Mucroporin-M1 analogues: synthesis, conformation and structure-activity correlation

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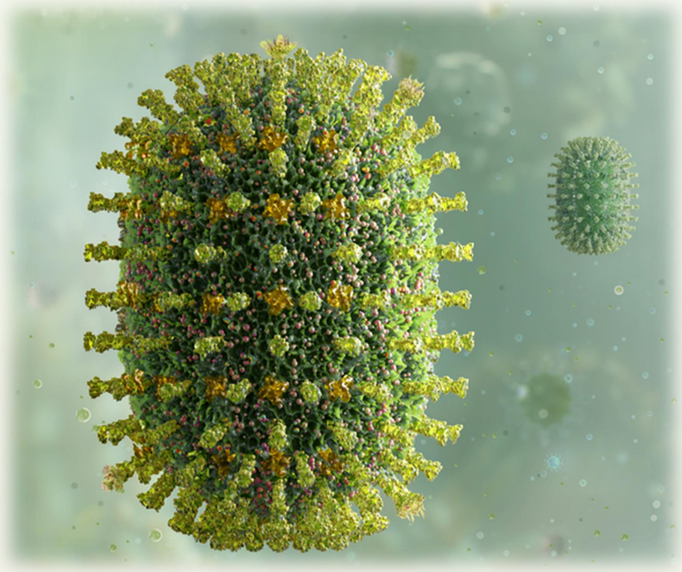
INTRODUCTION and AIM

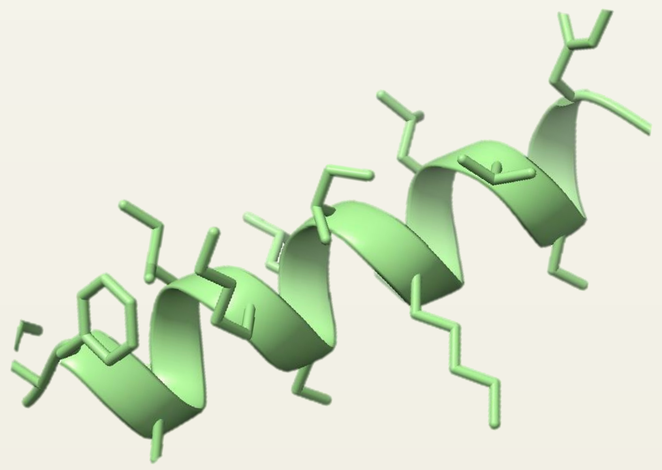
Mucroporin is a peptide of 17 amino acids deriving from the scorpion venom *Lychas mucronatus* with known antimicrobial properties (active against gram-positive bacteria). *Li et al.* [1] optimized the *Mucroporin* sequence by proposing **M*ucroporin-M1*** sequence with some amino acid substitutions (G3R, P6K, G10K and G11R). These modifications improved peptide activity at lower concentrations respect to the native peptide. Substitutions increase the net positive charge of the peptide, strengthening the amphipathic character of the peptide helix.
Previous studies have shown that Mucroporin-M1 also possesses antiviral activity: it interacts directly with viral particles and that, when the peptide attaches to the virus, its strong electrostatic affinity increases the interaction and destruction of the viral envelope [1-3].
The **aim** of this work was to further improve the **activity** of Mucroporin-M1 and develop its **analogues** by suitably modifying the amino acid sequence and studying the conformation of the synthesized compounds and the structure-activity relationship.



SEQUENCES

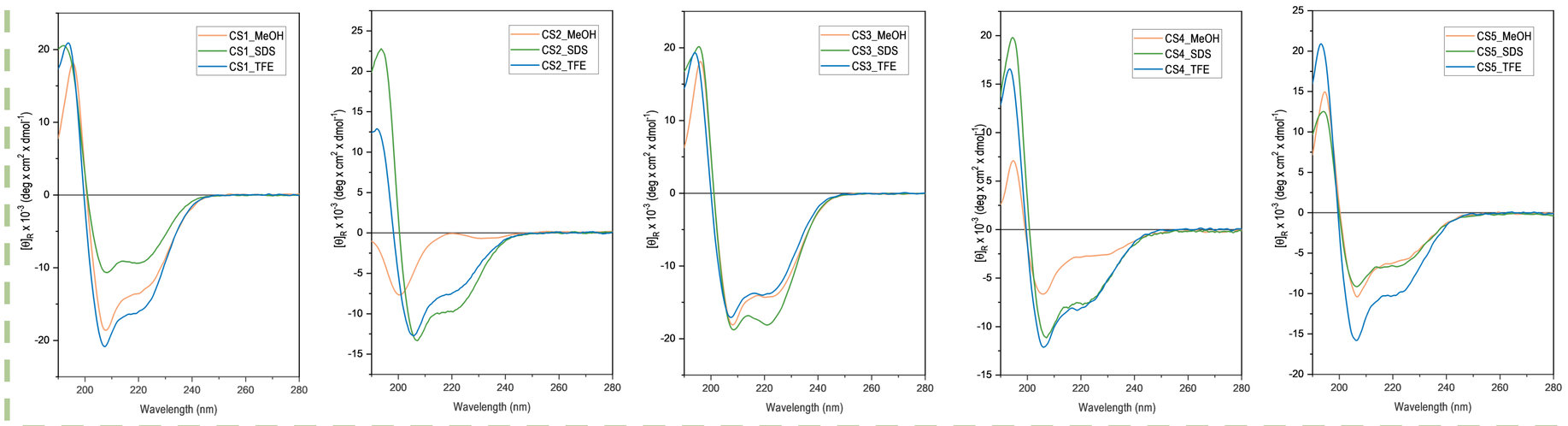
The summarized sequences are shown in the table. They were obtained by SPPS (Solid-Phase Peptide Synthesis), purified by preparative HPLC and characterized by HPLC-MS.

