FROM STRUCTURE TO ACTIVITY: A COMPREHENSIVE REVIEW OF THE ANTIBACTERIAL PEPTIDE ANOPLIN

Heba Mzik, Nelly Georgieva, Dancho Danalev

Department of Biotechnology, University of Chemical Technology and Metallurgy
8 Kliment Ohridski Blvd., Sofia 1797
Bulgaria, hebamzik@gmail.com (H.M.); neli@uctm.edu (N.G.).

Received 03 April 2026
Accepted 09 May 2026
DOI: 10.59957/jctm.v61.i4.2026.1

ABSTRACT

Biologically active peptides are a promising alternative of the existing medical drugs used in the medicinal practice. Especially, antimicrobial peptides are such an alternative to conventional antibiotics considering the growing prevalence of antimicrobial resistance. Anoplin, a short decapeptide isolated from the venom of the solitary wasp Anoplius samariensis, has attracted attention due to the simple structure and membrane - targeting feature. This review summarizes current knowledge on the synthesis, structural characteristics, and biological activity of anoplin, with emphasis on structure - activity relationships and modification strategies. The activity of anoplin is closely related to its cationic and amphipathic nature. This allows interaction with bacterial membranes and leads to membrane disruption and cell death. Various modification approaches, including amino acid substitution, lipidation, and structural stabilization, have been applied to improve anoplin's antimicrobial activity, selectivity, and stability. In addition, environmental factors such as ionic strength, pH, and proteolytic degradation significantly affect biological performance and bioavailability. Although anoplin shows relatively low susceptibility to resistance development, limitations related to stability and cytotoxicity is still a main disadvantage. Therefore, further optimization is required to enhance its therapeutic potential. Anoplin can serve as a useful model for the rational design of new antimicrobial peptides and peptide - based therapeutic agents.

Keywords: Anoplin, antimicrobial peptides, structure - activity relationship, peptide modification.

INTRODUCTION

In recent years, society around the world has been subjected to enormous health - related pressures, starting with the Covid - 19 pandemic in 2020 [1, 2], moving through the continuous increase in tumor and cardiovascular diseases [3, 4], and reaching pressure on the social and healthcare systems of individual countries [5]. The increasing prevalence of bacterial resistance to conventional antibiotics has become a major challenge in modern healthcare, as many treatments that were once effective are gradually losing their ability to control infectious diseases [6, 7]. Bacterial resistance can be broadly defined as the capacity of microorganisms to survive exposure to antibiotics that would normally inhibit their growth or lead to cell death [8]. This phenomenon arises through several mechanisms, typically classified as intrinsic, acquired, or adaptive resistance [9, 10]. One of the main factors contributing to this issue is the extensive and often inappropriate use of antibiotics, which imposes selective pressure and promotes the survival of resistant populations. As a result, multidrug - resistant pathogens are emerging at an increasing rate, posing significant challenges for both clinical treatment and the development of new therapeutic agents [11 - 13]. In the light of these limitations, antimicrobial peptides (AMPs) have gained considerable attention as potential alternatives, particularly due to their ability to target bacterial membranes through mechanisms distinct from those

of conventional antibiotics [14, 15]. Many examples exist in the scientific literature for such AMPs like $(KLAKLAK)_2$ - NH_2 sequence [16, 17] and Temporin's family [18 - 20]. In parallel with AMPs, other synthetic small bioactive molecules and amino acid derivatives have also been explored as antimicrobial candidates [21, 22].

Antimicrobial peptides are typically short molecules, composed of 10 - 50 amino acid residues. Most of them are cationic and exhibit an amphipathic organization, features that enable efficient interaction with microbial membranes, including those of drug - resistant bacteria [23, 24]. Their activity is largely determined by physicochemical parameters such as charge distribution, structural conformation, peptide length, and hydrophobicity, as well as the composition of the target membrane. These factors collectively influence membrane binding and subsequent disruption, ultimately leading to loss of membrane integrity and cell death [24, 25].

Animal venoms provide a diverse source of bioactive molecules, containing a wide range of compounds with potent biological activities [26, 27]. They are produced by various organisms, including insects (such as bees and wasps), arachnids (e.g., spiders and scorpions), as well as amphibians and snakes [26, 27]. Within this group, anoplin, a peptide originally isolated from wasp venom, has drawn particular interest due to its relatively simple structure and promising antibacterial properties (Fig. 1) [28].

This review has two main goals divided into two main sections. The first one is to outline the molecular and structural features of anoplin, whereas the second focuses on the antibacterial activity and the mechanisms responsible for its action against pathogenic bacteria.

MOLECULAR AND STRUCTURAL CHARACTERISTICS OF ANOPLIN

Origin and primary structure

Anoplin is considered one of the smallest naturally occurring linear antimicrobial peptides, consisting of only ten amino acid residues and possessing a C - terminal amidation (GLLKRIKTLL - NH_2, Fig. 2). The peptide was originally isolated from the venom sacs of the female solitary wasp *Anoplius samariensis* by Konno et al., who first identified and characterized it as a short amidated decapeptide with antimicrobial activity [28].

The increasing interest in short - chain AMPs, such as anoplin, is driven by several practical and biological advantages. Short peptides are generally associated with lower production costs and are more amenable to structural optimization through amino acid substitutions. This flexibility facilitates the development of peptide analogues with improved antimicrobial activity and selectivity, while also enabling convenient chemical manipulation for therapeutic applications [16 - 20, 29 - 35].

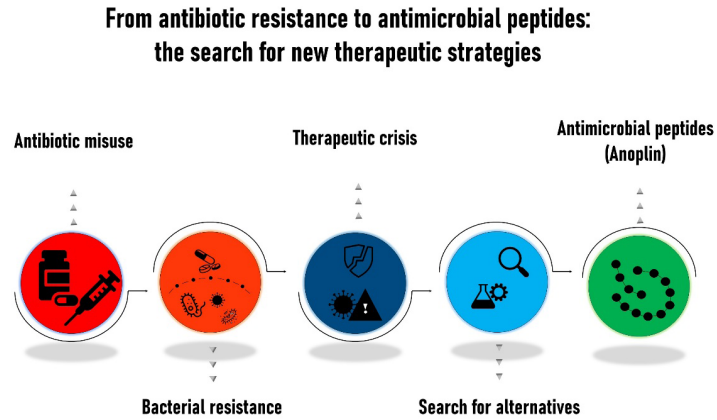
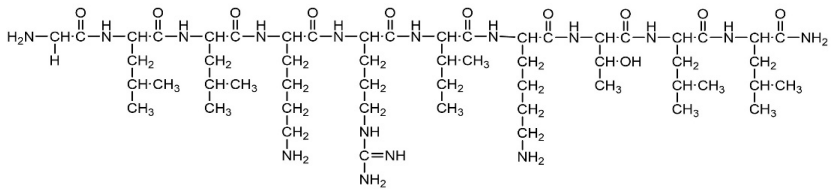


Fig. 1. Factors driving the exploration of antimicrobial peptides as alternatives to conventional antibiotics.



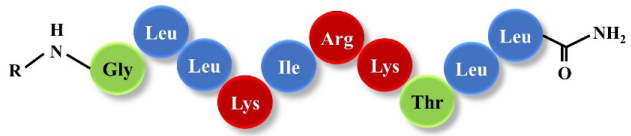


Fig. 2. Primary structure of the antimicrobial peptide anoplin.

