https://doi.org/10.17952/37EPS.2024.P2040

Cyclic Antimicrobial Peptides Guanidino-based Analogues of Temporin L

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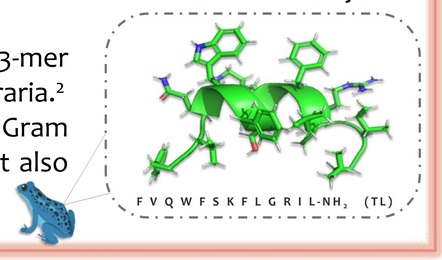
Introduction

Antimicrobial Resistance (**AMR**) occurs when microorganisms such as bacteria, viruses, fungi and parasites change in ways that render ineffective the medications used to cure the infections they cause.

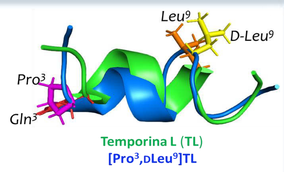
Antimicrobial peptides (**AMPs**) have recently been identified as promising targets for novel drug development, due to their properties: *i)* short sequence length, *ii)* activity against a wide range of pathogens, *iii)* additional chemotactic activity and immunomodulatory effects.[1].

Among these, Temporin L (TL) peptide is a 13-mer cationic peptide derived from skin of Rana Temporaria.[2] TL has high antimicrobial potency against Gram negative and Gram positive bacteria strains, but it also has hematotoxic effect.



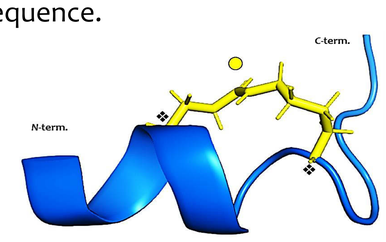
Aim

Local modifications, *stereoinversion* have been made on **TL**, subsequently, different cyclization were made to optimize antimicrobial activity but especially to reduce toxicity. [2,3]



Temporina L (TL)
[Pro³,DLeu⁹]TL

Based on previous results, a new library of ***cyclic derivatives*** was designed and synthesized, which bear a **guanidino group** in key positions of the TL peptide sequence.



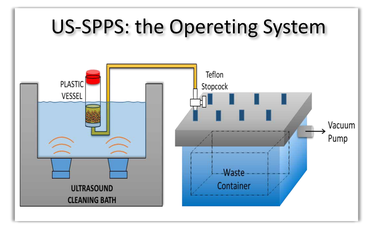
F V P W F *c*[K K F / E] R I L **(1)**

- Lactam bridge or guanidino bridge
- ❖ *D*-amino acids
- ■ Guanidino group in lateral chain

FVPWF[KKF/*le*]RIL **(2)**
$(H_2N)_2C$=N-FVPWF[KKF/*le*]RIL **(3)**
$(H_2N)_2C$=N-$_\beta$Ala-FVPWF[KKF/*le*]RIL **(4)**
$(H_2N)_2C$=N-GABA-FVPWF[KKF/*le*]RIL **(5)**
F[*p*-NHC(=NH)NH_2]VPWF[KKF/*le*]RIL **(6)**
FVP[*p*-NHC(=NH)NH_2]WF[KKF/*le*]RIL **(7)**
FVPWF[*p*-NHC(=NH)NH_2][KKF/*le*]RIL **(8)**
FVPWF[KRF/*le*]RIL **(9)**
FVPWF[KKF[*p*-NHC(=NH)NH_2]/*le*]RIL **(10)**
FVPWF[KKF/K]RIL **(11)**
FVPWF[KKF/*k*]RIL **(12)**
FVPWF[OrnKF/Orn]RIL **(13)**

