Bioorganic Chemistry 90 (2019) 103038

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Repurposing the scorpion venom peptide VmCT1 into an active peptide against Gram-negative ESKAPE pathogens

Cibele Nicolaski Pedron[a], Iris Araújo[a], Pedro Ismael da Silva Junior[c], Fernanda Dias da Silva[a], Marcelo Der Torossian Torres[d,e,*], Vani Xavier Oliveira Junior[a,b,*]

[a] Universidade Federal do ABC, Centro de Ciências Naturais e Humanas, 5001 Avenida dos Estados, Santo André, 09210580 SP, Brazil
[b] Universidade Federal de São Paulo, 100 Rua 3 de Maio, 04044020 São Paulo, SP, Brazil
[c] Instituto Butantan, Laboratório Especial de Toxinologia Aplicada, 1500 Avenida Vital Brasil, 05503900 São Paulo, SP, Brazil
[d] Departments of Psychiatry and Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia 19104, PA, United States
[e] Department of Bioengineering, University of Pennsylvania, Philadelphia 19104, PA, United States

ARTICLE INFO

Keywords:
Antimicrobial peptide
VmCT1
Structure-activity relationship
Scorpion venom peptide

ABSTRACT

VmCT1 is a cationic antimicrobial peptide (AMP) from the venom of the scorpion *Vaejovis mexicanus*. VmCT1 and analogs were designed with single substitutions for verifying the influence of changes in physicochemical features described as important for AMPs antimicrobial and hemolytic activities, as well as their effect on VmCT1 analogs resistance against proteases action. The increase of the net positive charge by the introduction of an arginine residue in positions of the hydrophilic face of the helical structure affected directly the antimicrobial activity. Arg-substituted analogs presented activity against Gram-negative bacteria from the ESKAPE list of pathogens that were not observed for VmCT1. Additionally, peptides with higher net positive charge presented increased antimicrobial activity with values ranging from 0.39 to 12.5 μmol L^{-1} against Gram-positive and Gram-negative bacteria and fungi. The phenylalanine substitution by glycine (position 1), and the valine substitution by a proline residue (position 8) led to analogs with lower hemolytic activity (at concentrations 50 and 100 μmol L^{-1}, respectively). These results revealed that it is possible to modulate the biological activities of VmCT1 derivatives by designing single substituted-analogs as prospective therapeutics against bacteria and fungi.

1. Introduction

Antimicrobial resistance is a major healthcare problem, therefore the search for the development of new antimicrobial agents has become crucial. The multi-drug resistance against Gram-positive and Gram-negative bacteria is a challenging obstacle, considering the availability of treatment with conventional antibiotics are losing effectiveness. Versatile biomolecules, such as peptides, emerge as promising alternatives to slow down the evolution and the spread of antibiotic resistance [1].

AMPs are biologically active molecules produced by a wide variety of organisms as an essential component of the innate immune response [2,3]. AMPs are usually small, cationic, and amphipathic structures that exhibit a heterogeneous amino acid composition, frequently classified according to their secondary structures [4,5]. AMPs have a broad-spectrum activity ranging Gram-positive and Gram-negative bacteria [6], fungi [7], enveloped viruses [8], and parasites [9]. Toxicity against human cell lines, such as human erythrocytes [2,10], is one of the limitations for the application of AMPs as therapeutics.

Erythrocytes and microbial cell membranes present different lipid content, which influences AMPs-membranes interaction and mode of action. Mammalian erythrocyte cell membranes contain zwitterionic phospholipids, as sphingomyelin and phosphatidylcholine (PC), besides sterols, such as cholesterol that regulates membrane fluidity [11]. Differently, the cytoplasmic membrane of bacteria is constituted by anionic lipids, such as phosphatidylglycerol (PG), cardiolipin, and phosphatidylserine, along with zwitterionic lipid, as phosphatidylethanolamine (PE), which are important for the membrane organization [11]. The lipid content is different from one species to another. Frequently, Gram-negative bacteria show higher PE content

* Corresponding authors at: Departments of Psychiatry and Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia 19104, PA, United States (M.D.T. Torres). Universidade Federal do ABC, Centro de Ciências Naturais e Humanas, 5001 Avenida dos Estados, Santo André, 09210580 SP, Brazil (V.X.Jr. Oliveira).
E-mail addresses: marcelot@pennmedicine.upenn.edu (M.D.T. Torres), vani.junior@ufabc.edu.br (V.X. Oliveira Junior).

https://doi.org/10.1016/j.bioorg.2019.103038
Received 14 March 2019; Received in revised form 28 May 2019; Accepted 3 June 2019
Available online 08 June 2019
0045-2068/

than Gram-positive bacteria. However, Gram-positive bacteria exhibit a higher amount of anionic lipids (PG and cardiolipin) [12]. The presence of charged portions in lipids favors the electrostatic interactions with cationic AMPs, followed by insertion of peptides in the lipid bilayer. In most of the cases AMPs destabilize the membrane making it permeable or disrupting it. Additionally, fungal membranes contain neutral sterols, such as ergosterol, and their cell walls are constituted by polysaccharides, such as chitin and β-glucan [7], possibly AMPs promote the disruption of membrane which results in the leakage of intracellular content [13].

Cationic and amphipathic AMPs are among the most active components of scorpion venoms. They are usually 13–56 amino acid residues-long, what favors these AMPs to undergo helix-coil transitions in the presence of helical inducer environments, such as biomembranes [14,15]. VmCT1 (Phe-Leu-Gly-Ala-Leu-Trp-Asn-Val-Ala-Lys-Ser-Val-Phe-NH_2) is an α-helical scorpion venom AMP isolated from *Vaejovis mexicanus smithi*, which exhibited antimicrobial activity against Gram-positive and Gram-negative bacteria ranging from 5 to 25 μmol L^{-1}, besides of hemolytic activity at 12.5 μmol L^{-1} [16]. Pedron et al. described VmCT1 analogs aiming to verify the effect of changes in physicochemical features in the antimicrobial and hemolytic activities with single and double substitutions at the hydrophilic and hydrophobic portions of the amphipathic structure of VmCT1. The most promising antimicrobial activity results were displayed by Lys-substituted analogs with high helical content [17]. However, VmCT1 Lys-substituted analogs were more hemolytic than Glu-substituted analogs, which presented lower helical tendency, and consequently, lower antimicrobial activity than Lys-substituted analogs.

Therefore, we leveraged a rational design strategy of AMPs described by Torres et al. [18,19], where the authors analyzed how the most well-known physicochemical features affect the antimicrobial activity of small cationic peptides. As a result, we proposed single-substituted VmCT1 analogs targeting to improve antimicrobial activity and suppress hemolytic activity. To achieve this, we promoted changes in helical content, net positive charge and hydrophobicity-related features (Fig. 1).

The influence of net positive charge in the biological activities was verified with Arg-substituted analogs. The variants were designed with Arg residues substitutions in positions Gly3, Asn7 and Ser11 in the VmCT1 hydrophilic face, which was shown as the most susceptible region of the sequence for the replacements by basic residues according to Pedron et al. [17].

The first step of AMPs interaction with biomembranes is through electrostatic interactions of the hydrophilic face of the amphipathic structure after the hydrophobic face interacts with the nonpolar membrane portion [20]. Lys residues are frequently found in scorpion venom peptide [21–23], so we proposed to verify whether the Arg side chain (guanidyl group) influences in activities of this peptide's family. Additionally, we designed analogs with substitutions in positions Phe1, Val8, Ala9, and Val12 (hydrophobic face), aiming to analyze the influence of changes in hydrophobicity-related features, such as mean hydrophobicity and mean resultant hydrophobic moment in the biological activities (Supplementary Table 1).

Gly substitution at position 1 was chosen to decrease hydrophobicity and hydrophobic moment values, besides increasing flexibility in the *N*-terminal extremity of the peptide, modifying its helical content and positive charge availability [24]. The Pro residue was chosen for the substitution at position 8 because of its structure disruptor features. The replacement by Pro aimed verifying the importance of helical structure in the biological activities. Leu and Phe residues for the substitutions at position 9 were chosen to increase hydrophobicity and hydrophobic moment mean values, whereas Leu and Tyr residues at position 12 were selected aiming to promote decreased hydrophobicity values for the analogs compared to the wild-type. Besides, Leu and Phe are residues frequently found in scorpion venom peptides, while Gly (at position 1), Tyr and Pro do not occur with frequency (Supplementary Table 1) [25–27].