Physicochemical properties

The antimicrobial activity of anoplin is closely associated with its physicochemical characteristics, particularly its net positive charge, hydrophobic residues, and amphipathic organization. These features play a central role in governing the interaction of the peptide with bacterial membranes and strongly influence its antimicrobial activity [29, 30].

Anoplin possesses a net positive charge (+ 4), primarily due to the presence of basic amino acid residues, including two lysine residues and one arginine residue within its sequence. In addition, the positively charged N - terminal amino group contributes to the overall cationic nature of the peptide, while C - terminal amidation neutralizes the negative charge that would otherwise be present at the carboxyl terminus [28, 36]. This cationic character promotes electrostatic attraction between the peptide and negatively charged components of bacterial membranes [37]. In particular, Gram - negative bacteria contain lipopolysaccharides (LPS), whereas Gram - positive bacteria possess teichoic and lipoteichoic acids, both of which contribute to the overall negative surface charge of bacterial cell envelopes [38, 39]. Consequently, these electrostatic interactions facilitate the initial binding of anoplin to bacterial membranes and represent a crucial step in the mechanism of antimicrobial action [37]. In addition to the cationic nature, anoplin contains several hydrophobic residues, particularly four leucine residues and one isoleucine residue within the sequence, which contribute to the formation of a hydrophobic face within the peptide structure.

In amphipathic α - helical peptides such as anoplin, hydrophobic amino acid side chains are spatially separated from hydrophilic or charged residues, creating two distinct molecular faces. This amphipathic arrangement enables anoplin to interact efficiently with biological membranes, where the hydrophobic residues associate with the lipid core of the membrane while the cationic residues remain exposed to the aqueous interface and interact with negatively charged lipid head groups. Consequently, this structural organization facilitates stable peptide - membrane interactions and contributes significantly to the antimicrobial activity of anoplin [37, 40]. Such amphipathic organization is commonly associated with the ability of AMPs to adopt ordered secondary structures upon interaction with membrane environments.

Secondary structure

Anoplin has been shown to adopt an α - helical secondary structure (Fig. 3) upon interaction with membrane - mimetic environments, a common structural motif among many membrane - active AMPs [37, 41, 42].

In aqueous solution, the peptide typically exhibits a more flexible or disordered conformation. However, the presence of lipid membranes or membrane - mimicking systems promotes the formation of an amphipathic α - helix. Structural studies, including circular dichroism (CD) spectroscopy and nuclear

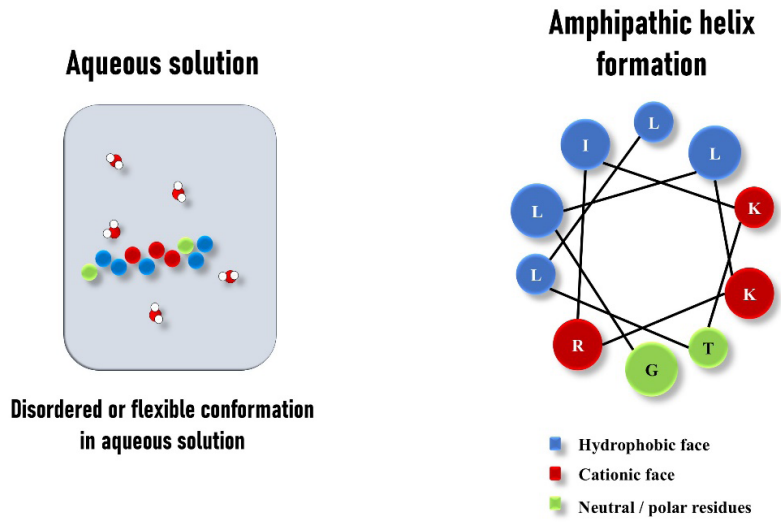


Fig. 3. Conformational transition of anoplin from a disordered structure in aqueous solution to an amphipathic α - helical conformation upon interaction with membrane environments.

magnetic resonance (NMR), have demonstrated that this conformational transition facilitates the spatial segregation of hydrophobic and cationic residues, thereby stabilizing the amphipathic α - helical structure characteristic of many membrane - active AMPs [37, 40].

Several studies have suggested that anoplin molecules may associate within membrane environments to form transient oligomeric structures, which could contribute to membrane permeabilization and ion channel - like activity [37].

Despite its remarkably short length, anoplin exhibits several structural features that contribute to the antimicrobial activity and distinguish the anoplin from many longer AMPs. Notably, anoplin is capable to adopt a stable amphipathic α - helical conformation upon interaction with membrane environments, despite consisting of only ten amino acid residues. This structural organization is supported by a balanced distribution of cationic residues, resulting in an overall net charge of + 4, and hydrophobic residues, which account for approximately half of the sequence and include a relatively high proportion of leucine residues.

Together, these characteristics promote efficient interactions with bacterial membranes and facilitate membrane disruption. Due to its structural simplicity and well - defined amphipathic organization, anoplin has become a valuable model peptide for structure - activity relationship studies, where even minor sequence modifications can lead to significant changes in antimicrobial activity [28, 29, 36 - 38, 40].

CHEMICAL SYNTHESIS AND EXPERIMENTAL CHARACTERIZATION OF ANOPLIN

Synthesis of anoplin using Solid - Phase Peptide Synthesis (SPPS)

Anoplin is commonly synthesized using Fmoc - based solid - phase peptide synthesis (SPPS) (Fig. 4) [32, 33].

This method represents a widely used strategy for the stepwise assembly of peptides on a solid support. In this method, the peptide chain is elongated through repetitive cycles of Fmoc deprotection and amino acid coupling reactions, typically performed on resins, in the case of anoplin, such as Rink amide resin. The Fmoc protecting group is generally removed using 20 % piperidine / N, N - dimethylformamide (DMF) solution, followed by coupling of the next protected amino acid using standard mixture of reagents such as N - [(lH - benzotriazole - l - yl) (dimethylammo) methylene] - N - methylmethanaminium hexafluorophosphate N - oxide (HBTU), N - [(lH - benzotriazole - l - yl) (dimethylamino) methylene] - N - methylmethanaminium tetrafluoroborate N - oxide (TBTU) or benzotriazole - 1 - yloxytris (pyrrolidino) phosphonium hexafluorophosphate (PyBop) in combination with 1 - hydroxybenzotriazole

