Original Article

The Interaction of Antiviral Drugs with SARS-Cov-2 3CL Protease

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

Abstract

SARS COV2 is one of the most destructive pandemics the world has faced and led to extreme economic losses. For its clinical therapy, SARS-COV-2 3CL Protease (3CLpro) is considered a target because of its crucial role in replication. Inhibition of this 3CLpro can lead to a decrease in viral load and infection. Different studies used various compounds and tested their inhibiting activity. Among them, flavonoids, serine derivatives, Chalcones, and alpha-keto amides were proven to have inhibitory effects. Many in-vitro tests were done to check the binding and inhibition abilities of such compounds. In vivo, some studies are done, but more is needed to prove this discovery. As far as research is concerned, therapeutic drugs against COVID-19 can be made by using such inhibitors. More in vivo studies and animal model experimentation should be done to confirm the findings.

Keywords: Inhibitors, SARS-Cov-2, COVID-19, SARS-Cov-2 3CL-Protease.

Introduction

3CLpro is a viral protease vital for the replication of the virus (Figure 1). Many small molecules (3CLpro) have been reported to inhibit SARS COV2 replication in cell cultures (Dampalla et al., 2021; V'kovski et al., 2021). 3CLPro is not present in the host cells, so it can be tested as an excellent target for antiviral agents. During the last five years, different inhibitors have been constructed for 3CLPro based on its structure. Other inhibitors include peptide mimetics and small molecules (He et al., 2020). Nigella sativa has high anti-SARS-CoV activity and could be a good source for developing new coronavirus antiviral treatments (Idrees et al., 2021; Saadh et al., 2022).

Consequently, the objective of this study is to examine inhibitors of SARS-CoV-2 3CL-protease as prospective therapeutics for the treatment of people infected with SARS-CoV-2, providing patients and healthcare professionals with new hope in the battle against SARS-CoV-2.

Access this article online

Quick Response Code:

Website: www.pnrjournal.com

DOI: 10.47750/pnr.2022.13.03.129

3CLpro Inhibitors and their Mechanism of Actions

Alpha-Ketoamide inhibitors

This inhibitor was designed using conserved active sites of the main coronavirus protease. To find the best compromise for various sizes of the S2 pocket of 3CLpro, the functional group at the p2 site of alpha-keto amides was most important. The most effective inhibition of SARS-CoV-2 was achieved by the designed compound 1. The 3CLPro enzymes of SARS-CoV-2 and compound 1 have the highest structural similarity, showing that compound 1 is the best antiviral candidate against SARS-CoV-2 (He et al., 2020).

Address for correspondence: Monica Preethi R
Meenakshi Academy of Higher Education and Research, Kanchipuram
Email: monicapreethi8@gmail.com

How to cite this article: Monica Preethi R, Archana S, Eswari V, To Estimate The Prevalence Of Adenomyosis And Its Correlation With Benign Endometrial Lesions In Tertiary Care Center, J PHARM NEGATIVE RESULTS 2022;13: 865-868.

Serine Derivatives

Tetrapeptide inhibitor 3 was used to derive serine derivatives and the D-Serine derivative 4, which was constructed and tested against 3CLpro R1881 mutant protease. No cytotoxicity was displayed by compound 5 towards HeLa cells (He et al., 2020; Mengist et al., 2021).

Flavonoids

A library of flavonoids containing ten scaffolds was checked for their inhibitory effectiveness and herbacetin, pectolinarin, and rhoifolin showed the most inhibitory effect. Compounds 6, 7 and 8 (PDB ID: 4WY3) were tested for docking. Four H-bonds were formed by the herbacetin at the S2 site. There was a difference in the binding modes of rhoifolin and pectolinarin (He et al., 2020; Jo et al., 2020).

There are 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 3CLpro in vitro (Saadh et al., 2021).

Chalcones

From chalocones, compound 14 (IC50 = 11.4 ± 1.4 µM) was suggested as being the most effective inhibitor. This could be due to the per hydroxyl group that might be most vital for binding to 3CLpro. Compound 14 (PDB ID: 2ZU) in docking studies showed a hydrogen bond between His163, Se144, and carbonyl and hydroxyl groups (He et al., 2020; Mathpal et al., 2022). Dampalla et al. used various variants of deuterated aldehyde compounds to test their inhibitory effects. In addition, alpha ketoamide based on compound 1 and compounds 3, 4, 8, and 11 prodrug variations of aldehydes bisulfite adduct were prepared for testing, with bisulfite adduct displaying 50% inhibitory concentrations in the enzyme assay that matched their aldehyde counterparts. Visible lower potency was shown by Alpha-Ketoamide derivative, i.e., compound 5, in the enzyme assay. Significant increased IC50 values were noted in prodrug counterparts, i.e., compounds 3, 4, 8, and 11 in contrast to their bisulfite and aldehyde precursors.

