

Original Article

Rational Design and Machine Learning-Guided Screening of Short α -Helical Antimicrobial Peptides Derived from Dendrocin-ZM1

Monir Ebadi Moghvan¹, Sima Sadat Seyedjavadi², Azam Haddadi¹, Naser Harzandi¹, Mehdi Goudarzi^{3*}

¹Department of Microbiology, Ka.C., Islamic Azad University, Karaj, Iran

²Department of Mycology, Pasteur Institute of Iran, Tehran, Iran

³Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Received: 23 June, 2026; Accepted: 19 July, 2026

DOI: 10.22037/nbm.v14i4.52578

Abstract

Background: The increasing prevalence of multidrug-resistant bacterial pathogens has created an urgent need for new antimicrobial strategies with improved efficacy and safety profiles. Antimicrobial peptides (AMPs) are promising therapeutic candidates due to their membrane-targeting mechanisms and broad antimicrobial potential. This study aimed to computationally design and screen shortened analogs of Dendrocin-ZM1 to identify potential antimicrobial peptide candidates against *Staphylococcus aureus*.

Materials and Methods: Dendrocin-ZM1, a 33-amino-acid α -helical antimicrobial peptide, was selected as the parental template for rational peptide redesign. A series of shortened and sequence-engineered analogs was generated by retaining selected cationic and hydrophobic residues associated with antimicrobial peptide activity. The designed peptides were evaluated based on physicochemical properties, including peptide length, net charge, hydrophobicity, and predicted α -helical structural characteristics. Antimicrobial potential was assessed using the CAMPR4 platform with Support Vector Machine, Random Forest, and Artificial Neural Network classifiers. Additional screening was performed using the DBAASP prediction platform, focusing on predicted activity against *S. aureus*. Potential toxicity and hemolytic effects were evaluated using ToxinPred and HemoPIV, respectively, and selected peptides were subjected to structural modeling to assess predicted α -helical conformations.

Results: The designed peptide analogs exhibited favorable predicted physicochemical characteristics, including reduced length, retained cationic character, and modified hydrophobicity. CAMPR₄ analysis indicated high antimicrobial prediction scores across multiple machine learning classifiers. DBAASP screening identified P5, P6, and P7 as computationally predicted active candidates against *S. aureus*. Structural modeling suggested predominantly α -helical conformations among these peptides, consistent with structural features commonly observed in membrane-active AMPs. Toxicity prediction indicated low toxicity, while hemolysis analysis suggested favorable hemocompatibility profiles. Among the evaluated candidates, P7 was prioritized based on its integrated computational profile, combining predicted anti-*S. aureus* activity with favorable toxicity, hemolysis, and structural predictions.

Conclusion: This study presents a computational workflow that integrates rational peptide shortening and sequence engineering with machine learning-assisted screening to prioritize potential antimicrobial peptide candidates derived from Dendrocin-ZM1. P7 was identified as a promising computationally prioritized candidate; however, experimental validation, including antimicrobial activity and safety evaluation, is required to confirm its therapeutic potential.

Keywords: Antimicrobial peptide, Dendrocin-ZM1, rational design, machine learning, *Staphylococcus aureus*, Computational screening, Toxicity prediction, Hemolysis, α -helical peptide.

***Corresponding Author:** Dr. Mehdi Goudarzi, Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran; Email: goudarzim@yahoo.com

Please cite this article as: Ebadi Moghvan M, Seyedjavadi SS, Haddadi A, Harzandi N, Goudarzi M. Rational Design and Machine Learning-Guided Screening of Short α -Helical Antimicrobial Peptides Derived from Dendrocin-ZM1. *Novel Biomed.* 2026;14(4):189-97.