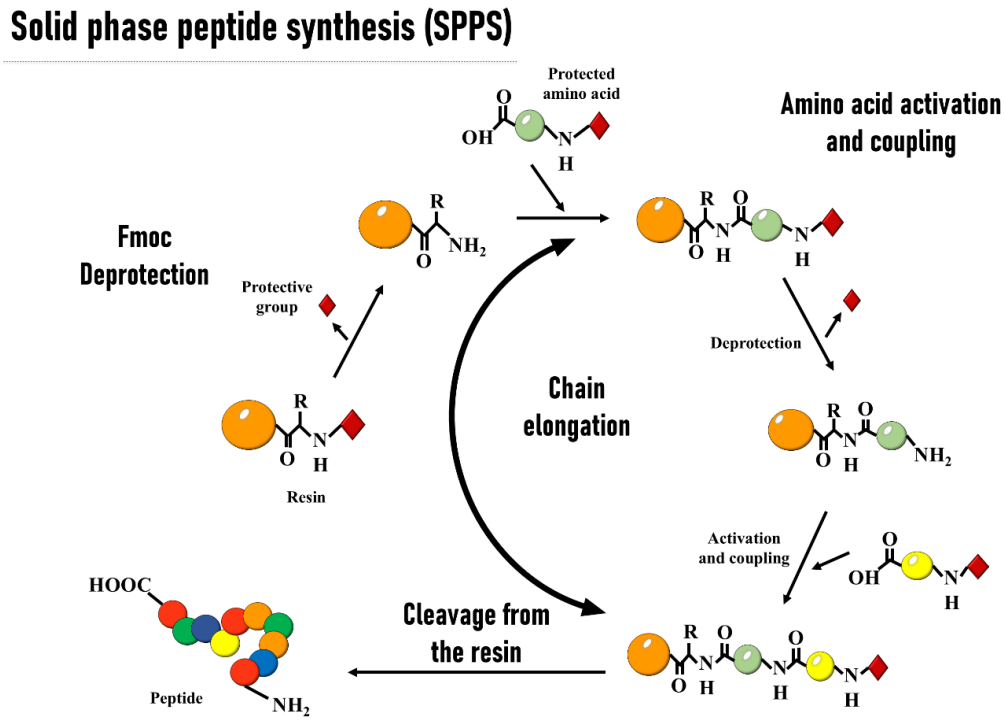


Fig. 4. Overview of the Fmoc solid - phase peptide synthesis (SPPS) cycle for C - terminal amide synthesis.

(1 - HOBt) and N, N - diisopropylethylamine (DIPEA) or 1, 3 - diisopropylcarbodiimide (DIC) with 1 - HOBt. This approach enables efficient synthesis of short peptides and facilitates the rapid generation of multiple analogues through systematic amino acid substitutions [16 - 20, 34, 35, 43].

The relatively short length of anoplin makes it particularly suitable for SPPS, as the peptide can typically be synthesized with high efficiency and yield compared with longer antimicrobial peptides [28, 32]. Moreover, the solid - phase strategy facilitates the rapid preparation of numerous anoplin analogues through systematic residue substitution or terminal modification, which has been extensively exploited in structure - activity relationship studies of anoplin [29, 32, 33, 36]. In several studies, after completion of chain assembly, the peptide is cleaved from the resin using trifluoroacetic acid - based cleavage mixtures (typically trifluoroacetic acid (TFA) / H_2O / triisopropylsilane (TIS), 95:2.5:2.5) that simultaneously remove the side - chain protecting groups, generating crude peptide products for subsequent purification and characterization [43].

From a methodological perspective, the relatively simple sequence and synthetic accessibility of anoplin have been one of the key factors contributing to its widespread use as a model AMP, since SPPS allows precise control of sequence modifications and rapid generation of derivatives designed to optimize antimicrobial activity, stability, or selectivity [29, 32, 33].

However, the crude products obtained after cleavage often contain truncated sequences and protecting - group - derived impurities, which necessitate purification by reverse - phase HPLC prior to biological evaluation. In addition, the preservation of C - terminal amidation is crucial, as deamidation has been reported to significantly reduce the biological activity of anoplin [37].

Purification and analytical characterization of anoplin

After synthesis and cleavage from the resin, the crude peptide is typically subjected to purification by reverse - phase high - performance liquid chromatography (RP - HPLC). This technique allows efficient separation of the target peptide from synthesis - related impurities

present in the crude product. RP - HPLC purification is commonly performed on C18 columns using gradients of water and acetonitrile containing TFA as an ion - pairing agent. Purified fractions are collected and subsequently lyophilized to obtain the final peptide product suitable for structural and biological studies [37, 44, 45].

Following purification, the identity and molecular weight of anoplin are generally confirmed using mass spectrometry, most commonly matrix - assisted laser desorption / ionization time - of - flight (MALDI - TOF MS) or electrospray ionization mass spectrometry (ESI - MS). These techniques provide accurate determination of peptide molecular weight and confirm the identity of the synthesized sequence corresponds to the expected molecular composition [32, 33, 37].

In addition to confirming peptide identity, circular dichroism (CD) spectroscopy is frequently employed to investigate the secondary structure of anoplin under different environmental conditions. CD studies have shown that anoplin typically adopts a disordered conformation in aqueous solution but forms a characteristic amphipathic α - helical structure in membrane - mimetic environments such as lipid vesicles or trifluoroethanol - containing solutions. These analyses provide important insights into the relationship between peptide conformation and antimicrobial activity [28, 37].

Accurate purification and structural characterization are essential to ensure that the observed antimicrobial activity is attributed to the correctly synthesized peptide rather than to synthesis - related impurities.

MODIFICATIONS AND OPTIMIZATION STRATEGIES FOR ANOPLIN ANALOGS SYNTHESIS

Over the past two decades, numerous structural modifications of anoplin have been explored in order to improve its antimicrobial potency, enhance physicochemical stability, reduce cytotoxicity toward mammalian cells, and better understand the structure - activity relationships governing this short AMP (Fig. 5) [32, 33, 38].

Building on the diverse design strategies reported in the literature, modifications of anoplin can be broadly classified into several key categories, each reflecting a distinct approach to modulating peptide activity, selectivity, and structural behaviour:

Amino acid substitutions

Amino acid substitution has been one of the most extensively explored approaches for optimizing anoplin while preserving its decapeptide framework. Early structure - activity studies showed that truncation

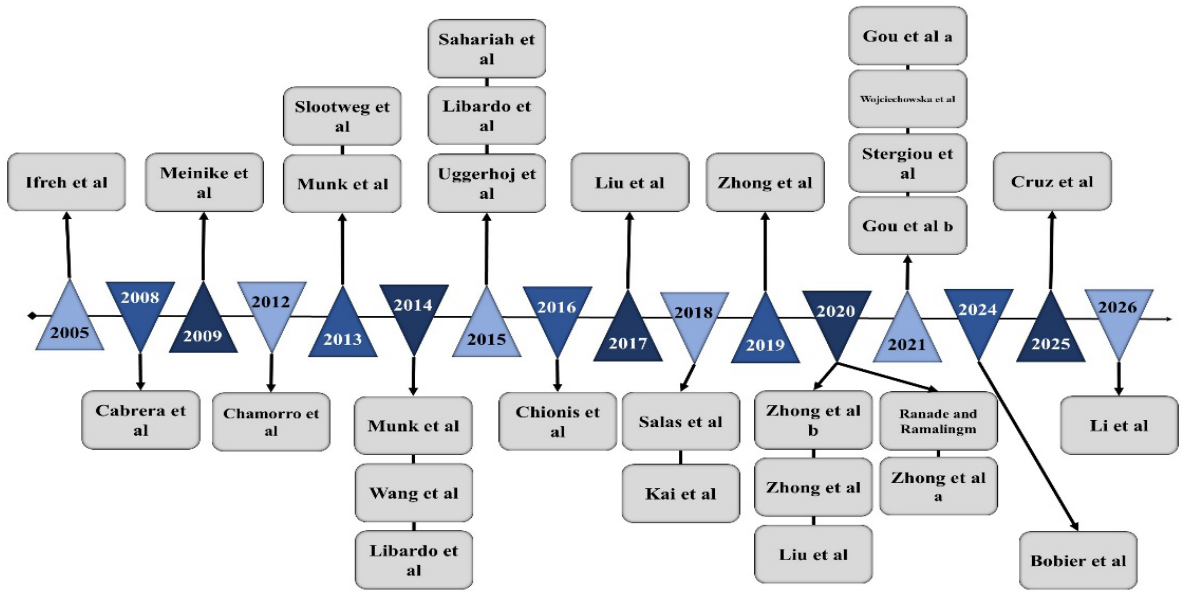


Fig. 5. Timeline of previously published studies on anoplin and its analogues from 2005 to 2026.

reduced biological activity, whereas residue substitutions within the 10 - residue sequence could markedly alter antimicrobial potency, hemolysis, and selectivity [46]. In particular, changes affecting charge distribution, hydrophobicity, and residue positioning were found to influence peptide activity in a highly sensitive manner [32, 40, 47].

