Amphiphilic Cell-Penetrating Peptides Containing Natural and Unnatural Amino Acids as Drug Delivery Tools and Antimicrobial Agents

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BACKGROUND AND HYPOTHESIS

The cellular delivery of cell-impermeable and water-insoluble compounds is a major challenge. Cell-penetrating peptides (CPPs) have been reported to have a great potential to solve the problem of cell impermeability. The previous data showed that cyclic peptide $[WR]_5$ containing alternative tryptophan (W) and arginine (R) residues improved the cellular uptake of cell-impermeable compounds across the phospholipid bilayer. Herein, we investigated peptides containing alternate arginine and unnatural hydrophobic residues as a molecular transporter of cell-impermeable compounds.

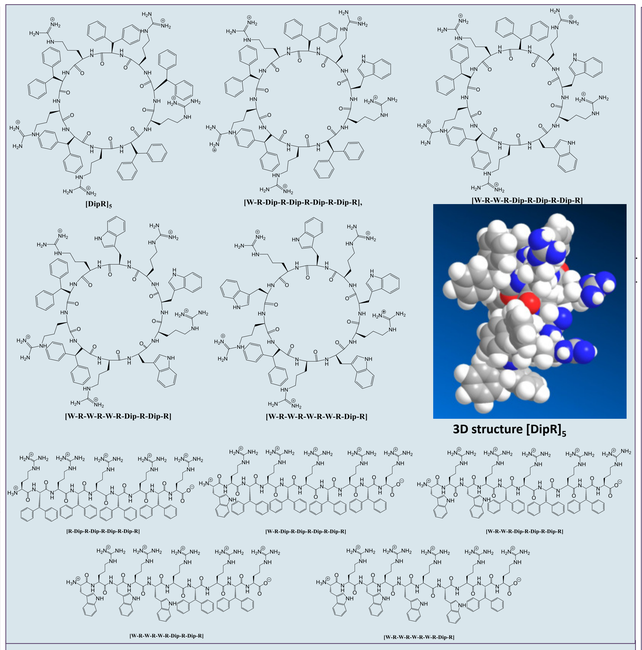
The **hypothesis** of this work is that a series of cyclic and linear peptides containing arginine, tryptophan and 3,3-diphenylalanine (Dip) residues, $[(DipR)_X(WR)_Y]$ (X and Y = 0-5), can act as molecular transporters and/or antimicrobial agents.

OBJECTIVES

1. To synthesize $[(DipR)_X(WR)_Y]$ (X and Y = 0-5) and their corresponding linear counterparts
2. To evaluate their cytotoxicity and molecular transporter properties
3. To determine the antimicrobial activities
4. To examine the nanoparticle formation with transmission electron microscopy (TEM) , dynamic light scattering (DLS), and circular dichroism (CD)

METHODS

- The peptides $[(DipR)_X(WR)_Y]$ (X and Y = 0-5) were synthesized through Fmoc solid-phase chemistry, cyclized in solution phase chemistry, purified with RP-HPLC, and lyophilized to afford a pure powder .
- *In vitro* cytotoxicity of the peptides were evaluated by MTS assay using T lymphoblast peripheral blood cells (CCRF-CEM), epithelial ovary cells (SK-OV-3), epithelial mammary gland/breast (MB-468) and epithelial kidney cells (LLCPK), and epithelial uterus cells (MES/SA).
- The cellular uptake studies were performed by the flow cytometry technique to measure the uptake of fluorescent-labeled phosphopeptide (F'-GpYEEI) and drugs after mixing with synthesized peptides.
- The microbroth dilution protocol was used for antibiotic assays. The linear and cyclic peptides were incubated around 24 hours with *Methicillin-Resistant Staphylococcus aureus* (MRSA), *Staphylococcus aureus* (*S. aureus*), *Enterococcus faecium* (*E. faecium*), *Enterococcus faecalis* (*E. faecalis*), *Streptococcus pneumoniae* (*S. pneumoniae*), *Bacillus subtilis*, *Klebsiella pneumoniae* (KPC), *Pseudomonas aeruginosa* (PSA), and *Escherichia coli* (E.Coli) to record the results.
- TEM of $[DipR]_5$ (1 mM) was determined by staining with uranyl acetate 2%. CD spectroscopy was measured for $[DipR]_5$ (75 µM) dissolved in water, and DLS data was acquired for $[DipR]_5$ (10 µM) in 5% DMSO and water.



RESULTS

In Vitro Cytotoxicity Studies

$[DipR]_5$ was not significantly toxic against SK-OV-3, MB-468, and LLCPK1 cells at a concentration of 10 µM after 24 h while it was toxic against CCRF-CEM, MES/SA and SK-OV3 cells after 72 h incubation.

