Virginia Commonwealth University
VCU Scholars Compass

Graduate Research Posters | Graduate School

2021

Peptidomimetic and Non- Peptidomimetic Derivatives as Possible SARS-CoV-2 Main Protease (Mpro) Inhibitors

Mohammed A. Al Awadh
Virginia Commonwealth University

Mohini S. Ghatge Ph.D
Virginia Commonwealth University

Mona A. Al Khairi
Virginia Commonwealth University

See next page for additional authors

Follow this and additional works at: https://scholarscompass.vcu.edu/gradposters

 Part of the Medicinal and Pharmaceutical Chemistry Commons

Downloaded from

Al Awadh, Mohammed A.; Ghatge, Mohini S. Ph.D; Al Khairi, Mona A.; Musayev, Faik N.; Donkor, Akua K.; AL Mughram, Mohammed H.; Omar, Abdelsattar M. Ph.D; El-Araby, Moustafa M. Ph.D; and Safo, Martin K. Ph.D, "Peptidomimetic and Non- Peptidomimetic Derivatives as Possible SARS-CoV-2 Main Protease (Mpro) Inhibitors" (2021). *Graduate Research Posters.* Poster 107.
https://scholarscompass.vcu.edu/gradposters/107

This Poster is brought to you for free and open access by the Graduate School at VCU Scholars Compass. It has been accepted for inclusion in Graduate Research Posters by an authorized administrator of VCU Scholars Compass. For more information, please contact libcompass@vcu.edu.

Authors

Mohammed A. Al Awadh, Mohini S. Ghatge Ph.D, Mona A. Al Khairi, Faik N. Musayev, Akua K. Donkor, Mohammed H. AL Mughram, Abdelsattar M. Omar Ph.D, Moustafa M. El-Araby Ph.D, and Martin K. Safo Ph.D

This poster is available at VCU Scholars Compass: https://scholarscompass.vcu.edu/gradposters/107

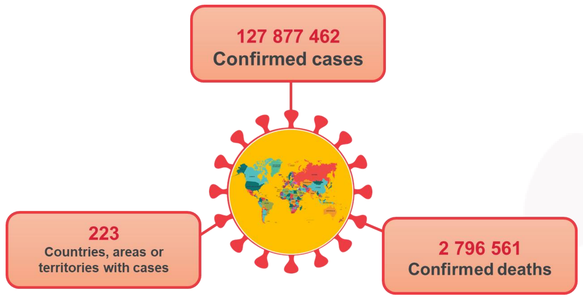
Peptidomimetic and Non- Peptidomimetic Derivatives as Possible SARS-CoV-2 Main Protease (Mpro) Inhibitors

Mohammed A. Al Awadh, Mohini S. Ghatge, Mona A. Al Khairi, Faik N. Musayev, Akua K. Donkor, Mohammed H. AL Mughram, Abdelsattar M. Omar, Moustafa M. El-Araby, Martin K. Safo

Introduction

- **Background**

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of coronavirus disease 2019 (COVID-19), a pandemic that has resulted in nearly 2.8 M deaths and more than 127.8 M confirmed cases worldwide.[1] There is an urgent need for novel drugs that target SARS-CoV-2 and other pathogenic coronaviruses. The COVID-19 main protease (Mpro) plays a critical role in the viral life cycle by releasing essential polypeptides for viral replication and transcription.[2] Furthermore, Mpro has several distinguishing characteristics that make it an appealing candidate for drug development: 1) shared among all members of the Coronavirus family, 2) absence of closely related homologous in humans, and 3) a conserved active site.[3]



- **Research Objective**

The main objective of this study is to identify potent, oral small molecule inhibitors of Mpro activity to prevent the devastating sequelae of severe COVID-19.

Methods

- **Development of SARS-CoV-19 Mpro Inhibitors**

A fragment-based drug design approach, based on recently reported α-ketoamide inhibitors of Mpro, was employed for designing and synthesizing several peptidomimetic and non-peptidomimetic compounds in 5 to 10 mg quantities.[2] Following, a stock solution of 100 mM concentration of each compound was prepared using Dimethyl Sulfoxide (DMSO) for the studies.

