Design and Evaluation of Cyclic and Linear Amphiphilic Peptides Against Multidrug Resistant Bacterial Pathogens

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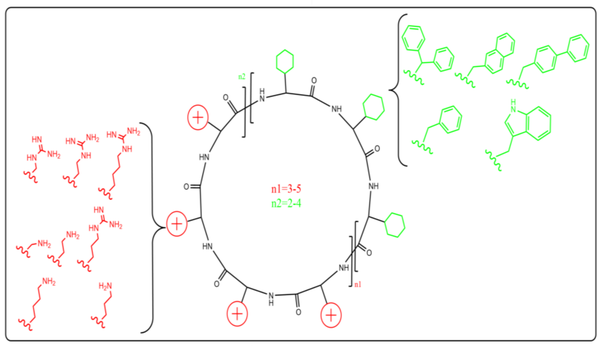
ABSTRACT

We have designed and synthesized a series of small amphiphilic peptides by incorporating various non-genetically coded hydrophobic amino acids, followed by positively-charges amino acids on the opposite side. To identify the optimum balance of positive charge and hydrophobicity, the number and position of both positive charge and hydrophobic residues were modified. Antibacterial screening results revealed the broad-spectrum activity of lead peptides with predominant activity against most of the Gram-positive bacteria, including the drug-resistant strains like methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococci* (VRE) with MIC values in the range of 1.5-3.1 µg/mL. Moderate activity was observed against Gram-negative bacteria with MIC values of 12.5 to 50 µg/mL. Negligible changes in MICs of lead peptides was observed against *S. aureus* and *E. coli* in the presence of serum and other physiologically relevant cationic salts (NaCl, KCl, NH_4Cl, $MgCl_2$, or $CaCl_2$), reflecting their therapeutic compatibility in the intended biological environment. We evaluated the toxicity of the compounds on human red blood cells (hRBCs), and the lead peptides were found to be significantly less hemolytic (HC_{50}>200 µg/mL) when compared with other known antibacterial peptides. These results highlight the therapeutic potential of newly designed amphiphilic peptides as the next generation of peptide-based antibiotics.