ABBREVIATION	COMPOUND	SEQUENCE
CS1	Mucroporin-M1 Long	LFRLIKSLIKRLVSAFK-NH_2
CS2	Mucroporin-M1 Short	LIKRLVSAFK-NH_2
CS3	Mucroporin-M2 Long	LFRLUKS LUKR LUSAFK-NH_2
CS4	Mucroporin-M2 Short	LUKR LUSAFK-NH_2
CS5	Palm-Mucroporin-M2 Short	Palm-LUKR LUSAFK-NH_2



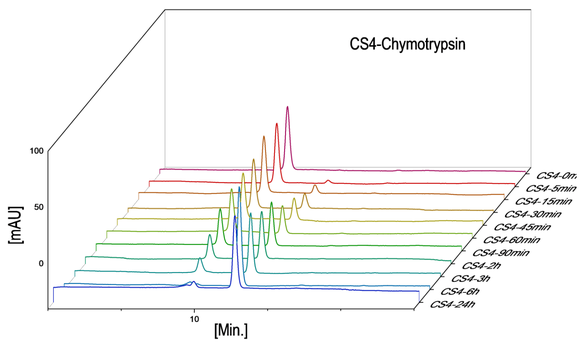
Mucroporin-M1

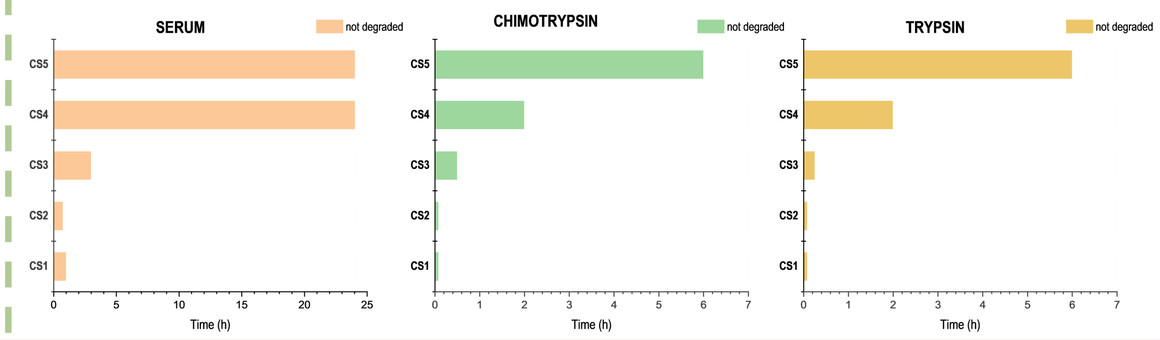
RESULTS

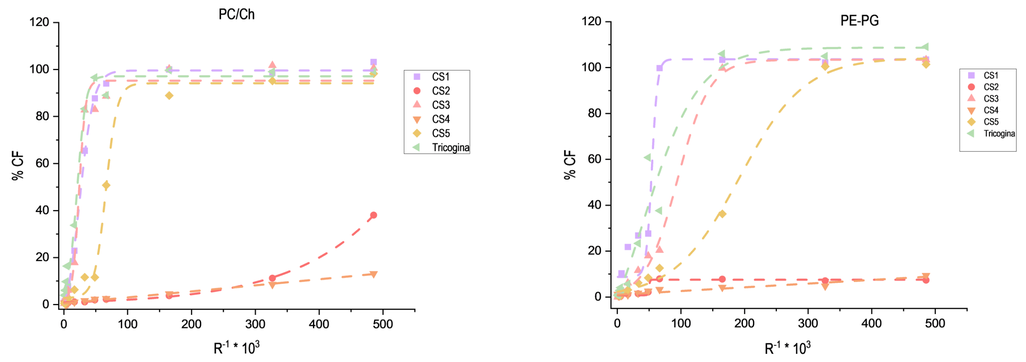


Conformational studies were conducted by **CD** in three different solvents: **MeOH** (organic solvent), **SDS 100 mM** (surfactant that forms micelles, which mimic cell membranes) and **TFE** (helix inducer solvent). Conformational studies by **NMR** are in progress.

In order to study resistance to enzymatic degradation, the synthesised peptides were tested with **Trypsin** (cut on the carboxyl of Lysine and Arginine), **Chymotrypsin** (cut on the carboxyl of apolar amino acids) and **Serum**. The enzymatic degradation reactions were followed by HPLC.







The **cytotoxicity** of the peptides was determined by MTT assay in 3 different cell lines (vero cell, vero E6 cell and MDCK cell). Preliminary **activity** studies were also conducted by leakage assays from liposomes to determine the ability to interact with membranes.

Peptide	Cytotoxicity , CC_{50} (µg/mL)		
	Vero cells (72h)	Vero E6 cells (72h)	MDCK cells (48h)
CS1	49	93	>100
CS2	>100	>100	>100
CS3	38	79	>100
CS4	>100	>100	>100
CS5	33	50	49

CONCLUSIONS

From the **structural studies**, we can observe that the helical structure is maintained in different solvents by the modified sequences, and that CS4 remains helical in MeOH, unlike CS2.
The **enzymatic degradation studies** show that the proposed modifications increase the products' resistance to enzymatic degradation, thereby enhancing their stability in serum.
Regarding **cytotoxicity**, it is noteworthy that the modified and shortened CS4 peptide is not cytotoxic in different cell lines. In-cell **activity studies** are currently underway. For now, a preliminary study has been conducted using leakage tests, which indicate a decrease in the ability to breach membranes as the peptide chain length decreases.

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