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Design methods for antimicrobial peptides with improved performance

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ABSTRACT

The recalcitrance of pathogens to traditional antibiotics has made treating and eradicating bacterial infections more difficult. In this regard, developing new antimicrobial agents to combat antibiotic-resistant strains has become a top priority. Antimicrobial peptides (AMPs), a ubiquitous class of naturally occurring compounds with broad-spectrum antipathogenic activity, hold significant promise as an effective solution to the current antimicrobial resistance (AMR) crisis. Several AMPs have been identified and evaluated for their therapeutic application, with many already in the drug development pipeline. Their distinct properties, such as high target specificity, potency, and ability to bypass microbial resistance mechanisms, make AMPs a promising alternative to traditional antibiotics. Nonetheless, several challenges, such as high toxicity, lability to proteolytic degradation, low stability, poor pharmacokinetics, and high production costs, continue to hamper their clinical applicability. Therefore, recent research has focused on optimizing the properties of AMPs to improve their performance. By understanding the physicochemical properties of AMPs that correspond to their activity, such as amphipathicity, hydrophobicity, structural conformation, amino acid distribution, and composition, researchers can design AMPs with desired and improved performance. In this review, we highlight some of the key strategies used to optimize the performance of AMPs, including rational design and *de novo* synthesis. We also discuss the growing role of predictive computational tools, utilizing artificial intelligence and machine learning, in the design and synthesis of highly efficacious lead drug candidates.

Keywords: Antimicrobial resistance; Antimicrobial peptides; Design methods; Peptidomimetics; Artificial intelligence

INTRODUCTION

With increasing concerns regarding untreatable infections, researchers are racing to develop novel antimicrobial agents to combat the growing epidemic of antimicrobial resistance (AMR). The severity of AMR as a global crisis cannot be underestimated. It poses a considerable threat to public health, healthcare systems, and the effectiveness of antimicrobial treatments. A recent report from Lancet Medical indicated that AMR currently kills more than 1.2 million people yearly and will be responsible for 10 million deaths annually by 2050 if the growing threat is not solved (Murray et al., 2022). Of concern, many pathogens, including viruses, fungi, and gram-negative and gram-positive bacteria, are increasingly becoming unresponsive to available antimicrobial agents, resulting in increased morbidity and mortality rates, with the death toll due to AMR surpassing those of HIV and malaria (Breijyeh et al., 2020; Jubeh et al., 2020). Under this trend, a growing number of researchers and pharmaceutical companies are evaluating potential alternatives to combat superbugs, especially those related to nosocomial infections from vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus* (MRSA) (Grundmann et al., 2006),

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carbapenem-resistant strains of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (Ramadan et al., 2018), and third-generation cephalosporin-resistant bacterial strains (Fridkin et al., 2001). In this regard, antimicrobial peptides (AMPs) have shown excellent potential as next-generation antimicrobial agents due to their ability to bypass microbial resistance mechanisms (Erdem Büyükkiraz & Kesmen, 2022). These small, naturally occurring molecules exhibit extensive microbicidal activity against bacteria, fungi, and viruses. Owing to their unique mechanism of action, primarily disruption of microbial membranes or interference with essential intracellular processes, AMPs present an excellent opportunity to discover and develop novel therapeutics and antimicrobial agents. Furthermore, AMPs have demonstrated remarkable potential as alternatives to conventional antibiotics due to their multimodal functionalities and low tendency to select for the development of resistance (Benfield & Henriques, 2020).

To date, more than 3 500 AMPs from various sources have been cataloged in the "Antimicrobial Peptide Database" (APD) and evaluated for their potential therapeutic application (Figure 1) (Erdem Büyükkiraz & Kesmen, 2022; Kumar et al., 2018; Tornesello et al., 2020; Wang et al., 2016, 2021b). The extensive phylogenetic distribution of AMPs underscores the rich evolutionary history of these molecules and their importance in innate defenses. AMPs have been isolated from organisms across the animal kingdom, including mammals, amphibians, insects, crustaceans, and several other invertebrates (Wang et al., 2016). The rich repertoire of AMPs derived from animal sources provides a veritable treasure trove of bioactive molecules with significant potential for the development of novel therapeutic agents (Mwangi et al., 2019a). With the increasing occurrence of drug-resistant infections, isolating AMPs from animals has become a promising strategy to bolster our antimicrobial arsenal. AMPs are ubiquitous in mammalian gastrointestinal, respiratory, and urogenital tracts, providing chemical barriers against infection (Radek & Gallo, 2007). Furthermore, the skins and mucosal surfaces of frogs contain over 300 identified AMPs, offering protection against microbes. For example, Li et al. (2007) discovered 372 cDNA sequences encoding AMPs from the skin of a single *Odorrana grahami* frog. These sequences were organized into 30 diverse groups, 24 of which were previously undescribed, resulting in the identification of 107 novel AMPs. These AMPs were further categorized into structural-based families, including brevinins, esculentins, palustrins, and gaegurins. Further sequence analysis revealed that the considerable AMP diversification arose via mutations, domain shuffling, and splicing, thereby generating diverse families of AMPs descended from common ancestors. The peptides also displayed a broad spectrum of antimicrobial activities and immune functions, including mast cell activation, bacterial membrane disruption, pore formation, and intracellular effects (Li et al., 2007). Overall, this research significantly expanded existing knowledge of sequence and functional diversity of amphibian AMPs, offering key insights into their evolutionary origins and defensive functions.

Yang et al. (2012) conducted extensive peptidomic and cDNA analyses to elucidate the diversity of AMPs in frog skin secretions. They first purified 198 high-performance liquid chromatography (HPLC) fractions with antimicrobial activity from the skin secretions of nine odorous frog species. Further peptidomic analysis identified 80 native AMPs belonging to several known families, including brevinins and esculentins, while cDNA cloning identified the precursors for 728 mature AMPs, including 662 novel sequences, thereby highlighting the remarkable diversity of AMPs. Contrary to expectations of species-specific uniqueness, identical AMPs were distributed across multiple odorous frog species. The AMPs demonstrated considerable diversity in size, structure, charge, and disulfide bonding, with 71% containing a conserved C-terminus cyclic motif. While the native peptides exhibited potent antimicrobial activities, synthesized variants showed enhanced immune modulating effects. The AMPs exhibited

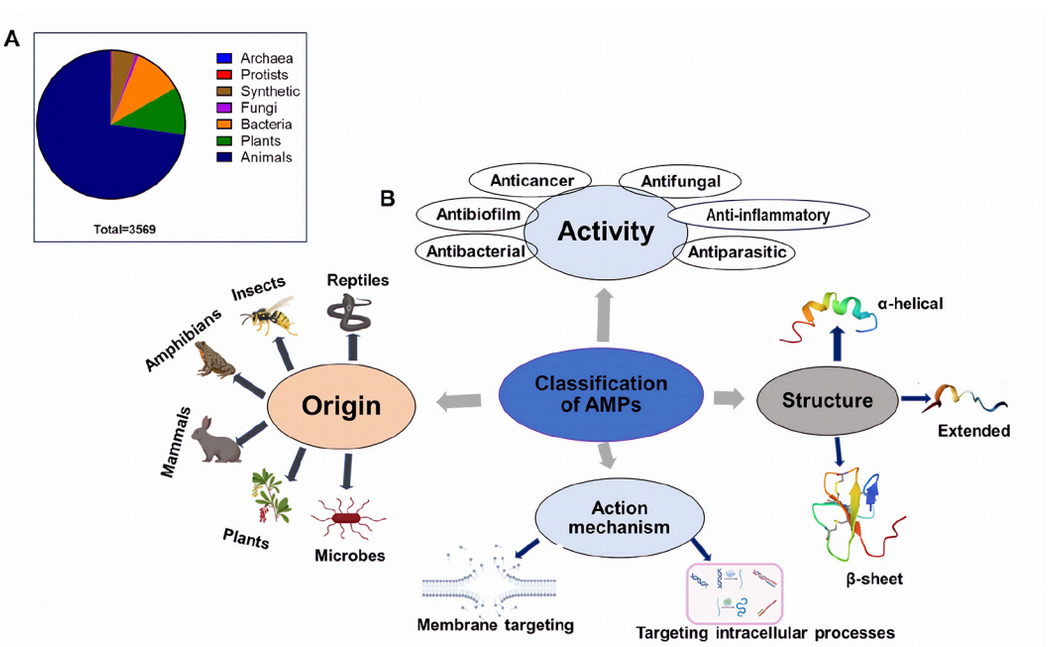


Figure 1 Graphical summary of origin and classification of antimicrobial peptides

A: Statistics showing number of AMPs from different sources cataloged in the Antimicrobial Peptide Database (APD: https://aps.unmc.edu/AP/) as of June 2023 (Wang et al., 2016). B: Schematic representation summarizing various parameters used to classify AMPs.

also strong antimicrobial activity against common bacterial and fungal pathogens, thus highlighting the importance of amphibians and other animals as an important source of novel antimicrobial compounds.

Several AMPs, noted for their remarkable therapeutic potential, are already in the drug development pipeline. However, despite their inherent antimicrobial properties, not all AMPs possess the characteristics necessary for next-generation antimicrobials or clinical translation. Notably, various challenges, including high toxicity, lability to proteolytic degradation, low stability, poor pharmacokinetics, and high production costs, continue to constrain their clinical applicability (Zeng et al., 2021). As such, the design of AMPs with enhanced performance has become a focal point of research. Numerous methodologies and strategies have been developed over the years to optimize the efficacy, specificity, and therapeutic potential of both natural and synthetic AMPs, including rational design, combinatorial chemistry, and structural modification. Furthermore, an increased understanding of the physicochemical-activity relationship of AMPs has driven the rapid development of peptidomimetics, a group of synthetic compounds that mimic both the structural and functional characteristics of natural AMPs (Croft & Purcell, 2011; Lachowicz et al., 2020; Méndez-Samperio, 2014). However, the design process for AMPs is complex and requires a thorough understanding of their structure-activity relationship. Furthermore, elucidating how these properties can be manipulated to optimize AMPs to generate lead drug candidates with desired qualities is essential. Several excellent studies and reviews have provided detailed discussions on the classification, properties, mechanisms of action, and structures of AMPs. Therefore, this review will focus on the design methods used to increase AMP performance.

The ability to engineer and optimize AMP performance opens new avenues for drug discovery, especially in an era where AMR has become a serious global threat. This review aims to offer valuable insights for researchers, clinicians, pharmaceutical companies, and scientists interested in designing and developing AMPs with improved performance, ultimately contributing to the advance of novel and effective antimicrobial therapies.

This review provides an overview of the diverse approaches and design methods used to enhance the performance of AMPs. Both conventional (chemical modification) and cutting-edge techniques (computational techniques, artificial intelligence (AI), and machine learning (ML) approaches) utilized by researchers to improve the stability, activity, selectivity, and safety profiles of new peptide sequences are critically explored. Overall, this review highlights key factors contributing to the successful design and optimization of high-performance AMPs and attempts to shed light on future directions and prospects in this dynamic field.

DESIGNING ANTIMICROBIAL PEPTIDES

Given their unique properties, AMPs hold remarkable potential as next-generation antimicrobial agents. Nevertheless, their clinical applicability is hampered by several drawbacks, including low stability in certain physiological conditions (Zhao et al., 2016), low specificity (Pachón-Ibáñez et al., 2017), high toxicity and hemolytic side effects, high production costs and technical issues, and proteolytic degradation (Huan et al., 2020). Therefore, overcoming these challenges is an active area of research in the development of high-performance AMPs. Designing AMPs is a complex process involving the selection of appropriate amino acid sequences, optimization of physicochemical properties, and evaluation of activity against target microorganisms (Lee et al., 2022). AMPs possess unique physical and chemical properties that can be optimized through various design approaches to enhance their therapeutic application potential (Huan et al., 2020). For example, the cationic nature of many AMPs due to the presence of positively charged amino acids with basic side chains facilitates their initial electrostatic attraction to negatively charged microbial cell surfaces, leading to bacterial membrane disruption and subsequent cell death (Mwangi et al., 2019a). Thus, the incorporation of positively charged amino acids, such as lysine (Lys) and arginine (Arg), can improve the cationicity of AMPs, thus enhancing their selectivity for microbial membranes (Jin et al., 2016).

Furthermore, the amphipathic nature of many AMPs, characterized by spatially segregated hydrophobic and hydrophilic faces within their molecular structure (Huan et al., 2020), is crucial for their antimicrobial activity. This amphipathicity enables insertions into microbial membranes while retaining solubility in aqueous environments (Cheng & Zeng, 2022). Another key property of AMPs is their concise sequence length, typically ranging from 10 to 50 amino acid residues. Maintaining a sequence length under 50 amino acids offers advantages in terms of cost-effective chemical synthesis, enhanced stability, and reduced immunogenicity, without compromising activity.

AMPs also have diverse amino acid compositions, allowing for flexibility in the design of peptides with specific properties, such as enhanced stability or selectivity against certain types of pathogens (Huan et al., 2020). Many AMPs are resistant to proteolytic degradation, enabling the maintenance of activity in the presence of enzymes that would typically degrade peptides or antibiotics. This property contributes to their longevity in the host and their ability to persist in hostile environments. Furthermore, AMPs also exhibit low selection toward mammalian cells, which is crucial for minimizing host harm while targeting pathogens.

Understanding the physicochemical properties of AMPs can help overcome the challenges associated with their design for therapeutic and biotechnological applications. Researchers continue to explore and optimize these properties to develop novel AMPs for various clinical and industrial purposes. Rational optimization of these key physicochemical properties through various design approaches, such as sequence modifications, conformational constraints, and lipidation, can improve the drug-like properties and therapeutic applicability of AMPs.

Rational design: Chemical modification of antimicrobial peptides

Rational design involves the design of AMPs using structural and bioinformatic tools to predict the most effective amino acid sequences and physicochemical properties for targeting specific microorganisms. This may include altering existing peptide sequences chemically or creating new peptide mimetics with structural and functional similarities to known peptides (peptidomimetics). Generally, rational design focuses on several key attributes, including amino acid composition, chain length, hydrophobicity, net positive charge, secondary structure, and amphiphilicity, as investigated in multiple

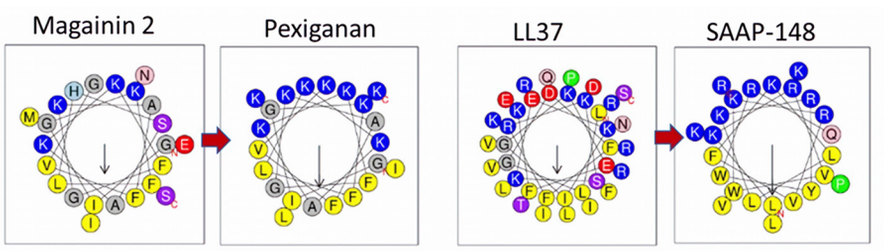
studies (Mwangi et al., 2019a; Zhang et al., 2019).

Chemical modification techniques, which include amino acid substitution, halogenation, acetylation, cyclization, nanoparticle conjugation, and peptidomimetics, are commonly used to modify AMPs to improve their performance (Chan et al., 2013; Datta et al., 2016; Gottler & Ramamoorthy, 2009; Guan et al., 2019; Han et al., 2021; Jerala, 2007; Méndez-Samperio, 2014). Peptide sequences of naturally occurring AMPs are typically composed of several amino acid residues, which can hinder translation to clinical application due to immunogenicity, toxicity, and high cost of synthesis (Elnagdy & Alkhazindar, 2020; Gan et al., 2021; Han et al., 2021; Haney et al., 2019). Therefore, rational design enables researchers to modify various parameters of AMPs to address their inherent limitations. For example, incorporating non-natural amino acids or using D-amino acids in place of L-amino acids in peptide sequences can reverse the stereochemistry of natural peptides, impacting their stability and antimicrobial activity (Zhong et al., 2020). AMPs also feature an optimal balance of hydrophobic and hydrophilic amino acids, which contributes to their preferential interaction with typically negatively charged microbial cell membranes (Giangaspero et al., 2001; Jin et al., 2016; Lei et al., 2019; Leptihn et al., 2010). Additionally, cationic residues, such as Lys and Arg, provide a net positive charge, which increases the affinity of AMPs to microbial membranes (Dennison et al., 2012; Edwards et al., 2016; Mura et al., 2016). Modifying these parameters can impact the structural conformation, physicochemical properties, and antimicrobial activity of AMPs. For example, the incorporation of Lys, His, and tryptophan (Trp) in peptide analogs of mastoparan (a primary component of wasp venom) significantly enhances their activity (Avram et al., 2012). Consistently, we previously showed that Trp, Arg, and Lys amino acid residues can increase the antimicrobial potency of designed peptides (Jin et al., 2016; Mwangi et al., 2019b).