BACKGROUND

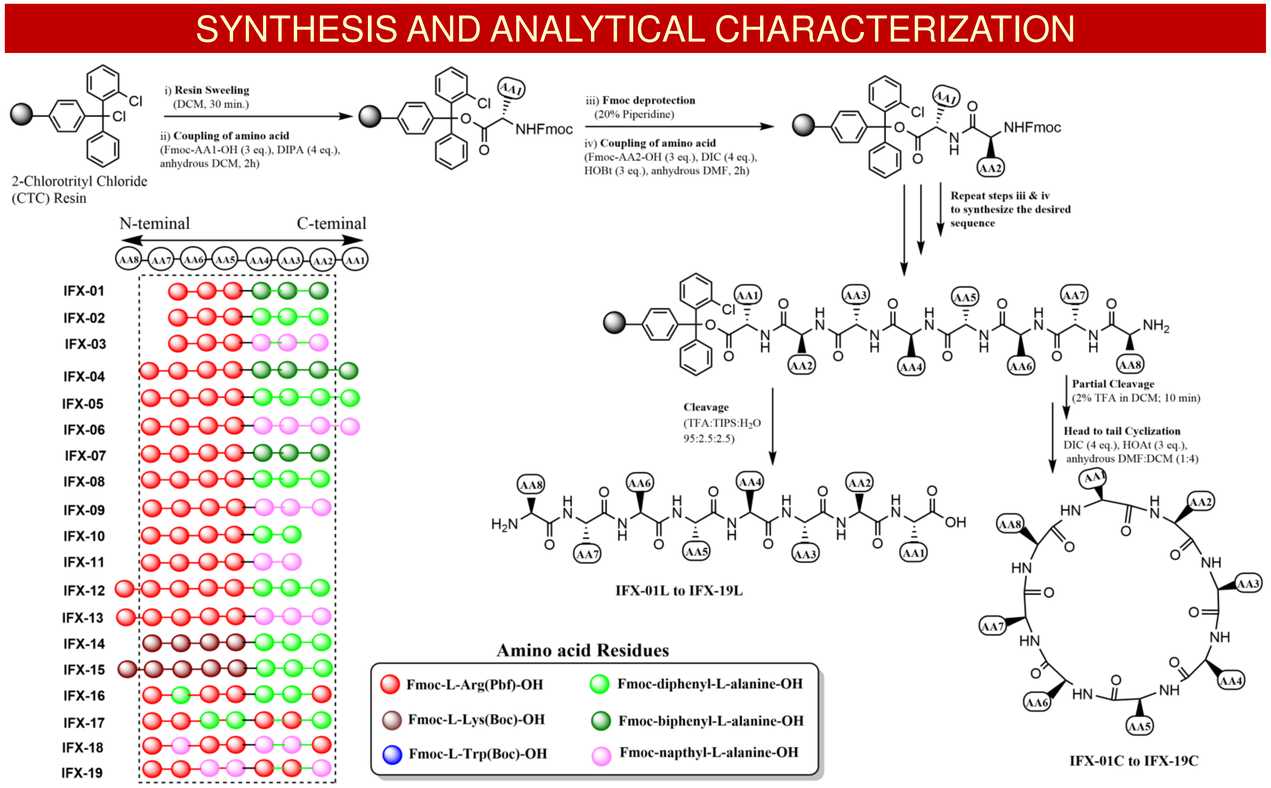
Antimicrobial peptides (AMPs), a vital component of innate immunity, are widespread in nature and have been isolated essentially from all multi-cellular organisms ranging from insects to humans. All forms of life use these native peptide templates as weapons to ward off pathogenic microbes in order to survive and thrive on this planet [1,2]. Structurally, native AMPs are fairly large molecules ranging from 12 to 50 amino acid residues and adopt amphipathic conformation in close proximity of biological membranes [3]. Previously, we have reported octameric cyclic antimicrobial peptide [W_4R_4] composed of four amino acid residues of arginine (R) and tryptophan (W) showed broad-spectrum of activity against drug resistant pathogens including MRSA [4].

