The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

A New Furin-based Peptidomimetic as an Inhibitor of NS2B-NS3 Protease from Zika Virus (ZIKV)

Érica E. S. Rodrigues,[1] Hannah Maus,[2] Stefan Josef Hammerschmidt,[2] João X. Araújo-Júnior, [1,3] Tanja Schirmeister,[2] and Edeildo F. Silva-Júnior[1] *

[1] Institute of Chemistry and Biotechnology, Federal University of Alagoas, Campus AC. Simões, Lourival Melo Mota Avenue, 57072-970 Maceió, Brazil.
[2] Institute of pharmaceutical and Biomedical Science, Johannes Gutenberg-University Mainz, Staudingerweg 5, D-55128 Mainz, Germany.
[3] Institute of Pharmaceutical Sciences, Federal University of Alagoas, Campus A.C. Simões, Lourival Melo Mota Avenue, 57072-970 Maceió, Brazil.

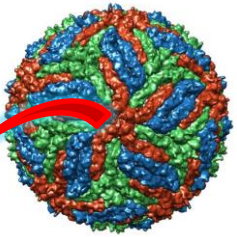
* Corresponding author: edeildo.junior@iqb.ufal.br

LABORATÓRIO DE QUÍMICA MEDICINAL

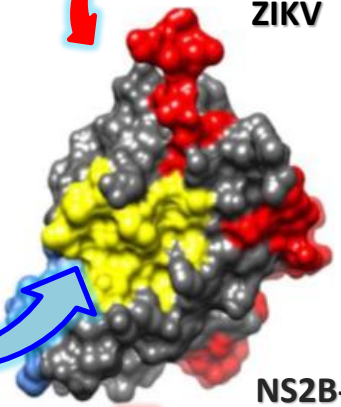
UFAL

JOHANNES GUTENBERG UNIVERSITÄT MAINZ

A New Furin-based Peptidomimetic as an Inhibitor of NSB2-NS3 Protease from Zika Virus (ZIKV)

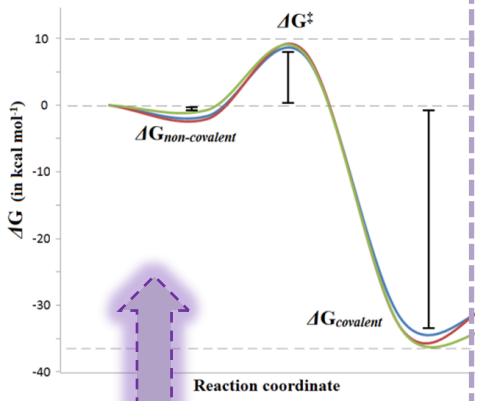


ZIKV

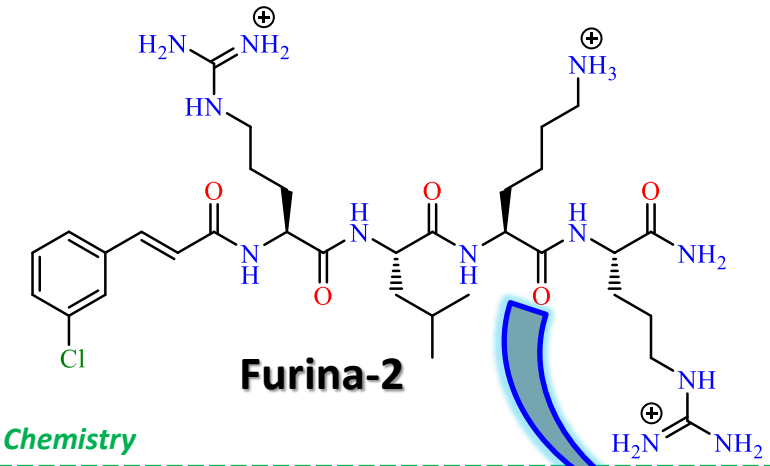


NS2B-NS3

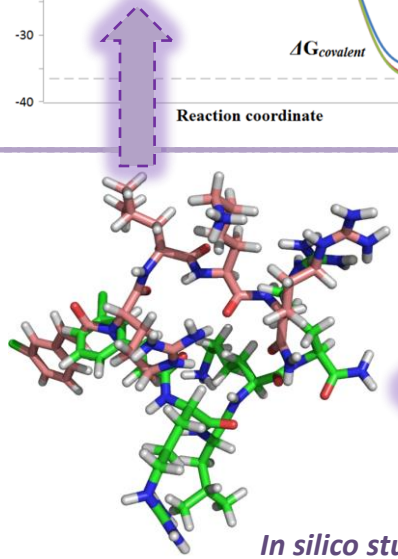
DFT/B3LYP Calculations

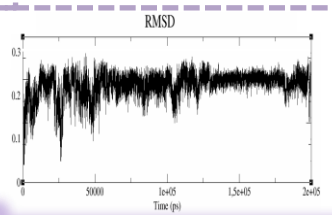


Chemistry



Furina-2

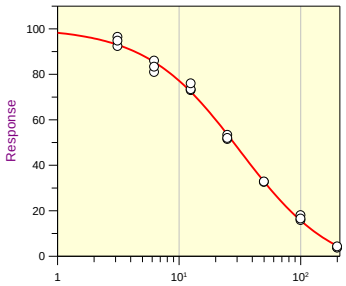




Dynamics simulations at 200 ns

In silico studies

Biological Evaluation



IC_{50} on ZIKV NS2B-NS3

Enzymatic assays

The 7th International Electronic Conference on Medicinal Chemistry

01–30 NOVEMBER 2021 | ONLINE

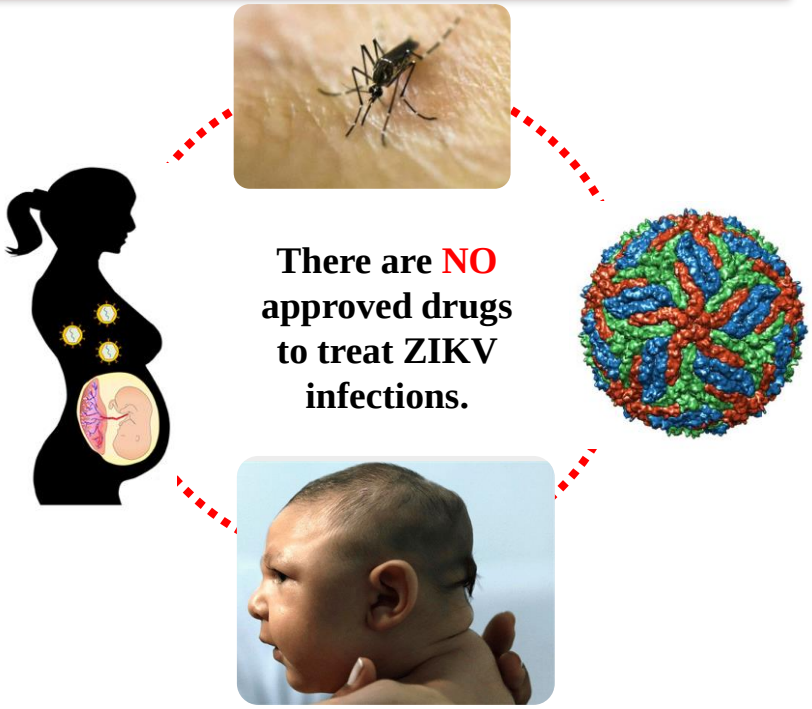
Abstract: The first report of Zika virus (ZIKV) outbreak was from the Yap Island (Micronesia) in 2007 then spreading across the world, being responsible for neutrotropic Guillain-Barré syndrome, neonatal microcephaly, and death cases. Currently, there are no licensed vaccines or even specific approved drugs to treat ZIKV infections. Thus, developing novel antiviral agents is crucial. The replication of this flavivirus depends on NS2B-NS3 protease complex, which represents a promising target for searching new drug candidates. Herein, we describe the solid-phase synthesis of a new furin-based peptidomimetic (ArC=CCO-Arg-Leu-Lys-Arg-NH_2), biological screening and *in silico* studies, involving docking using ChemPLP algorithm, molecular dynamics (MD), and DFT/B3LYP calculations. Fluorometric assays revealed the selective inhibition of ZIKV NS2B-NS3 (IC_{50}: 31.5 ± 1.9 µM), when compared to other serine and cysteine proteases. Moreover, docking analyses displayed that the best binding conformation (FitScore: 83.36) interacts with 12 amino acid residues, including Asn^{152} (hydrophobic) and Ser^{135} (H-bond) from the catalytic triad. Regarding MD simulations, we verified that the ligand-target complex remains stable from 50 to 200 ns. After the clusters' evaluation, the most stable complex conformation was retrieved (at ~150 ns) for determining the Gibbs free-energy value by DFT calculations. Finally, we verified that our inhibitor exhibits a ΔG value of −67.42 kcal/mol, suggesting it has an efficient binding mode at the catalytic site of ZIKV NS2B-NS3 protease.

