and demographics, underscored the virus's severe impact, especially among high-risk populations, such as the elderly and people with pre-existing health issues (Greene et al., 2020; Nicola et al., 2020). Despite remarkable advancements in medical treatments and the development of effective vaccines, the pandemic inflicted profound global losses, exacerbating unemployment, intensifying social distress, and disrupting healthcare systems and economies on an unprecedented scale (Fahriani et al., 2021). Moreover, the sustained evolution of SARS-CoV-2 has made current antiviral treatments insufficient, highlighting the urgent need for innovative and effective therapies (Dhama et al., 2023; Ren et al., 2022).

SARS-CoV-2, identified as a single-stranded, positive-sense RNA virus, is a member of the Coronaviridae family and the Betacoronavirus genus. Its diameter is approximately 80–120 nm. This virus features an average of 24–40 spike proteins on its surface, facilitating host cell entry. The RNA genome, about 30 kilobases (kb), encodes all the proteins necessary for viral replication and assembly (V'kovski et al.,



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2020). SARS-CoV-2 replication occurs in four key stages. First, the virus recognizes and binds to the host cell receptor, angiotensin-converting enzyme 2 (ACE2), via the spike protein, enabling attachment to the cell membrane. Next, the virus enters the cell via endocytosis and releases its genomic RNA into the cytoplasm, which is processed by the host's ribosomes to generate sixteen non-structural proteins (NSPs). Subsequently, replication-transcription complexes (RTCs) are formed and participate in viral genome replication and mRNA transcription. Finally, newly synthesized structural proteins encapsulate the replicated genomic RNA, and the fully assembled virions are transported out of the host cell through exocytosis (Chaudhary et al., 2021).

Multiple open reading frames (ORFs) exist in the SARS-CoV-2 RNA genome. ORF1ab encodes the NSPs essential for viral replication. One of the most critical proteases in this process is NSP5, also referred to as the main protease (Mpro). NSP5 is critical in the cleavage of polyproteins, assisting the maturation of viral proteins and eventually promoting viral replication (Yoshimoto, 2021). Since NSP5 has no homologous counterpart in mammalian hosts, it was identified as a key candidate for antiviral drug research due to this high specificity (Yadav et al., 2021). Current strategies for designing NSP5 inhibitors include peptidomimetic covalent inhibitors, non-covalent inhibitors, natural product-derived inhibitors, and fragment-based screening approaches (Yan & Gao, 2021).

Since 2019, several NSP5 inhibitors have been developed, with some achieving regulatory approval such as Pfizer's Paxlovid and Shionogi's Xocova. Paxlovid (Nirmatrelvir) is a covalent inhibitor that irreversibly binds to the catalytic Cys145 residue of NSP5, thereby blocking its protease activity (Owen et al., 2021). For clinical use, it is administered in combination with ritonavir, which inhibits the CYP3A4 metabolic enzyme (Pfizer, 2023). Xocova (Ensitrelvir), a production of Shionogi & CO., LTD, is a non-covalent inhibitor administered orally. It replaces traditional peptidomimetic chains with a rigid benzothiazole scaffold, enhancing metabolic stability (Unoh et al., 2022). These inhibitors have demonstrated significant efficacy in clinical trials, substantially reducing severe disease outcomes and mortality rates (Mukae et al., 2023; Yotsuyanagi et al., 2024). Other NSP5 inhibitors under development include Frontier Biotech (FB2001) (Shang et al., 2022), Simcere Pharmaceutical (SIM0417) (Wang et al., 2023), Enanta Pharmaceuticals (EDP-235) (Rhodin et al., 2024), Rigel Pharmaceuticals (RAY1216) (Wang et al., 2023), and Sorrento Therapeutics (STI-1558) (Mao et al., 2024). These investigational drugs aim to address the limitations of current NSP5 inhibitors, such as toxicity, drug resistance, and restricted patient populations, through structural optimization and technological innovation, offering improved options for next-generation coronavirus treatments.