Rational peptide design also involves a combination of several techniques and technologies, such as computational modeling, structural analysis, experimental validation, and bioinformatics tools, to design potent lead compounds. Computational tools can be used to predict the three-dimensional (3D) structure, hydrophobicity, hydrophilicity, and net charge of peptides (Cesaro et al., 2022). For example, the online webserver HeliQuest (https://heliquest.ipmc.cnrs.fr/) enables users to obtain the helical wheel projections and physicochemical properties of their peptide sequences, as well as mutate peptide sequences either manually or automatically to obtain peptide analogs with desired physicochemical properties (Gautier et al., 2008). Moreover, other techniques, such as nuclear magnetic resonance (NMR) and circular dichroism (CD) spectroscopy, have been employed to predict the secondary and 3D structures of new compounds. These methodologies are crucial for the discovery, design, and modification of peptide drugs (Cesaro et al., 2022; de Breij et al., 2018).

Amino acid substitution

Modifying specific properties of AMPs, such as hydrophobicity, amphipathicity, sequence length, distribution, and cationic charge, can significantly impact their activity (Edwards et al., 2016; Yin et al., 2012). As only a small fraction of key amino acids contributes to the antimicrobial properties of AMPs, other residues can be changed or removed without significant impact on their activity. Substituting single or multiple amino acids alters the physicochemical properties (e.g., hydrophobicity, net charge, and amphipathicity) of AMPs, affecting their antimicrobial potency (Figure 2). For instance, although the well-studied human cathelicidin has a primary sequence of 37 amino acid residues, only the C-terminal region (residues 17–29) has been shown to be antimicrobially active and has been used as a template for the generation of many potent peptide variants (de Breij et al., 2018; Rajasekaran et al., 2017; Wang, 2008). For example, replacing specific amino acid residues in the FK13 region of the LL-37 peptide gives rise to multiple analogs, which exhibit strong antimicrobial efficacy against multidrug-resistant pathogens, including MRSA and vancomycin-resistant *Enterococcus faecium* (VREF) (Rajasekaran et al., 2017). Similarly, the KR-12 peptide generated by substituting Phe at the 17th position of LL-37 displays potent antimicrobial activity against *Escherichia coli* with minimal toxicity to animal cells (Wang, 2008). Several other variants of LL-37 have shown potent antimicrobial, antibiofilm, anticancer, and wound-healing properties. For example, de Breij et al. (2018) synthesized several analogs through random substitution of amino acids from the C-terminal chain of LL-37, of which SAAP-148 exhibited remarkable (micromolar range) antimicrobial activity against a wide range of antibiotic-resistant bacteria (MRSA and MDR *A. baumannii*), as well as



Peptide	Sequence	Length	Net Charge	Hydrophobicity
Magainin 2	GIGKFLHSAKKFGKAFVGEIMNS	23	3	0.373
Pexiganan	GIGKFLKKAKKFGKAFVKILKK	22	9	0.241
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	37	6	0.201
SAAP-148	Ac-LKRVWKRVFKLLKRYWRQLKKPVR-NH_2	24	11	0.301

Figure 2 Helical wheel projections of magainin 2, LL-37, and their derivatives (pexiganan and SAAP-148, respectively)

Chemical modification of AMPs through amino acid substitutions can alter their physicochemical properties (e.g., net charge and hydrophobicity), leading to reduced toxicity and increased antimicrobial activity.

antibiofilm activity, persister cell eradication, and wound-healing properties *ex vivo*.

In a prior study, we identified a 30-amino-acid AMP (cathelicidin-BF) derived from the venom of the snake species *Bungarus fasciatus* (Wang et al., 2008). While this peptide exhibited significant activity against many pathogens, especially gram-negative bacteria, its applicability was somewhat limited due to its long amino acid sequence and hemolytic activity. Through subsequent optimization, including library screening and structure-based design, we generated a series of shorter peptides (15–17 amino acids in length) with enhanced antimicrobial and therapeutic properties. ZY13 (VKRWKKWRWKWKKWV-NH2), a tryptophan and lysine/arginine-rich cationic peptide, showed high efficacy against antibiotic-resistant clinical isolates of *Candida albicans* with minimal hemolysis (Jin et al., 2016b). Subsequent studies showed that ZY13 inhibited ZIKA virus infection *in vitro* and in mice models. The peptide directly inactivated ZIKV and reduced the *in-vivo* replication of infectious virions (Xing et al., 2020a). Furthermore, ZY4, a cyclic 17-amino-acid peptide stabilized with a cysteine-cysteine disulfide bridge, showed potent antimicrobial activity against common and antibiotic-resistant isolates of *A. baumannii* and *P. aeruginosa*, as well as robust antibiofilm and anti-persister cell activities, leading to the complete eradication of preformed biofilms within a few hours (Mwangi et al., 2019b).

Naturally occurring AMPs generally show a positive (+2 to +13) charge (Erdem Büyükkiraz & Kesmen, 2022; Huan et al., 2020). Altering the total net charge via cationic amino acid residue substitution may impact their affinity and interactions with negatively charged microbial membranes, thus influencing their antimicrobial activity (López Cascales et al., 2018; Omardien et al., 2016; Singh et al., 2016). For example, a more positively charged aurein 1.2 (GLFDIIKKIAESF-NH_2), a short and potent peptide derived from the skin of the *Litoria aurea* frog, significantly enhances its antibacterial activity (Ramezanzadeh et al., 2021). Furthermore, aurein M2 with a +5 net charge obtained through the substitution of Glu 11 and Asp 4 of aurein 1.2 shows increased antibacterial activity against *P. aeruginosa*, *S. aureus*, and *E. coli* compared to its parent peptide, which has a net positive charge of +1 (Ramezanzadeh et al., 2021). However, although increasing the cationic charge of AMPs can enhance their antimicrobial activities, exceeding a certain threshold has been shown to increase their toxicity (Singh et al., 2016).

Capping (terminal/side chain modification)

Post-translational modifications are commonly applied techniques in peptide-drug engineering. Generally, natural AMPs, such as cathelicidins, defensins, and aureins, are amidated (–NH_2) at the C-terminal, which is correlated with their structural conformity and antimicrobial activity (Kumar et al., 2018). The secondary structure or conformation of an AMP is crucial for its interactions with microbial cell membranes (Pastore et al., 2020; Qian et al., 2008; Smirnova et al., 2004; Yokoo et al., 2021). For example, the RANA box (a cyclic heptapeptide at the C-terminal containing a conserved disulfide bond) found in most AMPs obtained from ranid frogs has been shown to enhance their antibacterial properties (Bao et al., 2018; Dennison et al., 2015; Zhang et al., 2019). Capping involves the modification or addition of specific motifs to the terminal or side chains of AMPs and is an effective strategy for enhancing the stability and efficacy of AMPs (Brinckerhoff et al., 1999; Dennison et al., 2015; Sforça et al., 2004; Urnukhsaikhan et al., 2021). Common AMP capping techniques include C-terminal modification (amidation) and N-terminal modification (acetylation, methylation, and lipidation) (Figure 3). These techniques enhance the conformational stability of AMPs and protect them from enzymatic degradation while maintaining or enhancing their antimicrobial potency (Brinckerhoff et al., 1999; Croft & Purcell, 2011; Li et al., 2021; Sandín et al., 2021; Zhang et al., 2019). In the process of amidation, the C-terminal end of a peptide —the carboxyl group (–COOH) is converted into a carboxamide group (–CONH2) by replacing a hydroxyl group with a nitrogen atom. This process can be achieved by chemically or enzymatically adding an amide group to the C-terminal end of the peptide and has been shown to increase the stability and lower the toxicity (Mura et al., 2016; Sforça et al., 2004). For example, C-terminal carboxyl-amidated aurein 2.5 (GLFDIVKKVVGAFGSL-CONH2) exhibits increased antimicrobial potency against *K. pneumoniae* compared to its C-terminal carboxylated (GLFDIVKKVVGAFGSL-COOH) analog (Dennison et al., 2012). C-terminal amidation increases the efficacy of peptides due to enhanced conformational stability (Mura et al., 2016; Yakimov et al., 2016). For example, the carboxyl-amidated peptide modelin-5-CONH2 displays greater helix stability and

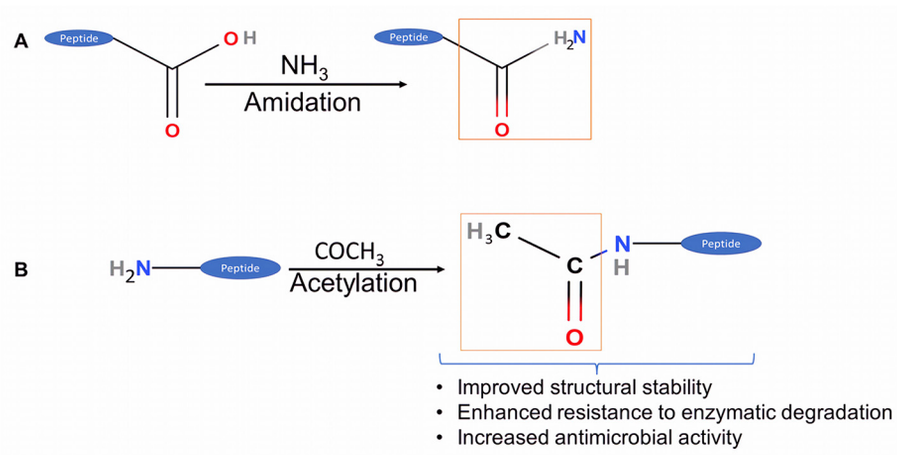


Figure 3 Representation of several techniques (C-terminal amidation and N-terminal modification acetylation) used to optimize AMP performance

efficacy against *E. coli* than its non-amidated analog modelin-5-COOH (Dennison & Phoenix, 2011).

In addition to C-terminal modification, side chain modifications are effective strategies for designing AMPs with improved antibacterial activity, enhanced stability under physiological conditions, and increased resistance to proteolytic degradation. For example, lipidation of the N-terminal domain (addition of non-natural amino acids such as lipids) can reduce cytotoxicity, increase resistance to proteolytic degradation, and enhance antibacterial activity (Datta et al., 2016; Jerala, 2007; Malina & Shai, 2005). Lipidation increases the potency of AMPs by strengthening the interaction between the fatty acid-peptide conjugate and microbial plasma membrane (Rounds & Straus, 2020). Other N-terminal modifications, such as methylation and acetylation, can also enhance resistance to enzymatic degradation and improve antimicrobial activity (Han et al., 2021; Nevitt et al., 2018). Methylation involves adding a methyl group to the N-terminus group, especially Lys and Arg residues, which can change the hydrophobicity and conformational structure of the peptide, thus affecting its activity (Chen & Kashina, 2021). For example, a methylated analog of anoplin—a peptide isolated from the venom of the solitary wasp *Anoplius samariensis*—retains its microbicidal activity even under harsh physiological conditions and exhibits increased resistance to protease degradation (Liu et al., 2020). Similarly, acetylation, the addition of an acetyl group ($-COCH_3$), can enhance the helicity of AMPs and promote resistance to proteolytic degradation while increasing antimicrobial potency (Kang et al., 2022; Li et al., 2021; Papanastasiou et al., 2009).

Halogenation

Halogenation is another valuable strategy for optimizing the properties of a wide range of bioactive compounds, including AMPs. This process involves the addition of one or more atoms, such as chlorine, fluorine, bromine, and iodine, into these compounds to achieve desired properties. Halogenation is considered a more effective approach than any other type of amino-acid substitution in improving drug performance, with more than a third of drugs under clinical trials being halogenated (Jiang et al., 2016; Shah et al., 2017; Shearer et al., 2022). Benedetto Tiz et al. (2022) provide several examples of halogenated drugs approved by the FDA. Halogenation can be applied to optimize the degradability of therapeutic agents, lipophilicity, catabolic stability, and membrane permeabilization properties (Huhmann & Koksch, 2018; Mardirossian et al., 2021). For example, fluorination (addition of fluorine atoms) is extensively used to enhance the pharmacokinetics of antimicrobial agents. Several drugs currently under clinical investigation and some approved for application, such as Ciprobay (antibacterial ciprofloxacin), Lipitor (cholesterol-lowering drug), and Prozac (antidepressant), contain fluorine ions (Müller et al., 2007). Due to the capacity of fluorine to alter the electrostatic properties of bioactive compounds, its addition provides an excellent opportunity for drug improvement. Notably, fluorine can alter the conformational structure of aliphatic halogenated amino acid-containing peptides, thereby influencing their pharmacological properties, such as resistance to proteolytic degradation (Asante et al., 2014; Berger et al., 2017; Robalo et al., 2017).

Halogenation exerts a transformative effect on the properties of AMPs, leading to enhanced stability, improved solubility, and increased antimicrobial activity, including under harsher physiological conditions, thereby improving their efficacy against target pathogens (Mardirossian et al., 2021). For example, the halogenation of jelleine-I, a short AMP purified from the venom of the royal jelly of honeybees (*Apis mellifera*), markedly enhances its therapeutic performance (Jia et al., 2019). The substitution of phenylalanine with its halogenated phenylalanine analog leads to a significant increase in the antibacterial and antibiofilm activities of peptide derivatives, as well as heightened stability (Jia et al., 2019). Jia et al. (2023) also found that halogenated jelleine-1 derivatives exhibit high efficacy against bacterial-associated colorectal cancer (CRC), outperforming the common antibiotic metronidazole. These findings highlight the effectiveness of halogenation as a strategy for enhancing the potency of therapeutic agents.

Cyclization

Cyclization involves the transformation of linear peptides into cyclic peptides to achieve desired effects, such as improved stability and resistance to proteolytic degradation (Li et al., 2021; Zhao et al., 2022). Cyclization can be achieved through various mechanisms. One approach involves forming a disulfide bond between two cysteine residues in the peptide sequence, typically achieved by oxidizing the thiol groups (–SH) of these cysteine residues to create a disulfide linkage (–S–S–) (Chan et al., 2013; Kwon et al., 2016; White et al., 2022). An alternative strategy employs head-to-tail cyclization by introducing a beta-lactam moiety that serves as a connecting bridge between the N- and C-termini of the peptide. This beta-lactam bridge, a structural feature also observed in antibiotics such as cephalosporins and penicillins, comprises a four-membered ring with a carbonyl group (C=O) and a nitrogen atom (N), conferring a cyclic orientation to the peptide (Figure 4).

