Design and Evaluation of Metallacarborane-Peptide Conjugates as Novel Antimicrobial Agents

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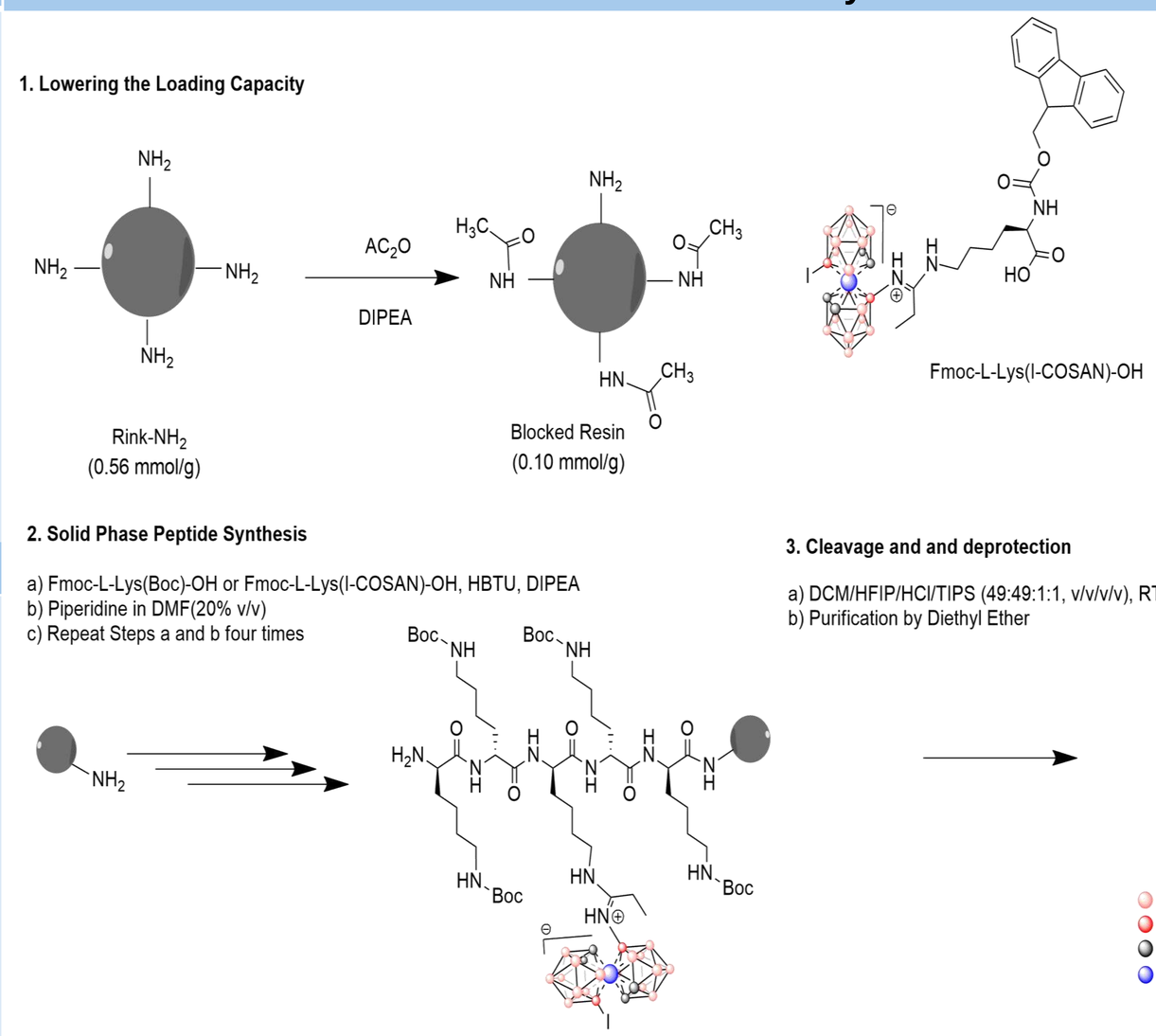
Introduction

Antimicrobial resistance constitutes a pressing global health threat, ranking bacterial infections second in worldwide mortality causes with >1.14 million annual deaths in 2019, projected to reach 2 million by 2050 [1]. Given the paucity of novel antibiotics in the clinical pipeline, wherein the 2025 WHO report identifies merely 15 truly innovative agents, there exists an imperative for novel therapeutic modalities [2]. Antimicrobial peptides exhibit potent antimicrobial activity through cationic–hydrophobic motifs that mediate electrostatic binding and disruptive insertion into bacterial membranes, yielding bactericidal outcomes with minimal resistance induction, while metallacarboranes such as cobalta bis(dicarbollide) (COSAN) independently manifest membrane-targeting antibacterial potency [3].To investigate their synergistic potential, we synthesized a series of ultrashort cationic peptide-metallacarborane conjugates designed as AMP mimics, in which COSAN-derived metallacarborane units were attached to the peptide scaffold, resulting in markedly enhanced antimicrobial efficacy surpassing that of the parent peptide scaffold or COSAN alone [4].