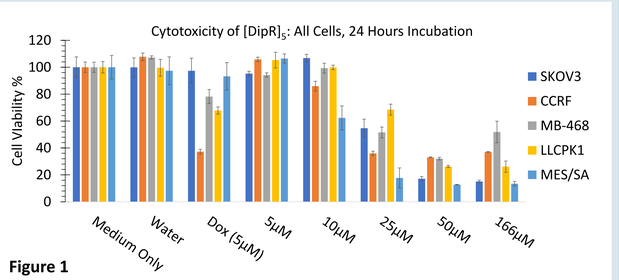
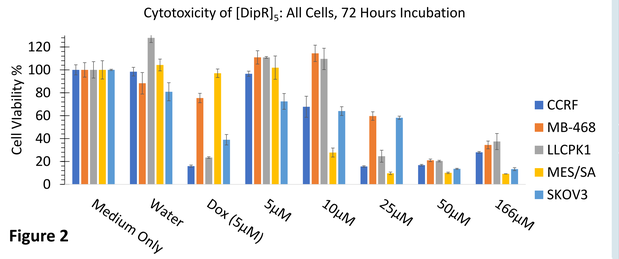


Figure 1



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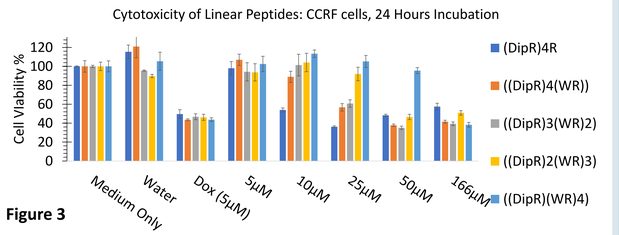

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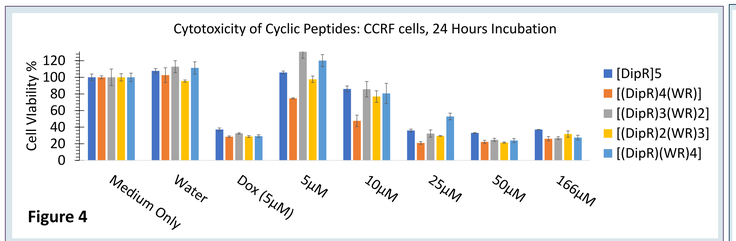
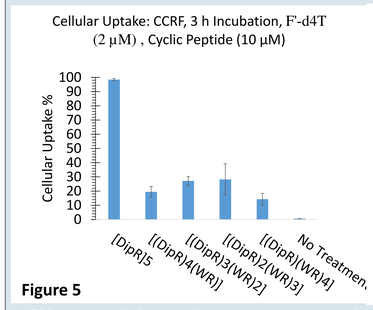


Figure 4

Cellular Uptake Studies

$[DipR]_5$ improved cellular uptake of F'-GpYEEI by approximately 110-folds.



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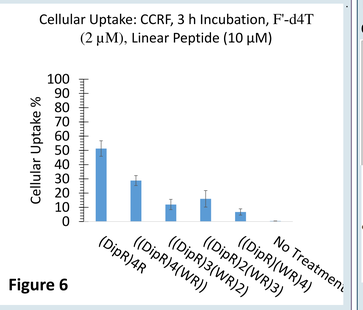

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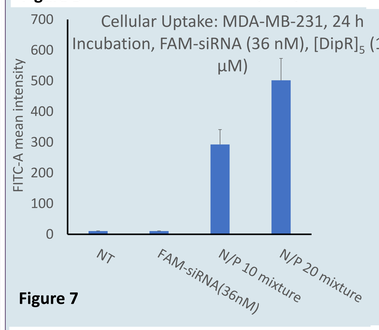

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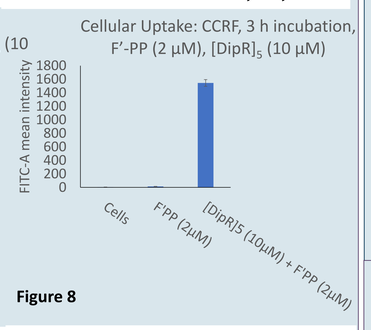

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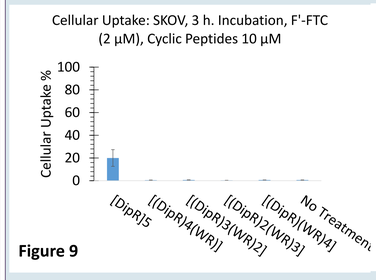