Increased hydrophobicity generally enhanced antimicrobial potency by strengthening membrane interactions, but this effect was often accompanied by increased hemolytic activity, indicating reduced selectivity toward mammalian cells [46]. More recent studies have shown that this limitation can be partially addressed through more careful tuning of physicochemical properties, allowing improved selectivity while maintaining biological activity in selected analogues [48].

In addition to charge and hydrophobicity, amphipathic balance and structural organization appear to play an important role of peptide performance. Fine adjustments in the distribution of polar and hydrophobic residues, as well as strategies that favour a more stable helical conformation, have been associated with improved activity in selected analogues [33, 49]. Taken together, these observations indicate that the biological behaviour of anoplin analogues is not governed by a single parameter, but rather by the combined influence of charge, hydrophobicity, amphipathicity, and structural stability [40, 46, 50].

Hydrophobic tuning and lipid modifications

Hydrophobic tuning and lipidation have been widely explored as effective strategies to improve the biological performance of anoplin analogues. While early efforts mainly focused on enhancing antimicrobial activity, more recent approaches have also addressed proteolytic stability, which is essential for maintaining peptide function under physiological conditions. In this context, combining N - methylation with lipidation has been shown to improve resistance to enzymatic degradation in anoplin analogues [51].

In general, increasing lipophilicity enhances peptide - membrane interactions and often leads to improved antimicrobial activity. However, this effect is not linear, as excessive hydrophobicity can negatively affect peptide behaviour [29, 38]. Early studies showed that N - terminal lipidation and the incorporation of lipophilic residues can enhance activity and facilitate membrane association, although the structural outcomes may vary depending on the specific analogue [29, 36, 52].

A key limitation of this approach is the loss of selectivity at higher hydrophobicity levels, indicating that increased potency alone is not sufficient for optimal peptide design [36, 38]. This trade - off is particularly evident in relation to fatty acid chain length, where medium - length chains (typically C8 - C12) tend to provide a more favourable balance between antimicrobial activity and selectivity than shorter or longer chains [38, 53]. Notably, increased structural complexity does not necessarily result in improved therapeutic performance, as simpler lipopeptides such as A - C8 have, in some cases, shown a more favorable therapeutic index than more complex dendrimerized analogues [38]. Combining lipidation with additional modification strategies, such as D - amino acid substitution or N - methylation, can further improve peptide stability and biological performance, highlighting the importance of optimizing multiple parameters simultaneously in anoplin design [51, 54].

Terminal modification

In addition to residue substitution and lipidation strategies, terminal modifications have been investigated to better understand the structural determinants of anoplin activity. Early studies demonstrated that truncation of either the N - or C - terminus leads to a significant reduction in antimicrobial activity, emphasizing the importance of maintaining the full - length decapeptide for optimal function [37, 46].

C - terminal amidation has been shown to play a key role in preserving the net positive charge and amphipathic character of anoplin. Loss of amidation alters charge distribution and weakens peptide - membrane interactions, resulting in reduced biological activity [37].

More recent work suggests that the effect of terminal modifications depends on the structural context of the peptide. In lipidated systems, certain truncated analogues were able to retain or even improve selectivity, indicating that terminal regions contribute not only to activity but also to the balance between antimicrobial potency and cytotoxicity [36].

In general, these observations highlight the importance of terminal regions in modulating peptide -

membrane interactions and biological performance, as even minor modifications at the peptide ends can lead to significant functional changes.

Structural stabilization strategies

Despite its potent antimicrobial activity, the relatively short length of anoplin inherently limits its structural stability, particularly in aqueous and physiologically relevant environments. This intrinsic flexibility can compromise its ability to maintain a stable amphipathic α - helical conformation, which is generally required for effective membrane interaction [37, 41]. To address these limitations, a range of structural stabilization strategies has been developed to enhance conformational rigidity and improve peptide stability, particularly under physiologically relevant conditions such as high salt concentrations and proteolytic environments [24, 25]. These approaches can be broadly grouped into several complementary strategies. First, conformational modifications, including residue substitution and, in some cases, peptoid incorporation, have been used to alter peptide structure and tune biological activity [33, 49, 55]. In contrast, more rigid stabilization strategies, such as peptide stapling and backbone bridging, aim to directly constrain peptide conformation and enhance structural stability [31, 44]. In addition, hybrid approaches combining lipidation with other modifications have been shown to improve both antimicrobial activity and proteolytic stability [53, 54]. Finally, more complex systems, including conjugates and multivalent designs, have been explored to further enhance peptide performance and expand therapeutic potential [51, 56, 57].

Multivalent and dendrimeric derivatives

Multivalent and dendrimeric strategies have emerged as advanced approaches for enhancing the biological performance of anoplin analogues by increasing the local concentration of active peptide units and strengthening interactions with bacterial membranes. In general, multivalent designs, including peptide dimers and dendrimeric structures, have been shown to improve antimicrobial activity compared with their monomeric counterparts [53, 55, 58]. In addition to activity enhancement, certain conjugation strategies have demonstrated the potential to improve selectivity and reduce cytotoxicity. For example, the conjugation of anoplin to polymeric systems such as chitosan has been reported to significantly decrease hemolytic activity while maintaining or enhancing antibacterial efficacy, highlighting the importance of multivalent presentation in improving therapeutic performance [59]. However, increasing structural complexity does not always result in improved biological outcomes. While dendrimerization and combined lipidation strategies can enhance antimicrobial potency, they may also lead to increased hemolysis and reduced selectivity in some cases [30, 38]. These results suggest that the benefits of multivalency depend strongly on the balance between enhanced membrane interaction and the preservation of selectivity. Overall, multivalent and dendrimeric designs represent a promising strategy for optimizing anoplin - based therapeutics, although careful control of structural parameters remains essential to achieve an optimal balance between activity and toxicity.

These strategies may bridge the gap between simple peptide design and clinically relevant antimicrobial systems.

ANTIBACTERIAL EFFECTS OF ANOPLIN

Antibacterial activity and spectrum

Anoplin exhibits antibacterial activity against both Gram - positive and Gram - negative bacteria, although its potency is generally moderate but tunable, depending on the bacterial strain and experimental conditions. Early characterization of native anoplin demonstrated measurable activity against representative Gram - positive species such as Staphylococcus aureus as well as Gram - negative bacteria including Escherichia coli, indicating a broad - spectrum antibacterial profile [28]. Subsequent studies further revealed that the antibacterial efficacy of anoplin is highly sensitive to sequence modifications and physicochemical properties. Synthetic analogues have been reported to display improved activity compared with the native peptide, with some derivatives showing enhanced potency against both Gram - positive and Gram - negative strains [32, 33, 40]. Comparable activity optimization has also been reported for other short cationic peptide analogues [60]. This supports the idea that the intrinsic activity of anoplin can be significantly optimized through targeted sequence modifications.

