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Short cationic lipopeptides as effective antibacterial agents: Design, physicochemical properties and biological evaluation

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ABSTRACT

The spread of drug-resistant bacteria has imparted a sense of urgency in the search for new antibiotics. In an effort to develop a new generation of antibacterial agents, we have designed *de novo* charged lipopeptides inspired by natural antimicrobial peptides. These short lipopeptides are composed of cationic lysine and hydrophobic lipoamino acids that replicate the amphiphilic properties of natural antimicrobial peptides. The resultant lipopeptides were found to self-assemble into nanoparticles. Some were effective against a variety of Gram-positive bacteria, including strains resistant to methicillin, daptomycin and/or vancomycin. The lipopeptides were not toxic to human kidney and liver cell lines and were highly resistant to tryptic degradation. Transmission electron microscopy analysis of bacteria cells treated with lipopeptide showed membrane-damage and lysis with extrusion of cytosolic contents. With such properties in mind, these lipopeptides have the potential to be developed as new antibacterial agents against drug-resistant Gram-positive bacteria.

1. Introduction

The rapid emergence of bacterial isolates resistant to most available antibiotics is of grave concern. Incidents associated with infection caused by drug-resistant Gram-positive bacteria, particularly MRSA (Methicillin resistant *Staphylococcus aureus*) and VRE (Vancomycin resistant Enterococcus), are on the rise in the community and in clinical settings.[1] Additionally, certain strains of Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are now resistant to most antibiotics and have caused an escalation in morbidity rates due to untreatable bacterial infection.[2] The declining rate of development of new antibiotics has further aggravated the ability to combat the escalation of superbugs. Antimicrobial peptides (AMPs), naturally occurring molecules in living organisms, are attractive candidates for the generation of new antibiotics due to their lethal and broad spectrum capabilities and their low propensity for resistance development.[3,4] Presently, more than 500 AMPs have been discovered and isolated from various life forms including human, plants and even bacteria.[5] The majority of naturally occurring AMPs possess 12–50 amino acids, comprising at least two positively charged amino acids and a certain proportion of hydrophobic residues. These peptides possess amphiphilic properties due to their nature of encompassing hydrophilic and hydrophobic moieties. AMPs are primarily known to exhibit their antibacterial activity by disrupting the bacterial cell membrane,[3] although variant modes of action such as interaction with intracellular targets[6] and immune modulation[7] have also been reported. The amphiphilic feature of AMPs is important for their interaction with the negatively charged surface of the bacterial cell membrane, as it enables permeation into the membrane's interior as part of its antibacterial activity.[8] However, drawbacks such as toxicity, lability against enzymatic degradation and high cost production have limited the clinical development of AMPs, especially for systemic application. Although overall resistance against AMPs is rare, some pathogens have been recognised to reduce AMPs' activity via, for example, bacterial surface modification or enzyme secretion.[9,10] One viable strategy to overcome these shortcomings is to develop *de novo* designed synthetic mimics of AMPs that replicate their essential biophysical characteristics; that is, are positively charged, possess hydrophobicity and have the ability to self-assemble.[11,12]

Recently, we have designed AMP peptidomimetics comprising an ultrashort peptide sequence of lysine and lipoamino acids

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(LAAs), referred to as 'lipopeptides'. Instead of using proteinogenic hydrophobic amino acids, we utilised unnatural amino acids, LAAs C_{12} (lipoamino acids with 12 carbon atoms) to modulate the lipophilicity of the molecules. The unique feature of LAAs is that they combine the structural property of lipids (aliphatic chain) and amino acids, thus allowing them to be easily incorporated into a peptide sequence, either as single or multiple copies. Additionally, the alkyl chain of LAAs can be modified to various lengths which confer different degrees of hydrophobicity. Previously, the lipopeptides were constructed in both cyclic and linear configurations,

