Targeting West Nile virus replicases: NS3 and heterodimers inhibitors

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Cristina Peggion [a] (supervisor), Riccardo Rigo [b] (co-supervisor), Mattia Sturlese [b]

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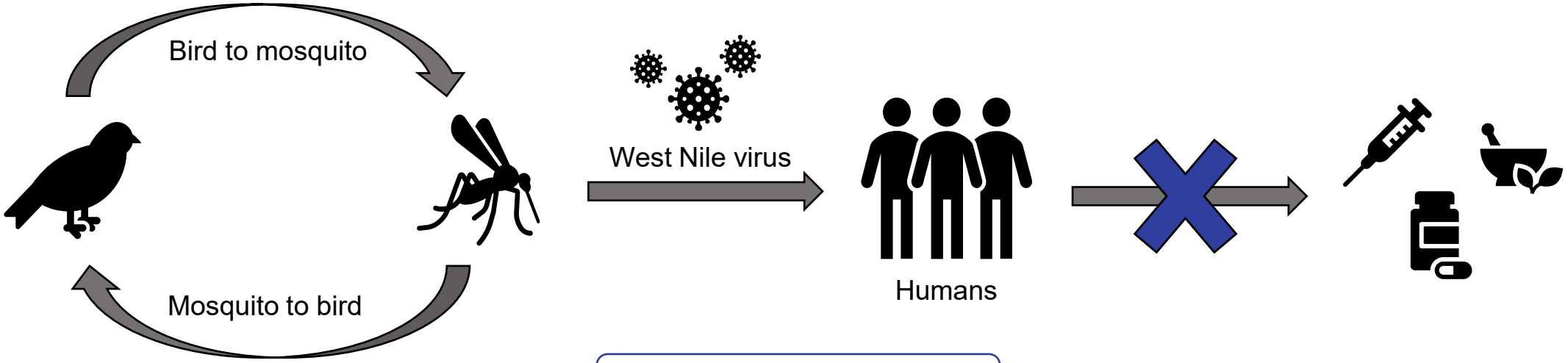
INTRODUCTION and AIM OF THE PROJECT

The **West Nile virus** (**WNV**) is a flavivirus of the Flaviviridae family. It circulates through a mosquito–bird–mosquito cycle and it is transmitted to humans through Culex mosquitoes. ⟶ Urgency for the **development of new WNV antiviral compounds**.



AIM OF THE PROJECT

A **direct approach** targeting the **active site of NS2B-NS3 protease.**

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Peptides/peptidomimetics as potential inhibitors.

An **indirect approach** targeting protein-protein interactions essential for NS3 activity.

↓

Peptides to disfavour NS2B-NS3 heterodimer.

NS2B-NS3 ACTIVE SITE INHIBITORS

- **Tri and tetra-peptide inhibitors**

Several peptides and peptidomimetics were synthesized as possible NS2B-NS3 inhibitors of WNV, tested and their IC_{50} value was evaluated.

Peptide name	Interaction	MW (g/mol)	Purity	IC_{50} (μM)
Nona-D-Arginine	NON – COVALENT	1422.73	87%	20.70 ± 3.60
PhAc-Lys-Lys-Arg-NH_2		547.71	97%	130.4 ± 8.3
Aun-Lys-Lys-Arg-NH_2		612.86	96%	34.43 ± 3.65
Palm-Lys-Lys-Arg-NH_2		667.99	95%	29.43 ± 3.81
PhAc-Lys-Lys-Arg-(cyclic-dehydro)		514.67	96%	**8.33** ± 0.33
Aun-Lys-Lys-Arg-(cyclic-dehydro)		579.83	98%	**8.34** ± 0.78
Palm-Lys-Lys-Arg-(cyclic-dehydro)		634.95	97%	**10.34** ± 1.71
PhAc-Lys-Lys-Arg-H	COVALENT	532.69	95%	**3.14** ± 0.19
Aun-Lys-Lys-Arg-H		597.85	97%	**7.05** ± 0.97

