Novel, non-covalent peptide furin inhibitors suppressing Zika virus replication

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https://doi.org/10.17952/37EPS.2024.P1095

Introduction

Furin is a subtilisin-like secretory **serine protease** widely distributed in various human tissues. It converts inactive precursor proteins into bioactive molecules (growth factors, neuropeptides or hormones).

Furin is also involved in the **development of various inflammatory diseases, cancers and pathogen infections (both viral and bacterial)**. Furin induces viral infections through processing of surface glycoproteins produced by numerous viruses (e.g. Zika, Dengue, SARS-CoV-2).

The **Zika virus (ZIKV)** infection results in relatively mild clinical symptoms, however it is associated with teratogenic effects on fetal development, so it is particularly dangerous for pregnant women. ZIKV is made up of 3 main structural proteins: capsid (C), membrane (M), which is created from its precursor premembrane (prM), and envelope (E). The host cell furin is involved in processing of prM and its transformation into mature M protein. This action is necessary to convert, immature, spiky, and noninfectious virus into a mature smooth, and infectious one. **Therefore, inhibition of furin seems to be a promising strategy to find new therapies against ZIKV infection.**

Fluorescein labeled inhibitors:

labeling peptides → biological tests

&[1]Lys-Arg-Arg-Cys(&[2])-Lys-Lys-Ser-Ile-Pro-Pro-Ile-Cys(&[2])-Phe&[1]

Fluo

&[1]Lys-Arg-Arg-Cys(&[2])-Lys-Lys-Ser-Ile-Pro-Pro-Ile-Cys(&[2])&[1]

Fluo

Fluo - 5(6)-Carboxyfluorescein

Concept and methods

&[1]Gly-Arg-Cys(&[2])-Thr-Lys-Ser-Ile-Pro-Pro-Ile-Cys(&[2])-Phe-Pro-Asp&[1]

Sunflower trypsin inhibitor, SFTI-1

X_l-X_k-Cys(&)-X_j-X_i-Ser-Ile-Pro-Pro-Ile-Cys(&)-Phe-NH_2

Peptide 1 was obtained by combinatorial chemistry followed by iterative deconvolution;

$X_i - X_l$ – variable amino acid residues

Our inhibitors

1. Arg-Arg-Cys(&)-Lys-Lys-Ser-Ile-Pro-Pro-Ile-Cys(&)-Phe-NH_2

Addition of basic residues (additional Arg or Lys residues - enhancement of the interaction of the inhibitor with furin) and additional cyclizations (increase proteolytic stability)

Gitlin-Domagalska A., Dębowski D., Maciejewska A., Samsonov S., Maszota-Zieleniak M., Ptaszyńska N., Łęgowska A., Rolka K., ACS Med. Chem. Lett., ***2023***, *14, 458−465*

2. Lys-Arg-Arg-Cys(&)-Lys-Lys-Ser-Ile-Pro-Pro-Ile-Cys(&)-Phe-NH_2
3. Arg-Arg-Arg-Cys(&)-Lys-Lys-Ser-Ile-Pro-Pro-Ile-Cys(&)-Phe-NH_2
4. &[1]Arg-Arg-Cys(&[2])-Lys-Lys-Ser-Ile-Pro-Pro-Ile-Cys(&[2])-Phe&[1]
5. &[1]Lys-Arg-Arg-Cys(&[2])-Lys-Lys-Ser-Ile-Pro-Pro-Ile-Cys(&[2])-Phe&[1]
6. &[1]Arg-Arg-Arg-Cys(&[2])-Lys-Lys-Ser-Ile-Pro-Pro-Ile-Cys(&[2])-Phe&[1]

Results

A) Peptides 3, 5, 6 – potent furin inhibitors

Inhibitor	K_i [nM]
3	0.27
5	0.21
6	0.25

B)

