Peptides derived from palindromic sequence RWQWRWQWR exert an additive effect with fluconazole in a resistant *C. albicans* strain.

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Research Group: Synthesis and Application of Peptidic Molecules

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AIMS

To obtain modified peptides or lipopeptides derived from the palindromic sequence RWQWRWQWR, that might present antibacterial or antifungal activity and determine possible antifungal-peptide synergy interactions.

EXPERIMENTAL DESIGN

Design and synthesis of the peptides → **Evaluation of the antimicrobial activity** → *E. coli* ATCC 11775; *E. coli* ATCC 25922; *S. aureus* ATCC 25923; *C. albicans* SC 5314; *C. auris* (435); *C.auris* (537); *C. albicans* (256)

Synergy Test ←

METHODS

1) Synthesis and characterization of the peptides

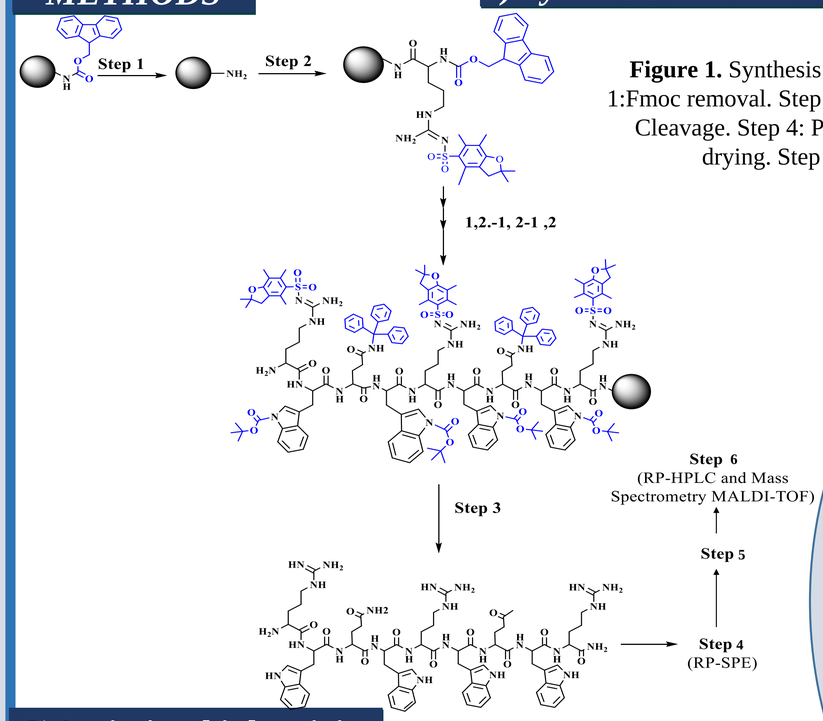
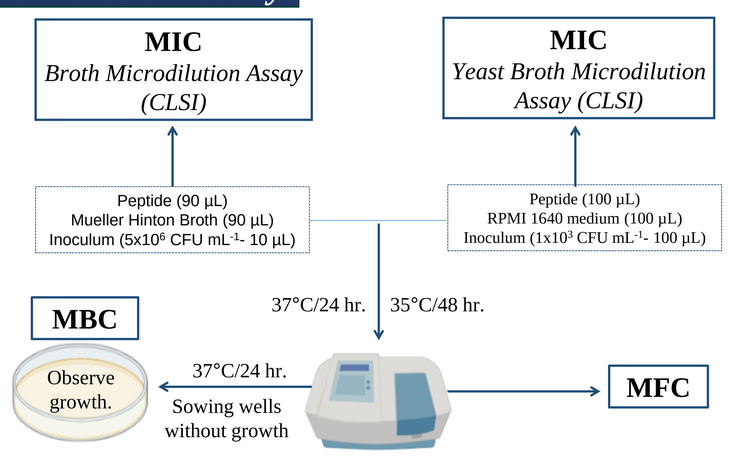


Figure 1. Synthesis of RWQWRWQWR. Step 1:Fmoc removal. Step 2: Coupling reaction. Step 3: Cleavage. Step 4: Purification. Step 5: freeze-drying. Step 6: Characterization

