Development of Lactam-based Inhibitors of SARS-CoV-2 M^{pro}

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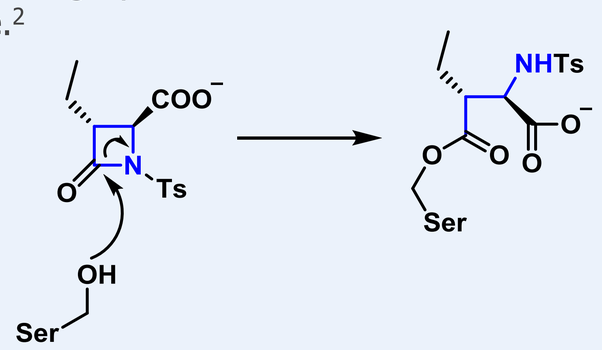
Abstract

The COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has highlighted an urgent need for development of new antiviral drugs. The main protease (M^{pro}) of SARS-CoV-2 plays an important role in viral replication and has become an attractive drug target for virus inhibition. The active site of SARS-CoV-2 M^{pro} contains Cys145 and His41, which allows to use structural subunits (or "warheads") for covalent binding to the thiol group of a cysteine residue in the active site of the enzyme.[1]

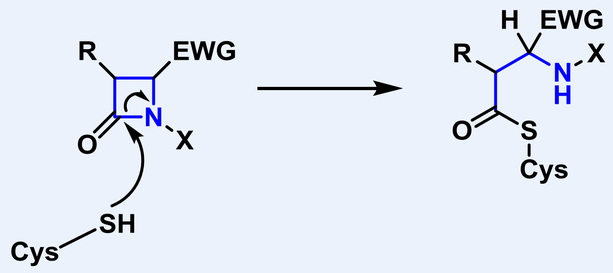
The aim of the project

▪ *β*-Lactams inhibit serine proteases with the mechanism of action involving the cleavage of *β*-lactam ring by serine residue in the active site of the enzyme.[2]



▪ The aim of our work is to use *β*-lactams for covalent binding to the thiol group of a cysteine residue in the active site of SARS-CoV-2 M^{pro}.

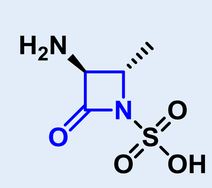
β-Lactams as cysteine protease inhibitors



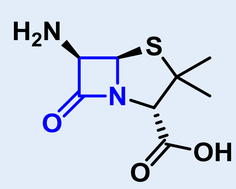
Fragment library of lactam-based inhibitors

▪ *β*- And γ-lactams and their structural analogs were chosen as "warheads" for covalent inhibition of SARS-CoV-2 protease M^{pro};

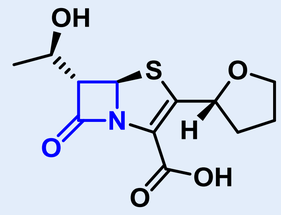
▪mechanism of action involves the cleavage of β-lactam ring by cysteine residue in the active site of the enzyme.



