ANTIMICROBIAL PEPTIDES AND TEMPORIN FAMILY IN THE CONTEXT OF RISING RESISTANCE - VIEW ON CURRENT DEVELOPMENT

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Received 25 February 2026
Accepted 23 March 2026 *DOI: 10.59957/jctm.v61.i3.2026.1*

ABSTRACT

Antimicrobial resistance has surfaced as a gradual yet progressively significant threat to global public health, frequently overshadowed by more widely pressing issues like cancer and cardiovascular disease. Nonetheless, the rapid proliferation of multidrug-resistant pathogens has gathered worldwide attention, prompting organizations like the World Health Organization to classify it as one of the most urgent challenges that modern medicine must face. The reduced efficacy of conventional antibiotics has led to increasing treatment failures, prolonged hospitalizations, and elevated mortality rates, highlighting the urgent need for novel therapeutic approaches. In response to this, there is an increasing interest in overcoming the limitations of conventional antibiotics by developing novel treatment alternatives. Among these alternatives, antimicrobial peptides have attracted considerable attention due to their broad-spectrum efficacy, which includes antibacterial, antiviral, antitumour activities, alongside a unique mechanism of action, coupled with reduced likelihood of inducing resistance. The Temporin family is one of the groups in this category. It was originally isolated from amphibian skin secretions and has shown significant potential as a new source of antibacterial, antiviral, and antifungal agents. Temporins show strong antibacterial properties, particularly against Gram-positive bacteria. Their simple structures make them attractive candidates for enhancement and therapeutic applications. Ongoing research on temporins and other AMPs holds great promise for finding a solution to the global problem of antimicrobial resistance and for shaping the next generation of infection-fighting therapeutics.

Keywords: antimicrobial resistance, antimicrobial peptides, temporin, temporin A, unnatural amino acids.

INTRODUCTION

The year 2020 brought forth extraordinary challenges as the world confronted the COVID-19 pandemic. Not only did the pandemic put enormous pressure on the medical systems and the medics around the world [1, 2], but also on the economy [3]. COVID-19 illustrated that optimization, combined with unique, novel approaches in the diagnosis and therapy are required, especially when dealing with complicated, though to treat infections and viruses. According to a study of Nandi et al. (2023) for the period of 2020-2022 in 71 countries, 75% of COVID-19 patients were prescribed antibiotics, even though bacterial coinfection rates were less than 10 %. The COVID-19 pandemic brought antibiotic abuse and antimicrobial resistance (AMR) back into the spotlight [4].

AMR is a major public health concern that is reaching a critical level. AMR refers to the ability of microorganisms to survive exposure to antimicrobial agents [5]. Antimicrobial-resistant organism infections are not only hard to cure, but they also carry a constant risk of serious following illness and even final lethal end is possible [6]. The large and frequent use of antibiotics has contributed to the development of resistance in certain strains of microorganisms. The World Health Organization agrees that the focus of research should be development of novel antimicrobial substances. For

the last 45 years, there have not been new antibiotics discovered, only derivatives or combinations of existing ones have been marketed [5]. In 2019, an estimated 1.27 million deaths worldwide were directly linked to AMR and for further 4.95 million deaths AMR was indirectly responsible. The perspectives suggest that, within the next 30 years, AMR could exceed cancer as a cause of mortality, potentially resulting in up to 10 million deaths annually in 2050 [7].

This review article aims to show antimicrobial peptides (AMPs) as a powerful candidate in the battle against AMR and to introduce the Temporin family as a promising group of AMPs, as well as to summarize the existing until the moment research in scientific literature on their characteristics, activity and possible uses.

