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A small molecule inhibitor of the main coronavirus protease 3CLPro based on a carbocyclic substituted peptidomimetic.

Ilya I. Yudin, Tatyana V. Grebennikova, Timur M. Garaev

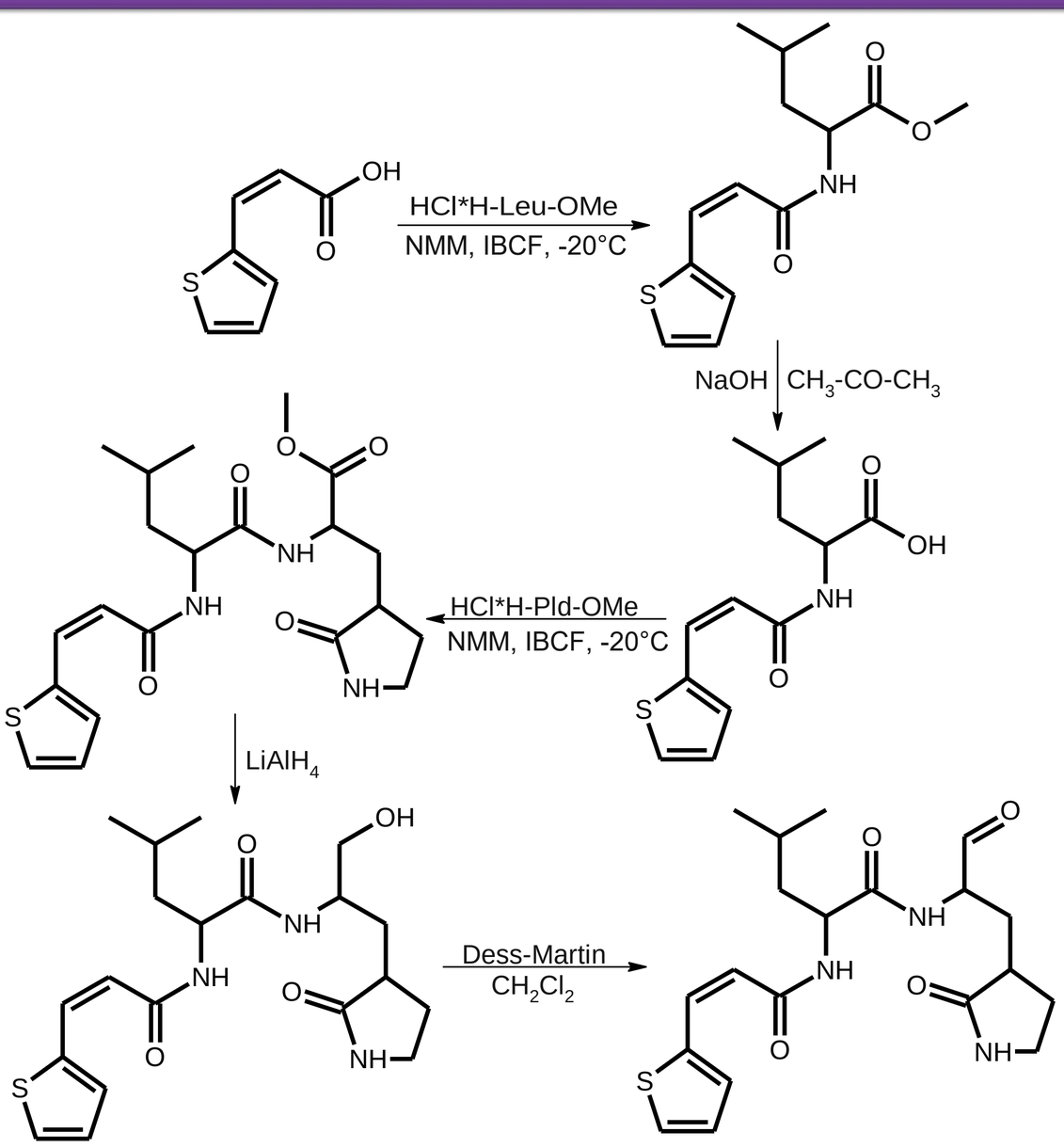
Gamaleya National Research Center for Epidemiology and Microbiology, Ministry of Health of Russian Federation, Moscow, 123098 Russia

INTRODUCTION & AIM

According to available data, the virus enters host cells and replicates in them with the participation of many molecular factors from both the host and the virus. One of the important molecular factors in SARS-CoV-2 replication is the main protease (Mpro), also known as the 3C-like protease (3CLpro).Mpro is necessary for the cleavage of the viral polyprotein pp1ab at 11 discrete sites, at the glutamine-serine bond in the Leu-Gln-Ser-Gly-Ala peptide motif. 3CLpro (nsp5) mediates the cleavage of nsp4 - nsp16. Thus, 3CLpro is the main target for the search for antiviral drugs against SARS-CoV-2.