Considering the pivotal role of NSP5 in viral replication and its potential for a drug target, this article presents systematic overview of its structural and functional characteristics, alongside recent advancements in NSP5 inhibitor development. This review also examines the structural biology of NSP5, while addressing challenges such as viral mutations that impact inhibitor efficacy. Furthermore, this review synthesizes recent breakthroughs, including novel inhibitors, new techniques (structure-based drug design, AI-driven approaches) and multi-target combination therapies. By consolidating existing knowledge and recent findings, this work aims to support ongoing efforts to establish effective antiviral strategies against these coronaviruses, contributing to the global management of COVID-19.

## 2 NSP5 STRUCTURE AND FUNCTION

NSP5 is discovered as a cysteine protease, and its molecular weight is close to 30 kDa. It is critical in cleaving viral polyproteins, an essential process for viral replication and maturation. Notably, the sequence identity between SARS-CoV-2 NSP5 and its SARS-CoV counterpart is 96%, although subtle variations in the S1/S4 subsites influence inhibitor binding efficiency and enzymatic activity. Its catalytic activity is driven by a Cys145-His41 dyad, and its substrate specificity is defined by the recognition sequence Leu-Gln (Ser/Ala/Gly). Nsp5 functions as a homodimer, as revealed by X-ray crystallography, with each monomer consisting of three domains (I–III), including two N-terminal domains that carry out the protease activity, and a C-terminal domain composed of α-helices. The substrate-binding pocket is located in a cleft between domains II and III, enabling precise substrate recognition and catalysis. The enzyme's sequence and structure are highly conserved across all known coronaviruses. The SARS-CoV-2 NSP5 can form a homodimer, which recognizes substrates that are about 10 amino acids long. However, it selectively cleaves only at four specific positions within the

polyprotein, exhibiting its tightly regulated enzymatic function (Roe et al., 2021). Beyond its role in processing viral proteins, NSP5 also contributes to immune evasion by suppressing the host's innate immune response, partly by degrading host protein factors (Rashid et al., 2022). Therefore, the main protease NSP5 does not directly participate in replication, it can be rephrased as NSP5 cleaves the polyprotein to release non-structural proteins, initiating genome replication. Due to this key function, NSP5 is a critical drug target for developing inhibitors (Jin et al., 2021).

# 3 CLINICALLY APPROVED NSP5 INHIBITORS

## 3.1 Nirmatrelvir (Paxlovid, PF-07321332)

Nirmatrelvir is a peptidomimetic covalent inhibitor, developed by Pfizer (Owen et al., 2021). It mimics the viral polyprotein substrate (e.g. Leu-Gln-Ser-Ala) and contains an α-ketoamide warhead(-CO-NH-) that can bind to the catalytic cysteine residue (Cys145) of NSP5, irreversibly blocking its protease activity. This compound is characterized by high activity and selectivity. The $IC_{50}$ for the SARS-CoV-2 NSP5 inhibition was 19.3 nM, with an efficiency rate of 1,930 /Ms. It had no significant inhibitory effect on human proteases, such as cathepsins. Nirmatrelvir has cross-inhibitory activity against NSP5 in MERS-CoV and SARS-CoV-1. In cellular assay, the $IC_{50}$ of this compound for SARS-CoV-2 replication inhibition was 74.5 nM. The activity against BA.1, BA.2 Omicron variants was maintained. In preclinical animal infection models, oral administration of Nirmatrelvir (300 mg/kg, twice daily) significantly reduced lung viral loads (>90%) and alleviated inflammatory damage. In the humanized ACE2 mouse model, survival rates increased to 100% with Nirmatrelvir treatment, compared to 40% in the control group, and the pathological damage in the lungs was significantly alleviated (Owen et al., 2021). Nirmatrelvir has low oral bioavailability and is rapidly metabolized by cytochrome P450 3A4 (CYP3A4). Therefor combination with a CYP3A4 inhibitor (Ritonavir)is required to keep the plasma concentration and therapeutic efficacy. Clinically, Nirmatrelvir is co-packaged with Ritonavir, called Paxlovid (Pfizer, 2023).

