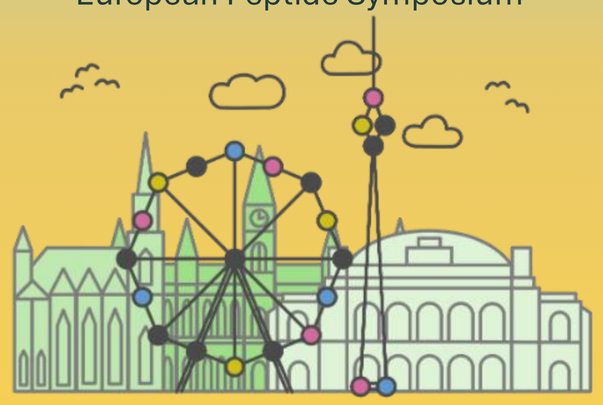
Design, Synthesis, and Biological Evaluation of Triazolyl-Bridged Antiviral Peptides Against Arboviruses

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Introduction

Global Threat: Arboviruses (DENV, WNV, ZIKV) cause severe neurotropic and visceral epidemics worldwide; no approved specific antiviral drugs exist.

Fusion Target: Entry is driven by the Envelope (E) glycoprotein. Endosomal low pH triggers a dimer-to-trimer transition, exposing the conserved hydrophobic fusion loop (FL) to initiate membrane fusion (Figures 1 & 2).[1]

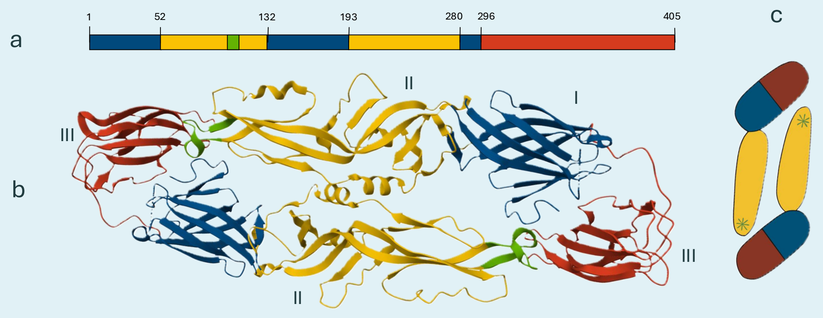


Figure 1. Structure of the dimer of the E protein.
(a) The three domains of E: Domain I is blue, Domain II is orange, Domain III is red, and FL is green.[2] (b) The conformation of E in solution above the fusion pH. (c) Neutral Prefusion Dimer (RCSB Protein Data Bank: 5JHM).

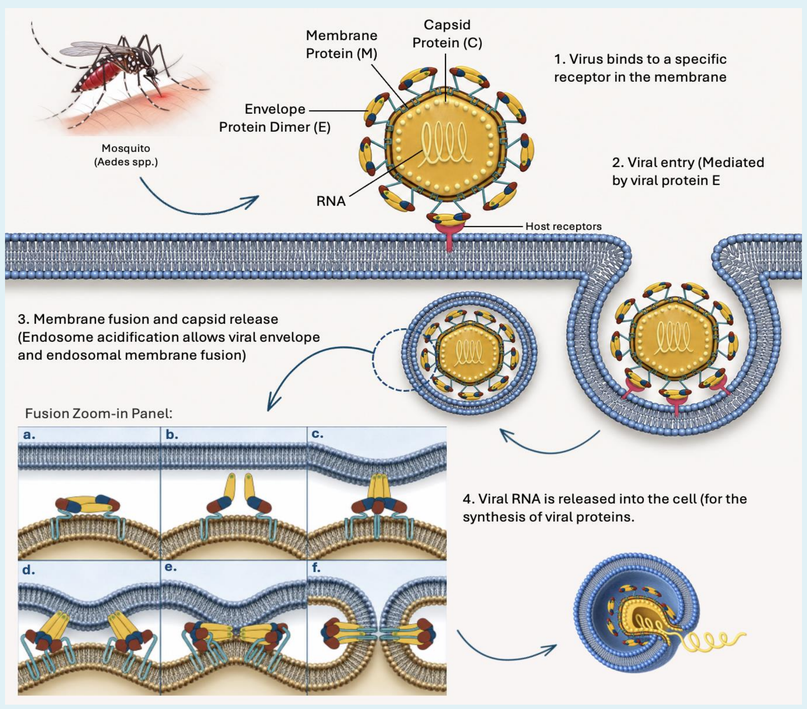
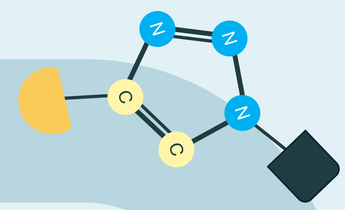


