International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Issue 6, 2022

Review Article

SARS-COV-2 3CL-PROTEASE INHIBITORS AS ANTIVIRAL AGENTS AGAINST COVID-19

MOHAMED J. SAADH[1*]

[1]Department of Pharmacy, Faculty of Pharmacy, Middle East University, Amman, Jordan
*Email: msaadeh@meu.edu.jo

Received: 29 Jul 2022, Revised and Accepted: 10 Sep 2022

ABSTRACT

The SARS-CoV-2 virus causes coronavirus, and the pandemic has led to efforts to develop appropriate drugs for treatment. Understanding the structure and function of SARS-CoV-2 3CL is crucial in unlocking ways of developing effective drugs. Some studies have described the structure of the protease at the DNA and protein levels. Notably, two important proteases help in the drug development process: PL^{pro} and $3CL^{pro}$. The $3CL^{pro}$, for instance, is helpful in viral replication alongside transcription. The PL is associated with NsP3, a multi-domain protein part of the viral replication and transcription complex which cleaves peptide bonds at specific sites. *In vitro* studies have shown that SARS-CoV-2 3CL-protease inhibitors can contribute to antiviral drug development, especially MG-132, boceprevir, telaprevir, and calpain, which are protein inhibitors with lethal dose values appropriate for drug development. In contrast, there are very limited studies *in vivo* reporting the appropriateness of protease inhibitors in antiviral drug development.

Keywords: Inhibitors, SARS-CoV-2, COVID-19, SARS-CoV-2 3CL-protease, Papain-like protease

DOI: https://dx.doi.org/10.22159/ijap.2022v14i6.46015. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), previously known as 2019-nCoV, causes the novel coronavirus disease 2019 (COVID-19). The disease was first identified following an outbreak of a respiratory illness in Wuhan, China [1]. COVID-19 became a global health emergency when the WHO declared it a global pandemic, the first such description since 2009 when H1N1 influenza was also declared a global pandemic. The structural features of the current virus particle are similar to related coronaviruses [2-4]. The coronavirus genome reveals an evolutionary relationship to the beta-coronavirus responsible for the severe acute respiratory syndrome (SARS) that was associated with bats as the causative agent [5]. Even though there has been research to develop antiviral agents against SARS-CoV, there is still no treatment.

Therefore, the purpose of this review is to evaluate inhibitors of SARS-CoV-2 3CL-protease as potential therapies for the treatment of individuals infected with SARS-CoV-2, providing an overview of several therapeutic options for the treatment of the CoV virus that can offer patients and healthcare professionals new hope in the fight against SARS-CoV-2.

Structure and function of sars-cov-2 3CL-protease

The SARS-CoV genome encodes over 20 proteins, including two proteases, namely $3CL^{pro}$ and PL^{pro}, which are critical for virus replication [5, 6]. The two cysteine proteases responsible for the proliferation cycle of the virus derive their names from the findings that they are papain-like protease (PL^{pro}) and chymotrypsin-like protease ($3CL^{pro}$) [6, 7]. The two proteases cleave the translated proteins Pp1a and Pp1b to form functional components [8-11]. There are 11 sites where the main protease usually cleaves in Pp1a and Pp1b through a sequence consensus, as PL^{pro} cleaves three sites through a recognition sequence consensus [7, 12]. The recognition sequence for SARS-CoV $3CL^{pro}$ is from leucine to glutamine with serine, alanine, and glycine and its own N-and-C-terminal for auto-processing sites, which recognize site P1 and also P1-4 [13]. SARS-CoV-2 is a single-stranded positive-sense RNA virus that relies on the cellular translation machinery post-infection to generate Pp1a and 1ab in a proteolytic cleavage process [1]. The main protease, 3CLpro, which is also called Mpro and looks like 3-chymotrypsin, is still the best drug target [5, 9]. The protease has been extensively studied to identify its capacity to aid therapeutics against the SARS-CoV virus alongside other disease-causing coronaviruses (fig. 1).

