



Targeting West Nile virus replicases: NS3 and heterodimers inhibitors

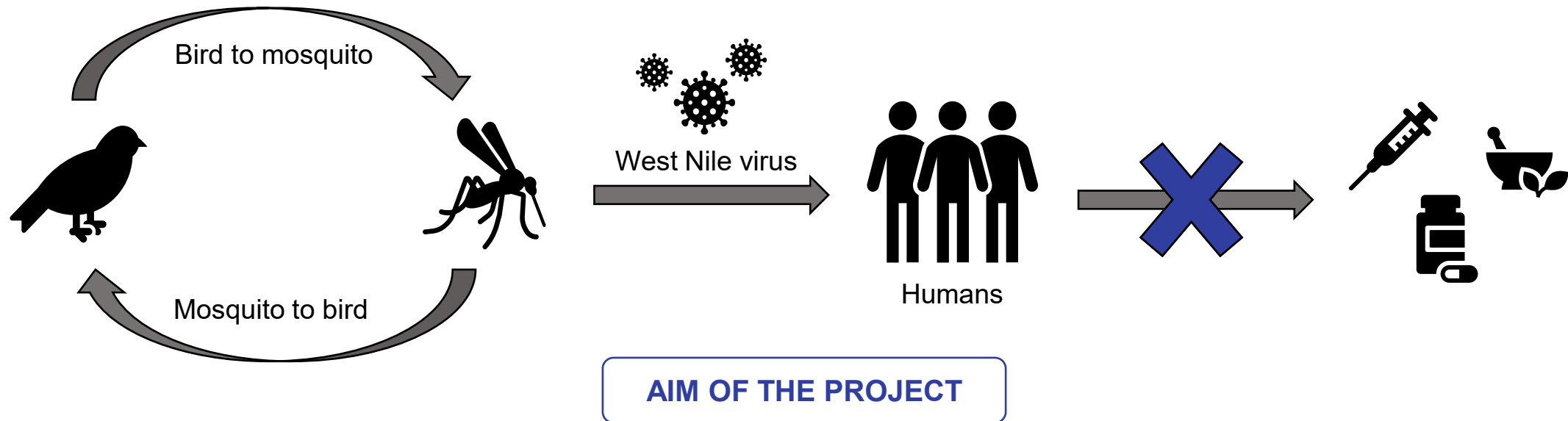
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The **West Nile virus (WNV)** is a flavivirus of the Flaviviridae family. It circulates through a mosquito–bird–mosquito cycle and it is transmitted to humans through *Culex* mosquitoes. —————> Urgency for the **development of new WNV antiviral compounds**.



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

↓
Peptides/peptidomimetics
as potential inhibitors.

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

↓
Peptides to disfavour NS2B-NS3 heterodimer.

☼ Tri and tetra-peptide inhibitors

Several peptides and peptidomimetics were synthesized as possible NS2B-NS3 inhibitors of WNV, tested and their IC₅₀ 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

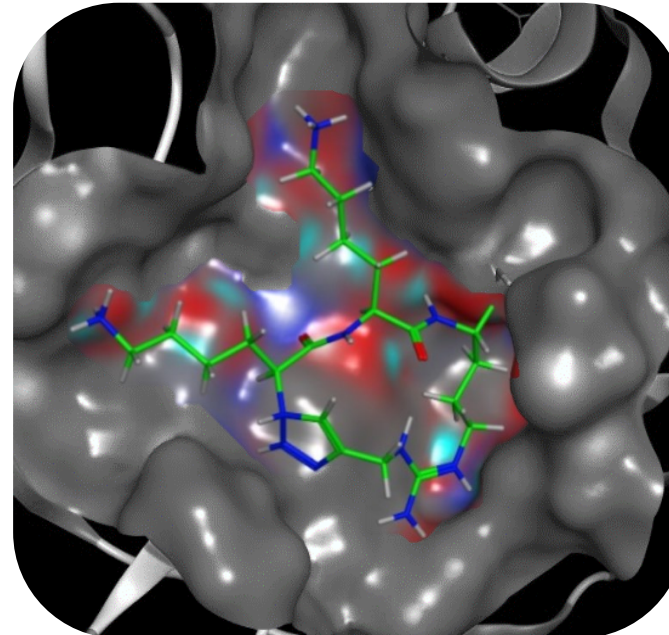
❁ Tri and tetra-peptide inhibitors

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} (μ M)
Nona-D-Arginine	NON – COVALENT	1422.73	87%	20.70 ± 3.60
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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.