Introduction

The rapid global emergence of antimicrobial resistance (AMR) poses a major public health challenge by reducing the effectiveness of existing antibiotics and complicating the treatment of bacterial infections. The World Health Organization has identified AMR as one of the major global health threats, emphasizing the urgent need for alternative antimicrobial strategies. Among clinically important resistant pathogens, *Staphylococcus aureus*, particularly methicillin-resistant *S. aureus* (MRSA), remains a significant concern due to its ability to develop resistance mechanisms and cause persistent community- and hospital-acquired infections^{1,2}.

Antimicrobial peptides (AMPs) have attracted considerable attention as potential alternatives to conventional antibiotics due to their diverse antimicrobial activities and unique mechanisms of action. These naturally occurring molecules are components of innate immunity and are found in various organisms, including microorganisms, plants, insects, and mammals **3**. Most AMPs are short, cationic, and amphipathic peptides enriched in hydrophobic and positively charged residues, characteristics that contribute to their interaction with microbial membranes. However, the antimicrobial activity and selectivity of AMPs are strongly influenced by their physicochemical properties, including peptide length, charge distribution, hydrophobicity, and structural organization^{4,5}.

Among different AMP structural classes, α -helical peptides are particularly important because their amphipathic organization enables the spatial separation of hydrophobic and cationic residues. This feature is commonly associated with selective interaction with negatively charged bacterial membranes. Nevertheless, excessive hydrophobicity or positive charge may increase nonspecific interactions with mammalian membranes and

contribute to cytotoxicity or hemolytic effects. Therefore, rational optimization of these parameters is essential for designing AMP candidates with improved predicted activity and safety profiles ^{6,7,8}.

Despite their therapeutic potential, the clinical translation of natural AMPs remains challenging due to several limitations, including susceptibility to degradation, production costs, sequence complexity, and potential toxicity. Peptide truncation has emerged as an effective strategy to overcome some of these limitations by generating shorter analogs that retain essential functional motifs while improving synthesis feasibility and potentially reducing undesirable effects. Identifying the optimal balance between sequence length, antimicrobial activity, and safety remains a major challenge in AMP engineering ^{9,10}.

Recent developments in computational biology and machine learning have provided valuable tools for accelerating AMP discovery and optimization. Databases such as CAMPR4 and DBAASP contain experimentally characterized antimicrobial peptide sequences and support the development of predictive models for sequence-based screening^{11,12}. Machine learning approaches, including Support Vector Machines (SVM), Random Forests (RF), and Artificial Neural Networks (ANN), have been applied to classify peptide sequences by antimicrobial potential using physicochemical and sequence-derived features ¹³. These computational strategies can reduce the number of candidates requiring experimental evaluation and facilitate the identification of promising peptide designs.

α -helical peptides remain particularly attractive in AMP design due to their predictable folding behavior and well-established structure–function relationships. Studies have shown that many natural and synthetic α -helical peptides adopt membrane-active conformations only upon interaction with lipid environments, suggesting that structural plasticity is a key determinant of antimicrobial activity¹⁴. In membrane-like environments, these peptides often undergo coil-to-

helix transitions, forming stable amphipathic helices that facilitate membrane disruption¹⁵.

Dendrocin-ZM1 is a 33-amino-acid α -helical antimicrobial peptide originally isolated from *Zataria multiflora* and has previously demonstrated antibacterial activity against *S. aureus*¹⁶. However, its relatively long sequence may pose challenges for peptide synthesis, stability, and optimization for therapeutic applications. Although Dendrocin-ZM1 represents a promising AMP template, systematic investigation of shortened derivatives with optimized physicochemical characteristics remains limited.

In this study, we developed a computational framework that combines rational sequence truncation and machine-learning-assisted screening to identify potential short α -helical AMP candidates derived from Dendrocin-ZM1. The workflow integrates physicochemical characterization, antimicrobial prediction using multiple machine learning classifiers, toxicity assessment, hemolysis prediction, and structural modeling. Rather than replacing experimental evaluation, this approach aims to prioritize peptide candidates for future validation and provide insights into the computational optimization of Dendrocin-ZM1-derived antimicrobial peptides.