- **Fluorescence Resonance Energy Transfer (FRET) assay**

The compounds were initially screened at a fixed concentration of 50 µM with a 384- well Microplate using FRET assay to evaluate their potential inhibition of Mpro. The study was conducted using the main protease, MBP-tagged (SARS-CoV-2) Assay Kit (BPS Bioscience, #79955-2) that contained Mpro, substrate, potent inhibitor (GC376) , and buffer. In this assay, the fluorescence due to enzymatic cleavage of the substrate by SARS-CoV-2 Mpro was detected and measured by BMG LABTECH CLARIOstar™, a fluorescent microplate reader, with an excited/emission wavelength of 360 nm/460 nm, respectively. For the top inhibitors, dose-dependent experiments were carried out to determine the IC50 by plotting the observed scores at each dose point using variable parameter nonlinear regression in Prism GraphPad Software.

- **Analysis of physiochemical and pharmacokinetic properties**

A computational analysis of the compounds' molecular properties and physicochemical profiles was performed using SwissADME to predict their pharmacokinetic properties and assess their suitability as possible orally active drug candidates.

- **Molecular Modeling study**

An *in-silico* molecular docking simulation of the SARS-CoV-2 Mpro crystal structure in complex with the identified compounds was conducted using GOLD2020 and sybyl X2.1 to investigate the crucial binding residues and potential binding modes of the compounds. The fitting scores were measured for the different poses of the compounds and compared to the known potent inhibitor (GC376) binding.

- **X-ray Crystallography**

One of the identified Mpro inhibitors, Compound 5 was co-crystallized with the protein and the structure determined at 2.5 Å.

Result

- **FRET assay**

The fragment-based drug design approach identified 168 potential Mpro inhibitors, which were all tested for their inhibitory activity against Mpro using FRET assay. The study showed 29 compounds to exhibit lower fluorescence compared to the negative control, indicating inhibitory activity, with three of the compounds (MCP-212, MCP-221 and MCP-256; Figure 1) exhibiting over 50% enzymatic inhibition.

A dose-dependent FRET assay was performed for MCP-212, 221, and 256 at concentrations of 1, 5, 25, 50, 50, 75, 100, 125, 250, 500, 750 and 1000mM, to calculate the IC50 (Figure 2), which is 131.6 µM, 57.7 µM, and 107.1 µM, respectively.

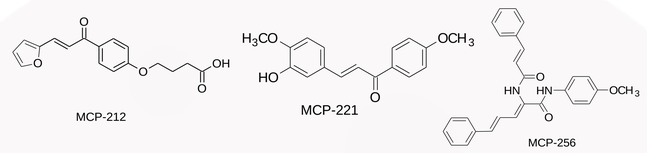


Figure 1: Chemical structures of compounds MCP-212, 221 and 256.

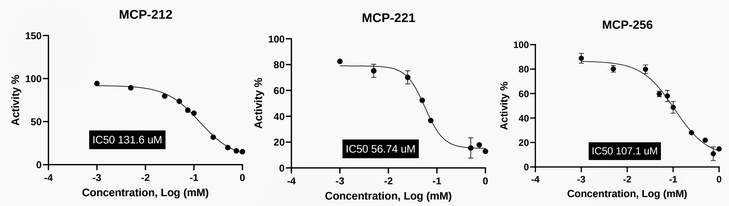


Figure 2: The IC50 plot of compounds MCP-212, 221 and 256.

- **Analysis of physiochemical and pharmacokinetic properties**

The *in-silico* evaluation of the pharmacokinetic properties revealed that all the tested compounds comply with Lipinski's rule of 5 (Lipinski violations = 0), where ClogP values ranged between 2.62 and 4.39 (<5), molecular weight (MW) range 284.31-424.49 (<500), number of H-bond acceptors (HBA) range 3-10 (≤ 10) and H-bond donors (HBD) range 1-2 (<5), suggesting that these compounds would have favorable oral bioavailability (Table 1)

Table 1: Drug likeness properties for oral bioavailability by Lipinski's rule of five

Compound	MW	#H-bond acceptors (HBA)	#H-bond donors (HBD)	Log P	Rule of 5 violations
MCP-212	300.31	5	1	2.62	0
MCP-221	284.31	4	1	3.01	0
MCP-256	424.49	3	2	4.39	0

- **Modeling Study**

From the *in-silico* molecular docking simulation study, MCP-256 gave the top score and best fit to the active site of Mpro that involves interactions with HIS 41, MET 49/165, GLU 166, PRO 168, ASP 187, ARG 188, and ALA 191 (Figure 3 and 4).

