Synthesis and Antimicrobial Activity of Cyclic Temporin L Analogues via Non-Conventional Guanidinium-Based Functionalization

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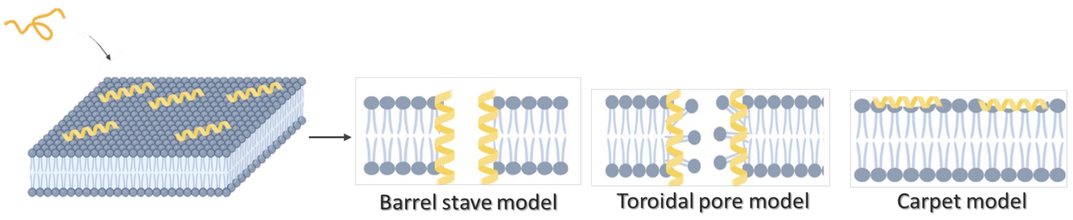
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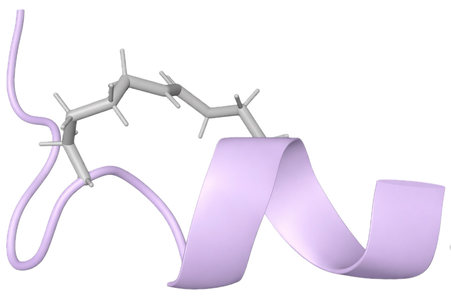
Background

Antimicrobial peptides (**AMPs**) represent versatile scaffolds for the development of novel antimicrobial agents, owing to the following properties: *i)* short sequence length, *ii)* activity against a wide range of pathogens, *iii)* additional chemotactic activity and immunomodulatory effects.[1]



Aim & Novel Design

Temporin L (TL) has undergone site-specific modifications, stereoinversion, and different cyclization strategies to optimize its biological profile.[2,3] Building on these findings, a new library of **cyclic TL derivatives** was designed and synthesized by introducing a **guanidino group** at selected positions along the peptide sequence.



F V P W F K K F *l* E R I L **(1)**

Peptide **12** in Bellavita et al, *J.Med.Chem.*, **2021**

Stereoinversion of cyclizing aa:

FVPWF[KKFl**e**]RIL **(2)**

PENDANT guanidino group:

$(H_2N)_2C$=N-FVPWF[KKFl**e**]RIL **(3)**

$(H_2N)_2C$=N-βAla-FVPWF[KKFl**e**]RIL **(4)**

$(H_2N)_2C$=N-GABA-FVPWF[KKFl**e**]RIL **(5)**

F[***p*-NHC(=NH)NH_2]**VPWF[KKFl**e**]RIL **(6)**

FV**P[4-NHC(=NH)NH_2]**WF[KKFl**e**]RIL **(7)**

FVPW**F[*p*-NHC(=NH)NH_2]**[KKFl**e**]RIL **(8)**

FVPWF[K**R**Fl**e**]RIL **(9)**

FVPW F[KK**F[*p*-NHC(=NH)NH_2]**l**e**]RIL **(10)**

BRIDGED guanidino group:

