INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Bactericidal Potential of a Dual-Target Synthetic Amphiphile

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Thesis Submitted to the Department/ ~~Center~~	:	Biosciences and Bioengineering
Date of completion of Thesis Viva-Voce Exam	:	30 August 2017
Key words for description of Thesis Work	:	Amphiphile, Antibacterial, Biofilm, Nanomaterial, MRSA, Efflux

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The growing menace of drug-resistant pathogenic bacteria underscores the need to develop efficient bactericidal agents that can act on irrefutable targets and defy the resistance mechanism. In this context, the present investigation reports the bactericidal activity and therapeutic potential of a rationally designed dual-target pyridinium-based synthetic amphiphile. The amphiphile referred to as C1 consisted of (i) a cationic pyridinium head group to initiate electrostatic interactions with anionic bacterial cells and DNA, (ii) a hydrophobic tail (12 carbon chain length) for membrane insertion and (iii) a fluorogenic pyrene group to facilitate intercalation with DNA and spectroscopic probing of interactions. Antibacterial screening experiments indicated that C1 exhibited broad-spectrum bactericidal activity, while the presence of a fluorogenic pyrene in C1 enabled probing of amphiphile-bacteria interactions and membrane-insertion. Florescence-based assays in conjunction with spectroscopic and molecular techniques demonstrated the membrane-directed bactericidal activity, intracellular transit, cellular DNA binding and intracellular plasmid DNA cleavage activity of C1. The bactericidal activity of C1 was retained in simulated gastric fluid (SGF), simulated intestinal fluid (SIF) and simulated body fluid (SBF). In a combinatorial regime, C1 rendered a substantial reduction of the minimal inhibitory concentration (MIC) of therapeutic antibiotics gentamicin and erythromycin against the target bacteria. An albumin-based nanocarrier loaded with C1 (C1-HNC) rendered facile release of the payload in *S. aureus* MTCC 96 biofilm matrix, which could subsequently cleave the extracellular DNA (eDNA) barrier and target the underlying cells resulting in dramatic annihilation of biofilm. Interestingly, C1-HNC could eradicate *S. aureus* biofilm from the surface of a model catheter and was non-toxic to HEK 293 cells in an *in vitro* assay. A C1-loaded Poly (lactic-co-glycolic acid) (PLGA) nanocarrier (C1-PNC) was developed, which could render significant reduction of the minimum biofilm eradication concentration ($MBEC_{90}$) of gentamicin and ciprofloxacin against a clinical strain of methicillin-resistant *Staphylococcus aureus* (MRSA). Mechanistic studies revealed that C1-PNC could enhance cellular uptake of gentamicin, while the propensity of C1-PNC to inhibit efflux pump activity resulted in higher cellular accumulation of ciprofloxacin and effective killing of MRSA cells. Interestingly, the combinatorial dosing regimen of C1-PNC and the antibiotics was non-toxic to cultured HEK 293 cells. The dual-target synthetic amphiphile described in the present investigation may serve as a prototype to the research community and provide a framework for development of synthetic antibacterials that can disable the resistance mechanism and hold therapeutic potential against antibiotic-resistant pathogenic bacteria.