Antimicrobial resistance

The discovery of antibiotics is a key milestone in modern medicine. The antibiotic era started with salvarsan and neosalvarsan in 1910, followed by prontosil and the discovery of penicillin from *Penicillium rubens* in 1928. Most current antibiotics were discovered between the 1940s and 1960s, marking the period as the "Golden Age". Since then, new discoveries have slowed down, but drug resistance is on the rise. This has been an issue that has been recognized since antibiotics first emerged [6, 8]. Methicillin was introduced in 1959 and within a year, methicillin-resistant strains of *Staphylococcus* were discovered. In 1958, the glycopeptide antibiotic vancomycin was proposed as a rescue therapy for infections caused by severe *Staphylococci* infections and after 1960 as a standard treatment against methicillin-resistant *Staphylococci*. Nevertheless, vancomycin-resistant coagulase-negative *Staphylococci* were documented by 1979. The β-lactam antibiotic cephalosporin was initially prescribed in 1964 to address penicillin-resistant cases. Since then, several cephalosporin generations have been developed. Emerging resistance has presented significant challenges for every previous generation. After tetracycline discovery in 1950, it had lost the ability to combat *Shigella* strains very fast till 1960 [6, 9].

Research studies indicate that certain antibiotics, including azithromycin, ciprofloxacin, and minocycline, exhibit additional properties such as antiviral, antitumor, or anticancer, highlighting their unforeseen nonantibiotic effects [10 - 13]. Some experimental data indicates that antimicrobial agents may exhibit similar selectivity and mechanisms of action. The similarity is due to the strong negative charge of the bacterial membranes. This is mostly due to the presence on the surface of anionic molecules like glycoconjugates and heparan sulfate [14 - 17]. AMPs are part of these substances that stand out. They are a promising pharmaceutical alternative because they can kill bacteria in various pathways and change the immune system in beneficial manners. In vitro tests for determining minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) show that AMPs can effectively target multidrug-resistant (MDR) bacteria [18].

Over the past 60 years, the widespread use and production of antibiotics have had a significant negative impact on the environment. These drugs are becoming more affordable, and used by people, often applied without a prescription. This is a big part of why microbes are becoming resistant to antibiotics. This resistance arises because people keep misusing and overusing antibiotics, which spreads through microbial populations all over the world [8]. According to Klein et al., antibiotic consumption, measured in defined daily doses (DDD), increased by 65 % between 2000 and 2015, while the rate of antibiotic consumption rose by 39 % during the same period [19]. Beyond human consumption, a significant contributing factor to the emergence and progression of antimicrobial resistance is their application as additives in agriculture, such as growth promoters for livestock [6, 20]. Bacteria that develop antibiotic resistance are also more likely to proliferate within humans, animals, and the environment [21]. Antimicrobial agents, such as disinfectants, preservatives, and antibiotics, have been extensively utilized throughout history and are anticipated to continue playing a crucial role in the management and eradication of microorganisms. Depending on the mechanism of their synthesis these agents can be classified as natural, semi-synthetic, or synthetic substances that can inhibit, impede, or terminate the growth and reproduction of various microbes. Their effectiveness can be related to mechanisms that interfere with physiological functions and microbial metabolism, such as translation, DNA replication, and cell wall biosynthesis [22]. Upon exposure to antimicrobial agents, bacteria can exhibit a range of biological and molecular responses. Bacterial resistance mechanisms can be categorized based on defined criteria. A particular

approach categorizes antimicrobial resistance into intrinsic and acquired types, depending on the source of the resistance characteristics. Intrinsic resistance pertains to the fundamental traits of a bacterium that provide resistance. For instance, Gram-negative bacteria demonstrate glycopeptide resistance because of the impermeability of their outer membrane within the cell envelope. On the other hand, acquired resistance arises when a bacterium that was once susceptible develops a resistance mechanism, either through genetic mutation or by acquiring new genetic material through horizontal gene transfer [9, 22, 23]. In their review, Zhou et al. identified three primary lines of defence based on the location of resistance: biofilm formation as the initial barrier; the cell wall and membrane, supplemented by efflux pumps, constituting the second line; and alterations in intracellular biochemistry and genetic responses upon entry of bactericides into bacterial cells representing the third mechanism of resistance [22].

