Reengineering the antimicrobial peptide from the scorpion venom of *Opisthacanthus madagascariensis* into highly active peptides with low toxicity

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Introduction

The poisons have several bioactive molecules and they are therefore considered a potential source of new drugs. In this work, we reprogrammed the cationic amphipathic antimicrobial peptide (AMP) IsCT1, derived from the scorpion venom Opisthacanthus madagascariensis, seeking to reduce the toxicity to human cells and enhance its intrinsic antimicrobial properties. In this attempt, synthetic variants with a net charge ranging from +3 to +6 were generated through the simultaneous replacement of 1 to 4 amino acid residues in the original sequence positions, resulting in 6 scorpion-derived antimicrobial peptide IsCT1.

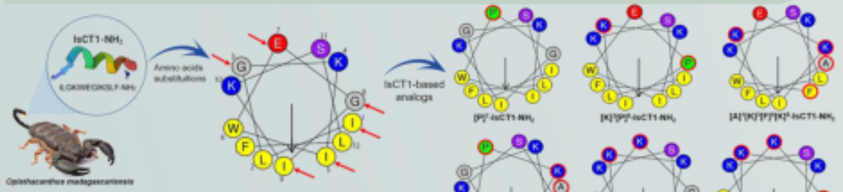

Figure 1. Helical wheel projections of IsCT1 and its analogs. Yellow, green and grey circles indicate hydrophobic amino acids, blue, purple and red, represent hydrophilic amino acids.

Table1. Peptide sequence, molecular weight and physiochemical properties

Peptide	Sequence	Molecular Weight (Da)		Purity (%)	H	μH	P/N	z
		Calculated	Observed					
IsCT1-NH$_2$	H_3N^+-ILGKIWEGIKSLF-CONH$_2$	1501.9	1503.0	98	0.783	0.776	0.857	+2
[P]^7-IsCT1-NH$_2$	H_3N^+-ILGKFW**P**GIKSLF- CONH$_2$	1469.9	1472.0	98	0.888	0.674	0.625	+3
[K]^3[P]^8-IsCT1-NH$_2$	H_3N^+-IL**K**KIWE**P**IKSLF- CONH$_2$	1613.0	1614.9	99	0.762	0.827	0.857	+3
[A]^1[K]^3[F]^5[K]^8-IsCT1-NH$_2$	H_3N^+-**A**LK**K**FWE**K**IKSLF-CONH$_2$	1582.0	1583.9	98	0.515	0.801	0.623	+4
[A]^1[F]^5[P]^7[K]^8-IsCT1-NH$_2$	H_3N^+-ALGK**F**W**P**K**I**KSLF- CONH$_2$	1532.9	1533.8	99	0.696	0.676	0.623	+4
[K]^3[K]^7[K]^9-IsCT1-NH$_2$	H_3N^+-IL**K**KIW**K**GI**K**KSLF- CONH$_2$	1587.0	1588.0	95	0.465	0.642	1.167	+6
[K]^3[K]^7[P]^8[K]^9-IsCT1-NH$_2$	H_3N^+-IL**K**KIW**KP**K**K**SLF- CONH$_2$	1627.1	1628.0	99	0.521	0.650	0.857	+6

H: hydrophobicity; μH: hydrophobic moment; P/N: polar – nonpolar residues proportion; z: net charge

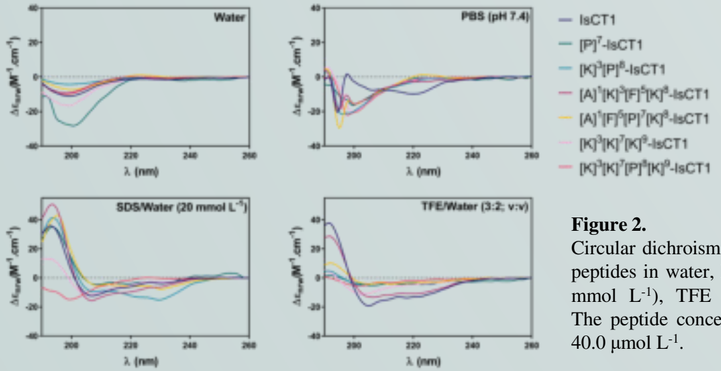

Figure 2. Circular dichroism spectra of the peptides in water, PBS, SDS (20 mmol L^{-1}), TFE Water (60%). The peptide concentrations were 40.0 μmol L^{-1}.

