The EuroBiotech Journal
RESEARCH ARTICLE
Medical Biotechnology

Antibacterial Activity of New Rimantadine Derivatives, Conjugated with Compact and Bulky Amino Acids

Radoslav Chayrov[1], Antoniya Stoymirska[1], Kiril Chuchkov[1], Veronica Nemska[2]*, Nelly Georgieva[2], Dancho Danalev[2], Ivanka Stankova[1]

Abstract

Rimantadine is an adamantane derivative, known for its antiviral activity against infections caused by influenza A viruses. The purpose of the present study was to synthesize 6 new rimantadine derivatives, containing short-chain (Gly, Ala, β-Ala) and bulky (Leu, Ile, Val) amino acids and to investigate their antimicrobial activity against the strains *Bacillus subtilis* NBIMCC 3562 and *Escherichia coli* NBIMCC 8785. All derivatives were successfully obtained in good yields by using the TBTU/TEA condensation system. Their antimicrobial properties were established by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) against both test strains. The MIC was obtained via a microdilution method, whereas the MBC – via a spread plate method. Most derivatives showed antimicrobial activity, with stronger effects against the Gram-positive strain *B. subtilis* NBIMCC 3562. Among them, *L*-Ile-Rim was the most effective derivative against both bacterial strains.

Keywords: rimantadine; adamantane derivative; antimicrobial activity; microdilution method.

Introduction

The excessive and often irrational use of antibiotics over the last 60 years has exerted very strong selective pressure for the emergence of resistant pathogenic strains [1]. Infections caused by them are often difficult or impossible to treat due to the ineffectiveness of the antibiotics and other medicines used. This results in increased mortality, morbidity, hospitalization length, and higher treatment costs [2]. This makes antimicrobial resistance one of the greatest global threats to human health. Tackling this problem has focused the attention of the scientific community on finding new alternative antimicrobial agents.

Rimantadine (α-methyl-1-adamantanemethylamine hydrochloride) is an adamantane derivative (Figure 1).

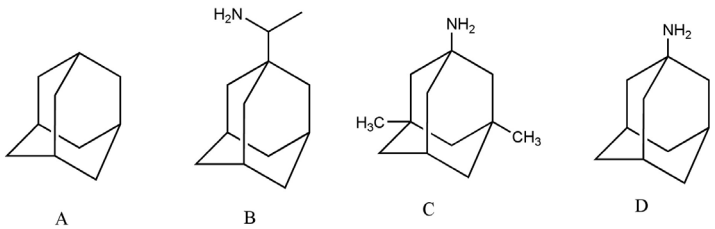

Figure 1. Chemical structures of: **A**. Adamantane, **B**. Rimantadine, **C**. Memantine, **D**. Amantadine

Along with memantine and amantadine, it belongs to the adamantane family [3]. In terms of structure, rimantadine is a tricyclic amine that consists of an adamantane backbone with an amino group substituted at one of the four methine positions. As an an-

[1]Department of Chemistry, South-West University "Neofit Rilski", Blagoevgrad, Bulgaria
[2]Department of Biotechnology, University of Chemical Technology and Metallurgy, Sofia, Bulgaria

***Corresponding author:**
Veronica Nemska
E-mail: vnemska@uctm.edu

DOI: 10.2478/ebtj-2026-0012

alogue of amantadine, rimantadine exhibits similar antiviral specificity and mechanism of action, along with a comparable risk for emergence of drug-resistant viral strains [4]. However, rimantadine is often preferred due to its better pharmacokinetic and pharmacodynamic properties, as well as fewer and milder side effects regarding the central nervous system [5]. Paintsil et al. [6] also reported that rimantadine is four to ten times more active than amantadine.

