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Rational Design of Melittin-Derived Peptides for Enhanced Biological Activity

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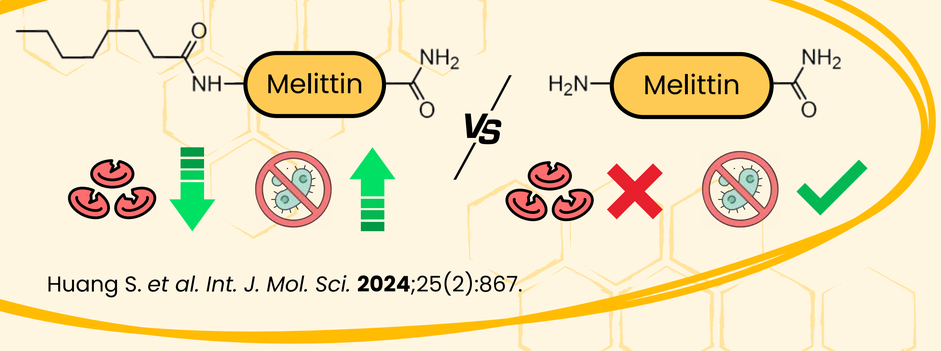
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Introduction

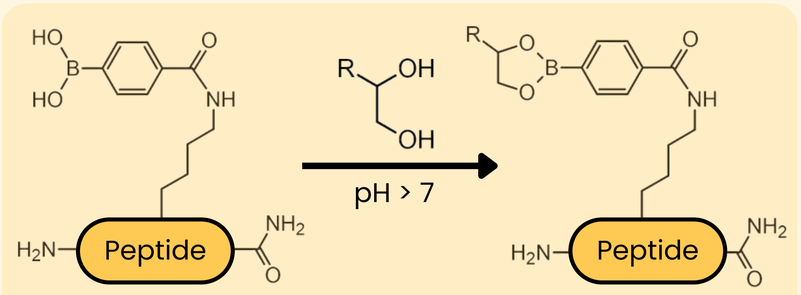
Melittin: H-G I G A V L K V L T T G L P A L I S W I K R K R Q Q -NH_2

MCFA: H-G L K K L I S W I K R A A Q Q G -NH_2

Subbalakshmi C. *et al. FEBS Lett.* **1999**;448(1):62.



Huang S. *et al. Int. J. Mol. Sci.* **2024**;25(2):867.



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Objectives

Synthesis of more selective MCFA analogs with lower cytotoxicity and enhanced proteolitic stability.

- Synthesis of 6 MCFA analogs modified with 4-carboxyphenylboronic acid and/or caprylic acid.
- Purification of all synthesized compounds by preparative HPLC.
- Evaluation of antimicrobial activity against Gram+ and Gram- bacteria.
- Evaluation of proteolytic stability against trypsin.