Methods

Peptide Template Selection: The antimicrobial peptide Dendrocin-ZM1, a 33-amino-acid α -helical peptide originally isolated from *Zataria multiflora*, was selected as the parental template for computational redesign. The amino acid sequence (TTLRLNTLAYKVAWLNVKAFWAAGRALKK VGR) was obtained from a previously published experimental study¹⁶. Initial sequence analysis was performed to evaluate peptide length, charge distribution, and hydrophobic residue composition to identify structural features potentially associated with antimicrobial activity.

Rational Design and Sequence Engineering: A rational truncation strategy was applied to generate shortened Dendrocin-ZM1-derived peptide analogs. The design objective was to retain sequence characteristics commonly associated with α -helical antimicrobial peptides while reducing peptide length. Candidate sequences were designed within a length

range of 15–25 amino acids based on previously reported characteristics of short cationic AMPs.

The initial design criteria included a peptide length of 15–25 amino acids, a net positive charge greater than +5, and a hydrophobic residue content above 60%. These criteria were selected based on previously described physicochemical features frequently observed in membrane-active α -helical AMPs^{5,6}. However, these parameters were considered screening criteria rather than definitive predictors of antimicrobial activity.

Hydrophobic residues, including leucine (Leu), valine (Val), alanine (Ala), phenylalanine (Phe), and tyrosine (Tyr), were preferentially retained to maintain hydrophobic character, whereas positively charged residues, mainly lysine (Lys) and arginine (Arg), were preserved to maintain cationic properties commonly associated with AMP activity.

Physicochemical Characterization: The physicochemical properties of designed peptide variants were calculated using the ExPASy ProtParam server (<https://web.expasy.org/protparam/>, accessed in 2025)¹⁷. Parameters including molecular weight, theoretical isoelectric point (pI), predicted net charge at physiological pH, grand average of hydropathicity (GRAVY), and amino acid composition were obtained. Peptide sequences that did not satisfy the predefined computational screening criteria were excluded from subsequent analyses.

Machine Learning-Based Antimicrobial Prediction: The antimicrobial potential of designed peptides was evaluated using the CAMPR4 prediction platform (<https://camp.bicnirrh.res.in/>)¹⁸. Three machine learning-based classifiers available within the platform, including Random Forest (RF), Support Vector Machine (SVM), and Artificial Neural Network (ANN), were applied to estimate the probability of antimicrobial activity.

Each classifier generated a prediction score representing the likelihood of antimicrobial activity. Peptides showing consistent prediction results across multiple classifiers were prioritized for further computational evaluation. Agreement among different classifiers was considered an additional criterion to reduce potential false-positive predictions, consistent with previous computational AMP screening studies¹⁹.

Secondary Validation Using DBAASP Prediction

Tool: Selected peptide candidates were further evaluated using the DBAASP linear antimicrobial peptide prediction tool (<https://dbaasp.org/tools?page=linear-amp-prediction>)²⁰. This platform estimates antimicrobial potential based on sequence characteristics and experimentally characterized AMP datasets. Predicted antimicrobial activity against different bacterial species was assessed, with particular emphasis on *S. aureus* due to its clinical importance. Where available, predicted MIC values were recorded to provide quantitative support for candidate prioritization.

Toxicity and Hemolysis Prediction: Potential toxicity of selected peptide candidates was evaluated using ToxinPred (<https://webs.iitd.edu.in/raghava/toxinpred/>)²¹, a machine learning-based prediction tool for identifying potentially toxic peptide sequences. Hemolytic potential was assessed using HemoPI (<https://crdd.osdd.net/raghava/hemopi/>)²², which predicts hemolytic activity based on sequence-derived characteristics and provides SVM prediction scores and estimated HC50 values.

Structural Modeling of Designed Peptides: The predicted three-dimensional structures of selected peptide candidates were generated using the PEP-FOLD3 server (<https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/>). Structural models were analyzed to evaluate the predicted presence of α -helical conformations and overall structural characteristics associated with α -helical AMP candidates.