2. Material and methods

2.1. Solid-phase peptide synthesis (SPPS), purification and analysis

The peptides were synthesized on a peptide synthesizer (PS3-Sync Technologies) using solid-phase peptide syntheses methodology and

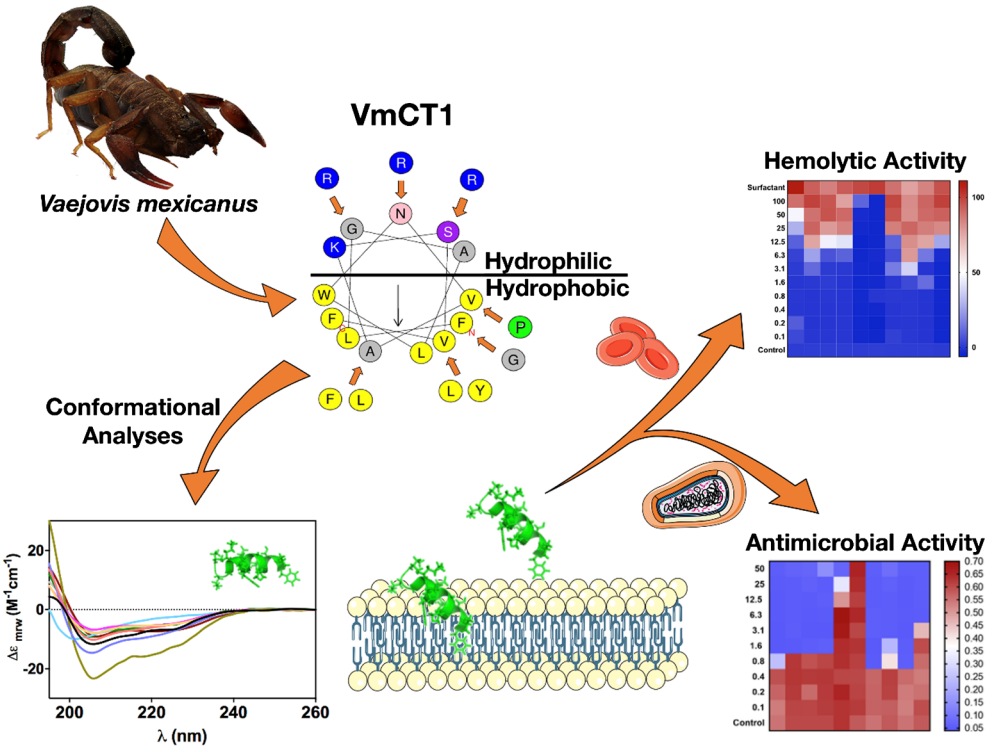

Fig. 1. Schematic of the structure-function-guided design used to generate biologically active AMPs derivatives. The scorpion venom derived AMP VmCT1 was subjected to structure-function analyses to evaluate the importance of the physicochemical features in secondary structure tendencies, antimicrobial and hemolytic activities.

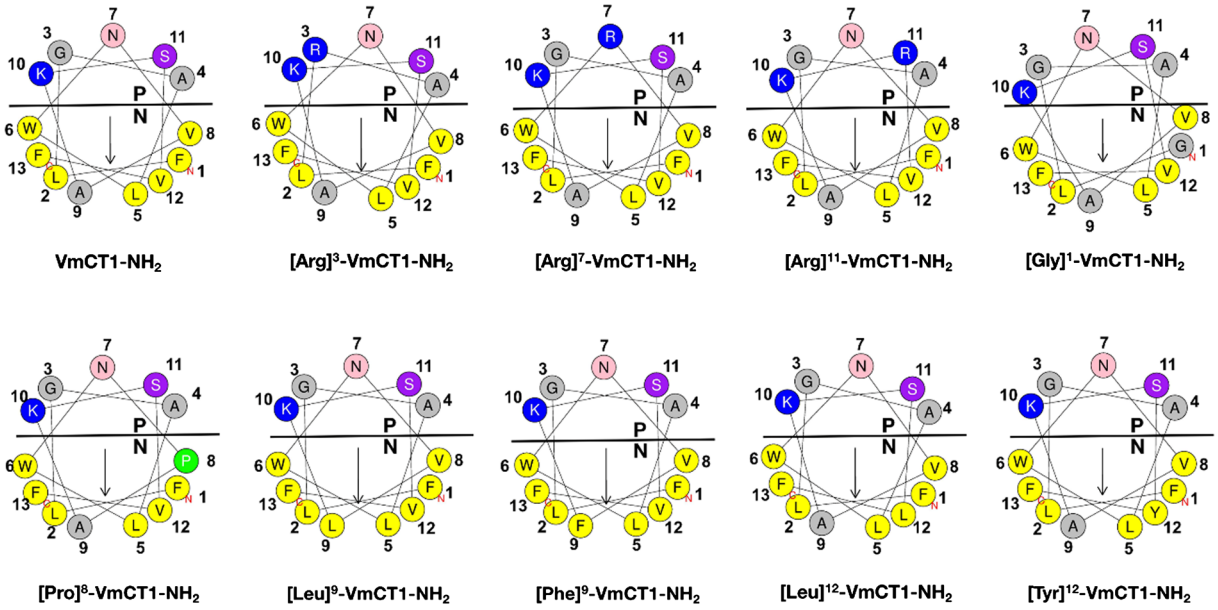

Fig. 2. Helical wheel projections of VmCT1 and its analogs. Yellow circles refer to aromatic and aliphatic hydrophobic residues; gray circles indicate residues with hydrophobicity close to zero; blue circles indicate basic positively charged residues; purple circles represent polar uncharged residues; red circles refer to acidic negatively charged amino acid residues; green circles represent the restricted pseudo amino acid proline; and pink circles represent polar uncharged amino acid residues. Black arrows indicate the direction and intensity of the hydrophobic moment [32]. P indicates hydrophilic face and N refers to the hydrophobic face of the amphipathic projection.

fluoromethyloxycarbonyl (Fmoc) strategy, detailed by Torres et al. [28].

The peptides purification assays were carried out by semi-preparative reverse-phase high-performance liquid chromatography (RP-HPLC) on a Delta Prep 600 (Waters Associates). Selected fractions containing the purified peptides were pooled and lyophilized. The purity was evaluated on an Alliance HPLC (Waters Associates system), and characterized by liquid-chromatography electrospray-ionization mass spectrometry (LC/ESI-MS) using a Model 6130 Infinity mass spectrometer coupled to a Model 1260 HPLC system (Agilent), according to Torres et al. [28].

2.2. Antimicrobial activity of VmCT1 and derivatives

The peptides were tested against *Escherichia coli* SBS 363, *Serratia marcescens* ATCC 4112, *Enterobacter cloacae* β-12, *Pseudomonas aeruginosa* ATCC 27853, *Micrococcus luteus* A270, *Staphylococcus aureus* ATCC 29213, *Bacillus megaterium* ATCC 10778, *Bacillus subtilis* ATCC 6633, *Candida albicans* MDM8, and *Candida tropicalis* IOC 4560. The microorganisms were obtained from the American Type Culture Collection (ATCC) and Instituto Butantan, São Paulo, Brazil.

Antimicrobial activity was determined by liquid growth inhibition assay, as previously described by Pedron et al. [17]. Briefly, it was used Peptone Broth and Potato Dextrose Broth (Invitrogen) for antibacterial and antifungal assays, respectively. Microorganisms were incubated with peptide two-fold serial dilutions (0.09–50 μmol L^{-1}) at 37 °C for 18 h. The microbial growth was assessed by measurements of the absorbance at 595 nm.

2.3. Hemolytic activity assays

Assays against freshly collected human erythrocytes were performed according to Pedron et al. [17]. Briefly, aliquots at a concentration of 0.1–100 μmol L^{-1} of the peptides were incubated with a suspension of erythrocytes. The surfactant SDS was used as positive control [29,30], and PBS buffer was used as the negative control. After that, the samples were incubated at room temperature for 1 h. The samples were centrifuged and the absorbance of supernatant was measured at 405 nm.

2.4. Circular dichroism (CD) spectroscopy

CD assays were performed by a Spectropolarimeter Jasco Mod. J-815 (JascoCorp.). CD spectra were obtained in Far-UV (195–260 nm), and analyzed in the following solutions: water, 2,2,2-trifluoroethanol (TFE) in water (3:2, v:v), palmitoyloleoylphosphatidylcholine (POPC – 10 mmol L^{-1}), and palmitoyloleoylphosphatidylcholine: palmitoyloleoylphosphatidylglycerol (POPC:POPG, 3:1, mol:mol, 10 mmol L^{-1}) at 50 μmol L^{-1}. Experiments conditions and detailed information are presented by Pedron et al. [17].

2.5. Stability assays

Stability assays were performed with GIBCO fetal bovine serum diluted to 25% in water according to Torres et al. [28]. Briefly, peptide solution was added to the serum solution and kept at 37 °C. The experiments were made in three independent replicates and aliquots were taken at 0, 0.5, 1, 2, 4 and 6 h. The degradation kinetics was monitored by liquid chromatography and the percentage of remaining peptide was calculated by integrating the peptide peak area.