Antimicrobial peptides (AMPs)

AMPs have emerged as a potentially useful alternative for the treatment of a broad-spectrum microorganism. In 1957, Robert Skarnes made the discovery that blood cells contain antimicrobial peptides. Since then, there have been over 1 200 AMPs discovered, and they have come from a wide variety of sources, including bacteria, plants, insects, and animals [5]. AMPs work differently than regular antibiotics. Firstly, they neutralize the charge on cell membranes before further interaction with them. After that, they enter the membranes and kill the bacteria. This specific mechanism of action makes it less likely to lead to bacterial resistance. AMPs are also effective against certain types of bacteria that are resistant to common antibiotics, and they are effective even at low concentrations. Such a typical example is thienamycin, active against *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* [24]. Furthermore, they can be used in conjugation with conventional antibiotics to achieve a synergistic effect on the condition being treated. In addition, they are thought to be safe for use, with fewer or no toxic side effects due to the natural mechanism of their metabolism. When compared to conventional antibiotics, they have the advantage of possessing antibacterial, antifungal, and antiviral properties that are broad-spectrum [18, 25, 26]. Natural peptides possess multifunctional activity, i.e. are being able to target several points of interest [5, 26]. Most AMPs display cationic and amphiphilic characteristics, usually possessing a positive charge. The length ranges from 10 to 60 amino acid residues, exhibiting an overall charge between +2 to +9. These peptides have positively charged amino acids like lysine and arginine, as well as hydrophobic residues [25 - 27]. A significant number of living organisms generate AMPs as part of their primary immune response, which are crucial for adaptive immunity, particularly in the context of autoimmune disorders and cancers, as well as offering protection against bacterial, viral, and fungal infections [18].

The diversity of natural AMPs is a problem when talking about their classification. However, they can still be clustered according to their origin, activity, and structural traits (Fig. 1) [26, 27].

Based on their origin, they can be divided into four groups: peptides from insects, microorganisms, amphibians, and mammals. The remarkable adaptability of insects can be ascribed to AMPs, produced in their blood cells and fat bodies. Cecropins, recognized as the most prominent family of AMPs and are identified in bees, guppy silkworms, and *Drosophila*. Bacteria and fungi have the potential to produce antimicrobial peptides as well. Some examples of these peptides include nisin and gramicidin, which originate from *Bacillus subtilis*, *Lactococcus lactis* and *Bacillus brevis*. Amphibian defense mechanisms against pathogens rely heavily on antimicrobial peptides. Frogs serve as one of the main sources of antimicrobial peptides with magainin being the most studied one. The mammalian AMPs are primarily composed of host defense peptides (HDPs) like cathelicidins and defensins. Lactoferricin B, cathelicidin LL-37, and human β-defensin 2 are notable examples from this category [26, 27].

A summary of their activities includes peptides with antitumour, antiviral, antifungal, antibacterial, and antiparasitic properties. Nisin, defensins, and cecropins are just a few of the many AMPs that have been shown to work against both Gram-positive and Gram-negative bacteria. Antifungal peptides are a type of AMPs that are designed to fight fungal infections that are difficult to treat with traditional drugs. Brevinine, cecropin, and ranatuerin are some examples of this group. Antiviral peptides represent a significant category