2)Antimicrobial activity



3) Synergy Test

Chessboard test

	1	2	3	4	5	6	7
A	2MIC						
B	MIC						
C	0,5MIC						
D	0,25MIC						
E	0,12MIC						
F	0,06MIC						
G	Strain	0,06MIC	0,12MIC	0,25MIC	0,5MIC	MIC	2MIC

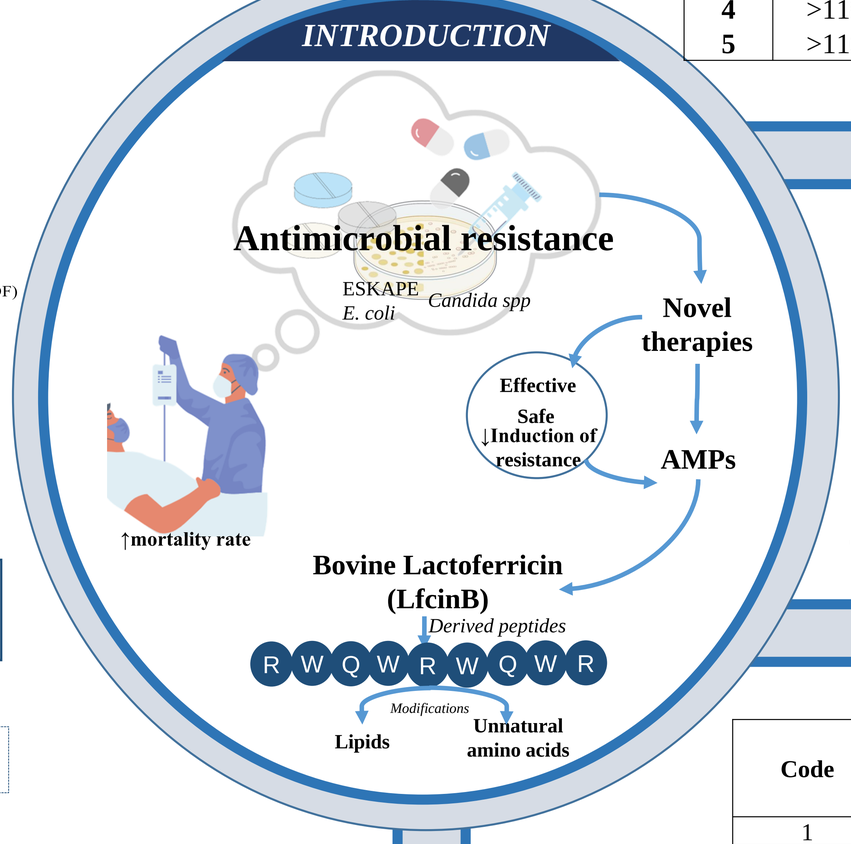
ANTIFUNGAL

PEPTIDE

Peptide and antibiotic
RPMI medium
Inoculum (2.5x10³ CFU mL⁻¹)

35°C/48 hr. → **FIC**

INTRODUCTION



Characterization

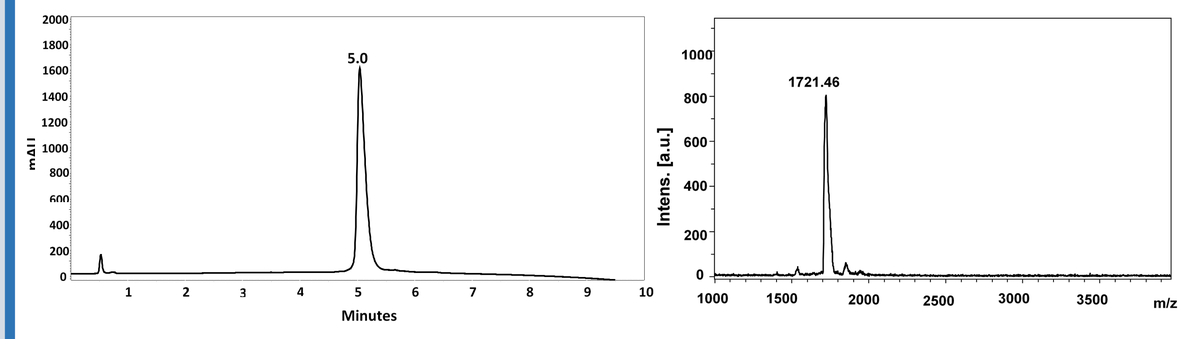


Figure 2. *Chromatogram (left) and mass spectrum (right) of pure lipopeptide (5).*

