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Design, Synthesis and *in vitro* Biological Evaluation of Acrylamide Derivatives Against Chikungunya Virus

Edeildo F. Silva-Júnior [1,*], Gabriel F.S. Passos [1], Matheus G.M. Gomes [1], Thiago M. Aquino [1], Stephannie J.M. Souza [2], João P.M. Cavalcante [2], Elane C. Santos [2], Ênio J. Bassi [2], and João X. Araújo-Júnior [1]

[1] Laboratory of Medicinal Chemistry, Institute of Pharmaceutical Sciences, Federal University of Alagoas, Maceió, Brazil;

[2] Laboratory of Research in Virology and Immunology, Institute of Biological Sciences and Health, Federal University of Alagoas, Maceió, Brazil.

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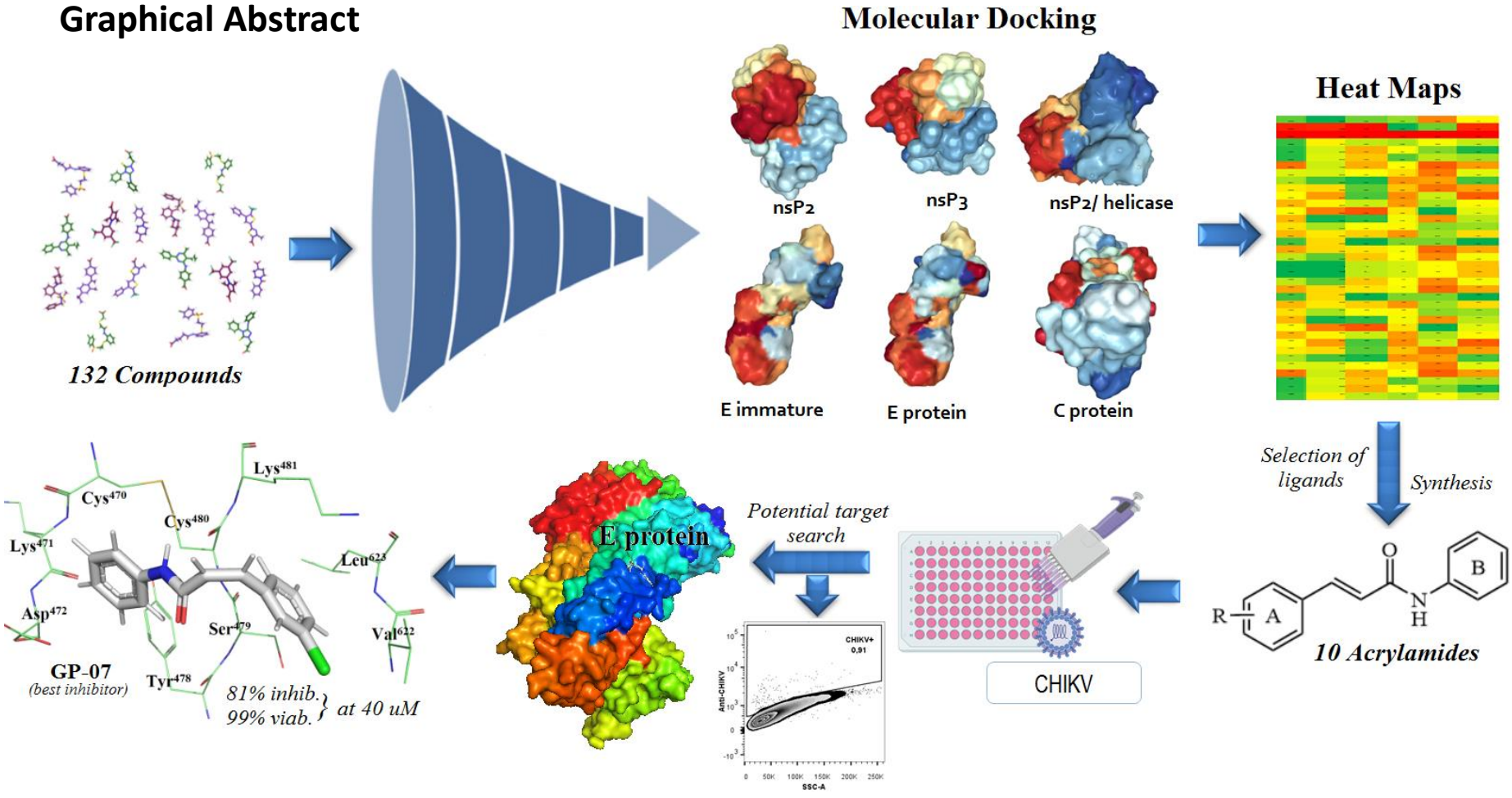
* Corresponding author: edeildo.junior@esenfar.ufal.br

Design, Synthesis and *in vitro* Biological Evaluation of Acrylamide Derivatives Against Chikungunya Virus

Graphical Abstract



Abstract: Chikungunya virus (CHIKV) causes an infectious disease characterized by inflammation and pain of the musculoskeletal tissues accompanied by swelling in the joints and cartilage damage. Currently, there are no licensed vaccines or chemotherapeutic agents to prevent or treat CHIKV infections. In sense, this research aims to explore the potential *in vitro* anti-CHIKV activity of acrylamide derivatives. *In silico* techniques were applied to 132 acrylamides toward the six most important biological targets from CHIKV. Subsequently, ten most promising acrylamides were selected and synthesized. From cytotoxicity MTT assay was verified that **GP03**, **07**, and **09** demonstrate cell viability higher than 94%. Additionally, **GP03** and **09** exhibited weak viral inhibition values (50 and 32% at 40 µM, respectively). In contrast, **GP07** displayed a significant *in vitro* anti-CHIKV activity, with inhibition of 81%. Thus, docking simulations were performed to suggest a potential CHIKV-target for **GP07**. It was observed that the **GP07** has a high affinity towards E protein. Moreover, **GP07** reduced the percentage of CHIKV-positive cells from 74.07 to 0.88%, 48h post-treatment on flow cytometry. In conclusion, all virtual simulations corroborated with experimental results, and **GP07** could be used as a promising anti-CHIKV scaffold for designing new drugs in the future.

Keywords: Virtual screening; acrylamides; Chikungunya; antiviral; molecular docking; E protein.

Introduction – Chikungunya virus key facts

Chikungunya virus (CHIKV) is an Alphavirus transmitted to humans by infected *Aedes aegypti* and *Ae. albopictus* mosquitoes. It causes fever, severe joint pain, and cartilage damage.[1]