Keywords: Zika, NS2B-NS3, peptidomimetic, antiviral, selectivity.

Introduction- Zika virus (ZIKV) key facts

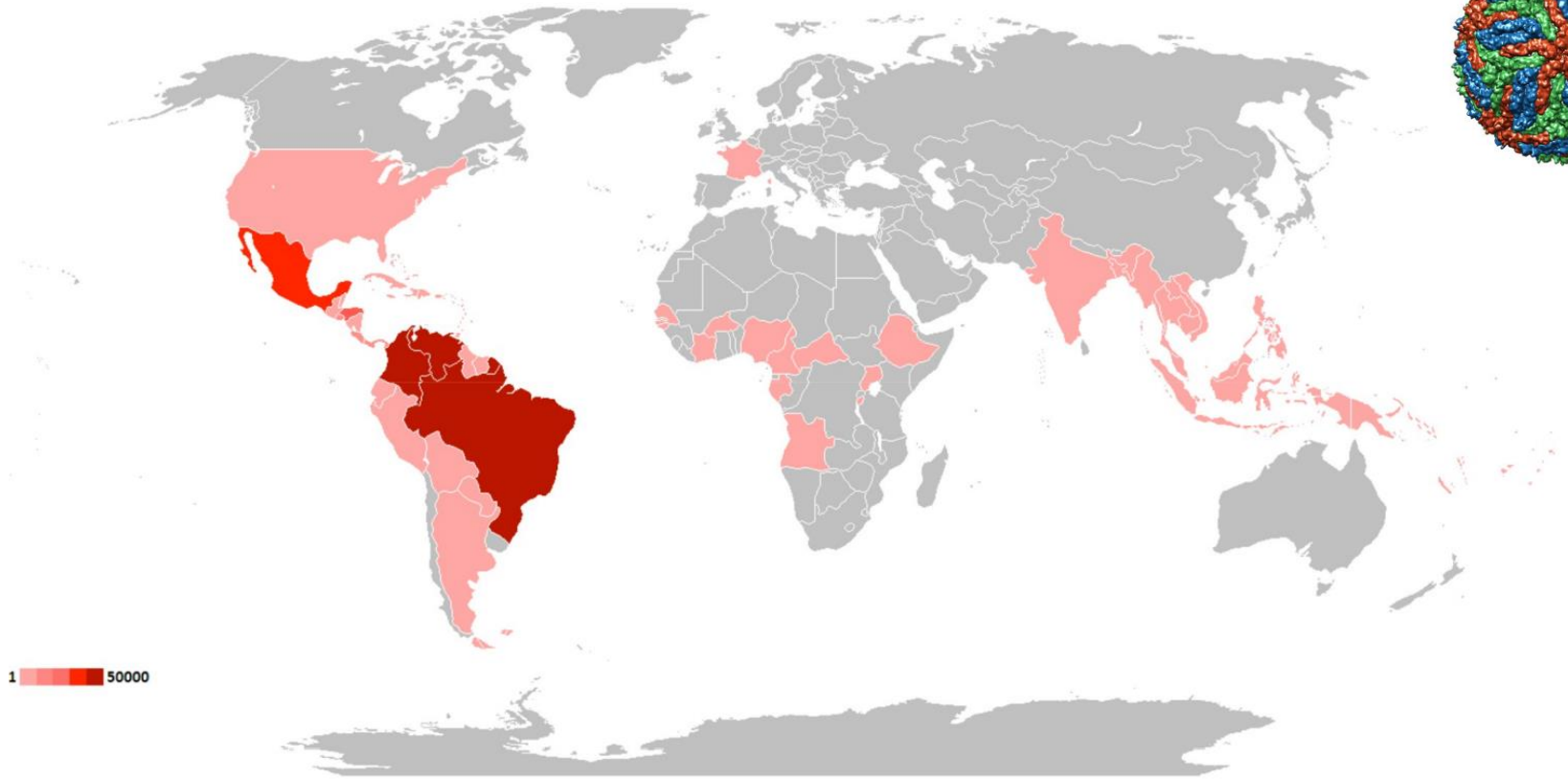
ZIKV is a member of the mosquito-borne Flavivirus genus, which causes skin rash, fever, non-purulent conjunctivitis, arthralgia, headache, non-intense myalgia mainly in the hands and feet joints, edema, and vomiting.[1,2]

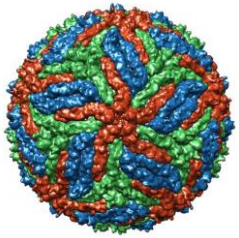
- **86** Countries have reported mosquito-transmitted Zika infection;[3]
- Its transmission is mediated by mosquitoes from *Aedes* genus;[1]
- It is transmitted vertically in pregnant women, or from person to person through sexual fluids;[1,2]
- **Infection with ZIKV during pregnancy can cause microcephaly in newborns.[1,2]**



[1] Rodrigues *et al*. The Medicinal Chemistry of Zika Virus. Book: Human Viruses: Diseases, Treatments and Vaccines. doi: 10.1007/978-3-030-71165-8_13.
[2] Kang et al. Trends in Microbiology, October 2017, Vol. 25, No. 10. doi: 10.1016/j.tim.2017.07.001.
[3] WHO. https://www.who.int/news-room/fact-sheets/detail/zika-virus

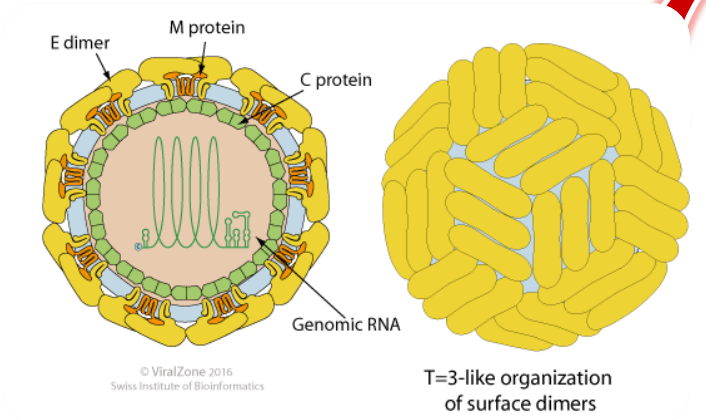
Introduction- Global distribution of ZIKV