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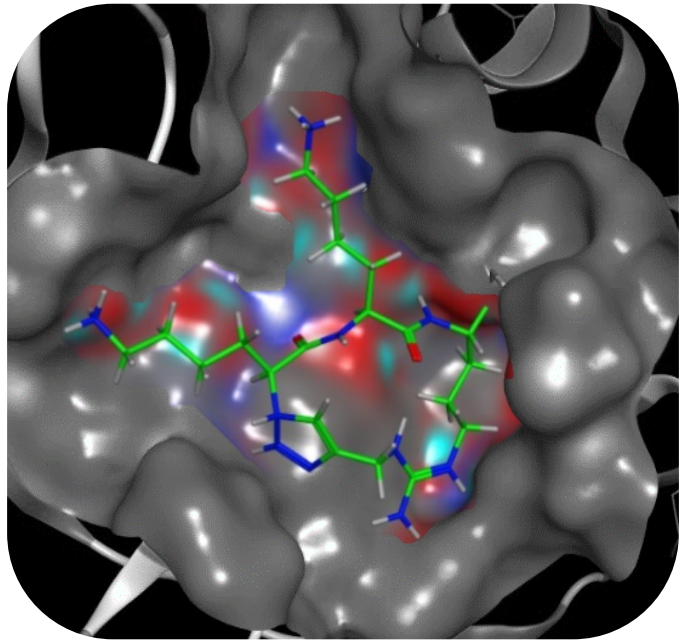
Cyclic peptide inhibitor

Cyclization of the tripeptides to increase the inhibitory efficacy and to disfavor their degradation.



Superimposition of cyclic tripeptide on the active site pocket of NS2B-NS3 protease of WNV.

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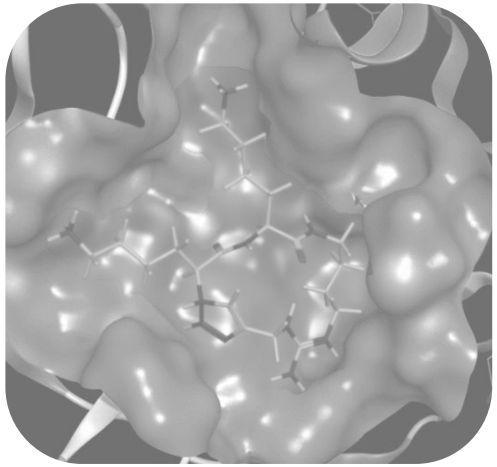
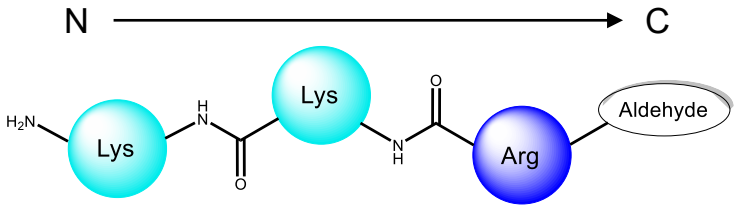
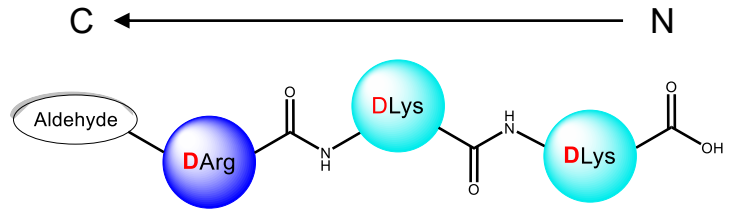


Retro-inverse peptide inhibitors

To increase the inhibitory efficacy and to disfavor its degradation.



Reference peptide

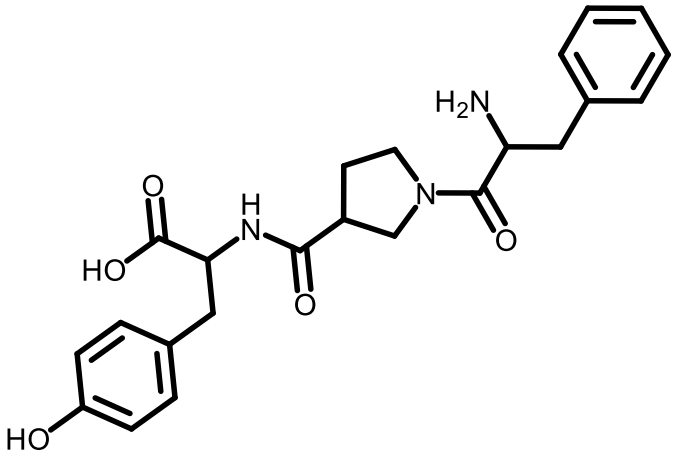


Retro-inverso peptide

NS2B-NS3 ALLOSTERIC SITE INHIBITORS

We designed **different tripeptides** that mimic the structure of an already known reversible allosteric inhibitor (SID:852843) of the formation of the functional conformation of the NS3 protease of WNV and we studied these **possible allosteric inhibitors by docking**.