3. Results and discussion

Microorganisms that are resistant to antibiotics are a global health problem, which can be associated with high morbidity and mortality rates [31]. The development of new alternatives to the treatment of these infections is crucial, and several authors recognized AMPs as a promising alternative [4].

We considered membrane content to choose VmCT1 as a template for structure-guided analyzes. VmCT1 is an AMP isolated from the venom of the *Vaejovis mexicanus smithi* scorpion, with reported antimicrobial activity against Gram-positive and Gram-negative bacteria [16]. Single-substituted VmCT1 analogs were designed (Fig. 2) to evaluate the effect of changes in physicochemical properties on antimicrobial and hemolytic activities, as well as resistance to degradation when exposed to serum peptidases.

Pedron et al. described VmCT1 analogs with single and double substitutions in the hydrophilic and hydrophobic portions of the amphipathic structure of VmCT1. The most active peptides were obtained by inserting a Lys residue in the hydrophobic face. CD measurements showed that Lys-substituted analogs presented higher helical content in TFE/water solution, zwitterionic (POPC 10 mmol L^{-1}) and negatively charged (POPC:POPG, 3:1, mol:mol, 10 mmol L^{-1}) lipid vesicles.

Furthermore, the authors described a study on the effect of Glu-substitutions along both hydrophobic and hydrophilic portion of VmCT1 original sequence. $[Glu]^4$-VmCT1-NH_2 analog was described as the less hemolytic peptide designed by them, confirming the importance of the helical structure of these peptides to hemolytic and antimicrobial activities. This behavior was not observed for $[Glu]^7$-VmCT1-NH_2, the peptide presented hemolytic activity values at the same range of concentrations of other toxic analogs. $[Glu]^7$-VmCT1-NH_2 presented antimicrobial activity was higher than $[Glu]^4$-VmCT1-NH_2; although, it was higher than the wild-type molecule.

Additionally, Pedron et al. observed the effect of hydrophobicity-related features evaluated through the substitution of residues from the original sequence by Trp residues. The Trp-substitution led to higher antimicrobial activities. The increase of hydrophobicity and hydrophobic moment generated by the substitution by a Trp residue led to higher antimicrobial activities compared to Glu-substituted analogs, and similar to MIC values observed for the wild-type molecule. $[Trp]^9$-VmCT1-NH_2 and $[Glu]^4[Trp]^9$-VmCT1-NH_2 analogs presented lower helical content in the presence of vesicles, however, their hemolytic activity was similar to the model molecule [17]. The authors also attributed the higher toxicity against erythrocytes to the higher hydrophobicity of Trp-substituted analogs compared to the wild-type.

In this study, the peptides were designed to improve the balance between antimicrobial activity and cytotoxicity. Supplementary Information Table 1 presents important considerations for the design of each one of the analogs synthesized in this work. The main physicochemical features of AMPs, such as net charge, hydrophobicity and amphipathicity were appraised for the design of the analogs.

We proposed to evaluate the effects of net positive charge by designing Arg-substituted analogs (Table 1). The modifications were planned according to the original helical structure of the model molecule at positions 3 (Gly), 7 (Asn), and 11 (Ser) in the hydrophilic face. The Arg residue was chosen as a result of its guanidyl group interactions with water molecules, dragging them inside the membrane and destabilizing the lipid bilayer. The guanidyl group also interacts with lipid head-groups by the formation of multiple hydrogen bonds that can lead to disruption and permeabilization of membranes [33].

The importance of net positive charge to peptide-membrane electrostatic interactions was evaluated by antimicrobial (Fig. 3) and hemolytic (Fig. 4) assays. $[Arg]^3$-VmCT1-NH_2, $[Arg]^7$-VmCT1-NH_2 and $[Arg]^{11}$-VmCT1-NH_2 analogs presented higher activity against *C. albicans* (1.6–3.1 μmol L^{-1}), *C. tropicalis* (1.6 μmol L^{-1}), *E. cloacae* (6.3–12.5 μmol L^{-1}), and *P. aeruginosa* (6.3–25 μmol L^{-1}) than the wild-type. Furthermore, $[Arg]^3$-VmCT1-NH_2 and $[Arg]^7$-VmCT1-NH_2 analogs were equipotent than VmCT1 against *S. marcescens* (0.4 μmol L^{-1}), *S. aureus* (1.6 μmol L^{-1}), *E. coli* (1.6 μmol L^{-1}), and *B. subtilis* (0.8 μmol L^{-1}). The exception was $[Arg]^{11}$-VmCT1-NH_2 against the Gram-positive bacteria: *M. luteus* (3.1 μmol L^{-1}), *B. megaterium* (3.1 μmol L^{-1}), *S. aureus* (3.1 μmol L^{-1}), and *B. subtilis* (1.6 μmol L^{-1}). The antimicrobial activity of Arg-substituted peptides against the Gram-negative bacteria *E. cloacae* and *P. aeruginosa* was of particular interest. The model molecule and the other analogs proposed in this work were not active against these bacteria that are part of ESKAPE list of bacteria (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.). The ESKAPE pathogens are a group of bacteria that are resistant to a diversity of antibiotics and responsible for relevant nosocomial infections [34,35]. These results point the net positive charge of this family of peptides coupled to physicochemical properties of the Arg guanidyl side chain group as the most relevant properties for the generation of VmCT1 analogs that can be use as therapeutics against Gram-negative ESKAPE pathogens. Supplementary Information Table 2 lists the antibiotic-resistance profile for the bacterium genera used by us. Despite the higher antimicrobial activities, the Arg-substituted peptides also exhibited high hemolytic activity (1.6, 3.1, and 3.1 μmol L^{-1} for $[Arg]^3$-VmCT1-NH_2, $[Arg]^7$-VmCT1-NH_2, and $[Arg]^{11}$-VmCT1-NH_2, respectively) (Supplementary Information Table 3). These results corroborate with the ones reported by Pedron *et al.* [17], demonstrating that the introduction of one positive charge unit to VmCT1 original sequence was important to favor not only the interaction with microbial membrane but with mammalian membranes as well.

Arg-substitutions also led to peptides with lower helical content (Table 2) when compared to the Lys-substituted analogs described previously [17]. Arg-substituted analogs presented lower helical content in the presence of zwitterionic vesicles (Fig. 5 and Table 2) and helical inducer medium compared to VmCT1. However, two of the analogs, $[Arg]^3$-VmCT1-NH_2 and $[Arg]^7$-VmCT1-NH_2, presented higher helical content than the wild-type molecule when exposed to negatively charged vesicles (Fig. 5 and Table 2). The same behavior was not observed for the $[Arg]^{11}$-VmCT1-NH_2 analog, probably due to the intramolecular stabilization effect of the hydroxyl group from the side chain of the Ser residue that was suppressed by the Arg residue substitution.

Table 1
Peptides sequence, molecular characterization and physiochemical properties.

Peptide	Sequence	Molecular Weight (Da)		H	μH	θ	z
		Theoretical	Observed				
VmCT1	Phe-Leu-Gly-Ala-Leu-Tep-Asn-Val-Ala-Lys-Ser-Val-Phe-NH_2	1450.7	1450.9	0.82	0.58	83.3	+2
$[Arg]^3$-VmCT1-NH_2	Phe-Leu-**Arg**-Ala-Leu-Trp-Asn-Val-Ala-Lys-Ser-Val-Phe-NH_2	1549.9	1550.8	0.65	0.65	73.0	+3
$[Arg]^7$-VmCT1-NH_2	Phe-Leu-Gly-Ala-Leu-Trp-**Arg**-Val-Ala-Lys-Ser-Val-Phe-NH_2	1492.8	1492.9	0.71	0.60	81.0	+3
$[Arg]^{11}$-VmCT1-NH_2	Phe-Leu-Gly-Ala-Leu-Trp-Asn-Val-Ala-Lys-**Arg**-Val-Phe-NH_2	1519.8	1520.9	0.66	0.62	85.9	+3
$[Gly]^1$-VmCT1-NH_2	**Gly**-Leu-Gly-Ala-Leu-Trp-Asn-Val-Ala-Lys-Ser-Val-Phe-NH_2	1359.8	1360.8	0.59	0.48	101.4	+2
$[Pro]^8$-VmCT1-NH_2	Phe-Leu-Gly-Ala-Leu-Trp-Asn-**Pro**-Ala-Lys-Ser-Val-Phe-NH_2	1447.8	1449.8	0.70	0.55	89.0	+2
$[Leu]^9$-VmCT1-NH_2	Phe-Leu-Gly-Ala-Leu-Trp-Asn-Val-**Leu**-Lys-Ser-Val-Phe-NH_2	1492.8	1492.3	0.85	0.67	82.7	+2
$[Phe]^9$-VmCT1-NH_2	Phe-Leu-Gly-Ala-Leu-Trp-Asn-Val-**Phe**-Lys-Ser-Val-Phe-NH_2	1526.8	1527.0	0.86	0.67	82.4	+2
$[Leu]^{12}$-VmCT1-NH_2	Phe-Leu-Gly-Ala-Leu-Trp-Asn-Val-Ala-Lys-Ser-**Leu**-Phe-NH_2	1464.7	1465.3	0.78	0.61	75.0	+2
$[Tyr]^{12}$-VmCT1-NH_2	Phe-Leu-Gly-Ala-Leu-Trp-Asn-Val-Ala-Lys-Ser-**Tyr**-Phe-NH_2	1513.8	1515.8	0.72	0.55	70.7	+2