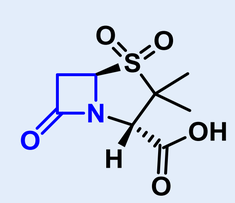
1 IC_{50} > 500 μM



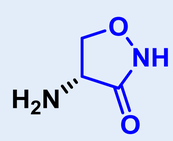
2 IC_{50} > 500 μM



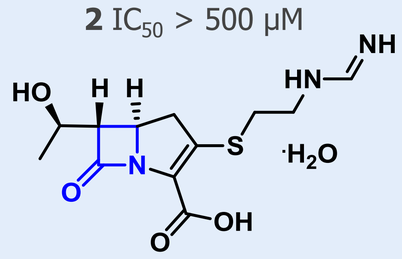
3 IC_{50} > 500 μM



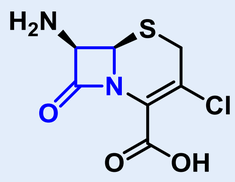
4 IC_{50} > 500 μM



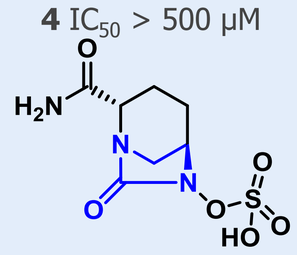
5 IC_{50} > 500 μM



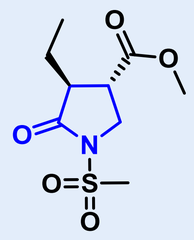
6 IC_{50} > 500 μM



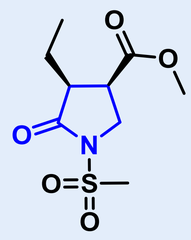
7 IC_{50} > 500 μM



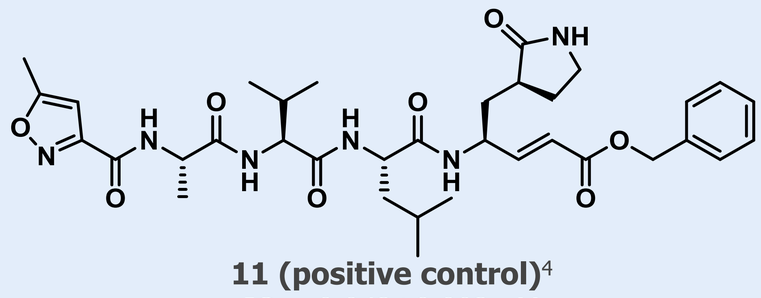
8 IC_{50} > 500 μM



9[3]**-rac-*cis***
IC_{50} > 500 μM



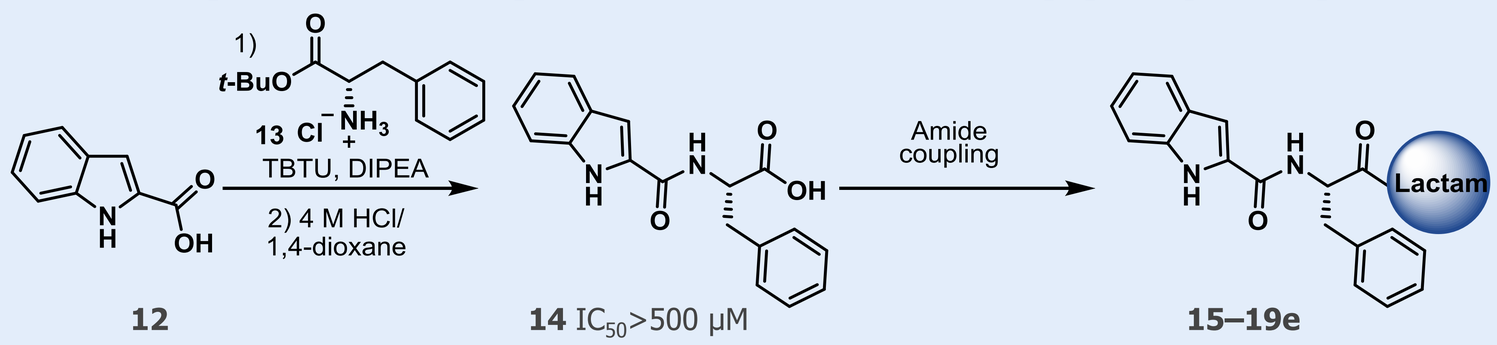
10[3]**-rac-*trans***
IC_{50} > 500 μM



11 (positive control)[4]
IC_{50}=0.042±0.002 μM

▪The ability of the "warheads" to inhibit M^{pro} was determined. None of the tested lactams **1**–**10** showed inhibitory activity. We propose that is due to size of designed fragments – the binding to the target protein M^{pro} and formation of a reversible protein-inhibitor complex doesn't occur; thereby formation a covalent protein-inhibitor complex isn't possible. To improve binding to the protein (1*H*-indole-2-carbonyl)-*L*-phenylalanine moiety **14** was introduced as a part of inhibitor.

Synthesis of (1*H*-indole-2-carbonyl)-*L*-phenylalanine derivatives 15–19e

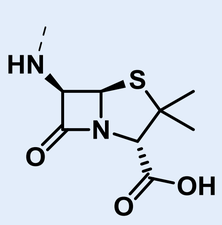


12

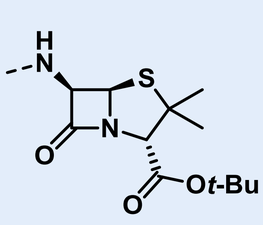
14 IC_{50}>500 μM

15–19e

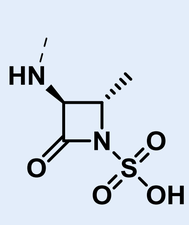
15
IC_{50}>500 μM



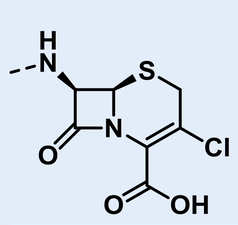
16
IC_{50}=134±8 μM



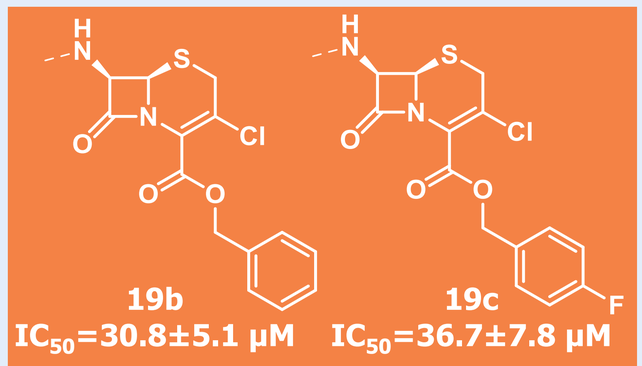
17
IC_{50}=32.4±1.7 μM



18
IC_{50}>500 μM



19a
IC_{50}=90.3±4.5 μM



19b
IC_{50}=30.8±5.1 μM

19c
IC_{50}=36.7±7.8 μM

19d
IC_{50}=104±8 μM

19e
IC_{50}=324±1 μM

▪ Attachment of the (1*H*-indole-2-carbonyl)-*L*-phenylalanine moiety **14** to the "warheads" resulted in increased inhibitory activity. Further work will focus on the inhibitory activity improvement of 7-amino-3-chloro cephalosporanic acid **7** derivatives.

References

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