Figure 2. Schematic Model of the Flavivirus Lifecycle and Class II Membrane Fusion Cascade
Receptor-mediated entry is followed by E-dimer dissociation, fusion loop insertion (a–c), and Domain III fold-back (d), driving membrane hemifusion (e) and pore opening (f) for genome release. (Adapted from Modis Y, et al. Nature, 2004).[2]

Aim of the work

This project aims to:



1 Design and Synthesis of Linear Precursor

Microwave-Assisted Solid-Phase Peptide Synthesis (SPPS) using Fmoc/tBu chemistry for the preparation of peptides derived from the Envelope (E) glycoprotein.

2 Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC)

Microwave-assisted intramolecular CuAAC click reaction using lysine azide and propargylglycine (Pra) to form a triazole bridge, stabilizing and mimicking the β-turn.

3 Biological Evaluation

Antiviral Activity: Plaque Reduction Assay (PRA) against ZIKV, WNV, and DENV in Vero E6 cells.
Cytotoxicity: MTT cell viability assay in Vero E6 cells.

Purification characterization

We initially selected a 13-mer E-protein fragment (PepE13), whose sequence cannot be disclosed for confidentiality reasons, and we performed Cu(I) catalyzed Azide-Alkyne Cycloaddition (CuAAC) to obtain progressively shortened cyclic and conformationally stable analogs.[3,4]

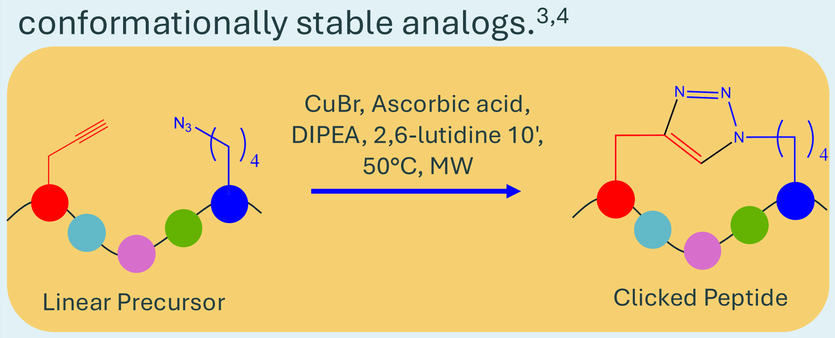


Figure 3. Schematic of the CuAAC

Table 1. List of selected peptides

Peptide	Sequence	LC gradient (% B)[a] Rt (min)	*HPLC purity (%)*
PepE13	Linear, 13 mer	10-90 3.62 min	>96
CycloPepE13	Cyclo(1-13), 13 mer	10-90 3.78 min	>96
CycloPepE11	Cyclo(1-11), 11 mer	10.90 3.88 min	>97
CycloPepE9	Cyclo(1-9), 9 mer	10.90 3.48 min	>97
CycloPepE7	Cyclo(1-7), 7 mer	10-90 2.90 min	>95

[a] *Eluents: 0.1% (v/v) TFA in H_2O (A) and 0.1% (v/v) TFA in ACN (B)*

Synthesis and Characterization

To analyze secondary structure, chirality, and conformational changes in different environments, CD spectra were recorded at 25 °C in phosphate buffer (PB) and increasing concentrations of trifluoroethanol (TFE), up to 50% (v/v).