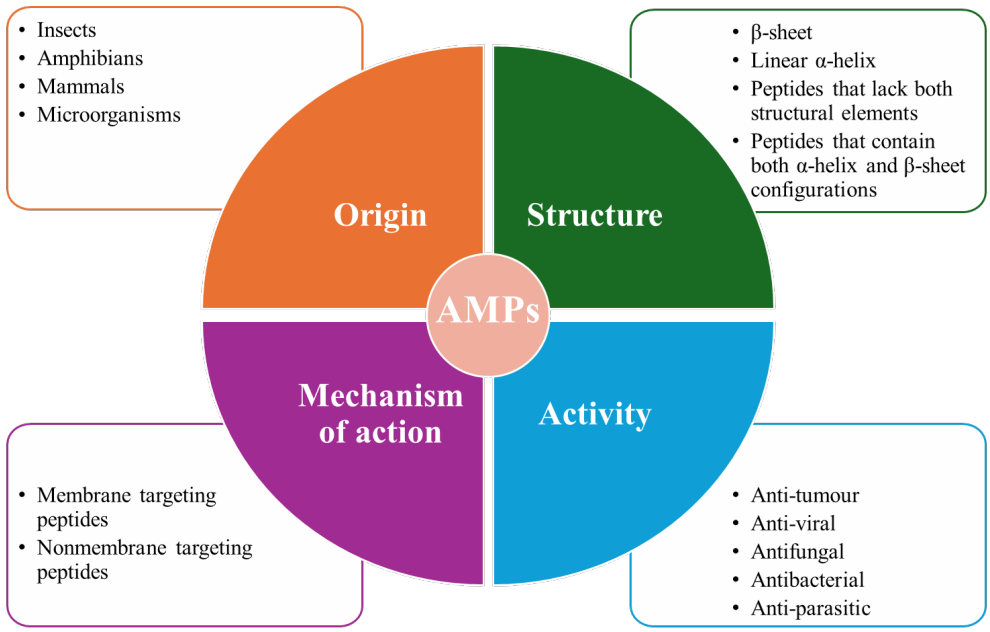

Fig. 1. Classification of antimicrobial peptides.

of AMPs that exhibit a strong efficacy in eliminating viruses. Antiparasitic peptides possess the capability to eliminate parasites that cause diseases such as malaria and leishmaniasis. The anticancer peptides exhibit their effects through several mechanisms: they recruit immune cells to eliminate tumour cells, induce necrosis or apoptosis in cancer cells, inhibit angiogenesis to prevent tumour growth and metastasis, and activate specific regulatory proteins to hinder the transcription and translation of tumour cell genes [26, 27].

AMPs can be categorized into four distinct groups according to their structures: β-sheet peptides, linear α-helix peptides, peptides that lack both structural elements, and those that contain both α-helix and β-sheet configurations. The α-helix conformational peptides are a fairly diverse and well-studied group of AMPs. Hundreds of different sequences have already been found in nature. The sequences usually have 12 to 40 amino acid residues, and they have a lot of helix-stabilizing residues like alanine, lysine and leucine. Each β-family AMP contains at least one pair of β-strands. Almost all the AMPs in this category contain cysteine residues, which have the ability to form one or more disulfide bridges, thereby accounting for their structural stability. The latter in solution is notably enhanced, and they do not substantially modify the structural composition of the membrane environment. AMPs featuring both α-helix and β-sheet structures have been documented across a diverse range of invertebrates and plants, as well as in humans and other mammals. The categorization of these AMPs is based on the different configurations of their three to five disulfide bonds. Certain AMPs are characterized as extended linear structures due to their lack of a defined 3D conformation in solution or upon interaction with membranes. The peptides in question are generally composed of enriched amino acids like glycine, proline, tryptophan, or histidine, and they do not contain α-helices or β-sheets [27, 28].

There are two distinct mechanisms for AMPs' antimicrobial effects - by targeting membrane, thus breaking down the cell membrane and those AMPs that do not target membranes and stop the production of nucleic acids, essential enzymes, and functional proteins [27, 29]. Some of the important physicochemical properties of AMPs that affect how they interact with membranes and ultimately compromise the integrity of the membrane are their net charge, amphipathicity, hydrophobicity, membrane curvature, and tendency to self-aggregate (Fig. 2). The mechanism of action of membrane-active AMPs is primarily elucidated through hydrophobic and cationic interactions [25, 29].

The initial binding of positively charged residues of AMPs to the negatively charged bacterial cell surface is primarily driven by electrostatic attraction. Complexes of