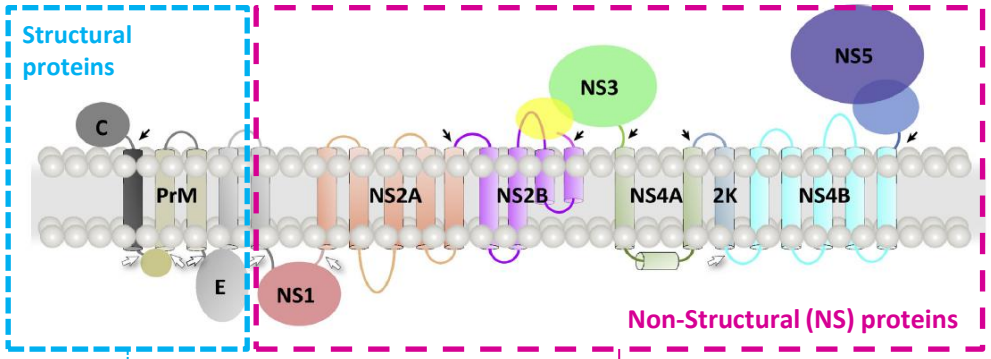
Puntasecca CJ, King CH, LaBeaud AD (2021) Measuring the global burden of chikungunya and Zika viruses: A systematic review. PLOS Neglected Tropical Diseases 15(3): e0009055. https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0009055

Introduction- Zika proteins



ZIKV is a lipid-enveloped Flavivirus having an RNA genome. SIB—Swiss Institute of Bioinformatics (https://viralzone.expasy.org/6756)

RNA is translated into a polyprotein encoding three structural proteins and seven non-structural (NS) proteins (scheme).[1]

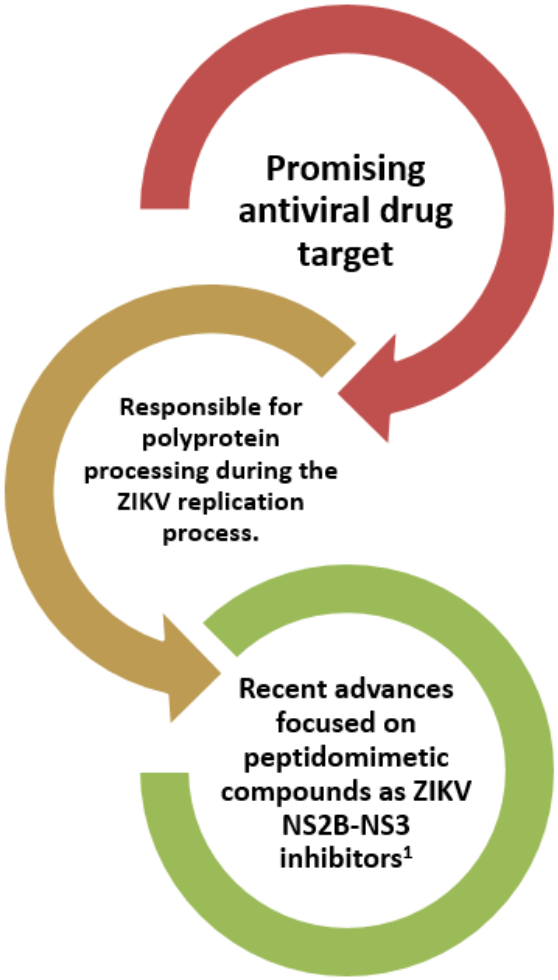


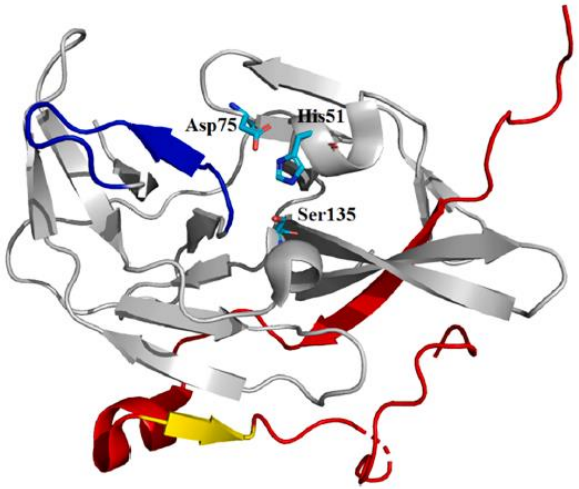
Virus entry, assembly, and release of new virions from the host cell.

Replication, assembly, and regulation of the host immune response to viral infection.

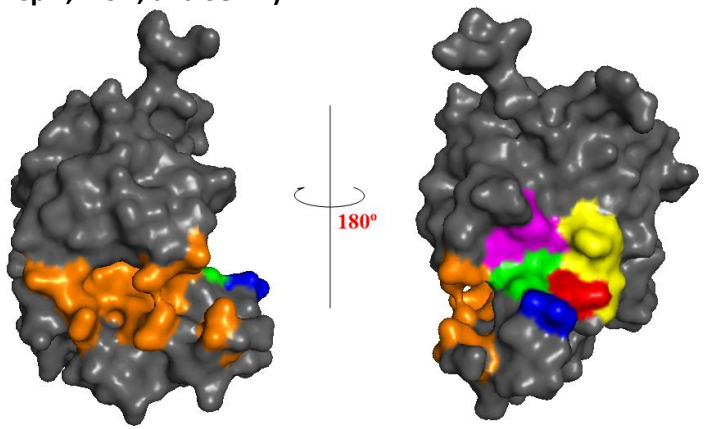
1. Kang et al. Trends in Microbiology, October 2017, Vol. 25, No. 10 http://dx.doi.org/10.1016/j.tim.2017.07.001

Introduction- ZIKV NS2B-NS3 Protease as a drug target



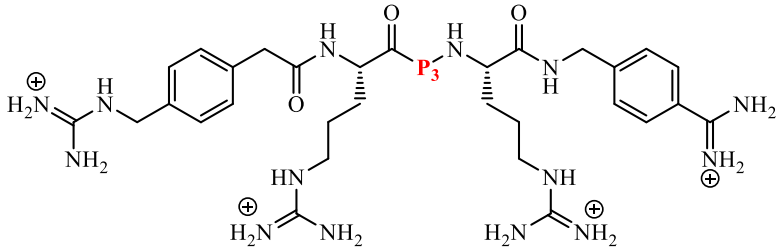


Crystal structure of Zika NS2B-NS3 protease (PDB id: 6KPQ), exhibiting its catalytic triad (Asp^{75}, His^{51}, and Ser^{135}).

