Bioorganic Chemistry 149 (2024) 107524

Contents lists available at ScienceDirect

Bioorganic Chemistry

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

Review Article

Important structural features of antimicrobial peptides towards specific activity: Trends in the development of efficient therapeutics

Fariba Fathi [a,1,2], Bahareh Alizadeh [b,1,3], Mohammad Vahid Tabarzad [b,4], Maryam Tabarzad [b,*,5]

[a] *Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[b] *Protein Technology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

ARTICLE INFO

Keywords:
Antimicrobial peptide
Amino acid sequence
Amphipathicity
Selective activity
Structure-activity relationship

ABSTRACT

Proteins and peptides, as polypeptide chains, have usually got unique conformational structures for effective biological activity. Antimicrobial peptides (AMPs) are a group of bioactive peptides, which have been increasingly studied during recent years for their promising antibacterial, antifungal, antiviral and anti-inflammatory activity, as well as, other esteemed bioactivities. Numerous AMPs have been separated from a wide range of natural resources, or produced *in vitro* through chemical synthesis and recombinant protein expression. Natural AMPs have had limited clinical application due to several drawbacks, such as their short half-life due to protease degradation, lack of activity at physiological salt concentrations, toxicity to mammalian cells, and the absence of suitable methods of delivery for the AMPs that are targeted and sustained. The creation of synthetic analogs of AMPs would both avoid the drawbacks of the natural analogs and maintain or even increase the antimicrobial effectiveness. The structure–activity relationship of discovered AMPs or their derivatives facilitates the development of synthetic AMPs. This review discovered that the relationship between the activity of AMPs and their positive net charge, hydrophobicity, and amino acid sequence and the relationship between AMPs' function and other features like their topology, glycosylation, and halogenation.

1. Introduction

Today, microbial infections are an important threat due to multidrug resistance, which should be seriously managed. Antimicrobial peptides (AMPs) have shown promising results in solving this problem. They are usually small amphipathic peptides with net positive charge. It has been found that the net charge and charge distribution are highly correlated with AMPs strength and are significantly different in terms of sequence and structure [1]. However, charge is not the only influential feature on AMPs activity. AMPs are usually small amphipathic peptides with less than 50 amino acids (rarely up to 100 amino acids) and a net positive charge of +2 to +9 at physiological pH and amphiphilic structures. It was confirmed that the presence of excessive charges can have a negative effect on AMPs activity by interfering with their structures. Although, the most members of AMPs are cationic, a number of anionic AMPs have also been identified in vertebrates, invertebrates and plants, and they are an integral part of the innate immune system [2,3].

A large number of AMPs are classified in the α-helix group (for example, cryptin-4, human alpha-defensins (HD-5 and 6), magainin 1 and 2, melittin, muricin) or β-sheet families (for example, beta- human defensins (hBD-1–6), lactoferricin B, protegrin-1, tachyplesin I) [4].

Several studies have investigated the structure-activities relationship of AMPs, which considered maximum efficacy through *in vivo* and *in vitro* analysis. It means that AMPs should be biocompatible, salt resistant, and more importantly, they should be sufficiently potent in antimicrobial activity. These properties can be achieved by rational design of peptides, which has performed by a good understanding of peptide structure–function relationships [5].

---

* Corresponding author at: Shahid Beheshti Faculty of Pharmacy, Protein Technology Research Center, Valiasr Street, PO Box 14155-6153, Tehran 19968-35113, Iran.

E-mail address: m_tabarzad@sbmu.ac.ir (M. Tabarzad).

[1] Both authors equally contributed.
[2] ORCID: https://orcid.org/0000-0002-3397-7083.
[3] ORCID: https://orcid.org/0000-0002-2353-9370.
[4] ORCID: https://orcid.org/0009-0001-6672-8315.
[5] ORCID: https://orcid.org/0000-0001-9923-0639.

https://doi.org/10.1016/j.bioorg.2024.107524
Received 18 December 2023; Received in revised form 29 April 2024; Accepted 2 June 2024

Moreover, a number of studies have also used machine learning and neural network methods in formulating the structure of known peptides and designing new antimicrobial peptides. For example, a study has used neural network-assisted methods to design ten novel AMPs, which were *de novo* generated. Two of these designed AMPS showed significant bacteriostatic effects against *Staphylococcus aureus* and *Escherichia coli* through *in vitro* study. However, none of them had bactericidal properties. Molecular dynamics simulations confirmed the presence of alpha helix structures in these AMPs. The results of this study emphasized the use of neural networks to guide the computer design of new peptides with desired properties [6]. Although, discussing about different computational methods in the design and development of novel AMPs is not the focus point of this review, however, a short segment will discussed these strategies in structure–activity relationship studies and designing the novel AMPs.

2. Methods

Although a number of reviews have been published on the antimicrobial peptides features and challenges, we tried to run a more focused narrow literatures review on the important features of AMPs structures, in order to deliver more recently findings and important issues that could be considered for the design of novel AMPs with more specific activities against particular microbial targtes. Here, a systematic review was performed based on PRISMA statements. PubMed and ScienceDirect were searched in the period of 2015-march 2023, using the following keywords: in PubMed; "antimicrobial peptide" AND "structure-activity relationship" AND "selectivity"; and in Science Direct; "antimicrobial peptide" AND "structure-activity relationship" AND "selectivity". Exclusion criteria were review articles and book chapters, in addition to the articles considered anti-cancers and anti-inflammatory activities, the articles that were not accessible by our institute subscription in their full length, and the articles that only introduced a novel peptide without specific structure activity analysis. Selected articles are generally original articles that are open access or are accessible through our institute subscription.

3. Results and discussion

A summary of the data extracted from the assessed articles is presented in Table 1. Analysis of structure–activity relationships have been published so far for the AMPs are discussed in the following sections:

3.1. Antimicrobial peptides physicochemical features

3.1.1. Cationic structure and positive charge enhanced the anti-bacterial activity of AMPs

In fact, positive charge of peptide sequences can generally enhance the antimicrobial activity of AMPs, such as reported for phylloseptins derivatives, in which increasing the net charge and the number of positively charged Lysine (Lys, K) in peptide sequence resulted in the enhanced membrane binding of AMP due to the electrostatic interactions and as a result, improved antibacterial activity [27]. In addition, it was shown that charge clustering at one terminus of AMPs sequence could reduce the mammalian cell toxicity of AMPs [36]. Previously, a study showed that the balance between AMPs positive charge and hydrophobicity, if did not affect the alpha-helical structure could improve antifungal activity without the decrease of antibacterial activity [37]. In contrary, it was also supposed that there was no direct correlation between high level of positive charges with antimicrobial activity, however, at least one positive charged residue is essential for bioactivity of AMPs [38].

Here, among the recently published literatures we retrieved from PubMed and Science Direct, the following data were achieved. Park *et al.* created analog peptides based on an original peptide, Hylin a1, in order to reduce toxicity, while preserving antibacterial activity. Alanine and lysine were replaced in the analog peptides. It was discovered that the presence of lysine increased the net charge value. Therefore, lysine-substituted analogues, Hylin a1-11 K and Hylin a1-15 K (with 4 and 5 positive net charges, respectively), exhibited a wide-spectrum antibacterial and anti-biofilm potentials, as well as, they specifically active against carbapenem-resistant *Acinetobacter baumannii*. While template peptide, Hylin a1, had only the two positive charges. This research demonstrated that by enhancing electrostatic attraction via the positively charged lysine residues, the binding between AMPs and the negatively charged bacterial membrane would increase [8]. Moreover, GV30, which was firstly isolated from the epidermal secretion of *Hylarana guentheri,* and then, it was recombinantly produced by ''shotgun'' cloning in a separate study, was identified as a member of the brevinin-2 peptide family. According to the strong broad-spectrum antibacterial properties of this AMP, it was assumed that six net positive charges could facilitate the binding of this peptide to negatively charged bacterial membrane [12].

Another study has provided evidence that human neutrophil peptide 4 (HNP4) exhibits a considerable preference for *E. coli* over *S. aureus*. This distinctive functional characteristic can be attributed to three closely located cationic residues, namely Arg10, Arg11, and Arg15, with a particular emphasis on the Arg10-Arg11 combination. These residues are in the loop region between positions 1 and 2 of HNP4. The cationic cluster, a unique molecular hallmark of HNP4, has various activities, including inhibiting *S. aureus* growth, suppressing anthrax fatal factor (LF), and binding to gp120, among others [14]. In addition, the antimicrobial mechanism of Gaduscidins derivatives, specifically Gad-1 and Gad-2, as illustrative instances of histidine-rich AMPs was also studied in more details. Gad-1 is a peptide consisting of 21 amino acid residues, of which five are histidine residues. In contrast, the Gad-2, as 19-residue peptide, has four histidine residues within its sequence. The study indicated that Gad-2, possessing a charge of +5 at pH 5, exhibits a little lesser impact on acyl chain order compared to Gad-1, which has a charge of +3 at pH 7. This implies that factors other than electrostatic interactions, like hydrophobic interactions, may significantly determine Gad peptide/lipid association. It can be inferred that the level of membrane disruption is influenced by factors other than charge alone [10]. The amount and location of hydrophobic and positively charged amino acid residues appear to be crucial in regulating the structural characteristics (such as α-helicity and amphipathicity) of amphipathic α-helical cationic AMPs [30].

3.1.2. Amphipathicity and hydrophobicity of AMPs modulate the antibacterial activity

Amphipathicity of the helical structures in AMPs is also important in their activity. Fig. 1 shows how hydrophobicity play a role in the antimicrobial activity of AMPS. Increasing the amphipathicity results in non-selective membrane disruption and more toxicity against mammalian cells. Increasing the hydrophobicity resulted in enhanced hemolytic activity. In the study of phylloseptin-PHa (PSPHa) derivatives, it was observed that the hydrophobicity should be maintained below the threshold of 0.73 at net positive charge of +6, in order to have no significant hemolytic activity [27].

Here, among the literatures we retrieved from PubMed and Science Direct, the following data were achieved. In the case of PSPHa derivatives, increasing the hydrophobicity enhanced the antibacterial activity against *S. aureus*, decreased the activity against *E. coli* and *K. pneumonia,* and no activity changes were observed against *C. albicans* and MRSA. Authors discussed that analogs with more hydrophobicity have not well reached the cell membrane of Gram-negative bacteria due to the lipopolysaccharide (LPS) layer of outer membrane, which resulted in the loss of antimicrobial activity against Gram-negative bacteria in more hydrophobic analogues. However, in Gram-positive bacterial targets, the hydrophilicity of peptidoglycan at cell wall, which has different thickness between bacteria, could reduce the binding of hydrophobic peptides and therefore, reduce the antimicrobial activity against

Table 1
A summary of literatures reviewed in this study.

Title	Structural characteristics	Chemical modifications	Features	AMP sequence feature	Ref.
Investigations into the membrane activity of arenicin antimicrobial peptide AA139	Arenicin peptides (GFCWYVCVYRNGVRVCYRRCN), β-hairpin AMPs, cationic and amphipathic	Minor amino acid mutations: valine residues are substituted with alanine and tyrosine is converted into arginine	Hydrophobicity reduced,with increased positive charge (by the arginine mutation) and amphipathicity (resulted in significant increase in the therapeutic value)	**AA139** (GFCWYVCARRNGARVCYRRCN)	[7]
Bactericidal activities and action mechanism of the novel antimicrobial peptide Hylin a1 and its analog peptides against *Acinetobacter baumannii* infection	Hylin a1 Amphipathic α-helical structures	amino acid mutations (alanine and lysine were substituted)	Cationic, hydrophobic (destroy the membrane through electrostaticinteraction) and α-helical properties	**Hylin a1-11K:** IAKAILPLALKALKNLIK-NH_2 **Hylin a1-15K:** IAKAILPLALKALKKLIK-NH_2	[8]
Selection and redesign for high selectivity of membrane-active antimicrobial peptides from a dedicated sequence/function database	Dadapin peptides	Modifying the Mutator algorithm	Bridged, β-sheet. inactivate bacteria by disrupting their membranes	**Dadapin-1:** GLLRASSKWGRKYYVDLAGCAKA **Dadapin-2:** GLLRASSKWGRKYYVDLAGCLKA **Dadapin-3:** GLFGKSSKWGRKYYVDLAGCAKA **Dadapin-4:** GL**S**GKSSVWG**V**KYYVDLAGCAKA **Dadapin-5:** GLFKKSSVQGRKYYVDLAGCAKA **Dadapin-6:** FLPKLFKKITKKNMAHIL **Dadapin-7:** FLPKLFQKITKKNMAHIL **Dadapin-8:** AAKKGCWTVSIPPKPCF-NH_2	[9]
Roles of histidine charge and cardiolipin in membrane disruption by antimicrobial peptides Gaduscidin-1 and Gaduscidin-2	Gaduscidins (Gads) Helical, histidine-rich	changes in pH, and hence changes in histidine charge	Helical structure in presence of lipid bilayers and detergents Their structures are remarkably plastic. Weakly activity change by changes in pH, and changes in histidine charge.	**Gad-1:** FIHHIIGWISHGVRAIHRAIN-**.**NH_2 **Gad-2:** FLHHIVGLIHHGLSLFGDR—**.**NH_2	[10]
Manipulating turn residues on de novo designed β-hairpin peptides for selectivity against drug-resistant bacteria	BTT β-hairpin	mutations of only 2 to 4 amino acids at the turn region	Structural flexibility is instrumental in potentiating membrane-permeabilizing interactions	**BTT1:** LKLKLKLTGKLKLKL-NH_2 **BTT2:** LKLKLKVDPPTKLKLKL-NH_2 **BTT3:** LKLKLK DPGKLKLKL-NH_2 **BTT4:** LKLKLKINGKKLKLKL-NH_2 **BTT5**: LKLKLKNGKLKLKL-NH_2 **BTT6**: LKLKLK DPNKLLKL-NH2 BTT7: LKLKLKRDGKLKLKL-NH_2	[11]