Results

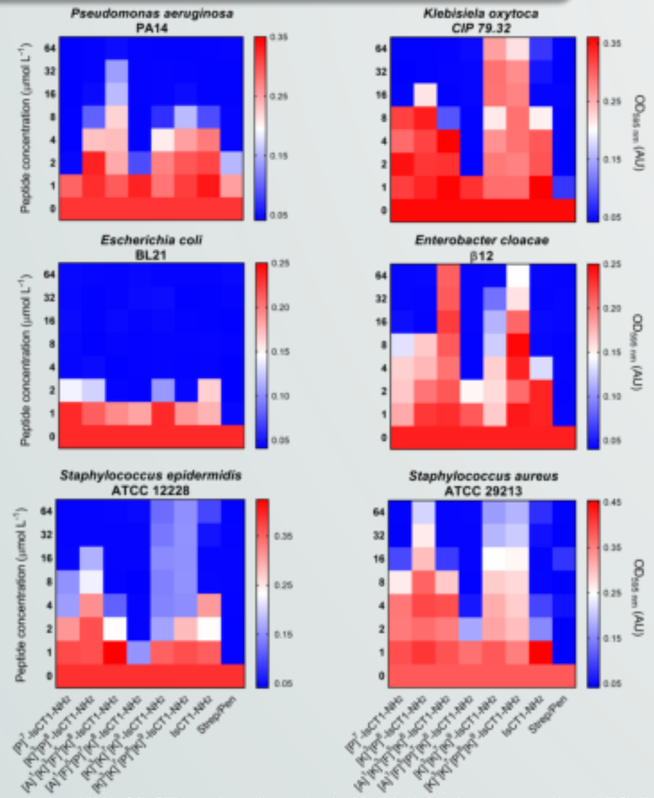

Figure 3. Antimicrobial of IsCT1 and analogs. Antimicrobial activity expressed as MIC (Minimal inhibitory concentration). The concentration range of the peptides was 64.0 to 1.0 μmol L^{-1}.

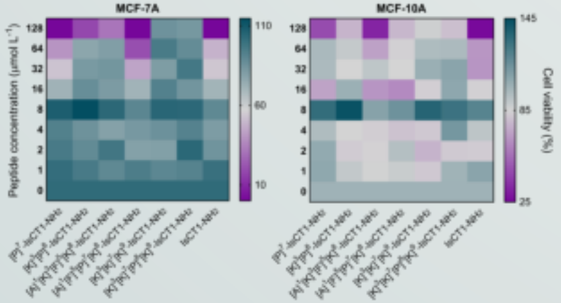

Figure 4. Antitumoral and Cytotoxicity of IsCT1 and analogs. Activity expressed as percentage of viable cells by peptide concentration (range of the peptides was 128.0 – 1.0 μmol L^{-1}).

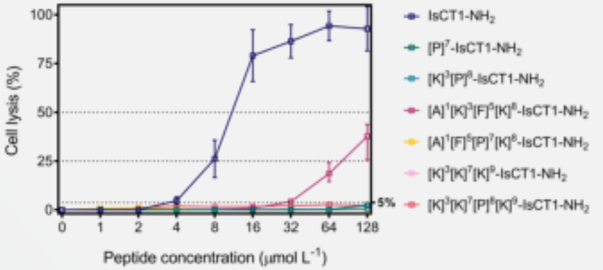

Figure 5. Hemolytic activities of IsCT1 and analogs. The concentration range of the peptides was 128 – 0.1 μmol L^{-1}.

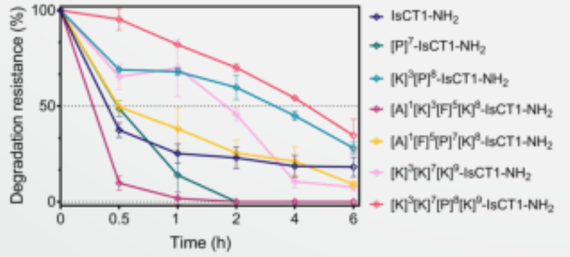

Figure 6. Degradation resistance assays of IsCT1 and its analogs in fetal bovine serum. Analyses were performed at 0, 0.5, 1, 2, 4 and 6 h.

Discussion and conclusion

The resulting synthetic peptides showed increased antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and Gram-negative bacteria (*Enterobacter cloacae, Escherichia coli, Klebsiella oxytoca* and *Pseudomonas aeruginosa*) and provoked a decreasing in the hemolytic activity compared to the native molecule. Glutamic acid (position 7) was shown to be important in the interaction with the erythrocyte membrane, with a reduction in hemolysis when replaced by another amino acid residue. It was also observed that several peptides have anti-cancer activity due to their ability to target the human breast cancer cell line MCF-7, without activity against healthy cell line MCF-10A. In general, we show a mutation-based approach to manipulate the peptide structure influencing its biological function, enabling new therapeutic properties.

Acknowledgements

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