Despite this broad - spectrum activity, reported

minimum inhibitory concentration (MIC) values vary considerably across studies, reflecting differences in peptide structure, bacterial membrane composition, and experimental conditions. In several cases, Gram - negative bacteria have been found to exhibit comparable or even higher susceptibility to anoplin and its derivatives, which may be attributed to the presence of negatively charged lipopolysaccharides (LPS) that facilitate electrostatic interactions with the cationic peptide. A comprehensive overview of reported MIC values and antibacterial profiles has been recently summarized, highlighting the variability in activity while confirming the overall antibacterial potential of anoplin [61]. Taken together, these results suggest that the antibacterial performance of anoplin is not fixed but strongly influenced by both intrinsic peptide properties and external environmental conditions, emphasizing the importance of structural optimization in enhancing its biological activity.

Interaction with bacterial membranes

The interaction of anoplin with bacterial membranes represents the initial and critical step in its antimicrobial mechanism. This process is primarily driven by electrostatic attraction between the cationic peptide and negatively charged components of bacterial membranes, facilitating binding to the cell surface [25]. Gram - negative bacteria contain negatively charged LPS, which enhance peptide - membrane interactions and promote initial surface association. Following this initial attraction, anoplin associates with the membrane interface and adopts an orientation that is influenced by its amphipathic nature. The spatial segregation of hydrophobic and cationic residues allows the peptide to align at the membrane surface, where hydrophobic side chains interact with lipid components while positively charged residues remain exposed to the aqueous environment [42]. This arrangement facilitates stable membrane binding and surface association at the membrane interface.

Notably, the interaction of anoplin with bacterial membranes is not governed solely by electrostatic forces. The efficiency of membrane binding is also strongly influenced by peptide conformation, hydrophobic balance, and overall structural organization [62], which together determine how effectively the peptide associates with the lipid bilayer [61]. In this context, the relatively short length of anoplin suggests that its initial interaction is predominantly surface - associated, preceding deeper membrane insertion. Experimental studies have further demonstrated that membrane interaction is closely linked to specific structural features of anoplin, including C - terminal amidation, which plays a critical role in stabilizing peptide - membrane interactions and enhancing biological activity [37]. Collectively, these observations indicate that the interaction of anoplin with bacterial membranes is a dynamic and multifactorial process involving electrostatic attraction, amphipathic alignment, and structural adaptation, ultimately preparing the peptide for subsequent membrane disruption. Related short amphipathic peptides have likewise been investigated using biomimetic membrane models [63].

Membrane disruption and bactericidal mechanism

Following membrane binding, anoplin disrupts bacterial membranes through mechanisms that compromise membrane integrity and ultimately lead to cell death (Fig. 6). This process is generally associated with increased membrane permeability, resulting in the leakage of intracellular components and the loss of essential ion gradients, which are critical for bacterial viability [37, 64].

Several models have been proposed to describe the membrane - disruptive activity of antimicrobial peptides, including pore formation, toroidal pore structures, and carpet - like mechanisms, all of which involve destabilization of the lipid bilayer [64, 65]. In the case of anoplin, experimental evidence suggests a channel - like mode of action, where peptide assembly within the membrane leads to increased permeability and loss of membrane function [37]. It is important to note that, membrane disruption is not a uniform or instantaneous event but rather a dynamic process influenced by peptide concentration, structural properties, and membrane composition. As the presence of peptide at the membrane surface increases, the extent of bilayer destabilization becomes more pronounced, ultimately resulting in extensive membrane damage [65, 66]. Altogether, this suggests that the bactericidal activity of anoplin primarily arises from its ability to destabilize bacterial membranes through a combination of permeabilization, structural disruption, and loss of membrane integrity, rather than through specific intracellular targets.

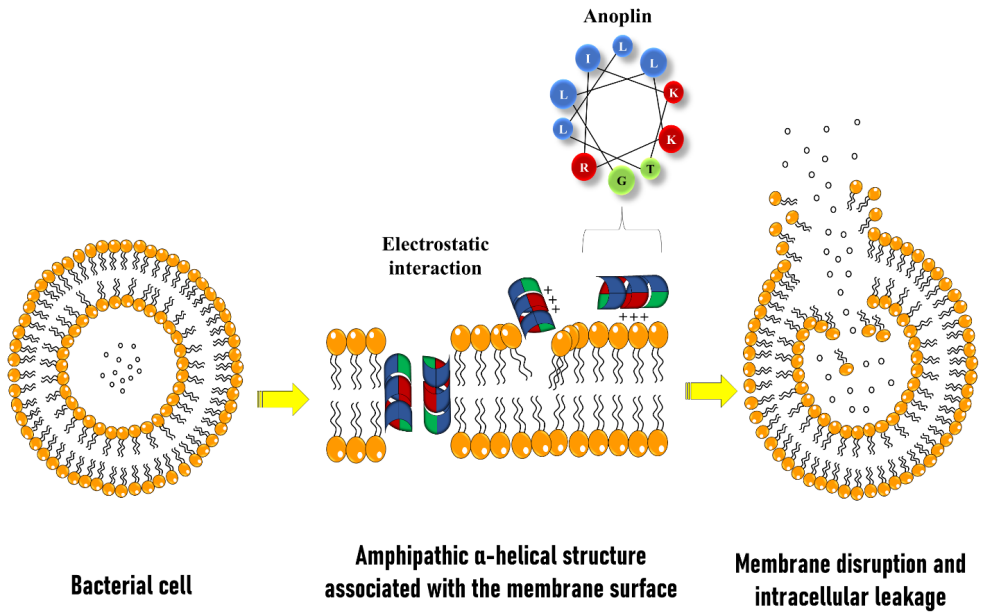


Fig. 6. Mechanism of action of anoplin, illustrating membrane binding, α - helical structuring, and subsequent membrane disruption leading to intracellular leakage.

Intracellular targets and secondary mechanisms

Although membrane disruption is widely considered the primary mechanism of action of anoplin, limited evidence suggests that antimicrobial peptides may also exert additional intracellular effects. Certain peptides have been reported to interact with intracellular targets such as ATP synthase, thereby interfering with bacterial energy metabolism. In the case of anoplin, this effect has been associated with inhibition of the F_1F_0 ATP synthase complex, leading to reduced ATP production and contributing to bacterial cell death [67]. However, such intracellular effects remain less well characterized and are generally considered secondary, likely occurring following membrane permeabilization that facilitates peptide entry into the cell. Accordingly, membrane disruption remains the dominant mechanism underlying the antibacterial activity of anoplin, while intracellular targeting may play a minor or complementary role under specific conditions [61].

Factors affecting antimicrobial activity

The antimicrobial activity of anoplin is strongly influenced by environmental and physicochemical factors that can modulate its interaction with bacterial membranes. In particular, ionic strength plays a critical role, as high salt concentrations can weaken electrostatic interactions between the cationic peptide and negatively charged bacterial membranes, leading to reduced activity [61]. Similarly, the presence of serum components may decrease peptide efficacy through binding interactions or proteolytic degradation, thereby limiting its stability under physiological conditions [14, 51]. In addition, pH variations can affect peptide charge distribution and membrane affinity, further influencing antimicrobial performance. These effects are closely linked to the structural properties of anoplin, as modifications that enhance stability - such as increased hydrophobicity, backbone alterations, or incorporation of non - natural residues - can partially mitigate the impact of these environmental factors [24, 51]. In general, these observations highlight that the biological activity of anoplin is not solely determined by its intrinsic structure, but also by external conditions that influence peptide stability, membrane interaction, and overall effectiveness.