Cyclization grants significant advantages upon AMPs, particularly in terms of increased structural stability both *in vitro* and *in vivo*. Cyclic AMPs are more resistant to enzymatic digestion, which can enhance their bioavailability and half-life *in vivo*. Cyclic AMPs are also less susceptible to conformational changes that can lead to loss of antimicrobial potency. Additionally, cyclization can enhance antimicrobial activity due to increased membrane penetration and disruption of peptide activity (Chan et al., 2013; Cheneval et al., 2014; Durek et al., 2018; Hellinger et al., 2021; Kwon et al., 2016; White et al., 2022; Zhao et al., 2022). For example, cyclic analogs of tachyplesin I, II, and III, AMPs with anticancer and antibacterial activities isolated from horseshoe crabs, exhibit reduced hemolytic activity and improved stability compared to their parent peptides (Vernen et al., 2019). In a prior study, we demonstrated that the disulfide-bridge-cyclized AMP ZY4 displayed significant *in vitro* and *in vivo* stability, resistance to enzymatic degradation for over 10 h, and heightened antimicrobial activity against multidrug-resistant nosocomial pathogens (Mwangi et al., 2019c).The heightened efficacy of cyclic peptides compared to linear peptides is due to the formation of more compact structures (Zhao et al., 2022). Various modes of cyclization, such as head-to-cyclization, disulfide bond formation, and internal side-chain bonding, have been observed in natural AMPs (Huan et al., 2020). For example, naturally occurring cyclic AMPs, such as defensins and magainins from mammals, plants, and insects, consistently exhibit potent antimicrobial activity.

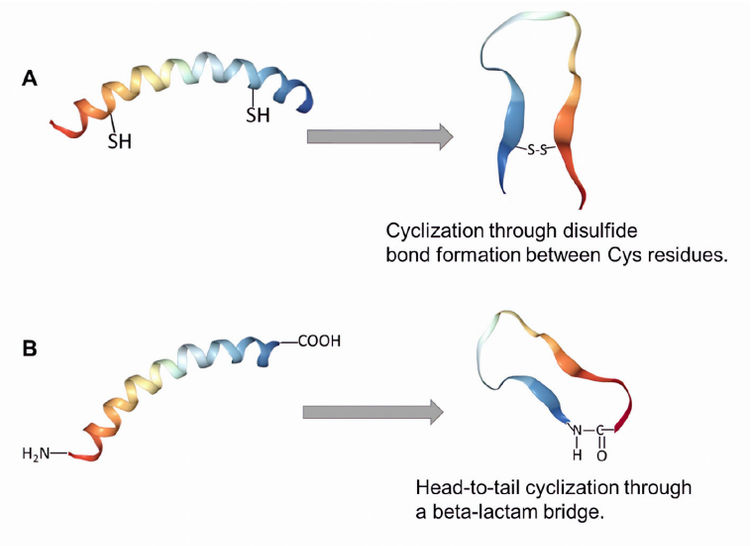


Figure 4 Two linear AMP cyclization techniques (disulfide bridge formation and head-to-tail beta-lactam ring formation)

Cyclization enables the design and synthesis AMPs with desired structural and functional properties. By choosing amino acid distribution and cyclization position, researchers can modify the physicochemical properties of cyclic AMPs to optimize conformational stability, antimicrobial activity, and pharmacokinetic properties. However, in some cases, cyclization can lower AMP activity due to interference by AMP-target interactions (Kumar et al., 2018). Moreover, the high-cost of AMP cyclization and chemical synthesis hinders large-scale production.

Conjugation with nanoparticles

Nanotechnology is a rapidly evolving field involving the generation and manipulation of nanoparticles (nanoscale materials or devices) in science, engineering, and medicine. Nanomedicine, a relatively new field of science, combines nanotechnology with drugs to improve pharmacological properties, such as drug delivery, stability, target specificity, and uptake (Fadaka et al., 2021; Natan & Banin, 2017; Thapa et al., 2021; Whitesides & Boncheva, 2002). Several forms of nanoparticles, such as ceramics, carbon-based, lipid-based, and semiconductors, are increasingly used to design novel AMPs with improved performance (Figure 5) (Biswaro et al., 2018; Fadaka et al., 2021; Natan & Banin, 2017; Teixeira et al., 2020). Incorporating AMPs into nanoparticles can enhance their stability and antimicrobial activity (Biswaro et al., 2018). Integrating nanotechnology in AMPs involves two main approaches, namely passive and direct delivery. In passive delivery, the peptide targets are encapsulate in nanoparticles without any surface modification (Teixeira et al., 2020), while direct delivery involves the conjugation of AMPs to surface-modified nanoparticles to facilitate targeted delivery to intended sites. Both approaches have proven to be effective in developing antimicrobial agents with improved performance. Biswaro et al. (2018) provide several examples of antimicrobial agents that have been formulated with nanoparticles.

Due to their small size, nanoparticles have a large specific surface area for loading drug molecules. Metallic nanoparticles, such as silver and zinc oxide, exhibit antimicrobial properties, thus complementing the microbicidal activity of AMPs (Xie et al., 2011; Yin et al., 2020). Silver nanoparticles (AgNPs) demonstrate both *in vitro* and *in vivo* activity against several bacterial pathogens (Bruna et al., 2021; Urnukhsaikhan et al., 2021). The conjugation of AMPs with AgNPs has also been shown to enhance their antimicrobial activity. For example, encapsulation of the AMP Tet-213 with AgNPs resulted in synergistic antibacterial and wound-healing activity (Jin et al., 2021). Combining the AMP P-13 with AgNPs not only improved the stability and bacterial killing effect of the compound but also reduced its cytotoxicity (Gao et al., 2020). Similarly, gold nanoparticles (AuNPs) complement the potency of AMPs, reduce their toxicity, and increase their serum stability (Rai et al., 2016; Rajchakit & Sarojini, 2017). For example, the conjugation of the AMP esculentin-1a derived from frog skin not only enhances its membrane permeabilization activity and resistance to proteolytic degradation but also increases its wound healing activity (Casciaro et al., 2017). Compared to other metallic NPs, AuNPs are attractive due to their comparatively higher tissue and cell compatibility, higher stability, more inert nature, and lower toxicity (Han et al., 2021; Yafout et al., 2021).

Other nanoparticles, such as polymeric NPs (poly lactic-co-glycolic acid (PLGA)) and magnetic NPs, have also been incorporated into AMPs. PLGA is characterized by low toxicity, controlled and sustained-release properties, and high biocompatibility, making it an effective drug delivery system (Tabatabaei Mirakabad et al., 2014). For example, the formulation of Smarter PLGA-Based Nanocrystal Carriers (SGNCs) with gliclazide, a Biopharmaceutics Classification System (BCS) class II drug for treating type 2 diabetes mellitus, overcame drug delivery and therapeutic challenges, showing significantly improved solubility, dissolution rate, and bioavailability in a type-2 diabetes rat model compared to pure gliclazide (Panda et al., 2019). Other studies have demonstrated that GIBIM-P5S9K AMPs encapsulated with PLGA exhibit significantly greater antimicrobial activity (20 times) against *E. coli*, MRSA, and *P. aeruginosa* than non-encapsulated analogs (Cruz et al., 2017; Gómez-Sequeda et al., 2020).

The development of peptide-based nanomedicines has emerged as an important research area, especially the conjugation of peptides with nanomaterials to control drug release and targeting (Lee et al., 2019). Notably, the conjugation of AMPs with NPs presents an attractive

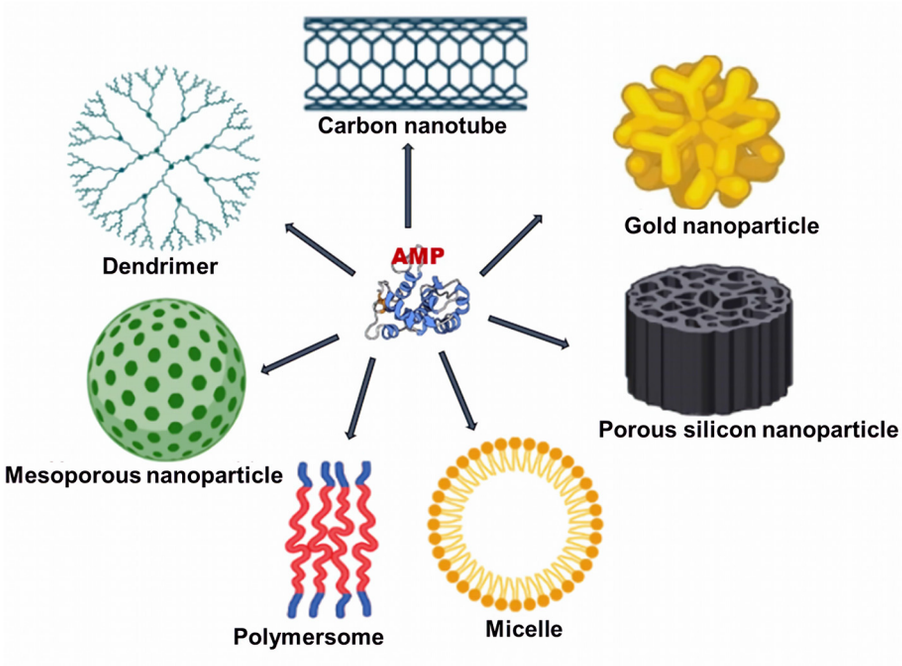


Figure 5 Conjugation or encapsulation of AMPs with different nanoparticles to optimize performance

opportunity to design self-assembling compounds with increased stability and antimicrobial activity (Lombardi et al., 2019). Peptide self-assembly involves the spontaneous formation of stable structures, such as nanofibers, nanotubes, and vesicles, driven by non-covalent interactions, such as electrostatic interactions, hydrogen bonding, and van der Waals forces, within the peptide (Whitesides & Boncheva, 2002). Based on these interactions, peptide self-assembly can be controlled to obtain supramolecular compounds that can disassemble upon contact with a target (Lombardi et al., 2019). Self-assembling AMPs display several advantages, such as biocompatibility, improved stability in diverse physiological conditions, lower toxicity, high resistance to proteolytic degradation, and improved antimicrobial activity (Whitesides & Boncheva, 2002; Ye et al., 2019; Yu et al., 2016). For example, the self-assembled AMP dhvar2 (KRLFKELLFSLRKY), a derivative of histatin 5, exhibited improved antibacterial activity compared to its parent peptide (Schnaider et al., 2020). Furthermore, the self-assembly of supramolecules of human α-defensin 5 through myristylation of the C-terminal can significantly improve its microbicidal activity against MRSA and *E. coli*, with minimal toxicity or hemolytic activity than non-assembling analogs (Lei et al., 2018).

Peptidomimetics

AMPs have emerged as attractive candidates for drug discovery; however, their inherent limitations continue to hinder their clinical applications. Therefore, peptidomimetics, a class of synthetic molecules engineered to mimic the structural and functional properties of known AMPs, offer a promising solution to overcome these challenges (Kuppusamy et al., 2019; Méndez-Samperio, 2014). Peptidomimetics display similar biological activities as their parent peptides but with enhanced structural stability and pharmacokinetic properties (Kuppusamy et al., 2019). These synthetic molecules have garnered increasing interest due to their significant potential for application in drug discovery, including the treatment of various diseases, AMR-associated infections, autoimmune disorders, cancers, and viral infections (Lenci & Trabocchi, 2020). Several drugs developed using peptidomimetics have already been approved for clinical application. For example, liraglutide (Victoza), a peptidomimetic drug that mimics the action of the hormone glucagon-like peptide-1 (GLP-1) (Jackson et al., 2010), enhances the secretion of insulin while suppressing glucagon release, and has been approved for the treatment of type 2 diabetes (Iepsen et al., 2015). Additionally, compared to the GLP-1 parent, liraglutide has a longer half-life, allowing for once-daily dosing (Jackson et al., 2010). Enfuvirtide (Fuzeon), a peptidomimetic drug used as an antiviral therapy for HIV, mimics a specific region of the HIV-1 gp41 protein, preventing viral attachment to host cell receptors and entry into the cells (Kitchen et al., 2008). Oritavancin (Orbactiv®), a peptidomimetic antibiotic approved for treating certain microbial infections, such as skin structure and acute bacterial skin infections (Brade et al., 2016), mimics the structure of the peptide antibiotic vancomycin and demonstrates potent activity against gram-positive bacteria (Syed & Scott, 2015). Maraviroc (Selzentry®), a peptidomimetic medication approved for the treatment of HIV-1 infection (Vandekerckhove et al., 2009), is a CCR5 antagonist that mimics the structure of the natural ligand of the CCR5 receptor to inhibit HIV from entering immune cells, thus reducing viral replication and infection progression (Lopalco, 2010; Mohamed et al., 2022). Ibrutinib (Imbruvica®) is a small molecule drug used for the treatment of chronic lymphocytic leukemia (CLL) and mantle cell lymphoma in adults (Parmar et al., 2014).

A key advantage of peptidomimetics is their improved stability and performance compared to parent peptides. More importantly, peptidomimetics hold significant promise in the fight against AMR as they exhibit enhanced antimicrobial potency against broad-spectrum microorganisms and can circumvent various mechanisms that make pathogens resistant to conventional antibiotics and AMPs (Kuppusamy et al., 2019; Molchanova et al., 2017; Qvit et al., 2017).

Several peptidomimetics with unique structural scaffolds, such as peptoids, α-peptides, peptoid hybrids, and β-peptoids, have been reported (Flint & Davis, 2022; Ghosh & Haldar, 2015; Karlsson et al., 2010; Kuppusamy et al., 2019; Makobongo et al., 2012; Molchanova et al., 2017; Olsen et al., 2007; Srinivas et al., 2010). Several approaches are used to generate peptidomimetics (Figure 6), enabling researchers to design and synthesize compounds with peptide-like characteristics but improved properties, such as enhanced stability, bioavailability, or target selectivity.

Substitution is the most commonly applied technique in the modification of AMPs. Substituting specific amino acids in an AMP sequence with structurally similar or modified amino acids can improve the properties of the peptide. Native amino acids can be substituted with non-natural amino acids to introduce unique chemical and structural properties into the peptide mimetic. Such non-natural amino acids may possess different functional groups or modified side chains, thus creating novel interactions or enhanced stability. For example, replacing a native amino acid with a non-natural amino acid, such as a halogen (fluoride), N-methyl amino acid, or β-amino acid, can affect peptide conformation and resistance to enzymatic degradation (D'Souza et al., 2021; Kumar et al., 2018). Similarly, substituting a L-amino acid with a D-amino acid can increase the conformational stability of a peptide, thus increasing its resistance to proteolytic degradation and its pharmacokinetic properties (Cai & Wei, 2021; Won et al., 2011). D-amino acids are less susceptible to enzymatic degradation and can enhance stability while maintaining overall peptide conformation and antimicrobial activity. Danalexin, a derivative of the AMP ranalexin derived from the skin of the American bullfrog (*Rana catesbeiana*), demonstrates superior pharmacokinetic properties in mouse models and, due to its exclusive composition of D-amino acids, offers enhanced stability and antimicrobial potency compared to its parent sequence (Domhan et al., 2019).

In addition to substitutions, peptidomimetics can be designed by altering the physicochemical properties of peptides (e.g., cationic charge, hydrophobicity, and amphipathicity) by changing the ratio of hydrophobic and hydrophilic amino acid residues in the peptide sequence (Edwards et al., 2016). Increasing the hydrophobicity and net positive charge of peptides by introducing hydrophobic building blocks can increase electrostatic interaction with microbial cells, thus enhancing its antimicrobial potency (Lachowicz et al., 2020; Matthyssen et al., 2022). Replacing hydrophobic building blocks with more hydrophilic amino acids can reduce the aggregation propensity of peptides, thus enhancing their penetration across microbial cell membranes as well as their antimicrobial potency (Al Musaimi et al., 2022). For example, the addition of hydrophobic amino acids, such as Trp, at strategic positions of the peptide sequence can promote peptide-microbial membrane interactions, thus increasing biological activity (Jin et al., 2016; Zhu et al., 2014). Similarly, backbone modifications of peptide mimetics by incorporating non-peptide linkers or cyclization can alter the overall hydrophobicity-hydrophilicity balance and the physicochemical properties of peptidomimetics. Cyclization of peptides through side-chain (cysteine-cysteine) disulfide bridge formations or other ring-forming strategies (N- to C-terminal beta-lactam rings) can promote conformational stability and antimicrobial activity of peptide mimetics. For example, in our prior work, introducing a disulfide bond to cyclize a linear AMP significantly enhanced its resistance to proteolytic enzymes, such as elastase, trypsin, and α-chymotrypsin, allowing it to withstand degradation for up to 10 h while maintaining its antimicrobial activity, compared to the linear peptide, which was degraded within 30 min (Mwangi et al., 2019d).