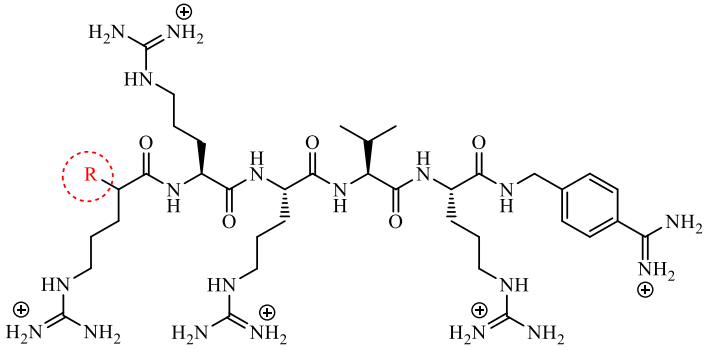


1. Silva-Júnior & Araújo-Júnior. Bioorganic & Medicinal Chemistry, 27 (2019) 3963–3978. doi: 10.1016/j.bmc.2019.07.038

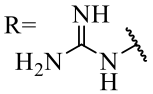
Introduction- Furin inhibitors with antiviral activity



(2) P3= Val **(3)** P3= Tle
Furin inhition Ki= 7.6 and 5.5 pM

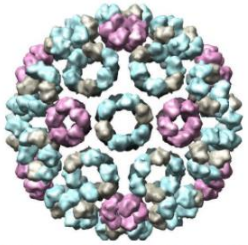


(9) R=H Furin inhibition Ki=28.3 ± 9.7pM

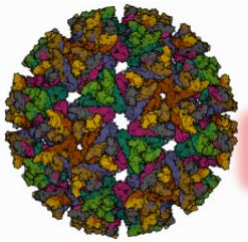
(16) R=  Furin inhibition Ki= 6.2 ± 1.0 pM

Hardes et al, ChemMedChem. 2017, 20; 12(8): 613–620. doi: 10.1002/cmdc.201700108

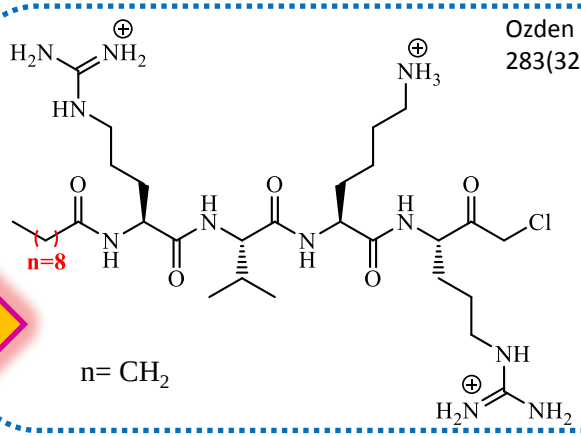
Alphaviruses' Inhibitors



SEMLIKI FOREST VIRUS (SFV)



CHIKUNGUNYA VIRUS

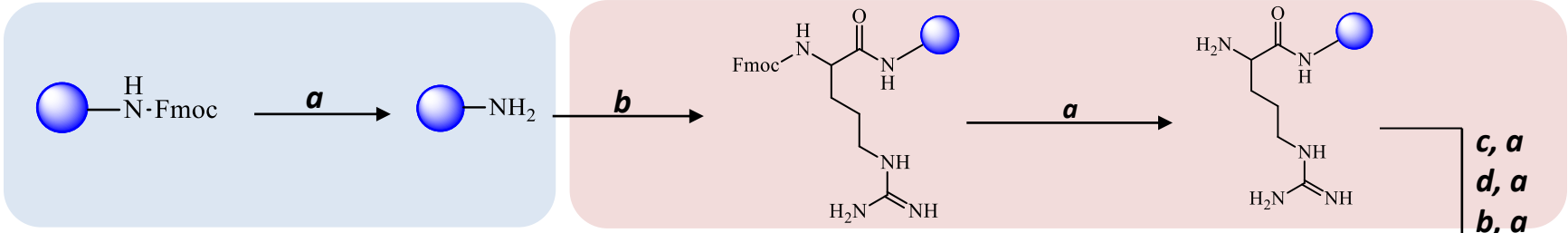


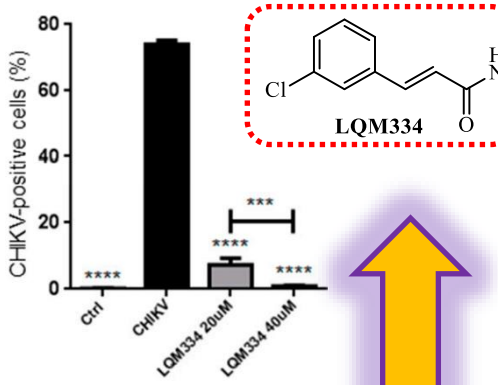
n= CH_2

Ozden et al, The Journal of Biological Chemistry. 2008, 283(32), 21899–21908. doi: 10.1074/jbc.M802444200

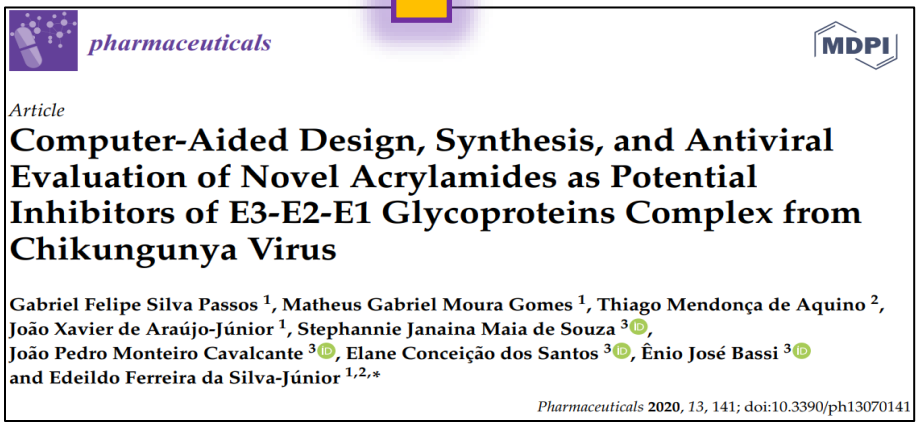
Selected a.a. Sequence

Results and discussion- Synthesis of a new Furin-based Peptidomimetic





a) 1-Methyl-piperazine, DMF b) Fmoc-Arginine, TBTU, DIPEA, DMF, r.t ; c) Fmoc-Lysine, TBTU, DIPEA, DMF, r.t d) Fmoc-Leucine, TBTU, DIPEA, DMF, r.t; e) (E)-3-(3-chlorophenyl)acrylic acid, TBTU, DIPEA, rt, f) TFA, H_2O, TIPS (95:4:1 v/v, 2h, r.t)



pharmaceuticals MDPI

Article

Computer-Aided Design, Synthesis, and Antiviral Evaluation of Novel Acrylamides as Potential Inhibitors of E3-E2-E1 Glycoproteins Complex from Chikungunya Virus