- **Active tripeptide allosteric inhibitor**



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IC_{50} = **48.30** ± 11.07 μM

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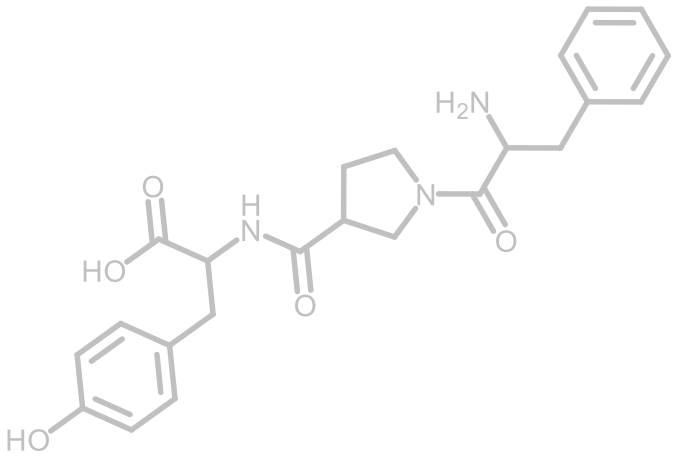

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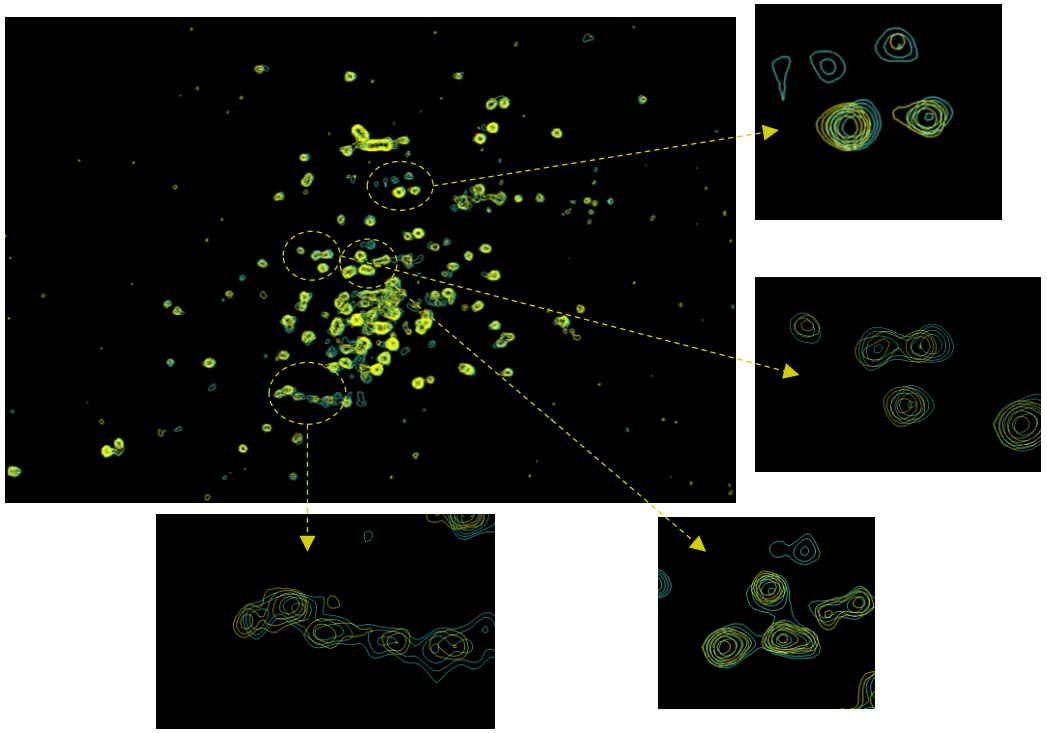
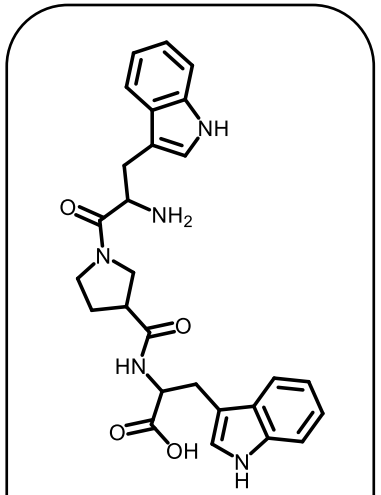
Protein-peptides interactions by NMR studies

2D NMR BEST-TROSY spectrum of the Trp-βPro-Trp peptide, which interacts with the NS2B-NS3 protease, as evidenced by Chemical Shift Perturbations.





Trp-βPro-Trp-OH

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