Selection Criteria for Final Candidates: Final candidate prioritization was performed using an integrated computational scoring strategy. The selection criteria included physicochemical properties (peptide length, net charge, and hydrophobicity), consistent antimicrobial predictions across machine-learning models, favorable DBAASP-based predictions, predicted low toxicity and low hemolytic potential, and preservation of α -helical structural characteristics.

Overall Computational Workflow: The computational workflow consisted of four major stages: (1) selection of Dendrocin-ZM1 as the parental peptide template, (2) rational sequence truncation and

physicochemical optimization, (3) machine learning-based antimicrobial prediction and secondary computational screening, and (4) prioritization of promising peptide candidates based on integrated computational criteria.

The overall workflow for peptide design, computational screening, and candidate selection is summarized in Figure 1.

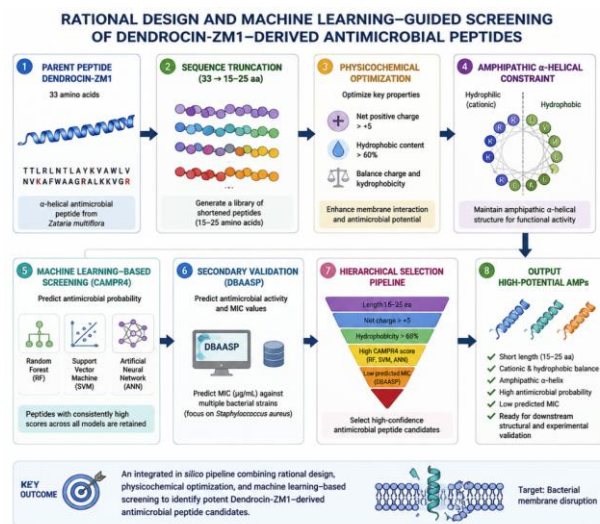


Figure 1. Schematic representation of the computational workflow for the rational design and machine learning-guided screening of Dendrocin-ZM1-derived antimicrobial peptide candidates.

Results

Design and Initial Screening of Dendrocin-ZM1-Derived Peptides: The antimicrobial peptide Dendrocin-ZM1 (33 amino acids; sequence: TTLRLNTLAYKVAWLNVNKAFAAGRALKKVGR) was selected as the parental template for rational peptide redesign. The native peptide exhibited a net positive charge of +7 and a hydrophobicity value of approximately 55%, indicating physicochemical characteristics commonly associated with α -helical antimicrobial peptides.

To generate shorter derivatives, a library of nine truncated analogs (P1–P9) was designed with peptide lengths ranging from 19 to 21 amino acids. The redesign strategy aimed to retain positively charged residues, including lysine and arginine, while maintaining an appropriate balance of hydrophobic residues to preserve physicochemical characteristics

frequently observed in antimicrobial peptides. and Artificial Neural Network (ANN) (Table 2).

Table 1. Physicochemical properties of peptides P1–P9.

Peptide Code	Sequence	Length	Net Charge	Hydrophobicity (%)
--------------	----------	--------	------------	--------------------

Table 2. Antimicrobial activity of selected peptides using CAMPR4 and DBAASP.

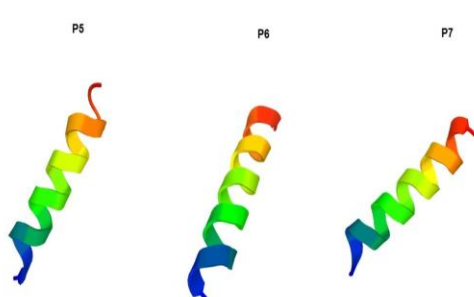
Peptide Code	Seq. ID.	CAMPR4					DBAASP
		SVM	RF		ANN	Anti- <i>S.aureus</i>	
	P5	0.931	AMP	0.991	AMP	0.998	Not active
	P6	0.927	AMP	0.993	AMP	0.998	Not active
	P7	0.919	AMP	0.994	AMP	0.997	Not active
		0.952	AMP	0.992	AMP	0.998	Not active
		0.914	AMP	0.974	AMP	0.999	Active
		0.935	AMP	0.979	AMP	0.998	Active
		0.889	AMP	0.989	AMP	0.998	Active

Figure 2. Three-dimensional structures of the designed antimicrobial peptides P5, P6, and P7.