Antifungal activity

Code	*C. albicans*		*C. auris*	
	SC 5314	*256*	*435*	*537*
	MIC/MFC			
1	67/67	67/67	>135/>135	>135/>135
2	64/64	64/64	>127/>127	>127/>127
3	>120/>120	>120/>120	>120/>120	>120/>120
4	>118/>118	>118/>118	>118/>118	>118/>118
5	>116/>116	>116/>116	>116/>116	>116/>116

Tabla 2. *Antifungal activity of the molecules(µM).*

Synergy Test

Code	MIC_A (µg/mL)	MIC_P (µg/mL)	[A] (µg/mL)	[P] (µg/mL)	FIC	MIC_A/[A]
2	32	100	16	50	1.0	2
			4	100	1.1	8
			8	100	1.3	4
			16	100	1.5	2
3	32	400	8	200	0.8	4
			16	200	1.0	2

Table 3. *Evaluation of the synergistic effect against C. albicans (256) between fluconazole (A) and molecule (P)*

Antibacterial activity.

Code	*E. coli*		*S. aureus*
	ATCC 25922	*ATCC 11775*	*ATCC 25923*
	MIC/MBC		
1	17/34	17/17	135/135
2	16/64	16/64	64/127
3	>120/>120	>120/>120	>120/>120
4	>118>118	>118>118	>118>118
5	>116>116	>116>116	>116>116

Table 4. *Antibacterial activity of the molecules (µM)*

CONCLUSION

Using the SPPS-Fmoc / tBu strategy, it was possible to obtain four derivatives of LfcinB $(21\text{-}25)_{Pal}$, one peptide and three lipopeptides, which have not been reported in the consulted literature. The synthesis, purification and characterization methods developed made the obtaining of the molecules possible, thus recognizing their synthetic viability.

Synergy was assessed for *C. albicans (256)*, a fluconazole resistant clinical isolate, between fluconazole and molecule (2 and 3), finding reductions of two to eight times in the MIC of the antifungal, a positive contribution in the development of combined antibiotic therapies

RESULTS

Characterization

Code	Sequence	Characterization			
		RP-HPLC		MALDI-TOF $[M+H]^+$	
		t_R (min)	Purity (%)	Theorical	Experimental
1	RWQWRWQWR	5,8	99	1485,76	1486,85
2	Aib-RWQWRWQWR	5,8	76	1570,82	1570,34
3	Lac-RWQWRWQWR	8,5	80	1667,93	1657,14
4	MyA-RWQWRWQWR	4,3	94	1695,96	1688,46
5	Pam-RWQWRWQWR	5,0	99	1723,99	1721,46

Aminoisobutyric acid (Aib), Lauric acid (Lac), Myristic acid (MyA), and Palmitic acid (Pam)

Table 1. Characterization of the synthesized molecules .

References

- Vargas-Casanova Y, Carlos Villamil Poveda J, Jenny Rivera-Monroy Z, et al., Palindromic Peptide LfcinB (21-25)Pal Exhibited Antifungal Activity against Multidrug-Resistant Candida. *ChemistrySelect* (5) *7236–7242*. (2020)
- Ardila-Chantré N, Hernández-Cardona AK, Pineda-Castañeda HM, et al., Short peptides conjugated to non-peptidic motifs exhibit antibacterial activity, *RSC Adv.* (10) *29580–29586.* (2020)
- A. Makovitzki, D. Avrahami, Y. Shai, Ultrashort antibacterial and antifungal lipopeptides. *Proceedings of the National Academy of Sciences*, 103(43), *15997–16002.*(2006)
- E. Berkow, S. Lock, Fluconazole resistance in *Candida* species: a current perspective. *Infect Drug Resist*, (10), 237-245 (2017).
- 46. M. Bondaryk, M. Staniszewska, P. Zielińska,Z. Urbańczyk-Lipkowska, Natural Antimicrobial Peptides as Inspiration for Design of a New Generation Antifungal Compounds. *Journal of Fungi*, 3(3), *46* (2017)

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