Resistance potential

Compared with conventional antibiotics, antimicrobial peptides such as anoplin are generally

considered less prone to inducing bacterial resistance, primarily due to their membrane - targeting mechanism, which involves interactions with fundamental structural components of the cell. Unlike antibiotics that act on specific intracellular targets, this mode of action reduces the likelihood of resistance development [25, 65]. However, resistance cannot be completely excluded, as some bacterial species can modify membrane composition, surface charge, or lipid organization to reduce peptide binding and activity. These adaptive responses may decrease susceptibility under certain conditions, although they are typically less efficient than classical resistance mechanisms [65]. Collectively, these observations suggest that while anoplin exhibits a relatively low propensity for resistance development, continuous monitoring and optimization remain important for its potential therapeutic application [61].

Therapeutic potential and applications

Anoplin possesses unique physicochemical properties, including its small size, cationic nature, and amphipathic structure, make it an attractive candidate for the development of novel antimicrobial agents. Its ability to disrupt bacterial membranes is a key factor in its activity. In addition, its relatively low propensity for resistance development highlights its potential as an alternative to conventional antibiotics [14, 61]. However, despite these promising features, several limitations must be addressed before clinical application. In particular, issues related to proteolytic instability, potential cytotoxicity, and reduced activity under physiological conditions remain significant challenges [51]. To overcome these limitations, a wide range of modification strategies - including amino acid substitution, lipidation, and structural stabilization - have been explored to enhance peptide stability, selectivity, and overall therapeutic performance [24]. Beyond its direct antimicrobial activity, anoplin has also shown potential as a functional scaffold for the design of advanced therapeutic systems. Its membrane - interacting properties enable its use in conjugated or hybrid systems, including advanced modified and multivalent antimicrobial systems [38], thereby expanding its applicability beyond traditional antimicrobial roles. In addition, studies on other peptide bioconjugates suggest that multifunctional systems with antioxidant - related properties may further broaden potential biomedical applications [68, 69]. In general, these findings suggest that while anoplin itself may require further optimization, it represents a valuable template for the rational design of next - generation antimicrobial peptides and peptide - based therapeutics [61].

CONCLUSIONS

Anoplin is a short antimicrobial peptide with a well - defined membrane - targeting mechanism, primarily driven by electrostatic interactions and amphipathic structure. Its antibacterial activity arises mainly from its ability to disrupt bacterial membranes, leading to loss of membrane integrity and cell death. Numerous studies have shown that the biological performance of anoplin can be significantly improved through rational modification strategies, including amino acid substitution, lipidation, and structural stabilization. These approaches allow fine - tuning of key properties such as activity, selectivity, and stability under physiological conditions. Although membrane disruption remains the dominant mechanism of action, additional intracellular effects may also contribute under certain conditions, highlighting the multifaceted nature of its antimicrobial activity. At the same time, factors such as ionic strength, serum components, and proteolytic degradation continue to represent important challenges that may limit its effectiveness in vivo. Overall, anoplin represents a valuable model peptide for understanding structure - activity relationships and serves as a promising scaffold for the development of next - generation antimicrobial agents. Continued optimization and deeper mechanistic insights will be essential to fully realize its therapeutic potential.

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' contributions

H.M., D.D.: Data collection and Original draft preparation; N.G., D.D.: Review, editing and supervision.

REFERENCES

1. E. Naseva, P. Krasteva, Additional hospital burden due to COVID - 19 - an assessment from the perspective of the emergency department, Health Policy and Management, 2023, 23, 1, 26-30, (in Bulgarian).
2. P. Krasteva, M. Tarein, M. Staneva, Professional overheating among emergency medical workers. Results of our own study, Medical Magazine, 07.2024, 126, 30-35, (in Bulgarian).
3. N. Nwosu, Cancer: A Disease of Modern Times?. Cureus 16, 11, 2024, e74666. DOI 10.7759/cureus.74666.
4. H. Yang, Q. Ma, L. Han, H. Liu, A global prediction of cardiovascular disease from 2020 to 2030, Front. Cardiovasc. Med., 12, 2025, 1462705. doi: 10.3389/fcvm.2025.1462705.
5. P. Romanova, Ecological textiles - best practices and economic prospects, Sustainable Development, 2025, 4, 28-34.
6. W.H. Organization, Who statement on widespread antibiotic resistance, 2025 24 February 2026. Available from: https://www.who.int/news/item/13-10-2025-who-warns-of-widespread-resistance-to-common-antibiotics-worldwide.
7. W.H. Organization, Global antibiotic resistance surveillance report 2025, 2025, Geneva. p. 114.
8. D.C. Nwobodo, M.C. Ugwu, C.O. Anie, M.T. Al - Ouqaili, J.C. Ikem, U.V. Chigozie, M. Saki, Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace, 2022.
9. M.E. Elshobary, N.K. Badawy, Y. Ashraf, A.A. Zatioun, H.H. Masriya, M.M. Ammar, N.A. Mohamed, S. Mourad, A.M. Assy, Combating antibiotic resistance: Mechanisms, multidrug - resistant pathogens, and novel therapeutic approaches: An updated review, Pharmaceuticals, 18, 3, 2025, 402.
10. F. Tavares - Carreon, K. De Anda - Mora, I.C. Rojas - Barrera, A. Andrade, Serratia marcescens antibiotic resistance mechanisms of an opportunistic pathogen: A literature review, PeerJ, 11, 2023, e14399.
11. M.E. Palmer, L.J. Andrews, T.C. Abbey, A.E. Dahlquist, E. Wenzler, The importance of pharmacokinetics and pharmacodynamics in antimicrobial drug development and their influence on the success of agents developed to combat resistant gram negative pathogens: A review, Frontiers in Pharmacology, 13, 2022, 888079.
12. C.H. Wang, Y.H. Hsieh, Z.M. Powers, C.Y. Kao, Defeating antibiotic - resistant bacteria: Exploring alternative therapies for a post - antibiotic era, Int. J. Molec. Scie., 21, 3, 2020, 1061.
13. W.H. Organization, Global antimicrobial resistance and use surveillance system (glass) report :Antibiotic use data for 2022, 2025, p. 28.
14. C.H. Chen, T.K. Lu, Development and challenges of antimicrobial peptides for therapeutic applications, Antibiotics, 9, 1, 2020, 24.
15. M.S. Zharkova, D.S. Orlov, O.Y. Golubeva, O.B. Chakchir, I.E. Eliseev, T.M. Grinchuk, O.V. Shamova, Application of antimicrobial peptides of the innate immune system in combination with conventional antibiotics - a novel way to combat antibiotic resistance?, Frontiers in Cellular and Infection Microbiology, 9, 2019, 128.
16. S. Jaber, I. Iliev, T. Angelova, V. Nemska, I. Sulikovska, E. Naydenova, N. Georgieva, I. Givechev, I. Grabchev, D. Danalev, Synthesis, antitumor and antibacterial studies of new shortened analogues of $(KLAKLAK)_2$ - NH_2 and their conjugates containing unnatural amino acids, Molecules, 26, 4, 2021, 898.
17. S. Jaber, V. Nemska, I. Iliev, E. Ivanova, T. Foteva, N. Georgieva, I. Givechev, E. Naydenova, V. Karadjova, D. Danalev, Synthesis and biological studies on $(KLAKLAK)_2$ - NH_2 analog containing unnatural amino acid β - Ala and conjugates with second pharmacophore, Molecules, 26, 23, 2021, 732.
18. D. Dimitrova, N. Georgieva, Antimicrobial peptides and temporin family in the context of rising resistance - view on current development, J. Chem. Technol. Metall., 61, 3, 2026, 391-401.
19. D. Dimitrova, V. Nemska, T. Foteva, I. Iliev, N. Georgieva, D. Danalev, Synthesis and biological studies of new temporin a analogs containing unnatural amino acids in position 7, Pharmaceutics, 16, 6, 2024, 716.
20. D. Dimitrova, V. Nemska, I. Iliev, S. Petrin, N. Georgieva, D. Danalev, New temporin a analogues modified in positions 1 and 10 - synthesis and biological studies, Pharmaceutics, 17, 4, 2025, 396.