PF-07321332 is emerging as a promising oral delivery second-generation clinical candidate (Vandyck et al., 2021). Compound 4, GC376 and MAC-5576 were identified as 3CLpro inhibitors. Protease biochemical inhibition was displayed by each of the mentioned compounds, along with inhibition of virus, in the cell-based assay by GC376 and compound 4, whereas it was not shown by MAC-5576. The crystal structures of these compounds were made in complex forms with the proteases to confirm their crystal inhibition. Similar interactions were depicted by compounds four and GC376 as mimetic inhibitors. MAC-5576 interactions were identical to those of the already identified 3CLpro small molecule inhibitor (Iketani et al., 2021; Resnick et al., 2021).

Recently, GC376 has been reported as an inhibitor of 3CLpro. The results of this study confirm the previous findings as similar interactions were observed in this study as well. The GC376 benzyl groups are bound to asymmetrical units of 3CLpro protomers and are also joined in the hydrophobic pocket constructed by Leu167, Gln 192, and M et165. In this region, weaker electronic density is observed; along with previous findings, it can be concluded that modifications in this subsite can be done to improve the inhibitor (Iketani et al., 2021). More than 40 types of tanshinone molecules have been identified to show clinical applications such as antiviral activity (Mandal et al., 2021).

Lead compound injected in mouse with Middle East respiratory syndrome (MERS) infection caused survival increase from 0 to 100 percent and reduction of lungs viral titers. Other potential compounds include nigelledine, hederagenin, thymohydroquinone, alpha hederin, thymoquinone, and hederagenin are the compounds isolated from Nigella sativa, have a role in inhibiting the viral replication and binding to host cell receptors. Other study reported 23 small molecules that acts as inhibitors, and they have been identified with IC50 ranging from 0.26 to 0.28.85 µM (Zhu et al., 2020).

Another study found that theaflavin, a chemical found in black tea, could dock in the catalytic site of SARS-CoV-2 RNA-dependent polymerase. Scutellaria baicalensis ethanol extract has been shown to stop the activity of SARS-CoV-2 main proteinase (3CL Mpro) and in vitro SARS-CoV-2 replication (Hasegawa et al., 2021). Baicalein binds to the substrate-binding site of the virus by the interaction between 2 catalytic residues and stops the peptide substrate from binding to the active site. Andquinadoline B, Scedapin, norquinadoline A, and the polyketide isochaetochromin D1 have higher affinity for the target proteins 3CLpro, nsp15, grp78 (spiking domain), and RdRp. An acoustic liquid-handling apparatus was used for the screening of 32,033 chemical samples, and eight compounds exhibited antiviral potential in the cell-based assay (Hasegawa et al., 2021).

Hu et al., used a deep learning model and an enzymatic assay for the inhibitor identification, and six novel potent inhibitors of 3CLpro were identified. Bung et al., used a de novo synthesis technique to design the molecules for the inhibition activity and were successful in the testing and designing. For in-vitro evaluation, 18 hits were selected for in-vitro evaluation, and results show that five compounds as inhibitors of viral protease were influential in terms of inhibition. After this, docking was done in detail for the study of the inhibition mechanism. For energetic hit generation, a pharmacophore model was generated using co-crystallized ligand and active hits (Abdallah et al., 2021). Intense inhibitory activity was depicted by glycosylated flavonoids that bound more strongly than the N3 co-crystallized inhibitors. The binding of the flavonoids to the protease active site is dependent on the structure. With increased strength, flavanols have mono or disaccharide substituted at position C3. NMR was used to identify inhibitors, and compound 6 exhibited 75% inhibitory activity against SARS-CoV-2 main proteinase (3CL Mpro) (Zubair et al., 2021; Klemm et al., 2021).