The $3CL^{pro}$ (M^{pro}) of the SARS-CoV-2 virus is a potential drug repurposing target because of its roles in coronavirus replication and transcription [9]. Among coronaviruses, M^{pro} has been projected as a cysteine protein with a highly conserved structure without human proteome analogs in recognition sequences or cleavage sites [9]. Therefore, $3CL^{pro}$ is a good target for drug candidates with small molecules. Aldehyde and ketone-based SARS-CoV-2 ^{pro} covalent inhibitors interact with Cys145 at the enzyme's active site [9]. The inhibitors of antiviral drugs developed previously have peptide scaffolds alongside functional groups supporting reversible binding.

The PL^{pro} is an NsP3 domain with 1945 residues in SARS-CoV-2 [7]. NsP3 is a multi-domain protein and an important component of the enzyme replicase-transcriptase complex (RTC) [7]. Importantly, the enzyme resides in NsP3 between the unique and nucleic acid binding domains. The highly conserved enzyme is also found in coronaviruses; usually, two copies are represented as PL pro 1 and 2. The cysteine protease usually cleaves peptide bonds formed between Nsp1 and 2, Nsp2 and 3, and Nsp3 and 4, to release three proteins, Nsp1, 2, and 3 [7]. The recognition sequence LXGG motif situated in Pp1a and 1ab normally corresponds to cysteine protease substrate positions P4 to P1 and is necessary for cleavage alongside recognition by PL^{pro} [7]. The Nsp1 is a protein of 180 residues that interacts with the 80S ribosome while inhibiting translation of the host. On the other hand, Nsp2 is a protein of 638 residues that has been proposed to modulate the survival of host cells. Efforts for SARS-CoV-2 antivirals have mostly focused on three NSP proteins, namely, $Nsp3PL^{pro}$, $NSP5M^{pro}$, and Nsp12-RNA-dependent polymerase, which have been projected as the main drug targets [14]. The Nsp3 domain, or PL^{pro} is an enzyme found in MERS-CoV, SARS-CoV, Swine Acute Diarrhea Syndrome coronaviruses, and Murine Hepatitis Virus, among other viruses. Notably, the structure, functional conservation, and even sequence of PL^{pro} indicate the possibility of therapeutics for SARS-CoV-2 being effective against other viruses that have PL^{pro} [7, 14]. The enzyme is structurally characterized with more than 40 PL^{pro} proteases of viral structures now available, particularly from SARS-CoV, which can be used in drug discoveries [15].

Alongside processing pp1a and 1ab, SARS-CoV has deubiquitination of disassembling various chains: mono, di, and branched polyubiquitin. Additionally, SARS-CoV has interferon activity induced by gene 15 (ISG15). Both ISG15 and ubiquitin proteins have a C-termini for carrying the PL^{pro} used in motif recognition [16]. Therefore, it is likely that removing changes from host cells affects how hosts respond to infection by viruses. Moreover, PL^{pro} blocks

the signaling of NF-kappaB, inactivates TBK1, prevents IRF3 translocation, and inhibits the TLR7 signaling pathway [17]. Nonetheless, PL^{pro} is a protein with many functions and is essential in viral polyprotein processing, maturation, and RTC assembly, and may disrupt the response of the host-virus to support the proliferation and replication of viruses [16, 17]. Therefore, the central role that PL^{pro} plays in viral replication makes it a potential target for therapy (fig. 1).

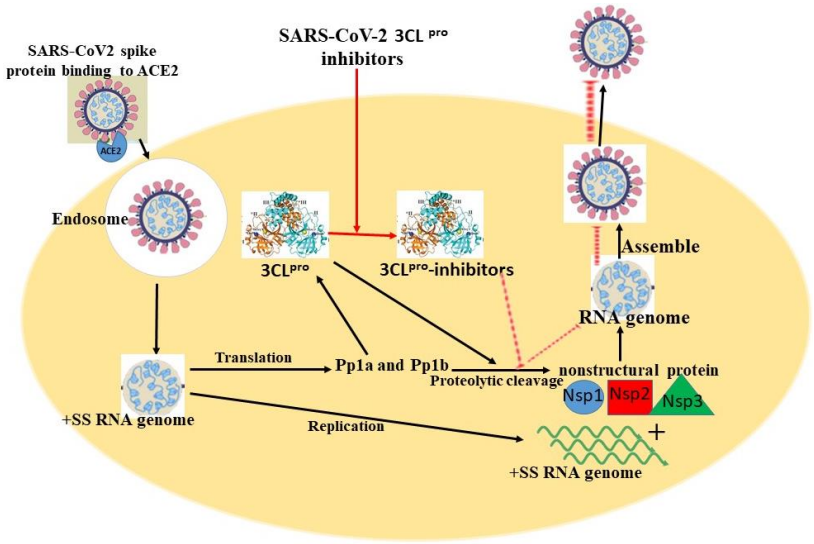

Fig. 1: The The $3CL^{pro}$ (M^{pro}) of the SARS-CoV-2 virus is a key antiviral drug target