Figure 2. Three-dimensional structures of the designed antimicrobial peptides P5, P6, and P7.

Physicochemical Filtering and Initial Selection: The physicochemical properties of all designed peptides are summarized in Table 1. Initial screening was performed according to predefined criteria, including peptide length, net charge, and hydrophobicity.

Peptides with reduced positive charge or unfavorable hydrophobicity profiles were excluded from further computational evaluation. Based on these criteria, peptides P1–P7 were selected as primary candidates for subsequent antimicrobial prediction and safety assessment.

Machine Learning-Based Antimicrobial Prediction: All selected peptides were evaluated using the CAMPR4 prediction platform. The results indicated that all candidates were classified as antimicrobial peptides across the three applied machine learning classifiers, including Support Vector Machine (SVM), Random Forest (RF),

Table 3. Prediction results of toxicity and hemolytic activity of the selected peptides P5, P6, and P7.

Peptide Code	Toxinpred		HempPI2 (HC50(μM))
	Prediction	SVM Score	
P5	Non-Toxin	-1.23	102.265
P6	Non-Toxin	-1.13	108.591
P7	Non-Toxin	-1.32	115.23

Further evaluation using the DBAASP prediction platform demonstrated differences in predicted activity against *S. aureus*. Among the evaluated sequences, peptides P5, P6, and P7 showed predicted antimicrobial activity against *S. aureus*, whereas the remaining peptides were classified as inactive.

These findings indicate that although CAMPR4 provided consistent AMP classification across candidates, secondary filtering using DBAASP contributed to the prioritization of peptides with higher predicted pathogen-specific activity.

Structural Modeling and Conformational Analysis: The predicted three-dimensional structures of selected peptides (P5, P6, and P7) were generated using the PEP-FOLD3 server. As shown in Figure 2, all selected peptides predominantly adopted predicted α-helical conformations, consistent with structural features commonly observed in α-helical antimicrobial peptides. P5, P6, and P7 demonstrated similar predicted helical organizations, although minor differences in terminal flexibility were observed among the generated models. These structural predictions supported the selection of these peptides for further computational safety evaluation

Toxicity and Hemolysis Activity Prediction: All selected peptides were predicted to be non-toxic using ToxinPred, with negative SVM scores supporting this

classification (Table 3). Among them, P7 exhibited the lowest SVM score (−1.32), suggesting the lowest predicted toxicity. Hemolytic potential was evaluated using HemoPI, where all peptides showed relatively high HC50 values (102.265–115.23 μ M), indicating low hemolytic activity. P7 demonstrated the highest HC50 value (115.23 μ M), suggesting the most favorable hemolytic safety profile.

Final Candidate Selection: Based on integrated computational evaluation, including predicted antimicrobial activity, physicochemical properties, structural characteristics, toxicity prediction, and hemolysis assessment, P7 was prioritized as the leading candidate among the designed peptides. However, experimental validation is required to confirm its antimicrobial efficacy and safety profile.

Discussion

The increasing prevalence of antimicrobial resistance (AMR) has intensified the need for alternative antimicrobial strategies beyond conventional antibiotics. In this context, antimicrobial peptides (AMPs) have attracted considerable attention due to their diverse mechanisms of action and potential to reduce the selective pressure associated with conventional antibiotics. Unlike many traditional antibiotics that target specific cellular pathways, AMPs commonly interact with bacterial surfaces through physicochemical properties including charge distribution, hydrophobicity, and amphipathic structure^{3,5}. In the present study, a rational design and machine learning-assisted screening strategy was applied to generate and prioritize shortened derivatives of the α -helical antimicrobial peptide Dendrocin-ZM1.

