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Structure-Activity Relationship Study of β-Hairpin Peptides with Antibiotic Activity

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Introduction

- The increasing resistance of pathogenic microbes to antibiotics poses a major threat and calls for methods to improve the pace of antibiotic discovery.[1] Antimicrobial peptides (AMPs) are effective against antibiotic-resistant bacteria in many cases.
- The peptide SAJO-2 developed by Sarojini and coworkers represents a tryptophan zipper-like motif involving a central D-Phe-Abz unit which is a peptidomimetic beta turn including the conformationally inflexible ortho-aminobenzoic acid. [2]
- By fine-tuning the intrinsic hydrophobicity of SAJO-2 by distinctive degrees of fluorination, Chowdhary and coworkers enhanced antimicrobial activity; however, undesired proteolytic degradation by β-trypsin was also enhanced. [3]

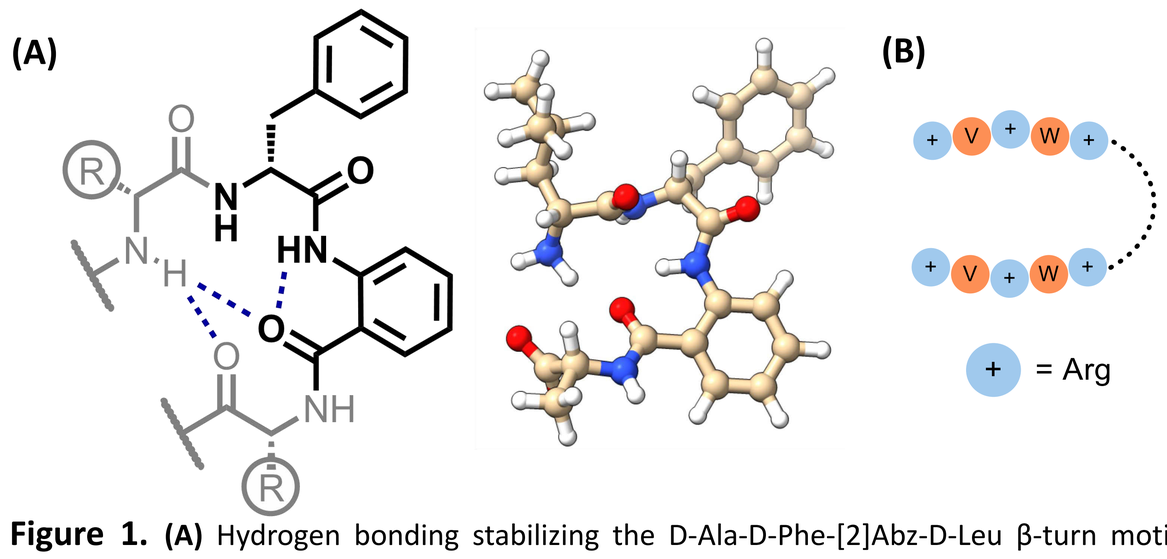
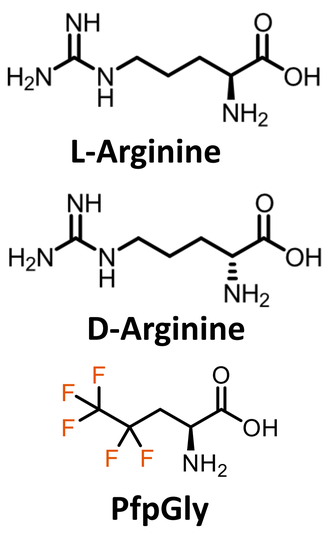


Figure 1. (A) Hydrogen bonding stabilizing the D-Ala-D-Phe-[2]Abz-D-Leu β-turn motif **(PDB Code: 6ANM), (B)** Cartoon of SAJO-2 peptide with positive charge denoting arginine residue and V and W denoting valine and tryptophan, respectively.