Peptidomimetics can also be designed using computational techniques, such as molecular modeling and computer-aided drug design (CADD) (Farhadi & Hashemian, 2018). Computer-aided tools and algorithms are regularly used to identify physicochemical properties, predict 3D structures, assess binding affinities, and evaluate potential pharmacokinetic characteristics of compounds (Farhadi & Hashemian, 2018;

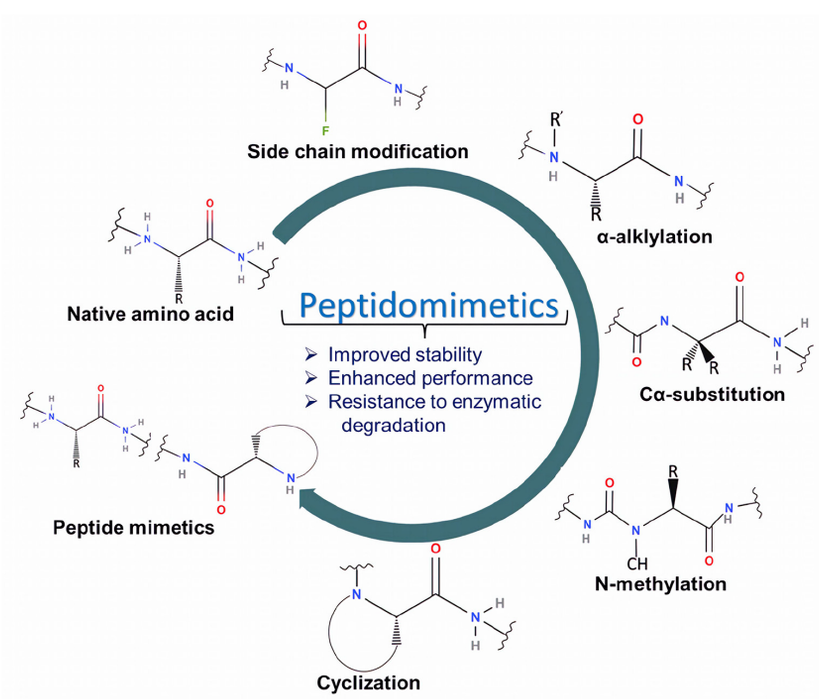


Figure 6 Representation of several techniques used to generate peptidomimetics

Prieto-Martínez et al., 2019). For example, the DeepCubist molecular generator, which uses 3D scaffolds to design novel peptides, was recently developed to precisely mimic bioactive peptide conformations (Umedera et al., 2023). Generally, computer-aided tools can be used to screen large libraries of small molecules and identify those that mimic the structure and activity of known peptides. These techniques can also be used to predict the affinity for and binding sites of the target peptide, and further optimize the specificity of the peptidomimetic to that site (Mitchell, 2014; Prieto-Martínez et al., 2019). Once peptidomimetic candidates have been designed, they can be evaluated for therapeutic potential, specificity, and toxicity using *in vitro* and *in vivo* assays, with the most promising candidates subsequently selected for further development and clinical application.

Despite the potential of peptidomimetics, the field is still in its infancy and designing and developing these peptides remain a considerable challenge, requiring a thorough understanding of their structure-activity relationships and pharmacokinetic properties. Furthermore, the design and development of peptidomimetics as therapeutic agents is still a new field, and further research is needed to fully grasp their potential and limitations.

De novo design

De novo design employs computational tools, including AI and ML, to generate novel AMP sequences with specific properties based on amino acid position preference, composition, and frequency (Figure 7) (Mouchlis et al., 2021; Schneider & Schneider, 2016), yielding sequences with significant amino acid diversity, structural conformations, and modes of action. A thorough understanding of the physicochemical properties and structure-activity relationship of AMPs has enabled researchers to predict and design new peptide sequences with desired qualities. This involves using computational tools and algorithms to predict the structure-activity relationship of desired peptides based on several properties unique to AMPs and then evaluate their potential antimicrobial activities (Yan et al., 2022).

Recently, cutting-edge technologies have accelerated the design and discovery of new lead drug candidates. AI has increasingly been used to design high-efficacy AMPs by screening millions of available peptide sequences and identifying similar functional motifs and properties, as well as develop and train ML models that predict the potential antimicrobial activity of a given peptide against a specific pathogen based on its physicochemical properties. Using large datasets of known AMPs, these models can be trained to predict various properties of new peptide sequences, allowing for the rapid design of novel AMPs with high efficacy (Figure 8) (Nagarajan et al., 2018; Wang et al., 2021a). For example, the "quantitative structure-activity relationship" (QSAR) ML model, which predicts the pharmacological potential of peptides using physicochemical descriptors, has become a valuable tool for designing potent antimicrobial agents (Mitchell, 2014; Taboureau, 2010). By correlating chemical properties, such as electrostatic charge and molecular weight, the QSAR tool can not only predict the biological activity or toxicity of compounds based on their molecular structure but can also identify lead compounds for drug development (Keyvanpour & Shirzad, 2021; Lévêque et al., 2022).

Based on large datasets of AMPs available in several databases, researchers can use AI and ML models to rapidly screen through millions of peptide sequences and select those with potent antimicrobial activity. Several AMP databases, such as APD3 (Wang et al., 2016), DRAMP v2.0 (Kang et al., 2019), and CAMP (Thomas et al., 2010), as well as online tools such as Ensemble-AMPPred (Lertampaiporn et al., 2021) and dbAMP v2.0 (Jhong et al., 2022), can help researchers identify known AMPs and generate novel peptides sequences. Several ML models have also been used to design and optimize novel peptide sequences.

Support vector machines (SVMs)

SVMs, a class of supervised ML algorithms, have become a powerful approach to predict the antimicrobial activity of novel AMPs against various target pathogens (Maltarollo et al., 2019). SVM algorithms work by analyzing large datasets to find the optimal hyperplane that separates positive and

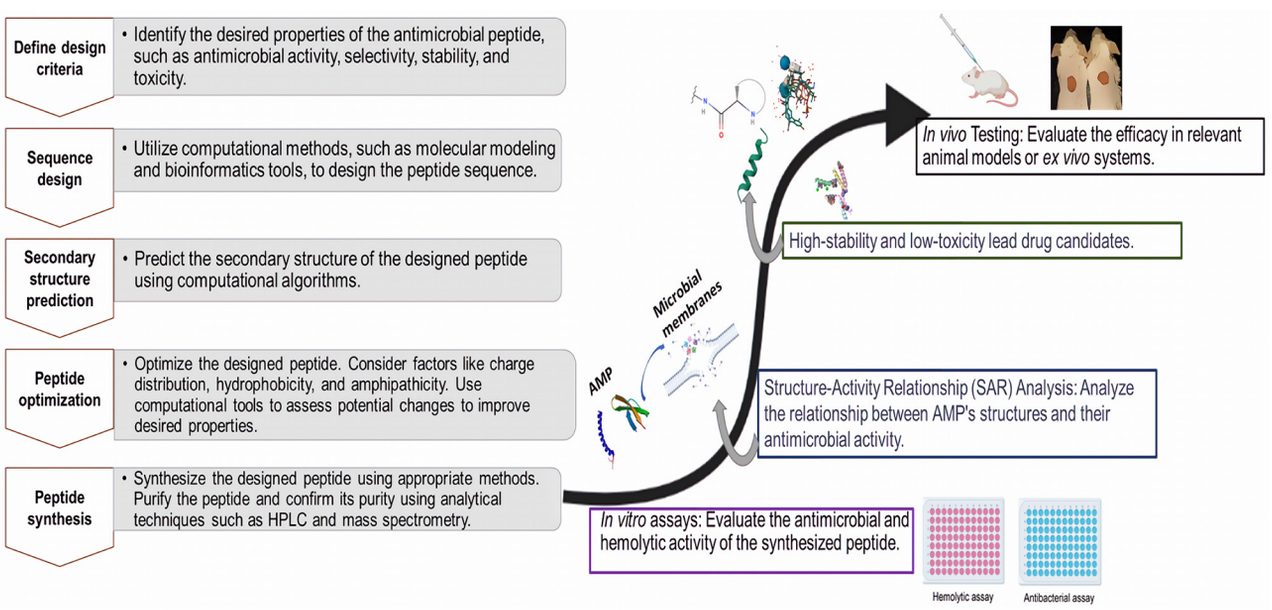


Figure 7 Schematic representation of multiple steps involved in *de novo* design, synthesis, and evaluation of therapeutic potential of novel peptide sequences

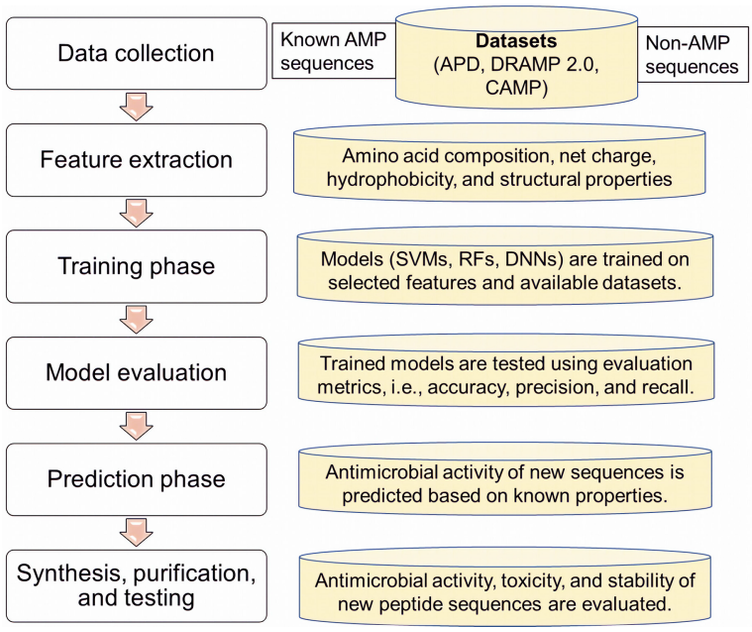


Figure 8 General overview of AMP design using artificial intelligence and machine learning models
For abbreviations see text.

negative examples in the training data (Cervantes et al., 2020). SVMs are used in AMP design to predict peptide structural scaffolds or amino acid compositions with the highest affinity and activity against specific targets or pathogens. In this approach, SVM ML models are trained using available datasets of AMPs with known activity against specific pathogens (Spänig & Heider, 2019). Such training incorporates various attributes that contribute to their antimicrobial activity, such as amino acid composition, distribution, net charge, and hydrophobicity. SVM models can also be trained to predict AMPs with high selectivity to target pathogens but with minimal harm to host cells or normal flora. These models enable the design of high-performing peptides, with the most promising candidates subsequently selected for further optimization and testing, thus accelerating drug discovery. Several user-friendly web server models that use SVMs have been trained and developed to enhance peptide activity prediction. For example, ClassAMP (http://www.bicnirrh.res.in/classamp/) is a ML algorithm that uses SVMs to predict the potential antibacterial, antifungal, and antiviral properties of new peptide sequences with high accuracy (Joseph et al., 2012). The web-based tool *i*AMPpred (http://cabgrid.res.in:8080/amppred/) (Meher et al., 2017) is trained on a dataset of 4 496 AMPs and 3 409 non-AMPs, allowing for the prediction of potential peptide activity with high accuracy (Meher et al., 2017). AntiBP2 (https://webs.iiitd.edu.in/raghava/antibp2/), another web-based tool, uses SVMs to predict the activity of novel AMPs with an overall accuracy of 92.14% based on amino acid composition (Lata et al., 2010). CAMPR3 (www.camp3.bicnirrh.res.in) (Waghu et al., 2016), LAMP (http://biotechlab.fudan.edu.cn/database/lamp) (Zhao et al., 2013), and DBAASP (http://www.biomedicine.org.ge/dbaasp/) (Gogoladze et al., 2014) are further examples.

Random forest

Random forest ML algorithms can be applied to a wide range of data analysis tasks, including the study and design of AMPs. The random forest algorithm, which uses an ensemble learning approach combining multiple decision trees to make predictions based on existing data, is one of the most commonly used ML algorithms, whereby each decision tree is built based on randomly selected subsets of training data and selected features (Cutler et al., 2012; Liu et al., 2012). Final prediction is based on the majority vote of the individual trees in the random forest, resulting in more accurate predictions. In the context of AMPs, random forests are trained on a dataset of known AMPs, based on their physicochemical properties, structures, and activities against specific microbial targets, to develop models that can predict the efficacy of novel AMPs. This provides a powerful approach for screening large libraries for potential antimicrobial agents, allowing researchers to focus on the most promising drug candidates (Lee et al., 2017; Spänig & Heider, 2019). A significant advantage of random forests is that they can identify key structural features strongly associated with activity, thus guiding the design of new AMPs with optimized target specificity and activity. Several models have been trained using random forests, enabling the rapid development of new lead drug candidates (Lee et al., 2017). Many of these models are available as user-friendly webservers, allowing researchers to predict the potential antibacterial, antifungal, and antiviral activities of peptide sequences (Vishnepolsky et al., 2018; Yan et al., 2022). For example, Bhadra et al. (2018) trained the amPEP RF model using a dataset of 3 268 AMPs based on amino acid distribution along the peptide sequences and achieved 87.2% accuracy in predicting the antimicrobial properties of AMPs.

Deep neural networks (DNNs)

DNNs are a type of deep learning approach used for various ML tasks, including the prediction of antimicrobial activity, hemolytic activity, and therapeutic potential of AMPs (Lin et al., 2021b; Yan et al., 2022). DNNs can be trained on large datasets of known AMPs to generate new peptide sequences by predicting their activity against specific pathogens. A significant advantage of DNNs over artificial neural networks (ANNs) is that they have multiple layers between input and output layers, enabling them to learn more complex patterns in the data, thereby predicting the antimicrobial and therapeutic properties of new peptide sequences with greater accuracy (Ahmad et al., 2021; Müller et al., 2017; Timmons & Hewage,

2021a, 2021b). For example, Lin et al. (2021c) developed a user-friendly DNN model AI4AMP (http://symbiosis.iis.sinica.edu.tw/PC_6/) and achieved 90% precision in predicting the antimicrobial potential of AMP sequences. Lin et al. (2021b) also trained the deep convolutional generative adversarial network (GAN) on known AMP datasets and generated novel peptides with broad-spectrum antimicrobial activities against drug-resistant pathogens, including carbapenem-resistant *P. aeruginosa* and MRSA.

Convolutional neural networks (CNNs)

CNNs have been widely applied in various research fields, including bioinformatics and drug discovery (Jing et al., 2018). CNNs represent a class of deep learning models designed to automatically learn and extract hierarchical patterns and features from input data, making them highly effective in completing complex computer functions, such as image classification, segmentation, and object detection (Yamashita et al., 2018). One key feature that makes CNN models highly effective for deep learning is their ability to represent data hierarchically. Typically, as data flows through the network, multiple convolutional layers with increasing receptive fields extract increasingly complex features (Bhatt et al., 2021; Dargan et al., 2020). This means that the lower-level layers capture simple features, like edges or textures, while the higher-level layers capture more abstract and high-level representations, thus enabling CNNs to learn from input data automatically. For example, Xiao et al. (2013) developed a two-level multilabel predictor, called iAMP-2L (http://www.jci-bioinfo.cn/iAMP-2L), for predicting AMPs, which employed CNN to extract features from datasets of known AMPs and successfully characterized novel peptide sequences as AMPs or non-AMPs. Notably, CNNs have shown remarkable performance in various applications, especially in peptide design and drug discovery. In AMP design, CNN models have been utilized to analyze peptide sequences and identify important functions and features (Veltri et al., 2018; Yan et al., 2022). CNN models are trained on a dataset of known AMPs to learn and recognize motif regions or amino acid combinations that can yield high-performance AMPs (Gupta et al., 2022). This enables researchers to predict the potential antimicrobial and hemolytic activity of novel peptide sequences (Yan et al., 2022). For example, Li et al. (2022) used AMPlify, an attentive deep learning model, to successfully predict and design a panel of AMPs from the *Lithobates catesbeiana* (bullfrog) genome with potent activity against World Health Organization (WHO) priority pathogens.