A major finding of this study was that systematic truncation of the parent peptide yielded shorter derivatives with favorable predicted antimicrobial properties while retaining key physicochemical features associated with AMP activity. This observation agrees with previous studies showing that many antimicrobial peptides are relatively short sequences in which activity depends on the appropriate balance between cationicity, hydrophobicity, and structural organization rather than peptide length alone^{3,23}. Peptide shortening may also improve synthetic feasibility and facilitate

optimization of peptide-based candidates by reducing sequence complexity and production requirements²⁴.

The physicochemical analysis demonstrated that the designed peptides maintained a balanced distribution of positively charged and hydrophobic residues. Cationic amino acids, particularly lysine and arginine, are frequently associated with electrostatic attraction toward negatively charged bacterial surfaces, whereas hydrophobic residues contribute to interactions with lipid environments¹⁰. However, excessive hydrophobicity may increase nonspecific interactions with mammalian membranes and potentially contribute to cytotoxicity or hemolytic effects⁶. Therefore, the screening strategy applied in this study emphasized a balanced physicochemical profile rather than maximizing individual parameters.

Machine learning-based screening using CAMPR4 classified all designed peptides as antimicrobial candidates across multiple prediction models. However, subsequent evaluation using DBAASP provided additional discrimination among candidates by identifying peptides with predicted activity specifically against *S. aureus*. Differences between general AMP prediction models and organism-specific prediction tools may arise from variations in training datasets and predictive features, highlighting the importance of integrating multiple computational approaches for peptide prioritization^{8,9}. In this study, only P5, P6, and P7 demonstrated predicted anti-*S. aureus* activity, suggesting that relatively small sequence modifications can influence computationally predicted antimicrobial properties.

Structural modeling using PEP-FOLD3 indicated that the selected peptides predominantly adopted predicted α -helical conformations. This finding is consistent with the structural characteristics commonly observed in many α -helical AMPs, where amphipathic organization is considered an important feature associated with antimicrobial function¹². Although these predicted structures support the retention of α -helical characteristics after truncation, experimental structural characterization and membrane interaction studies will be necessary to confirm their functional relevance.

Among the evaluated candidates, P7 demonstrated the most favorable overall computational profile based on predicted antimicrobial activity, toxicity, hemolysis, and structural characteristics. The favorable profile of

P7 may be related to its optimized balance of charged and hydrophobic residues, which represents a commonly observed design principle in selective AMP engineering²⁵. However, these findings represent computational prioritization rather than experimental confirmation, and the biological activity of P7 requires validation through antimicrobial assays and safety evaluation.

The toxicity and hemolysis prediction results further supported the computational prioritization of the designed peptides. All evaluated candidates were predicted as non-toxic by ToxinPred and showed relatively favorable HemoPI-based profiles. P7 exhibited the highest predicted HC50 value among the selected peptides, indicating a favorable predicted hemocompatibility profile. Nevertheless, computational toxicity and hemolysis predictions should be interpreted as preliminary indicators that require confirmation with experimental assays²⁶.

Compared with previously characterized antimicrobial peptides, the Dendrocin-ZM1-derived candidates developed in this study feature shorter peptide architectures while retaining physicochemical features commonly associated with antimicrobial activity, such as positive charge and amphipathic organization^{3, 4, 27}. However, experimental studies are required to determine whether these computationally optimized characteristics translate into improved antimicrobial performance or safety profiles.

Overall, this study demonstrates the utility of integrating rational peptide truncation with machine-learning-assisted screening to prioritize potential AMP candidates. Similar computational frameworks have increasingly helped reduce the experimental screening burden and accelerate early-stage peptide discovery²⁸. However, computational predictions are influenced by model training datasets and algorithmic assumptions; therefore, experimental validation remains essential.