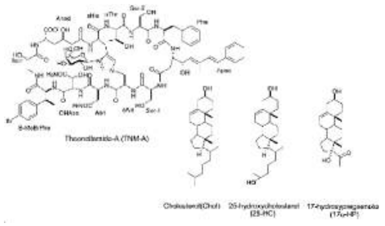
(*continued on next page*)

Table 1 (*continued*)

Title	Structural characteristics	Chemical modifications	Features	AMP sequence feature	Ref.
				BTT8: LKLKLKNPDGKKLKL-NH_2 **BTT9**: LKLKLKEGNKKLKL-NH_2 BTT10: LKLKLKRDNKLKLKL-NH_2	
Generation of truncated derivatives through in silico enzymatic digest of peptide GV30 target MRSA both *in vitro* and *in vivo*	GV30 α-helical structure	Truncated GV30 (removing the “Rana box” from the C-terminus at a cleavage site of trypsin)	Positively charged Removing “Rana box” loop could significantly improve the bactericidal speed on MRSA. Enhanced bacteria cell membrane permeabilisation is one of the antibacterial mechanisms of GV30 and its derivatives	**GV30** GVIFNALKGVAKTVAAQLLKKARCKLDKSC **GV23** GVIFNALKGVAKTVAAQLLKKAR –NH_2 **GV21** (GVIFNLKGVAKTVAAQLLKK-NH_2 **GV20** GVIFNALKGVAKTVAAQLLK –NH_2 **GV12** GVIFNALKGVAK-NH_2 **GV8** GVIFNALK-NH_2 **GV18** TVAAQLLKKARCKLDKSC **GV11** TVAAQLLKKAR –NH_2	[12]
A comparison of activity, toxicity, and conformation of tritrpticin and two TOAC-labeled analogues. Effects on the mechanism of action	Tritrpticin α-helical structure	Labeling with the paramagnetic amino acid TOAC	The mechanism of action is very sensitive to peptide primary sequence Replacement of PRO^5 by TOAC led to conformational changes both in aqueous solution and in TFE-water mixtures that resulted in a non-pore mechanism of action	**$TOAC^0$-TRP3:** TOAC-VRRFPWWWPFLRR (TOAC at the *N*-terminus, prior to V^1) **$TOAC^5$-TRP3**: VRRF-TOAC-WWWPFLRR (substituting P^5 with TOAC)	[13]
Systematic mutational analysis of human neutrophil α-defensin HNP4	human α-defensins (HNP1-3) cationic antimicrobial peptides	Mutations (mutate any of the nine conserved residues in α-defensins: Cys2, Cys4, Cys9, Cys19, Cys29, Cys30, Arg5, Glu13, and Gly17)	Stable tertiary structure was important for antimicrobial activity Random coils reduced antimicrobial activity	**HNP-4:** VCSCRLVFCRRTELRVGNCLIGGVSFTYCCTRV	[14]
Epimers L- and D-Phenylseptin: How the relative stereochemistry affects the peptide-membrane interactions	Phenylseptins linear cationic α-helical AMPs,	D-phenylalanine in sequence	The role of the *D*-phenylalanine in the increase of the antimicrobial activity: Specific aromatic interactions involving the phenylalanine side chains of D-Phe, located in the hydrophobic face of the helix, increased amphipathicity and resulted in anchoring of D-epimer into the lipid bilayer and increasing its affinity to the membrane. **carpet-like mechanism**	L-Phenylseptin and D-Phenylseptin peptides (FFFDTLKNLAGKVIGALT-NH_2)	[15]

(*continued on next page*)

Table 1 (*continued*)

Title	Structural characteristics	Chemical modifications	Features	AMP sequence feature	Ref.
Theonellamide A, a marine-sponge-derived bicyclic peptide, binds to cholesterol in aqueous DMSO: Solution NMR-based analysis of peptide-sterol interactions using hydroxylated sterol	Theonellamide A (TNM-A) Bicyclic dodecapeptides	Lipidation	The TNM-Cholesterol complex, where the hydrophobic cavity of TNM probably incorporates Chol, resulting in a greater accumulation of the peptide in membrane and enhances membrane disruption		[16]
Tachyplesin Causes Membrane Instability That Kills Multidrug-Resistant Bacteria by Inhibiting the 3-Ketoacyl Carrier Protein Reductase FabG	Tachyplesin Cationic β-hairpin antimicrobial peptide	Cyclization	FabG catalyzes the NADPH dependent reduction of 3-keto-acyl-ACP during fatty acid elongation, thus enabling lipid supply for production and maintenance of the cell envelope. Cyclization could not improve potency; however, cyclization improved AMPs stability in human serum as well as their hemolytic activity	**Tachyplesin I:** KWCFRVCYRGICYRRCR **Tachyplesin II:** RWCFRVCYRGICYRKCR **Tachyplesin III:** KWCFRVCYRGICYRKCR	[17,18]
The Dolphin Proline-Rich Antimicrobial Peptide Tur1A Inhibits Protein Synthesis by Targeting the Bacterial Ribosome	Tur 1A & B: Proline-rich antimicrobial peptides: dolphin *Tursiops truncatus*	Identified based on genome Sequence (sequence variations within the cathelicidin family of PrAMPs)	**Tur1A:** Inhibits bacterial growth by targeting the ribosome. **Tur1B:** Modest inhibitory effect on translation.	**Tur1A:** RRIRFRPPYLPRPGRRPRFPPPF—PIPRIPRIP **Tur1B:** RRIPFWPPNWPGPWLPPWSPPDF—RIPRILRKR	[19]
Bactericidal activity of cyclotides where phosphatidylethanolamine-lipid selectivity determines antimicrobial spectra	cyclotides	Cysteine knot embedded in a macrocyclic backbone	The factors influencing antimicrobial activity: 1) Absence of rich growth media, increased antibacterial activities. 2) Cyclotides exhibited a positive correlation between the presence of PE-lipids and membrane permeabilization.	**cycloviolacin O2:** G–IP-CGESCVWIPC-ISSAIGCSC-KSKVC-Y-RN **cycloviolacin O3:** G–IP-CGESCVWIPC-LTSAIGCSC-KSKVC-Y-RN **cycloviolacin O19:** G-TLP-CGESCVWIPC-ISSVVGCSC-KSKVC-Y-K_D **Cter B:** G–VP-CAESCVWIPCTVTALLGCSC-KDKVC-Y-LN **Cter E:** G–IP-CAESCVWIPCTVTALLGCSC-KDKVC-Y-LD **Cter G:** G–LP-CGESCVFIPC-ITTVVGCSC-KNKVC-Y-NN **Cter R:** G–IP-CGESCVFICTVTALLGCSC-KDKVC-Y-KN **tricyclon A:** GGTIFDCGESCFLGTC-YT-K-GCSCGEWKLC-YGTN **varv A:** G–LPVCGETCVGGTC-NT–PGCSC-SWPVC-*T*-RN **kalata B1** G–LPVCGETCVGGTC-NT–PGCTC-SWPVC-*T*-RN **kalata B2** G–LPVCGETCFGGTC-NT–PGCSC-TWPIC-*T*-RD **kalata B13:**	[20]

(*continued on next page*)

Table 1 (*continued*)

Title	Structural characteristics	Chemical modifications	Features	AMP sequence feature	Ref.
				G–LPVCGETCFGGTC-NT–PGCACDPWPVC-*T*-RD **kalata B7:** G–LPVCGETCTLGTC-YT–QGCTC-SWPIC-K-RN **kalata B7-Y15F:** G–LPVCGETCTLGTC-FT–QGCTC-SWPIC-K-RN **kalata B7-Y15S:** G–LPVCGETCTLGTC-ST–QGCTC-SWPIC-K-RN **kalata B7-Y15A:** G–LPVCGETCTLGTC-AT–QGCTC-SWPIC-K-RN **MCoTI–I:** G–GV-CPKILQRCRRDSDCPGACICRGNGYCGSGSD **MCoTI–II:** G–GV-CPKILKKCRRDSDCPGACICRGNGYCGSGSD	
Effect of tacticity-derived topological constraints in bactericidal peptides	designing amphipathic peptide systems	Stereochemical engineering of the peptide	Conformational locking by changing the stereochemical sequence offers the possibility of fixing the structure of the designed peptide, thus modulating its specificity and activity.	**MS1:** KrKiFlRtKiLv (LDLDLDLDLDLD) **MS2:**kRkIfLrTkIlV (DLDLDLDLDLDL) **MS3**:vLiKtRlFiKrK (DLDLDLDLDLDL) **MS4:**VlIkTrLfIkRk (LDLDLDLDLDLD) **MS5:** KRKIFLRTKILV (LLLLLLLLLLLL) **MS6:**VLIKTRLFIKRK (LLLLLLLLLLLL)	[21]
Biophysical and biological properties of small linear peptides derived from crotamine, a cationic antimicrobial/antitumoral toxin with cell penetrating and cargo delivery abilities	Crotamine (YKQCHKKGGHCFPKEKICLPPSSDFGKMDCRWRWKCCKKGSG)	Truncation of peptide and modified sequence (Cys replaced by Ser)	the presence of cysteine residues and reactive thiol group in the structure of linear peptides highly influenced the antifungal activity, and replacement by Ser residues suppress the antimicrobial activity against fungi strains.	**C1:** (Crot$_{2–18}$) KQCHKKGGHCFPKEKIC **C2:** (Crot$_{27–39}$) KMDCRWRWKCCKK **P1**: (Crot$_{2–18}$) KQSHKKGGHSFPKEKIS **P2**: (Crot$_{27–39}$) KMDSRWRWKSSKK (P: Cys residues were replaced by Ser)	[22]
Selective amino acid substitution reduces cytotoxicity of the antimicrobial peptide mastoparan	Mastoparan: (INLKALAALAKKIL)	Ala-substitution in positions 5 and 8 ((I^5, R^8) MP)	[I^5, R^8] MP is able to form an active amphipathic α-helix conformation and the pore-forming activity leads to cell death.	**(I^5, R^8)MP:** INLKILARLAKKIL	[23]
Structure–activity relationship of mastoparan analogs: Effects of the number and positioning of Lys residues on	Mastoparan (INLKALAALAKKIL)	Change in the amino acid sequence	Lys residues at positions 4, 5, 7, 8, 9, and 11of the tetradecapeptide chain are important to	**MK578:** INWLKAKKVAMI L-NH_2 **MK89**:INWLAIAKKVA GML-NH_2 **MK9:**INWLAIAAKVAGML-NH_2	[24]

(*continued on next page*)

Table 1 (*continued*)