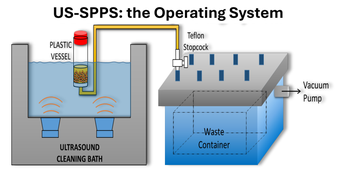
FVPWF[**K**KFl**K**]RIL **(11)**

FVPWF[**K**KFl**k**]RIL **(12)**

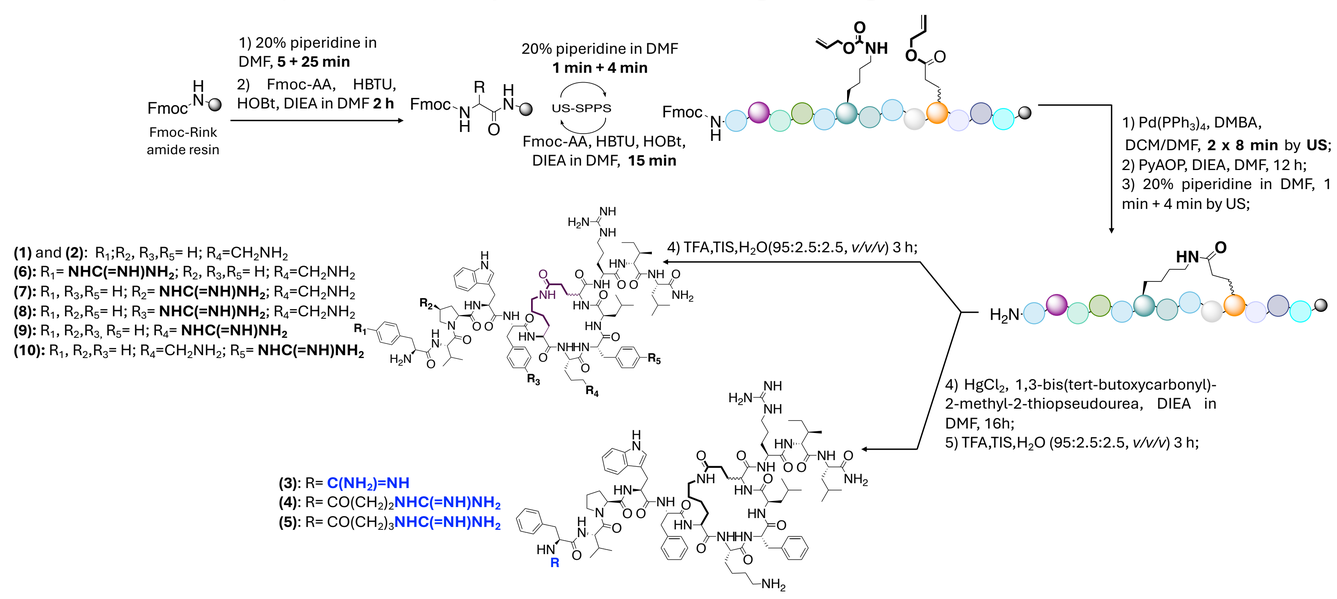
FVPWF[**Orn**KFl**Orn**]RIL **(13)**

PEPTIDE SYNTHESIS

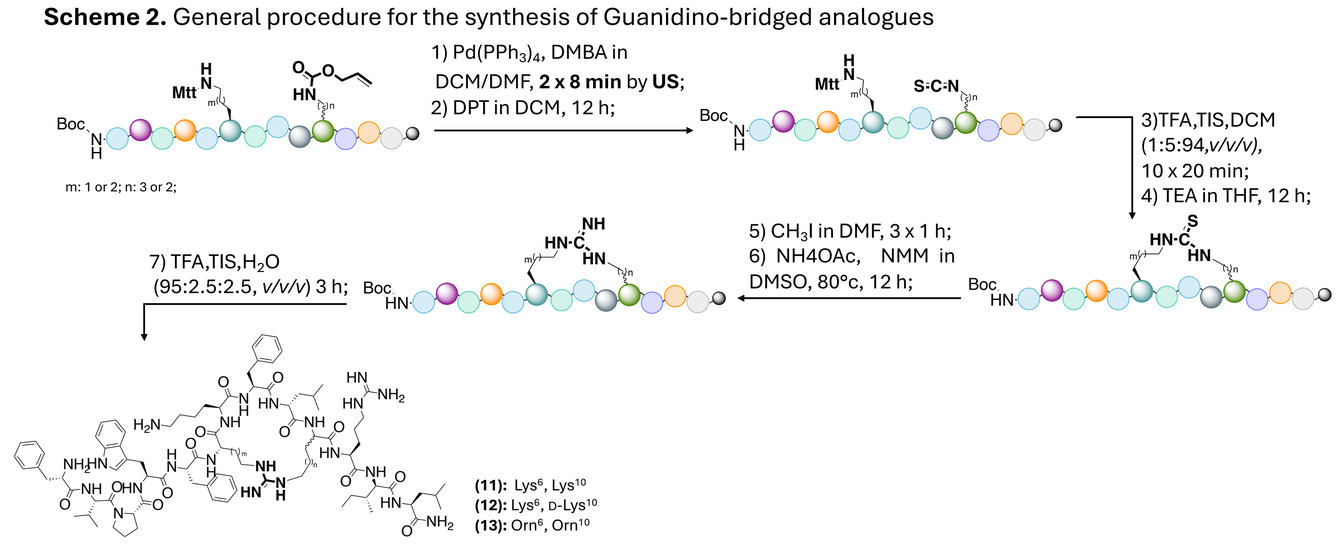
Linear peptides were assembled by ultrasonic-assisted solid-phase peptide synthesis (**US-SPPS**).[4] Peptides **1-10** were cyclized through lactam bridge formation using an orthogonal protection strategy (Scheme **1**), while peptide **11-13** underwent *side chain-to-side chain* cyclization according to Scheme **2**.