H (hydrophobicity), μ_H (hydrophobic moment), θ (polar angle°), z (net charge) according to Heliquest helical wheel projection [32]. Mass obtained under the following conditions: Phenomenex Gemini C18 column (2.0 mm × 150 mm, 3.0 μm particles, 110 Å pores). Solvent A was 0.1% TFA in water, and solvent B was 90% ACN in solvent A. Elution with a 5–95% B gradient was performed over 20 min, 0.2 mL min^{-1} flow and peptides were detected at 220 nm. Mass measurements were performed in a positive mode with the following conditions: mass range between 100 and 2500 *m/z*, ion energy of 5.0 V, nitrogen gas flow of 12.0 L min^{-1}, solvent heater of 250 °C, multiplier of 1.0, capillary of 3.0 kV and cone voltage of 35 V.

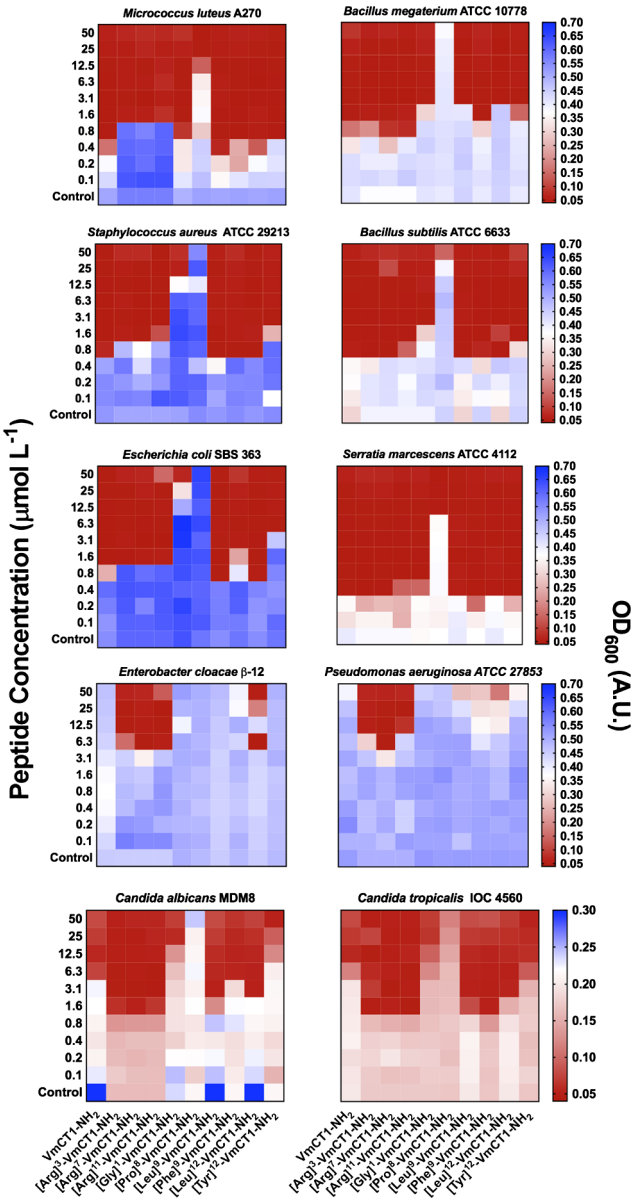

Fig. 3. Antimicrobial activities of VmCT1 and its analogs against fungi, Gram-positive and Gram-negative bacteria. Experiments were made in three independent replicates. Absorbance values in red represent maximal inhibition of microbial growth. Absorbance values related to microbial growth are presented in blue.

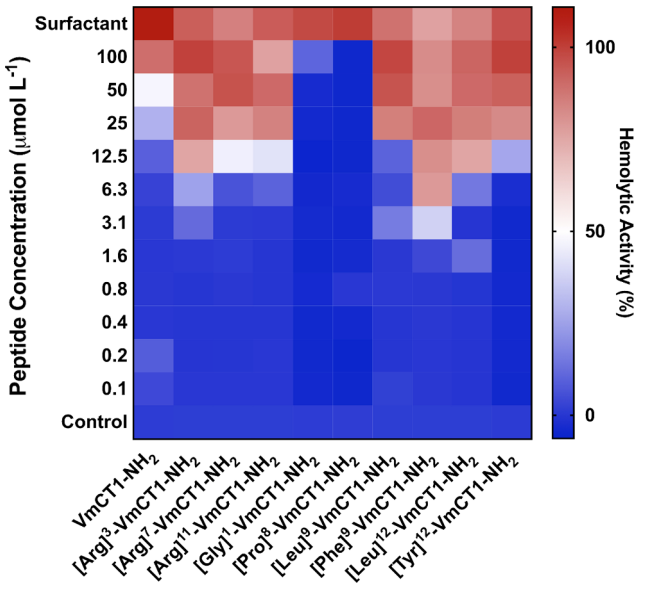

Fig. 4. Hemolytic activities of VmCT1 and analogs against human red blood cells in different peptide concentrations (0.1–100 μmol L^{-1}), in PBS at room temperature for 1 h. Experiments were performed in three independent replicates. Surfactant (1% SDS in PBS) was used to ensure complete hemolysis and PBS was used as control preserving erythrocyte integrity. Absorbance values in red represent the hemolytic activity. Absorbance values related to non-hemolytic activity are presented in blue.

Table 2
Helical fraction of the wild-type and analogs in four different media calculated used Lifson-Roig helix-coil theory.[55]

Peptide	Helical fraction (f_H)			
	Water	60% TFE/Water	POPC	POPC:POPG
VmCT1	0.05	0.30	0.42	0.25
$[Arg]^3$-VmCT1-NH_2	0	0.23	0	0.35
$[Arg]^7$-VmCT1-NH_2	0	0.22	0.03	0.43
$[Arg]^{11}$-VmCT1-NH_2	0.02	0.25	0.03	0.19
$[Gly]^1$-VmCT1-NH_2	0.02	0.37	0.08	0.27
$[Pro]^8$-VmCT1-NH_2	0.02	0.12	0.05	0.08
$[Leu]^9$-VmCT1-NH_2	0.03	0.62	0.31	0.04
$[Phe]^9$-VmCT1-NH_2	0.01	0.32	0.28	0.30
$[Leu]^{12}$-VmCT1-NH_2	0.02	0.23	0.05	0.26
$[Tyr]^{12}$-VmCT1-NH_2	0.02	0.24	0.28	0.88

The Gly residue was chosen for substituting Phe in position 1 of VmCT1 original sequence in the hydrophobic face because of its tendency to increase the flexibility of helical structures, besides slightly altering the helical propensity of peptides [24]. This substitution led to decreased hydrophobicity and hydrophobic moment values (Table 1) that might be responsible for $[Gly]^1$-VmCT1-NH_2 lower helical content in the presence of zwitterionic vesicles compared to VmCT1 (f_H: 0.08 and 0.42, respectively, Fig. 5 and Table 2). The substitution by Gly at the *N*-terminal extremity of AMPs is well-known for increasing availability of a formal positive charged leading to higher interactions in negatively charged molecules, such as phosphate groups from biomembrane phospholipids [18,24]. Contrarily, the helical content values of the Gly-substituted analog were similar to the wild-type in the presence of helical inducer medium (f_H: 0.37 and 0.30, respectively) and negatively charged vesicles (f_H: 0.27 and 0.25). This effect is usually observed for AMPs with the Gly residue in the first position of the sequence. As the α-amino group net positive charge is more exposed to intermolecular interactions. As a result of the higher helical tendency in negatively charged and hydrophobic environments, such as biomembranes, which contain phospholipids with polar head group and aliphatic tail. Gly residue is the most common first residue of AMP sequences [24], as examples we highlight AMPs such as anoplin [36], aurein [37] and StCT2 [38].

$[Gly]^1$-VmCT1-NH_2 exhibited activity against fungus *C. albicans* and *C. tropicalis* at 25 μmol L^{-1}, and antimicrobial activity ranging from 1.56 to 3.12 μmol L^{-1} against the Gram-positive bacteria *M. luteus*, *B. subtilis* and *B. megaterium*, except against *S. aureus*, where its MIC value was 25 μmol L^{-1}. However, against the Gram-negative bacteria *E. cloacae* and *P. aeruginosa*, the Gly-substituted analog was not active within the concentration range tested, and its MIC values against *S. marcescens* and *E. coli* were 0.8 μmol L^{-1} and 50 μmol L^{-1}, respectively (Supplementary Information Table 3). $[Gly]^1$-VmCT1-NH_2 was not hemolytic in the concentration range assessed. The low toxicity against erythrocytes was most likely correlated with the decreased hydrophobicity, which is tied to peptides affinity for the erythrocyte membrane [39,40].