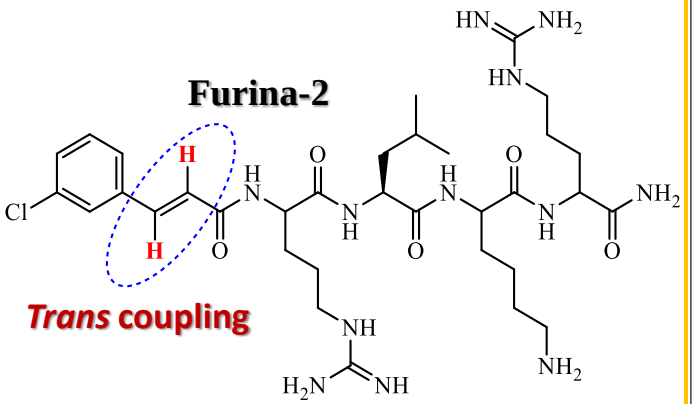
Gabriel Felipe Silva Passos [1], Matheus Gabriel Moura Gomes [1], Thiago Mendonça de Aquino [2], João Xavier de Araújo-Júnior [1], Stephannie Janaina Maia de Souza [3], João Pedro Monteiro Cavalcante [3], Elane Conceição dos Santos [3], Ênio José Bassi [3] and Edeildo Ferreira da Silva-Júnior [1,2,*]

Pharmaceuticals **2020**, *13*, 141; doi:10.3390/ph13070141

***Winner of the keynote speak award at the 5th International Electronic Conference on Medicinal Chemistry (2019)**

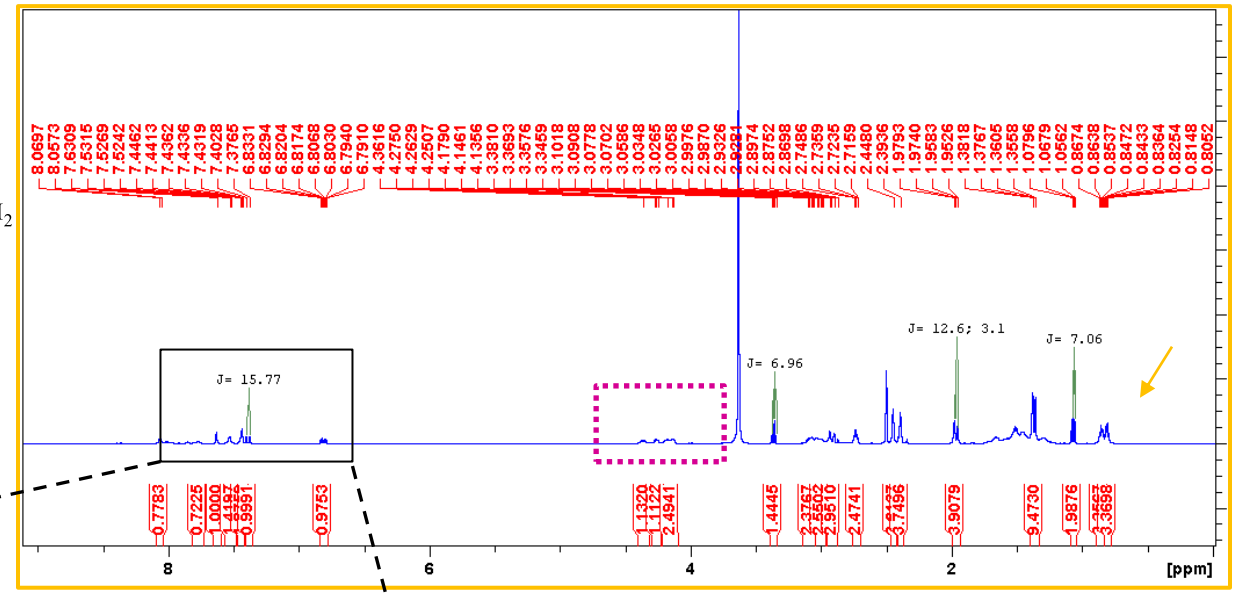
doi: 10.3390/ECMC2019-06379

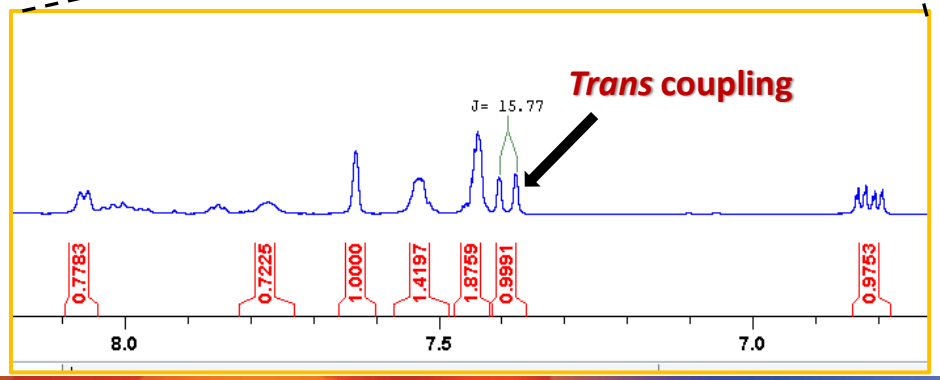
Results and discussion- NMR Spectroscopy



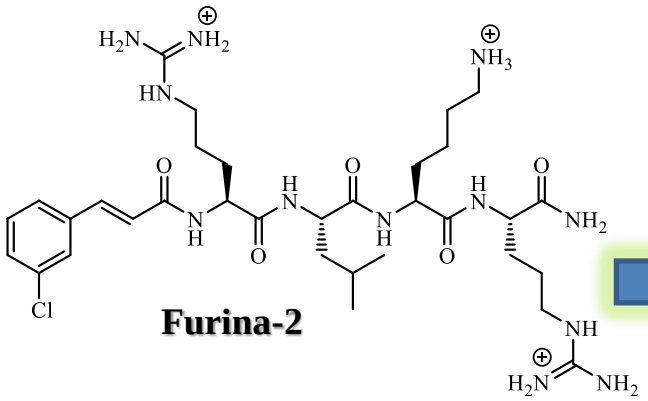
Purity: > 95%

^{1}H NMR spectrum (DMSO-d_6) 600 MHz

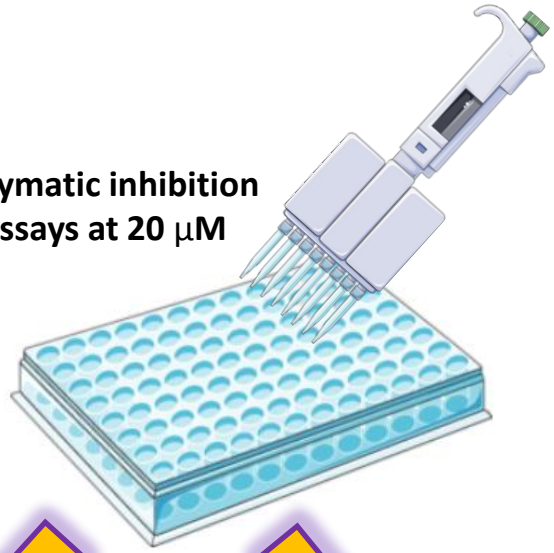


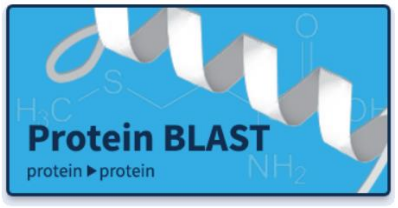


Results and discussion- Inhibition assays towards DENV-2 and ZIKV NS2B-NS3 proteases