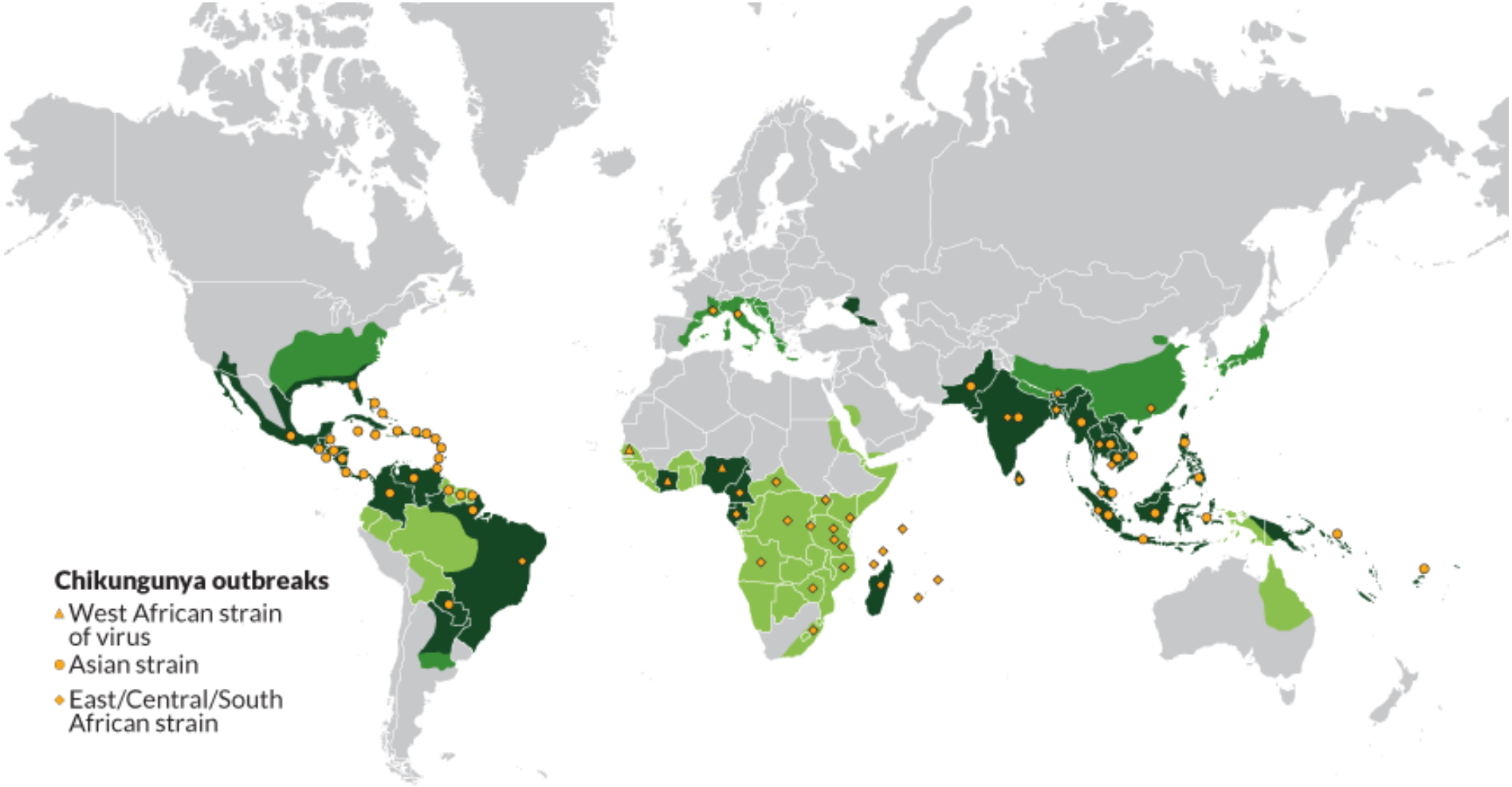
- ✓ CHIKV shares some clinical signs with Dengue and Zika, and can be misdiagnosed;[2,3]
- ✓ Joint pain is often debilitating and can vary in duration;[3]
- ✓ There are no licensed vaccines or chemotherapeutic agents to treat or prevent this infectious disease;[1,3]
- ✓ CHIKV has been identified in over 60 countries in Europe, Asia, and the Americas.[3]

CHIKV

Silva-Júnior *et al.* 2017. **Bioorg & Med Chem**, 25(16), 4219-4244. (adap.)

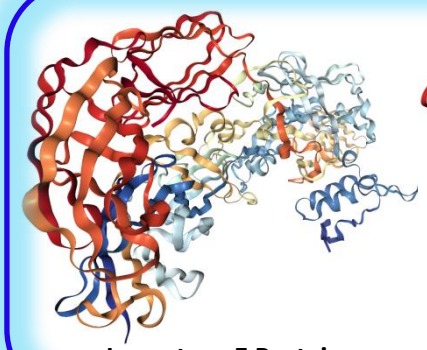
[1] Silva-Júnior *et al.* 2017. **Bioorg & Med Chem**, 25(16), 4219-4244.
[2] Silva-Júnior *et al.* **Bioorg & Med Chem**, 27(18), 3963-3978.
[3] WHO. 2014. Protect yourself from vector-brone diseases.

Introduction – Global distribution for Chikungunya virus

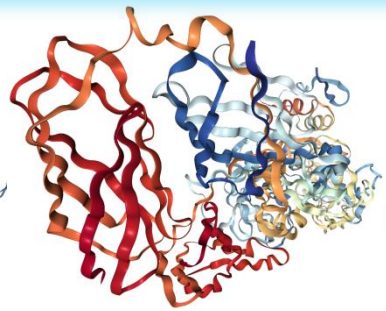


https://www.sciencenews.org/article/chikungunya-move

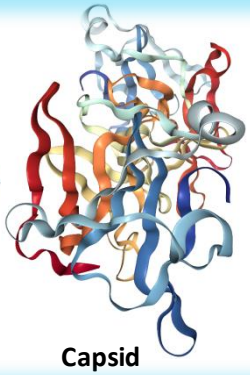
Introduction – Key macromolecular targets from CHIKV



Immature E Protein



E Protein



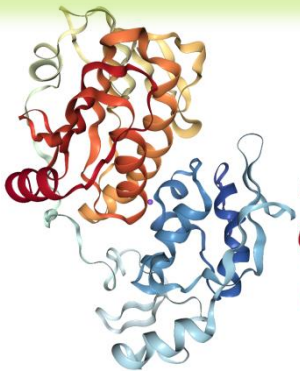
Capsid

CHIKV Structural Proteins

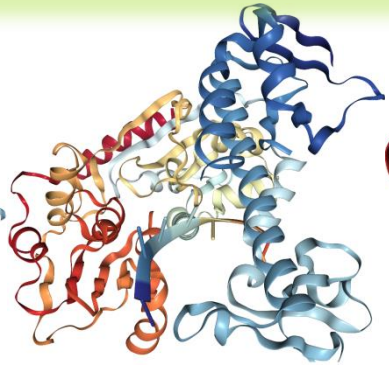
These proteins are related to viral structure, adsorption and host cell entry.

These proteins are associated with the virus replication.