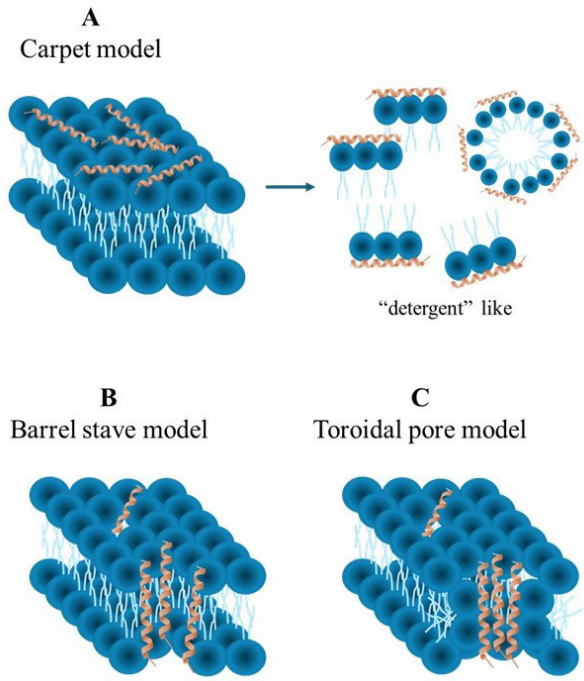

Fig. 2. AMP extracellular action models: (a) Carpet model: AMPs gather on the surface and destroy the cell membrane like "detergent"; (b) Barrel stave model: several AMPs come together and enter the cell membrane bilayer to form a channel; (c) Toroidal pore model: AMPs vertically embed in cell membrane and bend to form ring hole [27].

peptides or lipid - peptide arise when the concentration of antimicrobial peptides bound to the membrane increases. Upon reaching a critical concentration of AMPs on the membrane, they penetrate the hydrophobic core of the bilayer, resulting in the formation of transmembrane pores in the cytoplasmic membrane. The barrel - stave model suggests that AMP molecules undergo self-assembly upon adsorption to the membrane surface, facilitated by interactions with hydrophilic peptide regions. The peptide bulks undergo a perpendicular rotation relative to the plasma membrane once the accumulated peptide monomers attain a certain density on the membrane. Finally, the hydrophilic surface of the channel is oriented inward, while the peptide bulks create a channel along the hydrophobic section of the bilayer. The action mechanism of the toroidal model indicates that, unlike the barrel-stave model, peptides are inserted perpendicularly into the bilayer to create a peptide-lipid complex instead of engaging in peptide-peptide interactions. A toroidal pore is generated due to the conformation of the peptides, promoting a distinct membrane curvature that is partially encircled by phospholipid head groups and partially by the peptides themselves. The interaction between the negatively charged polar phospholipid heads and the positively charged cationic peptides results in the adherence of antimicrobial peptides parallel to the membrane surface, as described in the carpet model. The peptides come together to reach a certain concentration, then they flip their orientation to form micelles with a hydrophobic core inside the membranes. This causes the membranes to dissolve [25, 29].

The nonmembrane-targeting peptides can move into the cell membrane without causing any ruptures to it. They then inhibit essential cellular functions by engaging with intracellular targets. To this point, numerous mechanisms have been outlined, including the inhibition of the synthesis of protein and nucleic acid, as well as the enzymes and proteins degradation [29].

Temporin family

In 1996, Simmaco et al. isolated from the skin of frogs in the *Rana* genus homologous antimicrobial peptides that are short in length and were named temporins [30]. Because of certain resemblance to the short peptides found in the venom of wasps from the genus *Vespa*, temporins were called "Vespa-like." Temporins and Vespa peptides have some things in common, including being hydrophobic, having low molecular weights, and being able to kill bacteria. Nevertheless, Temporins are not hemolytic like the Vespa peptides [30, 31]. Wade et al. have obtained a primary consensus sequence, FLP(I/L)IASLL(G/S)KLL-NH_2, for 30 frog skin peptides (Table 1). But there were two places where it was unclear, which led to four possible sequence variations. $X_1X_2X_3X_4X_5X_6Y_7X_8X_9Y_{10}Y_{11}{}^{+}X_{12}X_{13}$-$NH_2$ is the general amino acid sequence by type, where X correspond to a hydrophobic amino acid, Y to a hydrophilic one, and Y+ a charged amino acid. The most prevalent amino acid in the sequence of temporins is leucine. With that the hydrophobic residues make up 70 % of the peptide. Most peptides from this family have a charge of +1 due to the presence of a single basic residue, usually Lys [31]. The majority of temporins, especially those with a positively charged amino acid and a net charge of +2,

Table 1. Temporins and their amino acid sequence, isolated from *Rana temporaria* [30].