Enzymatic inhibition
Assays at 20 μM



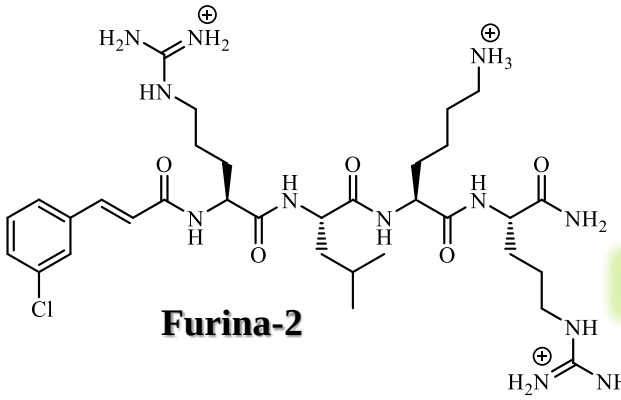


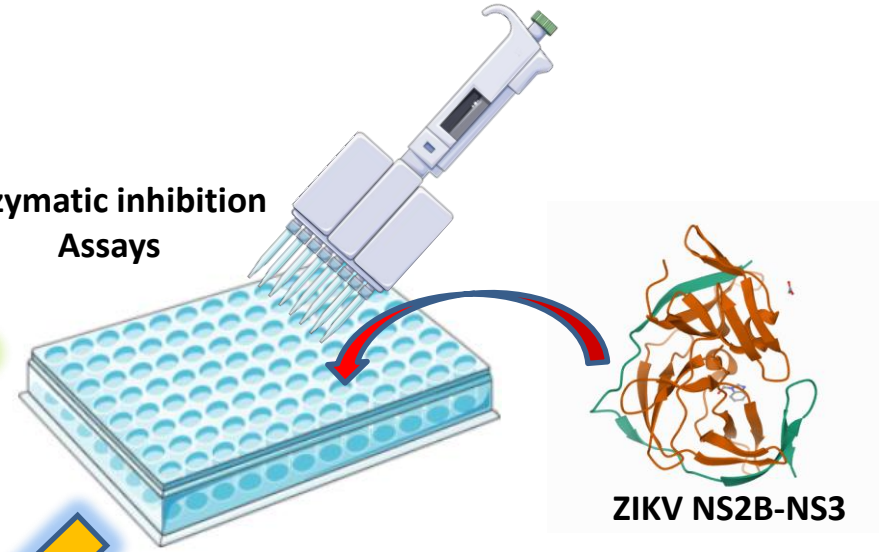
Identity: 43.42%
Accession: 6KK3_A
https://blast.ncbi.nlm.nih.gov/Blast.cgi

DENV-2 NS2B-NS3

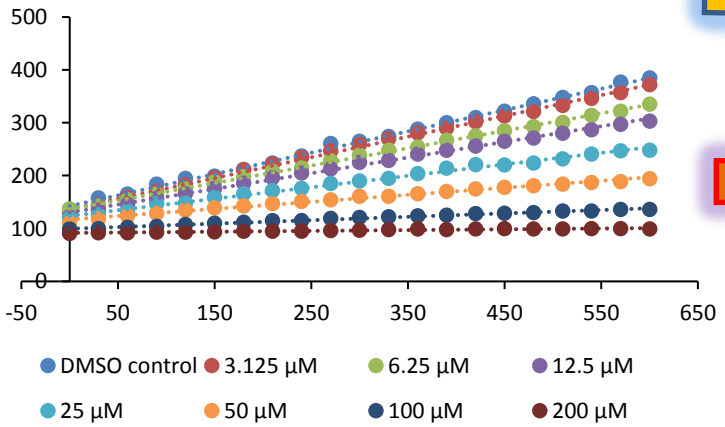
ZIKV NS2B-NS3
45% inhibition

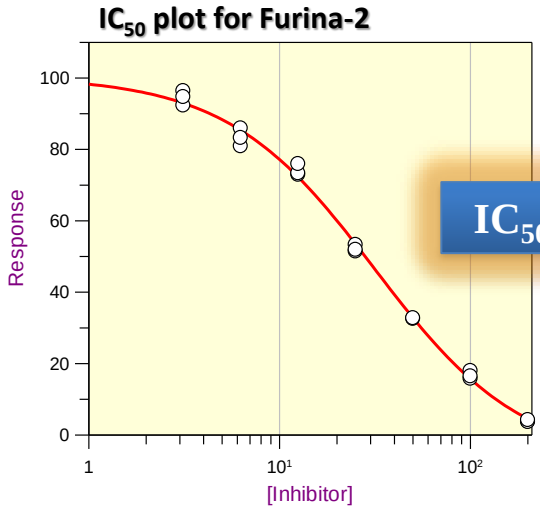
Results and discussion- IC_{50} determination towards ZIKV NS2B-NS3 protease





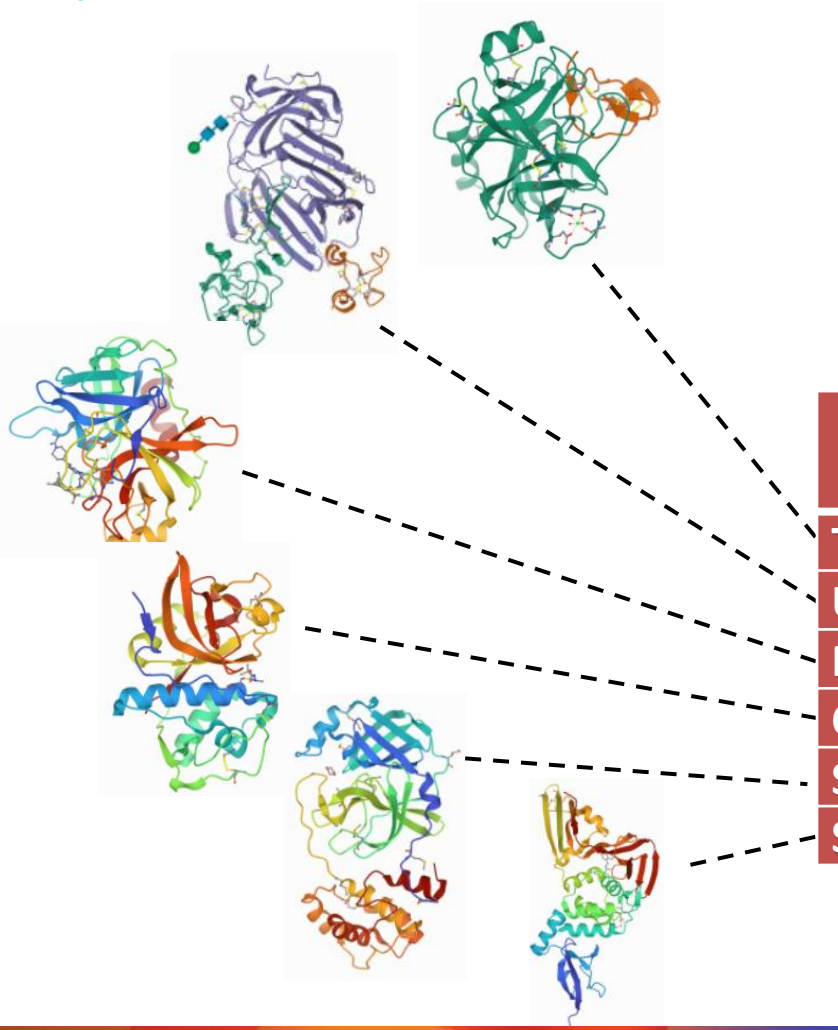
Inhibition of ZIKV NS2B-NS3 protease by different **Furina-2** concentrations

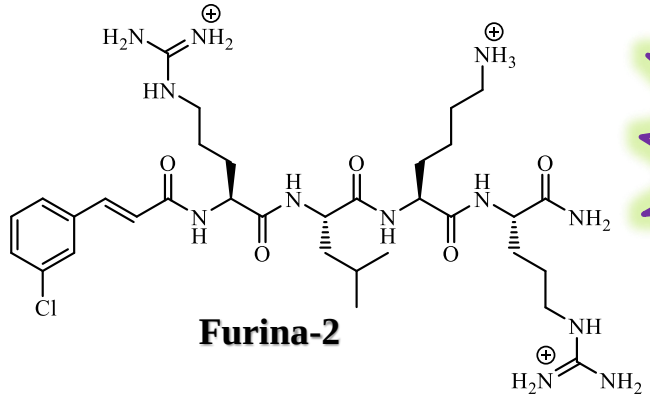




IC_{50}: 31.54 ± 1.94 µM

Results and discussion- Selectivity assays for Furina-2 towards other proteases





Selective!