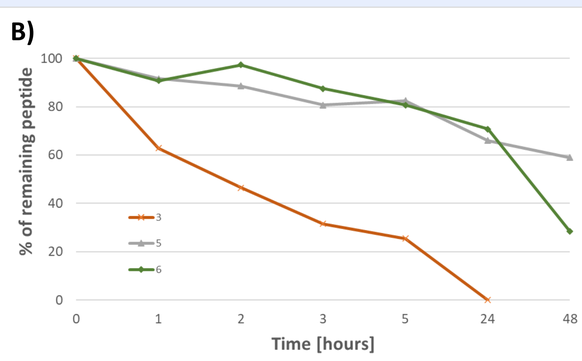

Fig. B) Stabilities of selected inhibitors in human serum

Results of biological tests of inhibitor 5

- Good membrane permeability
- Non-toxic at the concentration range 0.01-500 µM (Vero E6, A549 cells)
- Reduction of infectious virus progeny (Fig. A) as well as viral proteins level: E, pr and M (Fig. B)
- IC_{50} values were lower than those reported for commercial, covalent furin inhibitor decanoyl-Arg-Val-Lys-Arg-chloromethylketone (CMK) (Fig. E-G)

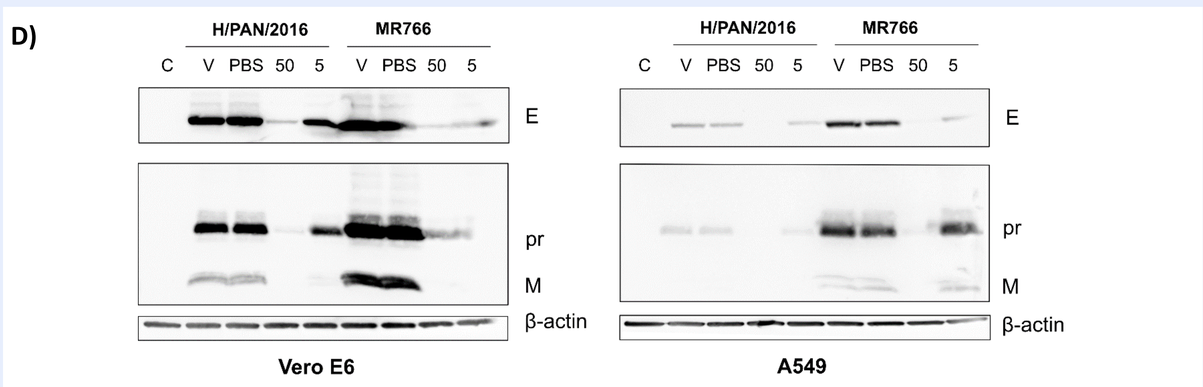

Fig. D) Western blot analysis of viral proteins (prM/M and E) in cell lysates after infection and after treatment with inhibitor 5 (50 µM and 5 µM)/PBS for 48 hours. C - uninfected cells; V - infected cells with ZIKV (MOI 0.1)

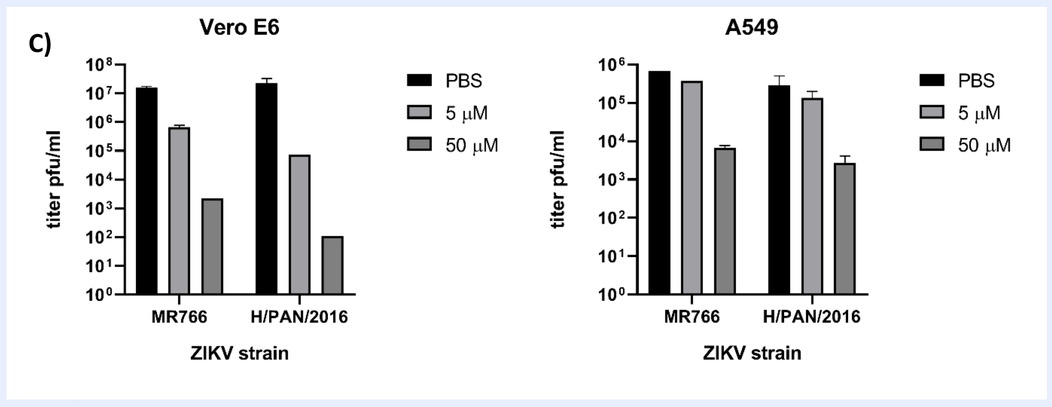

Fig. C) Antiviral assessment of inhibitor 5 against ZIKV. Vero E6 and A549 cells were infected with ZIKV (two strains: MR766 and H/PAN/2016) at 0.1 MOI and then incubated with inhibitor 5 (50 µM and 5 µM)/PBS for 48 hours. The virus was released into culture medium and then it was titrated on Vero E6 cells using the plaque method