The EPIC-HR (NCT04960202) trial, non-hospitalized adult patients receive Paxlovid BID for 5 days at 300 mg/100 mg. For the primary endpoint, the relative risk reduction for Paxlovid group was 86% (95%CI: 72%, 93%) compared to placebo group. When administered within three days of symptom onset, Paxlovid reduced hospitalization or death by 88%, and by 85% when administered within 5 days. On day 5, the Paxlovid group showed a significantly higher viral load reduction (10-fold difference) than the placebo group. The virus clearance time is shortened by about 2-3 days (Hammond et al., 2022). In addition, its efficacy maintained against early variants (e.g. Delta), though reduced against BA.2, BA.5 Omicron subvariants due to viral evolution and pre-existing immunity. Paxlovid remained effective in reducing hospitalization risks by 52% during the 2022-2023 Omicron wave, according to data from Hong Kong (Wong et al., 2022).

Paxlovid is generally well-tolerated, with common adverse effect including dysgeusia, diarrhea, nausea, and vomiting. Paxlovid treatment led to a 2.0% discontinued rate, lower than the rate in the placebo group (Hammond et al., 2022). Avoid drug-drug interactions is the main shortage of Paxlovid. Ritonavir's inhibition of CYP3A4 leads to severe interactions with medications metabolized by this enzyme, such as statins, anticoagulants (e.g. apixaban, rivaroxaban) and immunosuppressants (e.g. tacrolimus). Co-administration with drugs that strongly induce CYP3A4 (e.g. rifampin) may reduce nirmatrelvir efficacy (Pfizer, 2023). In addition, approximately 5-10% of patients experienced a rebound in viral load (Paxlovid rebound) after discontinuation, potentially linked to insufficient treatment duration or immune response deficiencies (Wang et al., 2022). Paxlovid is not advised for critically ill patients in hospital (Pfizer, 2023).

## 3.2 Ensitrelvir (S-217622, Xocova)

Ensitrelvir is an orally non-covalent SARS-CoV-2 Mpro inhibitor developed by Shionogi. It reversibly binds to the protease active site, potentially reducing susceptibility to resistance mutations (e.g. E166V). The structure of Ensitrelvir is precisely to achieve high affinity for NSP5. The S1 pocket is occupied by a 6-chloro-2-methyl-2H-indazole moiety to form strong hydrophobic interactions. The 2,4,5-trifluorobenzylic moiety occupies S2 pocket and interacts with His41 (a catalytic residue) to enhance the binding affinity. Ensitrelvir effectively inhibited NSP5 enzymatic activity in SARS-CoV-2 with $IC_{50}$ at 24.1 nM. It also presented antiviral activity against

HCoV-OC43, MERS-CoV and HCoV-229E with $EC_{50}$ at 0.074 μM, 1.4μM and 5.5 μM respectively. The inhibitory effect on multiple Omicron variants, including XBB.1.5 and BA.1/BA.2/BA.5, was well-preserved ($IC_{50}$ ~20-100 nM). The oral bioavailability of this compound is optimized through scaffold optimization. A single dose regimen can maintain effective plasma drug concentration, enabling once - daily dosing. In a hamster infection model, Ensitrelvir (30 mg/kg once a day) decreased the viral load in the lungs by 3-$\log_{10}$, associated with alleviated pneumonia severity. Prophylactic administration (24 hours pre-exposure) completely blocked viral replication. In hACE2 transgenic mice, Ensitrelvir achieved a 100% survival rate, whereas 40% in the control group, with undetectable viral loads three days post- dosing (Unoh et al., 2022).