Peptide	Structure
A	FLPLIGRVLSGIL-NH_2
B	LLPIVGNLLKSLL-NH_2
C	LLPILGNLLNGLL-NH_2
D	LLPIVGNLLNSLL-NH_2
E	VLPIIGNLLNSLL-NH_2
F	FLPLIGKVLSGIL-NH_2
G	FFPVIGRILNGIL-NH_2
H	LSPNLLKSLL-NH_2
K	LLPNLLKSLL-NH_2
L	FVQWFSKFLGRIL-NH_2

have antibacterial properties. An activity loss happens when an asparagine takes the place of a basic residue, as is the case in Temporins C, D, and E. Even though a basic residue (Temporins H and K) is kept, the activity is lost with the shortening of the chain length with three residues. Thirteen residues appear to be the bare minimum required for antibacterial activity, along with a net basic charge [30]. Temporins are the largest group of AMPs found in amphibians, with over 40 members. There can be up to ten distinct isoforms in a single specimen [32].

Temporins A, B, and L are the most studied peptides in the group for their antimicrobial properties. Their amino acid sequences are mostly made up of hydrophobic residues, and they have an α-amidated C-terminal end due to a post-translational enzymatic reaction [31, 33]. The majority are non-toxic to human red blood cells at the concentrations required to eliminate bacteria [33, 34]. These amphipathic α-helical antimicrobial peptides (AMPs), ranging from 10 to 14 amino acids in length, exhibit a net charge of 0 to +3 and demonstrate significant activity against Gram-positive bacteria, categorizing them as some of the smallest AMPs identified in nature. This family includes one significant exception, temporin L, which demonstrates high efficacy against both Gram-positive and Gram-negative strains. Membrane penetration, while not inherently lethal, alters the permeability of microbial membranes, allowing the passage of both small and large molecules [32].

Temporin A

Temporin A (FLPLIGRVLSGIL-NH_2) is a basic, highly hydrophobic, C-terminal amidated member of the Temporin family, that exhibits variable antimicrobial activity against a wide range of microorganisms [35]. According to Wade et al, its antibacterial effectiveness was greatly affected by the existence of a N-terminal hydrophobic residue and bulky residues in positions 5 and 12. Temporin A exhibits modest hemolytic activity [34].

The cytotoxicity studies done by Swithenbank et al. on Temporin A, demonstrated the highest potential as an anticancer agent by effectively inducing substantial cell death in non-small cell lung carcinoma cell lines while sparing normal epithelial cell lines from significant cell death [36].

Ghiselli et al. have worked on *Staphylococci*-induced infections, which are prevalent pathogens responsible for biomaterial infections. They have tested Temporin A against both methicillin-susceptible and methicillin-resistant *Staphylococcus epidermidis*. It showed comparable antibacterial *in vitro* activity against the two strains. The *in vivo* findings indicate its potential application in preventing direct graft contamination when administered alongside parenteral vancomycin hydrochloride [35]. Simonetti et al. have investigated the impact of emporin A on wound healing, specifically its efficacy in conjunction with the conventional antibiotic teicoplanin, which is currently regarded as the gold standard for treating staphylococcal infections. The microbiological data indicated that the combination of Temporin A with teicoplanin exhibits a bactericidal effect on methicillin-resistant strains of *Staphylococcus aureus* that surpasses the efficacy of parenteral teicoplanin alone [37].

Temporin A analogues

Wade et al. have worked on modifying the primary and/or secondary structure of the Temporin A molecule. In their papers on Temporin A analogues, they have reported 11 new analogues and studied their antibacterial activity (Table 2) [34, 38].

The structure-activity studies of Wade et al. revealed that in position 5 a bulky and hydrophobic side chain of the amino acid is a requisite for the antibacterial activity. The enantiomeric analogues are just as effective, revealing that the acting mechanism is not based on chirality. In addition, replacement of Phe with Lys in

Table 2. Temporin A analogues with amino acid sequences [34, 38].