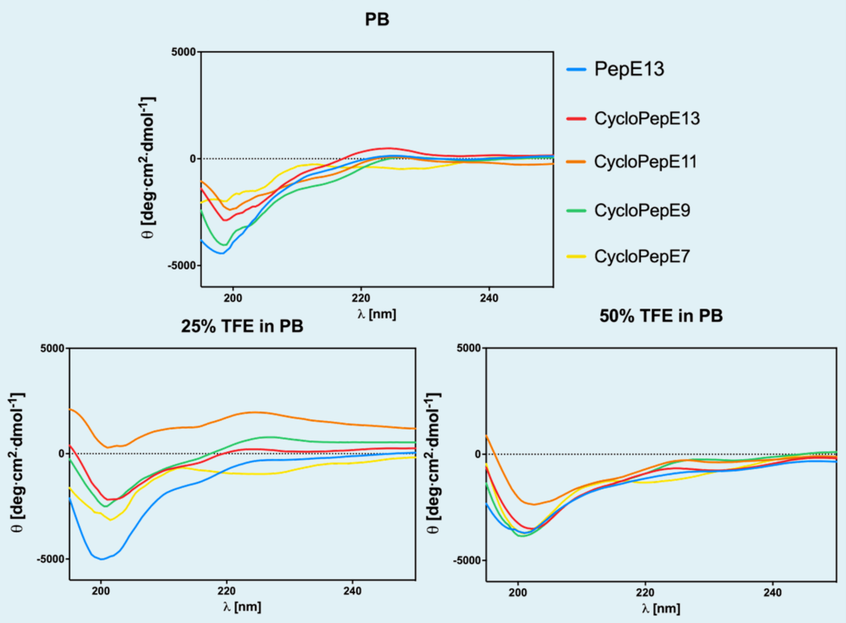


Figure 4. CD spectra of novel antiviral peptides in 10mM phosphate buffer (PB), pH 7.4

CD analysis shows that the linear E-protein mimic (PepE13) adopts a random-coil conformation, characterized by a prominent minimum at ~200 nm. In contrast, a red shift of the negative minimum and the appearance of characteristic features around 205 and 218 nm are observed, most clearly in the final peptide variant (CycloPepE7), indicating the stabilization of a β-turn conformation.[5]

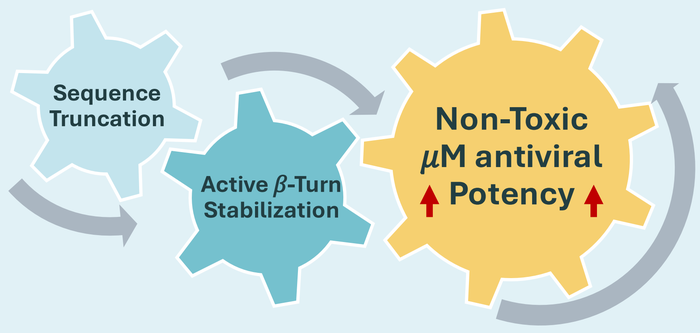
Circular Dichroism (CD)

Table 2. Inhibitory and cytotoxic activity of novel peptides

Peptide	ZIKA		WNV		DENV	
	IC50 μM	CC50 μM	IC50 μM	CC50 μM	IC50 μM	CC50 μM
PepE13	4.93 ± 2.20	>100	1.96 ± 1.60	>100	0.78 ± 0.31	>100
CycloPepE13	0.64 ± 0.60	>100	0.37 ± 0.30	>100	0.28 ± 0.13	>100
CycloPepE11	0.45 ± 0.40	>100	0.21 ± 0.2	>100	0.08 ± 0.03	>100
CycloPepE9	0.17 ± 0.10	>100	0.17 ± 0.05	>100	0.11 ± 0.02	>100
CycloPepE7	0.14 ± 0.04	>100	0.08 ± 0.02	>100	0.21 ± 0.30	>100

Triazole Safety: Triazole stapling showed an excellent safety profile (CC_{50} > 100 μM).
Enhanced Potency: Truncation to a 7-mer maximized activity, achieving micromolar potency against WNV (0.08 μM) and ZIKV (0.14 μM).

Antiviral activity



Conclusion

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