Methodology

A rationally designed library of six metallacarborane–peptide conjugates was synthesized by Fmoc-SPPS on Rink amide resin using amino acid derivatives of metallacarboranes, including one β-alanine-based and five lysine-based derivatives. Resin loading was reduced from 0.56 to 0.10 mmol g^{-1} by acetylation to minimize steric effects and aggregation. The conjugates were cleaved using DCM/HFIP/37% HCl/TIPS (49:49:1:1), precipitated with diethyl ether, purified to >95% purity, and characterized by mass spectrometry and NMR spectroscopy. Antibacterial activity was evaluated by broth microdilution according to CLSI M07 guidelines, and time-kill kinetics were performed against *E. coli* PCM300 and *S. aureus* PCM2602 at 1×–10× MIC over 24 h. Cytotoxicity was assessed using the SRB assay, while hemolytic activity was measured using human red blood cells to calculate EC_{50} values.

Scheme of Synthesis



Cpd	Sequence
Cpd.1	βAla(I-COSAN)-KKKK-NH_2
Cpd.2	Lys(I-COSAN)-KKKK-NH_2
Cpd.3	K-Lys(I-COSAN)-KKK-NH_2
Cpd.4	KK-Lys(I-COSAN)-KK-NH_2
Cpd.5	KKK-Lys(I-COSAN)-K-NH_2
Cpd.6	KKKK-Lys(I-COSAN)-NH_2

Antimicrobial Activity MIC[µM]

Cpd.	*S.aureus* PCM 2602	*MRSA* PCM 3144	*E.faecalis* DSMZ 12155	*E.coli* PCM 2646	*E.coli* PCM 300	*E.coli* PCM 307	*A.baumannii* PCM 2740
Cpd.1	2.5	2.5	6.20	5	10	10	40
Cpd.2	2.5	1.25	6.20	5	5	10	10
Cpd.3	1.25	1.25	6.20	10	5	20	40
Cpd.4	1.25	1.25	6.20	5	5	20	20
Cpd.5	1.25	1.25	6.20	10	5	40	40
Cpd.6	1.25	1.25	6.20	5	2.5	40	40
KKKK	>40	>40	>40	>40	>40	>40	>40
COSAN	>40	>40	>40	>40	>40	>40	>40
I-COSAN	1.25	1.25	>40	>40	>40	>40	>40
VAN	0.31	0.62	0.8uM	-	-	-	-
PMB	-	--	--	0.31	0.15	0.15	>40

Fig. 1. Minimum inhibitory concentrations (MIC) for all compounds and controls against Gram-positive and Gram-negative bacterial strains.

Safety Profiling

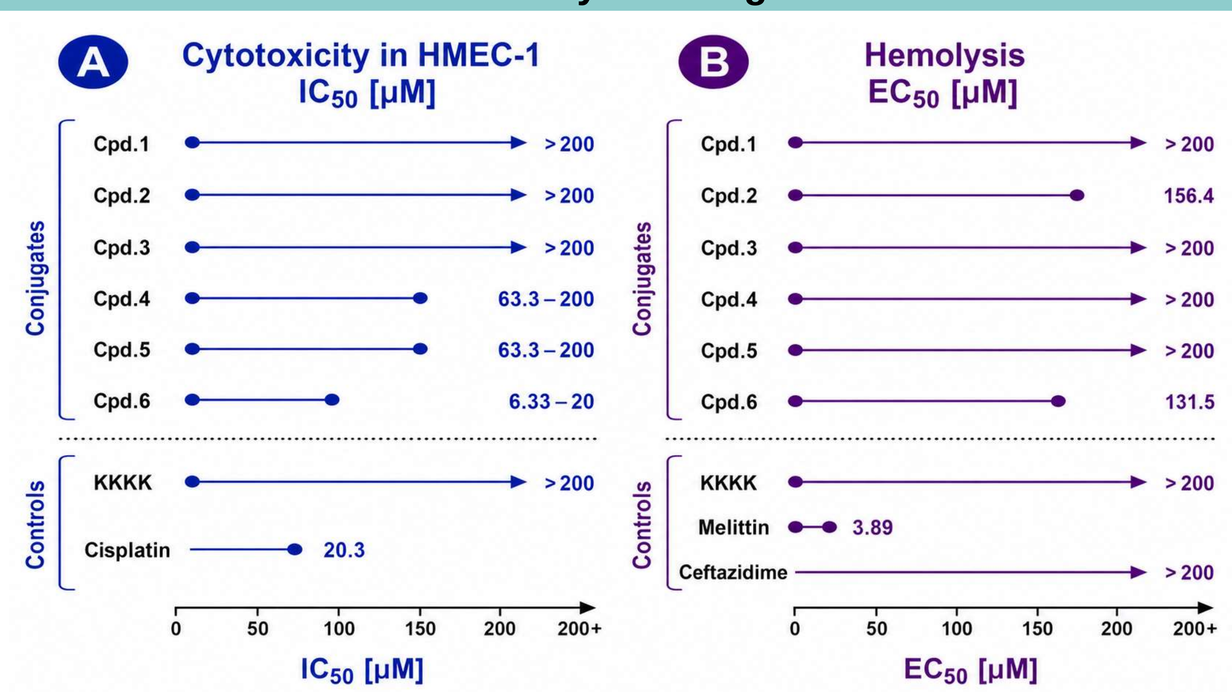


Fig. 3. Cytotoxicity (IC_{50}) and hemolysis (EC_{50}) of metallacarborane-conjugated peptide derivatives and controls, showing representative HMEC-1 cell line data.