Title	Structural characteristics	Chemical modifications	Features	AMP sequence feature	Ref.
secondary structure,interaction with membrane-mimetic systems and biological activity			optimize the antibacterial activity of mastoparans.	**MK5**:INWLKIAAAVA GML-NH_2 **MK0:**INWLAIAAAVA GML-NH_2 **MK4589**INWKKI AKKVA GML-NH_2 **MK5789** INWLKI KKKVA GML-NH_2 **MK58911** INWLKI AKKVK GML-NH_2 **MK45789** INWKKI KKKVA GML-NH_2 **MK4578911**:INWKKI KKKVK GML-NH_2	
Proline-15 creates an amphipathic wedge in maculatin 1.1 peptides that drives lipid membrane disruption	maculatin 1.1 (Mac-1), caerin 1.1 (Cae-1)	amino acid residues were either substituted or deleted in the region surrounding the proline residues.	the specific sequence in terms of charge distribution and amphipathicity plays a dominant role in antimicrobial activity of AMP	**Mac-1:** GLFGVLAKVAAHVVPAIAEH F-NH_2 **Mac-2:** GLFGVLAKVAAKVVPAIAEHF-NH_2 **Mac-3:** GLFGVLAKVAKHVVPAIAEHF-NH_2 **Mac-4:** GLFGVLAKHVVPAIAEHF-NH_2 **Mac-5:** GLFGVLAKVAAIAEHF-NH_2 **Mac-6:** GLFGVLAKIAEHF-NH_2 **Cae-1:** GLLSVLGSVAKHVLPHVVPVIAEHL-NH_2 **Cae-2:** GLLSVLGSVAKHVVPVIAEHL-NH_2	[25]
Effect of acyl chain length on therapeutic activity and mode of action of the CX-KYR-NH2 antimicrobial lipopeptide	KYR (LysTyrArg-NH_2)	Peptide lipidation	the extent and kinetics of inner membrane penetration and depolarization were dependent on the acyl chain length.By adding an acyl chain to modify the ultrashort peptide, antibacterial activity increased: hydrophobicity is a key parameter dictating the biological activity of peptides.	**C_{10}-KYR-NH_2**: Decanoyl-LysTyrArg-NH_2 **C_{12}-KYR-NH_2**: Lauroyl–LysTyrArg-NH_2 **C_{14}-KYR-NH_2**: Myristoyl-LyTyrArg-NH_2 **C_{16}-KYR-NH_2** Palmitoyl-LysTyrArg-NH_2	[26]
Structure–activity relationship of an antimicrobial peptide, Phylloseptin-PHa: balance of hydrophobicity and charge determines the selectivity of bioactivities	Phylloseptins: cationic peptides	Altering primary structure: Lys was applied to manipulate the cationicity and Ile was employed to adjust the hydrophobicity of peptides,	enhanced cationicity increased the antimicrobial activity (the positively charged Lys enhances binding of the peptide to the bacterial membrane.) Increasing the hydrophobicity, particularly on the hydrophobic face, correlated with enhanced membrane permeabilization	**PSPHa**: FLSLIPAAISAVSALANHF-NH_2 **PSPHa1**: FLSLIPKAISAVSALANHF-NH_2 **PSPHa2:** FLSLIPKAISAISALANHF-NH_2 **PSPHa3:** FLSLIPKAISAISALINHF-NH_2 **PSPHa4:** FLSLIPKIISAISALINHF-NH_2 **PSPHa5**: FLSLIPKIISAISALIKHF-NH_2	[27]
Activity and characterization of a pH-sensitive antimicrobial peptide	C18G, ALWKKLLKKLLKSAKKLG	Replacing the original Lys residues with His.	charge-state of the histidine, and the peptide in general, is an important driver for antibacterial activity.	C18G-His ALWHHLLHHLLHSAHHLG	[28]

(*continued on next page*)

Table 1 (*continued*)

Title	Structural characteristics	Chemical modifications	Features	AMP sequence feature	Ref.
	Cationic, neutral pH and helical conformation	The effect of pH on antimicrobial activity was investigated	the C18G-His peptide clearly demonstrates pH-dependent antibacterial activity the depth and orientation of the peptide when bound is significantly different between the electrostatic-enhanced binding (low pH, anionic lipids) and the neutral form (neutral pH, zwitterionic lipids) .		
Molecular Basis of Selectivity and Activity for the Antimicrobial Peptide Lynronne-1 Informs Rational Design of Peptide with Improved Activity	Lynronne-1: LPRRNRWSKIWKKVVTVFS)	Varied in the size of the hydrophobic helical face/polar angle of the APH(amphipathic helix).	a direct relationship between antimicrobial activity and the size of the hydrophobic helical face.	**Lynronne-1:** LPRRNRWSKIWKKVVTVFS)	[29]
Antimicrobial Peptide Modifications against Clinically Isolated Antibiotic-Resistant Salmonella	BmKn-2amphipathic α-helical cationic structure (to be a template for further modifications)	a peptide template modification	increased cationicity especiallywith arginine (**R**) stretched *N*-terminus with hydrophobic aromatic amino acids whether phenylalanine (**F**) or tryptophan (**W**) is correlated with enhanced antibacterial potency, increased anti-Salmonella potency is a result of an increased number of net positive charges.	**Kn2-5R:** FIRRIARLLRRIF **Kn2-5R-NH$_2$:** FIRRIARLLRRIF-NH$_2$ **dKn2-5R-NH$_2$:** firriarllrrif-NH$_2$ **2F-Kn2-5R-NH$_2$:** FFFIRRIARLLRRIF-NH$_2$	[30]
A Safe and Multitasking Antimicrobial Decapeptide: The Road from De Novo Design to Structural and Functional Characterization	1018-K6 (12-residue cathelicidin-related antimicrobial peptide)	shortpeptides (truncating 2-amino-acids at the *N*-terminus of 1018-K6 and by introducing selective and specific amino acid substitutions in the parental sequence)	Two tryptophan residues in peptide sequence play a critical role in antimicrobial activity.	**RiLK1** (decametic peptide) RLKWVRIWRR	[31]
Rational design and synthesis of Oreoch-2 analogues as efficient broad-spectrum antimicrobial peptides	Oreoch-2 (25-aminoacid peptide)	truncation strategy	Cationic amino acids and high hydrophobicity (GLFSAG) was closely related to the antibacterial activity of Oreoch-2.	**ZN-1** (12-aminoacid peptide): KAIHRLIRRRRR **ZN-2** (12-aminoacid peptide): FIHHIIGRRRRR **ZN-3** (24-aminoacid peptide): FIHIIGGLFSAGKAIHRLIRRRRR **ZN-4** (23-aminoacid peptide): FHIIGGLFSAGKAIHRLIRRRRR **ZN-5** (22-aminoacid peptide): FIIGGLFSAGKAIHRLIRRRRR **ZN-6** (18-aminoacid peptide): FLFSAGKAIHRLIRRRRR	[32]

(*continued on next page*)

Table 1 (*continued*)

Title	Structural characteristics	Chemical modifications	Features	AMP sequence feature	Ref.
Design of Heptad Repeat Amphiphiles Based on Database Filtering and Structure–Function Relationships to Combat Drug-Resistant Fungi and Biofilms	*In silico* design based on a typical heptad repeat sequence (abcdefg), such as melittin using K, R, L, A and C	heptad repeat sequences based on anantimicrobial peptide database (APD)	**K** residues provided basic cations for peptide sequences, side chains of **R** residues improved the penetration of the peptide sequence, **C** residues can enhance antimicrobial activity and selectivity, **A** promotes helix formation. The presence of complete hydrophobic faces and cationic faces increase toxicity. Increasing the number of repeat units, enhanced stability of peptide. The sequence length and hydrophobic/cationic face integrity affected peptide activity and cell selectivity	**CAR2:** LKKLCARLKKLCAR-NH$_2$ **CAR3**: LKKLCARLKKLCARLKKLCAR-NH$_2$ **CAR4**: LKKLCARLKKLCARLKKLCARLKKLCAR-NH$_2$ **ACR2:** LKKLACRLKKLACR-NH$_2$ **ACR3:** LKKLACRLKKLACRLKKLACR-NH$_2$ **ACR4**: LKKLACRLKKLACRLKKLACRLKKLACR-NH$_2$	[33]
Design of therapeutically improved analogue of the antimicrobial peptide, indolicidin, using a glycosylation strategy	Indolicidin (ILPWKWPWWPWRR-NH$_2$)	glycosylation strategy	Sugar moiety decreased toxicity of glycosylated analogue and enhanced its solubility glycosylated analogue displayed reduced cytotoxicity against Murine cell lines.	**[T9,K7] indolicidin:** ILPWKWKWTPWRR-NH2 **[βGlc-T9,K7] indolicidin:** ILPWKWKW*T*PWRR-NH2 (*T* = βGlc-T) glycosylated analogue	[34]
C-terminal modification of a *de novo* designed antimicrobial peptide via capping of macrolactam rings	namely zp80 (IIRR)$_4$-NH$_2$	capping a macrolactam ring at the *C*-terminus of a *de novo*-designed peptide, (aspartic acid-rich macrolactam rings) Group I: Cyclization via the ε-amino group of lysine *K* Group II: Cyclization via the α-amino group of lysine *K*	Destroy the integrity of bacterial envelope, leading to cytoplasm leakage and rapid dissipation of membrane potential The C-terminal capping of peptide zp80 with a macrolactam ring enhanced cellular selectivity.	**Group I: zp83** (IIRR)$_4$*K*DaDG **zp87** (IIRR)$_4$*K*DdDG **zp88** (IIRR)$_4$*K*DeDG **zp84** (IIRR)$_4$*K*DADG **zp71** (IIRR)$_4$*K*KIKK **zp73** (IIRR)$_4$*K*KKKK **zp75** (IIRR)$_5$*K*KIKK **zp69** (IIRR)$_4$*K*IKK **zp70** (IIRR)$_3$*K*KIKK **zp85** (IIRR)$_4$*K*DGDG **zp86** (IIRR)$_4$*K*DkDG **zp68** (IIRR)$_3$*K*IKK **zp76** (IIRR)$_6$*K*KIKK **zp81** *K*DADG **zp82** *K*DGDG **Group II:** **zp90** (IIRR)4*K*aDGD **zp91** (IIRR)4*K*DGDa **zp89** (IIRR)4*K*DaDG **zp92** (IIRR)4*K*GDaD **zp72** (IIRR)4*K*KIKK	[35]

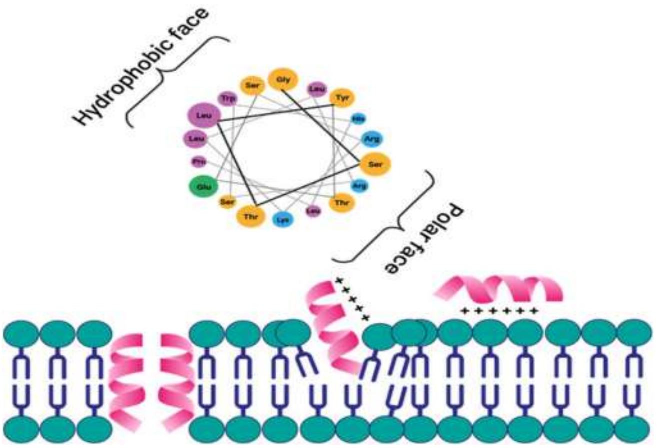


Fig. 1. Antimicrobial peptides (AMPs) interaction with cell membrane. AMPs close the cell by their polar surface and then, intercalate through the phospholipid bilayer via their hydrophobic residues. Thus, AMPs amphipathicity is important for cell membrane activity.

resistant Gram-positive bacteria that have thicker peptidoglycan layer [27].

Moreover, the findings propose that increasing the hydrophobicity of peptide reduced their ability to discriminate bacterial and mammalian cells. Generally, cell membrane compositions and associated surface charges make difference between these two types of cells, in a way that the bacterial cells are more negatively charged, while mammalian cells are neutral. Consequently, it can be concluded that the action of AMPs on mammalian cells is mainly due to hydrophobic interactions, rather than electrostatic interactions [30].

Nevertheless, the investigation of Hylin a1 analog peptides reveals that analogous peptides containing hydrophobic alanine and positively charged lysine residues exhibit the formation of the α-helix structure and possess significant antibacterial properties. As previously stated, a lysine residue with a positive charge enhanced electrostatic interactions, let to a reinforcing of interaction between AMP and the bacterial membrane, with negative charges [8]. This highlights the significance of maintaining an equilibrium between positive charge and hydrophobicity in order to sustain antibacterial efficacy, while ensuring non-toxicity towards mammalian cells.

The hydrophobicity of antimicrobial peptides not only influences their antibacterial action, but also contributes to the stability of these peptides. The results of a study showed that ZN-5 and ZN-6, two similar compounds to Oreoch-2, were very effective against a wide range of gram-positive bacteria. Furthermore, it was noticed that they exerted no hemolysis, indicating a significant level of selectivity towards bacteria. However, in contrast to ZN-6, ZN-5 exhibited greater stability across diverse environmental conditions. Even though both ZN-5 and ZN-6 peptides have the same net positive charge, the researchers came to the conclusion that the difference in how stable they are in different situations, especially in the physiological salt environment, was due to differences in their hydrophobic properties. ZN-5 was more hydrophobic than ZN-6 and therefore, was more stable in a wide range of saline conditions, which reduced the chance of cytoplasmic precipitation. Consequently, ZN-5 possesses enhanced potential for effective integration into the cell membrane [32].

3.1.3. Conformational structure

The α-helices and β-sheets are the primary, regular secondary structures observed in antimicrobial peptides [39]. Here, among the literatures we retrieved from PubMed and Science Direct, the following data were achieved. In naturally occurring proteins or antimicrobial peptides, the α-helical structure commonly exhibits a characteristic heptad repeat sequence (*abcdefg*), as observed in BMAP-27, melittin, BMAP-28, and other examples. The aforementioned model assumes a significant function in preserving cytotoxicity, rendering it a suitable model for advancing α-helical peptide pharmaceuticals. The hydrophobic contact between residues in *a* and *d* positions of heptapeptide would be the driving force that facilitates the cohesion of amphipathic α-helices in this particular model system [33].