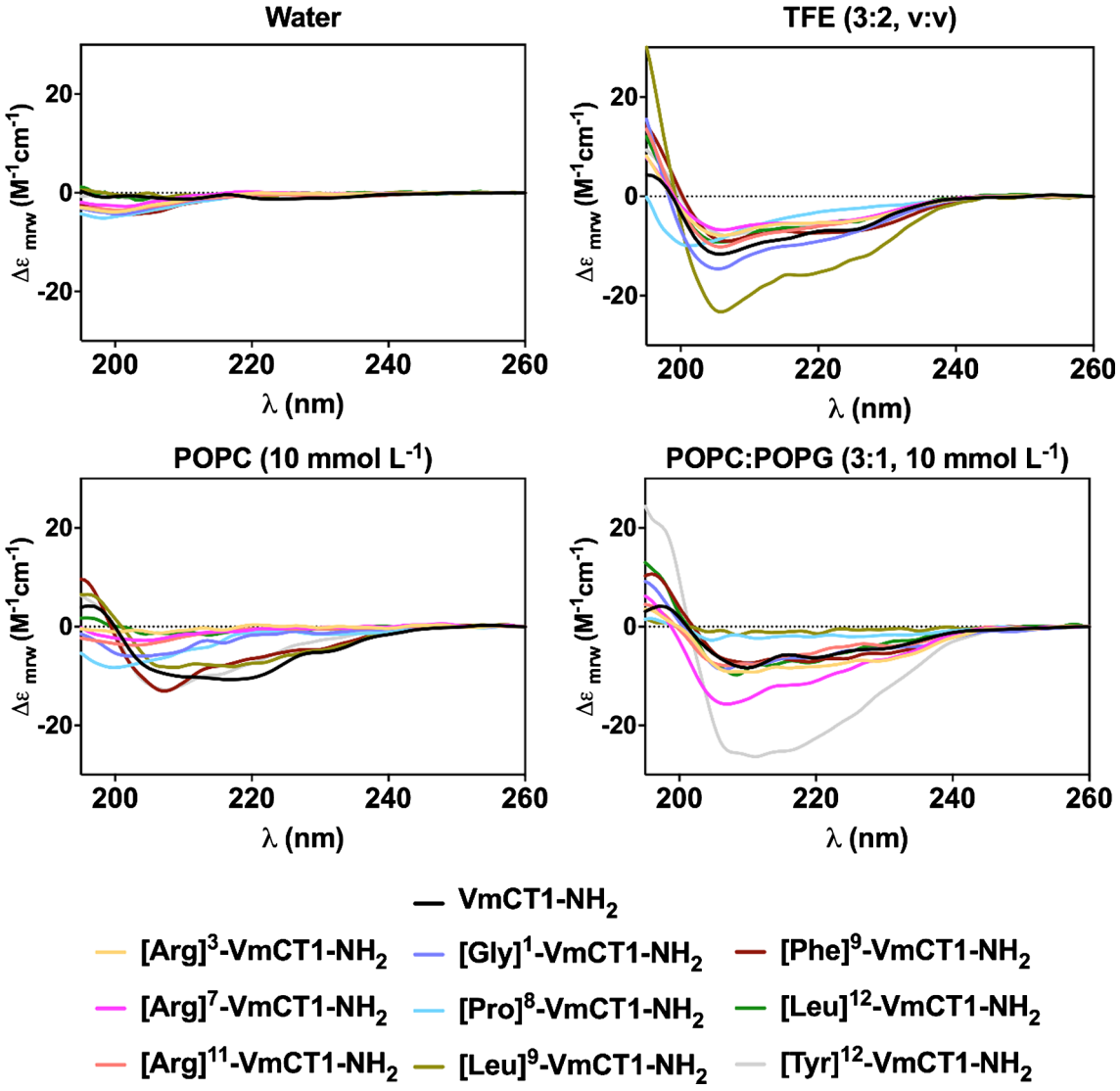

Fig. 5. Circular dichroism spectra of the peptides in water, TFE/Water (3:2, v:v), POPC (10 mmol L^{-1}) and POPC:POPG (3:1, mol:mol – 10 mmol L^{-1}). CD were recorded after four accumulations at 20 °C, using a 1 mm path length quartz cell, between 195 and 260 nm at 100 nm min^{-1}, with a band width of 0.5 nm. Peptide concentration at 50 μmol L^{-1}.

The substitution of the Val residue at position 8 by a Pro residue, led to a peptide with low hemolytic activity (MHC > 100 μmol L^{-1} – Fig. 4). This position was chosen based on results obtained previously by a Pro residue substitution in the scorpion venom AMP IsCT that presents high homology to VmCT1. The insertion of a Pro residue in the middle of the original sequence of IsCT, led to maintenance of antimicrobial activity and lower hemolytic activity, despite lower helical content [41]. The Pro-substituted VmCT1 analog presented decreased hydrophobicity compared to the model molecule (Table 1). The Pro amino acid is a well-known structure disruptor that leads to the distortion of the helical conformation, because of the presence of its pyrrolidine side chain group, which is responsible for the lack of hydrogen bond forming proton in addition to the rigid five-membered ring [42,43]. This effect was confirmed by the lower helical fraction observed for this analog in all media studied here. The lower helical content of the $[Pro]^8$-VmCT1-NH_2 analog affected directly its antimicrobial activity at the concentration range tested, exposing the direct correlation of this peptide family's antimicrobial activity with its helical content (Supplementary Information Table 3).

Except for $[Pro]^8$-VmCT1-NH_2 and $[Gly]^1$-VmCT1-NH_2 analogs, all the other peptides presented high hemolytic activity (ranging from 0.8 to 6.3 μmol L^{-1}), similar to the wild-type molecule. This outcome is closely related to the strategy adopted for designing the peptides, where the physicochemical features were tuned to favor peptides interactions with negatively charged membranes [28].

Pedron *et al.* also substituted the Alanine residue at position 9 by a Trp residue [17] to increase the mean hydrophobicity of the analogs compared to the model peptide. This change led to increased antimicrobial activity. However, the Trp-substituted peptide presented higher hemolytic activity, which was an expected behavior as Trp-containing peptides present intrinsic toxicity [28,44]. The Trp residue plays an important role in the hydrophobic interactions with the acyl chains of phospholipids, destabilizing the lipid bilayers [45].

As the helical content of the peptides proved to be important for VmCT1 analogs activity, the Trp residue at position 9 reported previously by Pedron et al. was substituted by a Phe residue. The Phe residue was chosen because of its similar helical penalty values compared to Trp (0.54 and 0.49 kcal mol^{-1}, respectively) [46] and the preservation of the hydrophobic side chain which leads to stronger interactions with the lipid portion of the membrane.

The Phe-substitution led to slightly increased mean hydrophobicity and mean hydrophobic moment values compared to the model

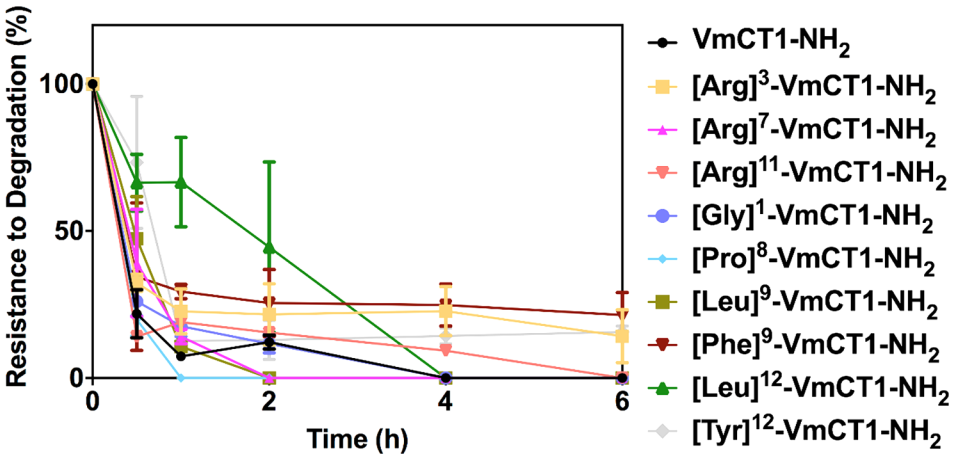

Fig. 6. Resistance to degradation of the wild-type and designer analogs in fetal bovine serum for 6 h. Experiments were performed in three independent replicates.