In the enzyme assay, Vero E6 and A549-ACE2 were the cell lines used to test the deuterated compounds that are more potent as compared to GC378. When tested effective concentration values (50%) of deuterated compounds were compared to those in cell lines, they were lower by 2.67-3.38 folds when compared to GC376 in Vero E6 cells. Up to 100µ, every compound, including GC376, doesn't show any cytotoxicity (Dampalla et al., 2021).

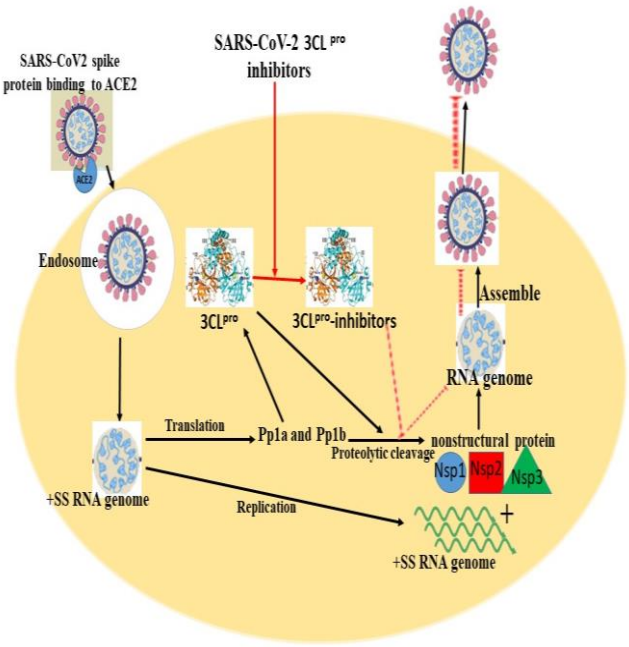

Figure 1: The The 3CL pro (Mpro) of the SARS-CoV-2 virus is a key antiviral drug target.

Conclusions

It can be concluded that many different chemicals from natural sources can be potent treatments for coronavirus infection. In terms of sequence, the catalytic domain of SARS-CoV-2 main proteinase (3CL Mpro) for COV-1 and COV-2 viruses is identical. The naturally occurring compounds found to be effective include Chalcone, flavonoids, Andquinadoline B, Scedapin, norquinadoline A, and polyketide Isochaetochromin D1. These compounds were very successful in in vitro studies and in vivo, although more in vivo studies are required. Naturally occurring compounds and their derivatives are the best options, as synthetic compounds are costly and will require further research.

Acknowledgements

The author is grateful to the Middle East University (MEU), Amman, Jordan, for the financial support granted to cover the publication fee of this research article.