21. V. Nemska, S. Yaneva, N. Georgieva, D. Danalev, Antimicrobial profile of extracts from the fungus species *Lepista personata*, J. Chem. Technol. Metall., 58, 3, 2023, 572-576.
22. A. Tencheva, I. Stankova, T. Angelova, V. Nemska, N. Georgieva, D. Danalev, Antimicrobial activity of amino acid derivatives of memantine, J. Chem. Technol. Metall., 57, 2, 2022.
23. J.K. Boparai, P.K. Sharma, Mini review on antimicrobial peptides, sources, mechanism and recent applications, Protein and Peptide letters, 27, 1, 2020, 4-16.
24. P. Gagat, M. Ostrówka, A. Duda - Madej, P. Mackiewicz, Enhancing antimicrobial peptide activity through modifications of charge, hydrophobicity, and structure, Int. J. Molec. Sci., 25, 19, 2024, 10821.
25. A.H. Benfield, S.T. Henriques, Mode - of - action of antimicrobial peptides: Membrane disruption vs. Intracellular mechanisms, Frontiers in Medical Technology, 2, 2020, 610997.
26. Y. Utkin, Animal venoms and their components: Molecular mechanisms of action, Toxins, 13, 6, 2021, 415.
27. A.N. de Oliveira, A.M. Soares, S.L. Da Silva, Why to study peptides from venomous and poisonous animals?, Int. J. Pept. Res. Ther., 29, 5, 2023, 76.
28. K. Konno, M. Hisada, R. Fontana, C.C. Lorenzi, H. Naoki, Y. Itagaki, A. Miwa, N. Kawai, Y. Nakata, T. Yasuhara, Anoplin, a novel antimicrobial peptide from the venom of the solitary wasp anoplius samariensis, Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology, 1550, 1, 2001, 70-80.
29. K. Chionis, D. Krikorian, A.I. Koukkou, M. Sakarellos-Daitsiotis,E. Panou-Pomonis, Synthesis and biological activity of lipophilic analogs of the cationic antimicrobial active peptide anoplin, J. Pept. Sci., 22, 11 - 12, 2016, 731-736.
30. S. Gou, B. Li, X. Ouyang, Z. Ba, C. Zhong, T. Zhang, L. Chang, Y. Zhu, J. Zhang, N. Zhu, Novel broad - spectrum antimicrobial peptide derived from anoplin and its activity on bacterial pneumonia in mice, J. Med. Chem., 64, 15, 2021, 11247-11266.
31. M. Bobier, A. Joseph Villaraza, P. Mahal Sabido, Bismaleimidohexane - stapled anoplin analogs, in J. Pept. Sci., Wiley 111 river st., hoboken 07030 - 5774, NJ USA, 2024.
32. J.K. Munk, L.E. Uggerhøj, T.J. Poulsen, N. Frimodt - Møller, R. Wimmer, N.T. Nyberg, P.R. Hansen, Synthetic analogs of anoplin show improved antimicrobial activities, J. Pept. Sci., 19, 11, 2013, 669-675.
33. Y. Wang, J. Chen, X. Zheng, X. Yang, P. Ma, Y. Cai, B. Zhang, Y. Chen, Design of novel analogues of short antimicrobial peptide anoplin with improved antimicrobial activity, J. Pept. Sci., 20, 12, 2014, 945-951.
34. S. Jaber, V. Nemska, I. Iliev, E. Ivanova, T. Foteva, N. Georgieva, I. Givechev, D. Tanev, E. Naydenova, D. Danalev, Synthesis, antiproliferative and antimicrobial activities of $(KLAKLAK)_2$ - NH_2 analogue containing nor - Leu and its conjugates with a second pharmacophore, Biotechnology & Biotechnological Equipment, 37, 1, 2023, 151-158.
35. N. Angelova, I. Iliev, V. Nemska, T. Dzimbova, N. Georgieva, D. Danalev, E. Naydenova, Design, synthesis, and biological evaluation of new analogs of aurein 1.2 containing non - proteinogenic amino acids, Molecules, 30, 9, 2025, 2050.
36. R.L. Salas, J.K.D. Garcia, A.C.R. Miranda, W.L. Rivera, R.B. Nellas, P.M.G. Sabido, Effects of truncation of the peptide chain on the secondary structure and bioactivities of palmitoylated anoplin, Peptides, 104, 2018, 7-14.
37. M.P. Dos Santos Cabrera, M. Arcisio-Miranda, S.T. Broggio Costa, K. Konno, J.R. Ruggiero, J. Procopio, J. Ruggiero Neto, Study of the mechanism of action of anoplin, a helical antimicrobial decapeptide with ion channel-like activity, and the role of the amidated C - terminus, J. Pep. Sci.: An Official Publication of the European Peptide Society, 14, 6, 2008, 661-669.
38. S. Gou, B. Li, X. Ouyang, Z. Ba, C. Zhong, J. Ni, Tuning the activity of anoplin by dendrimerization of lysine and lipidation of the N - terminal, ACS omega, 6, 33, 2021, 21359-21367.
39. J. Jittikoon, Short native α - helical cationic antimicrobial peptides: Promising alternative antibiotics, The Thai J. Pharmaceut. Sci., 39, 1, 2015, 1-9.
40. J.K. Munk, C. Ritz, F.P. Fliedner, N. Frimodt - Møller, P.R. Hansen, Novel method to identify the optimal antimicrobial peptide in a combination