Peptide	Structure*
TA	FLPLIGRVLSGIL-NH_2
D-TA	flpligrvlsgil-NH_2
K1TA	**K**LPLIGRVLSGIL-NH_2
DK1-TA	klpligrvlsgil-NH_2
K7TA	FLPLIG**K**VLSGIL-NH_2
A512TA	FLPL**A**GRVLSG**A**L-NH_2
L512TA	FLPL**L**GRVLSG**L**L-NH_2
W1-TA	**W**LPLIGRVLSGIL- NH_2
G3-TA	FL**G**LIGRVLSGIL- NH_2
E7-TA	FLPLIG**E**VLSGIL- NH_2
L,D-TA	FlPlIGRvLsGiL- NH_2
Rev-TA (reserved TA)	LIGSLVRGILPLF- NH_2

**D-amino acids are presented in a lowercase.*

position 1 has led to a significant loss of antibacterial activity. Reversal of the Temporin A sequence has resulted in a two-fold loss of activity [34, 38].

Our research team has developed 8 analogues of Temporin A with modification in positions 1, 7 or 10 and examined their effect on the antibacterial activity, antiproliferative activity and hydrolytic stability (Table 3). We have incorporated non-proteinogenic amino acids such as ornithine (Orn), citrulline (Cit), 2,4-diaminobutyric acid (Dab), and 2,3-diaminopropionic acid (Dap) and fluorinated Phenylalanine (Phe(4F)). In addition , we have investigated the effect of the hydroxyl function in the molecule - primary, secondary, or aromatic [39, 40].

From our studies, we have concluded that a longer, bulkier and more basic side chain of the amino acid at position 7 is a requisite for achieving a lesser MIC value in broth microdilution testing. In contrast to that, a smaller and not so basic amino acid like Dab produced larger inhibition zones in the disk-diffusion assay, highlighting the differences between the two methods. The ideal lateral chain length for good antibacterial effectiveness was determined to be two methylene groups, such as the Dab-containing analogue. The elimination of the positive charge in the side chain resulted in a shortfall of the antibacterial properties of the DTCit analogue.

Table 3. Temporin A analogues and their amino acid sequences [39, 40].

Peptide	Structure
DTCit	FLPLIG-**Cit**-VLSGIL-NH_2
DTOrn	FLPLIG-**Orn**-VLSGIL-NH_2
DTDab	FLPLIG-**Dab**-VLSGIL-NH_2
DTDap	FLPLIG-**Dap**-VLSGIL-NH_2
DTThr	FLPLIGRVL-**T**-GIL-NH_2
DTTyr10	FLPLIGRVL-**Y**-GIL-NH_2
DTTyr1	**Y**-LPLIGRVLSGIL-NH_2
DT4F	**Phe(4F)**-LPLIGRVLSGIL-NH_2

This analogue, however, demonstrated commendable antiproliferative activity, accompanied by relatively low cytotoxicity and absence of phototoxicity [39].

The structure-activity relationship data obtained by Dimitrova et al. for position 10, indicate that the most promising compound in the series was DTTyr10, in which the Ser residue was substituted with a more hydrophobic, also hydroxyl-containing Tyr residue. This analogue also exhibited significant antiproliferative activity with no cytotoxicity or phototoxicity, as well as complete hydrolytic stability across all tested pH systems. Regrettably, this analogue exhibited a bacteriostatic effect at a slightly elevated MIC of 320 μg mL^{-1} against the tested strains, indicating its impracticality as a dual antiproliferative and antimicrobial agent. The analogue with the fluorinated phenylalanine at position 1, DT4F, exhibited the most significant antiproliferative effect against the evaluated tumour cell lines, alongside with notable antibacterial activity. However, it demonstrated greater cytotoxicity and phototoxicity compared to the parent peptide Temporin A and no stability at pH 9 over a 24 h duration. This aligns completely with previously published findings of Danalev et al. that the incorporation of fluorine into a peptide enhances activity, while reducing the hydrolytic stability [40, 41].

The own contributions in the area of AMPs studies could be summarized in several directions:

- Incorporation of unnatural amino acids to enhance antibacterial activity;
- Incorporation of fluorinated amino acids to enhance antibacterial and antiproliferative activity in position 1, resulting however in higher photo- and

cytotoxicity and decreased hydrolytic stability at pH 9;

- A positive charge in the side chain in position 7 of the Temporin A molecule is crucial for the antibacterial property;
- The optimal length for antibacterial activity of the side chain in position 7 was determined to be two methylene groups;
- A more hydrophobic OH-containing residue in position 10 results in better antiproliferative activity [39, 40].