In the pivotal clinical trials, mild-to-moderate COVID-19 patients were given Ensitrelvir (125 mg, once daily) over 5 days. Symptom relief occurred 24 hours earlier in the Ensitrelvir group (median time: 167.9 hours vs. 192.2 hours) (Yotsuyanagi et al., 2024). The time for fever relief in the treatment group was shortened by approximately 30 hours. On the 4th day, Ensitrelvir decreased the viral load by –1.4 to –1.5 log10 copies/ mL, while that in the placebo group decreased by 0.6-$\log_{10}$. The rate of viral negativity after Ensitrelvir treatment for 5 days was 68% (35% in the placebo group) (Mukae et al., 2023). A retrospective study in Japan in 2023 reported greater viral titer change and lower total score of symptoms after Ensitrelvir treatment during Omicron wave (Mukae et al., 2023).

Ensitrelvir avoids the potential long-term toxicity risks of covalent inhibitors. Due to its excellent pharmacokinetics (PK), the single - drug oral regimen significantly simplifies clinical use and broadens the applicable population, such as patients with complex co-medications. Currently, its indication is limited to mild-to-moderate patients, and its efficacy in hospitalized patients has not been verified.

# 4 DEVELOPMENT STRATEGIES FOR NSP5 INHIBITORS

## 4.1 Computer-Aided Drug Design

### 4.1.1 Structure-based Drug Design

SBDD utilizes the three-dimensional structure of the protease to identify and optimize lead compounds. Cryo-electron microscopy (cryo-EM) and X-ray crystallography were employed to reveal the high-resolution structure of substrate-binding pockets and active site. These structural data formed the foundation for molecular docking simulations, which predict the binding affinity and orientation of potential inhibitors within the protease's active site (Dai et al., 2020). Additionally, molecular dynamics (MD) simulations allow for dynamic exploration, revealing conformational changes and key interactions between NSP5 and inhibitors over time (Amorim et al., 2023). By integrating these computational approaches, it is possible to design compounds with enhanced binding efficiency, specificity, and stability, thereby improving drug development efficiency.

### 4.1.2 Ligand-Based Drug Design (LBDD)

LBDD complements SBDD by focusing on the chemical properties of known inhibitors to identify novel candidates. Machine learning (ML) algorithms trained on datasets of experimentally validated NSP5 inhibitors can predict compounds with potential antiviral activity (Mohamed et al., 2021). These models analyze molecular descriptors, including hydrophobicity, hydrogen bonding capacity, and molecular weight to identify promising candidates. The integration of SBDD and LBDD allows for a comprehensive approach to inhibitor discovery, facilitating the design of NSP5-targeted compounds with improved potency and reduced off-target effects.

## 4.2 Lead Compound Optimization

The lead compounds optimization is a crucial step in NSP5 inhibitor development, aiming to enhance selectivity, bioavailability, and metabolic stability. Structural modifications, such as the introduction of cyclopropyl groups or the replacement of labile functional groups, can improve the binding affinity and pharmacokinetic properties. For example, incorporating non-natural amino acids or peptidomimetic scaffolds can significantly improve oral bioavailability while maintaining strong interactions with NSP5.

Chemical modifications also fulfill an essential role in optimizing NSP5 inhibitors. The addition of electron-withdrawing groups or the modulation of steric hindrance can fine-tune binding interactions between inhibitors and the protease, leading to improved inhibitory potency. Additionally, prodrug strategies, which convert inactive precursors into

active inhibitors in vivo, can address challenges related to poor absorption or rapid metabolism. These optimization processes are guided by computational predictions and validated through functional assays to ensure that the optimized compounds retain high antiviral activity while minimizing toxicity and adverse effects.

### 4.3 Preclinical Efficacy Evaluation

The development of NSP5 inhibitors involves rigorous *in vitro* and *in vivo* evaluations to determine antiviral potency, pharmacokinetics, and safety. *In vitro* studies are the initial step in evaluating the antiviral potency and cytotoxicity of candidate compounds. Cell-based assays using Vero E6 and HEK293T cells are commonly employed to measure the inhibition of viral replication and the compound's effect on host cell viability. These experiments provide essential preliminary data on the potency and selectivity of inhibitors, guiding further structural modifications and compound optimization.