Results

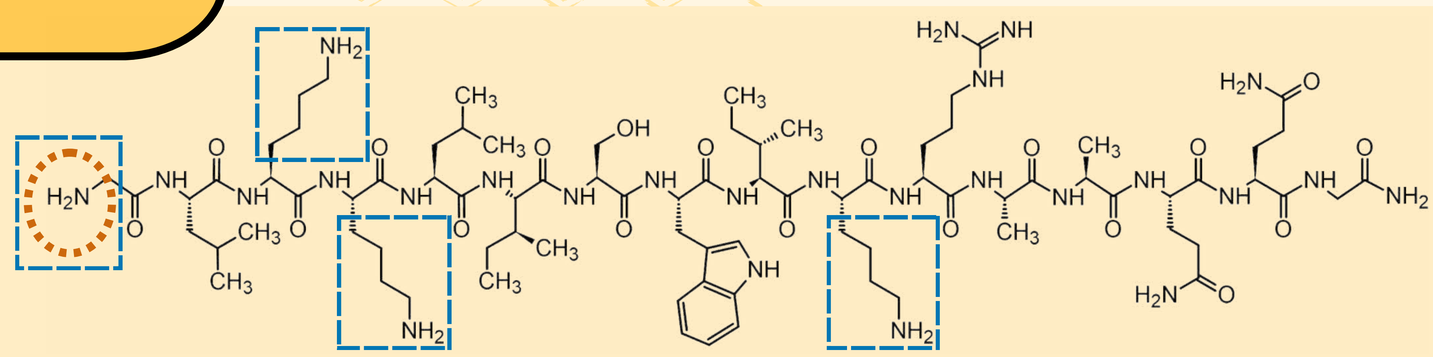
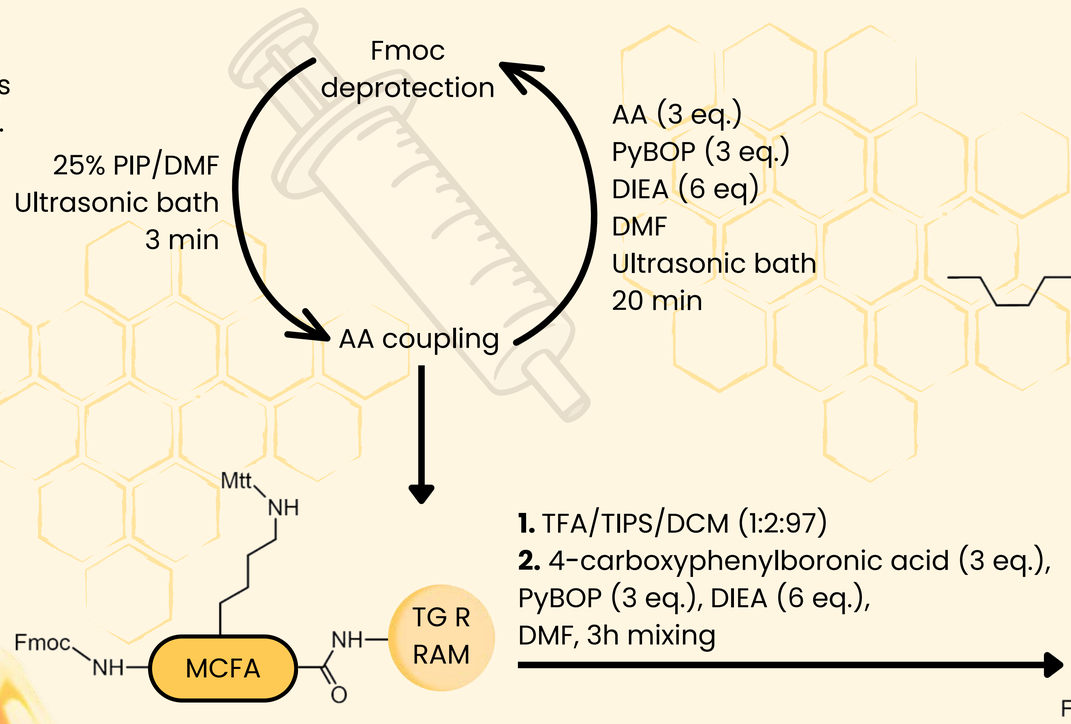


Fig. 1. Structure of MCFA with marked sites of post-synthetic modifications with 4-carboxyphenylboronic acid and caprylic acid.

Tab. 1. *In vitro* hemolytic activity of peptides determined as 50% hemolytic concentration.

Compound	HC_{50} [µg/mL]
MCFA	16
MCFA-C8	128
MCFA-PBA(2.Lys)	n/d
MCFA-PBA(2.Lys)-C8	64
MCFA-2PBA(1.3.Lys)-C8	32
MCFA-2PBA(1.2.Lys)-C8	Not tested yet
MCFA-2PBA(1.2.Lys)-PBA	Not tested yet



Tab. 2. *In vitro* antibacterial activity determined as a minimal inhibitory concentration (MIC). MCFA-2PBA(1.2.Lys)-C8 and MCFA-2PBA(1.2.Lys)-PBA have not yet been tested.

Strain	MIC [µg/mL]				
	MCFA	MCFA-C8	MCFA-PBA(2.Lys)	MCFA-PBA(2.Lys)-C8	MCFA-2PBA(1.3.Lys)-C8
S. aureus	8	4	4	16	8
S. epidermidis	32	16	8	32	16
E. coli	16	8	32	4	8
K. pneumoniae	4	4	16	4	4
K. rhinoscleromatis	4	2	2	8	2