Additionally, CNNs can be used for structure-activity relationship analysis to aid in predicting and designing high-performance AMPs. Key structural information, such as secondary structure and physicochemical properties of AMPs, can be incorporated into input data, enabling the CNN model to learn from data patterns and features contributing to the efficacy of novel peptide sequences (Veltri et al., 2018). In addition to predicting AMP activity, CNNs can be utilized to classify new peptide sequences into different categories based on their properties and antimicrobial activities. CNN models trained on a labeled dataset of peptides with known activity can learn to distinguish between AMPs and non-AMPs. This can help researchers to screen and prioritize candidate peptides with potential antimicrobial properties. For example, the two-level iAMP-CA2L model (http://www.jci-bioinfo.cn/) uses CNN to predict whether a peptide sequence is an AMP or not and whether it belongs to one or more functional types (Xiao et al., 2021).

CURRENT STATUS AND APPLICATION OF AMPs

In recent years, AMP research has witnessed remarkable progress, revealing the potential of these compounds as powerful alternatives to traditional antibiotics. Due to their diverse mechanisms of action and broad-spectrum antimicrobial activity, AMPs provide new possibilities for combating multidrug-resistant infections. Therefore, continued research and development, combined with collaborative efforts among industry partners, researchers, and clinicians, are vital for harnessing the full potential of AMPs and their effective translation into clinical interventions. Notably, AMPs have shown excellent potential as therapeutic agents and are increasingly being adopted for clinical application. For example, the first AMP-based drug, gramicidin D—a mixture of three antibiotic compounds, gramicidins A, B, and C —isolated from soil bacteria (*Bacillus subtilis*) and commercially produced in the 1950s is still being used as a topical drug for the treatment of superficial wound, throat, eye, and nose infections (Guan et al., 2019; Sergiev, 1944).

Due to their potent biological properties, many AMPs are currently in clinical trials to evaluate their efficacy as therapeutic agents. For example, eight AMPs cataloged in the "Data Repository of Antimicrobial Peptides" (DRAMP, http://dramp.cpu-bioinfor.org/), including polymixin B, colistin, vancomycin, gramicidin, bacitracin, daptomycin, telaprevir, and enfuvirtide, have been approved for clinical application by the US Food and Drug Administration (FDA) (Wang et al., 2021b). Additionally, 70 peptide drugs are currently in the drug development pipeline, including 27 in clinical trials and 33 in the pre-clinical stage (Shi et al., 2022). Table 1 provides a summary of AMPs at various stages of clinical trials and their mechanisms of action. These discoveries highlight the significant advancement in AMP research. Cathelicidin BF, a noteworthy AMP derived from the venomous secretions of the banded krait (*Bungarus fasciatus*), has garnered considerable interest in medical research due to its excellent antimicrobial properties. First identified in 2008, cathelicidin BF was initially noted for its potent broad-spectrum activity against drug-resistant bacteria and *C. albicans* (Wang et al., 2008). Structure-activity relationship studies revealed that the peptide exhibited an α-helical conformation that enabled microbial membrane disruption. Further optimization of the peptide, including cyclization, non-natural amino acid incorporation, and post-translation modifications, yielded several analogs with significantly enhanced potency, stability, and pharmacokinetics (Mwangi et al., 2019b; Wang et al., 2011; Xing et al., 2020). Moreover, extensive preclinical testing demonstrated excellent efficacy of the optimized peptide in mouse models of vaginitis (Jin et al., 2016). Due to its excellent therapeutic potential in the treatment of colpitis, cathelicidin BF was approved for Phase I clinical trials by the Chinese National Medical Products Administration (Approval number: CXHL1700235) in 2018, marking a key milestone toward clinical translation. The decade-long journey from discovery to approval for clinical trials exemplifies the extensive work required to translate natural peptide antimicrobials into approved human therapeutics.

The discovery and characterization of novel peptide sequences from diverse sources, including animals, plants, and microorganisms, has significantly expanded our

Table 1 Summary of various AMPs at different stages of clinical trials and their mechanism of action

Name	Origin	Description	Design strategy	Administration	Target	Mechanism of action	Phase	Trial ID	References
LL-37	Human cathelicidin	A-helical peptide	Naturally occurring AMP	Topical	Leg ulcers	Membrane disruption and immunomodulation	II	EUCTR2012-002100-41	Mahlapuu et al., 2021
Dusquetide (SGX942)	Synthetic peptide	First-in-class innate defense regulator	Quantitative structure-activity relationship modeled to optimize human IDR-1	IV infusion	Oral mucositis and bacterial infections	Modulates the innate immune response and reduces inflammation	III	NCT03237325	Kudrimoti et al., 2017
Brilacidin	Synthetic peptide mimetic	Amphiphilic α-helix	Designed to mimic host defense proteins	Topical and Oral	Treatment of skin and oral infections	Disrupts bacterial cell membranes and interferes with bacterial DNA replication	II	NCT04784897	Lin et al., 2021a
Colistin (Polymyxin E)	Bacterium *Paenibacillus polymyxa*	Cyclic polypeptide	Naturally occurring AMP	Intravenous	Gram-negative bacteria	Membrane disruption and immunomodulation	III	NCT02573064	Michalopoulos et al., 2005
Gramicidin	Soil bacterium *Bacillus brevis*	Polycyclic peptide	Natural	Topical	Wound and ulcers	Membrane disruption and immunomodulation	III	NCT00534391	Lipsky & Hoey, 2009
Pexiganan (MSI-78)	African clawed frog	Analog of magainin 1	Substitution of amino acids to enhance activity and stability	Topical	Diabetic foot ulcers	Membrane permeabilization	III	NCT01594762 NCT01590758	Flamm et al., 2015
Murepavadin (POL7080)	Synthetic peptide	Cyclic beta-hairpin peptidomimetic of protegrin 1	Discovered by screening natural microbial peptides. Optimized through structure-activity relationship studies	Intravenous	*K. pneumoniae*, *P. aeruginosa*	Binding to LptD	II	EUCTR2017-003933-27-EE	Wach et al., 2018
Iseganan (IB-367)	Analog of protegrin-1	Cyclic derivative of morphine protegrin-1	Synthetic protegrin analog modeled after porcine leukocyte AMPs	Topical	Oral mucositis, Pneumonia	Membrane permeabilization	III	NCT00022373 NCT00118781	Bellm et al., 2002
Omiganan (MBI-226)	Bovine neutrophils	Linear derivative of indolicidin	Optimized by substituting residues to reduce hemolysis	Topical	Antisepsis	Immunomodulation /membrane disruption	III	NCT00608959	Sader et al., 2004
OP-145	Synthetic peptide of human LL-37	Amphiphilic α-helix	Optimized for activity against *Pseudomonas*	Ear drops	Ear infection	Immunomodulation /membrane disruption	II	ISRCTN84220089	Peek et al., 2020
NVB-302	Semisynthetic antibiotic	Aminoheptylamido peptide	Designed using plasma proteomics to identify naturally abundant Arg-rich peptides.	Oral	*C. difficile*	Cell wall synthesis inhibition	I	ISRCTN40071144	Crowther et al., 2013
Delmitide (RDP58)	Synthetic peptide	D-amino acid decapeptide	Mimics natural mitochondrial peptide. Designed by substituting amino acids to improve stability and activity.	Topical	Inflammatory bowel disease	Immunomodulation	II	ISRCTN84220089	Travis et al., 2005
LFF571	Semi-synthetic peptide	Cyclic lipopeptide antibiotic	Computer modeling	Oral	*C. difficile*	Inhibition of protein synthesis	II	NCT01232595	Mullane et al., 2015
Ghrelin	Synthetic peptide	Endogenous peptide	Natural peptide hormone	Intravenous	Chronic respiratory infection	Immunomodulation	II	NCT00763477	Kodama et al., 2008

Continued

Name	Origin	Description	Design strategy	Administration	Target	Mechanism of action	Phase	Trial ID	References
GSK1322322 (Lanopepden)	Synthetic hydrazide	Peptide deformylase inhibitor	Designed by modifying platelet factor IV, optimizing its antibacterial and anti-inflammatory properties.	Oral	Bacterial skin infection	Inhibit peptide deformylase	II	NCT01209078	Corey et al., 2014
NP213 (Novexatin)	Synthetic peptide	Cyclic polypeptide	Derived from human neutrophil peptide 1. Optimized to enhance antibacterial potency and selectivity	Topical	Onychomycosis	Membrane disruption	I	NCT02933879	Mercer et al., 2020
P113 (PAC-113)	Derivative of human saliva protein histatin 5	Hydrophilic α-helical peptide	Designed using quantitative structure-activity relationship to optimize candidacidal potency	Mouth wash	Oral candidiasis	Immunomodulation /membrane disruption	II	NCT00659971	Jang et al., 2008

understanding of these molecules. Furthermore, high-throughput screening techniques, bioinformatics tools, and multiomics analyses have further facilitated the identification and classification of new peptides with distinct sequences, structures, and therapeutic potentials. In our previous research, we identified short-designed peptides derived from cathelicidin BF-30 (a cathelicidin-class peptide) with broad-spectrum antimicrobial activity against multidrug-resistant pathogens and biofilms (Mwangi et al., 2019b; Yuan et al., 2022; Zhang et al., 2013), antimalarial activity (Fang et al., 2019), antiviral activity (Xing et al., 2020), and antifungal activity (Jin et al., 2016). Moreover, the identification and in-depth characterization of AMPs offer valuable strategies for addressing the current AMR crisis. These peptides hold significant potential for biomedical application, particularly the treatment of bacterial, fungal, and viral infections, including those associated with antibiotic-resistant strains. Additionally, AMPs show remarkable promise in wound healing, eradication and inhibition of biofilm formation, and as coatings for medical devices to mitigate the risk of infections. For instance, de Breij et al. (2018) found that a hypromellose ointment containing SAAP-148, a peptide derivative of LL-37, completely eradicates established MRSA and MDR *A. baumannii* biofilms and exhibits wound healing properties. Targeting bacterial biofilms is an effective therapeutic strategy, as biofilms contribute to antibiotic resistance by providing a protective environment for microbial cells to proliferate and exchange genetic material (Shahrour et al., 2019; Sharma et al., 2019; Stewart, 2002). Biofilm-enclosed cells are also 10–1 000 times less susceptible to antibiotics than planktonic cells (Bowler et al., 2020; Sharma et al., 2019; Stewart, 2002). Notably, AMPs demonstrate remarkable antibiofilm activity compared to traditional antibiotics, as evidenced by the inclusion of 83 antibiofilm AMPs in the current APD (https://aps.unmc.edu/database/anti).

Although AMPs have a low propensity to induce resistance compared to traditional antibiotics, the development of resistance to AMPs should not be ignored (Assoni et al., 2020). While AMPs can kill a broad range of microorganisms, certain bacteria have developed resistance mechanisms to AMPs to evade their killing effects. Experimental analysis has revealed diverse mechanisms of bacterial resistance to AMPs, including alteration of surface charge, expression of active efflux pumps, modification of intracellular targets, and activation of proteolytic enzymes (Mishra et al., 2018; Nizet, 2006). Several pathogens can alter their cell surface charge to prevent or reduce initial electrostatic interactions with cationic AMPs, while other bacteria can express efflux pumps that actively pump out AMPs from the cells (Nizet, 2006). Additionally, certain bacteria can produce proteases that degrade AMPs (Nawrocki et al., 2014; Nizet, 2006). For example, *S. aureus* can resist LL-37 by secreting aureolysin, which cleaves peptide bonds in the C-terminus (Sieprawska-Lupa et al., 2004). Aureolysin, which belongs to the metalloprotease family and exhibits proteolytic activity against various compounds, including LL-37 and other AMPs, recognizes and cleaves LL-37 at specific amino acid residues, resulting in peptide degradation (Moravej et al., 2018).

To overcome these resistance mechanisms, researchers have developed several strategies. One approach is to modify the structure of AMPs to make them more resistant to degradation by bacterial proteases or more effective at penetrating bacterial membranes (Zhang et al., 2021). For example, the incorporation of non-natural amino acids, cyclization, and alternative delivery strategies (i.e., nanoparticle formulation) can enhance the stability of AMPs. The cationic charge of peptides can also be enhanced by incorporating positively charged amino acids, such as Lys and Arg, to increase the affinity of AMPs to the altered negative microbial surface charges (Cheng & Zeng, 2022). Combination therapy with two or more AMPs or with an AMP and an antibiotic can increase the killing efficacy and reduce the likelihood of resistance development (Sheard et al., 2019). The use of adjuvants may also enhance the activity of AMPs by disrupting bacterial membranes or inhibiting bacterial efflux pumps (Douafer et al., 2019). Resistance to AMPs threatens

to undermine their efficacy, but rational and *de novo* engineering strategies leveraging emerging computation technologies show considerable promise in resensitizing bacteria to AMP therapies. Furthermore, understanding the survival strategies of bacteria will help realize the full clinical potential of AMPs. While much research has focused on optimizing the antimicrobial efficacy, stability, selectivity, and safety profiles of AMPs, further rational peptide design, structure-activity relationship studies, molecular modeling approaches, and computational techniques are necessary to modify peptide sequences and optimize their performance. Additionally, combination therapies involving AMPs with conventional antibiotics or synergistic peptides need to be further explored to combat multidrug-resistant pathogens (Sheard et al., 2019). Synergistic interactions can enhance antimicrobial efficacy, reduce the likelihood of resistance development, and broaden the spectrum of activity. For instance, combining the antibiotic chloramphenicol with the AMP LL-17-27 induces synergistic effects and significantly enhances antimicrobial activity against multidrug-resistant *P. aeruginosa* and MRSA (Rajasekaran et al., 2017).

CONCLUDING REMARKS

Despite significant progress in the field of AMP research, several challenges remain, including potential toxicity, peptide stability, high production costs, and large-scale manufacturing complexity. Addressing these challenges requires concerted efforts, multidisciplinary collaborations, technological innovations, and a better understanding of peptide-target interactions. Moreover, establishing regulatory frameworks is essential for the safe and efficacious clinical translation of AMP-based therapies. Given that AMPs represent one of the most promising solutions to the current AMR crisis, future research should focus on enhancing AMP activity. Innovative strategies, such as targeted delivery systems, nanoparticle conjugation, self-assembling supramolecular architectures, and combination therapies with conventional antibiotics warrant continued exploration to optimize AMP performance. The accelerating pace of knowledge accumulation, facilitated by expanding online databases and rapidly evolving computational methodologies, suggests that an increasing number of AMPs will advance to clinical trials in the near future.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

J.M., P.M.K., and R.C.T. prepared and wrote the manuscript. R.L. reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

REFERENCES

Ahmad A, Akbar S, Khan S, et al. 2021. Deep-AntiFP: prediction of antifungal peptides using distanct multi-informative features incorporating with deep neural networks. *Chemometrics and Intelligent Laboratory Systems*, **208**: 104214.

Al Musaimi O, Lombardi L, Williams DR, et al. 2022. Strategies for improving peptide stability and delivery. *Pharmaceuticals*, **15**(10): 1283.

Asante V, Mortier J, Wolber G, et al. 2014. Impact of fluorination on proteolytic stability of peptides: a case study with α-chymotrypsin and pepsin. *Amino Acids*, **46**(12): 2733−2744.