molecule (Table 1). [Phe]^{9}-VmCT1-NH_2 exhibited similar helical content than the wild-type in TFE/water solution (f_H: 0.32 and 0.30, respectively – Fig. 5 and Table 2), and in negatively charged vesicles (f_H: 0.30 and 0.25, respectively). While it presented lower helical content than VmCT1 in the presence of zwitterionic vesicles (f_H: 0.28 and 0.42, respectively). The lower helical content was expected once the original Ala residue at this position is described as a well-known helical stabilizer. [Phe]^{9}-VmCT1-NH_2 analog showed similar antimicrobial activity compared to the wild-type against *M. luteus* (0.8 μmol L^{-1}) and *S. marcescens* (0.4 μmol L^{-1}). However, the Phe-substituted analog presented higher antimicrobial activity against *C. tropicalis* (1.6 μmol L^{-1} and 12.5 μmol L^{-1}) and *S. aureus* (0.8 μmol L^{-1} and 1.6 μmol L^{-1}) compared to the wild-type (Supplementary Information Table 3). Although its hemolytic activity was higher (0.8 μmol L^{-1}) than the model molecule (6.3 μmol L^{-1}), it is likely related to the higher mean hydrophobicity values caused by the phenyl side chain group insertion, causing higher number of hydrophobic interactions with vesicles composed by zwitterionic lipids that mimic erythrocytes membranes [47]. The hemolytic activity was comparable to the Trp-substituted analog previously reported by Pedron et al. [17]

In order to compare the effects of different hydrophobicity at position 9, we designed a Leu-substituted analog that would not impair peptide helical tendency, and at the same time, increase mean hydrophobicity and mean hydrophobic moment values (Table 1). Leu was chosen over Ile as its symmetric aliphatic side chain group is deeply inserted into the acyl chain region of the lipids. Additionally, Leu residues are recognized as strong helix formers [48], what was proved by structure analyses in helical inducer medium where the Leu-substituted analog showed higher helical content than the wild-type (f_H: 0.62 and 0.30, respectively – Fig. 5 and Table 2). However, in the presence of both vesicle formulations, the helical content values were lower than the VmCT1 (zwitterionic vesicle f_H: 0.31 and 0.42, and negatively charged vesicle f_H: 0.04 and 0.25, respectively) (Supplementary Information Table 3).

The second Leu-substitution was proposed at position 12, replacing the original Val residue. This modification led to a decreased mean hydrophobicity value (Table 1), and lower helical contents in helical inducer medium (f_H: 0.23 and 0.30, Fig. 5 and Table 2) and zwitterionic vesicles (f_H: 0.05 and 0.42) if compared to VmCT1 (0.30 and 0.42, respectively). Although, [Leu]^{12}-VmCT1-NH_2 presented equivalent helical content in the presence of negatively charged vesicles compared to VmCT1 (f_H: 0.26 and 0.25, respectively). The antimicrobial activities of both leucine-substituted analogs were similar to the activity of the wild-type molecule. The analogs presented higher activity against *C. albicans* (3.1 μmol L^{-1}), *C. tropicalis* (3.1 μmol L^{-1}), *E. coli* (0.8 μmol L^{-1}) and *S. aureus* (0.8 μmol L^{-1}), and equipotent activity against *M. luteus* (0.8 μmol L^{-1}) and *S. marcescens* (0.4 μmol L^{-1}) (Supplementary Information Table 3).

The Val residue at position 12 was also substituted by a Tyr residue. This substitution was designed to generate an analog with decreased mean hydrophobicity compared to VmCT1 (Table 1). The Tyr residue was chosen based on its aromatic side chain with the presence of a hydroxyl group. The Tyr residue presents similar helical propensity to Trp and Phe residues [46], but its side chain phenol group has a greater affinity for membrane polar/nonpolar interfaces than Phe and Trp side chains [49]. [Tyr]^{12}-VmCT1-NH_2 analog presented the lower hemolytic activity (6.3 μmol L^{-1}) among the aromatic-substituted analogs proposed here and also the ones described by Pedron et al. [17]. Its antimicrobial activity was equipotent to VmCT1 against *M. luteus* (0.8 μmol L^{-1}), *S. marcescens* (0.4 μmol L^{-1}) and *C. tropicalis* (12.5 μmol L^{-1}). However, the Tyr-substituted analog presented the higher helical content in the presence negatively charged vesicles (f_H: 0.88) compared to the wild-type. Although, [Tyr]^{12}-VmCT1-NH_2 presented lower helical content compared to the model molecule in the presence of zwitterionic vesicles (f_H:0.28 and 0.42) and helical inducer medium (f_H:0.24 and 0.30) (Supplementary Information Table 3).

Peptides resistance to degradation was assessed by adding the designed peptides and the template molecule to a fetal bovine serum solution from 0 to 6 h (Fig. 6). Essentially, VmCT1 and analogs were not stable in the presence of serum proteases, common behavior of natural small linear peptides [50,51]. [Tyr]^{12}-VmCT1-NH_2, [Leu]^{9}-VmCT1-NH_2, and [Leu]^{12}-VmCT1-NH_2 analogs were the most resistant peptides, with more than 50% of remaining peptide after 30 min of exposure. However, less than 20% of the peptides remained after 1 h of the start of the experiment (Supplementary Information Table 3). Cyclization [52,53], modification of the terminal regions [37], replacement of $_L$-amino acids for $_D$-amino acids [54] and utilization of unusual amino acids [51] are the most common approaches to overcome serum proteases activity, and can be coupled to our rational design methodology for the design of future protease-resistant VmCT1 analogs.

As well as for other well-known AMPs, the helical content is not the only feature closely related to their antimicrobial activity. For example, Decoralin analogs with higher net positive charge and hydrophobicity presented higher activity when compared to the wild-type. However, Decoralin most active analogs besides of increased net charge and hydrophobicity presented higher helical content than the wild-type, showing that the antimicrobial activity depends on the combination of the main physicochemical properties of AMPs [28].

We showed that by means of a structure-activity relationship study, it is possible to identify important physicochemical features or combinations of features that might help to turn cytotoxic AMPs into selective peptides. We envision that the principles and approaches exploited here

can be applied to other structure-activity studies in order to rationalize and better understand the role of physicochemical features and which approaches fit better to each family of peptides.

4. Conclusions

VmCT1 analogs were designed and synthesized to verify the effect of changes in physicochemical parameters such as net positive charge, mean hydrophobicity, and mean hydrophobic moment on the antimicrobial and hemolytic activities.

The increase of the net positive charge from +2 to +3 by introducing Arg residues in the hydrophilic face of VmCT1 resulted in higher antimicrobial activity values compared to the wild-type. Arg-substituted analogs showed activity against Gram-negative bacteria from the ESKAPE pathogens list, while VmCT1 and other analogs were not active at the concentration range tested.

[Gly]^{1}-VmCT1-NH_2 analog was less cytotoxic against human erythrocytes and it preserved the antimicrobial activity of the wild-type for most of the strains tested in this study, except against *E. coli*, *E. cloacae*, and *P. aeruginosa*. [Pro]^{8}-VmCT1-NH_2 analog was not active in the concentrations range tested against most of the microorganisms. However, it presented the lower hemolytic activity, showing that the Pro residue helical destabilization effect was important to confirm that the antimicrobial and hemolytic activities of VmCT1 and analogs is closely related to its secondary structure.

The replacement of Ala (position 9) and Val (position 12) by aliphatic and hydrophobic amino acid residues showed that the Leu-substituted analogs presented similar antimicrobial and hemolytic activities compared to the model molecule. Although, Phe-substituted peptides were more hemolytic activity than other analogs and maintained the antimicrobial activity, most likely because of the favored hydrophobic interactions of the side chains aromatic rings with erythrocyte membranes. The Tyr-substituted analogs were not as hemolytic as the Phe-substituted derivatives.

The approaches used here are useful tools for designing and predicting small cationic AMPs biological activities for the generation of synthetic peptides candidates to antimicrobial therapeutics. Some of the peptides designed in this work outranked the activity of the model molecule and presented activity at low concentrations against pathogenic bacteria that are responsible for serious nosocomial infections, pointing us to the direction of new analogs that might be used as versatile antibiotics.

Declaration of Competing Interest

The authors declare that they do not have conflict of interest.

Acknowledgements

We thank Profa. Dra. Katia Regina Perez for the donation of the vesicles, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (Finance Code 001 – CAPES) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for funding (VXO #2017/03046-2 and MDTT #2014/04507-5) and Multiuser Central Facilities (UFABC) for the experimental support.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103038.

References

[1] C. de la Fuente-Nunez, M.D. Torres, F.J. Mojica, T.K. Lu, Next-generation precision antimicrobials: towards personalized treatment of infectious diseases, Curr. Opin. Microbiol. 37 (2017) 95–102, https://doi.org/10.1016/j.mib.2017.05.014.