Methods

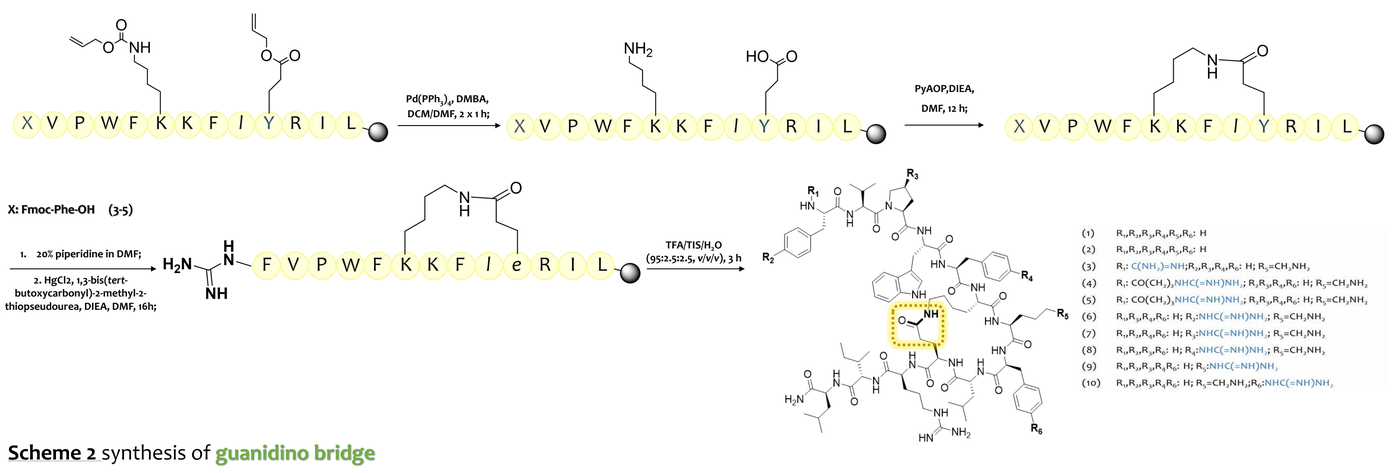
Peptides were assembled by an ultrasonic-assisted solid phase peptide synthesis (US-SPPS) approach.[4] The cyclization of peptides **1-10** occurs via the formation of the lactam bridge (Scheme **1**), due to orthogonal protection of the residues involved. For peptides **11-13**, the synthesis of the side chain-to-side chain guanidino bridge proceeded according to Scheme **2.**