SARS-COV-2 3CL-protease inhibitors as antiviral agents

In vitro compound library screening alongside molecular docking using SARS-CoV crystal structures has shown inhibitors with different action modes against the SARS-CoV-2 3CL. The compounds include zinc metal conjugates such as zinc pyrithione; natural products from the isoflavone family, such as 5,7,3,4-tetrahydroxy-2-(3,3-dimethylallyl) isoflavone; approved drugs, such as carmofur and disulfiram; and drug candidates, such as ebselen [1]. There are novel potential SARS CoV-2 3CL-pro inhibitors, such as bonaphthone, calpeptin, myricetin, CR8-(R0), ML311, thioguanosine, and MLS0315771, which could be investigated further [1]. The compounds contain known scaffolds, such as flavonoids, kinase inhibitors, protease inhibitors, and peptide derivatives. In particular, the peptide-like proteasome inhibitor MG-132 with IC_{50} of 7.4 µM with less potency on 3CL than the anticytopathic with IC_{50} of 0.4 µM and an antiviral replication of IC_{50} of 0.1 µM [1, 18]. The higher potency witnessed in the antiretroviral properties of MG-132 may include polypharmacology rather than be solely mediated by 3CL function inhibition. Compound MG-132 is active against coronaviruses, thereby warranting further *in vivo* chemoproteomic alongside confirmatory studies [1, 6, 19]. Moreover, *in vitro* screens have shown that boceprevir with IC_{50} of 1.6 µM alongside telaprevir with IC_{50} of 55 µM both act against the SARS-CoV-2 3CL after forming a covalent bond with sulfur of Cys 145 [1, 20-22]. Other *in vitro* studies on the antiviral activity of calpain inhibitors revealed that the drugs strongly inhibit the replication of the SARS-CoV-2 virus in cell culture, yielding EC50 values of between 0.49 and 3.37 µM [2, 21, 22]. These findings reveal many compounds which could act as *in vitro* antiviral agents and offer effective treatment. Also, the combination of punicalagin and Zn^{+2} particles inhibits the SARS-CoV-2 3CL-protease *in vitro* [23, 24].

There are commercialized actives with promising candidates that could be used in clinical development processes. Antiviral riodoxol, apomorphine, rabeprazole, and benserazide are among the actives that could be studied further [1, 25]. It is possible to administer all the molecules orally with acute controlled toxicity of over 5 mg per kilogram of dose [1, 22]. For instance, apomorphine and benserazide can cause brain exposure, while riodoxol is already a disinfectant, an ointment, and an antiseptic in treating skin infections. *In vitro* assays determining the compounds' replication are unlikely to provide a full reflection of infection pathology.

SARS-COV-2 3CL-protease inhibitors as antiviral agents *in vivo*

Some of the drugs that have been approved for Human Immunodeficiency Virus, such as ritonavir, lopinavir, carmofur, compounds containing aldehyde, 6e, peptidomimetic alpha ketoamide inhibitors, ebelesen, and Michael acceptor 3, have exhibited effective *in vivo* antiviral activity [5]. The drugs bind to a cleft on the M^{pro} where they inhibit any further activity, thereby halting viral replication or infection. However, it is worth noting that the effectiveness of the drugs on human beings has not been established. For instance, there is still no *in vivo* potency for 13a and b, while Ebselen, alongside N3, is a promising drug that targets M^{pro} and effectively inhibits SARS-CoV-2 replication [5, 22, 25]. Ebselen has demonstrated potency with an EC50 value of 5 µM, while n3 has a high antiviral activity with an EC50 value of more than 16 µM [1, 22, 25]. Overall, data on *in vivo* pharmacokinetic studies of drugs against SARS-CoV is limited, with only a few outcomes reported.