Several limitations should be acknowledged. First, all analyses were performed computationally, and antimicrobial activity, cytotoxicity, and hemolytic effects require experimental confirmation. Second, molecular docking, molecular dynamics simulations, and membrane interaction studies were not performed and should be considered in future investigations to

provide additional mechanistic insights. Future studies combining computational refinement with experimental validation will be necessary to evaluate the therapeutic potential of Dendrocin-ZM1-derived peptide candidates.

Conclusion

In conclusion, this computational study demonstrated that rational truncation and sequence optimization of Dendrocin-ZM1 can generate shorter peptide analogs with favorable physicochemical characteristics and predicted antimicrobial potential. By integrating physicochemical screening, machine learning-based antimicrobial prediction, DBAASP-based evaluation against *S. aureus*, structural modeling, and computational safety assessment, peptide P7 was prioritized as the most promising candidate among the designed sequences.

P7 exhibited a favorable computational profile, including balanced cationic and hydrophobic properties, a predicted α -helical conformation, and predicted anti-*S. aureus* activity, low predicted toxicity, and favorable hemolysis prediction. However, these findings are based exclusively on in silico analyses, and experimental validation is required to confirm antimicrobial efficacy, cytotoxicity, and hemocompatibility.

Overall, this study highlights the potential of integrating rational peptide engineering with machine learning-assisted screening as an efficient approach for prioritizing antimicrobial peptide candidates. Further investigations, including experimental assays and advanced computational analyses such as molecular docking and membrane interaction studies, are necessary to evaluate the therapeutic potential of the identified candidates.

Acknowledgment

None.

Conflict of Interest

The authors declare no conflicts of interest related to this study.

Funding

This work is financially supported by a research grant from the Research Deputy of Shahid Beheshti University of Medical Sciences, Tehran, Iran [Grant No. 43014381]. The funding agency has no role in the design of the project, execution of the work, analysis, interpretation of the data, or manuscript writing and submission.