Protease	%inhibition of Furina-02 at 20 μM concentration
Trypsin	N.A.
Urokinase	N.A.
Matriptase	9%
Cathepsin L	29%
SARS-CoV-2 M^{pro}	N.A.
SARS-CoV-2 PL^{pro}	5%

N.A.: not active.

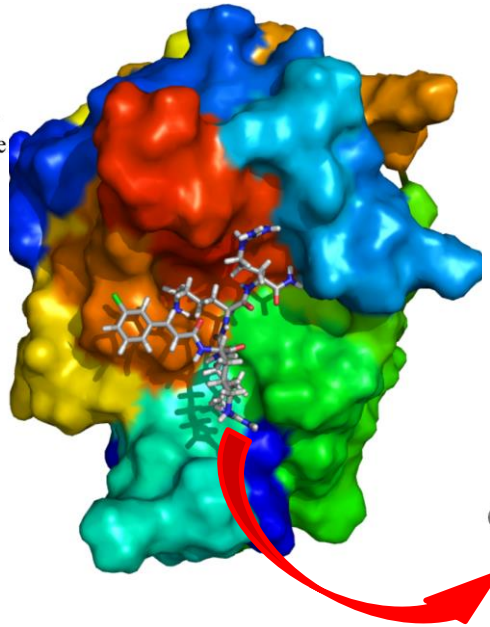
Results and discussion- Molecular docking studies

*ChemPLP
*GoldScore
*ChemScore
*ASP

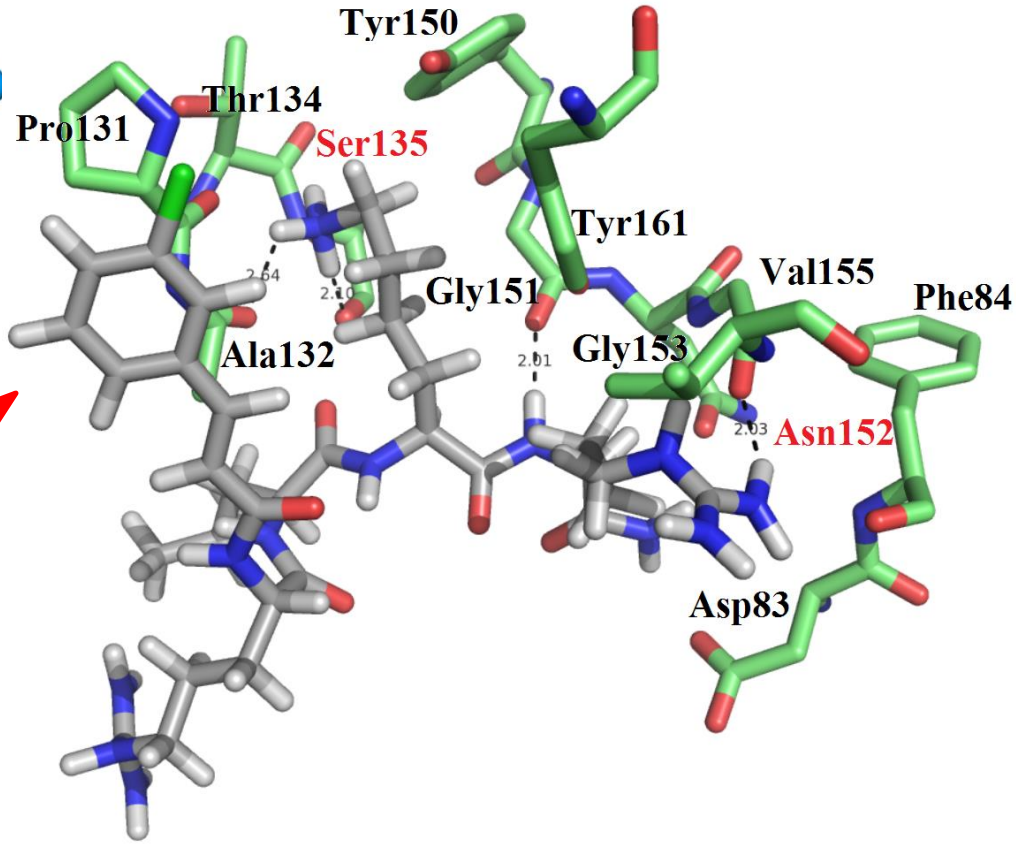
GOLD v. 5.8.1

AutoDock 4

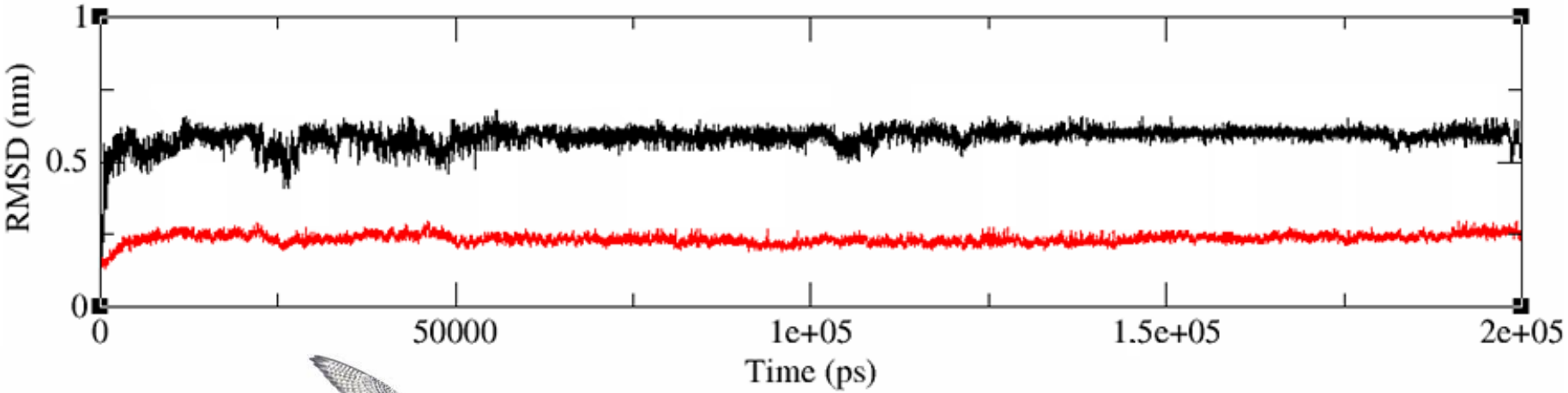
PyMOL



ZIKV NS2B-NS3
(PDB: 6KPQ)