Assoni L, Milani B, Carvalho MR, et al. 2020. Resistance mechanisms to antimicrobial peptides in gram-positive bacteria. *Frontiers in Microbiology*, **11**: 593215.

Avram S, Buiu C, Borcan F, et al. 2012. More effective antimicrobial mastoparan derivatives, generated by 3D-QSAR-Almond and computational mutagenesis. *Molecular BioSystems*, **8**(2): 587−594.

Bao KF, Yuan WY, Ma CB, et al. 2018. Modification targeting the "rana box" motif of a novel nigrocin peptide from *Hylarana latouchii* enhances and broadens its potency against multiple bacteria. *Frontiers in Microbiology*, **9**: 2846.

Bellm L, Giles FJ, Redman R, et al. 2002. Iseganan HCl: a novel antimicrobial agent. *Expert Opinion on Investigational Drugs*, **11**(8): 1161−1170.

Benedetto Tiz D, Bagnoli L, Rosati O, et al. 2022. New halogen-containing drugs approved by FDA in 2021: an overview on their syntheses and pharmaceutical use. *Molecules*, **27**(5): 1643.

Benfield AH, Henriques ST. 2020. Mode-of-action of antimicrobial peptides: membrane disruption vs. Intracellular mechanisms. *Frontiers in Medical Technology*, **2**: 610997.

Berger AA, Völler JS, Budisa N, et al. 2017. Deciphering the fluorine code-the many hats fluorine wears in a protein environment. *Accounts of Chemical Research*, **50**(9): 2093−2103.

Bhadra P, Yan JL, Li JY, et al. 2018. AmPEP: sequence-based prediction of antimicrobial peptides using distribution patterns of amino acid properties and random forest. *Scientific Reports*, **8**(1): 1697.

Bhatt D, Patel C, Talsania H, et al. 2021. CNN variants for computer vision: history, architecture, application, challenges and future scope. *Electronics*, **10**(20): 2470.

Biswaro LS, da Costa Sousa MG, Rezende TMB, et al. 2018. Antimicrobial peptides and nanotechnology, recent advances and challenges. *Frontiers in Microbiology*, **9**: 855.

Bowler P, Murphy C, Wolcott R. 2020. Biofilm exacerbates antibiotic resistance: is this a current oversight in antimicrobial stewardship?. *Antimicrobial Resistance & Infection Control*, **9**(1): 162.

Brade KD, Rybak JM, Rybak MJ. 2016. Oritavancin: a new lipoglycopeptide antibiotic in the treatment of Gram-positive infections. *Infectious Diseases and Therapy*, **5**(1): 1−15.

Breijyeh Z, Jubeh B, Karaman R. 2020. Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules*, **25**(6): 1340.

Brinckerhoff LH, Kalashnikov VV, Thompson LW, et al. 1999. Terminal modifications inhibit proteolytic degradation of an immunogenic MART-$1_{27–35}$ peptide: implications for peptide vaccines. *International Journal of Cancer*, **83**(3): 326−334.

Bruna T, Maldonado-Bravo F, Jara P, et al. 2021. Silver nanoparticles and their antibacterial applications. *International Journal of Molecular Sciences*, **22**(13): 7202.

Cai JF, Wei LL. 2021. Editorial of special column "novel peptides and peptidomimetics in drug discovery". *Acta Pharmaceutica Sinica B*, **11**(9): 2606−2608.

Casciaro B, Moros M, Rivera-Fernández S, et al. 2017. Gold-nanoparticles coated with the antimicrobial peptide esculentin-1a(1-21)NH_2 as a reliable strategy for antipseudomonal drugs. *Acta Biomaterialia*, **47**: 170−181.

Cervantes J, Garcia-Lamont F, Rodríguez-Mazahua L, et al. 2020. A comprehensive survey on support vector machine classification: applications, challenges and trends. *Neurocomputing*, **408**: 189−215.

Cesaro A, Der Torossian Torres M, de la Fuente-Nunez C. 2022. Methods for the design and characterization of peptide antibiotics. *Methods in Enzymology*, **663**: 303−326.

Chan LY, Zhang VM, Huang YH, et al. 2013. Cyclization of the antimicrobial peptide gomesin with native chemical ligation: influences on stability and bioactivity. *ChemBioChem*, **14**(5): 617−624.

Chen L, Kashina A. 2021. Post-translational modifications of the protein

termini. *Frontiers in Cell and Developmental Biology*, **9**: 719590.
Cheneval O, Schroeder CI, Durek T, et al. 2014. Fmoc-based synthesis of disulfide-rich cyclic peptides. *The Journal of Organic Chemistry*, **79**(12): 5538−5544.
Cheng QP, Zeng P. 2022. Hydrophobic-hydrophilic alternation: an effective pattern to de novo designed antimicrobial peptides. *Current Pharmaceutical Design*, **28**(44): 3527−3537.
Corey R, Naderer OJ, O'riordan WD, et al. 2014. Safety, tolerability, and efficacy of GSK1322322 in the treatment of acute bacterial skin and skin structure infections. *Antimicrobial Agents and Chemotherapy*, **58**(11): 6518−6527.
Croft NP, Purcell AW. 2011. Peptidomimetics: modifying peptides in the pursuit of better vaccines. *Expert Review of Vaccines*, **10**(2): 211−226.
Crowther GS, Baines SD, Todhunter SL, et al. 2013. Evaluation of NVB302 versus vancomycin activity in an *in vitro* human gut model of *Clostridium difficile* infection. *Journal of Antimicrobial Chemotherapy*, **68**(1): 168−176.
Cruz J, Flórez J, Torres R, et al. 2017. Antimicrobial activity of a new synthetic peptide loaded in polylactic acid or poly(lactic-co-glycolic) acid nanoparticles against Pseudomonas aeruginosa, Escherichia coli O157: H7 and methicillin resistant Staphylococcus aureus (MRSA). *Nanotechnology*, **28**(13): 135102.
Cutler A, Cutler DR, Stevens JR. 2012. Random forests. *In*: Zhang C, Ma YQ. Ensemble Machine Learning: Methods and Applications. New York: Springer, 157–175.
Dargan S, Kumar M, Ayyagari MR, et al. 2020. A survey of deep learning and its applications: a new paradigm to machine learning. *Archives of Computational Methods in Engineering*, **27**(4): 1071−1092.
Datta A, Kundu P, Bhunia A. 2016. Designing potent antimicrobial peptides by disulphide linked dimerization and N-terminal lipidation to increase antimicrobial activity and membrane perturbation: structural insights into lipopolysaccharide binding. *Journal of Colloid and Interface Science*, **461**: 335−345.
de Breij A, Riool M, Cordfunke RA, et al. 2018. The antimicrobial peptide SAAP-148 combats drug-resistant bacteria and biofilms. *Science Translational Medicine*, **10**(423): eaan4044.
Dennison SR, Morton LHG, Phoenix DA. 2012. Effect of amidation on the antimicrobial peptide aurein 2.5 from Australian southern bell frogs. *Protein & Peptide Letters*, **19**(6): 586−591.
Dennison SR, Mura M, Harris F, et al. 2015. The role of C-terminal amidation in the membrane interactions of the anionic antimicrobial peptide, maximin H5. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, **1848**(5): 1111−1118.
Dennison SR, Phoenix DA. 2011. Influence of C-terminal amidation on the efficacy of modelin-5. *Biochemistry*, **50**(9): 1514−1523.
Domhan C, Uhl P, Kleist C, et al. 2019. Replacement of L-amino acids by D-amino acids in the antimicrobial peptide ranalexin and its consequences for antimicrobial activity and biodistribution. *Molecules*, **24**(16): 2987.
Douafer H, Andrieu V, Phanstiel IV O, et al. 2019. Antibiotic adjuvants: make antibiotics great again! *Journal of Medicinal Chemistry*, **62**(19): 8665–8681.
D'Souza AR, Necelis MR, Kulesha A, et al. 2021. Beneficial impacts of incorporating the non-natural amino acid azulenyl-alanine into the Trp-rich antimicrobial peptide buCATHL4B. *Biomolecules*, **11**(3): 421.
Durek T, Cromm PM, White AM, et al. 2018. Development of novel melanocortin receptor agonists based on the cyclic peptide framework of sunflower trypsin inhibitor-1. *Journal of Medicinal Chemistry*, **61**(8): 3674−3684.
Edwards IA, Elliott AG, Kavanagh AM, et al. 2016. Contribution of amphipathicity and hydrophobicity to the antimicrobial activity and cytotoxicity of β-hairpin peptides. *ACS Infectious Diseases*, **2**(6): 442−450.
Elnagdy S, Alkhazindar M. 2020. The potential of antimicrobial peptides as an antiviral therapy against COVID-19. *ACS Pharmacology & Translational Science*, **3**(4): 780−782.
Erdem Büyükkiraz M, Kesmen Z. 2022. Antimicrobial peptides (AMPs): a promising class of antimicrobial compounds. *Journal of Applied Microbiology*, **132**(3): 1573−1596.
Fadaka AO, Sibuyi NRS, Madiehe AM, et al. 2021. Nanotechnology-based delivery systems for antimicrobial peptides. *Pharmaceutics*, **13**(11): 1795.
Fang YQ, He XQ, Zhang PC, et al. 2019. In vitro and in vivo antimalarial activity of LZ1, a peptide derived from snake cathelicidin. *Toxins*, **11**(7): 379.
Farhadi T, Hashemian SM. 2018. Computer-aided design of amino acid-based therapeutics: a review. *Drug Design, Development and Therapy*, **12**: 1239−1254.
Flamm RK, Rhomberg PR, Simpson KM, et al. 2015. *In vitro* spectrum of pexiganan activity when tested against pathogens from diabetic foot infections and with selected resistance mechanisms. *Antimicrobial Agents and Chemotherapy*, **59**(3): 1751−1754.
Flint AJ, Davis AP. 2022. Vancomycin mimicry: towards new supramolecular antibiotics. *Organic & Biomolecular Chemistry*, **20**(39): 7694−7712.
Fridkin SK, Edwards JR, Courval JM, et al. 2001. The effect of vancomycin and third-generation cephalosporins on prevalence of vancomycin-resistant enterococci in 126 U. S. adult intensive care units. *Annals of Internal Medicine*, **135**(3): 175−183.
Gan BH, Gaynord J, Rowe SM, et al. 2021. The multifaceted nature of antimicrobial peptides: current synthetic chemistry approaches and future directions. *Chemical Society Reviews*, **50**(13): 7820−7880.
Gao JY, Na H, Zhong RB, et al. 2020. One step synthesis of antimicrobial peptide protected silver nanoparticles: the core-shell mutual enhancement of antibacterial activity. *Colloids and Surfaces B:Biointerfaces*, **186**: 110704.
Gautier R, Douguet D, Antonny B, et al. 2008. HELIQUEST: a web server to screen sequences with specific α-helical properties. *Bioinformatics*, **24**(18): 2101−2102.
Ghosh C, Haldar J. 2015. Membrane-active small molecules: designs inspired by antimicrobial peptides. *ChemMedChem*, **10**(10): 1606−1624.
Giangaspero A, Sandri L, Tossi A. 2001. Amphipathic α helical antimicrobial peptides. *European Journal of Biochemistry*, **268**(21): 5589−5600.
Gogoladze G, Grigolava M, Vishnepolsky B, et al. 2014. DBAASP: database of antimicrobial activity and structure of peptides. *FEMS Microbiology Letters*, **357**(1): 63−68.
Gómez-Sequeda N, Ruiz J, Ortiz C, et al. 2020. Potent and specific antibacterial activity against *Escherichia coli* O157: H7 and methicillin resistant *Staphylococcus aureus* (MRSA) of G17 and G19 peptides encapsulated into poly-lactic-Co-glycolic acid (PLGA) nanoparticles. *Antibiotics*, **9**(7): 384.
Gottler LM, Ramamoorthy A. 2009. Structure, membrane orientation, mechanism, and function of pexiganan-a highly potent antimicrobial peptide designed from magainin. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, **1788**(8): 1680−1686.
Grundmann H, Aires-de-Sousa M, Boyce J, et al. 2006. Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat. *The Lancet*, **368**(9538): 874−885.
Guan QK, Huang SH, Jin Y, et al. 2019. Recent advances in the exploration of therapeutic analogues of gramicidin S, an old but still potent antimicrobial peptide. *Journal of Medicinal Chemistry*, **62**(17): 7603−7617.
Gupta S, Azadvari N, Hosseinzadeh P. 2022. Design of protein segments and peptides for binding to protein targets. *BioDesign Research*, **2022**: 9783197.
Han YJ, Zhang ML, Lai R, et al. 2021. Chemical modifications to increase the therapeutic potential of antimicrobial peptides. *Peptides*, **146**: 170666.
Haney EF, Straus SK, Hancock REW. 2019. Reassessing the host defense peptide landscape. *Frontiers in Chemistry*, **7**: 43.
Hellinger R, Muratspahić E, Devi S, et al. 2021. Importance of the cyclic

cystine knot structural motif for immunosuppressive effects of cyclotides. *ACS Chemical Biology*, **16**(11): 2373−2386.

Huan YC, Kong Q, Mou HJ, et al. 2020. Antimicrobial peptides: classification, design, application and research progress in multiple fields. *Frontiers in Microbiology*, **11**: 582779.

Huhmann S, Koksch B. 2018. Fine-tuning the proteolytic stability of peptides with fluorinated amino acids. *European Journal of Organic Chemistry*, **2018**(27-28): 3667−3679.

Iepsen EW, Torekov SS, Holst JJ. 2015. Liraglutide for type 2 diabetes and obesity: a 2015 update. *Expert Review of Cardiovascular Therapy*, **13**(7): 753−767.

Jackson SH, Martin TS, Jones JD, et al. 2010. Liraglutide (victoza): the first once-daily incretin mimetic injection for type-2 diabetes. *P & T:A Peer-Reviewed Journal for Formulary Management*, **35**(9): 498−529.

Jang WS, Li XS, Sun JN, et al. 2008. The P-113 fragment of histatin 5 requires a specific peptide sequence for intracellular translocation in *Candida albicans*, which is independent of cell wall binding. *Antimicrobial Agents and Chemotherapy*, **52**(2): 497−504.

Jerala R. 2007. Synthetic lipopeptides: a novel class of anti-infectives. *Expert Opinion on Investigational Drugs*, **16**(8): 1159−1169.

Jhong JH, Yao LT, Pang YX, et al. 2022. dbAMP 2.0: updated resource for antimicrobial peptides with an enhanced scanning method for genomic and proteomic data. *Nucleic Acids Research*, **50**(D1): D460−D470.

Jia FJ, Yu Q, Wang RL, et al. 2023. Optimized antimicrobial peptide jelleine-I derivative Br-J-I inhibits fusobacterium nucleatum to suppress colorectal cancer progression. *International Journal of Molecular Sciences*, **24**(2): 1469.

Jia FJ, Zhang Y, Wang JY, et al. 2019. The effect of halogenation on the antimicrobial activity, antibiofilm activity, cytotoxicity and proteolytic stability of the antimicrobial peptide Jelleine-I. *Peptides*, **112**: 56−66.

Jiang SQ, Zhang LJ, Cui DB, et al. 2016. The important role of halogen bond in substrate selectivity of enzymatic catalysis. *Scientific Reports*, **6**: 34750.

Jin L, Bai XW, Luan N, et al. 2016. A designed tryptophan- and lysine/arginine-rich antimicrobial peptide with therapeutic potential for clinical antibiotic-resistant *Candida albicans* vaginitis. *Journal of Medicinal Chemistry*, **59**(5): 1791−1799.

Jin Y, Yang YD, Duan W, et al. 2021. Synergistic and on-demand release of Ag-AMPs loaded on porous silicon nanocarriers for antibacteria and wound healing. *ACS Applied Materials & Interfaces*, **13**(14): 16127−16141.

Jing YK, Bian YM, Hu ZH, et al. 2018. Deep learning for drug design: an artificial intelligence paradigm for drug discovery in the big data era. *The AAPS Journal*, **20**(3): 58.