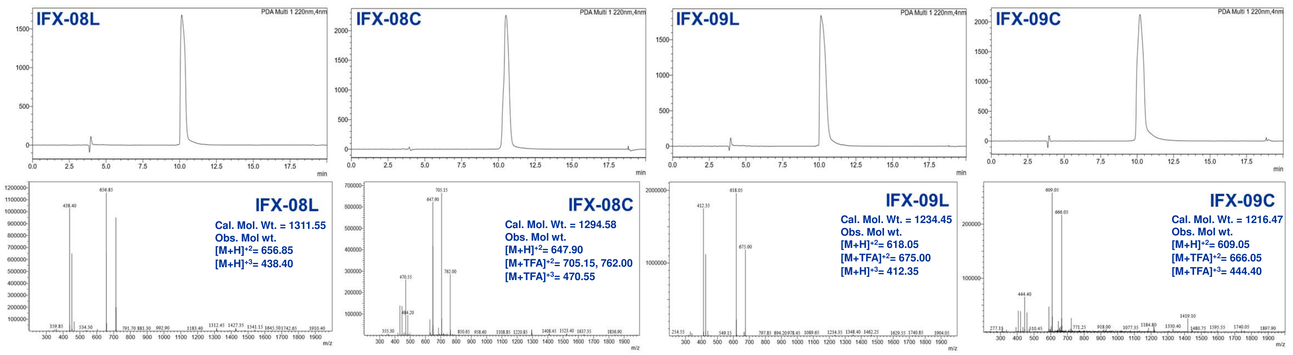
HYPOTHESIS



Pharmacophore Model

SYNTHESIS AND ANALYTICAL CHARACTERIZATION



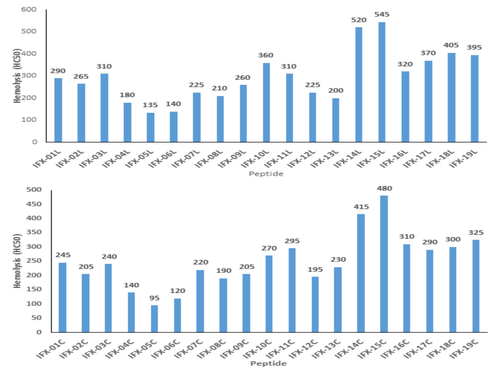


RESULTS: Antibacterial Activity

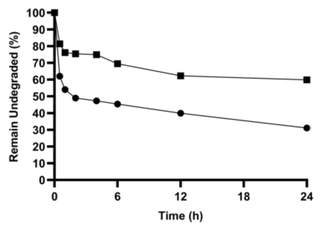
Peptide Code	Minimum Inhibitory Concentration (µg/mL)			
	S. aureus ATCC 33592	*S. aureus* ATCC 29213	*P. aeruginosa* ATCC BAA-1744	*E. Coli* ATCC BAA-2471
IFX-01L	50	50	>100	>100
IFX-02L	3.1	3.1	25	6.2
IFX-03L	3.1	3.1	25	16
IFX-04L	>100	>100	>100	>100
IFX-05L	6.2	6.2	50	50
IFX-06L	6.2	3.1	>100	50
IFX-07L	6.2	6.2	25	>100
IFX-08L	3.1	3.1	6.2	6.2
IFX-09L	3.1	3.1	12.5	12.5
IFX-10L	12.5	12.5	50	50
IFX-11L	6.2	6.2	>100	50
IFX-12L	3.1	3.1	50	25
IFX-13L	3.1	3.1	25	25
IFX-14L	25	25	>100	>100
IFX-15L	50	50	>100	>100
IFX-16L	25	12.5	>100	25
IFX-17L	12.5	12.5	25	25
IFX-18L	25	12.5	50	25
IFX-19L	25	12.5	25	25
Daptomycin	0.7	0.7	>50	>50

Peptide Code	Minimum Inhibitory Concentration (µg/mL)			
	S. aureus ATCC 33592	*S. aureus* ATCC 29213	*P. aeruginosa* ATCC BAA-1744	*E. Coli* ATCC BAA-2471
IFX-01C	50	50	>100	>100
IFX-02C	3.1	3.1	12.5	12.5
IFX-03C	6.2	3.1	50	25
IFX-04C	>100	50	>100	>100
IFX-05C	6.2	3.1	25	25
IFX-06C	6.2	3.1	50	25
IFX-07C	6.2	3.1	25	>100
IFX-08C	1.5	1.5	12.5	12.5
IFX-09C	1.5	1.5	12.5	25
IFX-10C	6.2	6.2	25	50
IFX-11C	6.2	6.2	50	50
IFX-12C	6.2	6.2	25	25
IFX-13C	6.2	6.2	25	50
IFX-14C	12.5	12.5	>100	>100
IFX-15C	12.5	6.2	>100	50
IFX-16C	6.2	6.2	50	>100
IFX-17C	12.5	6.2	>100	>100
IFX-18C	6.2	6.2	>100	25
IFX-19C	6.2	6.2	50	50
Ciprofloxacin	1.5	1.5	0.7	0.3

Hemolytic assay



Human Plasma Stability



CONCULSION

- A closer examination of activity results revealed the predominantly anti-staphylococcal action of newly designed peptides
- The antibacterial activity results clearly indicate the effectiveness of unnatural amino acids Dip and Nal for the designing of antimicrobial peptides as compared to Bip, which has similar hydrophobicity bulk with two aromatic rings.
- Cyclic peptide was found to be more stable in human plasma as compared to its linear counterpart.

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