Scheme 1 synthesis of **lactam bridged**

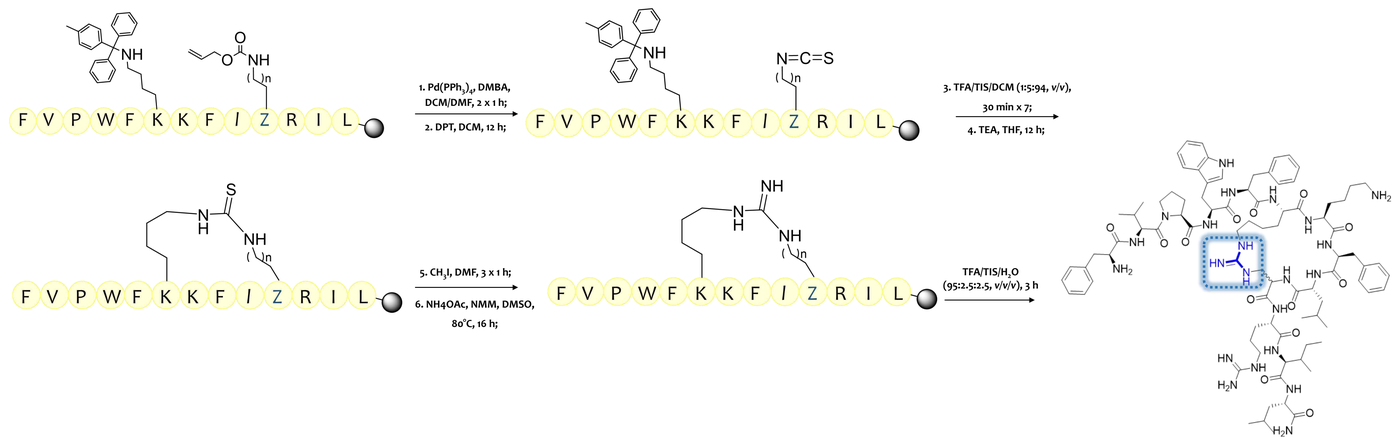
X: Boc-Phe-OH or Fmoc-Phe-OH

Y: Fmoc-Glu(OAll)-OH or Fmoc-D-Glu(OAll)-OH



Scheme 2 synthesis of **guanidino bridge**

Z: Fmoc Lys(Alloc) or Fmoc-D-Lys(Alloc) or Fmoc-Orn(Alloc)-OH



Results

☑ Minimum Inhibitory Concentration

Biological assays on selected bacterial strains suggest the major activity, especially, against **Gram negative** bacteria when the **guanidino group** is located in the *N*-term region or when it is involved in the cyclization bridge.

MIC (μM)

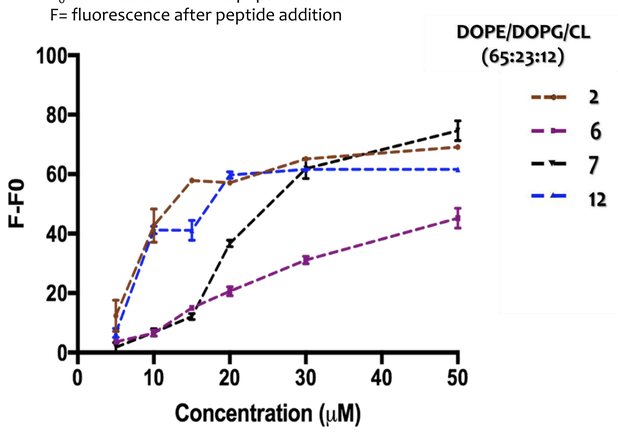
ID	Gram positive			Gram negative		
	S. aureus ATCC 25923	*S. epidermidis* ATCC 12228	*Bac.megaterium* Bm11	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*Ac. baumannii* ATCC 19606
(1)	3.12	3.12	1.56	25	100	3.12
(2)	*n.a*	3.12	*n.a.*	6.25	50	6.25
(3)	6.25	3.12	0.78	6.25	100	3.12
(4)	3.12	1.56	0.78	3.12	25	3.12
(5)	3.12	3.12	0.78	6.25	50	3.12
(6)	25	3.12	0.78	6.25	25	3.12
(7)	6.25	3.12	0.78	3.12	12.5	3.12
(8)	25	6.25	0.78	12.5	50	6.25
(9)	6.25	3.12	1.56	6.25	100	3.12
(10)	100	12.5	0.78	6.25	50	6.25
(11)	3.12	1.56	1.56	6.25	50	1.56
(12)	6.25	1.56	0.39	6.25	12.5	1.56
(13)	6.25	1.56	0.39	6.25	50	1.56

☑ Membrane Interaction

- Thioflavin T assay studied the **peptide aggregation** on LUVs (large unilamellar vesicles) composed by DOPE/DOPG/CL, mimicking Gram negative membrane, is significant for peptide **12**, also at low concentration.
- Laurdan assay studied the membrane fluidity by calculating the GP value in the presence of LUVS mimicking Gram negative bacteria. The value increased for all tested peptides at at 50 μM, indicating a shift towards more ordered membranes.