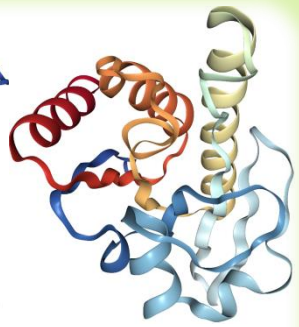
CHIKV Non-Structural Proteins



nsP2



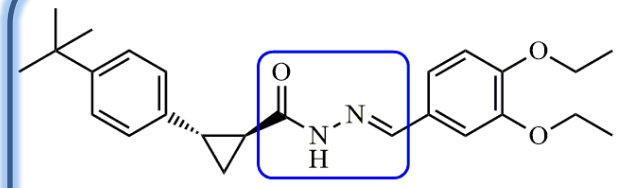
nsP2/helicase



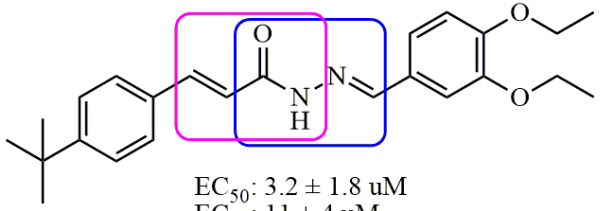
nsP3

Introduction – Anti-CHIKV scaffolds found in the literature

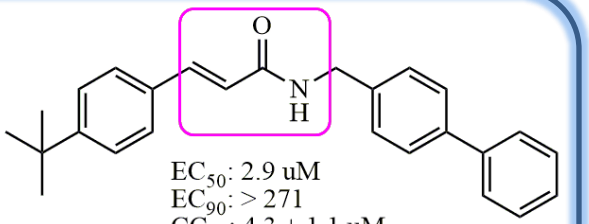
* Acylhydrazones
* Acrylamides
* Acylhydrazines



EC_{50}: 5 ± 0.2 uM
EC_{90}: 6.4 ± 0.5 uM
CC_{50}: 72 ± 20 uM
SI: 14

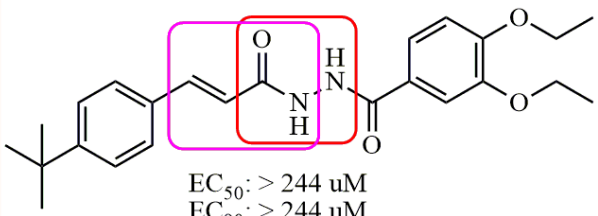


EC_{50}: 3.2 ± 1.8 uM
EC_{90}: 11 ± 4 uM
CC_{50}: 101 ± 50 uM
SI: 32

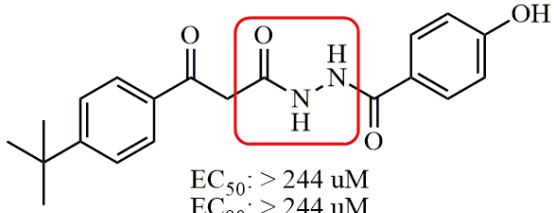


EC_{50}: 2.9 uM
EC_{90}: > 271
CC_{50}: 4.3 ± 1.1 uM
SI: n.d.

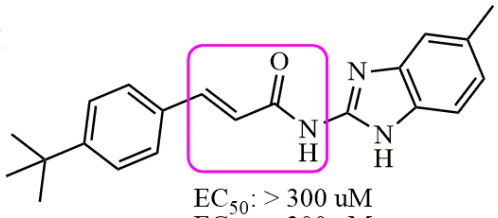
Tardugno et al., 2018. ***Bioorg & Med Chem****, 26(4), 869-874.*



EC_{50}: > 244 uM
EC_{90}: > 244 uM
CC_{50}: n.d.
SI: n.d.



EC_{50}: > 244 uM
EC_{90}: > 244 uM
CC_{50}: 81 ± 53 uM
SI: n.d.

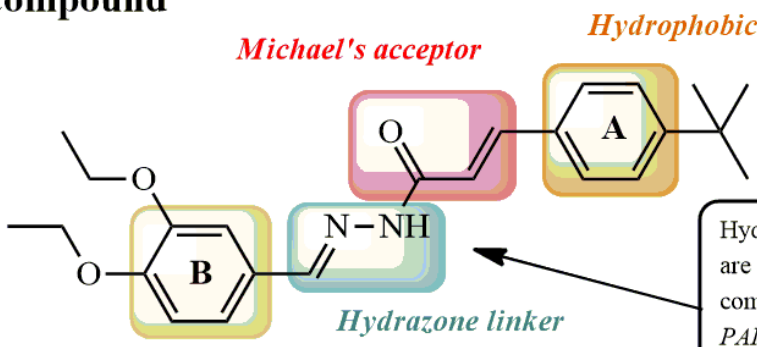


EC_{50}: > 300 uM
EC_{90}: > 300 uM
CC_{50}: n.d.
SI: n.d.

Giancotti et al., 2018. ***Eur J Med Chem****, 149, 56-68.*

Results and discussion – Rational design for acrylamides

Hit compound

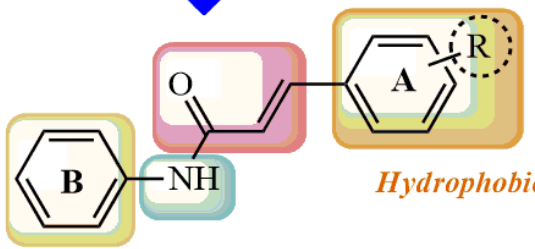


EC_{50}: 3.2 microM
EC_{90}: 11 microM
CC_{50}: 101 microM
SI: 32

Tardugno et al. ***Bioorg & Med Chem****, 2018, 24(6), 869-874.*

Hydrazone and acylhydrazone derivatives are frequently reported as promiscous compounds (*Pan-Assay Interference Scaffold - PAINS*), e.g. *Verma et al.* ***J Pharm Biollied Sci****. 2014, 6(2); 69-80. Pouliot and Jeanmart.* ***J Med Chem****. 2016, 59, 497-503.*

Replacement of hydrazone group with amide group

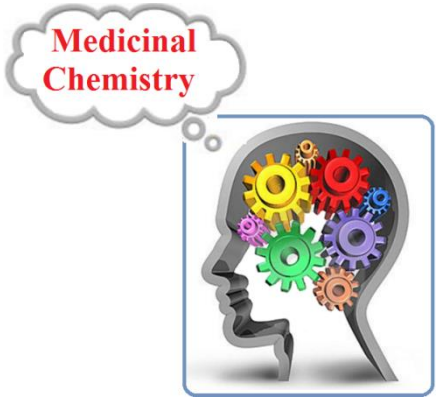


132 new analogs

Dynamics & Docking

Ligands' selection for synthesis and antiviral evaluation*

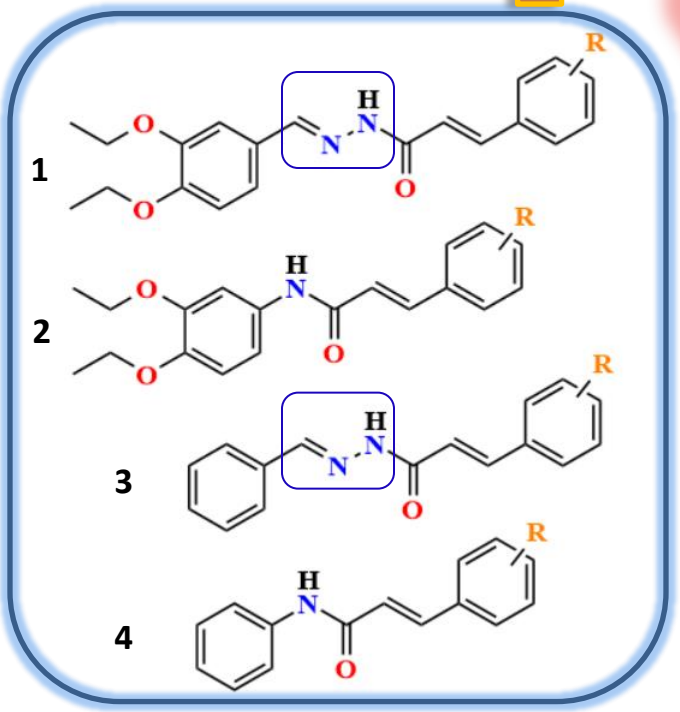
Acrylamide - The most promising scaffold



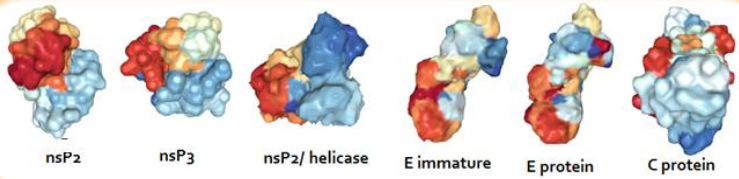
* Molecular targets: nsP2, nsP2/helicase, nsP3, immature E protein, E protein, and capsid.