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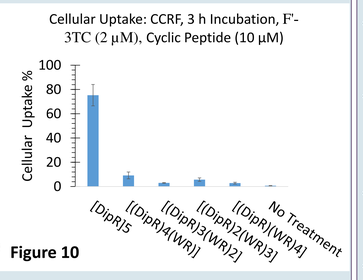
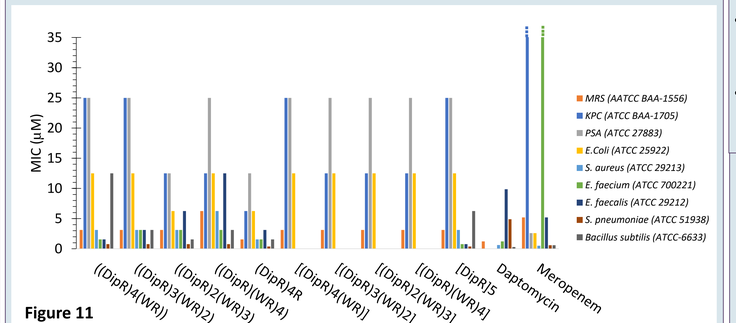


Figure 10

Antimicrobial activity

$[DipR]_5$ showed promising results against bacteria strains.



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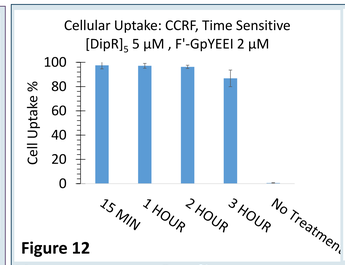

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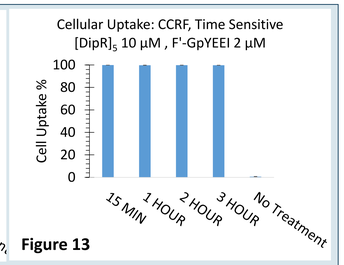
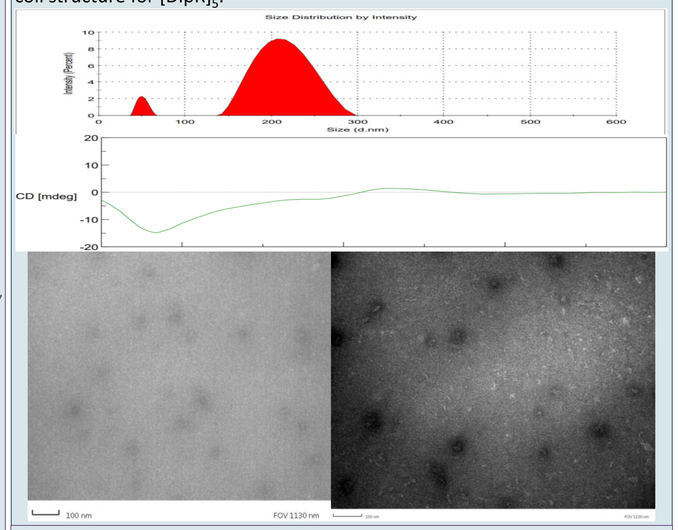


Figure 13

Nanostructure Studies

TEM and DLS showed formation of nanoparticles while CD exhibited random coil structure for $[DipR]_5$.



CONCLUSIONS

- $[DipR]_5$ showed toxicity at the concentration more than 25 µM. However, the toxicity at 10 µM concentration was minimal in a few cells after 24 h incubation.
- The cellular uptake studies showed that $[DipR]_5$ significantly improved the cellular uptake of fluorescence-labeled phosphopeptide by 110-folds after approximately 3 h incubation. $[DipR]_5$ was able to penetrate an average of 99.84% of cells.
- Peptide/Fluorescence-labeled siRNA complex with N/P ratios of 10 and 20 were found to be 90-folds and 140-folds higher respectively when they are compared with cells exposed to only siRNA.
- $[DipR]_5$ showed promising MIC values of 0.39-6.25 µM (0.74-11.9 µg/mL) against Gram-positive bacteria strains: Methicillin-Resistant *Staphylococcus aureus*, Staphylococcus aureus, *Enterococcus faecium*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, and *Bacillus subtilis* bacteria.
- On the other hand, $[DipR]_5$ showed moderate MIC values of 12.5-25 µM (23.78-47.56 µg/mL) against Gram-negative strains: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa,* and *Escherichia coli.*
- DLS and TEM showed formation of nanoparticles.

REFERENCE

1. Hanna, S.E., Mozaffari, S., Tiwari, R.K., Parang, K., *ACS Omega*, **2018**, 3, 16281-16291.
2. Mandal, D., Nasrolahi Shirazi, A., Parang, K. *Angew. Chem. Int. Ed.* **2011,** *50*, 9633-9637.