References

- World Health Organization. Antibacterial agents in clinical development: an analysis of the antibacterial clinical development pipeline, including tuberculosis. World Health Organization; 2017.
- Parsons JB, Mourad A, Conlon BP, Kielian T, Fowler Jr VG. Methicillin-resistant and susceptible *Staphylococcus aureus*: tolerance, immune evasion and treatment. *Nature Reviews Microbiology*. 2026;24(2):127-45.
- Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nature biotechnology*. 2006;24(12):1551-7.
- Jenssen H, Hamill P, Hancock RE. Peptide antimicrobial agents. *Clin Micro-biol Rev*. 2006; 19 (3): 491–511 [Internet].
- Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?. *Nature reviews microbiology*. 2005;3(3):238-50.
- Shai Y. Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by α -helical antimicrobial and cell non-selective membrane-lytic peptides. *Biochimica et Biophysica Acta (BBA)-Biomembranes*. 1999;1462(1-2):55-70.
- Chen J, Wang W, Hu X, Yue Y, Lu X, Wang C, Wei B, Zhang H, Wang H. Medium-sized peptides from microbial sources with potential for antibacterial drug development. *Natural Product Reports*. 2024;41(8):1235-63.
- Dathe M, Wieprecht T. Structural features of helical antimicrobial peptides: their potential to modulate activity on model membranes and biological cells. *Biochimica et Biophysica Acta (BBA)-Biomembranes*. 1999;1462(1-2):71-87.
- Lei J, Sun L, Huang S, Zhu C, Li P, He J, Mackey V, Coy DH, He Q. The antimicrobial peptides and their potential clinical applications. *American journal of translational research*. 2019;11(7):3919.
- Fjell CD, Hiss JA, Hancock RE, Schneider G. Designing antimicrobial peptides: form follows function. *Nature reviews Drug discovery*. 2012 Jan;11(1):37-51.
- Gabere MN, Noble WS. Empirical comparison of web-based antimicrobial peptide prediction tools. *Bioinformatics*. 2017;33(13):1921-9.
- Pirtskhalava M, Amstrong AA, Grigolava M, Chubinidze M, Alimbarashvili E, Vishnepolsky B, Gabrielian A, Rosenthal A, Hurt DE, Tartakovsky M. DBAASP v3: database of antimicrobial/cytotoxic activity and structure of peptides as a resource for development of new therapeutics. *Nucleic acids research*. 2021;49(D1):D288-97.
- Szymczak P, Szczurek E. Artificial intelligence-driven antimicrobial peptide discovery. *Current Opinion in Structural Biology*. 2023;83:102733.
- Sani MA, Separovic F. How membrane-active peptides get into lipid membranes. *Accounts of chemical research*. 2016 Jun 21;49(6):1130-8.
- Bechinger B, Gorr SU. Antimicrobial peptides: mechanisms of action and resistance. *Journal of dental research*. 2017;96(3):254-60.
- Seyedjavadi SS, Razzaghi-Abyaneh M, Nasiri MJ, Hashemi A, Goudarzi H, Haghighi M, Dadashi M, Goudarzi M, Zare-Zardini H, Pourhossein B. Isolation and chemical characterization of an alpha-helical peptide, dendrocin-ZM1, derived from *zataria multiflora* boiss with potent antibacterial activity. *Probiotics and antimicrobial proteins*. 2022;14(2):326-36.
- Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A. ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic acids research*. 2003;31(13):3784-8.
- Gawde U, Chakraborty S, Wagh FH, Barai RS, Khanderkar A, Indraguru R, Shirsat T, Idicula-Thomas S. CAMPR4: a database of natural and synthetic antimicrobial peptides. *Nucleic Acids Research*. 2023;51(D1):D377-83.
- Aronica PG, Reid LM, Desai N, Li J, Fox SJ, Yadahalli S, Essex JW, Verma CS. Computational methods and tools in antimicrobial peptide research. *Journal of Chemical Information and Modeling*. 2021;61(7):3172-96.
- Gogoladze G, Grigolava M, Vishnepolsky B, Chubinidze M, Duroux P, Lefranc MP, Pirtskhalava M. DBAASP: database of antimicrobial activity and structure of peptides. *FEMS microbiology letters*. 2014;357(1):63-8.
- Gupta S, Kapoor P, Chaudhary K, Gautam A, Kumar R, Open Source Drug Discovery Consortium, Raghava GP. In silico approach for predicting toxicity of peptides and proteins. *PloS one*. 2013;8(9):e73957.
- Kumar V, Kumar R, Agrawal P, Patiyal S, Raghava GP. A method for predicting hemolytic potency of chemically modified peptides from its structure. *Frontiers in pharmacology*. 2020 Feb 20;11:54.
- Fry DE. Antimicrobial peptides. *Surgical infections*. 2018;19(8):804-11.
- Oliveira Júnior NG, Souza CM, Buccini DF, Cardoso MH, Franco OL. Antimicrobial peptides: structure, functions and translational applications. *Nature Reviews Microbiology*. 2025;23(11):687-700.
- Shai Y. Mode of action of membrane active antimicrobial peptides. *Peptide Science: Original Research on Biomolecules*. 2002;66(4):236-48.

26. Zelezetsky I, Tossi A. Alpha-helical antimicrobial peptides—Using a sequence template to guide structure–activity relationship studies. *Biochimica et Biophysica Acta (BBA)-Biomembranes*. 2006;1758(9):1436-49.
27. Wimley WC, Hristova K. Antimicrobial peptides: successes, challenges and unanswered questions. *Journal of Membrane Biology*. 2011;239:27–34.
28. Rossetti P, Trollmann MF, Wichmann C, Gutschmann T, Eggeling C, Böckmann RA. From membrane composition to antimicrobial strategies: experimental and computational approaches to AMP design and Selectivity. *Small*. 2026;22(16):2411476.