Results and discussion – Virtual screening for acrylamides

132 possible compounds
(33 R-substituents x 4 scaffolds)



6 molecular targets from CHIKV



Dynamics *(Gromacs)*

Docking *(Gold)*

Molecular modeling

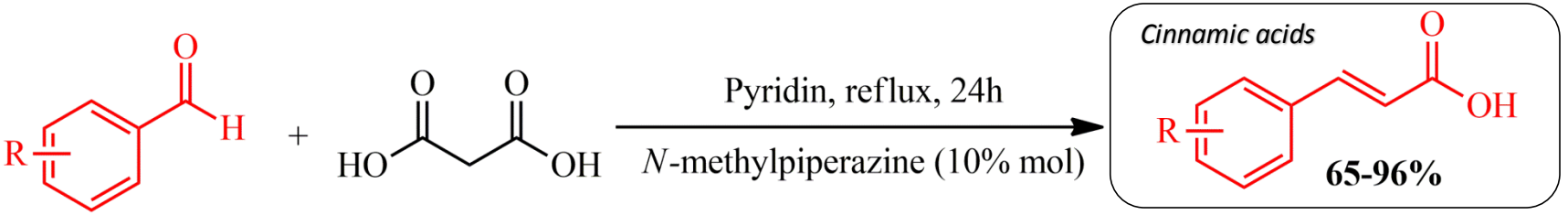
R:

3,4-Cl	2,4-Cl
4-Phenyl	3-Cl
4-CF_3	3,4-OCH_3
2,3-Cl	2-OCH_3
4-F	2-Phenyl

The 10 most promising R-substituents

* Hydrazone compounds **(1** and **3)** were considered into virtual screening steps to identify chemical characteristics from these molecular class, such as interactions, fitscore values, among others. Molecular targets: nsP2 (PDB: 3TRK), nsP2/helicase (PDB: 6JIM), nsP3 (PDB: 3GPO), immature E protein (PDB: 3N40), E protein (PDB: 3N41), and capsid (PDB: 5H23). In sense, dynamics simulations were performed by using Gromacs (10 ns) and molecular dockings using Gold software (ChemPLP genetic agorithm).

Results and discussion – Synthesis of new antiviral acrylamides

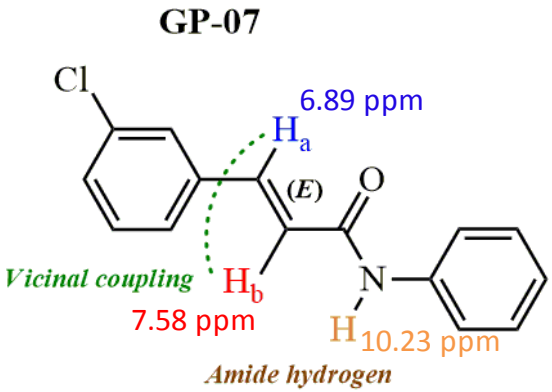


R:

3,4-Cl **(GP-01)**	2,4-Cl **(GP-06)**
4-Phenyl **(GP-02)**	3-Cl **(GP-07)**
4-CF_3 **(GP-03)**	3,4-OCH_3 **(GP-08)**
2,3-Cl **(GP-04)**	2-OCH_3 **(GP-09)**
4-F **(GP-05)**	2-Phenyl **(GP-10)**

Initially, cinnamic acids were synthesized by Knoevenagel/Doebner modification reaction using malonic acid (1 eq) and the corresponding aldehydes (1 eq). Cinnamic acids were purified by filtration and washing with concentrated HCl (37%), and collected powders were dryied under high-vaccum. Subsequently, the final compounds **(GP's)** were obtained by TBTU-coupling reaction between aniline (1 eq) and the corresponding cinnamic acids (1.1 eq), in DMF at room temperature *(overnight),* and DIPEA as catalyst base. All purifications were performed by filtration and washing with a satured $NaHCO_3$ solution and destilled water, respectively. In some cases, it was necessary to recrystallize the product from an acetone/water (1:2) mixture.

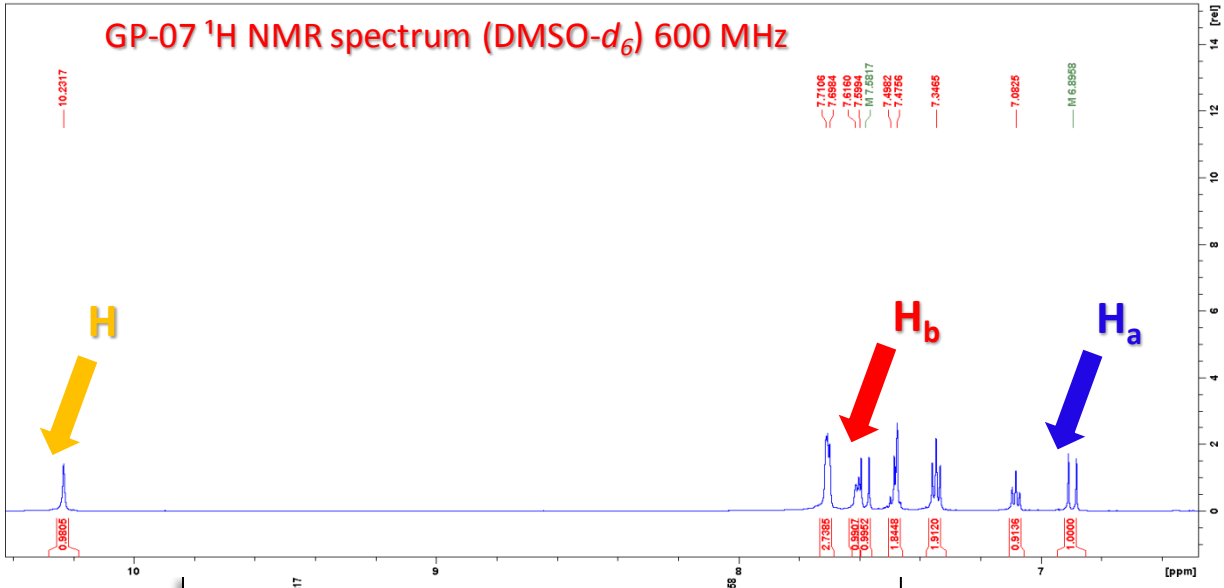
Results and discussion – Chemical characterization

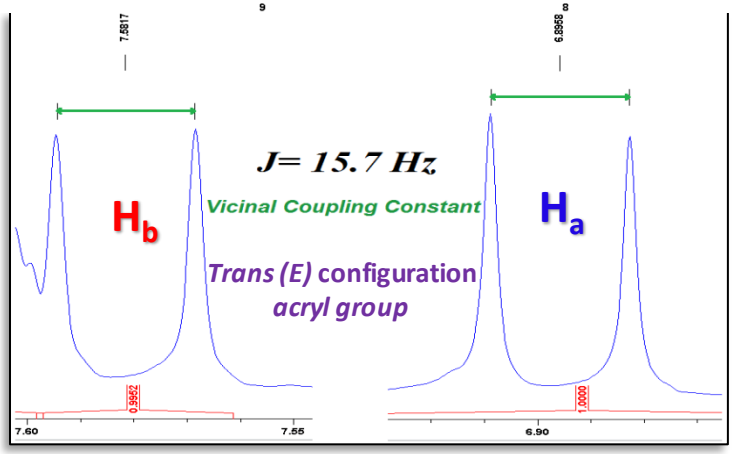


GP compounds*

Physico-chemical parameter	Values (range)
Molecular Mass	241.09 – 299.13 g/mol
State	Solids
Retention Time (R_T)	2.9 – 3.88
Purity	96.3 – 99.9%
Melting Point (M_p)	123 – 246 °C
Degradation Point (D_p)	> 300 °C

*The table shows values for all compounds.

