INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	**BASU BHATTACHARJEE**
Roll Number	:	166106018
Programme of Study	:	Ph.D.

Thesis Title: Studies on the Adjuvant Potential of Synthetic Ligands for Targeting MRSA in Combination Therapy

Name of Thesis Supervisor(~~s~~)	:	Prof. Aiyagari Ramesh
Thesis Submitted to the Department/ ~~Center~~	:	Biosciences and Bioengineering
Date of completion of Thesis Viva-Voce Exam	:	22 November 2022
Key words for description of Thesis Work	:	MRSA, Antimicrobial, Adjuvant, Combination Therapy, Adhesion, Bone Cell Infection,

SHORT ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major healthcare concern as the pathogen is not only associated with hospital-acquired infections but also holds implications in community-acquired infections. Mitigation of MRSA infection is an arduous task as the number of therapeutic antibiotics effective against clinical strains of MRSA are limited. This crisis underpins a critical need to develop antagonistic agents that can counter the resistance mechanism and resensitize the pathogen against therapeutic antibiotics. To address this pertinent healthcare issue, the current work highlights the adjuvant potential of rationally designed synthetic efflux pump inhibitor (EPI) and membrane-targeting antibacterials to counter the core resistance mechanism in MRSA and restore susceptibility of the pathogen to low doses of a therapeutic antibiotic. As a first objective, the potential of urea-based synthetic ligands (C1-C8) as an EPI was studied. Amongst the ligands, C8 could significantly inhibit efflux pump activity, downregulate expression of *norA* gene, reduce the minimum inhibitory concentration (MIC) of ciprofloxacin (CPX) by 16-fold and prevent emergence of CPX resistance in a clinical strain of MRSA till 120 generations. The therapeutic potency of C8 was leveraged by generating a C8-loaded PLGA nanocarrier (C8-PNC), which displayed EPI activity and could potentiate the efficacy of CPX against MRSA. Further, the payload nanocarrier (C8-PNC) was non-toxic to HEK-293 cells and could effectively hinder adhesion of MRSA cells onto collagen in combination with CPX. It is acknowledged that the bacterial cell membrane is a formidable permeability barrier for antibiotics. Hence, it was conceived that antibacterials that can breach the membrane hold significant prospect against MRSA. Amongst a set of quinoxaline-based synthetic ligands (C1-C4), the ligand C2 could remarkably impede MRSA cell growth, with an MIC of 32 µM, render dose-dependent membrane-directed activity and inhibit MRSA biofilm formation. A quantitative real-time PCR analysis indicated that C2 could influence the expression of the regulator element *agrC* and the adhesin genes *fnbA* and *cnbA*, which are implicated in MRSA biofilm formation. The membrane-targeting C2 could also be effectively leveraged as an adjuvant molecule to heighten the potency of CPX, deter the emergence of CPX resistance trait in MRSA cells over 360 generations and effectively counter MRSA invasion in an *in vitro* bone cell infection model. Interestingly, the adjuvant potential of C2 was also leveraged by developing a biocompatible C2-loaded HSA nanocarrier (C2-HNC), which in combination with CPX could thwart the invasion of MRSA onto titanium wire, which was used in *in vitro* experiments as a model orthopaedic implant. The payload nanocarrier C2-HNC was also non-toxic to cultured bone cells. The rational design of the adjuvants described in the Ph.D. thesis work is an illustration of judicious medicinal chemistry to address a pertinent global healthcare problem. It is envisaged that the nanomaterials developed in the current study can serve as prototypes of therapeutic adjuvants to alleviate MRSA infections in soft tissue and bone implants.