Temporin B

Temporin B (LLPIVGNLLKSLL-NH_2) possesses a single positively charged residue, and exhibits activity against Gram-positive bacteria and fungi, while demonstrating negligible hemolysis and toxicity to normal human cells. Zhang et al. have examined morphological alterations of planar bilayer membranes, consisting of a mixture of zwitterionic and anionic phospholipids, stimulated by Temporin B and Temporin L. When subjected to amphipathic environments (water-gas interface and lipid bilayer surface), both peptides adopt α-helical conformations, with the hydrophilic and hydrophobic residues segregated into two distinct facets. At low peptide concentrations, the peptides predominantly interact with the lipid head groups on the membrane surface rather than penetrating deeply into the bilayer. At elevated peptide concentrations, temporins are prone to aggregate via hydrophobic interactions upon membrane adsorption, resulting in the formation of peptide-rich domains on the membrane surface. Upon comparison of Temporin B and Temporin L, it was observed that Temporin L exhibits marginally superior antimicrobial activity at low peptide concentrations [42].

Its membrane activity and potency against gram-positive and gram-negative bacteria are improved by increasing the cationicity of the C-terminal end of Temporin B and the hydrophobicity of the N-terminal end. On the other hand, increasing the N-terminal cationicity makes it less effective against *Staphylococcus aureus* and eliminates its capacity to initiate channel conductance, but it still increases its potency against *Escherichia coli* [43].

Temporin L

Temporin L (FVQWFSKFLGRIL-NH_2) has strong antibacterial properties, particularly against gram-negative bacteria and fungal infections. It is the only Temporin to possess a tryptophan residue in its chain and has the greatest cationic charge. Rinaldi et al. have studied Temporin L against over 20 bacterial strains, as well as different fungal strains, and have found that Temporin L has the highest bioactivity of any temporin that has been investigated to date, against human erythrocytes and bacterial and fungal strains. They reached the conclusion that against every Gram-positive strain tested, temporin L was at least ten times more effective than temporin B. Additionally, it was active against Gram-negative strains, which sets it apart from other temporins [44].

The study of Rosenfeld et al. elucidates the lack of activity of Temporins A and B against Gram-negative bacteria. The two peptides oligomerize upon contact with the outer membrane, as indicated by the rhodamine fluorescence dequenching measurements done, following the addition of purified *E. coli* lipopolysaccharides (LPS). This contrasts with the highly active antibacterial Temporin L, which disassembles upon contact with LPS. These findings correlate with other studies that the oligomerization of antimicrobial peptides (AMPs) significantly diminishes their antimicrobial efficacy against Gram-negative bacteria, as their increased size hinders efficient diffusion through the cell wall. According to Rosenfeld et al., the synergistic effect of temporin L when paired with either temporin A or B occurs at the outer membrane of Gram-negative bacteria and is associated with the capacity of temporin L to facilitate the passage of temporins A and B through the LPS layer [32].

CONCLUSIONS

Antimicrobial peptides (AMPs) represent a promising novel approach to combat the escalating issue of antimicrobial resistance. Their unique advantages, including significant antibacterial effectiveness, relatively low toxicity, and diverse functions such as antioxidant, antiviral, and antitumor properties, make them strong contenders for next-generation therapeutics. Antimicrobial peptides possess significant potential to assist or even supplant conventional antibiotics in promoting health; however, further research is required to address issues related to stability, delivery, and large-scale production. With focus only on two positions in

the molecule of Temporin A, our group have shown that AMPs and in particular Temporin A are a subject worth being a focus of study in the search of novel therapeutics for combating a serious threat to human health.

Acknowledgments

This study was supported by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0002, "BiOrgaMCT".

Authors thank to Prof. D. Danalev for the support constructive notes.

Authors' contributions

D.D.: Original draft preparation; N.G.: review, editing and supervision.

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