[2] O.G. Travkova, H. Moehwald, G. Brezesinski, The interaction of antimicrobial peptides with membranes, Adv. Colloid Interface Sci. 247 (2017) 521–532, https://doi.org/10.1016/j.cis.2017.06.001.

[3] M. Pushpanathan, P. Gunasekaran, J. Rajendhran, Antimicrobial peptides: Versatile biological properties, Int. J. Pept. 2013 (2013), https://doi.org/10.1155/2013/675391.

[4] J. Cruz, C. Ortiz, F. Guzmán, R. Fernández-Lafuente, R. Torres, Antimicrobial peptides: promising compounds against pathogenic microorganisms, Curr. Med. Chem. 21 (2014) 2299–2321, https://doi.org/10.2174/0929867321666140217110155.

[5] M. Mahlapuu, J. Håkansson, L. Ringstad, C. Björn, Antimicrobial peptides: an emerging category of therapeutic agents, Front. Cell. Infect. Microbiol. 6 (2016) 1–12, https://doi.org/10.3389/fcimb.2016.00194.

[6] A. De Breij, M. Riool, R.A. Cordfunke, N. Malanovic, L. De Boer, R.I. Koning, E. Ravensbergen, M. Franken, T. Van Der Heijde, B.K. Boekema, P.H.S. Kwakman, N. Kamp, A. El Ghalbzouri, K. Lohner, S.A.J. Zaat, J.W. Drijfhout, P.H. Nibbering, The antimicrobial peptide SAAP-148 combats drug-resistant bacteria and biofilms, Sci. Transl. Med. 10 (2018), https://doi.org/10.1126/scitranslmed.aan4044.

[7] B. Durnaœ, U. Wnorowska, K. Pogoda, P. Deptuła, M. Watek, E. Piktel, S. Głuszek, X. Gu, P.B. Savage, K. Niemirowicz, R. Bucki, Candidacidal activity of selected ceragenins and human cathelicidin LL-37 in experimental settings mimicking infection sites, PLoS One 11 (2016) 1–21, https://doi.org/10.1371/journal.pone.0157242.

[8] J.M.C. Monteiro, M.D. Oliveira, R.S. Dias, L. Nacif-Marçal, R.N. Feio, S.O. Ferreira, L.L. Oliveira, C.C. Silva, S.O. Paula, The antimicrobial peptide HS-1 inhibits dengue virus infection, Virology 514 (2018) 79–87, https://doi.org/10.1016/j.virol.2017.11.009.

[9] J.F.C. Vinhote, D.B. Lima, R.R.P.P.B. de Menezes, C.P. Mello, B.M. de Souza, A. Havt, M.S. Palma, R.P. dos Santos, E.L. de Albuquerque, V.N. Freire, A.M.C. Martins, Trypanocidal activity of mastoparan from Polybia paulista wasp venom by interaction with TcGAPDH, Toxicon 137 (2017) 168–172, https://doi.org/10.1016/j.toxicon.2017.08.002.

[10] I.A. Edwards, A.G. Elliott, A.M. Kavanagh, J. Zuegg, M.A.T. Blaskovich, M.A. Cooper, Contribution of amphipathicity and hydrophobicity to the antimicrobial activity and cytotoxicity of β-hairpin peptides, ACS Infect. Dis. 2 (2016) 442–450, https://doi.org/10.1021/acsinfecdis.6b00045.

[11] V. Teixeira, M.J. Feio, M. Bastos, Role of lipids in the interaction of antimicrobial peptides with membranes, Prog. Lipid Res. 51 (2012) 149–177, https://doi.org/10.1016/j.plipres.2011.12.005.

[12] R.M. Epand, R.F. Epand, Domains in bacterial membranes and the action of antimicrobial agents, Mol. Biosyst. 5 (2009) 580–587, https://doi.org/10.1039/b900278m.

[13] M. Rautenbach, A.M. Troskie, J.A. Vosloo, Antifungal peptides: to be or not to be membrane active, Biochimie 130 (2016) 132–145, https://doi.org/10.1016/j.biochi.2016.05.013.

[14] A. Almaaytah, Q. Albalas, Scorpion venom peptides with no disulfide bridges: a review, Peptides 51 (2014) 35–45, https://doi.org/10.1016/j.peptides.2013.10.021.

[15] P.L. Harrison, M.A. Abdel-Rahman, K. Miller, P.N. Strong, Antimicrobial peptides from scorpion venoms, Toxicon 88 (2014) 115–137, https://doi.org/10.1016/j.toxicon.2014.06.006.

[16] S. Ramírez-Carreto, V. Quintero-Hernández, J.M. Jiménez-Vargas, G. Corzo, L.D. Possani, B. Becerril, E. Ortiz, Gene cloning and functional characterization of four novel antimicrobial-like peptides from scorpions of the family Vaejovidae, Peptides 34 (2012) 290–295, https://doi.org/10.1016/j.peptides.2012.02.002.

[17] C.N. Pedron, M.D.T. Torres, J.A. da S. Lima, P.I. Silva, F.D. Silva, V.X. Oliveira, Novel designed VmCT1 analogs with increased antimicrobial activity, Eur. J. Med. Chem. 126 (2017) 456–463, https://doi.org/10.1016/j.ejmech.2016.11.040.

[18] M.D.T. Torres, C.N. Pedron, Y. Higashikuni, R.M. Kramer, M.H. Cardoso, K.G.N. Oshiro, O.L. Franco, P.I. Silva, F.D. Silva Junior, V.X. Oliveira Junior, T.K. Lu, C. de la Fuente-Nunez, Structure-function-guided exploration of the antimicrobial peptide polybia-CP identifies activity determinants and generates synthetic therapeutic candidates, Commun. Biol. 1 (2018) 221, https://doi.org/10.1038/s42003-018-0224-2.

[19] M.D.T. Torres, S. Sothiselvam, T.K. Lu, C. de la Fuente-Nunez, Peptide design principles for antimicrobial applications, J. Mol. Biol. (2019), https://doi.org/10.1016/j.jmb.2018.12.015.

[20] M.R. Yeaman, Mechanisms of antimicrobial peptide action and resistance, Pharmacol. Rev. 55 (2003) 27–55, https://doi.org/10.1124/pr.55.1.2.

[21] L. Dai, A. Yasuda, H. Naoki, G. Corzo, M. Andriantsiferana, T. Nakajima, IsCT, a novel cytotoxic linear peptide from scorpion Opisthacanthus madagascariensis, Biochem. Biophys. Res. Commun. 286 (2001) 820–825, https://doi.org/10.1006/bbrc.2001.5472.

[22] R. Yan, Z. Zhao, Y. He, L. Wu, D. Cai, W. Hong, Y. Wu, Z. Cao, C. Zheng, W. Li, A new natural α-helical peptide from the venom of the scorpion Heterometrus petersii kills HCV, Peptides 32 (2011) 11–19, https://doi.org/10.1016/j.peptides.2010.10.008.

[23] X.C. Zeng, L. Zhou, W. Shi, X. Luo, L. Zhang, Y. Nie, J. Wang, S. Wu, B. Cao, H. Cao, Three new antimicrobial peptides from the scorpion Pandinus imperator, Peptides 45 (2013) 28–34, https://doi.org/10.1016/j.peptides.2013.03.026.

[24] I. Zelezetsky, A. Tossi, Alpha-helical antimicrobial peptides-Using a sequence template to guide structure-activity relationship studies, Biochim. Biophys. Acta - Biomembr. 1758 (2006) 1436–1449, https://doi.org/10.1016/j.bbamem.2006.03.021.