Time-Kill Kinetics

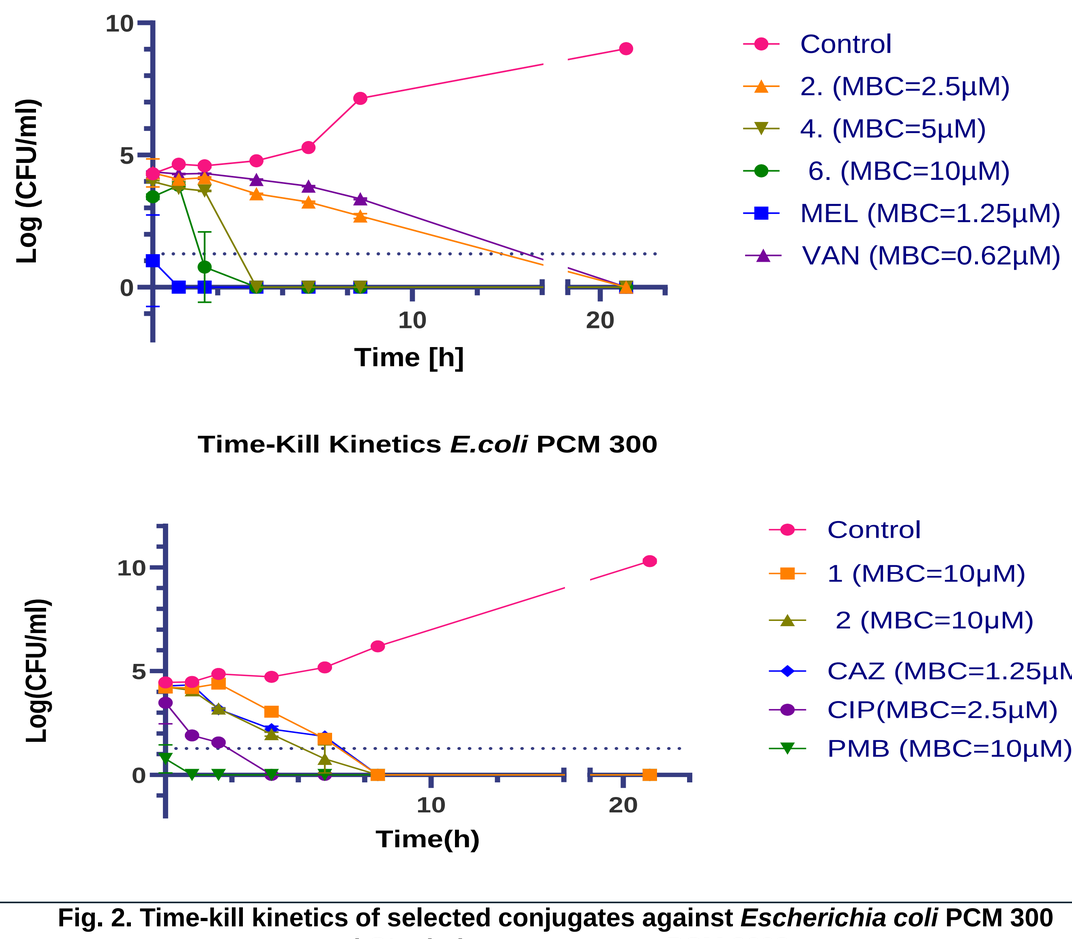


Fig. 2. Time-kill kinetics of selected conjugates against *Escherichia coli* PCM 300 and *Staphylococcus aureus* PCM 2602.

Conclusions

- **Position-Dependent Activity**
  Metallacarborane attachment site strongly modulated antimicrobial activity, killing kinetics, cytotoxicity, and hemolysis
- **Enhanced Antibacterial Potency:**
  Metallacarborane conjugation transformed the inactive KKKK scaffold into potent bactericidal conjugates
- **Gram-Positive Optimization:**
  Central and C-terminal metallacarborane positioning improved activity against *S. aureus* and MRSA, with MIC values reaching **1.25 µM**.
- **Gram-Negative Selectivity:**
  Near-N-terminal positioning favored Gram-negative activity, while C-terminal shifting reduced efficacy against *A. baumannii* and resistant *E. coli* 307.
- **Rapid Bactericidal Action:**
  Time-kill assays confirmed complete eradication of *S. aureus* PCM 2602 and *E. coli* PCM 300 by selected conjugates.
- **Favorable Safety Profile:**
  Cpd.1 and Cpd.3 emerged as the most promising conjugates, combining strong antibacterial activity with low hemolysis (**EC_{50} >200 µM**) and low HMEC-1 cytotoxicity (**IC_{50} >200 µM**).

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References

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