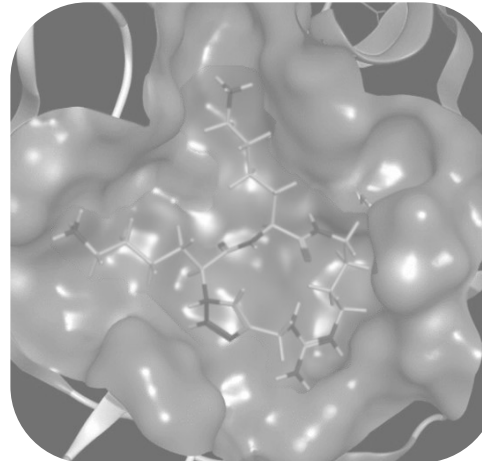
❁ Tri and tetra-peptide inhibitors

Several peptides and peptidomimetics were synthesized as possible NS2B-NS3 inhibitors of WNV, tested and their IC₅₀ value was evaluated.

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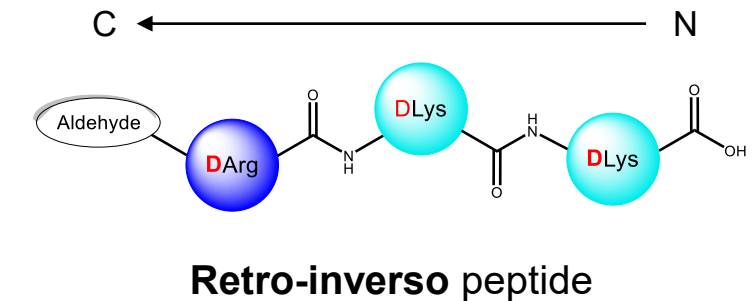
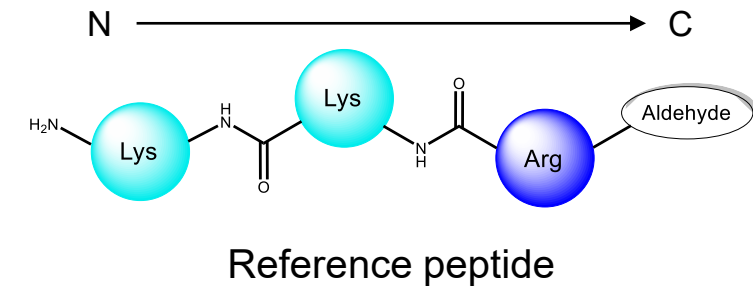
❁ Cyclic peptide inhibitor

Cyclization of the tripeptide to increase the inhibitory efficacy and to disfavor its degradation.



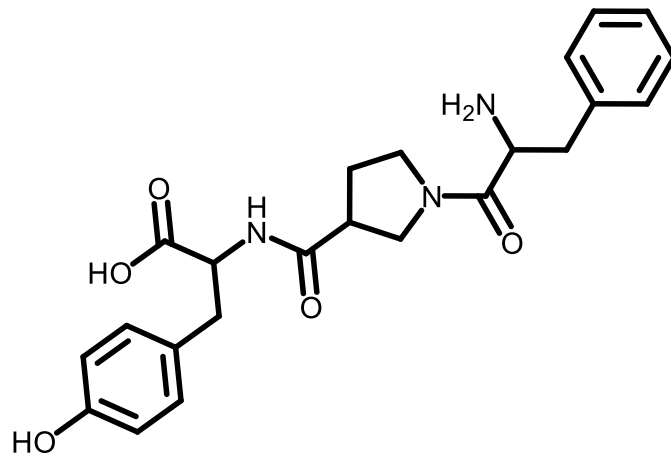
❁ Retro-inverse peptide inhibitors

To increase the inhibitory efficacy and to disfavor its degradation.

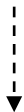


We designed **different tripeptides** that mimic the structure of an already known reversible allosteric inhibitor (SID:852843) of the formation of the functional conformation of the NS3 protease of WNV and we studied these **possible allosteric inhibitors by docking**.