Besides, a β-hairpin, a mere simplification of the β-sheet structure, comprises a single sequence folded back on itself to produce two antiparallel strands joined by a turn. To investigate the role of the β-hairpin peptide turn region, a set of de novo β-hairpin peptides with different turns but the same side strands was created. Using transplanted turn sequences selected after a comprehensive literature inquiry, alternate Leu- and Lys-repeat units on the side strands stabilized, and a new family of 14–16 residue-long hairpin peptides was developed from BTT-1 (LKLKLKLTGKLKLKL-NH2), known as BTT1–10. This study demonstrated that, independent of side-strand alterations, altering two to four residues in the turn region may substantially impact the antimicrobial efficacy of β-hairpin peptides, suggesting that it was a workable addition to the toolkit for AMP design. Numerous changes in conformation are involved in the membrane contacts with β-hairpin peptides. The permeabilizing interactions of peptide and membranes are improved by structural flexibility (Fig. 2). Despite the lipid A mutation, BTT1 remained highly active in strains of *Enterobacterales* that were mcr-1 positive, partly due to its hydrophobicity and affinity for binding to LPS [11].

In an in silico approach, the Mutator algorithm was employed in a study to choose and modify the high selectivity of membrane-active antimicrobial peptides derived from a specialized database of Anuran defense peptides (DADP). Out of the eight sequences suggested by the program, two peptides, namely Dadapin-1 (GLLRASSKWGRKKYYV-DLAGCAKA) and Dadapin-8 (AAKKGCWTVSIPPKPCF-NH_2), were selected for experimental evaluation. The findings obtained from circular dichroism (CD) spectroscopy indicated that Dadapin-1 exhibited promising interaction with bacterial membranes, while manifesting as a peptide with a partly helical structure. Conversely, Dadapin-8 has a notable presence of a β-hairpin-like conformation when exposed to anionic artificial membranes. The presence of two cysteine (Cys)

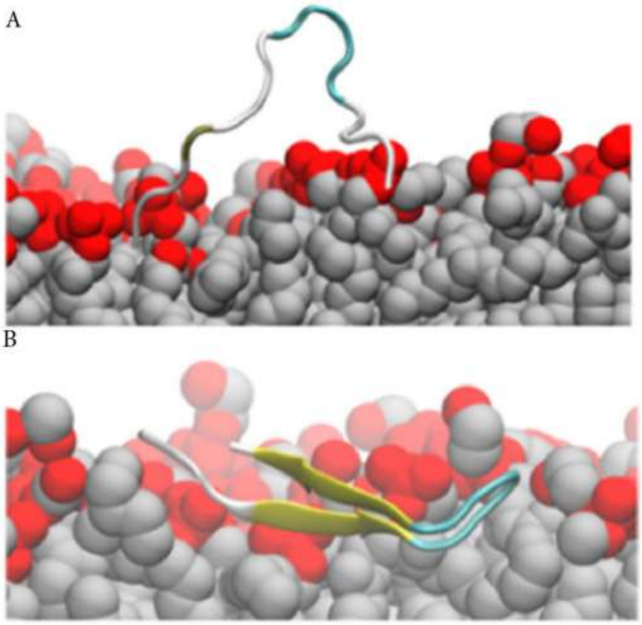


Fig. 2. β-hairpin structure of an antimicrobial peptide (BTT). (A) Random structure of monomer AMP before interaction with membrane and (B) formation of β-hairpin structure after peptide interaction with membrane [reprinted with permission from [11].

residues in Dadapin-8 seems to facilitate the creation of a disulfide bridge. This structural feature is expected to promote a β-hairpin conformation rather than the helical conformation. Both peptides exhibited moderate activity against Gram-negative bacteria, despite the fact that Dadapin-1 demonstrated higher potency. The diminished efficacy of Dedapin-8 may be attributed to structural dissimilarities in comparison to Dadapin-1 or its reduced charge. Considering the minimum bactericidal concentration (MBC) values that exhibited a general similarity to the minimum inhibitory concentration (MIC), it was also confirmed that these peptides possess bactericidal properties rather than bacteriostatic effects [9].

Unexpectedly, there are a number of studies that have underscored the significance and the function of structural conformation in the antibacterial action of AMPs. For instance, Aracin 1 is a member of proline rich AMPs (PrAMPs), isolated from small spider crab *Hyas araneus*. Structurally, aracin1 has rich proline-arginine segment at *N*-terminal, and four cysteine at C-terminal that form two di-sulphide bridges. It exhibited antimicrobial activity against Gram-negative and Gram-positive bacteria. It was shown that proline-rich region at *N*-terminal of arasin 1 is crucial for the antibacterial and antifungal activities. In addition, the study indicated that the first twenty-three amino acids of *N*-terminal sequence could exhibit the complete antimicrobial activity. Regard to the structural features, it was found that the disulfide bonds and the loop structure of the C-terminus augment the stability against proteolytic cleavage [40].

3.2. The effect of amino acid sequence on AMPs structure and function

The amino acid sequence is supposed to play a significant role in diverse activities that AMPs perform. A single amino acid substitution may alter an AMP's antimicrobial activities as well as other bioactivities. As an example, study of a mastoparan analog, which was made by substituting Ala in positions 5 and 8, resulted in a broad-spectrum antibacterial activity of the derivatives against bacteria and fungus, without being hemolytic or cytotoxic toward HEK-293 cells, even though original mastoparans have strong cytotoxic effects [23]. Furthermore, previous studies have provided evidence that linear peptides generated from crotamine have noteworthy antibacterial properties that was critically dependent on the presence of the reactive thiol group within the sequence of these peptides. However, when this thiol group was substituted with Ser residue, the antimicrobial activity against various strains of fungi was significantly diminished [22].

In particular cases, some AMPs may have a high content of specific amino acids, such as glycine, proline, cysteine, tryptophan, or histidine (e.g. Histatin-5 from human saliva with potent antifungal activity). The differences in amino acid composition, length, and net charge lead to the diverse mechanisms of actions. Two of these classifications including Pro-rich AMPs such as apidaecin (from bees and wasps) and pyrrhocoricin (from firebeetles), and Trp-rich AMPs such as indolicidin, tritrpticin, and the wheat puroindoline-based peptides, have been wide distributed among many organisms, and they can specifically target intracellular biomolecules [41]. In fact, it has been discovered that residue positions may have an impact on how well AMPs function. Take mast cell degranulation activity, for instance. The interaction between peptide and membrane-coupled G-protein receptor was directly responsible for this activity. Research revealed that mastoparan analogs need to have one or two Lys residues at positions eight and/or nine to meet the apparent structural requirements for maximizing this activity [24].

The presence of specific amino acids, such as tryptophan, arginine, and histidine, in AMPs sequence could insert particular forces for AMPs interaction with targets. For example, the indole ring of Trp delivers a strong negative electrostatic potential for the cation–π interaction. Besides, presence of Arg enhanced hydrogen bonds due to their side chain structure, in contrast to Lys, which highly contributed in cation–π interactions. Generally, it was proposed that specific AMPs sequences with well-arranged Arg and Trp residues could be more active than Lys and Trp arrangements. AMPs structures rich in Arg and Trp could lead to different structures rather than α-helices and β-sheets [42].

Here, among the literatures we retrieved from PubMed and Science Direct, the following data were achieved. One of the reported AMPs is C18G that was confirmed as a selective and broad-spectrum active agent against Gram-negative and Gram-positive bacteria. It has 7 Lys residues which introduced a net cationic charge of +8. In a study, the effect of the substitution of Lys with other natural or non-proteinogenic cationic amino acids including arginine, histidine, ornithine (Orn), or diaminopropionic acid on the AMP activity was investigated. These changes included variations in the side chain structure of residues and the identity and pKa of the cationic amino acids. Results showed that substitution with His significantly reduced the activity. However, those had Arg, or Orn substitution exhibited the most widely activity similar to the peptides contained Lys. Results of this study showed that the antibacterial activity of these AMPs was generally tolerant to variations of the cationic amino acids and they overally fold to helical structure in binding to the bacterial lipid membrane [43].

In a similar manner, the effect of hydrophobic amino acids substitution in C18G on activity was studied. This substitution did not considerably alter the secondary structure; however, antibacterial activity and binding to lipid membrane models were affected. Those containing Leu or Phe instead of Trp as the hydrophobic residue could bound more tightly to lipid bilayers and therefore, they were most effective antibacterial analogues. Analogues that had Ile showed intermediate activity and those had Val or α-aminoisobutyric acid (Aib) exhibited significantly reduced lipid binding and antibacterial activity. In general, the hydrophobic residue identity impact significantly the lipid binding and antibacterial activity of AMPs [44].

Previous studies have demonstrated that substituting the Arg residue with Lys in tritrpticin (TRP3), a 13-residue antimicrobial peptide with a net charge of +4 at pH 7.4 and an almost palindromic sequence (VRRFPWWWPFLRR), led to a reduction in hemolytic property and interaction with zwitterionic model membranes. However, the negatively charged membrane interaction, antimicrobial capacity, and conformation of the peptide stay unchanged. Furthermore, it has been shown that the interaction and permeabilization of peptide to membranes are contingent upon robust guanido-phosphate interactions between the side chains of Arg residues and lipid phosphate groups. The three successive Trp residues are essential for TRP3 membrane activity. The substitution of Pro residues led to notable alterations in both the activity and structural properties of TRP3. Replacing both Pro residues with Ala resulted in an enhanced antibacterial, hemolytic, and membrane permeabilization activity, along with notable modifications in the structural characteristics of the peptide. When a single proline residue was modified, the removal of Pro5 resulted in an elevation of antibacterial activity, while reduction in hemolytic activity, and modification in the peptide structure. Conversely, the replacement of Pro9 maintained the characteristics of the original peptide [13].

Similar to the importance of single amino acids substitution on AMPs bioactivities, peptides' motifs are also important in this regard. For example, an N- terminal motif of FLSLIP in the primary structure of phylloseptin-PHa (PSPHa), which was extracted from *Pithecopus hypochondrialis*, has shown important impact on the antimicrobial activity. This AMP with 19 amino acids has C- terminal amidation, and it is a Lys/His rich peptide with α-Helical structure [27]. In addition, it was reported that proline-rich antimicrobial peptides, which were widely identified in insects, crustaceans and mammalians, can affect intracellular targets. They interact with ribosomal exit tunnel and interfere with the process of protein synthesis during elongation phase of translation [45].

Through a recent study, the antibacterial activity of a peptide named P4 (RTKLWEMLVELGNMDKAVKLWRKLKR) was studied, which was designed based on alpha-helix fragments of M1 influenza virus protein and the cholesterol-recognizing amino-acid consensus (CRAC) amino acid motif. The results showed that the antimicrobial mechanism of the P4 peptide on the Gram-negative bacterium *E. coli* was related to the interaction of the CRAC motif with sterols present in the bacterial membrane, while in the case of the Gram-positive bacterium *B. subtilis*, which lacks sterols, the antimicrobial activity was based on membrane permeability through the interaction of the cationic domain of the peptide and the anionic lipids of the bacterial membrane [46]. Therefore, a single sequence of AMP containing various motifs could display different mechanisms of actions in different situations.

Another group have reported an increase in the activity of AMPs by adding copper-nickel binding unit (ATCUN) to the amino-terminal of peptide. They synthesized a membrane-active peptide, Anoplin, and two ATCUN-Anoplin derivatives and showed that the increased activity was actually due to the formation of reactive oxygen species (ROS) by the CuII-ATCUN complex. ATCUN-Anoplin peptides were up to four times more potent against standard test bacteria than Anoplin alone. Addition of the ATCUN motif did not result in a difference in microbial membrane disruption. When helical content was calculated, it was observed that ATCUN-Anoplin had less helical composition. They found that ATCUN-Anoplin was able to oxidatively damage lipids in the bacterial membrane and their activity is influenced by the rate of ROS formation by CuII-ATCUN complexes. This study showed that the addition of a metal-binding tripeptide motif is a simple strategy to increase the potency of AMPs by inducing a secondary function [47].

In another study, the active region of Aracin 1 was characterized through structure and activity studies using different peptide fragments derived from the sequence of Aracin 1. The results showed that the pharmacophore was located at the proline/arginine-rich NH_2 terminus of the peptide, and the Aracein 1(1–23) fragment was almost as active as the full-length peptide. At the minimal inhibitory concentration (MIC), Aracin 1 (1–23) was not bactericidal and had no membrane disrupting effect. In contrast, it was bactericidal at a concentration of 5 × MIC and above and interfered with membrane integrity. As a result, Aracin 1 (1–23) like Scropin P1 has a different mode of action compared to lytic peptides. Thus, a dual mode of action for Aracin 1 (1–23) can be proposed that tis peptide involved membrane disruption at peptide concentrations above the MIC, and an alternative mechanism of action, possibly interacting with intracellular targets, at its MIC [40].