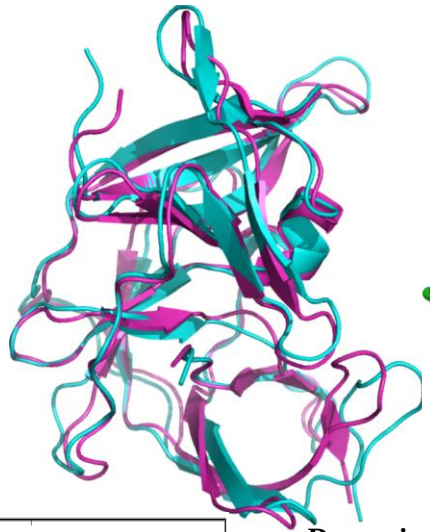


Results and discussion- Molecular dynamics simulations

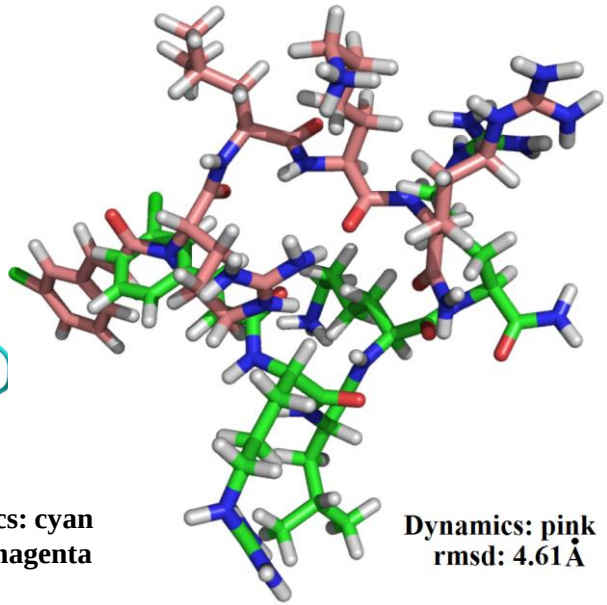


GROMACS
fast, flexible & free

UCSF Chimera

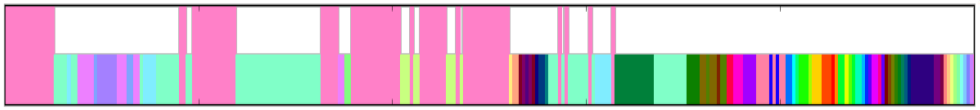


Dynamics: cyan
X-ray: magenta



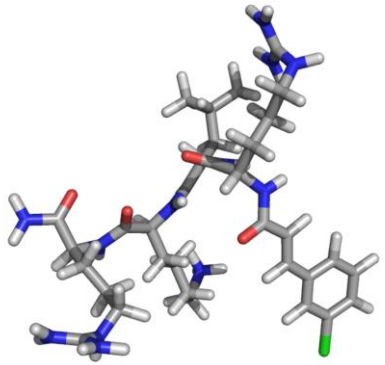
Dynamics: pink
rmsd: 4.61Å

Cluster analysis

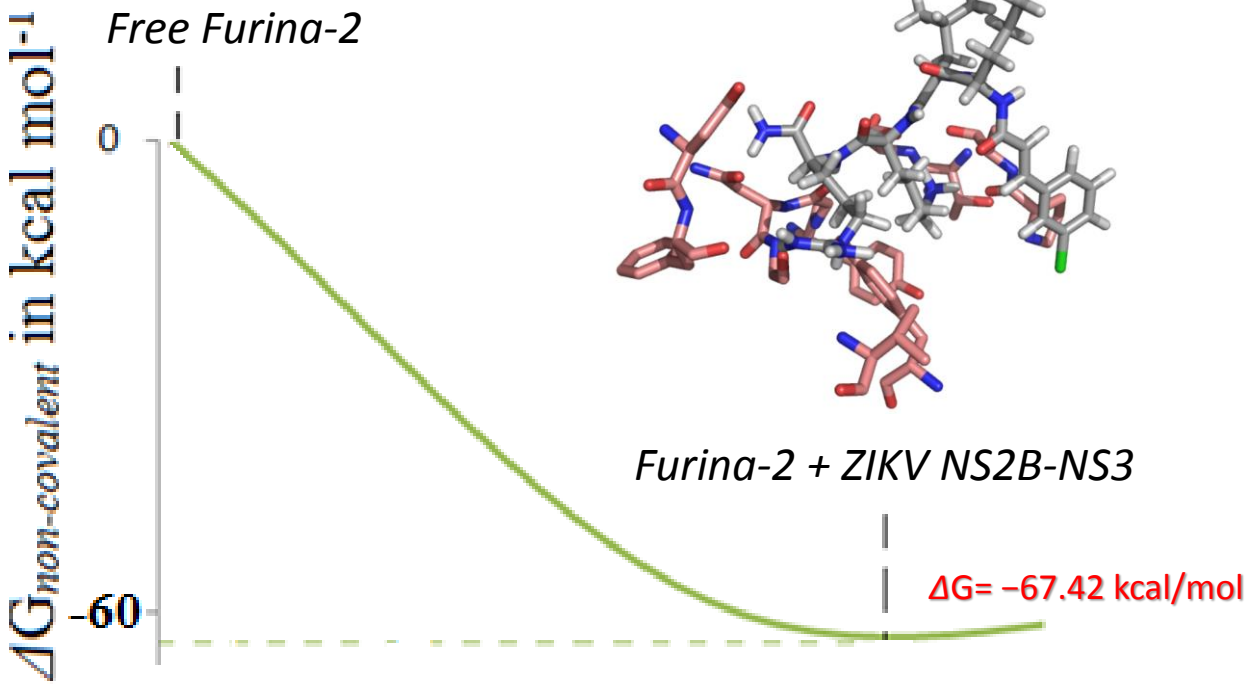


Results and discussion- DFT/B3LYP Calculations

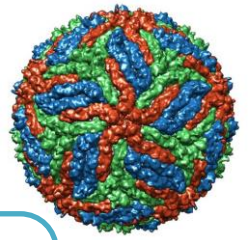
ORCA
V. 5.0.1



$$\Delta G_{bind} = \Delta G_{complex} - (G_{protein} + \Delta G_{ligand})$$



Conclusions



Chemistry

- **Solid-phase synthesis proved to be effective, providing the product in a satisfactory yield, with a high purity degree.**

Biological Assays

- **A new selective Alphavirus furin-based peptide was active against ZIKV NS2B-NS3 protease, being the first one reported in the literature. Additionally, it can be considered as a selective inhibitor.**

In silico

- **Molecular docking demonstrated that furina-2 binds at the catalytic site of ZIKV NS2B-NS3. Also, dynamics simulations revealed that the complex furina-2/protease is stable. Finally, DFT calculations suggested that such complex formation involves a non-covalent binding mode.**

Acknowledgments

** The authors still thank to the Prof. Frank Neese and his team for creating the ORCA program and make it open source; and to AutoDock Tools, UCSF Chimera, GROMACS and Schrödinger developers for their open source programs.*

*** Also, the authors thank the financial support given by CNPq/INCT/ RENNOFITO- grant number: N 465516/2014-0.*