CONCLUSION

The emergency of the SARS-CoV-2 virus has led to frantic research efforts to develop drugs for the treatment of the COVID-19 associated with the virus. The scientific community has extended studies to the DNA and protein levels to determine appropriate ways of developing effective drugs. Important discoveries of 3CL and PL have helped to understand how protease inhibitors could be used as antiviral treatment agents both in *in vitro* and *in vivo* studies. Research efforts have focused on novel and some of drugs that have been used before to treat HIV and hepatitis, among others. *In vitro* studies have shown numerous successes with many drugs, indicating lethal dose values that increase their chances for consideration. However, *in vivo* studies have remained limited, meaning more research still needs to occur in the domain.

ACKNOWLEDGMENT

Authors are grateful to the Middle East University, Amman, Jordan, for the financial support granted to cover the publication fee of this research article.

AUTHORS CONTRIBUTIONS

MJS, designed the study and drafted the manuscript and write the article. All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Kuzikov M, Costanzi E, Reinshagen J, Esposito F, Vangeel L, Wolf M. Identification of inhibitors of SARS-CoV-2 3CL-pro enzymatic activity using a small molecule *in vitro* repurposing screen. ACS Pharmacol Transl Sci. 2021 Mar;4(3):1096-110. doi: 10.1021/acsptsci.0c00216, PMID 35287429.
2. Cui W, Yang K, Yang H. Recent progress in the drug development targeting SARS-CoV-2 main protease as a treatment for COVID-19. Front Mol Biosci. 2020 Dec;7:616341. doi: 10.3389/fmolb.2020.616341, PMID 33344509.
3. Cherumanalil JM, Thayyil J. Pharmacological treatments of Covid-19– a review. Asian J Pharm Clin Res. 2020 Oct;13(10):16-22. doi: 10.22159/ajpcr.2020.v13i10.39055.
4. Mandeep S, Dhruv D. A recent review on: coronavirus disease 2019. Asian J Pharm Clin Res. 2022 Jul;15(7):17-24.
5. Mengist HM, Mekonnen D, Mohammed A, Shi R, Jin T. Potency, safety, and pharmacokinetic profiles of potential inhibitors targeting SARS-CoV-2 main protease. Front Pharmacol. 2020;11:630500. doi: 10.3389/fphar.2020.630500, PMID 33597888.
6. Park JY, Kim JH, Kim YM, Jeong HJ, Kim DW, Park KH. Tanshinones as selective and slow-binding inhibitors for SARS-CoV cysteine proteases. Bioorg Med Chem. 2012 Oct;20(19):5928-35. doi: 10.1016/j.bmc.2012.07.038, PMID 22884354.
7. Osipiuk J, Azizi SA, Dvorkin S, Endres M, Jedrzejczak R, Jones KA. Structure of papain-like protease from SARS-CoV-2 and its complexes with non-covalent inhibitors. Nat Commun. 2021 Feb;12(1):743. doi: 10.1038/s41467-021-21060-3, PMID 33531496.
8. Prajapat M, Sarma P, Shekhar N, Avti P, Sinha S, Kaur H. Drug targets for coronavirus: A systematic review. Indian J Pharmacol. 2020 Jan;52(1):56-65. doi: 10.4103/ijp.IJP_115_20, PMID 32201449.
9. Ge R, Shen Z, Yin J, Chen W, Zhang Q, An Y. Discovery of SARS-CoV-2 main protease covalent inhibitors from a DNA-encoded library selection. Slas Discov. 2022 Mar;27(2):79-85. doi: 10.1016/j.slasd.2022.01.001, PMID 35063690.
10. Imam SS, Imam ST, Mdwasifathar K, Kumar R, Ammar MY. Interaction between ace 2 and SARS-CoV2, and use of egcg and theaflavin to treat COVID 19 in initial phases. Int J Curr Pharm Sci. 2022:5-10. doi: 10.22159/ijcpr.2022v14i2.1945.