Thioflavin T assay

F_0= fluorescence before peptide addition
F= fluorescence after peptide addition



Laurdan assay

Membrane fluidity evaluation using the generalized polarization value (GP)

$$GP = \frac{I_{440} - I_{490}}{I_{440} + I_{490}}$$

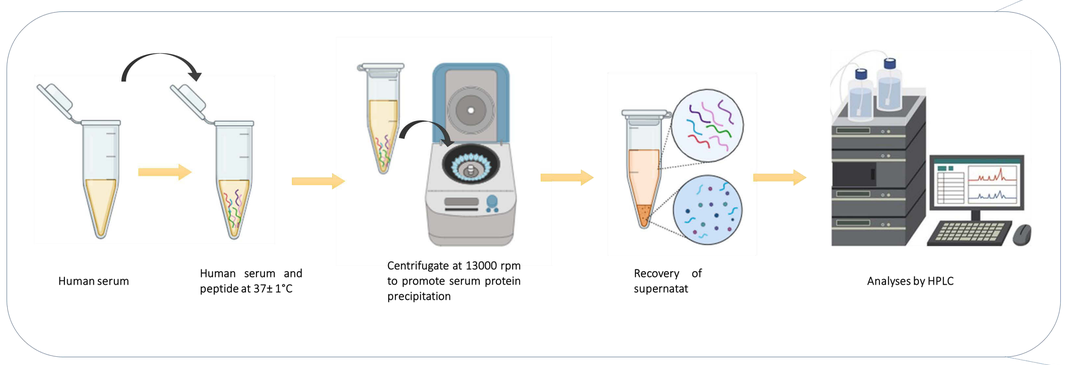
I_{440} and I_{490} are the emission intensities of Laurdan

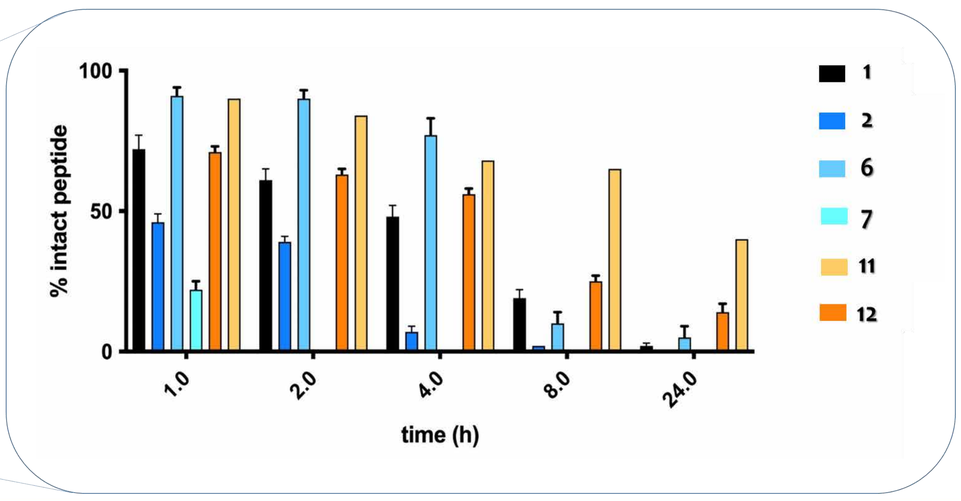
GP value

ID	Unloaded LUVs	LUVs + 20μM Compound	LUVs + 50μM Compound	LUVs + 100μM Compound
Gram negative mimicking membranes				
(2)	-0.21	-0.18	0.07	0.02
(6)	-0.22	-0.05	0.05	0.06
(7)	-0.20	-0.02	0.08	0.07
(12)	-0.18	-0.04	0.06	0.06

☑ Serum Stability

The **guanidino group**, like as pendant or bridge, confer greater stability to proteases, also after 4h or 8h (see analogues **6**, **11** and **12**).





Conclusions and Future Directions

The results suggest that the increase in antimicrobial activity is potentially related to the introduction of a further **positive charge**. The preliminary results of the **antimicrobial activity** and on **modes of interaction with membrans** obtained for these derivatives will guide the development of further cyclic analogues.

References

1. L.D. D'Andrea et al. *Int. J. Mol.* **2023**, 24, 5426.
2. F. Merlino et al., *Eur. J. Med. Chem.* **2017**, 139, 750–761.
3. R. Bellavita, et al. *J. Med.Chem.* **2021**, 64, 11675–11694.
4. F. Merlino et al., *Org. Lett.* **2019**, 21,6378–6382.

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