Rimantadine is well-known for its antiviral properties. Due to its effectiveness in treating and preventing influenza A infections in both adults and children in 1993, it was approved as an antiviral drug by the Food and Drug Administration. It is recommended to be administrated within the first 48 hours of the onset of symptoms [6]. Rimantadine binds to specific amino acids within the M2 channel, which are responsible for viral replication. Through this interaction, it blocks proton transport and disrupts viral uncoating [7]. Drug treatment shortens viral excretion and reduces fever and other systemic symptoms [6]. Rimantadine has also demonstrated effectiveness against other RNA-containing viruses like Saint Louis encephalitis, respiratory syncytial virus, Sindbis and parainfluenza viruses [8]. Rimantadine, like amantadine, exhibits N-methyl-D-aspartate receptor antagonistic activity and has been used in the treatment of Parkinson`s disease. However, neither drug is considered a first-line therapy and is generally reserved for cases that are less responsive to standard treatment [4]. In addition, rimantadine is also known to possess anti-tumor [9] and trypanocidal activity. It has been found to be highly effective in killing *Trypanosoma brucei* and *Trypanosoma cruzi,* the causative agents of the African sleeping sickness and Chagas` disease, respectively [10]. Contrariwise, due to the lack of the M2 protein in influenza B viruses, rimantadine shows little or no effect against this class of viruses. Unfortunately, in recent years, there is increasing resistance to adamantane derivatives, mainly regarding H1N1 and H3N2, because of which, since the mid-2000s, rimantadine is no longer recommended for routine treatment of influenza A infections [11].

Many research groups began exploring structural modifications of rimantadine to overcome emerging resistance. Thus, in 2003, Zoidis et al. [12] reported the synthesis of 2-rimantadine analogues, which were 2 times more potent than the drug rimantadine against influenza virus A H2N2, whereas their spirocyclobutane and spirocyclopentane conjugates showed equal activity to the parent drug. According to the same study, 2-methyl-2-rimantadine exhibited a fourfold higher potency compared with rimantadine against the H3N2 strain, whereas its spirocyclopentane analogue was about 1.5 times more active [12]. In another research, Stamatiou et al. [13] demonstrated that one piperidine derivative of rimantadine was four times more powerful than the drug rimantadine. Zoidis et al. [14] also reported novel heterocyclic rimantadine analogues that enhanced activity against influenza A H2N2 and H3N2 strains. In our previous work, a rimantadine derivative, conjugated with the amino acid Gly, exhibited the highest antiviral activity against influenza virus A H3N2 while maintaining low cytotoxicity [15].

Given the diverse and promising biological activity of rimantadine and its derivatives, a series of six new amino acid rimantadine derivatives was synthesized. These compounds were designed to explore amino acid modification as a strategy for developing new prodrug molecules with enhanced antimicrobial activity and potential to overcome microbial resistance. Based on our previous experience, rimantadine was conjugated with spatially compact (Gly, Ala, β-Ala) and bulky (Leu, Ile and Val) amino acids, and their antimicrobial potential was assessed against model Gram-positive and Gram-negative strains.

Materials-Methods

2.1. Chemical synthesis

Essentially, the chemical synthesis of the target compounds is the formation of an amide bond. For this purpose, the methodology described by Knorr et al. was used [16]. Briefly, rimantadine (1 eq.) was dissolved in 5 mL of dichloromethane (DCM), and triethylamine (TEA, 1 eq.) was added to the formed solution. The carboxyl component, a Boc-protected amino acid (1.1 eq.), was dissolved in 10 mL DCM in a second flask. Further, TEA (1.1 eq.) and O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU, 1.5 eq.) were added in the second solution. Thirty minutes later, both solutions were mixed. The reaction mixture was stirred at room temperature under nitrogen for 24 h. Further, the reaction was quenched and was treated twice with 5% $NaHCO_3$ solution in order to remove non-reacted excess of amino acid. Then the solution was dried with anhydrous Na_2SO_4 and the solvent was evaporated under vacuum. The Boc-protecting group of crude obtained oil was removed by treatment with DCM/TFA 50:50 (v/v) for 3 h. Both reactions of synthesis and Boc-protection removal were monitored by TLC. The obtained final compound was purified by flash chromatography on a silica gel column with an elution system DCM/MeOH 97:3 or Hex/EtOAc 5:4 ratios, depending on the polarity of the obtained compounds. All tested final compounds are previously described, and they have more than 95% chromatographic purity [15].

2.2. Antimicrobial assays

2.2.1. Test microorganisms and culture conditions

Test microorganisms, *B. subtilis* NBIMCC 3562 and *E. coli* NBIMCC 8785, were provided by the National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC, Sofia, Bulgaria). Exponential-phase cultures of both strains, grown in Nutrient broth or Luria-Bertani broth (HiMedia, Mumbai, India), were incubated in a shaker incubator ES-20/60 (Biosan, Latvia) at 30/37°C for 24 h, respectively. The cultures were subsequently adjusted to 0.5 McFarland turbidity standard using a Grant Bio DEN-1B Densitometer (Grant Instruments, Royston, United Kingdom).