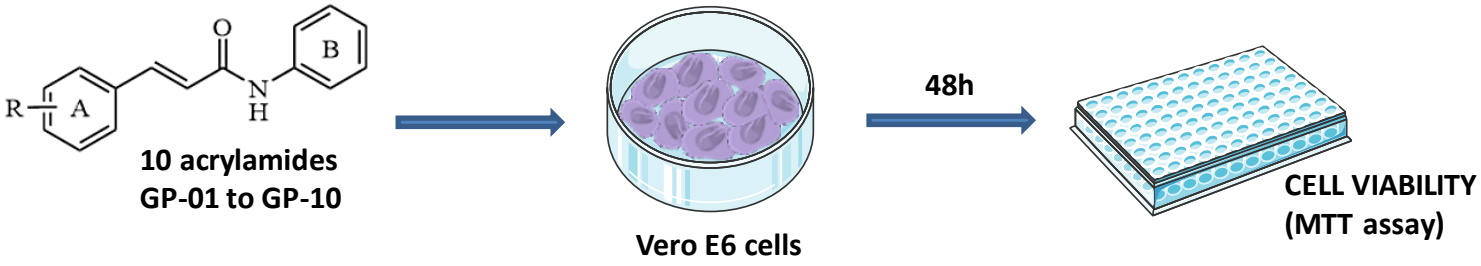


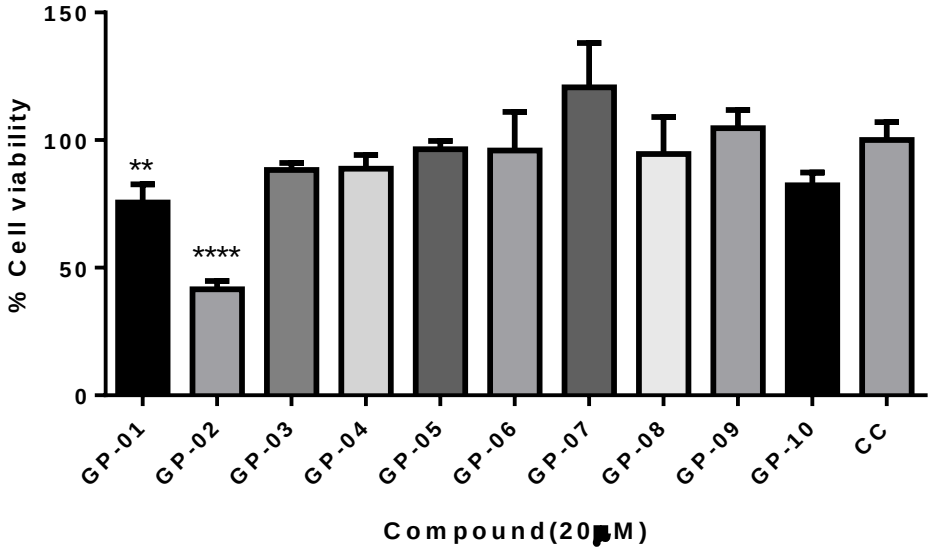


Zoom into the acryl peaks region

Results and discussion – Cell viability (MTT assay)

The cytotoxicity was performed *in vitro* for ten acrylamides (**GP01-10**) toward *Vero E6* cells at 20 µM concentration by MTT assay