References

1. Abdallah, H. M., El-Halawany, A. M., Sirwi, A., El-Araby, A. M., Mohamed, G. A., Ibrahim, S. R., ... & A. Elfaky, M. (2021). Repurposing of some natural product isolates as SARS-COV-2 main protease inhibitors via in vitro cell free and cell-based antiviral assessments and molecular modeling approaches. Pharmaceuticals, 14(3), 213.
2. Bung, N., Krishnan, S. R., Bulusu, G., & Roy, A. (2021). De novo design of new chemical entities for SARS-CoV-2 using artificial intelligence. Future medicinal chemistry, 13(06), 575-585.
3. Cherrak SA, Merzouk H, Mokhtari-Soulimane N. Potential bioactive glycosylated flavonoids as SARS-CoV-2 main protease inhibitors: A molecular docking and simulation studies. PLoS One. 2020 Oct 15;15(10):e0240653.
4. Dampalla, C. S., Zheng, J., Perera, K. D., Wong, L. Y. R., Meyerholz, D. K., Nguyen, H. N., ... & Chang, K. O. (2021). Postinfection treatment with a protease inhibitor increases survival of mice with a fatal SARS-CoV-2 infection. Proceedings of the National Academy of Sciences, 118(29), e2101555118.
5. Hasegawa, T., Imamura, R. M., Suzuki, T., Hashiguchi, T., Nomura, T., Otsuguro, S., ... & Kojima, H. (2022). Application of acoustic ejection MS system to high-throughput screening for SARS-CoV-2 3CL protease inhibitors. Chemical and Pharmaceutical Bulletin, 70(3), 199-201.
6. He, J., Hu, L., Huang, X., Wang, C., Zhang, Z., Wang, Y., ... & Ye, W. (2020). Potential of coronavirus 3C-like protease inhibitors for the development of new anti-SARS-CoV-2 drugs: Insights from structures of protease and inhibitors. International journal of antimicrobial agents, 56(2), 106055.
7. Hu, F., Wang, L., Hu, Y., Wang, D., Wang, W., Jiang, J., ... & Yin, P. (2021). A novel framework integrating AI model and enzymological experiments promotes identification of SARS-CoV-2 3CL protease inhibitors and activity-based probe. Briefings in bioinformatics, 22(6), bbab301.
8. Idrees, M., Khan, S., Memon, N. H., & Zhang, Z. (2021). Effect of the Phytochemical Agents against the SARS-CoV and Some of them Selected for Application to COVID-19: A Mini-Review. Current Pharmaceutical Biotechnology, 22(4), 444-450.
9. Iketani, S., Forouhar, F., Liu, H., Hong, S. J., Lin, F. Y., Nair, M. S., & Ho, D. D. (2021). Lead compounds for the development of SARS-CoV-2 3CL protease inhibitors. Nature communications, 12(1), 1-7.
10. Jo, S., Kim, S., Shin, D. H., & Kim, M. S. (2020). Inhibition of SARS-CoV 3CL protease by flavonoids. Journal of enzyme inhibition and medicinal chemistry, 35(1), 145-151.
11. Klemm, T., Ebert, G., Calleja, D. J., Allison, C. C., Richardson, L. W., Bernardini, J. P., ... & Komander, D. (2020). Mechanism and inhibition of the papain-like protease, PLpro, of SARS-CoV-2. The EMBO journal, 39(18), e106275.
12. Mandal, A., Jha, A. K., & Hazra, B. (2021). Plant products as inhibitors of coronavirus 3CL protease. Frontiers in Pharmacology, 12, 583387.
13. Mathpal, S., Joshi, T., Sharma, P., Pande, V., & Chandra, S. (2022). Assessment of activity of chalcone compounds as inhibitors of 3-chymotrypsin like protease (3CLPro) of SARS-CoV-2: in silico study. Structural chemistry, 1-17.
14. Mengist, H. M., Mekonnen, D., Mohammed, A., Shi, R., & Jin, T. (2021). Potency, safety, and pharmacokinetic profiles of potential inhibitors targeting SARS-CoV-2 main protease. Frontiers in pharmacology, 11, 630500.
15. Resnick, S. J., Iketani, S., Hong, S. J., Zask, A., Liu, H., Kim, S., & Chavez, A. (2021). Inhibitors of coronavirus 3CL proteases protect cells from protease-mediated cytotoxicity. Journal of Virology, 95(14), e02374-20.
16. Saadh, M. J., Almaaytah, A. M., Alaraj, M., Dababneh, M. F., Sa'Adeh, I., Aldalaen, S. M., & DAYYIH, W. (2021). Punicalagin and zinc (II) ions inhibit the activity of SARS-CoV-2 3CL-protease in vitro. Eur Rev Med Pharmacol Sci, 25(10), 3908-13.
17. Saadh, M. J. (2022). OMICRON SARS-CoV-2 VARIANT, B. 1.1. 529: SEVERITY, TRANSMISSION, MUTATION, AND EFFICACY OF CURRENT VACCINES. International Journal of Applied Pharmaceutics, 12-15.

18. V'kovski, P., Kratzel, A., Steiner, S., Stalder, H., & Thiel, V. (2021). Coronavirus biology and replication: implications for SARS-CoV-2. Nature Reviews Microbiology, 19(3), 155-170.
19. Vandyck, K., & Deval, J. (2021). Considerations for the discovery and development of 3-chymotrypsin-like cysteine protease inhibitors targeting SARS-CoV-2 infection. Current Opinion in Virology, 49, 36-40.
20. Zhu, W., Xu, M., Chen, C. Z., Guo, H., Shen, M., Hu, X., ... & Zheng, W. (2020). Identification of SARS-CoV-2 3CL protease inhibitors by a quantitative high-throughput screening. ACS pharmacology & translational science, 3(5), 1008-1016.
21. Zubair, M. S., Maulana, S., Widodo, A., Pitopang, R., Arba, M., & Hariono, M. (2021). GC-MS, LC-MS/MS, Docking and molecular dynamics approaches to identify potential SARS-CoV-2 3-chymotrypsin-like protease inhibitors from Zingiber officinale Roscoe. Molecules, 26(17), 5230.