A different study quantitatively analyzed the significance of amino acid sequence by creating a range of sequence variants for an artificial peptide, Lysette. Lysette is a truncated variant of staphylococcal δ-lysin, characterized by the absence of the first four amino acids in the complete peptide sequence. The Lysette and δ-lysin exhibited a significant affinity for lipid bilayer vesicles consisting of zwitterionic phosphatidylcholine, resulting in the prompt release of their contents. Compared to the full-length peptide, which exhibited no net charge at a pH of 7, Lysette and its sequence variations possess a net charge +2 under neutral pH conditions. This characteristic makes them more effective at emulating antimicrobial peptides. Furthermore, the intensified electrical charge decreased peptide aggregation compared to the original peptide, δ-lysin, in a solution. The Lysette variants were developed in a way that the hydrophobic moment remained consistent across all peptides. These variants maintained their overall charge and hydrophobicity due to the conservation of the amino acid composition. It was determined that the interaction with phosphatidylcholine bilayers was solely dependent on the helicity of the peptide during membrane binding, regardless of its pre-existing hydrophobic moment or its capability to create intramolecular salt bridges. The results obtained from molecular dynamics simulations conducted on two peptides indicate that the sequence of the peptides plays a crucial role in determining their insertion depth into the bilayer. The presence of two aspartate residues positioned at the C-terminus of lysette-2 resulted in a reduction in helical structure in the computational models. This reduction in helical content was associated with an accelerated detachment from the lipid bilayer in comparison to the Lysette [48].

Moreover, two naturally occurring AMPs, maculatin 1.1 (Mac-1) and caerin 1.1 (Cae-1), released from the skin of Australian tree frogs, were chosen as starting scaffolds in a different investigation to investigate the structure–activity relationship of these two peptides. Whereas caerin 1.1 has two Pro residues and 25 amino acids, maculatin 1.1 has 21 residues and a Pro residue, in which it has been demonstrated to be crucial for the bilayer breakdown. On the basis of these two cationic peptides, eight peptides with altered or deleted amino acid residues in the vicinity of proline residues were created. In the first category, Lys was substituted at residues 11 and 12 to provide Mac-1 with an extra charge. In the second group, either one or two helical turns of the α-helix were excised. The analysis focused on the interaction between the AMPs and two lipid membrane models. These models included a neutral or zwitterionic systems. One consisted of palmitoyl oleoyl phosphatidyl choline (POPC), which is a major phospholipid found in eukaryotic cell membranes. Additionally, a negatively charged system was examined, which consisted of POPC with 30 % palmitoyl oleoyl phosphatidyl glycerol (POPG). The investigation focused on examining the impact of peptide physicochemical qualities on the changes in secondary structure, adsorption onto lipid bilayers, and disruption of these bilayers, with specific consideration given to the membrane surface charge. The comparison of the sequence and activity of each peptide demonstrates the significance of the hydrophobic and charge distribution surrounding the pivotal Pro residue. This emphasizes the importance of the interaction between this Pro residue and the overall amphipathicity in the AMP activity. This research demonstrated that the permeation and lysis of a lipid bilayer by maculatin 1.1 were contingent upon the hydrophobic environment and the cationic charge position of Pro-15. The observed variations in the impacts on zwitterionic and anionic bilayers indicated that the manipulation of the topography of these residues in a way that selectively hindered binding and/or insertion based on the features of the bilayer had a great potential to control membrane selectivity [25].

Research conducted by Mangmee et al. on various BmKn-2 variants also revealed that variants with differing characteristics (such as charge, hydrophobicity, amphipathicity, and α-helicity) could show similar good antibiofilm activity. This finding may indicate that features that significantly impact antibacterial activity do not likewise affect antibiofilm activity. It is conceivable that the common amino acid sequence (FIRRIARLLRRIF) among all variations is the primary cause of the action [30]. Additionally, it has been revealed that this motif played a part in the penetrated peptides within the cell.

Moreover, the mechanism of action of arenicin-3 and its improved synthetic equivalent, AA139, was explored by a research group through examining its impacts on lipid bilayer models of membranes and *E. coli* bacterial cells. Arenicin-3 is an antimicrobial peptide with an amphipathic β-hairpin structure synthesized by the marine polychaete worm *Arenicola marina*. The amino acid residues of AA139 is derived from the parent sequence arenicin-3 through three specific amino acid substitutions, namely V8A, Y9R, and V13A. The modifications made to the amino acids resulted in a decrease in hydrophobicity and an increase in positive charge compared to arenicin-3. The alterations increased the antibacterial efficacy of AA139, surpassing that of arenicin-3 by a factor of 2–4. Conversely, the hemolytic potential of AA139 was approximately 15 times lower than that of arenicin-3. Upon evaluation of cytotoxicity against kidney cells (HEK-293), it was observed that AA139 exhibited a toxicity level that was three times lower than that of arenicin-3. The findings indicated that minor alterations in amino acid composition could significantly influence the physicochemical, biological and membrane selectivity characteristics of AMPs [7].

Furthermore, previous studies have demonstrated that the alteration in peptide length can impact its secondary structure when exposed to the bacterial membrane. An example of a peptide belonging to the Cathelicidin family, consisting of 12 residues, is 1018K-6. In the presence of bacterial membrane mimics, this peptide exhibited a conformation characterized by a combination of α-helical and β-sheet structures. Nevertheless, RiLK1, a shortened version of 1018K-6, is a peptide consisting of 10 amino acid residues primarily adopts a β-sheet shape, in the presence of SDS, as a mimic of the negatively charged outer layer of bacterial cell membranes. Based on the observed higher killing efficiency of RiLK1 compared to the parental peptide against *Listeria monocytogenes* and *Salmonella Typhimurium*, it can be inferred that alterations in conformation may contribute to the enhanced antibacterial potency of the peptide. This underscores the significance of the peptide's sequence and structural conformation in determining its functional attributes [31].

Concerning hemolytic activity, the number of amino acid residues from the C-terminal region of each peptide that is constantly embedded in the membrane of L-α-phosphatidylcholine vesicles could be used as a measure of how much the zwitterionic membrane has been disturbed, as this has a strong relationship with the amount of hemolysis. In a study of mastoparans analogs, the peptides MK9, MK89, MK58911, and MK5 had the highest number of amino acid residues in the C-terminal parts of their molecules that were consistently embedded in the membrane core, led to the most increased hemolytic activity [24].

3.3. The effect of structural features on antimicrobial activity

3.3.1. Facial amphiphilicity index

Facial amphiphilicity is a remarkable molecular structural characteristic of many antimicrobial peptides and polymers (Fig. 3). In recent years, the facial amphiphilicity index (FAI) was introduced as a metric used to assess the structural influence of a compound on the effectiveness of antibiotics and their cytotoxicity. The FAI was put forth as a way to relate chemical structures with antibacterial properties. The ratio of cationic charges to rings is known as the FAI. This parameters was studied for the design of several AMPs mimetic synthetic polymers. Cationic multicyclic molecules were utilized as representative systems to assess the impacts of FAIs. Result of a study showed that neither monocyclic nor bicyclic complexes with varying FAIs exhibit antibacterial activity. On the other hand, the influence of FAIs on tricyclic and tetracyclic systems was significant. Lower FAIs are associated with increased antibacterial efficacies, but the tendency of cytotoxicity was conversely seen. It can be concluded that the ideal FAIs can achieve a delicate equilibrium between exhibiting high antimicrobial activity and low cytotoxicity [49].

Here, among the literatures we retrieved from PubMed and Science Direct, the following data were achieved. In a study on AMPs, Peng Tan and his colleagues [33] used a heptad repeat sequence (abcdefg) model to create new peptides that were effective against fungi and biofilms. The designers strategically positioned Leu (L) residues at the "a" and "d" positions to create a hydrophobic core. This setup made it easier for peptides to enter the hydrophobic part of the fungal or bacterial lipid bilayer due to the induction of pore channels in membrane. Lys (K) was included as a cationic amino acid at positions "b" and "c" to add a positive charges. This modification ensured the peptides possessing an adequate driving force to establish an electrostatic connection with the negatively charged component of the fungal or bacterial cell wall. Subsequently, the three high-frequency amino acids, namely Cys (C), Ala (A), and Arg (R), are allocated to the vacant locations denoted by "e", "f", and "g", respectively. Two more heptad repeat sequence templates, namely $(LKKLACR)_n$ and $(LKKLCAR)_n$, were acquired based on the procedures as mentioned above. The findings demonstrated that the peptides' antimicrobial potential would be variable based on the number of repetition units. The antibacterial activity exhibited its highest level when the value of "n" equaled to 3, while each further increment in the number of repetition units resulted in a decrease in activity. Moreover, it was observed that the $(LKKLACR)_n$ series exhibited considerably higher activity compared to the $(LKKLCAR)_n$ series, despite both series of synthetic peptides having identical amino acid composition, peptide length, charge number, and hydrophobicity. Hence, the authors proposed that this outcome could be associated with amphipathicity. In contrast to the $(LKKLACR)_n$ series peptides, the $(LKKLCAR)_n$ series peptides exhibited enhanced hydrophobic and cationic characteristics on their surfaces. Numerous investigations have consistently demonstrated that the presence of a fully developed hydrophobic surface tends to diminish the overall activity of peptides. Moreover, the study's findings revealed that the existence of fully hydrophobic surfaces and positively charged surfaces was associated with an elevated level of toxicity [33].

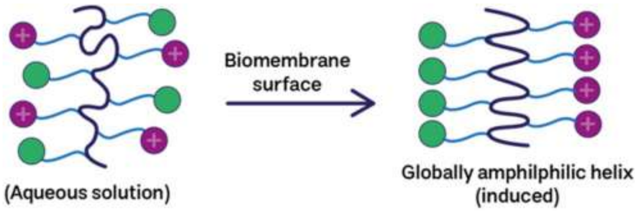


Fig. 3. Schematic presentation of global structures changes of amphiphilic antimicrobial peptides and polymers through interaction with membranes [adopted with redesigning from [49].

Furthermore, a study conducted by Jayawant et al. revealed that Lynronne-1, as an AMP with potential therapeutic applications, was in an unfolded state when exposed to aqueous circumstances. This unfolded conformation persisted even in the presence of membranes that mimic mammalian cells, resulting in an inability of Lynronne-1 to bind to these membrane effectively. Nevertheless, when exposed to detergents and anionic lipid vesicles containing phosphoglycerol, Lynornne-1 has a pronounced affinity for binding and undergoes a conformational change, adopting a highly stable α-helical shape spanning residues R3 to V15. This peptide sequence encompassed six cationic residues. Hence, it is imperative that the peptide needed particular lipid constituents in order to assume the suitable shape and engage with the membrane. Moreover, the presence of the α-helix structure is essential for the manifestation of antimicrobial activity. This study also proposed that the degree of α-helicity and the affinity for membrane attachment and lysis would be enhanced with an increase in the size of the hydrophobic helical surface. Nevertheless, it is important to highlight that there exists a correlation between the hydrophobicity of peptides and their hemolytic activity. This correlation arises from the fact that peptides possessing higher hydrophobicities have the capability to penetrate more deeply into the hydrophobic core of the erythrocyte membrane, hence leading to the occurrence of hemolysis [29].

3.3.2. Halogenation

The halogenation process exhibits a strong correlation with the activity, stability, and specificity of AMPs. According to literatures, halogenated α,α-disubstituted β-amino amides, amines, and guanidines have reported as promising antibacterial agents, particularly against difficult-to-treat Gram-negative multi-resistant clinical isolates. The compound 2,2-bis(3,5-dibromobenzyl)-3-guanidino-N-(2-guanidinoethyl) propenamide (referred to as 3,5-Br-Ph) is a diguanidine derivative. It has exhibited remarkably potent antibacterial properties against many bacterial strains, including those resistant to multiple antibiotics. Furthermore, it has exhibited no significant cytotoxic effects on human cell lines. Research conducted using liver microsomes has also demonstrated that the lipophilic side chains of 3,5-Br-Ph were resistant to Phase I oxidations. This characteristic suggested that these side chains may contribute to a high level of *in vivo* metabolic stability [50].