Scheme 1. General procedure for the synthesis of Lactam-bridged analogues



Scheme 2. General procedure for the synthesis of Guanidino-bridged analogues



RESULTS

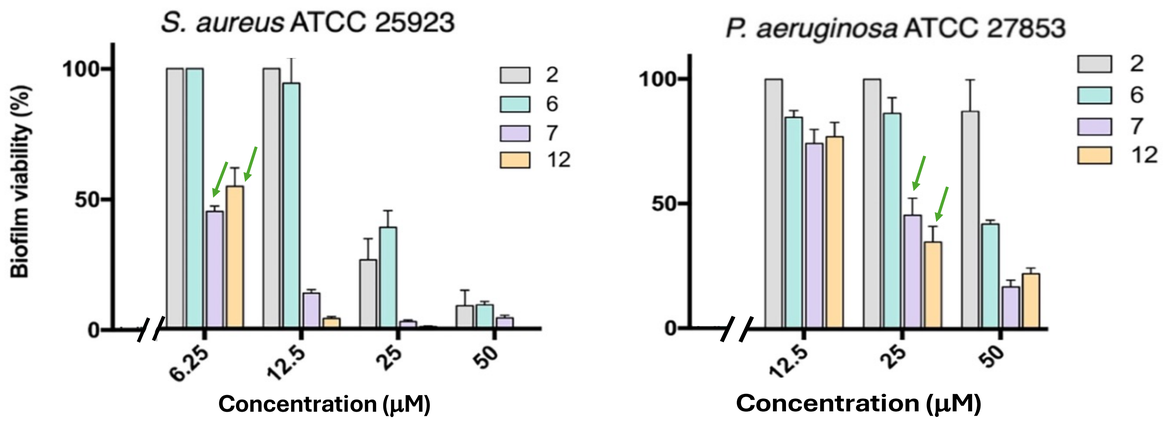
☑ Antimicrobial & AntiBiofilm Activity

Biological evaluation highlighted a favorable structure-activity relationship, particularly against Gram-negative bacteria when the **guanidino group** was located in the *N*-terminal region or incorporated into the cyclization bridge.

MIC (μM)

Peptides	Gram-positive			Gram-negative		
	S. aureus ATCC 25923	*S. epidermidis* ATCC 12228	*Bac.megaterium* Bm11	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*Ac. baumannii* ATCC 19606
(1)	3.12	3.12	1.56	25	100	3.12
(2)	12.5	3.12	0.78	6.25	50	6.25
(6)	25	3.12	0.78	6.25	25	3.12
(7)	6.25	3.12	0.78	3.12	12.5	3.12
(12)	6.25	1.56	0.39	6.25	12.5	1.56

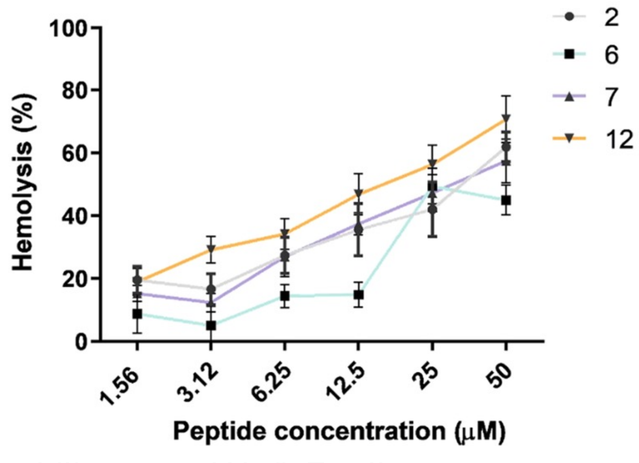
Peptides **7** and **12** emerged as the most active antibiofilm derivatives, reducing *S. aureus* biofilm viability by 50% at 6.25 μM and *P. aeruginosa* biofilm viability by >50% at 25-50 μM.



☑ Cytotoxicity

vs red blood cells

Lactam-bridged peptide **6** retained the most favorable hemolytic profile, showing limited hemolysis up to 25 μM. In contrast, guanidino-bridged peptide **12** displayed increased hemolytic activity from 12.5 μM.

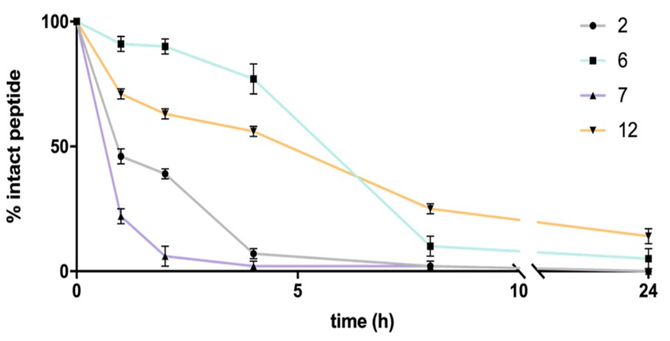


vs human keratinocytes (HaCaT)

All tested peptides maintained good tolerability toward HaCaT cells up to 12.5 μM, with peptide **6** showing the most favorable cytotoxicity profile and peptide **12** the lowest tolerability.

☑ Human Serum Stability

Peptides **6** and **12** showed the highest serum stability, retaining a high percentage of intact peptide over 4-8 h.



Conclusions & Future Outlook

These findings identify **guanidinium incorporation** into the cyclic peptide as a promising strategy to enhance TL antimicrobial activity. This structure-activity insight will guide the design of next-generation cyclic TL analogues toward an improved **activity-cytotoxicity balance**.

References: 1. I.Boccino et al., *ChemMedChem*. **2026**, *21*, e70397; 2. R. Bellavita et al., *J.Med.Chem*. **2021**, *64*, 11675-11694; 3. R. Bellavita, I. Boccino et al., *J.Med.Chem*. **2025**, *68*, 25038-25060; 4. F. Merlino et al., *Org. Lett*. **2019**, *21*, 6378–6382.