**Design, synthesis, in vitro and in vivo evaluation of unexplored trisindolines as potent anti-MRSA agents targeting cell membrane**

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**Abstract:** In our quest toward antimicrobial drug discovery to combat WHO-designated global priority pathogens, we designed, synthesized and comprehensively evaluated a series of trisindoline derivatives against Methicillin-resistant *Staphylococcus aureus* (MRSA). Trisindolines are a class of organic compounds composed of a central isatin core with two attached indole moieties, exhibiting a broad range of biological activities, including antimicrobial, antitubercular, antifungal, anticancer and antioxidant properties, making them potential candidates for drug development across various therapeutic areas. Their functions in diverse biological domains mainly depend on their specific chemical structure. However, their potential as antibacterial agents has remained largely unexplored, with existing studies limited to synthesis and preliminary activity screening.

Here, we present a comprehensive approach to trisindoline development, encompassing innovative chemical design, robust in vitro and in vivo evaluations, and mechanistic insights. The synthesized derivatives were first structurally confirmed using $^{1}$H NMR, $^{13}$C NMR and HRMS spectral analysis. Among the synthesized derivatives, eight compounds demonstrated potent inhibitory activity against MRSA, including their multidrug-resistant (MDR) clinical isolates (MIC ranging from 0.7-5µg/ml). In vitro cytotoxicity evaluations revealed a high safety profile for these compounds across various mammalian cell lines (SI index ≥ 10 fold of the MIC values). Notably, the derivatives displayed rapid bactericidal action and exceptional inhibitory efficacy against MRSA biofilm. Antimicrobial synergy assays with vancomycin highlighted several synergistic interactions, underscoring their potential role in combination therapy to counteract MRSA resistance. Additionally, these molecules showed promising intracellular MRSA-killing capability inside amalian cell lines. Further, mechanistic studies revealed that these compounds disrupt MRSA cell membrane integrity, contributing to their strong antibacterial effects. Finally, in vivo assessments of a lead compound demonstrated significant bacterial load reduction in a systemic MRSA infection model.

This work underscores the potential of trisindoline derivatives as a new class of antibacterial agents, addressing critical gaps in antimicrobial drug discovery. By integrating innovative chemical design with comprehensive biological evaluations, our findings provide a strong foundation for the development of effective therapies against MRSA, contributing to the global effort to combat antimicrobial resistance.

Keywords: Antimicrobial resistance (AMR)/ MRSA/ Trisindolines / In vitro and In vivo studies /Antimicrobial synergy/ Mechanism of Action/SAR

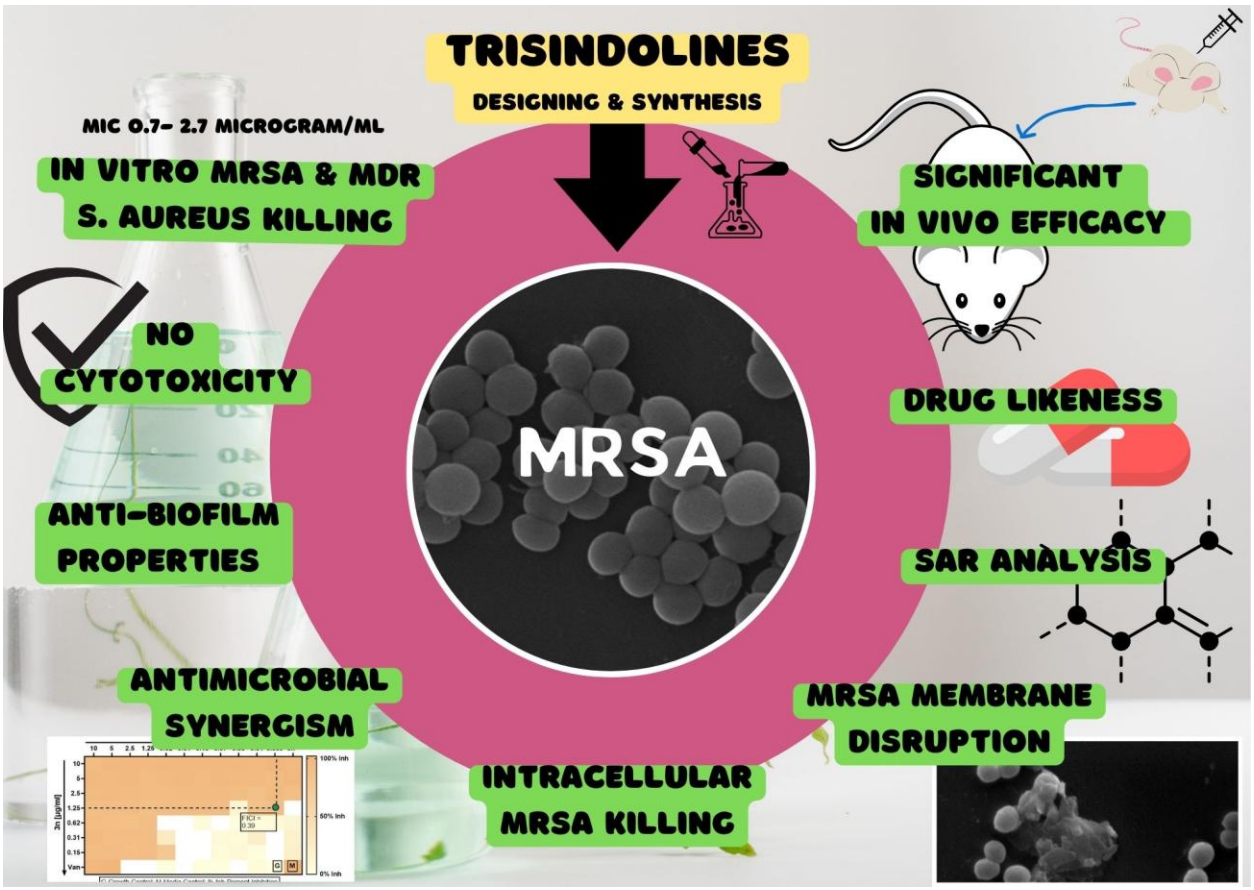


**Fig_1. Design, synthesis and anti-MRSA efficacy of trisindolines**