Joseph S, Karnik S, Nilawe P, et al. 2012. ClassAMP: a prediction tool for classification of antimicrobial peptides. *IEEE/ACM Transactions on Computational Biology and Bioinformatics*, **9**(5): 1535−1538.

Jubeh B, Breijyeh Z, Karaman R. 2020. Resistance of gram-positive bacteria to current antibacterial agents and overcoming approaches. *Molecules*, **25**(12): 2888.

Kang SJ, Nam SH, Lee BJ. 2022. Engineering approaches for the development of antimicrobial peptide-based antibiotics. *Antibiotics*, **11**(10): 1338.

Kang XY, Dong FY, Shi C, et al. 2019. DRAMP 2.0, an updated data repository of antimicrobial peptides. *Scientific Data*, **6**(1): 148.

Karlsson AJ, Flessner RM, Gellman SH, et al. 2010. Polyelectrolyte multilayers fabricated from antifungal β-peptides: design of surfaces that exhibit antifungal activity against *Candida albicans*. *Biomacromolecules*, **11**(9): 2321–2328.

Keyvanpour MR, Shirzad MB. 2021. An analysis of QSAR research based on machine learning concepts. *Current Drug Discovery Technologies*, **18**(1): 17−30.

Kitchen CM, Nuño M, Kitchen SG, et al. 2008. Enfuvirtide antiretroviral therapy in HIV-1 infection. *Therapeutics and Clinical Risk Management*, **4**(2): 433−439.

Kodama T, Ashitani JI, Matsumoto N, et al. 2008. Ghrelin treatment suppresses neutrophil-dominant inflammation in airways of patients with chronic respiratory infection. *Pulmonary Pharmacology & Therapeutics*, **21**(5): 774−779.

Kudrimoti M, Curtis A, Azawi S, et al. 2017. Dusquetide: reduction in oral mucositis associated with enduring ancillary benefits in tumor resolution and decreased mortality in head and neck cancer patients. *Biotechnology Reports*, **15**: 24−26.

Kumar P, Kizhakkedathu JN, Straus SK. 2018. Antimicrobial peptides: diversity, mechanism of action and strategies to improve the activity and biocompatibility in vivo. *Biomolecules*, **8**(1): 4.

Kuppusamy R, Willcox M, Black DS, et al. 2019. Short cationic peptidomimetic antimicrobials. *Antibiotics*, **8**(2): 44.

Kwon S, Bosmans F, Kaas Q, et al. 2016. Efficient enzymatic cyclization of an inhibitory cystine knot-containing peptide. *Biotechnology and Bioengineering*, **113**(10): 2202−2212.

Lachowicz JI, Szczepski K, Scano A, et al. 2020. The best peptidomimetic strategies to undercover antibacterial peptides. *International Journal of Molecular Sciences*, **21**(19): 7349.

Lata S, Mishra NK, Raghava GPS. 2010. AntiBP2: improved version of antibacterial peptide prediction. *BMC Bioinformatics*, **11**(S1): S19.

Lee EY, Lee MW, Fulan BM, et al. 2017. What can machine learning do for antimicrobial peptides, and what can antimicrobial peptides do for machine learning?. *Interface Focus*, **7**(6): 20160153.

Lee S, Trinh THT, Yoo M, et al. 2019. Self-assembling peptides and their application in the treatment of diseases. *International Journal of Molecular Sciences*, **20**(23): 5850.

Lee YCJ, Shirkey JD, Park J, et al. 2022. An overview of antiviral peptides and rational biodesign considerations. *BioDesign Research*, **2022**: 9898241.

Lei J, Sun LC, Huang SY, et al. 2019. The antimicrobial peptides and their potential clinical applications. *American Journal of Translational Research*, **11**(7): 3919−3931.

Lei RY, Hou JC, Chen QX, et al. 2018. Self-assembling myristoylated human α-defensin 5 as a next-generation nanobiotics potentiates therapeutic efficacy in bacterial infection. *ACS Nano*, **12**(6): 5284−5296.

Lenci E, Trabocchi A. 2020. Peptidomimetic toolbox for drug discovery. *Chemical Society Reviews*, **49**(11): 3262−3277.

Leptihn S, Har JY, Wohland T, et al. 2010. Correlation of charge, hydrophobicity, and structure with antimicrobial activity of S1 and MIRIAM peptides. *Biochemistry*, **49**(43): 9161−9170.

Lertampaiporn S, Vorapreeda T, Hongsthong A, et al. 2021. Ensemble-AMPPred: robust AMP prediction and recognition using the ensemble learning method with a new hybrid feature for differentiating AMPs. *Genes*, **12**(2): 137.

Lévêque L, Tahiri N, Goldsmith MR, et al. 2022. Quantitative Structure-Activity Relationship (QSAR) modeling to predict the transfer of environmental chemicals across the placenta. *Computational Toxicology*, **21**: 100211.

Li CK, Sutherland D, Hammond SA, et al. 2022. AMPlify: attentive deep learning model for discovery of novel antimicrobial peptides effective against WHO priority pathogens. *BMC Genomics*, **23**(1): 77.

Li DD, Yang YH, Li RF, et al. 2021. N-terminal acetylation of antimicrobial peptide L163 improves its stability against protease degradation. *Journal of Peptide Science*, **27**(9): e3337.

Li J, Xu X, Xu C, et al. 2007. Anti-infection peptidomics of amphibian skin. *Molecular & Cellular Proteomics*, **6**(5): 882−894.

Lin LM, Chi JY, Yan YL, et al. 2021a. Membrane-disruptive peptides/peptidomimetics-based therapeutics: promising systems to combat bacteria and cancer in the drug-resistant era. *Acta Pharmaceutica Sinica B*,

11(9): 2609−2644.
Lin TT, Yang LY, Lu IH, et al. 2021c. AI4AMP: an antimicrobial peptide predictor using physicochemical property-based encoding method and deep learning. *mSystems*, **6**(6): e0029921.
Lin TT, Yang LY, Wang CT, et al. 2021b. Discovering novel antimicrobial peptides in generative adversarial network. *BioRxiv*, doi: https://doi.org/10.1101/2021.11.22.469634.
Lipsky BA, Hoey C. 2009. Topical antimicrobial therapy for treating chronic wounds. *Clinical Infectious Diseases*, **49**(10): 1541−1549.
Liu TQ, Zhu NY, Zhong C, et al. 2020. Effect of N-methylated and fatty acid conjugation on analogs of antimicrobial peptide Anoplin. *European Journal of Pharmaceutical Sciences*, **152**: 105453.
Liu YL, Wang YR, Zhang J. 2012. New machine learning algorithm: random forest. *In*: Third International Conference on Information Computing and Applications. Chengde: Springer, 246–252.
Lombardi L, Falanga A, Del Genio V, et al. 2019. A new hope: self-assembling peptides with antimicrobial activity. *Pharmaceutics*, **11**(4): 166.
Lopalco L. 2010. CCR5: from natural resistance to a new anti-HIV strategy. *Viruses*, **2**(2): 574−600.
López Cascales JJ, Zenak S, García de la Torre J, et al. 2018. Small cationic peptides: influence of charge on their antimicrobial activity. *ACS Omega*, **3**(5): 5390−5398.
Mahlapuu M, Sidorowicz A, Mikosinski J, et al. 2021. Evaluation of LL-37 in healing of hard-to-heal venous leg ulcers: a multicentric prospective randomized placebo-controlled clinical trial. *Wound Repair and Regeneration*, **29**(6): 938−950.
Makobongo MO, Gancz H, Carpenter BM, et al. 2012. The oligo-acyl lysyl antimicrobial peptide C_{12}K-2β_{12} exhibits a dual mechanism of action and demonstrates strong *in vivo* efficacy against *Helicobacter pylori*. *Antimicrobial Agents and Chemotherapy*, **56**(1): 378–390.
Malina A, Shai Y. 2005. Conjugation of fatty acids with different lengths modulates the antibacterial and antifungal activity of a cationic biologically inactive peptide. *Biochemical Journal*, **390**(Pt 3): 695–702.
Maltarollo VG, Kronenberger T, Espinoza GZ, et al. 2019. Advances with support vector machines for novel drug discovery. *Expert Opinion on Drug Discovery*, **14**(1): 23−33.
Mardirossian M, Rubini M, Adamo MFA, et al. 2021. Natural and synthetic halogenated amino acids-structural and bioactive features in antimicrobial peptides and peptidomimetics. *Molecules*, **26**(23): 7401.
Matthyssen T, Li WY, Holden JA, et al. 2022. The potential of modified and multimeric antimicrobial peptide materials as superbug killers. *Frontiers in Chemistry*, **9**: 795433.
Meher PK, Sahu TK, Saini V, et al. 2017. Predicting antimicrobial peptides with improved accuracy by incorporating the compositional, physico-chemical and structural features into Chou's general PseAAC. *Scientific Reports*, **7**(1): 42362.
Méndez-Samperio P. 2014. Peptidomimetics as a new generation of antimicrobial agents: current progress. *Infection and Drug Resistance*, **7**: 229−237.
Mercer DK, Robertson JC, Miller L, et al. 2020. NP213 (Novexatin®): a unique therapy candidate for onychomycosis with a differentiated safety and efficacy profile. *Medical Mycology*, **58**(8): 1064−1072.
Michalopoulos AS, Tsiodras S, Rellos K, et al. 2005. Colistin treatment in patients with ICU-acquired infections caused by multiresistant Gram-negative bacteria: the renaissance of an old antibiotic. *Clinical Microbiology and Infection*, **11**(2): 115−121.
Mishra AK, Choi J, Moon E, et al. 2018. Tryptophan-rich and proline-rich antimicrobial peptides. *Molecules*, **23**(4): 815.
Mitchell JBO. 2014. Machine learning methods in chemoinformatics. *WIREs Computational Molecular Science*, **4**(5): 468−481.
Mohamed H, Gurrola T, Berman R, et al. 2022. Targeting CCR5 as a component of an HIV-1 therapeutic strategy. *Frontiers in Immunology*, **12**: 816515.
Molchanova N, Hansen PR, Franzyk H. 2017. Advances in development of antimicrobial peptidomimetics as potential drugs. *Molecules*, **22**(9): 1430.
Moravej H, Moravej Z, Yazdanparast M, et al. 2018. Antimicrobial peptides: features, action, and their resistance mechanisms in bacteria. *Microbial Drug Resistance*, **24**(6): 747−767.
Mouchlis VD, Afantitis A, Serra A, et al. 2021. Advances in de novo drug design: from conventional to machine learning methods. *International Journal of Molecular Sciences*, **22**(4): 1676.
Mullane K, Lee C, Bressler A, et al. 2015. Multicenter, randomized clinical trial to compare the safety and efficacy of LFF571 and vancomycin for *Clostridium difficile* infections. *Antimicrobial Agents and Chemotherapy*, **59**(3): 1435−1440.
Müller AT, Gabernet G, Hiss JA, et al. 2017. modlAMP: python for antimicrobial peptides. *Bioinformatics*, **33**(17): 2753−2755.
Müller K, Faeh C, Diederich F. 2007. Fluorine in pharmaceuticals: looking beyond intuition. *Science*, **317**(5846): 1881−1886.
Mura M, Wang JP, Zhou YH, et al. 2016. The effect of amidation on the behaviour of antimicrobial peptides. *European Biophysics Journal*, **45**(3): 195−207.
Murray CJL, Ikuta KS, Sharara F, et al. 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, **399**(10325): 629−655.
Mwangi J, Hao X, Lai R, et al. 2019a. Antimicrobial peptides: new hope in the war against multidrug resistance. *Zoological Research*, **40**(6): 488−505.
Mwangi J, Yin YZ, Wang G, et al. 2019b. The antimicrobial peptide ZY4 combats multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infection. *Proceedings of the National Academy of Sciences of the United States of America*, **116**(52): 26516−26522.
Nagarajan D, Nagarajan T, Roy N, et al. 2018. Computational antimicrobial peptide design and evaluation against multidrug-resistant clinical isolates of bacteria. *Journal of Biological Chemistry*, **293**(10): 3492−3509.
Natan M, Banin E. 2017. From Nano to Micro: using nanotechnology to combat microorganisms and their multidrug resistance. *FEMS Microbiology Reviews*, **41**(3): 302−322.
Nawrocki KL, Crispell EK, McBride SM. 2014. Antimicrobial peptide resistance mechanisms of gram-positive bacteria. *Antibiotics*, **3**(4): 461−492.
Nevitt C, Tooley JG, Schaner Tooley CE. 2018. N-terminal acetylation and methylation differentially affect the function of MYL9. *Biochemical Journal*, **475**(20): 3201−3219.
Nizet V. 2006. Antimicrobial peptide resistance mechanisms of human bacterial pathogens. *Current Issues in Molecular Biology*, **8**(1): 11−26.
Olsen CA, Bonke G, Vedel L, et al. 2007. α-peptide/β-peptoid chimeras. *Organic Letters*, **9**(8): 1549−1552.
Omardien S, Brul S, Zaat SAJ. 2016. Antimicrobial activity of cationic antimicrobial peptides against gram-positives: current progress made in understanding the mode of action and the response of bacteria. *Frontiers in Cell and Developmental Biology*, **4**: 111.
Pachón-Ibáñez ME, Smani Y, Pachón J, et al. 2017. Perspectives for clinical use of engineered human host defense antimicrobial peptides. *FEMS Microbiology Reviews*, **41**(3): 323−342.
Panda BP, Krishnamoorthy R, Bhattamisra SK, et al. 2019. Fabrication of second generation smarter PLGA based nanocrystal carriers for improvement of drug delivery and therapeutic efficacy of gliclazide in type-2 diabetes rat model. *Scientific Reports*, **9**(1): 17331.
Papanastasiou EA, Hua Q, Sandouk A, et al. 2009. Role of acetylation and charge in antimicrobial peptides based on human β-defensin-3. *APMIS*, **117**(7): 492−499.
Parmar S, Patel K, Pinilla-Ibarz J. 2014. Ibrutinib (imbruvica): a novel targeted therapy for chronic lymphocytic leukemia. *P & T:A Peer-Reviewed Journal for Formulary Management*, **39**(7): 483−519.

Pastore A, Raimondi F, Rajendran L, et al. 2020. Why does the Aβ peptide of Alzheimer share structural similarity with antimicrobial peptides?. *Communications Biology*, **3**(1): 135.

Peek NFAW, Nell MJ, Brand R, et al. 2020. Ototopical drops containing a novel antibacterial synthetic peptide: safety and efficacy in adults with chronic suppurative otitis media. *PLoS One*, **15**(4): e0231573.

Prieto-Martínez FD, López-López E, Eurídice Juárez-Mercado K, et al. 2019. Computational drug design methods —current and future perspectives. *In*: Roy K. In Silico Drug Design: Repurposing Techniques and Methodologies. London: Academic Press, 19–44.

Qian S, Wang WC, Yang L, et al. 2008. Structure of the alamethicin pore reconstructed by X-ray diffraction analysis. *Biophysical Journal*, **94**(9): 3512−3522.

Qvit N, Rubin SJS, Urban TJ, et al. 2017. Peptidomimetic therapeutics: scientific approaches and opportunities. *Drug Discovery Today*, **22**(2): 454−462.

Radek K, Gallo R. 2007. Antimicrobial peptides: natural effectors of the innate immune system. *Seminars in Immunopathology*, **29**(1): 27−43.

Rai A, Pinto S, Velho TR, et al. 2016. One-step synthesis of high-density peptide-conjugated gold nanoparticles with antimicrobial efficacy in a systemic infection model. *Biomaterials*, **85**: 99−110.

Rajasekaran G, Kim EY, Shin SY. 2017. LL-37-derived membrane-active FK-13 analogs possessing cell selectivity, anti-biofilm activity and synergy with chloramphenicol and anti-inflammatory activity. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, **1859**(5): 722−733.