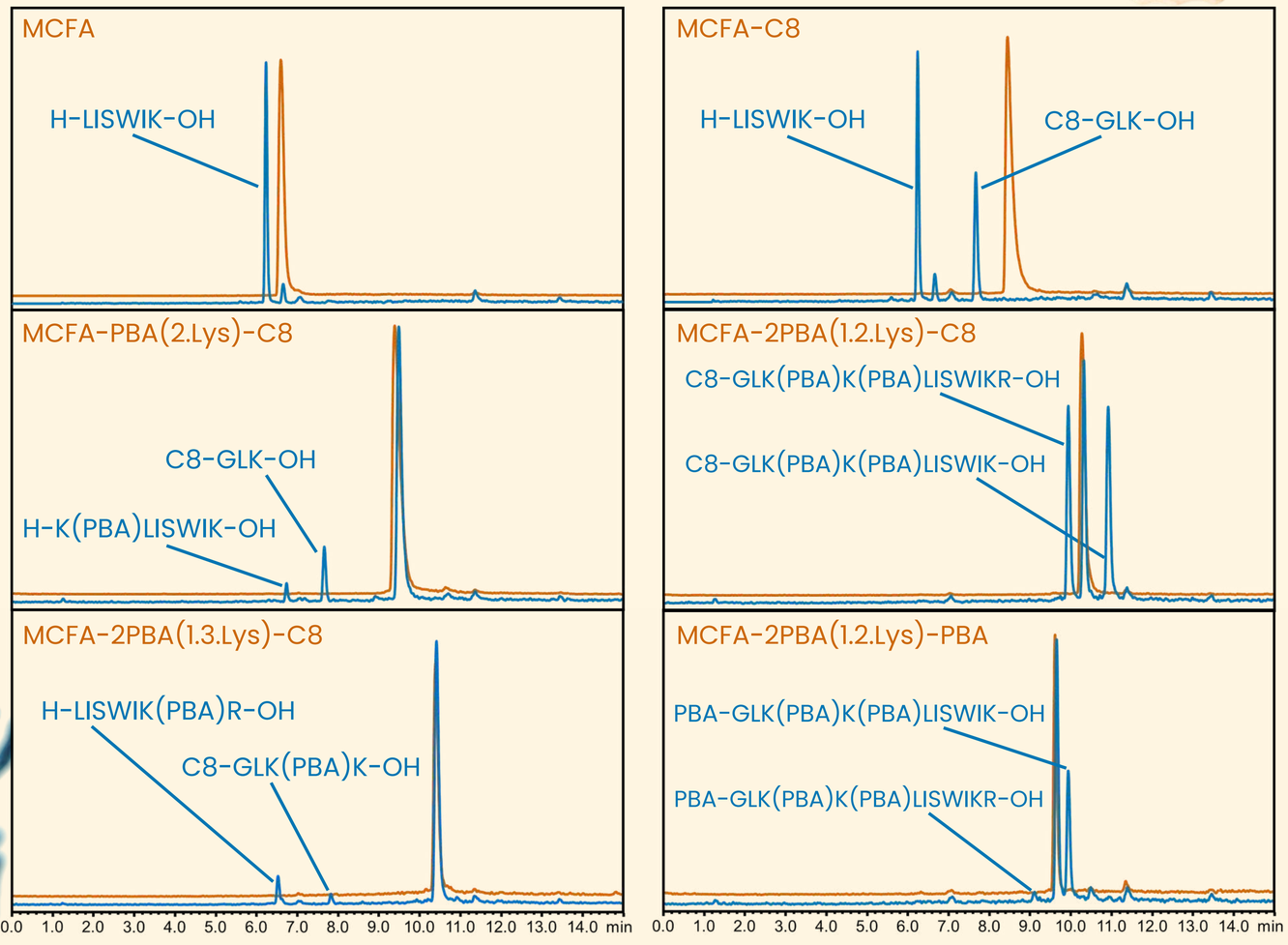


Fig. 2. LC-MS (TIC) of peptides before and after trypsin digestion. MCFA-PBA(2.Lys) has not yet been tested.

Conclusions

- PBA improved MCFA selectivity, with MCFA-PBA(2.Lys) showing no hemolysis.
- The strain-dependent activity of PBA-modified analogs suggests that differences in bacterial surface glycoconjugates may contribute to peptide recognition and selectivity.
- C8 lipidation maintained or enhanced antibacterial activity while substantially reducing hemolytic activity, consistent with previous reports on lipidated melittin analogs.
- Post-synthetic modifications altered the proteolytic degradation patterns of MCFA analogs, highlighting their potential to modulate peptide stability.

Acknowledgements

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