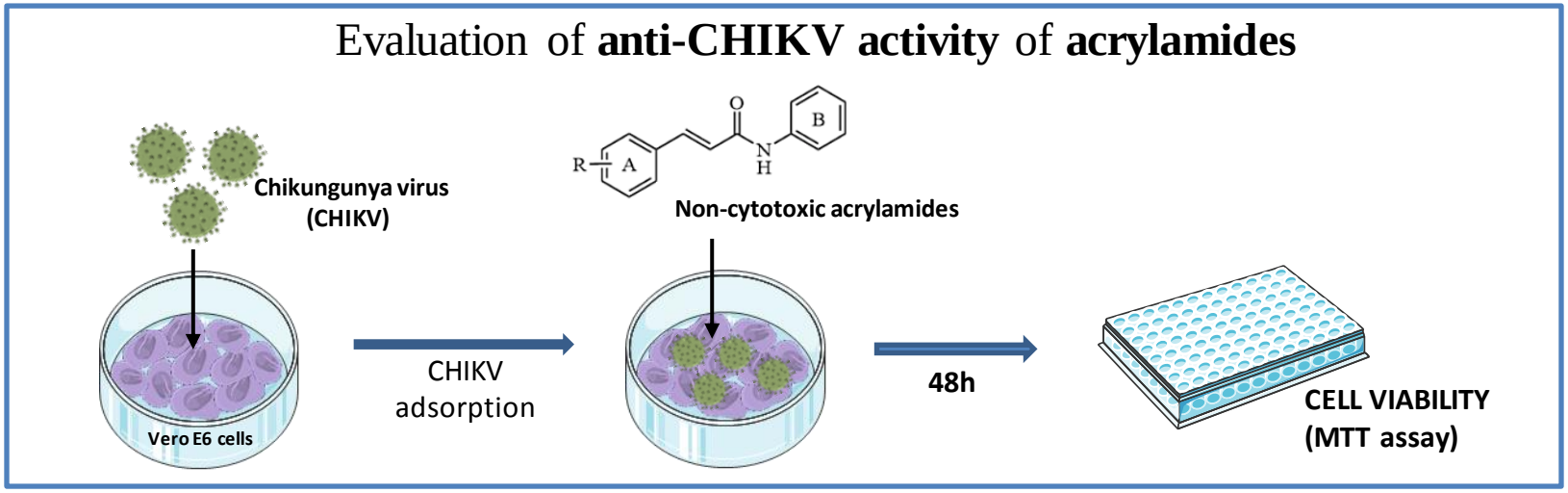


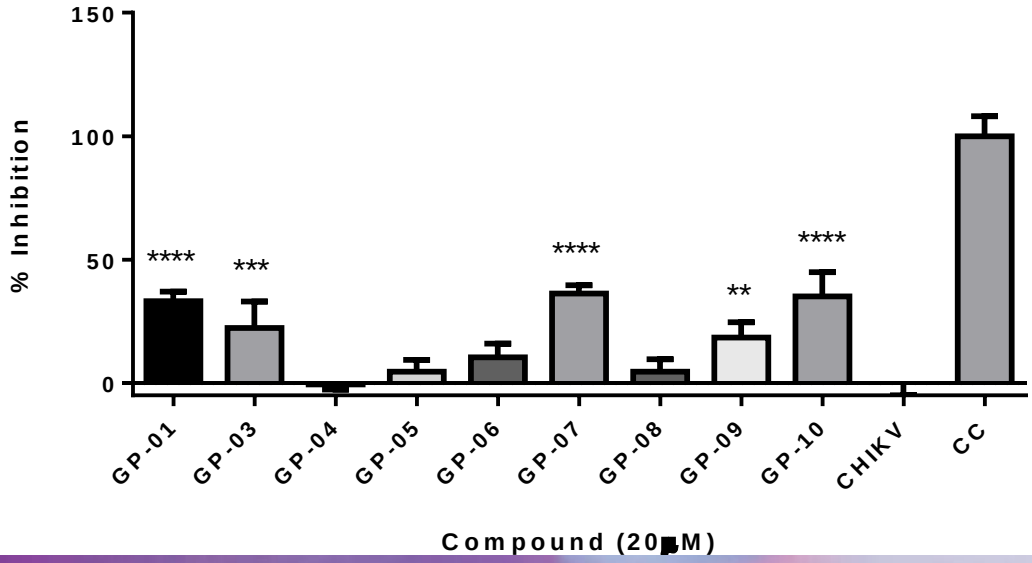


** p ≤ 0.01; *** ≤ 0.001 vs cell control (CC)

GP-02 acrylamides was cytotoxic (≤ 50%)

Results and discussion – *In vitro* antiviral screening

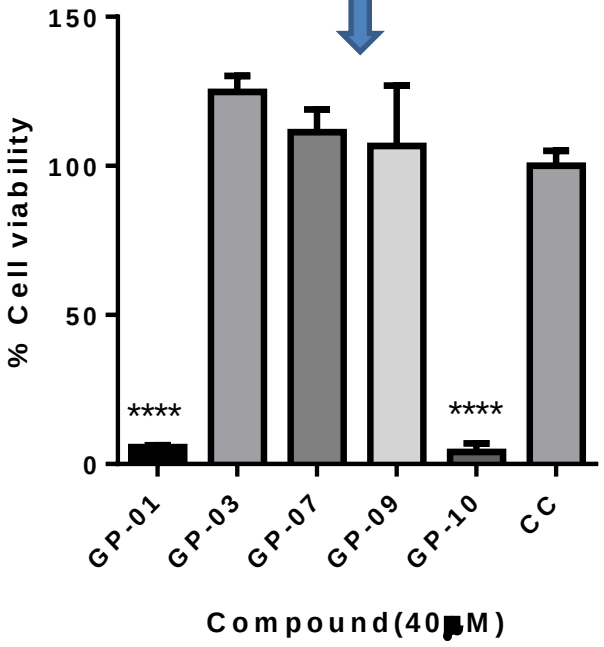




Anti-CHIKV activity was detected for GP-01, GP-03, GP-07, GP-09 and GP-10 acrylamides at 20 µM

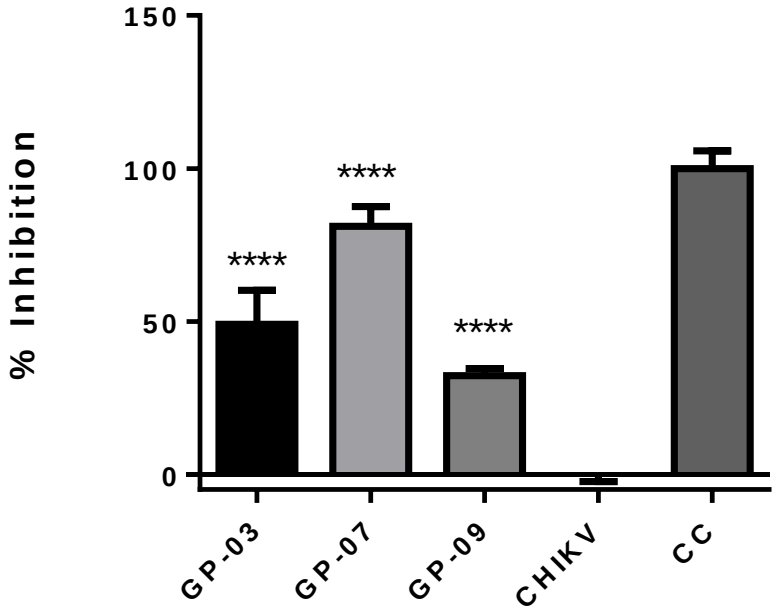
Results and discussion

The cytotoxicity was performed for five selected acrylamides at 40 µM concentration for 72h



✓ **GP-1 and GP-10** were **cytotoxic after 72h**

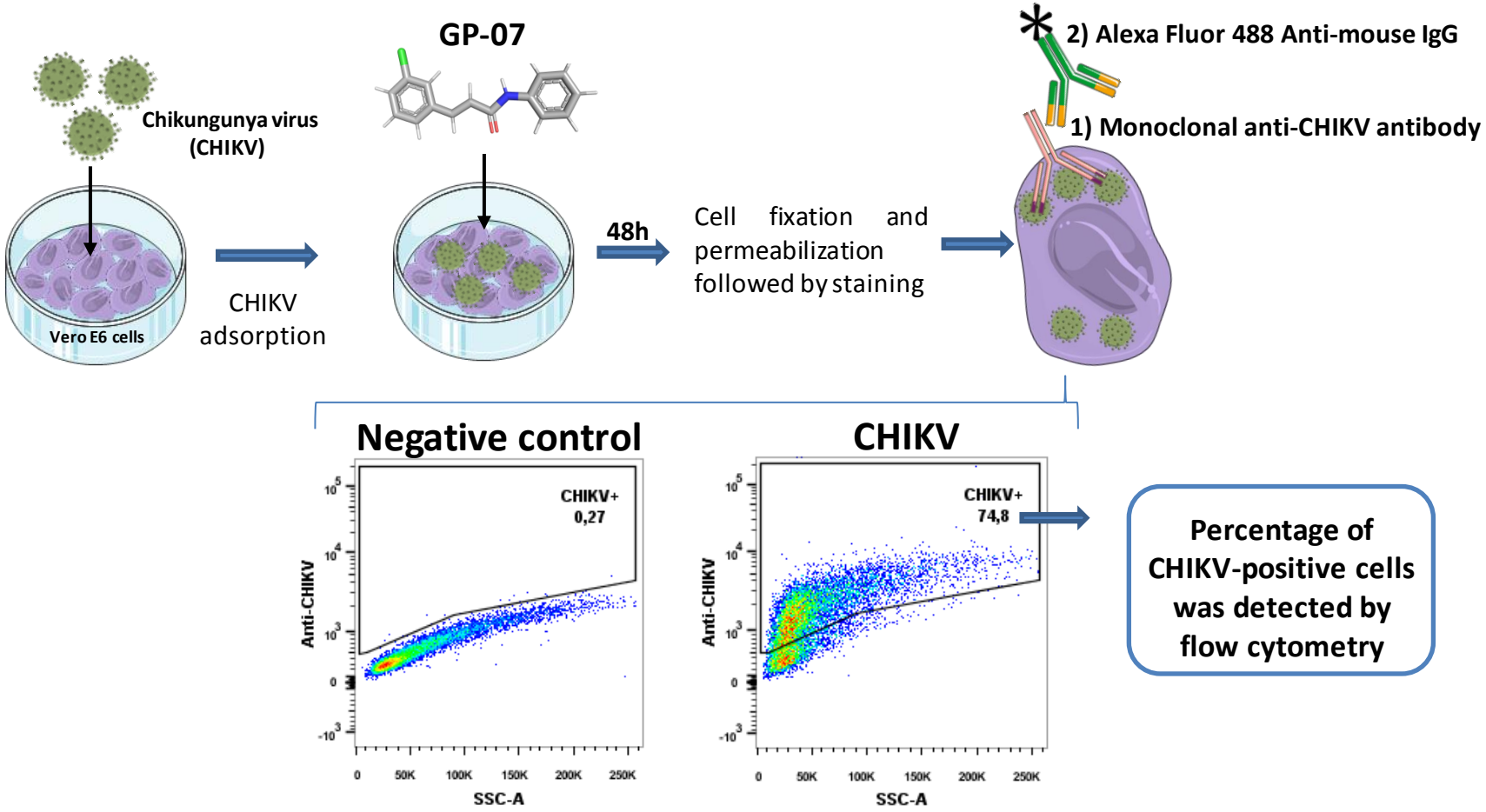
Evaluation of **anti-CHIKV activity** of **selected acrylamides at 40 µM**