2.2.2. Minimum inhibitory and minimum bactericidal concentration determination

The standard broth microdilution method was used to determine the MIC of the new rimantadine derivatives against *B. subtilis* 3562 and *E. coli* 8785, in accordance with established guidelines of the Clinical and Laboratory Standards Institute M07 [17] (Figure 2). Rimantadine derivatives were dissolved in 10% EtOH/H_2O, and stock solutions (10 mg/mL) were prepared and stored at -20°C. 0.5 McFarland bacterial suspensions were inoculated into 96-well polystyrene microplates (Deltalab S.L., Barcelona, Spain), containing varying concentrations of rimantadine derivatives (0, 10, 20, 40, 80, 160 and 320 µg/mL). In addition, 10% EtOH/H_2O control, diluted in the nutrient medium, was added to separate wells. The microplates were incubated at 30°C (*B. subtilis*) or 37°C (*E. coli*) for 24 h. The MIC was the lowest concentration of each derivative, that showed no bacterial growth visually. Aliquots of 20 µL of all samples that showed no visible bacterial growth were then seeded on LB/NB agar media and cultured for 24 h. The lowest concentration of each derivative, which resulted in more than 99.9% reduction of the initial inoculum, was regarded as MBC. Experiments were repeated in triplicate.

2.3. Statistical analysis

All measurements were carried out in triplicate. The means were presented for the averages of experiments ± standard deviation (SD) of three independent measurements.

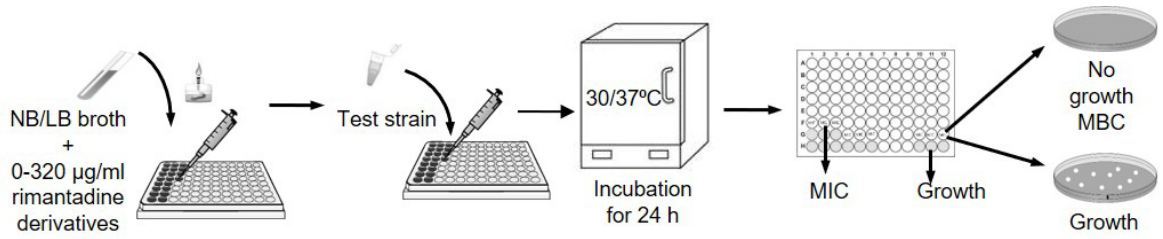

Figure 2. Scheme of work of the in vitro methods used for the determination of the MIC and MBC of the tested rimantadine derivatives against *E. coli* NBIMCC 8785 and *B. subtilis* NBIMCC 3562.

Results

The antimicrobial activity of the rimantadine derivatives was carried out by using the Gram-positive strain *B. subtilis* 3562 and the Gram-negative strain *E. coli* 8785. The susceptibility of the test microorganisms to the obtained derivatives was determined using the broth microdilution method, whereas their MBC was assessed via the spread plate technique. The experiments were performed in triplicate, and the obtained MIC and MBC values are summarised in Table 1.

The newly synthesized rimantadine derivatives showed relatively moderate antibacterial activity against *B. subtilis* NBIMCC 3562 and *E. coli* NBIMCC 8785, ranging from 160 to 320 µg/mL for MIC. The derivatives 5A, 6A and 11A, containing bulky amino acids Ile, Leu and Val, possessed the highest antibacterial activity against *B. subtilis* NBIMCC 3562 among the compounds tested (Table 1). The MIC and MBC values of derivatives 5A and 6A coincided. Only the derivative 11A showed a higher MBC (MBC = 320 µg/mL). Among the derivatives, containing amino acids with a short lateral chain, the derivative 1A showed weaker antibacterial activity (MIC = 320 µg/mL), whereas the derivative Gly-Rim did not show any effect against *B. subtilis* 3562. The derivative 12A, containing the unnatural amino acid β-Ala, showed no antibacterial activity against *B. subtilis* NBIMCC 3562.