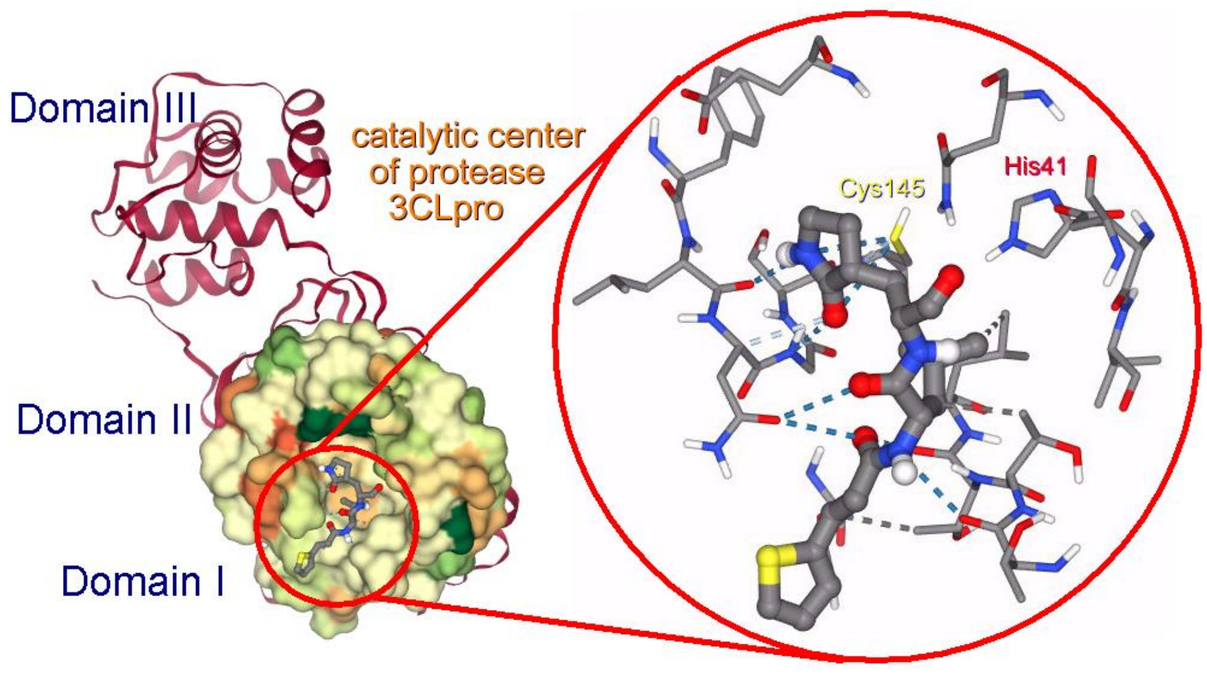
Structurally, inhibitors of the main coronavirus protease enzyme (3CLpro) are peptidomimetics that mimic natural 3CLpro peptide substrates and form various non-covalent bonds with amino acids in the active site of the protease, while the warhead is located very close to the catalytic center of the enzyme, and a covalent bond is formed with cysteine at position 145 (Cys145) by nucleophilic attack [1]. These warheads covalently bind to the Cys145 residue in the S1' pocket of 3CLpro, exerting an inhibitory effect. The coronavirus 3CLpro protease inhibitor PF-07321332 (Pfizer™) covalently binds directly to the catalytic center of the enzyme (His41 – Cys145), namely to the cysteine residue (Cys145). The attacking group in the PF-07321332 compound is a nitrile group that forms a covalent bond with the thiol group of Cys145. In the present study, an aldehyde warhead was used as the attacking group.

METHOD



The studies used the SARS-CoV-2 human coronavirus strain, passage 4, with an infectious activity of 106 TCID50/ml for Vero-E6 cells. The antiviral efficacy assessment experiment was conducted in the concentration range of 375.0...0.37 μg/ml. The antiviral activity of the compound was evaluated visually under a microscope 96 hours after infection by inhibiting the CPE of the virus in Vero E6 cell culture.

RESULTS & DISCUSSION



Molecular docking was performed using the Simple and Accessible Docking Web Server Interactive and Collaborative Online Docking (SeamDock) online service (https://seamless.rpbs.univ-paris-diderot.fr). This service uses AutoDock VINA for highly accurate prediction of protein-ligand interactions. The monomeric structure of 3CLpro SARS-CoV-2 (PDB code: 1z1i) was obtained from the RCSB PDB (https://www.rcsb.org/). The ligand structure was generated in HyperChem 8.0.8 (HeperCube, Ltd.).

In this study, we present a new small molecule inhibitor of the main coronavirus protease 3CLPro based on the carbocyclically substituted peptidomimetic N-(4-methyl-1-oxo-1- ((1-oxo-3-(2-oxopyrrolidin-3-yl)propan-2-yl)amino)pentan-2-yl)-(2Z)-3-(thiophen-2-yl)prop-2-enamide (TEA-Leu-Pld-CHO). The inhibitor demonstrated Si=32 (CC50 187.5; IC50 5.8 μg/mL). The inhibitor was not inferior to the reference drug Nirmatrevir (PF-07321332) and, in extended series of experiments (5-6 days of incubation), slightly superior in terms of inhibitory properties.A decisive step towards 3CLpro inhibition is the formation of a covalent bond between the cysteine residue of the active site (Cys145) and the aldehyde carbonyl of the inhibitor (see Fig. 1). The TEA-Leu-Pld-CHO inhibitor participates in several favorable binding interactions with the enzyme, including lipophilic interactions with Thr26, Thr25, Leu27 residues and hydrogen bonds with Asn142 and Cys145.

CONCLUSION

The proposed compound, given its high antiviral activity and low cytotoxicity, as well as its economic and synthetic availability, can be recommended as a candidate for preclinical and clinical trials to develop an etiotropic antiviral drug aimed at inhibiting an important enzyme—the main protease of the coronavirus, including modern strains of SARS-CoV-2.

FUTURE WORK / REFERENCES

1. Qiao J., Li Y.-S., Zeng R., Liu F.-L., Luo R.-H., Huang C., et.al. SARS-CoV-2 Mpro inhibitors with antiviral activity in a transgenic mouse model. *Science.* 2021: 371; 1374–1378