Subsequently, in vivo efficacy studies are conducted to evaluate the pharmacokinetics, therapeutic efficacy, and potential toxicity of lead compounds. Animal models, including mice and hamsters, serve as preclinical platforms for simulating virus infection and evaluating the potential therapeutic potency of NSP5 inhibitors. Parameters such as viral load reduction, immune response modulation, and organ toxicity are monitored to ensure that the selected compounds demonstrate both safety and efficacy. These preclinical studies are essential for selecting compounds with the highest potential for clinical translation.

## 5 FUTURE PROSPECTS AND CHALLENGES OF NSP5 INHIBITORS

### 5.1 Challenges in Research

The development of NSP5 inhibitors is confronted with several notable challenges. A major concern is the effect of viral mutations on inhibitor sensitivity. As a key protease in viral replication, NSP5 is subject to evolutionary changes that can modify its structural binding sites, potentially reducing the efficiency of current inhibitors. This mutation-driven resistance not only limits the applicability of existing inhibitors but also necessitates continuous optimization in drug design strategies to address rapidly evolving viral strains. Addressing these variations requires structural monitoring of NSP5 mutants and the development of inhibitors capable of maintaining activity.

Another major issue in NSP5 inhibitor development is the optimization of pharmacokinetics, particularly absorption, distribution, metabolism, and excretion (ADME) properties. Effective oral drugs must be sufficient bioavailability and stability to sustain therapeutic concentrations in vivo. However, many candidate compounds that demonstrate excellent activity in vitro fail in clinical trials due to poor ADME characteristics. Therefore, improving bioavailability through structural optimization and formulation enhancements is a central focus of current studies. Moreover, the assessment of safety and long-term toxicity poses another major challenge in the clinical translation of NSP5 inhibitors. While NSP5 inhibitors effectively suppress viral replication, they may also induce nonspecific toxicity in host cells, particularly with prolonged administration. Balancing antiviral efficacy with safety and systematically evaluating potential risks through toxicological studies are essential for advancing NSP5 inhibitors toward clinical application.

### 5.2 Future Directions

To address the challenges in NSP5 inhibitor research, future efforts should focus on several promising directions. First, the discovery of novel covalent and non-covalent inhibitors represents a critical strategy for improving the therapeutic window. Covalent inhibitors, which form irreversible bonds with NSP5, can provide sustained suppression of viral replication but require careful evaluation of potential toxicity due to their permanent binding mechanism. Non-covalent inhibitors, on the other hand, offer higher selectivity but may be more susceptible to resistance caused by viral mutations. Therefore, developing inhibitors that combine high efficacy with safety is a key priority. Second, multi-target synergistic therapy holds significant potential for enhancing antiviral efficacy. For example, combining NSP5 inhibitors with RNA-dependent RNA polymerase (RdRp) inhibitors or ACE2 receptor blockers could effectively disrupt viral replication and infection at multiple stages, thereby decreasing the risk of resistance and improving therapeutic potency. The development of such combination therapies requires a well-rounded understanding of the synergistic mechanisms between different targets and rigorous validation

through clinical trials. Finally, the application of artificial intelligence (AI) offers new opportunities to accelerate drug discovery. AI-driven virtual screening and molecular design can rapidly identify compounds with potential antiviral activity and optimize their pharmacokinetic properties. Moreover, AI can predict the impact of viral mutations on inhibitor sensitivity, guiding more effective drug design.

In summary, the discovery of the next generation of NSP5 inhibitor lies in the integration of novel inhibitor development, multi-target synergistic therapy, and AI-powered drug design. By addressing the challenges and leveraging computational and experimental techniques, the research on NSP5 inhibitors is poised to achieve groundbreaking advancements, providing more effective solutions for the development of broad-spectrum antiviral treatment.