All rimantadine analogues possessed antibacterial activity against *E. coli* NBIMCC 8785. Most of them were assigned a MIC of 320 µg/mL. The only exception was the derivative *L*-Ile-Rim, which showed a lower MIC (MIC = 160 µg/mL). MBCs against this strain were not reported, which indicates a very weak bacteriostatic effect of the studied derivatives.

Discussion

Some of the main features important for antimicrobial activity are the net charge, amphipathicity and hydrophobicity of the molecule, specific membrane properties of different types of microorganisms, self-organization potential, etc. Taking into account all observed structure-activity relationships, especially those that general interactions which are very important for antibacterial activity are hydrophobic and cationic interactions with the membrane surface of microorganisms [18-22], rimantadine was conjugated with spatially compact (Gly, Ala, β-Ala) and bulky (Leu, Ile and Val) amino acids that are able to interact with different forces to the microbial membrane. The amino acids used for conjugation to the rimantadine motif were selected based on our previous investigations with other adamantane derivatives [23-25].

The obtained data from the further antimicrobial activity assay revealed that most of the newly-synthesized rimantadine derivatives were more active against *B. subtilis* NBIMCC 3562 than *E. coli* NBIMCC 8785. The difference in microbial susceptibility is probably due to variations in the cell wall structure of the two test microorganisms. *B. subtilis* NBIMCC 3562, a Gram-positive bacterium, has a thick peptidoglycan layer but lacks the outer membrane, which is characteristic of

Table 1. MIC and MBC values of rimantadine derivatives against *E. coli* NBIMCC 8785 and *B. subtilis* NBIMCC 3562.

Rimantadine derivatives		*B. subtilis* NBIMCC 3562		*E. coli* NBIMCC 8785	
Code	**Corresponding compound**	**MIC, [μg/mL]**	**MBC, [μg/mL]**	**MIC, [μg/mL]**	**MBC, [μg/mL]**
1A	*L*-Ala-Rim	320 ± 0.38	NI	320 ± 0.35	NI
4A	Gly-Rim	NI*	NI	320 ± 0.29	NI
5A	***L*-Ile-Rim**	**160 ± 0.34**	**160 ± 0.38**	**160 ± 0.36**	**NI**
6A	*L*-Leu-Rim	160 ± 0.28	160 ± 0.32	320 ± 0.30	NI
11A	*L*-Val-Rim	160 ± 0.31	320 ± 0.32	320 ± 0.31	NI
12A	β-Ala-Rim	NI	NI	320 ± 0.28	NI

* NI – no inhibition monitored; Data are expressed as means ± SD (n=3).

the Gram-negative *E. coli* NBIMCC 8785. The outer membrane acts as an additional selective permeability barrier, protecting *E. coli* from the action of various antimicrobial agents [26].

Considering the MIC data, rimantadine analogues 5A, 6A and 11A, containing bulky amino acids, showed the highest antibacterial activity against *B. subtilis* NBIMCC 3562 (MIC=160 μg/mL). Their enhanced potency suggests that the presence of hydrophobic and sterically bulky side chains may facilitate penetration into the cell and interfere with essential intracellular processes. After the primary electrostatic attraction between positively charged peptides and negatively charged phosphate groups on the membrane surface, bulky hydrophobic side chains probably promote deeper insertion into the lipid bilayer. Their size and hydrophobicity may allow partitioning into the membrane core, disrupting lipid packing [27] and enabling the formation of transient pores or translocation across the membrane without complete lysis. This may allow the peptides to reach intracellular targets such as DNA, RNA, or essential proteins and inhibit processes like replication, transcription and translation [28].

The coinciding MIC and MBC values of analogues 5A and 6A indicate that they are not only effective in inhibiting bacterial growth but also possess strong bactericidal properties at the same concentration. This dual action highlights their potential as candidates for further development. In contrast, despite its notable antibacterial activity, analogue 11A required a higher concentration to achieve a bactericidal effect (MBC = 320 μg/mL). By comparison, analogues incorporating short-chain amino acids in their molecules exhibited weaker or no antibacterial activity, suggesting that shorter side chains lack the hydrophobic interactions necessary to support effective antibacterial activity. The lack of activity of analogue 12A, containing unnatural amino acid β-Ala, is consistent with our previous results, where the β-Ala-substituted analogue of memantine also demonstrated a lack of activity against *B. subtilis* 3562 [3].