11. Saadh MJ. Omicron sars-CoV-2 variant, B.1.1.529: Severity, transmission, mutation, and efficacy of current vaccines. Int J App Pharm. 2022:12-5. doi: 10.22159/ijap.2022v14i3.44376, b1.1.
12. Gorkhali R, Koirala P, Rijal S, Mainali A, Baral A, Bhattarai HK. Structure and function of major SARS-CoV-2 and SARS-CoV proteins. Bioinform Biol Insights. 2021 Jun;15:11779322211025876. doi: 10.1177/11779322211025876, PMID 34220199.
13. He J, Hu L, Huang X, Wang C, Zhang Z, Wang Y. Potential of coronavirus 3C-like protease inhibitors for the development of new anti-SARS-CoV-2 drugs: insights from structures of protease and inhibitors. Int J Antimicrob Agents. 2020 Aug;56(2):106055. doi: 10.1016/j.ijantimicag.2020.106055, PMID 32534187.
14. V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol. 2021 Mar;19(3):155-70. doi: 10.1038/s41579-020-00468-6, PMID 33116300.
15. Klemm T, Ebert G, Calleja DJ, Allison CC, Richardson LW, Bernardini JP. Mechanism and inhibition of the papain-like protease, PLpro, of SARS-CoV-2. EMBO J. 2020 Sep 15;39(18):e106275. doi: 10.15252/embj.2020106275, PMID 32845033.
16. McClain CB, Vabret N. SARS-CoV-2: the many pros of targeting PLpro. Signal Transduct Target Ther. 2020 Oct;5(1):223. doi: 10.1038/s41392-020-00335-z, PMID 33024071.
17. Li SW, Wang CY, Jou YJ, Yang TC, Huang SH, Wan L. SARS coronavirus papain-like protease induces Egr-1-dependent up-regulation of TGF-β1 via ROS/p38 MAPK/STAT3 pathway. Sci Rep. 2016 May;6(1):25754. doi: 10.1038/srep25754, PMID 27173006.
18. Costanzi E, Kuzikov M, Esposito F, Albani S, Demitri N, Giabbai B. Structural and biochemical analysis of the dual inhibition of MG-132 against SARS-CoV-2 main protease (Mpro/3CLpro) and human *cathepsin-L.* Int J Mol Sci. 2021 Oct;22(21):11779. doi: 10.3390/ijms222111779, PMID 34769210.
19. Ma C, Sacco MD, Hurst B, Townsend JA, Hu Y, Szeto T. Boceprevir, GC-376, and calpain inhibitors II, XII inhibit SARS-CoV-2 viral replication by targeting the viral main protease. Cell Res. 2020 Aug;30(8):678-92. doi: 10.1038/s41422-020-0356-z, PMID 32541865.
20. Su H, Yao S, Zhao W, Zhang Y, Liu J, Shao Q. Identification of pyrogallol as a warhead in the design of covalent inhibitors for the SARS-CoV-2 3CL protease. Nat Commun. 2021 Jun;12(1):3623. doi: 10.1038/s41467-021-23751-3, PMID 34131140.
21. Sacco MD, Ma C, Lagarias P, Gao A, Townsend JA, Meng X. Structure and inhibition of the SARS-CoV-2 main protease reveal strategy for developing dual inhibitors against Mpro and cathepsin L. Sci Adv. 2020 Dec;6(50):eabe0751. doi: 10.1126/sciadv.abe0751, PMID 33158912.
22. Ma C, Hu Y, Townsend JA, Lagarias PI, Marty MT, Kolocouris A. Ebselen, disulfiram, carmofur, PX-12, tideglusib, and shikonin are nonspecific promiscuous SARS-CoV-2 main protease inhibitors. ACS Pharmacol Transl Sci. 2020 Oct 9;3(6):1265-77. doi: 10.1021/acsptsci.0c00130, PMID 33330841.
23. Saadh MJ, Almaaytah AM, Alaraj M, Dababneh MF, Sa'Adeh I, Aldalaen SM, Kharshid AM, Alboghdadly A, Hailat M, Khaleel A, Al-Hamaideh KD. Punicalagin and zinc (II) ions inhibit the activity of SARS-CoV-2 3CL-protease *in vitro*. Eur Rev Med Pharmacol Sci. 2021 May;25(10):3908-13.
24. Saadh MJ, Almaaytah AM, Alaraj M. Sauchinone with zinc sulphate significantly inhibits the activity of sars-cov-2 3cl-protease. Pharmacologyonline. 2021;2:242-8.
25. Jo S, Kim S, Shin DH, Kim MS. Inhibition of SARS-CoV 3CL protease by flavonoids. J Enzyme Inhib Med Chem. 2020 Jan;35(1):145-51. doi: 10.1080/14756366.2019.1690480, PMID 31724441.