3.3.3. Lipid conjugation

The process of peptide lipidation has demonstrated its efficacy and cost-effectiveness in developing novel peptide-based pharmaceutical molecules for the future. Bacteria and fungi make natural lipopeptides (LiPs) by non-ribosomal processes during carbon source cultivation. The structure is formed by the covalent linkage of a hydrophobic lipid molecule with hydrophilic peptides consisting of 6–7 amino acids. Due to their lack of cell specificity, native liposomes have significant toxicity toward mammalian cells. Synthetic lipopeptides (LiPs) have garnered significant attention as a viable substitute for typical AMPs and native LiPs. This interest stems from their non-cytotoxic nature, remarkable effectiveness and persistence in living organisms, and the lowered expenses associated with their synthesis. The attachment of a carbon chain to the short AMP has been demonstrated to result in a significant amplification of antimicrobial efficacy, surpassing a 100-fold increase in activity for certain LiPs. Moreover, this modification has been found to decrease their harmful effects on mammalian cells compared to the original peptides or other antibacterial/antifungal substances [51].

Here, among the recently published literatures we retrieved, the following data were achieved. In a study, an investigation was conducted to examine the antimicrobial properties and mechanism of action of KYR-NH2, one of the shortest reported antimicrobial peptides. Additionally, the study examined the effects of lipid modification on KYR-NH2 by attaching four distinct fatty acids at the *N*-terminus. The antimicrobial activity of these changed forms was tested against four strains of pathogenic bacteria. The study highlights the robust antibacterial activity of the modified LiPs by introducing an acyl chain to change the ultrashort peptide. The maximum antibacterial activity was observed when an acyl chain consisting of 12–14 carbon atoms was added, suggesting that hydrophobicity plays a crucial role in determining the biological activity of this peptide. Significantly, this study also illustrated the essential collaboration between the aliphatic peptide component and hydrophobic lipid component of the LiPs in facilitating membrane destabilization, depolarization, and subsequent disintegration. These processes closely resembled the initial stages of the lethal mechanism observed in a typical antimicrobial peptide [26].

3.3.4. *Glycosylation*

Glycosylation represents an additional approach for addressing the limitations associated with antimicrobial peptides. Here, among the literatures we retrieved from PubMed and Science Direct, the following data were achieved. The compound [βGlc-T9, K7] indolicidin is a glycosylated peptide with strong antibacterial properties. It is structurally similar to indolicidin but possesses a lower level of toxicity towards red blood cells and macrophage cells compared to indolicidin. The peptide's manner of bacterial killing, LPS binding, functional stability, and cytokine inhibitory potential appeared to remain unchanged following glycosylation. However, the addition of sugar enhanced the solubility of the peptide [34].

3.3.5. *Topology*

The utilization of topology is crucial in assessing the structure–activity relationship during the development of physiologically active molecular structures. Moreover, topological characteristics play a crucial role in shaping the geometrical evolution of a prototypical protein structure. Stereochemistry has the ability to introduce topological diversity in otherwise identical molecules. Proteins are polymeric molecules that exhibit isotacticity, meaning they consist only of L-chiral amino acids as their monomeric components. Tacticity corresponds to the comparative stereochemistry of consecutive stereocenters within a polymeric macromolecule. A polymer chain is classified as an "isotactic" polymer if all of its stereocenters exhibit L-(S) or D-(R) configurational types solely. Syndiotactic molecules exhibit a pattern of alternating L- and D-stereocenters in a stereoregular manner. Conversely, if the stereocenters are arbitrarily dispersed in a non-uniform manner, the molecules are called 'heterotactic'. The majority of therapeutic peptides consist of asymmetric amino acids, exhibiting an L-chiral stereochemical configuration, so classifying them as stereoregular 'isotactic' heteropolymers. The observed stereo-specificity suggests the potential for expanding the fundamental amino acid sequence alphabet through the incorporation of D-enantiomers [21].

Here, among the literatures we retrieved from PubMed and Science Direct, the following data were achieved. A study examined the physiological effects of stereochemical mutation, specifically focusing on the topological variations it can produce. This was achieved by systematically designing a potential antimicrobial sequence. The results showed that the deliberate design of a heterotactic backbone leads to the imposition of topological constraints, effectively addressing the problem of nonspecific distribution and subsequent toxicity. Additionally, the syndiotactic peptides displayed unusual conformational rigidity and stability, allowing them to maintain the intended electrostatic environment when interacting with the membrane surface. All of the designed systems demonstrated bactericidal activity, highlighting the potential usefulness and specificity of stereo-engineered peptide systems in therapeutic applications [21].

The peptides L-Phenylseptin (L-Phes) and D-Phenylseptin (D-Phes), which both exist naturally, were found to have differential activity against *S. aureus* and *Xanthomonas axonopodis*. Specifically, the D-epimer of Phenylseptin had greater efficacy in comparison to the L-epimer against these bacterial strains. A study was conducted to evaluate the impact of D-phenylalanine in enhancing antibacterial activity. While the helical region from D4 to the C-terminus of both peptides exhibited structural similarity, notable structural distinctions were identified in the vicinity of the *N*-termini, which comprises the FFF motif. The maintenance of the F1-F3 (phenylalanine) residues on the hydrophobic face of the peptide, leading to an increased amphipathicity in comparison to the L-epimer, can be attributed to the involvement of specific aromatic interactions with the phenylalanine side chains of D-Phenylalanine. The enhanced capacity of D-Phenylalanine to effectively anchor within the hydrophobic core of the phospholipid bilayer suggests that the *N*-terminus plays a crucial role in promoting the contact between the D-peptide and the membrane interface, compared to its epimer [15].

3.4. *Receptor-specific activity of AMPs*

Some of AMPs act through interacting with specific targets. For example, proline-rich antimicrobial peptides (PrAMPs) such as insect oncocins, Tur1A from bottlenose dolphin *Tursiops truncates,* and bovine bactenecins (Bac7), target ribosome structure and interfere with protein synthesis [19,45]. Here, among the literatures we assessed, the following data were achieved. Drosocin (Dro) is one of the PrAMPs, which was extracted from fruit flies and can bind the ribosomal units, while it inhibited protein synthesis by a different mechanism of action. This mechanism has not been clearly known, however, a study showed that Dro may interact with sequestering class 1 release factors associated with the ribosome and therefore, it arrested ribosomes at stop codons. This mechanism of action had also been reported for apidaecin (Api) from honeybees. Scientist classified Api and Dro as the members of the type II PrAMP class. It was discovered that a few number of Api amino acids at C-terminal have important role in ribosome binding, in contrast, multiple amino acid residues distributed in the Dro sequence involved in its interaction with the ribosome, which a single-residue substitutions of them could significantly change the target-specific activity of Dro [52].

Another selective feature could be the specific binding of AMPs to the structural molecules of cells. For examples, Aracin 1 has shown the ability of binding to chitin, which is a component of fungal cell walls and the crustacean shell. The first 23 amino acid sequence of this peptide displayed chitin-binding features. Aracin 1 and its active and non-hemolytic fragment, Aracin 1 (1–23), were able to bind chitin [40].

Furthermore, it has been demonstrated that introducing a macrolactam ring at the C-terminal of a peptide can enhance both its antibacterial efficacy and selectivity towards bacterial cells. In this regard, a cyclic macrolactam ring was introduced at the C-terminal of a linear *de novo*-designed peptide called zp80. The peptide with a cyclic macrolactam ring, identified as KaDGD, has been designated zp90. The findings revealed that the ring had the potential to serve as a directing group, effectively controlling the cellular selectivity of the whole molecules through its preferred interaction with calcium ions. Consequently, it facilitated the aggregation of peptide zp90 specifically on the bacterial surface, while exhibiting no such effect on mammalian cells

[35].

Moreover, Theonellamides (TNMs) represent a notable illustration of peptides possessing a distinct receptor. *Theonella* sp. is the source of antifungal and cytotoxic bicyclic dodecapeptides. Theonellamides exhibited significant cytotoxic effects on P388 mouse leukemia cells, as well as antifungal properties in micromolar range, against *Candida*, *Trichophyton*, and *Aspergillus*. Using solid-state NMR experiments, it has been observed that TNM-A could exhibit direct interactions with 3β-hydroxysterols, such as cholesterol and ergosterol in phosphatidylcholine membranes. The affinity of TNM-A for membranes containing a mole ratio of 9:1 of POPC to cholesterol was 40 times higher compared to membranes without sterols [16].

Additionally, findings about the antimicrobial peptide tachyplesin demonstrated its efficacy against four distinct gram-negative pathogens, namely *E. coli, A. baumanni, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. These results indicated that tachyplesin exerted its antibacterial properties by inhibiting the FabG enzyme that is an essential enzyme for type II fatty acid biosynthesis in bacteria [17]. In a following study, cyclization of tachyplesin I, II and III was studied and the results showed that this strategy could not improve potency; however, cyclization improved AMPs stability in human serum, as well as, reduced their hemolytic activity. Type and orientation of these AMPs and their cyclic derivatives were different, but they all had similar potent antimicrobial and anticancer properties [18].

Moreover, several cyclotides have been investigated that exclusively bind to the lipids found in the phosphatidyleth anolamine (PE) membrane. This peptide-ligand affinity drives the permeabilization to the membranes including PE. The greater adsorption on PE-containing membranes due to PE-affinity, which was followed by a disintegration and depletion of PE-lipids, was ascribed to this selective lytic activity. Then, the membrane thins, becomes porous and vulnerable to peptide-induced leakage. Cyclotides are the only class of peptides that mediates the selective extraction of PE from membranes and has PE-specific lytic activity [20].

4. Computational methods using structural features in AMPs design

To use data of physicochemical and structural features in the design of novel potent AMPs, computational methods are the best choice. Recently, computational methods such as machine learning and molecular docking have been extensively applied to design and develop novel AMPs. This approach is the cause of less time, effort and cost that would be needed through *in silico* studies compared to experimental techniques. Aronica *et al.* summarize a relatively complete list of AMPs databases, prediction tools and machine learning algorithms used in antimicrobial peptide research [53]. Moreover, Franco *et al.* that was summarized the “Computer-Aided Design of Antimicrobial Peptides” until 2020, explained that two model of *de novo* designs would be applied including receptor-based and ligand-based using AMPs available structural information [54]. Some of tools and methods used in *in silico* design of AMPs were schematically presented in Fig. 4. In addition, computational approach has recently applied to detect novel AMPs within the organisms proteome using artificial intelligence tools [55]. Focusing of the computational techniques is not the aim of the current review, however, there are several valuable review in this regards [56,57]. As an example of considering physicochemical and structural features of AMPs in the design of novel AMP by computational approach, a group of researchers generated an AMP database (AMPad) of structure related features from frog-derived helical AMPs, in which the AMPs selectivity was defined by therapeutic index (TI). They concluded that the physicochemical descriptors related to sequences hydrophobic moments, could highly influence the selectivity of peptide antibiotics. Using different structural descriptors, they developed an AMP-Designer algorithm, which was tested by designing a short length glycine-rich AMP adepantin 1. The de novo designed AMP has a potent antibacterial activity against the *E. coli*, with a significantly superior selectivity (TI $>$ 200) than the best AMP of the AMPad database (TI $=$ 125) [58].

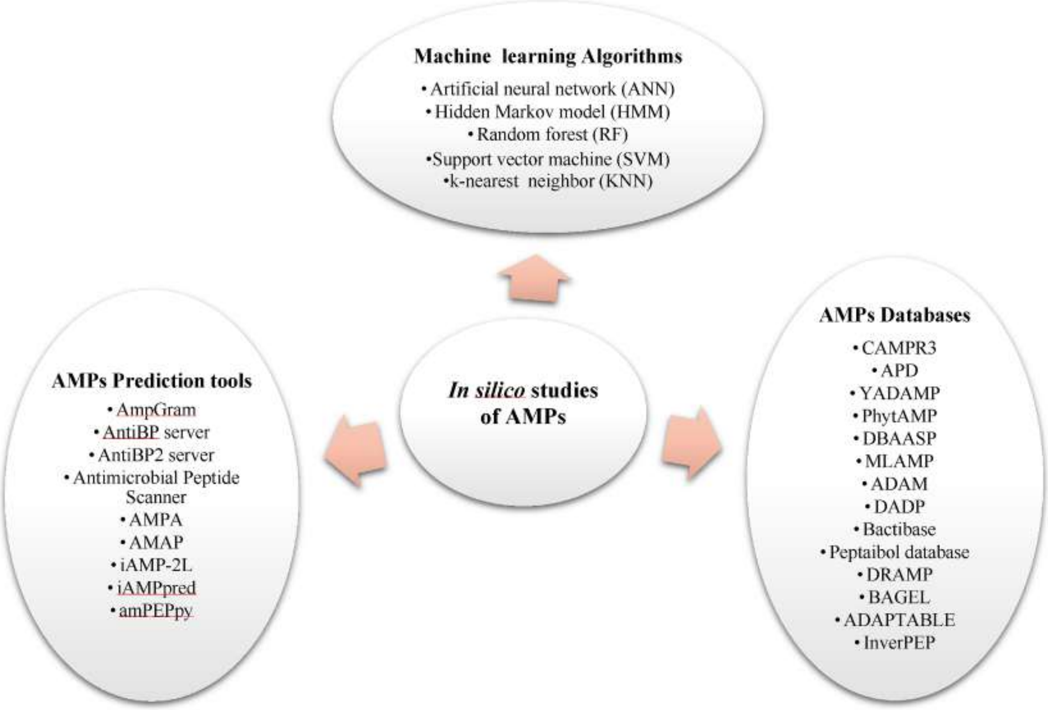


Fig. 4. A brief summary of databases, tools and algorithms used in in silico study of antimicrobial peptides. Data was extracted from [53,54,56].