All tested rimantadine analogues exhibited antibacterial activity against *E. coli* NBIMCC 8785, although their overall potency was low (MIC = 320 μg/mL). The exception was analogue 5A, with a MIC of 160 μg/mL, suggesting that the bulky, hydrophobic isoleucine side chain may enhance interaction with the Gram-negative bacterial cell and improve membrane penetration. The absence of reported MBC values for any of the derivatives suggests that they exert only weak bacteriostatic effects against *E. coli* NBIMCC 8785, in contrast to their stronger activity against *B. subtilis* NBIMCC 3562.

The results obtained provide a good preliminary evaluation for the antimicrobial potential of the newly synthesized rimantadine derivatives. However, further studies involving a broader range of clinically relevant and multidrug-resistant strains are necessary to better assess their therapeutic applicability.

Conclusions

The results revealed that all derivatives except Gly-Rim and β-Ala-Rim showed antimicrobial activity against both model strains. They possessed greater antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. Among the compounds tested, *L*-Ile-Rim demonstrated the highest antimicrobial activity against both strains, highlighting the importance of bulky amino acid residues in enhancing efficacy. Future analyses will focus on optimizing the antimicrobial activity of rimantadine derivatives against Gram-positive bacteria through the evaluation of other bulky amino acids. The most promising derivatives will be further tested against multidrug-resistant strains to identify potential candidates for overcoming antibiotic resistance.

Acknowledgements

This research was funded by the contract №: BG-RRP-2.004-

0002-C01, "BiOrgaMCT" (Bioactive organic and inorganic materials and clean technologies) By procedure: BG-RRP-2.004—Creation of a network of research universities in Bulgaria under the National Recovery and Resilience Plan.

Ethical Approval
Not applicable.

Conflicts of Interest
The authors declare no conflicts of interest.

Data Availability
The data presented in this study are available from the authors.