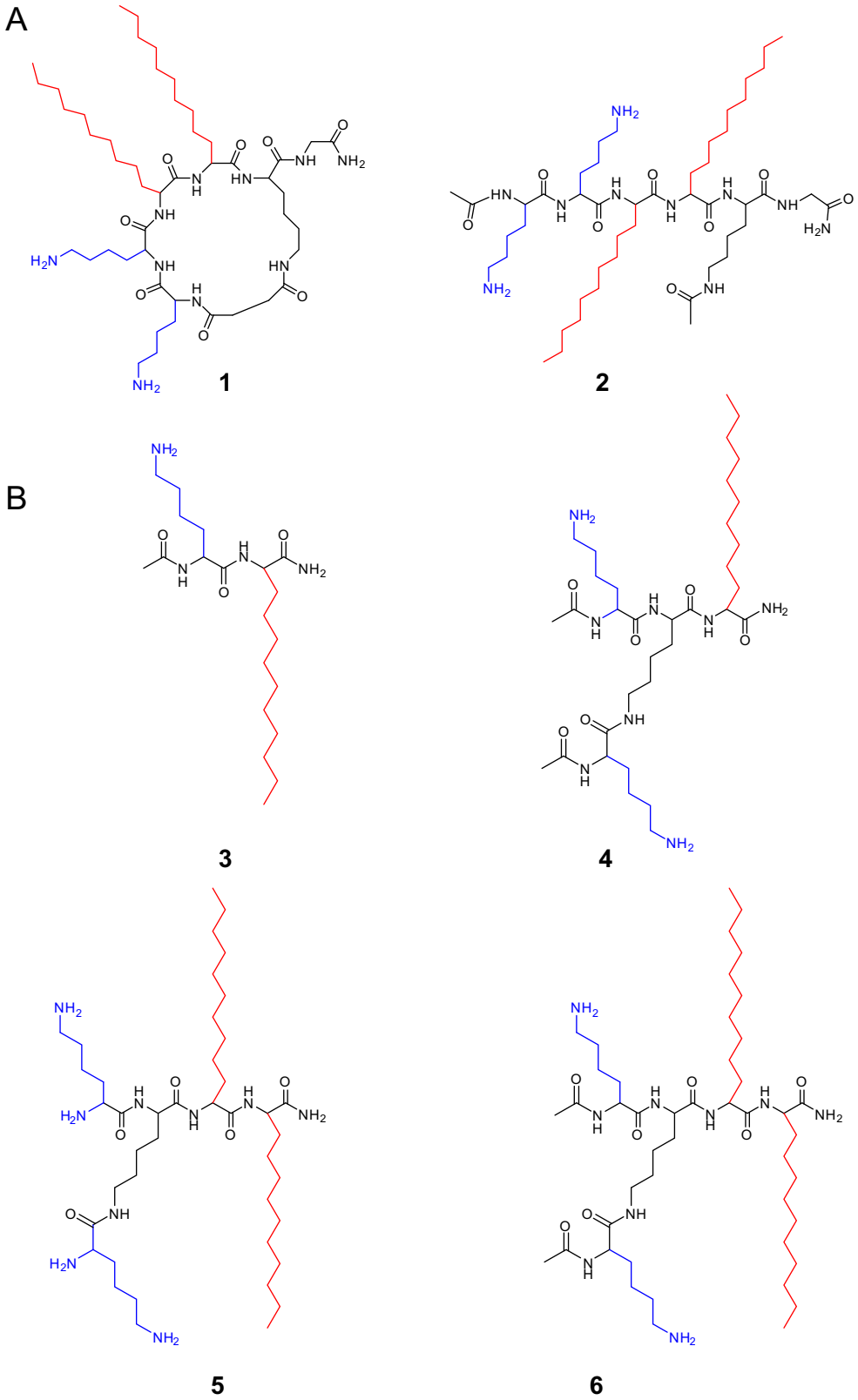

Figure 1. (A) Structures of the lead antibacterial compounds reported previously[14] and (B) structures of their simplified and branched analogues (**3**, **4**, **5** and **6**).