[25] L. Cao, C. Dai, Z. Li, Z. Fan, Y. Song, Y. Wu, Z. Cao, W. Li, Antibacterial activity and mechanism of a Scorpion venom peptide derivative in vitro and in vivo, PLoS One 7

(2012), https://doi.org/10.1371/journal.pone.0040135.
[26] X.C. Zeng, W.X. Li, S.X. Wang, S.Y. Zhu, F. Luo, Precursor of a novel scorpion venom peptide (BmKn1) with no disulfide bridge from Buthus martensii Karsch, IUBMB Life 51 (2001) 117–120, https://doi.org/10.1080/15216540152122120.
[27] B. Gao, P. Sherman, L. Luo, J. Bowie, S. Zhu, Structural and functional characterization of two genetically related meucin peptides highlights evolutionary divergence and convergence in antimicrobial peptides, FASEB J. 23 (2009) 1230–1245, https://doi.org/10.1096/fj.08-122317.
[28] M.D.T. Torres, C.N. Pedron, I. Araújo, P.I. Silva, F.D. Silva, V.X. Oliveira, Decoralin analogs with increased resistance to degradation and lower hemolytic activity, ChemistrySelect. 2 (2017) 18–23, https://doi.org/10.1002/slct.201601590.
[29] S. Shalel, S. Streichman, A. Marmur, The mechanism of hemolysis by surfactants: effect of solution composition, J. Colloid Interface Sci. 252 (2002) 66–76, https://doi.org/10.1006/jcis.2002.8474.
[30] L.H. Love, The hemolysis of human erythrocytes by sodium dodecyl sulfate, J. Cell. Comp. Physiol. 36 (2005) 133–148, https://doi.org/10.1002/jcp.1030360202.
[31] M. Frieri, K. Kumar, A. Boutin, Antibiotic resistance, J. Infect. Public Health 10 (2017) 369–378, https://doi.org/10.1016/j.jiph.2016.08.007.
[32] R. Gautier, D. Douguet, B. Antonny, G. Drin, HELIQUEST: a web server to screen sequences with specific α-helical properties, Bioinformatics 24 (2008) 2101–2102, https://doi.org/10.1093/bioinformatics/btn392.
[33] L. Li, I. Vorobyov, T.W. Allen, The different interactions of lysine and arginine side chains with lipid membranes, J. Phys. Chem. B 117 (2013) 11906–11920, https://doi.org/10.1021/jp405418y.
[34] Q. Lin, B. Deslouches, R.C. Montelaro, Y.P. Di, Prevention of ESKAPE pathogen biofilm formation by antimicrobial peptides WLBU2 and LL37, Int. J. Antimicrob. Agents (2018) 10–15, https://doi.org/10.1016/j.ijantimicag.2018.04.019.
[35] S. Hijazi, D. Visaggio, M. Pirolo, E. Frangipani, L. Bernstein, P. Visca, Antimicrobial activity of gallium compounds on ESKAPE pathogens, Front. Cell. Infect. Microbiol. 8 (2018) 1–11, https://doi.org/10.3389/fcimb.2018.00316.
[36] K. Konno, M. Hisada, R. Fontana, C.C.B. Lorenzi, H. Naoki, Y. Itagaki, A. Miwa, N. Kawai, Y. Nakata, T. Yasuhara, J. Ruggiero Neto, W.F. De Azevedo, M.S. Palma, T. Nakajima, Anoplin, a novel antimicrobial peptide from the venom of the solitary wasp Anoplius samariensis, Biochim. Biophys. Acta - Protein Struct. Mol. Enzymol. 1550 (2001) 70–80, https://doi.org/10.1016/S0167-4838(01)00271-0.
[37] T. Rozek, K.L. Wegener, J.H. Bowie, I.N. Olver, J.A. Carver, J.C. Wallace, M.J. Tyler, The antibiotic and anticancer active aurein peptides from the Australian Bell Frogs Litoria aurea and Litoria raniformis: the solution structure of aurein 1.2, Eur. J. Biochem. 267 (2000) 5330–5341, https://doi.org/10.1046/j.1432-1327.2000.01536.x.
[38] L. Cao, Z. Li, R. Zhang, Y. Wu, W. Li, Z. Cao, StCT2, a new antibacterial peptide characterized from the venom of the scorpion Scorpiops tibetanus, Peptides 36 (2012) 213–220, https://doi.org/10.1016/j.peptides.2012.04.010.
[39] Y. Chen, M.T. Guarnieri, A.I. Vasil, M.L. Vasil, C.T. Mant, R.S. Hodges, Role of peptide hydrophobicity in the mechanism of action of α-helical antimicrobial peptides, Antimicrob. Agents Chemother. 51 (2007) 1398–1406, https://doi.org/10.1128/AAC.00925-06.
[40] A. Hollmann, M. Martínez, M.E. Noguera, M.T. Augusto, A. Disalvo, N.C. Santos, L. Semorile, P.C. Maffía, Role of amphipathicity and hydrophobicity in the balance between hemolysis and peptide-membrane interactions of three related antimicrobial peptides, Colloids Surf. B Biointerfaces 141 (2016) 528–536, https://doi.org/10.1016/j.colsurfb.2016.02.003.
[41] S.L. Shin, Y. Kim, Y. Park, I.K. Jae, I.S. Park, K.S. Hahm, Y.S. Song, The role of the central L- or D-Pro residue on structure and mode of action of a cell-selective α-helical IsCT-derived antimicrobial peptide, Biochem. Biophys. Res. Commun. 334 (2005) 1329–1335, https://doi.org/10.1016/j.bbrc.2005.07.029.
[42] H.R. Wilman, J. Shi, C.M. Deane, Helix kinks are equally prevalent in soluble and membrane proteins, Proteins Struct. Funct. Bioinforma. 82 (2014) 1960–1970, https://doi.org/10.1002/prot.24550.
[43] N. Sal-Man, D. Gerber, Y. Shai, Proline localized to the interaction interface can mediate self-association of transmembrane domains, Biochim. Biophys. Acta - Biomembr. 1838 (2014) 2313–2318, https://doi.org/10.1016/j.bbamem.2014.05.006.
[44] S.C. Park, M.H. Kim, M.A. Hossain, S.Y. Shin, Y. Kim, L. Stella, J.D. Wade, Y. Park, K.S. Hahm, Amphipathic α-helical peptide, HP (2–20), and its analogues derived from Helicobacter pylori: Pore formation mechanism in various lipid compositions, Biochim. Biophys. Acta - Biomembr. 1778 (2008) 229–241, https://doi.org/10.1016/j.bbamem.2007.09.020.
[45] E. Lee, A. Shin, K.W. Jeong, B. Jin, H.N. Jnawali, S. Shin, S.Y. Shin, Y. Kim, Role of phenylalanine and valine10 residues in the antimicrobial activity and cytotoxicity of piscidin-1, PLoS One 9 (2014) 1–31, https://doi.org/10.1371/journal.pone.0114453.
[46] C.N. Pace, J.M. Scholtz, A helix propensity scale based on experimental studies of peptides and proteins, Biophys. J. 75 (1998) 422–427, https://doi.org/10.1016/S0006-3495(98)77529-0.
[47] J.K. Lee, S.C. Park, K.S. Hahm, Y. Park, Antimicrobial HPA3NT3 peptide analogs: placement of aromatic rings and positive charges are key determinants for cell selectivity and mechanism of action, Biochim. Biophys. Acta - Biomembr. 1828 (2013) 443–454, https://doi.org/10.1016/j.bbamem.2012.09.005.
[48] K.D. Saint Jean, K.D. Henderson, C.L. Chrom, L.E. Abiuso, L.M. Renn, G.A. Caputo, Effects of hydrophobic amino acid substitutions on antimicrobial peptide behavior, probiotics antimicrob, Proteins (2017) 1–12, https://doi.org/10.1007/s12602-017-9345-z.
[49] T. Abraham, E.J. Prenner, R.N.A.H. Lewis, C.T. Mant, S. Keller, R.S. Hodges, R.N. McElhaney, Structure-activity relationships of the antimicrobial peptide gramicidin S and its analogs: aqueous solubility, self-association, conformation, antimicrobial activity and interaction with model lipid membranes, Biochim. Biophys. Acta - Biomembr. 1838 (2014) 1420–1429, https://doi.org/10.1016/j.bbamem.2013.12.019.
[50] S.H. Lee, S.J. Kim, Y.S. Lee, M.D. Song, I.H. Kim, H.S. Won, De novo generation of short antimicrobial peptides with simple amino acid composition, Regul. Pept. 166 (2011) 36–41, https://doi.org/10.1016/j.regpep.2010.08.010.
[51] D. Knappe, P. Henklein, R. Hoffmann, K. Hilpert, Easy strategy to protect antimicrobial peptides from fast degradation in serum, Antimicrob. Agents Chemother. 54 (2010) 4003–4005, https://doi.org/10.1128/AAC.00300-10.
[52] A. Rozek, J.P.S. Powers, C.L. Friedrich, R.E.W. Hancock, Structure-based design of an indolicidin peptide analogue with increased protease stability, Biochemistry 42 (2003) 14130–14138, https://doi.org/10.1021/bi035643g.
[53] T. Unger, Z. Oren, Y. Shai, The effect of cyclization of magainin 2 and melittin analogues on structure, function, and model membrane interactions: implication to their mode of action, Biochemistry 40 (2001) 6388–6397, https://doi.org/10.1021/bi0026066.
[54] Y. Zhao, M. Zhang, S. Qiu, J. Wang, J. Peng, P. Zhao, R. Zhu, H. Wang, Y. Li, K. Wang, W. Yan, R. Wang, Antimicrobial activity and stability of the d-amino acid substituted derivatives of antimicrobial peptide polybia-MPI, AMB Express 6 (2016), https://doi.org/10.1186/s13568-016-0295-8.
[55] S. Lifson, A. Roig, On the theory of helix-coil transition in polypeptides, J. Chem. Phys. 34 (1961) 1963–1974, https://doi.org/10.1063/1.1731802.