5. Challenges in clinical application of AMPs

Due to the rapid spread of antimicrobial resistance to existing antimicrobials, the return of multidrug-resistant microorganisms, and the slow creation of new classes of antimicrobials, it is urgent that new classes of therapeutic molecules be made. A group of bioactive macromolecules called antimicrobial peptides (AMPs) are found in a variety of natural organisms and offer protection against microbial illnesses. They have been useful parts of the host defense system for a long time. Because microorganisms develop resistance to AMPs more slowly or later than they do to traditional antibiotics, AMPs have the potential to be valuable alternative therapies in the future. Numerous AMPs have been separated from a wide range of natural resources, such as humans, plants, animals, crustaceans and insects. Nevertheless, only some of them have been commercially translated for clinical consumption, so far; which was the result of a few intrinsic flaws in naturally occurring AMPs, such as their short half-life due to protease degradation, lack of activity at physiological salt concentrations, toxicity to mammalian cells, and the absence of suitable methods of targeted and sustained delivery of AMPs. The creation of synthetic analogs of AMPs, which would both avoid the drawbacks of their natural analogs and maintain or even increase their antibacterial efficacy, has become increasingly popular. Different studies confirmed that the chemical modifications of amino acids sequence, for example using of D-amino acid instead of L-amino acids, or using unnatural amino acid and cyclization could improve AMPs stability against enzymatic degradation. Moreover, glycosylation, PEGylation, lipidation, and grafting are the alternative strategies [59,60]. Another is the side-chain covalent bridge modification of AMPs that called stapled peptide and could result in an enhanced secondary structure stability, improved protease resistance and cell penetration, leading to more potent biological activity. These strategies can be performed by computational methods and artificial intelligence [61]. Additionally, conjugation with supramolecular polymers such as ureido-pyrimidinone (UPy) resulted in enhanced cell adhesion, and improved antimicrobial activity [62].

Another considerable challenge for clinical application of AMPs is their delivery. Wang et al. reviewed the formulation and delivery aspect of AMPs in 2021. They summarized the physical or chemical attachment of carries or targeting agent to improve the bioavailability, stability and targeted delivery of AMPs. This review presented a range of inorganic carriers, such as metal and silica based solid nanoparticles, and drug delivery systems consisting of bioorganic compounds like micelle, liposome and hydrogel [63].

6. Conclusion

Comprehending the structure–activity relationship of natural AMPs or their derivatives facilitates the development of novel synthetic AMPs. The studies have discovered that the relationship between the AMPs activity and their positive net charge, hydrophobicity, and amino acid sequence, as well as, the relationship between AMPs' function and other features like topology, lipophilicity, glycosyl functional groups, and halogens attachment. Research findings have indicated that the quantity and positioning of hydrophobic and positively charged amino acid residues play a critical role in inducing the structural attributes, such as α-helicity and amphipathicity, in amphipathic α-helical cationic AMPs. Consequently, these factors significantly influence the functionality of antimicrobial peptides. The point is that maintaining a delicate balance between positive charge and hydrophobicity is crucial in the design of AMPs that exhibit non-toxicity towards mammalian cells. Additionally, research has demonstrated that the hydrophobic nature of antimicrobial peptides plays a crucial role in their antibacterial efficacy and overall stability [32].

The presence of C-terminus loop structure and disulfide bonds are another structural characteristic that has been demonstrated to be crucial to the activity of AMPs. They strengthen the resistance to proteolytic cleavage. Furthermore, the amino acid sequence is believed to be important for the many other functionalities that AMPs carry out. A single amino acid substitution may change an AMP's capacity to act as an antibiotic or other therapeutic activity, according to certain research [22,23]. Variations in the length, content, and net charge of amino acids cause various mechanism of actions.

We also discussed that the topology is a critical tool for evaluating the structure–activity relationship when developing physiologically active molecule structures, thus, it needs adequate consideration when designing synthetic AMPs. Only a few research studies have examined how topological properties affect an AMP's antibacterial action, stability, and toxicity to mammalian cells. In general, topological limitations are imposed due to the intentional design of a heterotactic backbone, effectively addressing the issue of nonspecific dispersion and the following toxicity [64]. The syndiotactic peptides also exhibited peculiar structural stiffness and stability, which enabled them to interact with the membrane surface while preserving the desired electrostatic environment [21].

Other structural characteristics that need more attention are glycosylation and halogenation. According to certain research, there is a significant relationship between the halogenation process and AMP activity, stability, and specificity. Certain amines, guanidines, and halogenated α,α-disubstituted β-amino amides have been reported to exhibit promising antibacterial activity, especially against challenging Gram-negative multi-resistant clinical isolates [50]. Additionally, glycosylation is another way to overcome the restrictions related to antimicrobial peptides. The results demonstrate that the peptide's mechanisms of cytokine inhibitory capability, LPS binding, killing of bacteria, and functional stability seem to be unaffected by glycosylation. Nonetheless, the peptide's solubility is improved by the addition of sugar [34].

In conclusion, there are a number of structural features other than positive charge and hydrophobicity that should be considered when designing novel antimicrobial peptides in order to increase their antimicrobial potency and overcome the shortcomings of naturally occurring AMPs. More importantly, different researches have demonstrated that the combination of these structural properties determines the peptides performance, and individual structural elements can not significantly affect the peptides activity.

Funding

This manuscript was financially supported by Shahid Beheshti University of Medical Sciences, Tehran, Iran (Grant# 43003231).

Author contributions

F. Fathi, B. Alizadeh and M.V. Tabarzad have contributed in data search and gathering and prepared the initial draft. They equally contributed in preparing the manuscript. M. Tabarzad revised the initial drat and prepared the final manuscript. All authors read, reviewed and confirmed the final manuscript.

CRediT authorship contribution statement

Fariba Fathi: Writing – original draft, Data curation. **Bahareh Alizadeh:** Writing – original draft, Data curation. **Mohammad Vahid Tabarzad:** Writing – review & editing, Data curation. **Maryam Tabarzad:** Writing – review & editing, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] V. Lázár, A. Martins, R. Spohn, L. Daruka, G. Grézal, G. Fekete, M. Számel, P. K. Jangir, B. Kintses, B. Csörgő, Á. Nyerges, Á. Györkei, A. Kincses, A. Dér, F. R. Walter, M.A. Deli, E. Urbán, Z. Hegedűs, G. Olajos, O. Méhi, B. Bálint, I. Nagy, T. A. Martinek, B. Papp, C. Pál, Antibiotic-resistant bacteria show widespread collateral sensitivity to antimicrobial peptides, Nat. Microbiol. 3 (6) (2018) 718–731.

[2] F. Fathi, M. Ghobeh, M. Tabarzad, Anti-microbial peptides: strategies of design and development and their promising wound-healing activities, Mol. Biol. Rep. 49 (9) (2022) 9001–9012.

[3] P. Panahi Chegini, I. Nikokar, T. Hosseinabadi, M. Tabarzad, Concerns in the design and development of novel antimicrobial peptides, trends in peptide and protein, Sciences 1 (4) (2017) 135–143.

[4] H.B. Koo, J. Seo, Antimicrobial peptides under clinical investigation, Pept. Sci. 111 (5) (2019) e24122.

[5] R. Saravanan, X. Li, K. Lim, H. Mohanram, L. Peng, B. Mishra, A. Basu, J.-M. Lee, S. Bhattacharjya, S.S.J. Leong, Design of short membrane selective antimicrobial peptides containing tryptophan and arginine residues for improved activity, salt-resistance, and biocompatibility, Biotechnol. Bioeng. 111 (1) (2014) 37–49.

[6] A.T. Müller, A.C. Kaymaz, G. Gabernet, G. Posselt, S. Wessler, J.A. Hiss, G. Schneider, Sparse neural network models of antimicrobial peptide-activity relationships, Mol. Inf. 35 (11–12) (2016) 606–614.

[7] I.A. Edwards, S.T. Henriques, M.A. Blaskovich, A.G. Elliott, M.A. Cooper, Investigations into the membrane activity of arenicin antimicrobial peptide AA139, Biochim. Biophys. Acta (BBA)-Gen. Subj. 1866 (8) (2022) 130156.

[8] H.J. Park, H.K. Kang, E. Park, M.K. Kim, Y. Park, Bactericidal activities and action mechanism of the novel antimicrobial peptide Hylin a1 and its analog peptides against Acinetobacter baumannii infection, Eur. J. Pharm. Sci. 175 (2022) 106205.

[9] T. Rončević, D. Vukičević, L. Krce, M. Benincasa, I. Aviani, A. Maravić, A. Tossi, Selection and redesign for high selectivity of membrane-active antimicrobial peptides from a dedicated sequence/function database, Biochim. Biophys. Acta (BBA)-Biomembr. 1861 (4) (2019) 827–834.

[10] G. Sandhu, M.R. Morrow, V. Booth, Roles of histidine charge and cardiolipin in membrane disruption by antimicrobial peptides Gaduscidin-1 and Gaduscidin-2, Biochim. Biophys. Acta Biomembr. 1862 (11) (2020) 183444.

[11] N.D.T. Tram, V. Selvarajan, A. Boags, D. Mukherjee, J.K. Marzinek, B. Cheng, Z.-C. Jiang, P. Goh, J.-J. Koh, J.W.P. Teo, P.J. Bond, P.L.R. Ee, Manipulating turn residues on de novo designed β-hairpin peptides for selectivity against drug-resistant bacteria, Acta Biomater. 135 (2021) 214–224.

[12] Y. Ma, A. Yao, X. Chen, L. Wang, C. Ma, X. Xi, T. Chen, C. Shaw, M. Zhou, Generation of truncated derivatives through in silico enzymatic digest of peptide GV30 target MRSA both in vitro and in vivo, Comput. Struct. Biotechnol. J. 19 (2021) 4984–4996.

[13] J.C. Bozelli, L.C. Salay, M. Arcisio-Miranda, J. Procopio, K.C.T. Riciluca, P.I. Silva Junior, C.R. Nakaie, S. Schreier, A comparison of activity, toxicity, and conformation of tritrpticin and two TOAC-labeled analogues. Effects on the mechanism of action, Biochim. Biophys. Acta Biomembr. 1862 (2) (2020) 183110.

[14] H. Hu, B. Di, W.D. Tolbert, N. Gohain, W. Yuan, P. Gao, B. Ma, Q. He, M. Pazgier, L. Zhao, W. Lu, Systematic mutational analysis of human neutrophil α-defensin HNP4, Biochim. Biophys. Acta Biomembr. 1861 (4) (2019) 835–844.

[15] V.H.O. Munhoz, C.S. Ferreira, L.O. Nunes, T.L. Santos, C. Aisenbrey, R. Adão, A. F. de Carvalho Alcântara, M.T.Q. de Magalhães, D. Piló-Veloso, J.M. Resende, B. Bechinger, R.M. Verly, Epimers l- and d-Phenylseptin: How the relative stereochemistry affects the peptide-membrane interactions, Biochim. Biophys. Acta Biomembr. 1863 (11) (2021) 183708.

[16] K. Cornelio, R.A. Espiritu, S. Hanashima, Y. Todokoro, R. Malabed, M. Kinoshita, N. Matsumori, M. Murata, S. Nishimura, H. Kakeya, M. Yoshida, S. Matsunaga, Theonellamide A, a marine-sponge-derived bicyclic peptide, binds to cholesterol in aqueous DMSO: Solution NMR-based analysis of peptide-sterol interactions using hydroxylated sterol, Biochim. Biophys. Acta Biomembr. 1861 (1) (2019) 228–235.

[17] C. Liu, J. Qi, B. Shan, Y. Ma, Tachyplesin causes membrane instability that kills multidrug-resistant bacteria by inhibiting the 3-ketoacyl carrier protein reductase FabG, Front. Microbiol. 9 (2018) 825.

[18] F. Vernen, P.J. Harvey, S.A. Dias, A.S. Veiga, Y.H. Huang, D.J. Craik, N. Lawrence, S. Troeira Henriques, Characterization of tachyplesin peptides and their cyclized analogues to improve antimicrobial and anticancer properties, Int. J. Mol. Sci. 20 (17) (2019).

[19] M. Mardirossian, N. Pérébaskine, M. Benincasa, S. Gambato, S. Hofmann, P. Huter, C. Müller, K. Hilpert, C.A. Innis, A. Tossi, D.N. Wilson, The dolphin proline-rich antimicrobial peptide tur1a inhibits protein synthesis by targeting the bacterial ribosome, Cell Chem. Biol. 25 (5) (2018) 530–539.e7.