matrix, using anoplin as an example, Antimicrobial agents and chemotherapy, 58, 2, 2014, 1063-1070.
41. M. Zasloff, Antimicrobial peptides of multicellular organisms, Nature, 415, 6870, 2002, 389-395.
42. B. Bechinger, K. Lohner, Detergent - like actions of linear amphipathic cationic antimicrobial peptides, Biochimica et Biophysica Acta (BBA) - Biomembranes, 1758, 9, 2006, 1529-1539.
43. W. Chan, P. White, Fmoc solid phase peptide synthesis: A practical approach. Vol. 222: OUP Oxford, 1999.
44. B. Liu, H. Huang, Z. Yang, B. Liu, S. Gou, C. Zhong, X. Han, Y. Zhang, J. Ni, R. Wang, Design of novel antimicrobial peptide dimer analogues with enhanced antimicrobial activity in vitro and in vivo by intermolecular triazole bridge strategy, Peptides, 88, 2017, 115-125.
45. J. Macyszyn, M. Burmistrz, A. Mieczkowski, M. Wojciechowska, J. Trylska, Conjugates of aminoglycosides with stapled peptides as a way to target antibiotic - resistant bacteria, ACS omega, 8, 21, 2023, 19047-19056.
46. D. Ifrah, X. Doisy, T.S. Ryge, P.R. Hansen, Structure - activity relationship study of anoplin, J. Pept. Sci.: An Official Publication of the European Peptide Society, 11, 2, 2005, 113-121.
47. L.E. Uggerhøj, T.J. Poulsen, J.K. Munk, M. Fredborg, T.E. Sondergaard, N. Frimodt - Moller, P.R. Hansen, R. Wimmer, Rational design of alpha-helical antimicrobial peptides: Do's and don'ts, ChemBioChem, 16, 2, 2015, 242-253.
48. C. Zhong, Y. Zhu, N. Zhu, T. Liu, S. Gou, F. Zhang, J. Yao, J. Xie,J. Ni, Synthesis and anti - pseudomonal activity of new ß - ala modified analogues of the antimicrobial peptide anoplin, Int. J. Med. Microbiol., 310, 5, 2020, 151433.
49. K. Meinike, P. Hansen, Peptoid analogues of anoplin show antibacterial activity, Protein and Peptide Letters, 16, 9, 2009, 1006-1011.
50. K. Cruz Orozco, M.A. Ibarra Valencia, H. Barrios Camacho, G. Corzo Burguete, I. Arenas Sosa, Antimicrobial and antibiofilm activity of analogous synthetic peptides from anoplin, temporin, and aurein, Int. J. Pept. Res. Therap., 31, 2, 2025, 25.
51. T. Liu, N. Zhu, C. Zhong, Y. Zhu, S. Gou, L. Chang, H. Bao, H. Liu, Y. Zhang, J. Ni, Effect of n - methylated and fatty acid conjugation on analogs of antimicrobial peptide anoplin, Eur. J. Pharmac. Sci., 152, 2020, 105453.
52. J.C. Slootweg, T.B. van Schaik, H.L.C.Q. van Ufford, E. Breukink, R.M. Liskamp, D.T. Rijkers, Improving the biological activity of the antimicrobial peptide anoplin by membrane anchoring through a lipophilic amino acid derivative, Bioorganic & Medicinal Chemistry Letters, 23, 13, 2013, 3749-3752.
53. C. Zhong, N. Zhu, Y. Zhu, T. Liu, S. Gou, J. Xie, J. Yao, J. Ni, Antimicrobial peptides conjugated with fatty acids on the side chain of d - amino acid promises antimicrobial potency against multidrug-resistant bacteria, Eur. J. Pharm. Sci., 141, 2020, 105123.
54. C. Zhong, T. Liu, S. Gou, Y. He, N. Zhu, Y. Zhu, L. Wang, H. Liu, Y. Zhang, J. Yao, Design and synthesis of new n - terminal fatty acid modified - antimicrobial peptide analogues with potent in vitro biological activity, Eur. J. Med. Chem., 182, 2019, 111636.
55. M. Kai, W. Zhang, H. Xie, L. Liu, S. Huang, X. Li, Z. Zhang, Y. Liu, B. Zhang, J. Song, Effects of linker amino acids on the potency and selectivity of dimeric antimicrobial peptides, Chinese Chem. Lett., 29, 7, 2018, 1163-1166.
56. M. Wojciechowska, J. Miszkiewicz, J. Trylska, Conformational changes of anoplin, W - MreB1 - 9, and (KFF) 3K peptides near the membranes, Int. J. Molec. Sci., 21, 24, 2020, 9672.
57. S.S. Ranade, R. Ramalingam, Hydrogen bonds in anoplin peptides aid in identification of a structurally stable therapeutic drug scaffold, J. Molec. Model., 26, 6, 2020, 155.
58. C. Chamorro, M.A. Boerman, C.J. Arnusch, E. Breukink, R.J. Pieters, Enhancing membrane disruption by targeting and multivalent presentation of antimicrobial peptides, Biochimica et Biophysica Acta (BBA) - Biomembranes, 1818, 9, 2012, 2171-2174.
59. P. Sahariah, K.K. Sørensen, M.Á. Hjálmarsdóttir, Ó.E. Sigurjónsson, K.J. Jensen, M. Másson, M.B. Thygesen, Antimicrobial peptide shows enhanced activity and reduced toxicity upon grafting to chitosan polymers, Chem. Commun., 51, 58, 2015, 11611-11614.
60. S. Jaber, Y. Evstatieva, V. Nemska, D. Nikolova, E. Naydenova, N. Georgieva, D. Danalev, Antimicrobial activity of (KLAKLAK) - NH_2

analogs against pathogenic microbial strains, Curr. Res. Biotechnol., 8, 2024, 100236.
61. C. Zhong, J. Zou, W. Mao, P. Yang, J. Zhang, S. Gou, Y. Zhang, H. Liu, J. Ni, Structure modification of anoplin for fighting resistant bacteria, Eur. J. Med. Chem., 268, 2024, 116276.
62. V. Vitkova, A. Stoyanova - Ivanova, S. Jaber, E. Naydenova, D. Danalev, Bending elasticity of phospholipid bilayers containing an amphipathic peptide with low mammalian cytotoxicity, Comptes rendus de l'Acad´emie bulgare des Sciences, 75, 10, 2022, 1428-1436.
63. V. Vitkova, K. Antonova, O. Petkov, A. Stoyanova - Ivanova, S. Jaber, V. Ivanova, E. Naydenova, D. Danalev, Interaction of KLAKLAK-NH_2 and analogs with biomimetic membrane models, Pharmaceutics, 16, 3, 2024, 340.
64. Q.Y. Zhang, Z.B. Yan, Y.M. Meng, X.Y. Hong, G. Shao, J.J. Ma, X.R. Cheng, J. Liu, J. Kang, C.Y. Fu, Antimicrobial peptides: Mechanism of action, activity and clinical potential, Military Medical Research, 8, 1, 2021, 48.
65. Y. Shai, Mode of action of membrane active antimicrobial peptides, Peptide Science: Original Research on Biomolecules, 66, 4, 2002, 236 - 248.
66. S. Guha, J. Ghimire, E. Wu, W.C. Wimley, Mechanistic landscape of membrane - permeabilizing peptides, Chem. Rev., 119, 9, 2019, 6040-6085.
67. H. Syed, M. Tauseef, Z. Ahmad, A connection between antimicrobial properties of venom peptides and microbial atp synthase, Int. J. Biolog. Macromol., 119, 2018, 23-31.
68. Y. Stoyanova, S. Jaber, E. Naydenova, N. Georgieva, D. Danalev, Antioxidant capacity of $(KLAKLAK)_2$ bioconjugates assessed by the electron - transfer methods FRAP and CUPRAC, J. Chem. Technol. Metall., 60, 6, 2025, 913-920.
69. Y. Stoyanova, S. Jaber, Y. Dinev, E. Naydenova, N. Georgieva, D. Danalev, Radical - scavenging activity of $(KLAKLAK)_2$ bioconjugates with caffeic acid, J. Chem. Technol. Metall., 60, 6, 2025, 931-936.