# 6 CONCLUSION

The worldwide coronavirus pandemic has underscored the urgent request for effective antiviral treatments to fight SARS-CoV-2 and its continuously evolving variants. NSP5 has been proven to be a particularly attractive therapeutic target, because of its essential role in viral replication and the lack of homologous proteins in human cells. This article has provided a detailed insight of the structural and functional characteristics of NSP5. The development and clinical efficacy of approved NSP5 inhibitors, such as Paxlovid (Nirmatrelvir) and Xocova (Ensitrelvir), which have demonstrated significant reductions in severe disease outcomes and mortality rates have also been examined. These inhibitors, along with others in development, represent promising advancements in antiviral therapy, yet challenges such as viral mutations, drug resistance, and pharmacokinetic limitations remain.

The integration of advanced computational strategies, including SBDD, LBDD, and AI-driven approaches, has accelerated the discovery and optimization of NSP5 inhibitors. These technologies have enabled the identification of novel covalent and non-covalent inhibitors, as well as the exploration of multi-target combination therapies, which hold promise for overcoming resistance and improving therapeutic outcomes. Furthermore, rigorous preclinical evaluations have been instrumental in advancing lead compounds toward clinical translation, ensuring both efficacy and safety profiles are thoroughly validated.

Despite these achievements, several challenges still exist. The persistent evolution of SARS-CoV-2 necessitates ongoing efforts to address mutation-driven resistance and optimize drug design strategies. Additionally, improving the pharmacokinetic properties of NSP5 inhibitors, particularly enhancing their oral bioavailability and metabolic stability, remains a critical hurdle in drug development. The development of next-generation inhibitors must also prioritize safety, minimizing off-target effects and long-term toxicity while maintaining high antiviral efficacy.

Looking ahead, the future of NSP5 inhibitor research lies in the integration of innovative approaches, including the discovery of novel inhibitors, the development of multi-target synergistic therapies, and the application of AI technologies. These efforts will not only enhance our ability to combat SARS-CoV-2 but also contribute to global preparedness for future coronavirus outbreaks. By consolidating existing knowledge and leveraging cutting-edge technologies, this review aims to support ongoing research and development efforts, ultimately contributing to the global antiviral strategies and strengthening response to coronavirus and the mitigation of future pandemics.

# REFERENCES

Amorim, V.M.F., de Souza, R.F., Guzzo, C.R., & de Souza, A.S. 2023. Molecular dynamics simulations suggest SARS-CoV-2 3CLpro mutations in Beta and Omicron variants do not alter binding affinities for cleavage sites of non-structural proteins. Covid 3(4): 622-636. doi:10.3390/covid3040044

Chaudhary, J.K., Yadav, R., Chaudhary, P.K., Maurya, A., Roshan, R., Azam, F., et al. 2021. Host cell and SARS-CoV-2-associated molecular structures and factors as potential therapeutic targets. Cells 10(9). doi:10.3390/cells10092427

Dai, W., Zhang, B., Jiang, X.-M., Su, H., Li, J., Zhao, Y., et al. 2020. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. Science 368(6497): 1331-1335.

Dhama, K., Nainu, F., Frediansyah, A., Yatoo, M.I., Mohapatra, R.K., Chakraborty, S., et al. 2023. Global emerging Omicron variant of SARS-CoV-2: Impacts, challenges and strategies. Journal of Infection and Public Health 16(1): 4-14. doi:10.1016/j.jiph.2022.11.024

Fahriani, M., Ilmawan, M., Fajar, J.K., Maliga, H.A., Frediansyah, A., Masyeni, S., et al. 2021. Persistence of long COVID symptoms in COVID-19 survivors worldwide and its potential pathogenesis - a systematic review and meta-analysis. Narra J 1(2). doi:10.52225/narraj.v1i2.36

Greene, C.J., Burleson, S.L., Crosby, J.C., Heimann, M.A., & Pigott, D.C. 2020. Coronavirus disease 2019: International public health considerations. JACEP Open 1(2): 70-77. doi:10.1002/emp2.12040