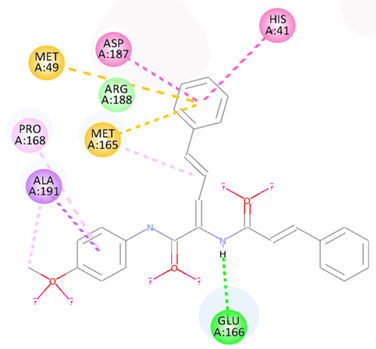


Figure 3: 2D diagram of putative binding of MCP-256 at the active site of COVID-19 Mpro.

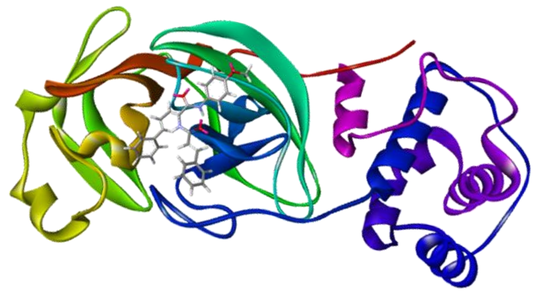


Figure 4: 3D representation of the putative binding of MCP-256 at the active site of COVID-19 Mpro.

- **X-ray Crystallography**

The electron density map from the co-crystal structure of Mpro with one of the identified inhibitors, Compound 5 showed potential binding of the inhibitors at the active site (Figure 5).

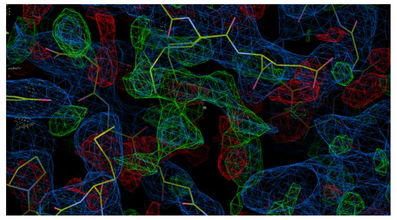


Figure 5: The electron density map of Mpro complexed with Compound 5 at 2.5 Å.

Discussion and Conclusion

- **Discussion**

We have identified several promising inhibitors of SARS-CoV-2 Mpro. Further studies, e.g., biological, biophysical are ongoing which would help future designing of more potent inhibitors.

- **Conclusion**

Although vaccines are critical in mitigating the impact of the emerging corona virus, genetic mutations can be a challenge.[4] The discovery of more strategies of inhibiting the virus, e.g., inhibition of main protease, is necessary to effectively target this virus.

Acknowledgment and References

- **Acknowledgment**

This project is supported by department of medicinal chemistry, VCU, King-Abdulaziz University (KAU) and Saudi Arabian Culture Mission.

- **References**

1. The World Health Organization (WHO), Health Emergency Dashboard. https://covid19.who.int/
2. Zhang, L.; Lin, D.; Sun, X.; Curth, U.; Drosten, C.; Sauerhering, L.; Becker, S.; Rox, K.; Hilgenfeld, R. Crystal Structure of SARS-CoV-2 Main Protease Provides a Basis for Design of Improved α-Ketoamide Inhibitors. *Science* **2020**, 368, 409–412.
3. Jin, Z.; Du, X.; Xu, Y.; Deng, Y.; Liu, M.; Zhao, Y.; Zhang, B.; Li, X.; Zhang, L.; Peng, C.; et al. Structure of Mpro from SARS-CoV-2 and Discovery of Its Inhibitors. *Nature* **2020**, 582, 289–293.
4. Ferrareze, P. A. G.; Franceschi, V. B.; Mayer, A. D. M.; Caldana, G. D.; Zimerman, R. A.; Thompson, C. E. E484K As an Innovative Phylogenetic Event for Viral Evolution: Genomic Analysis of the E484K Spike Mutation in SARS-CoV-2 Lineages from Brazil. **2021**.