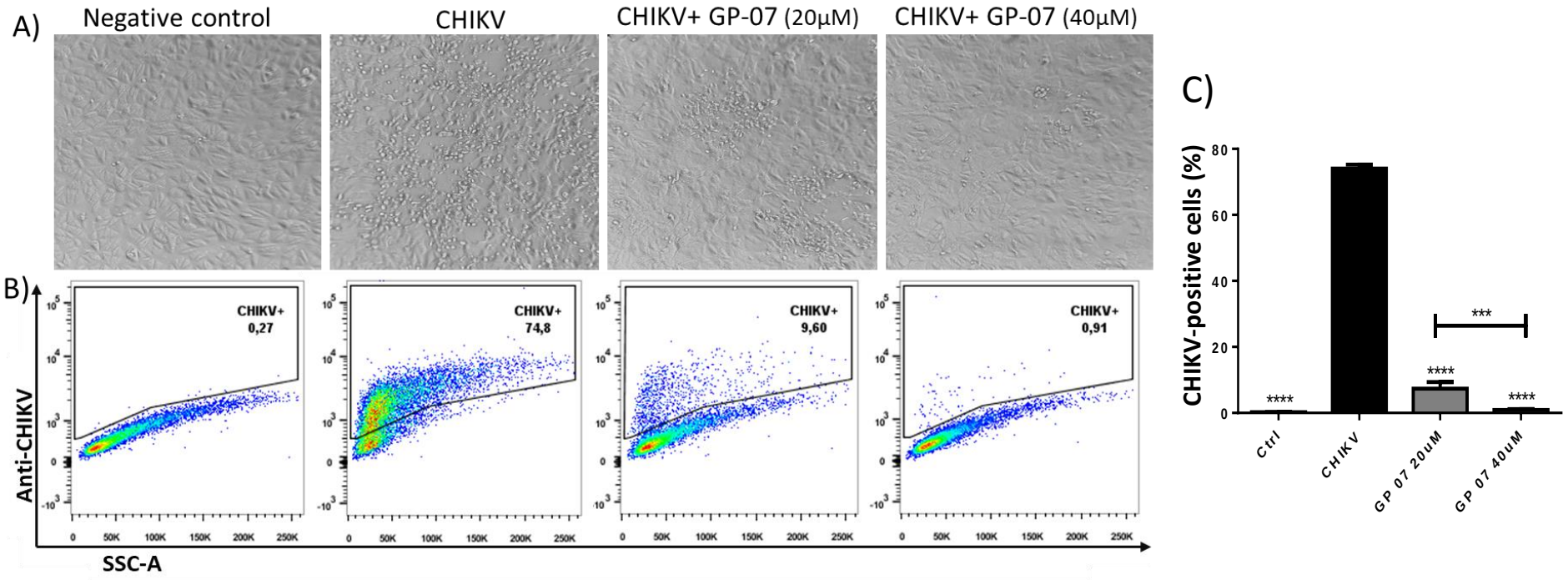
As result, it was observed that acrylamides **GP03** and **09** exhibited weak viral inhibition values (49 and 32%, respectively). In contrast, the acrylamide **GP07** displayed a **significant *in vitro* anti-CHIKV activity**, with **an inhibition value of 81% after 72h.**

Results and discussion – Detection of CHIKV-infected cells

In order to confirm the antiviral activity, **the intracellular labelling of CHIKV** was performed and **the percentage of CHIKV-positive cells was detected by flow cytometry**