Hammond, J., Leister-Tebbe, H., Gardner, A., Abreu, P., Bao, W., Wisemandle, W., et al. 2022. Oral nirmatrelvir for high-risk, nonhospitalized adults with COVID-19. New England Journal of Medicine 386(15): 1397-1408. doi:10.1056/NEJMoa2118542

Jin, Z., Wang, H., Duan, Y., & Yang, H. 2021. The main protease and RNA-dependent RNA polymerase are two prime targets for SARS-CoV-2. Biochemical and Biophysical Research Communications 538: 63-71. doi:10.1016/j.bbrc.2020.10.091

Lew, H.L., Oh-Park, M., & Cifu, D.X. 2020. The war on COVID-19 pandemic. American Journal of Physical Medicine & Rehabilitation 99(7): 571-572. doi:10.1097/phm.0000000000001460

Mao, L., Shaabani, N., Zhang, X., Jin, C., Xu, W., Argent, C., et al. 2024. Olgotrelvir, a dual inhibitor of SARS-CoV-2 Mpro and cathepsin L, as a standalone antiviral oral intervention candidate for COVID-19. Med 5(1): 42-61.e23. doi:10.1016/j.medj.2023.12.004

Mohamed, N.M., Ali, E.M.H., & AboulMagd, A.M. 2021. Ligand-based design, molecular dynamics and ADMET studies of suggested SARS-CoV-2 Mpro inhibitors. RSC Advances 11(8): 4523-4538. doi:10.1039/d0ra10141a

Mukae, H., Yotsuyanagi, H., Ohmagari, N., Doi, Y., Sakaguchi, H., Sonoyama, T., et al. 2023. Efficacy and safety of ensitrelvir in patients with mild-to-moderate coronavirus disease 2019: the Phase 2b part of a randomized, placebo-controlled, Phase 2/3 study. Clinical Infectious Diseases 76(8): 1403-1411. doi:10.1093/cid/ciac933

Nicola, M., Alsafi, Z., Sohrabi, C., Kerwan, A., Al-Jabir, A., Iosifidis, C., et al. 2020. The socio-economic implications of the coronavirus pandemic (COVID-19): a review. International Journal of Surgery 78: 185-193. doi:10.1016/j.ijsu.2020.04.018

Owen, D.R., Allerton, C.M.N., Anderso, A.S., Aschenbrenner, L., Avery, M., Berritt, S., et al. 2021. An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19. Science 374(6575): 1589-1593. doi:10.1126/science.abl4784

Pfizer. 2023. PAXLOVID. https://www.pfizer.com/news/press-release/press-release-detail/pfizer-amends-us-government-paxlovid-supply-agreement-and

Rashid, F., Xie, Z., Suleman, M., Shah, A., Khan, S., & Luo, S. 2022. Roles and functions of SARS-CoV-2 proteins in host immune evasion. Frontiers in Immunology 13. doi:10.3389/fimmu.2022.940756

Ren, S.-Y., Wang, W.-B., Gao, R.-D., & Zhou, A.-M. 2022. Omicron variant (B.1.1.529) of SARS-CoV-2: mutation, infectivity, transmission, and vaccine resistance. World Journal of Clinical Cases 10(1): 1-11. doi:10.12998/wjcc.v10.i1.1

Rhodin, M.H.J., Reyes, A.C., Balakrishnan, A., Bisht, N., Kelly, N.M., Gibbons, J.S., et al. 2024. The small molecule inhibitor of SARS-CoV-2 3CLpro EDP-235 prevents viral replication and transmission in vivo. Nature Communications 15(1). doi:10.1038/s41467-024-50931-8

Roe, M.K., Junod, N.A., Young, A.R., Beachboard, D.C., & Stobart, C.C. 2021. Targeting novel structural and functional features of coronavirus protease nsp5 (3CLpro, Mpro) in the age of COVID-19. Journal of General Virology 102(3). doi:10.1099/jgv.0.001558

Shang, W., Dai, W., Yao, C., Xu, L., Tao, X., Su, H., et al. 2022. In vitro and in vivo evaluation of the main protease inhibitor FB2001 against SARS-CoV-2. Antiviral Research 208. doi:10.1016/j.antiviral.2022.105450