✿ Active tripeptide allosteric inhibitor



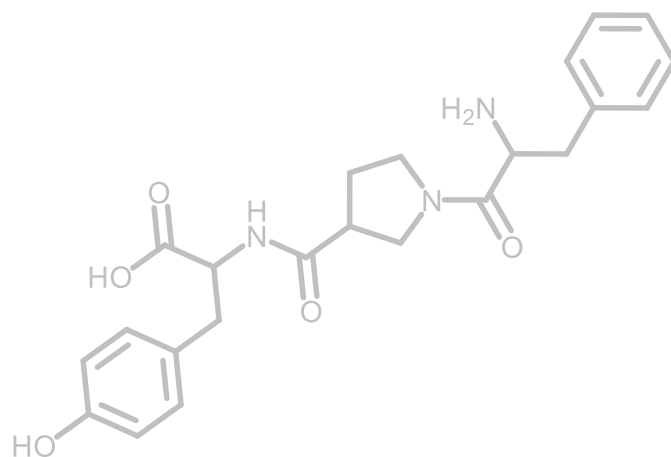
Phe-βPro-Tyr-OH



$$IC_{50} = 48.30 \pm 11.07 \mu M$$

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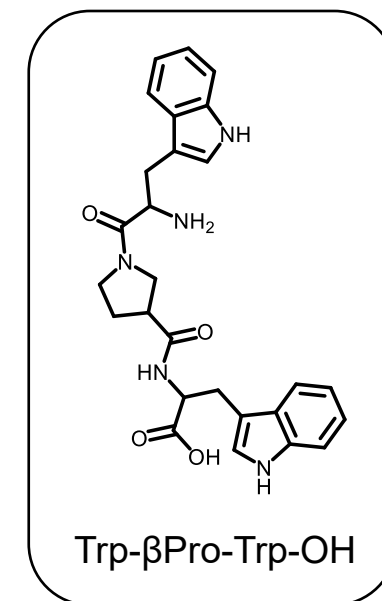
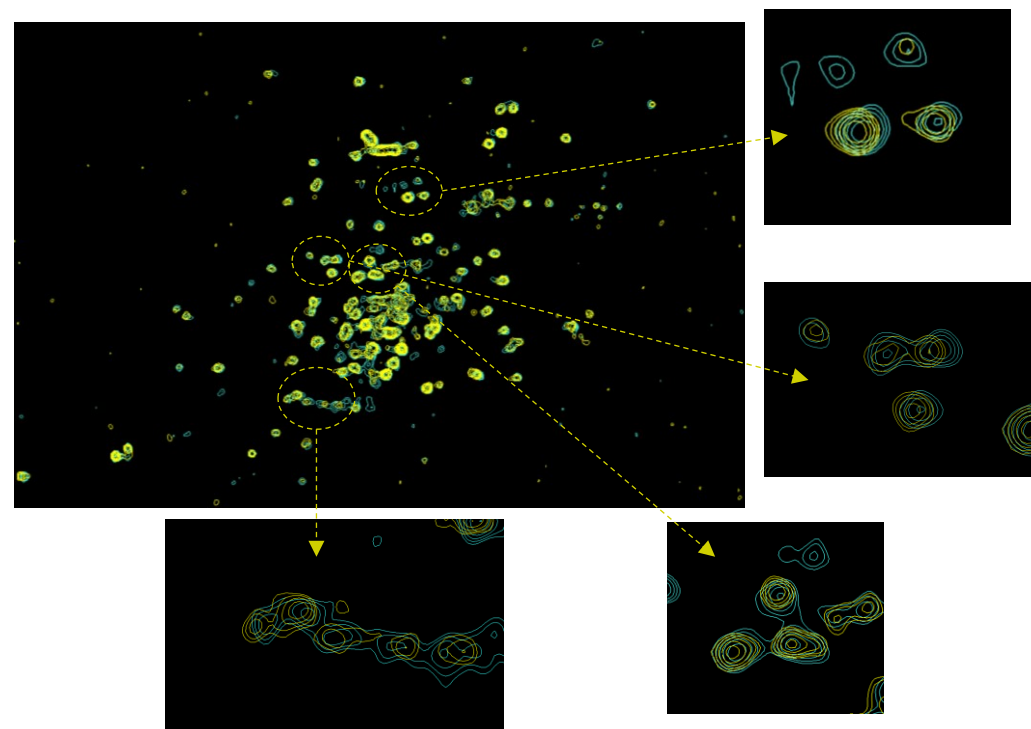


Phe-βPro-Tyr-OH

$$IC_{50} = 48.30 \pm 11.07 \mu M$$

✿ 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



AKNOWLEDGMENTS



Prof. **Cristina Peggion**: supervisor

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