Rajchakit U, Sarojini V. 2017. Recent developments in antimicrobial-peptide-conjugated gold nanoparticles. *Bioconjugate Chemistry*, **28**(11): 2673−2686.

Ramadan RA, Gebriel MG, Kadry HM, et al. 2018. Carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: characterization of carbapenemase genes and E-test evaluation of colistin-based combinations. *Infection and Drug Resistance*, **11**: 1261−1269.

Ramezanzadeh M, Saeedi N, Mesbahfar E, et al. 2021. Design and characterization of new antimicrobial peptides derived from aurein 1.2 with enhanced antibacterial activity. *Biochimie*, **181**: 42−51.

Robalo JR, Huhmann S, Koksch B, et al. 2017. The multiple origins of the hydrophobicity of fluorinated apolar amino acids. *Chem*, **3**(5): 881−897.

Rounds T, Straus SK. 2020. Lipidation of antimicrobial peptides as a design strategy for future alternatives to antibiotics. *International Journal of Molecular Sciences*, **21**(24): 9692.

Sader HS, Fedler KA, Rennie RP, et al. 2004. Omiganan pentahydrochloride (MBI 226), a topical 12-amino-acid cationic peptide: spectrum of antimicrobial activity and measurements of bactericidal activity. *Antimicrobial Agents and Chemotherapy*, **48**(8): 3112−3118.

Sandín D, Valle J, Chaves-Arquero B, et al. 2021. Rationally modified antimicrobial peptides from the N-Terminal domain of human RNase 3 show exceptional serum stability. *Journal of Medicinal Chemistry*, **64**(15): 11472−11482.

Schnaider L, Rosenberg A, Kreiser T, et al. 2020. Peptide self-assembly is linked to antibacterial, but not antifungal, activity of histatin 5 derivatives. *mSphere*, **5**(2): e00021−20.

Schneider P, Schneider G. 2016. De novo design at the edge of chaos. *Journal of Medicinal Chemistry*, **59**(9): 4077−4086.

Sergiev PG. 1944. Clinical use of gramicidin S. *The Lancet*, **244**(6327): 717−718.

Sforça ML, Oyama Jr S, Canduri F, et al. 2004. How C-terminal carboxyamidation alters the biological activity of peptides from the venom of the eumenine solitary wasp. *Biochemistry*, **43**(19): 5608−5617.

Shah MB, Liu JB, Zhang QH, et al. 2017. Halogen-π interactions in the cytochrome P450 active site: structural insights into human CYP2B6 substrate selectivity. *ACS Chemical Biology*, **12**(5): 1204−1210.

Shahrour H, Ferrer-Espada R, Dandache I, et al. 2019. AMPs as anti-biofilm agents for human therapy and prophylaxis. *Advances in Experimental Medicine and Biology*, **1117**: 257−279.

Sharma D, Misba L, Khan AU. 2019. Antibiotics versus biofilm: an emerging battleground in microbial communities. *Antimicrobial Resistance & Infection Control*, **8**: 76.

Sheard DE, O'Brien-Simpson NM, Wade JD, et al. 2019. Combating bacterial resistance by combination of antibiotics with antimicrobial peptides. *Pure and Applied Chemistry*, **91**(2): 199−209.

Shearer J, Castro JL, Lawson ADG, et al. 2022. Rings in clinical trials and drugs: present and future. *Journal of Medicinal Chemistry*, **65**(13): 8699−8712.

Shi GB, Kang XY, Dong FY, et al. 2022. DRAMP 3.0: an enhanced comprehensive data repository of antimicrobial peptides. *Nucleic Acids Research*, **50**(D1): D488−D496.

Sieprawska-Lupa M, Mydel P, Krawczyk K, et al. 2004. Degradation of human antimicrobial peptide LL-37 by *Staphylococcus aureus*-derived proteinases. *Antimicrobial Agents and Chemotherapy*, **48**(12): 4673−4679.

Singh J, Joshi S, Mumtaz S, et al. 2016. Enhanced cationic charge is a key factor in promoting staphylocidal activity of α-melanocyte stimulating hormone via selective lipid affinity. *Scientific Reports*, **6**: 31492.

Smirnova MP, Afonin VG, Shpen' VM, et al. 2004. Structure-Function relationship between analogues of the antibacterial peptide indolicidin. I. Synthesis and biological activity of analogues with increased amphipathicity and elevated net positive charge of the molecule. *Russian Journal of Bioorganic Chemistry*, **30**(5): 409−416.

Spänig S, Heider D. 2019. Encodings and models for antimicrobial peptide classification for multi-resistant pathogens. *BioData Mining*, **12**: 7.

Srinivas N, Jetter P, Ueberbacher BJ, et al. 2010. Peptidomimetic antibiotics target outer-membrane biogenesis in *Pseudomonas aeruginosa*. *Science*, **327**(5968): 1010–1013.

Stewart PS. 2002. Mechanisms of antibiotic resistance in bacterial biofilms. *International Journal of Medical Microbiology*, **292**(2): 107−113.

Syed YY, Scott LJ. 2015. Oritavancin: a review in acute bacterial skin and skin structure infections. *Drugs*, **75**(16): 1891−1902.

Tabatabaei Mirakabad FS, Nejati-Koshki K, Akbarzadeh A, et al. 2014. PLGA-based nanoparticles as cancer drug delivery systems. *Asian Pacific Journal of Cancer Prevention*, **15**(2): 517−535.

Taboureau O. 2010. Methods for building quantitative structure-activity relationship (QSAR) descriptors and predictive models for computer-aided design of antimicrobial peptides. *In*: Giuliani A, Rinaldi AC. Antimicrobial Peptides: Methods and Protocols. Totowa: Humana, 77–86.

Teixeira MC, Carbone C, Sousa MC, et al. 2020. Nanomedicines for the delivery of antimicrobial peptides (AMPs). *Nanomaterials*, **10**(3): 560.

Thapa RK, Diep DB, Tønnesen HH. 2021. Nanomedicine-based antimicrobial peptide delivery for bacterial infections: recent advances and future prospects. *Journal of Pharmaceutical Investigation*, **51**(4): 377−398.

Thomas S, Karnik S, Barai RS, et al. 2010. CAMP: a useful resource for research on antimicrobial peptides. *Nucleic Acids Research*, **38**(S1): D774−D780.

Timmons PB, Hewage CM. 2021a. ENNAACT is a novel tool which employs neural networks for anticancer activity classification for therapeutic peptides. *Biomedicine & Pharmacotherapy*, **133**: 111051.

Timmons PB, Hewage CM. 2021b. ENNAVIA is a novel method which employs neural networks for antiviral and anti-coronavirus activity prediction for therapeutic peptides. *Briefings in Bioinformatics*, **22**(6): bbab258.

Tornesello AL, Borrelli A, Buonaguro L, et al. 2020. Antimicrobial peptides as anticancer agents: functional properties and biological activities. *Molecules*, **25**(12): 2850.

Travis S, Yap LM, Hawkey C, et al. 2005. Rdp58 is a novel and potentially effective oral therapy for ulcerative colitis. *Inflammatory Bowel Diseases*, **11**(8): 713−719.

Umedera K, Yoshimori A, Chen HW, et al. 2023. DeepCubist: molecular

generator for designing peptidomimetics based on complex three-dimensional scaffolds. *Journal of Computer-Aided Molecular Design*, **37**(2): 107−115.

Urnukhsaikhan E, Bold BE, Gunbileg A, et al. 2021. Antibacterial activity and characteristics of silver nanoparticles biosynthesized from *Carduus crispus*. *Scientific Reports*, **11**(1): 21047.

Vandekerckhove L, Verhofstede C, Vogelaers D. 2009. Maraviroc: perspectives for use in antiretroviral-naive HIV-1-infected patients. *Journal of Antimicrobial Chemotherapy*, **63**(6): 1087−1096.

Veltri D, Kamath U, Shehu A. 2018. Deep learning improves antimicrobial peptide recognition. *Bioinformatics*, **34**(16): 2740−2747.

Vernen F, Harvey PJ, Dias SA, et al. 2019. Characterization of tachyplesin peptides and their cyclized analogues to improve antimicrobial and anticancer properties. *International Journal of Molecular Sciences*, **20**(17): 4184.

Vishnepolsky B, Gabrielian A, Rosenthal A, et al. 2018. Predictive model of linear antimicrobial peptides active against gram-negative bacteria. *Journal of Chemical Information and Modeling*, **58**(5): 1141−1151.

Wach A, Dembowsky K, Dale GE. 2018. Pharmacokinetics and safety of intravenous murepavadin infusion in healthy adult subjects administered single and multiple ascending doses. *Antimicrobial Agents and Chemother*, **62**(4): e02355−17.

Waghu FH, Barai RS, Gurung P, et al. 2016. $CAMP_{R3}$: a database on sequences, structures and signatures of antimicrobial peptides. *Nucleic Acids Research*, **44**(D1): D1094−D1097.

Wang C, Garlick S, Zloh M. 2021a. Deep learning for novel antimicrobial peptide design. *Biomolecules*, **11**(3): 471.

Wang C, Hong TT, Cui PF, et al. 2021b. Antimicrobial peptides towards clinical application: delivery and formulation. *Advanced Drug Delivery Reviews*, **175**: 113818.

Wang GS. 2008. Structures of human host defense cathelicidin LL-37 and its smallest antimicrobial peptide KR-12 in lipid micelles. *Journal of Biological Chemistry*, **283**(47): 32637−32643.

Wang GS, Li X, Wang Z. 2016. APD3: the antimicrobial peptide database as a tool for research and education. *Nucleic Acids Research*, **44**(D1): D1087−D1093.

Wang YP, Hong J, Liu XH, et al. 2008. Snake cathelicidin from *Bungarus fasciatus* is a potent peptide antibiotics. *PLoS One*, **3**(9): e3217.

Wang YP, Zhang ZY, Chen LL, et al. 2011. Cathelicidin-BF, a snake cathelicidin-derived antimicrobial peptide, could be an excellent therapeutic agent for acne vulgaris. *PLoS One*, **6**(7): e22120.

White JK, Muhammad T, Alsheim E, et al. 2022. A stable cyclized antimicrobial peptide derived from LL-37 with host immunomodulatory effects and activity against uropathogens. *Cellular and Molecular Life Sciences*, **79**(8): 411.

Whitesides GM, Boncheva M. 2002. Beyond molecules: self-assembly of mesoscopic and macroscopic components. *Proceedings of the National Academy of Sciences of the United States of America*, **99**(8): 4769−4774.

Won A, Khan M, Gustin S, et al. 2011. Investigating the effects of L- to D-amino acid substitution and deamidation on the activity and membrane interactions of antimicrobial peptide anoplin. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, **1808**(6): 1592−1600.

Xiao X, Shao YT, Cheng X, et al. 2021. iAMP-CA2L: a new CNN-BiLSTM-SVM classifier based on cellular automata image for identifying antimicrobial peptides and their functional types. *Briefings in Bioinformatics*, **22**(6): bbab209.

Xiao X, Wang P, Lin WZ, et al. 2013. iAMP-2L: a two-level multi-label classifier for identifying antimicrobial peptides and their functional types. *Analytical Biochemistry*, **436**(2): 168−177.

Xie YP, He YP, Irwin PL, et al. 2011. Antibacterial activity and mechanism of action of zinc oxide nanoparticles against *Campylobacter jejuni*. *Applied and Environmental Microbiology*, **77**(7): 2325–2331.

Xing MC, Ji MY, Hu JM, et al. 2020. Snake cathelicidin derived peptide inhibits zika virus infection. *Frontiers in Microbiology*, **11**: 1871.

Yafout M, Ousaid A, Khayati Y, et al. 2021. Gold nanoparticles as a drug delivery system for standard chemotherapeutics: a new lead for targeted pharmacological cancer treatments. *Scientific African*, **11**: e00685.

Yakimov AP, Afanaseva AS, Khodorkovskiy MA, et al. 2016. Design of stable α-helical peptides and thermostable proteins in biotechnology and biomedicine. *Acta Naturae*, **8**(4): 70−81.

Yamashita R, Nishio M, Do RKG, et al. 2018. Convolutional neural networks: an overview and application in radiology. *Insights into Imaging*, **9**(4): 611−629.

Yan JL, Cai JX, Zhang B, et al. 2022. Recent progress in the discovery and design of antimicrobial peptides using traditional machine learning and deep learning. *Antibiotics*, **11**(10): 1451.

Yang XW, Lee WH, Zhang Y. 2012. Extremely abundant antimicrobial peptides existed in the skins of nine kinds of Chinese odorous frogs. *Journal of Proteome Research*, **11**(1): 306−319.

Ye Z, Zhu X, Acosta S, et al. 2019. Self-assembly dynamics and antimicrobial activity of all L- and D-amino acid enantiomers of a designer peptide. *Nanoscale*, **11**(1): 266−275.

Yin IX, Zhang J, Zhao IS, et al. 2020. The antibacterial mechanism of silver nanoparticles and its application in dentistry. *International Journal of Nanomedicine*, **15**: 2555−2562.

Yin LM, Edwards MA, Li J, et al. 2012. Roles of hydrophobicity and charge distribution of cationic antimicrobial peptides in peptide-membrane interactions. *Journal of Biological Chemistry*, **287**(10): 7738−7745.

Yokoo H, Hirano M, Ohoka N, et al. 2021. Structure-activity relationship study of amphipathic antimicrobial peptides using helix-destabilizing sarcosine. *Journal of Peptide Science*, **27**(12): e3360.

Yu CY, Huang W, Li ZP, et al. 2016. Progress in self-assembling peptide-based nanomaterials for biomedical applications. *Current Topics in Medicinal Chemistry*, **16**(3): 281−290.

Yuan BQ, Lu XY, Yang M, et al. 2022. A designed antimicrobial peptide with potential ability against methicillin resistant *Staphylococcus aureus*. *Frontiers in Microbiology*, **13**: 1029366.

Zeng ZZ, Huang SH, Alezra V, et al. 2021. Antimicrobial peptides: triumphs and challenges. *Future Medicinal Chemistry*, **13**(16): 1313−1315.

Zhang F, Guo ZL, Chen Y, et al. 2019. Effects of C-terminal amidation and heptapeptide ring on the biological activities and advanced structure of amurin-9KY, a novel antimicrobial peptide identified from the brown frog. *Rana kunyuensis*. *Zoological Research*, **40**(3): 198−204.

Zhang QY, Yan ZB, Meng YM, et al. 2021. Antimicrobial peptides: mechanism of action, activity and clinical potential. *Military Medical Research*, **8**(1): 48.

Zhang ZY, Mu LX, Tang J, et al. 2013. A small peptide with therapeutic potential for inflammatory acne vulgaris. *PLoS One*, **8**(8): e72923.

Zhao JS, Ge G, Huang YB, et al. 2022. Butelase 1-mediated enzymatic cyclization of antimicrobial peptides: improvements on stability and bioactivity. *Journal of Agricultural and Food Chemistry*, **70**(50): 15869−15878.

Zhao XW, Wu HY, Lu HR, et al. 2013. LAMP: a database linking antimicrobial peptides. *PLoS One*, **8**(6): e66557.

Zhao YY, Zhang M, Qiu S, et al. 2016. Antimicrobial activity and stability of the D-amino acid substituted derivatives of antimicrobial peptide *polybia*-MPI. *AMB Express*, **6**(1): 122.

Zhong C, Zhu NY, Zhu YW, et al. 2020. Antimicrobial peptides conjugated with fatty acids on the side chain of D-amino acid promises antimicrobial potency against multidrug-resistant bacteria. *European Journal of Pharmaceutical Sciences*, **141**: 105123.

Zhu X, Ma Z, Wang JJ, et al. 2014. Importance of tryptophan in transforming an amphipathic peptide into a *Pseudomonas aeruginosa*-targeted antimicrobial peptide. *PLoS One*, **9**(12): e114605.