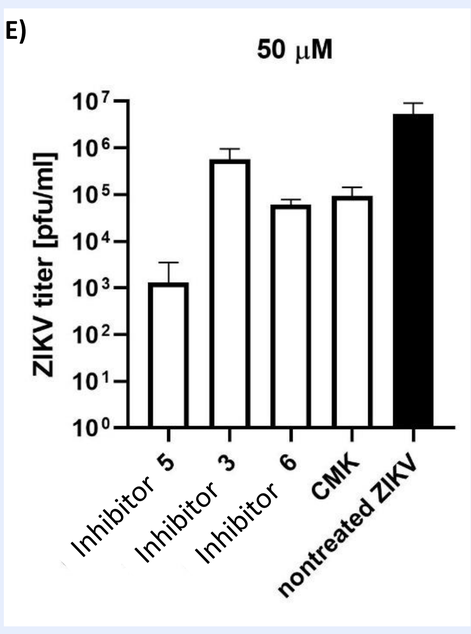

Fig. E) Comparsion inhibitory activity of inhibiotr 5 with other potent inhibitors (3 and 6) and comercial furin inhibitor (CMK). ZIKV titer in the culture medium after treatment with compounds (50 µM) of infected (MOI 0.1) Vero E6 cells for 48 hours

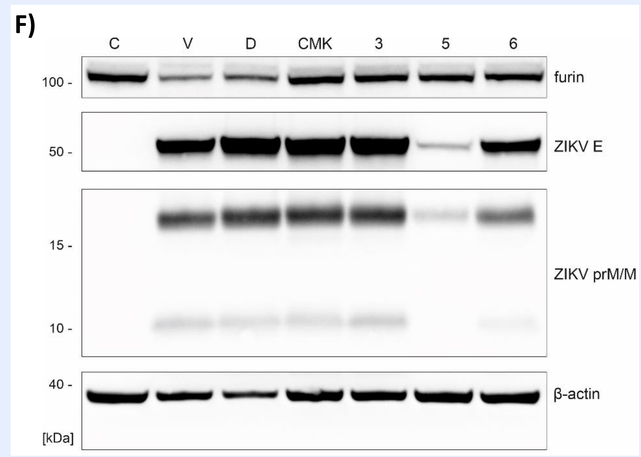

Fig. F) Expression of ZIKV proteins in cell lysates after 48 hours

G)

Inhibitor	IC_{50} [µM]	CC_{50} [µM]	SI
Dec-RVKR-CMK	9.06	615.2	**67.90**
3	22.25	707.3	**31.79**
5	3.69	1303	**353.12**
6	13.45	545.8	**40.58**

Fig. G) IC_{50} values of the tested inhibitors

Dec-RVKR-CMK – comercial furin inhibitor

IC_{50} – concentration of inhibitor necessary to reduce the number of infected cells by 50%

CC_{50} – cytotoxic concentration 50%

SI – selectivity index; SI = CC_{50} / IC_{50}

Conclusions

- We obtained 3 inhibitors more potent than the reference peptide (K_i = 0.49 nM; *Fittler, H. Depp, A. Avrutina, O. Dahms, S. O. Than, M. E., ChemBioChem 2015, 16 (17), 2441–2444*)
- Additional cyclization significantly improved the proteolytic stability of inhibitors
- Our inhibitors have extremely strong antiviral properties against ZIKV
- Inhibitor 5 is less cytotoxic than the comercial furin inhibitor (CMK) and its selectivity index (SI) is 5 times higher than SI value of CMK

Further plans

- Antiviral activity tests against other flaviviruses: tick-borne encephalitis virus (TBEV), West Nile Virus (WNV), Hepatitis C virus (HCV)
- Intestinal permeability *in vitro* tests: Parallel Artificial Membrane Permeability Assay (PAMPA test) and test based on the Caco-2 cell line
- Blood-brain barrier permeability test
- Synthesis of next generation inhibitors (improvement of their stability in proteolytic and reducing environment)

Acknowledgements

This work was supported by Ugrants-start-4 1220/158/2024