Results – Proteolytic Studies and Antimicrobial Susceptibility Testing

Amino acids employed



Scientific Aim

- Improve SAJO-2 stability against proteolytic degradation by the introduction of D-arginine or PfpGly amino acids
- Study the relationship between structural conformation and biological activity

ANTIMICROBIAL PEPTIDE	APPLIED ENZYMES
SAJO-2	**α- chymotrypsin:** Preferentially cleaves at aromatic residues at position P1 (Phenylalanine, Tryptophan, and Tyrosine)
SAJO-D	
SAJO-1D	**β- trypsin:** Specifically cleaves the C-terminal side of lysine and arginine residues
SAJO-2D	
SAJO-PfpGly-DArg	**Carboxypeptidase B:** Perform diverse physiological functions by removing C-terminal amino acids (preferably arginine) **Proteinase K:** Broad specificity and cleaves peptide bonds adjacent to the carboxylic group of aliphatic and aromatic amino acids

(A) Minimum Inhibitory Concentration (MIC) Assay

Antimicrobial Peptide	S. Typhimurium ATCC 14028	E. Coli ATCC 25922	S. Aureus ATCC 29213	P. Aeruginosa ATCC 27853	K. pneumoniae ATCC 700603	C. Albicans IMT 9655
SAJO - 2	32	16	64	64	64	32
SAJO - D	32	16	256	512	64	32
SAJO - 1D	32	32	128	1024	64	32
SAJO - 2D	16	32	256	1024	128	32
SAJO - PfpGly-DArg	32	32	64	512	64	32

(B) Proteolytic Digestion

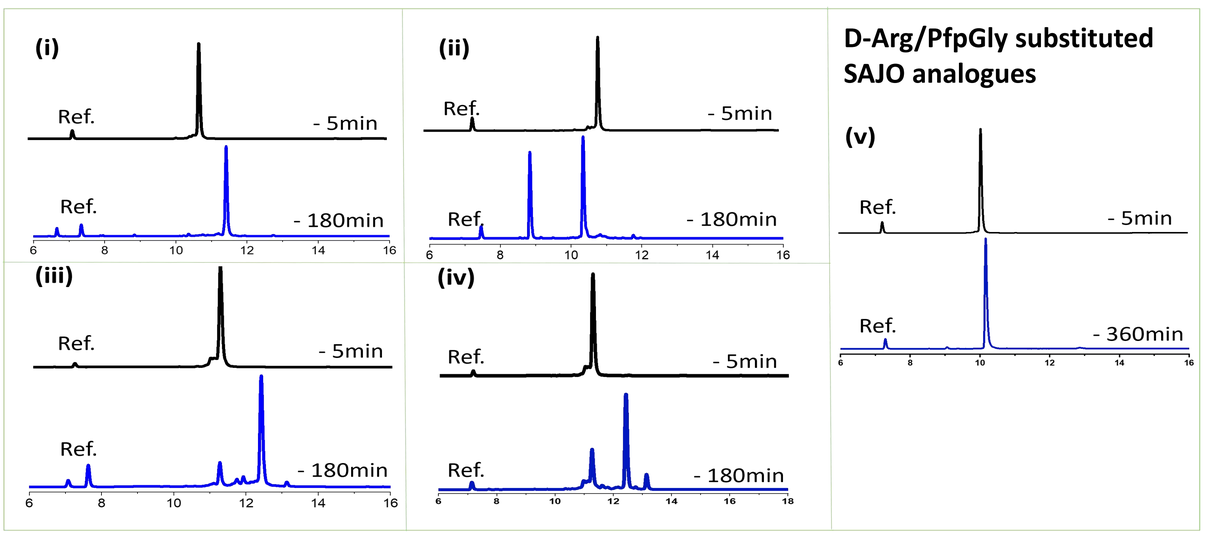


Figure 2. (A) MIC analysis of different library members, all experiments carried out in triplicate with three internal measurements **(B)** Proteolytic degradation profile of native peptide SAJO-2 (1mM stock concentration) observed by HPLC when incubated against **(i)** β-trypsin, **(ii)** α-chymotrypsin, **(iii)** Carboxypeptidase B and **(iv)** Proteinase K (All 20µM stock concentration) at 30°C for 3hours and **(v)** after introducing D-arginine and PfpGly amino acids (1mM stock concentration).

Conclusion

- A library of the turn motif SAJO-2 was synthesized with varying non-canonical amino acid substitutions
- All peptides were found to be stable against enzymatic degradation against β-trypsin and α-chymotrypsin due to altered stereochemistry that prohibits sufficient enzyme-substrate binding
- MIC values of D- analogues were comparable with their L counterparts against *E. coli*, *S. typhimurium,* and fungal pathogen *C. albicans*

Outlook

- Cytotoxicity and hemolysis studies
- Mode of action studies
- MIC assays with resistant strains of *S. typhimurium*
- Structural conformation studies with circular dichroism (CD) experiments

References
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[4] S. Chowdhary, T. Pelzer, M. Fulde, B. Koksch, "Peptide With Antimicrobial Activity", int. patent, EP4375289A1

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