Antimicrobial Potential and Mechanistic Insights of Clioquinol Derivatives Against Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT

Introduction: The development of penicillin-resistant Staphylococcus aureus within a few years of the introduction of penicillin marked the start of a long-term global problem, and over the past several decades, the overuse and misuse of antibiotics has led to the emergence of multidrug-resistant (MDR) and methicillin-resistant strains of S. aureus that are responsible for considerable burdens on healthcare systems worldwide and are associated with both hospital- and community-acquired infections, thus driving the need for new antimicrobial agents as resistance to existing antibiotics continues to decline.

Objectives: This study aimed to identify and characterize new inhibitory compounds with activity against MDR *S. aureus* strains, focusing on the potential of Clioquinol derivatives as alternative therapeutic candidates.

Methodology: 10 clioquinol-based compounds were screened for minimum inhibitory concentrations against MDR S. aureus strains using the microplate alamar blue assay, and the most active compounds were further subjected to a comprehensive mechanistic analysis using atomic force microscopy (AFM), scanning electron microscopy (SEM), and fluorescence microscopy; ex vivo hemolysis tests determined compatibility with human red blood cells, and membrane-potential tests evaluated effects on bacterial membrane function; and the MTT assay was used to measure cytotoxicity to determine the safety profile of the active compounds.

Key Findings: Six of the ten tested compounds demonstrated MIC values in the range of 30 to 100 µg/mL, indicating antibacterial activity, with both compounds 1 and 7 exhibiting strong and broad inhibitory effects on all tested MDR S. aureus strains. Microscopy revealed significant alterations in cell morphology, elasticity, and surface integrity after treatment. Membrane-potential evaluations indicate that these substances affect membrane stability. Importantly, none of the active ingredients caused hemolysis or cytotoxicity, suggesting that they may be candidates for further preclinical testing.

Conclusion: Clioquinol derivatives, particularly compounds 1 and 7, show strong promise as candidates for developing new antimicrobial agents targeting MDR *S. aureus*. Their combined efficacy and favorable safety profile highlight their potential for advancement into in vivo studies. Continued investigation is essential to further understand their pharmacodynamics and therapeutic applicability.

Keywords: Multidrug-Resistant *Staphylococcus aureus*, Clioquinol Derivatives, Minimum Inhibitory Concentration, AFM, SEM.

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