References

1. European Commission. Opinion of the scientific panel on additives and products or substances used in animal feed on the updating of the criteria used in the assessment of bacteria for resistance to antibiotics of human or veterinary importance. The EFSA J. 2005; 223: 1-12.
2. Oliveira M, Antunes W, Mota S, Madureira-Carvalho A, Dinis-Oliveira RJ, da Silva DD. An overview of the recent advances in antimicrobial resistance, Microorganisms 2024; 12: 1920.
3. Tencheva A, Stankova I, Angelova Ts, Nemska V, Georgieva N, Danalev D. Antimicrobial activity of amino acid derivatives of memantine. J Chem Technol Metall. 2022; 57: 286-290.
4. Mondal D. Rimantadine, Reference Module in Biomedical Sciences, Elsevier 2016, doi: 10.1016/B978-0-12-801238-3.99410-8.
5. Deyde VM, Xu X, Bright RA, Shaw M, Smith C.B, Zhang Y, Shu Y, Gubareva LV, Cox NJ, Klimov AI. Surveillance of Resistance to Adamantanes among Influenza A(H3N2) and A(H1N1) Viruses Isolated Worldwide. J Infect Dis. 2007; 196: 249–257.
6. Paintsil E, Cheng Y-C. Antiviral Agents, In Encyclopedia of Microbiology, 3rd ed.; Schaechter, M., Eds.; Academic Press, 2009; 223-257.
7. Vorobjev YN. An effective molecular blocker of ion channel of M2 protein as anti-influenza A drug, J. Biomol Struct Dyn. 2021; 39: 2352-2363.
8. Zlydnikov DM, Kubar OI, Kovaleva TP, Kamforin LE. Study of Rimantadine in the USSR: a review of the literature. Rev Infect Dis. 1981; 3: 408-421.
9. Miyauchi S, Sanders PD, Guram K, Kim SS, Paolini F, Venuti A, Cohen EEW, Gutkind JS, Califano JA, Sharabi AB. HPV16 E5 Mediates Resistance to PD-L1 Blockade and Can Be Targeted with Rimantadine in Head and Neck Cancer. Cancer Res. 2020; 15: 732-746.
10. Kelly JM, Miles MA, Skinner AC. The Anti-Influenza Virus Drug Rimantadine Has Trypanocidal Activity. Antimicrob. Agents Chemother. 1999; 43: 985-987.
11. CDC, 2023, Influenza antiviral medications: summary for clinicians. Available online: https://www.cdc.gov/flu/professionals/antivirals/summary-clinicians.html (accessed on 25 February 2026).
12. Zoidis G, Kolocouris N, Foscolos GB, Kolocouris A, Fytas G, Karayannis P, Padalko E, Neyts J, de Clercq E. Are the 2-Isomers of the Drug Rimantadine Active Anti-Influenza a Agents? Antivir Chem Chemother. 2003; 14: 153-164.
13. Stamatiou G, Foscolos G.B, Fytas G, Kolocouris A, Kolocouris N, Pannecouque C, Witvrouw M, Padalko E, Neyts J, De Clercq E. Heterocyclic rimantadine analogues with antiviral activity. Bioorg. Med Chem. 2003; 11: 5485–5492.
14. Zoidis G, Fytas C, Papanastasiou I, Foscolos GB, Fytas G, Padalko E, De Clercq E, Naesens L, Neyts J, Kolocouris N. Heterocyclic rimantadine analogues with antiviral activity. Bioorg. Med Chem. 2006; 14(10): 3341-3348.
15. Chayrov R, Parisis NA, Chatziathanasiadou MV, Vrontaki E, Moschovou K, Melagraki G, Sbirkova-Dimitrova H, Shivachev B, Schmidtke M, Mitrev Y, Sticha M, Mavromoustakos T, Tzakos AG, Stankova I. Synthetic Analogues of Aminoadamantane as Influenza Viral Inhibitors – In Vitro, In Silico and QSAR Studies. Molecules. 2020; 25: 3989.
16. Knorr R, Trzeciak A, Bannwarth W, Gillessen D. New coupling reagents in peptide chemistry. Tetrahedron Lett. 1989; 30: 1927–1930.
17. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 11th ed. CLSI standard M07. Wayne, PA: Clinical and Laboratory Standards Institute, 2018.
18. 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. Am J Transl Res. 2019; 11(7): 3919-3931.
19. Pushpanathan M, Gunasekaran P, Rajendhran J. Antimicrobial Peptides: Versatile Biological Properties. Int J Pept. 2013; 1-15.
20. Huan Y, Kong Q, Mou H, Yi H. Antimicrobial Peptides: Classification, Design, Application and Research Progress in Multiple Fields. Front Microbiol. 2020; 11: 582779.
21. Xuan J, Feng W, Wang J, Wang R, Zhang B, Bo L, Chen Z-S, Yang H, Sun L. Antimicrobial peptides for combating drug-resistant bacterial infections. Drug Resist Updat. 2023; 68: 100954.
22. Büyükkiraz ME, Kesmen Z., Antimicrobial peptides (AMPs): A promising class of antimicrobial compounds, J Appl Microbiol, 2022; 132(3): 1573-1596.
23. Stankova I, Chuchkov K, Chayrov R, Mukova L, Galabov A, Marinkova D, Danalev D. Adamantane derivatives containing thiazole moiety – synthesis, antiviral and antibacterial activity. Int J Pept Res Ther. 2020; 26(4): 1781-1787.

24. Stankova IG, Chayrov RL, Schmidtke M, Danalev DL, Ognichenko LN, Artemenko AG, Shapkin VA, Kuz'min VE. Quantitative structure-activity relationship modelling of influenza M2 ion channels inhibitors. J Serb Chem Soc. 2021; 86 (7-8): 625-637.
25. Tencheva A, Stankova I, Angelova T, Nemska V, Georgieva N, Danalev D. Antimicrobial activity of amino acid derivatives of memantine. J Chem Technol Metall. 2022; 57(2): 286-290.
26. Rohde M. The Gram-positive bacterial cell wall. Microbiol Spectr. 2019, 7: 10.
27. Nunes, LO. Predicting peptide orientation in membrane interations: a review on transmembrane and surface-bound states. Eur Biophys J. 2026; 55: 9-20.
28. Le C-F, Fang C-M, Sekaran SD. Intracellular targeting mechanisms by antimicrobial peptides, Antimicrob Agents Chemother. 2017; 61(4): e02340-16.