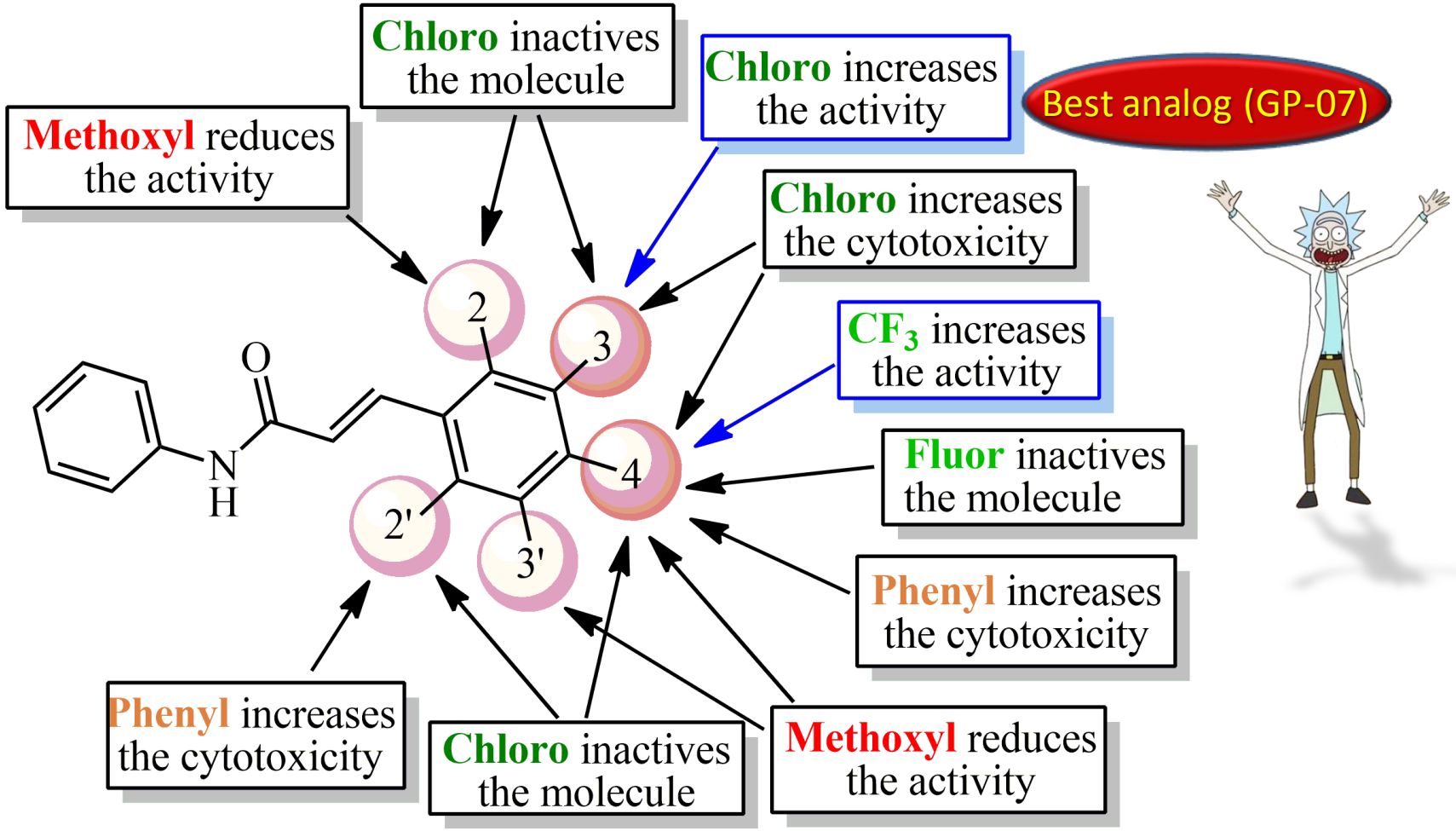
Results and discussion



GP-07 inhibited the CHIKV infection *in vitro*. (A) Representative micrographs (200x magnification) showing the cytopathic effect and (B) flow cytometry dot-plots of Vero E6 cells infected (CHIKV) or uninfected (Ctrl/negative control) with CHIKV. The cells were treated with GP-07 at 20 and 40 µM. Percentages of CHIKV-positive cells are shown. C) Mean ± SEM of CHIKV-positive cells (triplicate).

As result, **GP07** was able **to reduce the percentage of CHIKV-positive cells** from **74.07 to 0.88 %,** 48h post-treatment.

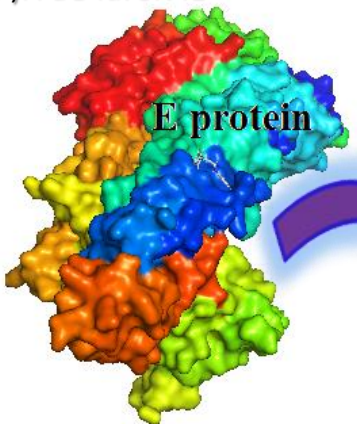
Results and discussion – Structure-Activity Relationship (SAR)



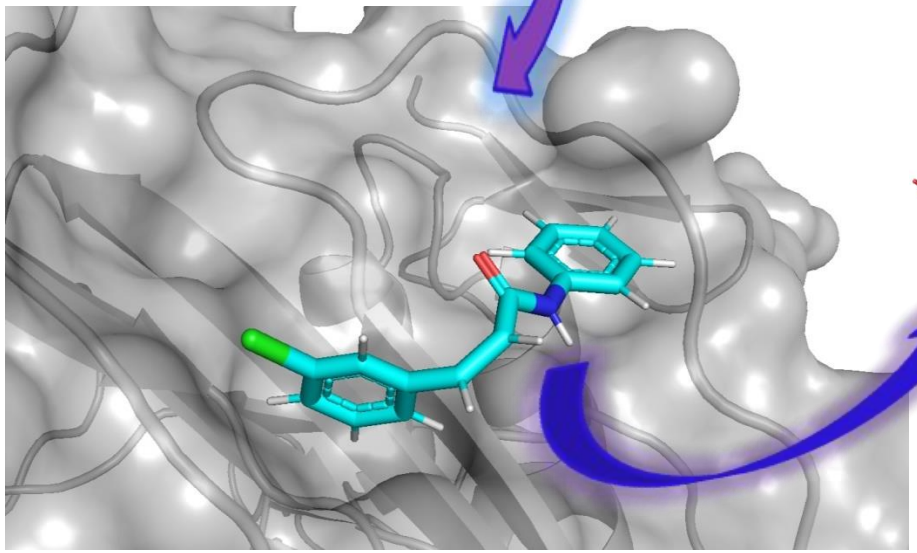
✓ *SAR at 40 μM concentration*

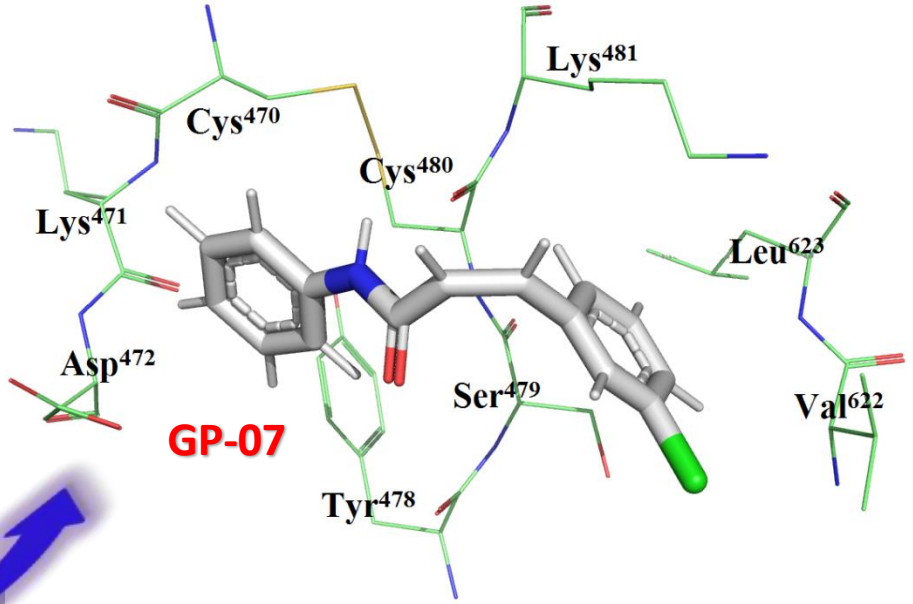
Results and discussion – Molecular target proposal for GP-07

Mature E protein glycoprotein complex (E1-E2-E3). PDB id: 3N41



After all molecular docking simulations, E protein was found to be the main target for **GP-07**. Furthermore, it was confirmed in our experimental results, in which was observed a protective effect on host cell towards CHIKV, suggesting that **GP-07** binds to the CHIKV viral surface and prevents its adhesion/adsorption to the cells.





In general, it was not verified H-bonds between **GP-07** and the binding site from the E protein, suggesting an essential role of hydrophobic groups in the complex formation.

Conclusions – Remarkable topics

Compound Selection and Synthesis	Cytotoxicity Assay	Viral Inhibition Assay	Target Proposal
• From 132 compounds, we have identified 10 promising compounds (acrylamides) to be synthesized	• 9 of 10 compounds presented good tolerance at 20 µM concentration	• **GP-07** was found to be the most active analog towards infected cell with CHIKV *in vitro*	• Docking simulations were able to predicted that **GP-07** binds to the CHIKV E protein.

Additional points & perspectives

- ✓ Theoretical data were corrobarated by experimental results;
- ✓ **GP-03** could be also considered as promising compound. However, it needs specific modifications to improve its potential anti-CHIKV activity;
- ✓ Based on **GP-07** results, it is possible to suggest that this compound could be used as a promising anti-CHIKV scaffold for designing new antiviral agents in the future.

Acknowledgments

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