[20] A.A. Strömstedt, S. Park, R. Burman, U. Göransson, Bactericidal activity of cyclotides where phosphatidylethanolamine-lipid selectivity determines antimicrobial spectra, Biochim. Biophys. Acta Biomembr. 1859 (10) (2017) 1986–2000.

[21] P.K. Hazam, G. Jerath, A. Kumar, N. Chaudhary, V. Ramakrishnan, Effect of tacticity-derived topological constraints in bactericidal peptides, Biochim. Biophys. Acta Biomembr. 1859 (8) (2017) 1388–1395.

[22] C. Dal Mas, D.A. Pinheiro, J.D. Campeiro, B. Mattei, V. Oliveira, E.B. Oliveira, A. Miranda, K.R. Perez, M.A.F. Hayashi, Biophysical and biological properties of small linear peptides derived from crotamine, a cationic antimicrobial/antitumoral toxin with cell penetrating and cargo delivery abilities, Biochim. Biophys. Acta Biomembr. 1859 (12) (2017) 2340–2349.

[23] L.N. Irazazabal, W.F. Porto, S.M. Ribeiro, S. Casale, V. Humblot, A. Ladram, O. L. Franco, Selective amino acid substitution reduces cytotoxicity of the antimicrobial peptide mastoparan, Biochim. Biophys. Acta Biomembr. 1858 (11) (2016) 2699–2708.

[24] B.M.d. Souza, M.P.d.S. Cabrera, P.C. Gomes, N.B. Dias, R.G. Stabeli, N.B. Leite, J. R. Neto, M.S. Palma, Structure–activity relationship of mastoparan analogs: Effects of the number and positioning of Lys residues on secondary structure, interaction with membrane-mimetic systems and biological activity, Peptides 72 (2015) 164–174.

[25] M.-A. Sani, T.-H. Lee, M.-I. Aguilar, F. Separovic, Proline-15 creates an amphipathic wedge in maculatin 1.1 peptides that drives lipid membrane disruption, Biochim. Biophys. Acta (BBA) Biomembr. 1848 (10, Part A) (2015) 2277–2289.

[26] S. Nasompag, P. Dechsiri, N. Hongsing, P. Phonimdaeng, S. Daduang, S. Klaynongsruang, T.A. Camesano, R. Patramanon, Effect of acyl chain length on therapeutic activity and mode of action of the CX-KYR-NH2 antimicrobial lipopeptide, Biochim. Biophys. Acta Biomembr. 1848 (10, Part A) (2015) 2351–2364.

[27] Y. Liu, Q. Du, C. Ma, X. Xi, L. Wang, M. Zhou, J.F. Burrows, T. Chen, H. Wang, Structure-activity relationship of an antimicrobial peptide, Phylloseptin-PHa: balance of hydrophobicity and charge determines the selectivity of bioactivities, Drug Des. Devel. Ther. 13 (2019) 447–458.

[28] M.A. Hitchner, L.E. Santiago-Ortiz, M.R. Necelis, D.J. Shirley, T.J. Palmer, K. E. Tarnawsky, T.D. Vaden, G.A. Caputo, Activity and characterization of a pH-sensitive antimicrobial peptide, Biochim. Biophys. Acta Biomembr. 1861 (10) (2019) 182984.

[29] E.S. Jayawant, J. Hutchinson, D. Gašparíková, C. Lockey, L. Pruñonosa Lara, C. Guy, R.L. Brooks, A.M. Dixon, Molecular basis of selectivity and activity for the antimicrobial peptide lynronne-1 informs rational design of peptide with improved activity, ChemBioChem 22 (14) (2021) 2430–2439.

[30] S. Mangmee, O. Reamtong, T. Kalambaheti, S. Roytrakul, P. Sonthayanon, Antimicrobial peptide modifications against clinically isolated antibiotic-resistant Salmonella, Molecules 26 (15) (2021) 4654.

[31] B. Agrillo, Y.T.R. Proroga, M. Gogliettino, M. Balestrieri, R. Tatè, L. Nicolais, G. Palmieri, A safe and multitasking antimicrobial decapeptide: the road from de novo design to structural and functional characterization, Int. J. Mol. Sci. 21 (18) (2020) 6952.

[32] N. Zhang, X. Gu, D. Song, P. Zhang, N. Zhang, W. Chen, S. Ji, Y. Qi, S. Ma, Rational design and synthesis of Oreoch-2 analogues as efficient broad-spectrum antimicrobial peptides, Bioorg. Chem. 119 (2022) 105583.

[33] P. Tan, Z. Lai, Q. Jian, C. Shao, Y. Zhu, G. Li, A. Shan, Design of heptad repeat amphiphiles based on database filtering and structure-function relationships to combat drug-resistant fungi and biofilms, ACS Appl. Mater. Interfaces 12 (2) (2020) 2129–2144.

[34] R. Dwivedi, P. Aggarwal, N.S. Bhavesh, K.J. Kaur, Design of therapeutically improved analogue of the antimicrobial peptide, indolicidin, using a glycosylation strategy, Amino Acids 51 (10–12) (2019) 1443–1460.

[35] P. Zeng, Q. Cheng, L. Yi, S. Shui Yee Leung, S. Chen, K.F. Chan, K.Y. Wong, C-terminal modification of a de novo designed antimicrobial peptide via capping of macrolactam rings, Bioorg. Chem. 130 (2023) 106251.

[36] T.A. Stone, G.B. Cole, D. Ravamehr-Lake, H.Q. Nguyen, F. Khan, S. Sharpe, C. M. Deber, Positive charge patterning and hydrophobicity of membrane-active antimicrobial peptides as determinants of activity, toxicity, and pharmacokinetic stability, J. Med. Chem. 62 (13) (2019) 6276–6286.

[37] S.Y. Hong, T.G. Park, K.-H. Lee, The effect of charge increase on the specificity and activity of a short antimicrobial peptide, Peptides 22 (10) (2001) 1669–1674.

[38] P.G. Lima, J.T.A. Oliveira, J.L. Amaral, C.D.T. Freitas, P.F.N. Souza, Synthetic antimicrobial peptides: Characteristics, design, and potential as alternative molecules to overcome microbial resistance, Life Sci. 278 (2021) 119647.

[39] L. Xu, S. Chou, J. Wang, C. Shao, W. Li, X. Zhu, A. Shan, Antimicrobial activity and membrane-active mechanism of tryptophan zipper-like β-hairpin antimicrobial peptides, Amino Acids 47 (2015) 2385–2397.

[40] V.S. Paulsen, H.-M. Blencke, M. Benincasa, T. Haug, J.J. Eksteen, O.B. Styrvold, M. Scocchi, K. Stensvåg, Structure-activity relationships of the antimicrobial peptide arasin 1 — and mode of action studies of the N-terminal, proline-rich region, PLoS ONE 8 (1) (2013) e53326.

[41] A.K. Mishra, J. Choi, E. Moon, K.-H. Baek, Tryptophan-rich and proline-rich antimicrobial peptides, Molecules 23 (4) (2018) 815.

[42] M. Pirtskhalava, B. Vishnepolsky, M. Grigolava, G. Managadze, Physicochemical features and peculiarities of interaction of AMP with the membrane, Pharmaceuticals (Basel) 14 (5) (2021) 471.

[43] E.M. Kohn, D.J. Shirley, L. Arotsky, A.M. Picciano, Z. Ridgway, M.W. Urban, B. R. Carone, G.A. Caputo, Role of cationic side chains in the antimicrobial activity of C18G, Molecules 23 (2) (2018) 329.

[44] 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 10 (3) (2018) 408–419.

[45] M. Graf, M. Mardirossian, F. Nguyen, A.C. Seefeldt, G. Guichard, M. Scocchi, C. A. Innis, D.N. Wilson, Proline-rich antimicrobial peptides targeting protein synthesis, Nat. Prod. Rep. 34 (7) (2017) 702–711.

[46] O. Koksharova, N. Safronova, A. Dunina-Barkovskaya, New antimicrobial peptide with two CRAC motifs: activity against Escherichia coli and Bacillus subtilis, Microorganisms 10 (8) (2022) 1538.

[47] M.D. Libardo, S. Nagella, A. Lugo, S. Pierce, A.M. Angeles-Boza, Copper-binding tripeptide motif increases potency of the antimicrobial peptide Anoplin via Reactive Oxygen Species generation, Biochem. Biophys. Res. Commun. 456 (1) (2015) 446–451.

[48] M.A. Cherry, S.K. Higgins, H. Melroy, H.S. Lee, A. Pokorny, Peptides with the same composition, hydrophobicity, and hydrophobic moment bind to phospholipid bilayers with different affinities, J. Phys. Chem. B 118 (43) (2014) 12462–12470.

[49] L.B. Kurnaz, Y. Luo, X. Yang, A. Alabresm, R. Leighton, R. Kumar, J. Hwang, A. W. Decho, P. Nagarkatti, M. Nagarkatti, Facial amphiphilicity index correlating chemical structures with antimicrobial efficacy, Bioact. Mater. 20 (2023) 519–527.

[50] H. Marianne, A. Dominik, B. Annette, E. Magnus, H. Terkel, H. Tor, Antimicrobial activity of amphipathic a, a-disubstituted b-amino amide derivatives against ESBL e CARBA producing multi-resistant bacteria; effect of halogenation, lipophilicity and cationic character, Eur. J. Med. Chem. 183 (2019) 111671.

[51] V. Posada, B.F. Espejo, S. Orduz, De novo design of short antimicrobial lipopeptides, An. Acad. Bras. Cienc. 93 (2021) e20210362.

[52] K. Mangano, D. Klepacki, I. Ohanmu, C. Baliga, W. Huang, A. Brakel, A. Krizsan, Y. S. Polikanov, R. Hoffmann, N. Vázquez-Laslop, A.S. Mankin, Inhibition of translation termination by the antimicrobial peptide Drosocin, Nat. Chem. Biol. (2023).

[53] P.G.A. Aronica, L.M. Reid, N. Desai, J. Li, S.J. Fox, S. Yadahalli, J.W. Essex, C. S. Verma, Computational methods and tools in antimicrobial peptide research, J. Chem. Inf. Model. 61 (7) (2021) 3172–3196.

[54] M.H. Cardoso, R.Q. Orozco, G. Rodrigues, K.G. Oshiro, E.S. Cândido, O.L. Franco, Computer-aided design of antimicrobial peptides: are we generating effective drug candidates? Front. Microbiol. 10 (2020) 501217.

[55] D. Monsalve, A. Mesa, L.M. Mira, C. Mera, S. Orduz, J.W. Branch-Bedoya, Antimicrobial peptides designed by computational analysis of proteomes, Antonie Van Leeuwenhoek 117 (1) (2024) 55.

[56] M. Kordi, Z. Borzouyi, S. Chitsaz, M.h. Asmaei, R. Salami, M. Tabarzad, Antimicrobial peptides with anticancer activity: Today status, trends and their computational design, Arch. Biochem. Biophys. 733 (2023) 109484.

[57] M. Nedyalkova, A.S. Paluch, D.P. Vecini, M. Lattuada, Progress and future of the computational design of antimicrobial peptides (AMPs): bio-inspired functional molecules, Digital Discovery 3 (1) (2024) 9–22.

[58] D. Juretić, D. Vukičević, N. Ilić, N. Antcheva, A. Tossi, Computational design of highly selective antimicrobial peptides, J. Chem. Inf. Model. 49 (12) (2009) 2873–2882.

[59] N. Chen, C. Jiang, Antimicrobial peptides: Structure, mechanism, and modification, Eur. J. Med. Chem. 255 (2023) 115377.

[60] S.B. Rezende, K.G.N. Oshiro, N.G.O. Júnior, O.L. Franco, M.H. Cardoso, Advances on chemically modified antimicrobial peptides for generating peptide antibiotics, Chem. Commun. 57 (88) (2021) 11578–11590.

[61] Y. You, H. Liu, Y. Zhu, H. Zheng, Rational design of stapled antimicrobial peptides, Amino Acids 55 (4) (2023) 421–442.

[62] S. Zaccaria, R.C. van Gaal, M. Riool, S.A.J. Zaat, P.Y.W. Dankers, Antimicrobial peptide modification of biomaterials using supramolecular additives, J. Polym. Sci. A Polym. Chem. 56 (17) (2018) 1926–1934.

[63] C. Wang, T. Hong, P. Cui, J. Wang, J. Xia, Antimicrobial peptides towards clinical application: Delivery and formulation, Adv. Drug Deliv. Rev. 175 (2021) 113818.

[64] P.K. Hazam, R. Akhil, G. Jerath, J. Saikia, V. Ramakrishnan, Topological effects on the designability and bactericidal potency of antimicrobial peptides, Biophys. Chem. 248 (2019) 1–8.