**Modification of a Nature Derived Antimicrobial Peptide into Superior Analogues with Improved Antimicrobial Activity and Protease Resistance**

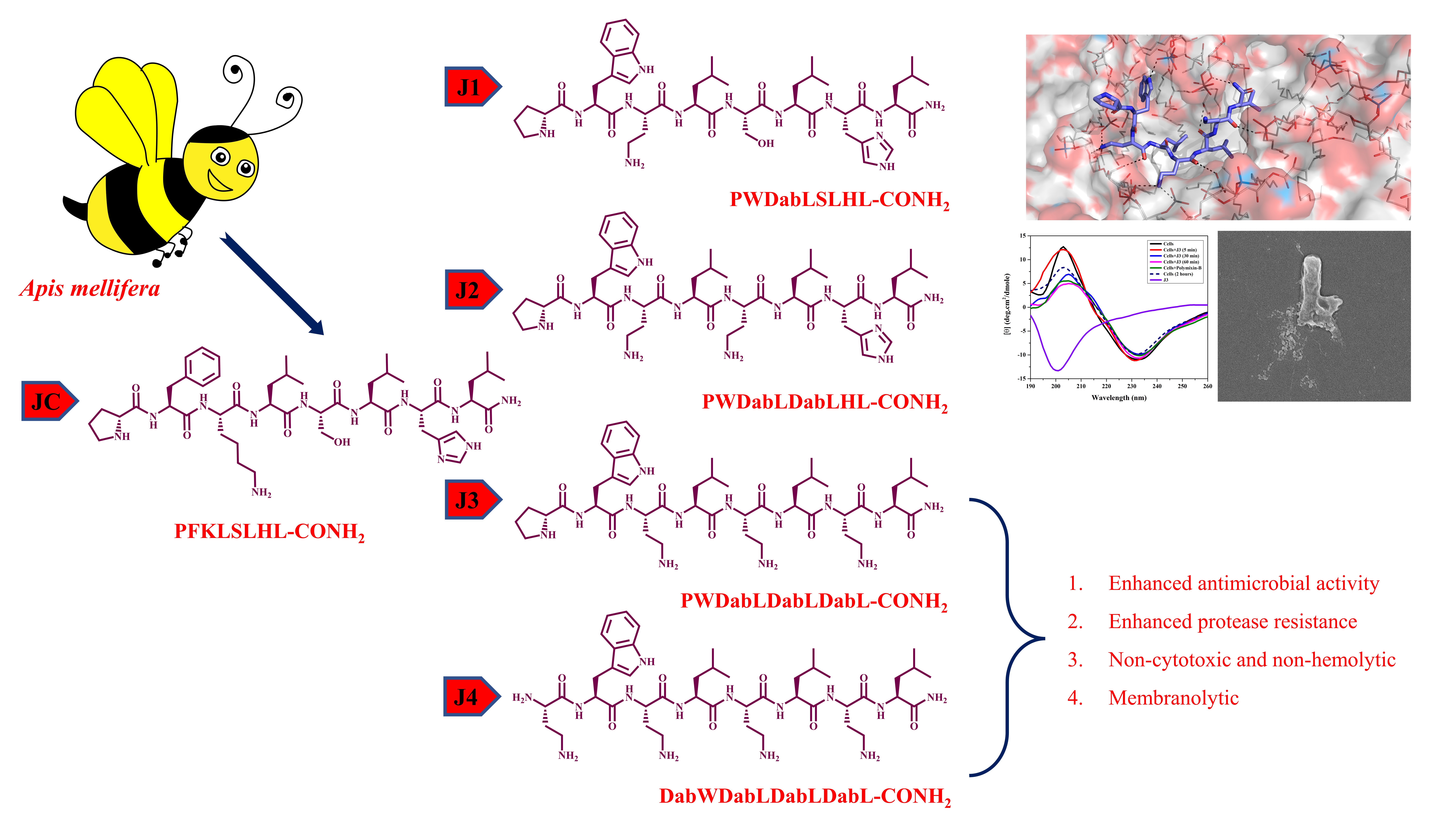
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Most of the antibiotics used for the treatment of microbial infections and diseases caused by pathogenic microbes have either become useless or are on the verge of becoming useless owing to their reckless and unnecessary usages. Microbes have evolved at rates faster than the rate of discovery of new antimicrobial drugs or antibiotics. Therefore, alternative drugs with lesser scope for the microbes to evolve or resist need to be developed. Antimicrobial peptides that are abundantly available in nature and constitute the primary line of defense in most of the living organisms may be considered as an alternative. However, low bioavailability due to serum instability, high cytotoxicity, and high cost of production limit their commercial production. Synthetic modifications involving sequence modification, incorporation of unnatural amino acids to combat serum degradation are some of the possible ways to tackle the limitations of the naturally obtained AMPs. Here in this work, we have designed and synthesized a series of peptides based on the sequence of a naturally obtained antimicrobial peptide Jelleine-I (JC), (1) incorporating tryptophan, (2) substituting lysine with its unnatural analogue diaminobutyric acid (Dab), (3) increasing positive charge. Out of the modified peptides, J3 (PWDabLDabLDabL-CONH2) and J4 (DabWDabLDabLDabL-CONH2) were found to have enhanced antimicrobial activity tested against a series of ESKAPE group gram-negative and gram-positive bacteria as well as fungus, and an improved serum stability compared to its parent sequence. Peptides J3 and J4 displayed excellent killing kinetics against *P. aeruginosa* at their respective MICs. Peptides were non-cytotoxic against two mammalian cell lines tested HDF and HeLa. Peptides were also non-hemolytic upto a very high concentration. Peptides were found to be membranolytic from FESEM imaging. CD experiments in the presence of membrane mimics as well as live cells implied specific bacterial selectivity of the modified peptides. A series of fluorescence studies involving both bacterial as well as mammalian membrane mimic also proved the bactericidal but non-cytotoxic nature of the peptides. Several studies like inner membrane permeation, outer membrane permeation, confocal microscopy further confirmed their membranolytic mode of actions. Studied involving interactions of the peptides with both bacterial as well as mammalian membrane mimic were assessed through ITC which also led to the conclusion of bacterial membrane selectivity of the peptides. Molecular dynamics simulations studies revealed strong interaction of the peptide J3 with model membrane mimicking *Pseudomonas aeruginosa* but not with model mimicking mammalian cells.

**Keywords:** Antimicrobial Peptides (AMPs), Minimum Inhibitory Concentration, Cytotoxicity, Protease Resistance.



**Figure1.** Schematic representation of the peptides modified from parent sequence Jelleine-I (JC).