Unoh, Y., Uehara, S., Nakahara, K., Nobori, H., Yamatsu, Y., Yamamoto, S., et al. 2022. Discovery of S-217622, a noncovalent oral SARS-CoV-2 3CL protease inhibitor clinical candidate for treating COVID-19. Journal of Medicinal Chemistry 65(9): 6499-6512. doi:10.1021/acs.jmedchem.2c00117

V'kovski, P., Kratzel, A., Steiner, S., Stalder, H., & Thiel, V. 2020. Coronavirus biology and replication: implications for SARS-CoV-2. Nature Reviews Microbiology 19(3): 155-170. doi:10.1038/s41579-020-00468-6

Wang, B., Li, H.-J., Cai, M.-M., Lin, Z.-X., Ou, X.-F., Wu, S.-H., et al. 2023. Antiviral efficacy of RAY1216 monotherapy and combination therapy with ritonavir in patients with COVID-19: a Phase 2, single-centre, randomised, double-blind, placebo-controlled trial. eClinicalMedicine 63. doi:10.1016/j.eclinm.2023.102189

Wang, F., Xiao, W., Tang, Y., Cao, M., Shu, D., Asakawa, T., et al. 2023. Efficacy and safety of SIM0417 (SSD8432) plus ritonavir for COVID-19 treatment: a randomised, double-blind, placebo-controlled, Phase 1b trial. The Lancet Regional Health - Western Pacific 38. doi:10.1016/j.lanwpc.2023.100835

Wang, L., Berger, N.A., Davis, P.B., Kaelber, D.C., Volkow, N.D., & Xu, R. 2022. COVID-19 rebound after Paxlovid and Molnupiravir during January-June 2022. doi:10.1101/2022.06.21.22276724

WHO. 2024. Number of COVID-19 cases reported to WHO. https://data.who.int/dashboards/covid19/cases

Wong, C.K.H., Au, I.C.H., Lau, K.T.K., Lau, E.H.Y., Cowling, B.J., & Leung, G.M. 2022. Real-world effectiveness of molnupiravir and nirmatrelvir plus ritonavir against mortality, hospitalisation, and in-hospital outcomes among community-dwelling, ambulatory patients with confirmed SARS-CoV-2 infection during the Omicron wave in Hong Kong: an observational study. The Lancet 400(10359): 1213-1222. doi:10.1016/s0140-6736(22)01586-0

Yadav, R., Chaudhary, J.K., Dhamija, P., Jain, N., Chaudhary, P.K., Sharma, A., et al. 2021. Role of structural and non-structural proteins and therapeutic targets of SARS-CoV-2 for COVID-19. Cells 10(821). doi:10.3390/cells

Yan, F., & Gao, F. 2021. An overview of potential inhibitors targeting non-structural proteins 3 (PLpro and Mac1) and 5 (3CLpro/Mpro) of SARS-CoV-2. Computational and Structural Biotechnology Journal 19: 4868-4883. doi:10.1016/j.csbj.2021.08.036

Yoshimoto, F.K. 2021. A biochemical perspective of the nonstructural proteins (NSPs) and the spike protein of SARS-CoV-2. The Protein Journal 40(3): 260-295. doi:10.1007/s10930-021-09967-8

Yotsuyanagi, H., Ohmagari, N., Doi, Y., Yamato, M., Bac, N.H., Cha, B.K., et al. 2024. Efficacy and safety of 5-day oral ensitrelvir for patients with mild to moderate COVID-19. JAMA Network Open 7(2). doi:10.1001/jamanetworkopen.2023.54991

Yotsuyanagi, H., Ohmagari, N., Doi, Y., Yamato, M., Fukushi, A., Imamura, T., et al. 2024. Prevention of post COVID-19 condition by early treatment with ensitrelvir in the Phase 3 SCORPIO-SR trial. Antiviral Research 229. doi:10.1016/j.antiviral.2024.105958