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SYNTHESIS AND CHARACTERIZATION OF FERROCENE-PEPTIDE CONJUGATES WITH ANTIMICROBIAL ACTIVITY

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INTRODUCTION

Over the past five years we have synthesized a variety of ferrocene (Fc)-peptide conjugates with the aim of studying how the peptide backbone affects charge/electron transfer, Fc being an easy-to-handle electrochemical probe.[1] In addition, Fc is also able to promote the generation of reactive oxygen species (ROS) under physiological conditions. High levels of ROS are associated with cytotoxic effects. Therefore, Fc-peptide conjugates were designed to create new anticancer drugs.[2]

AIM

Here, we extended the same approach to the antibacterial field. We selected a set of ultrashort, antimicrobial lipopeptides, characterized by the presence of a non-coded His structural isomer (Figure 1), which we recently published.[3] We connected an Fc moiety to them via spacers of different lengths to evaluate whether Fc is able to enhance the antibacterial action, as reported in the literature.[4] A representative example is reported in Figure 1. In addition, we planned to explore the role of His* chirality as in our previous report the two His* of each lipopeptide were of the same configuration. The amino acid sequences of the six newly synthesized lipopeptides and of two reference compounds, previously published,[3] are reported in Table 1.

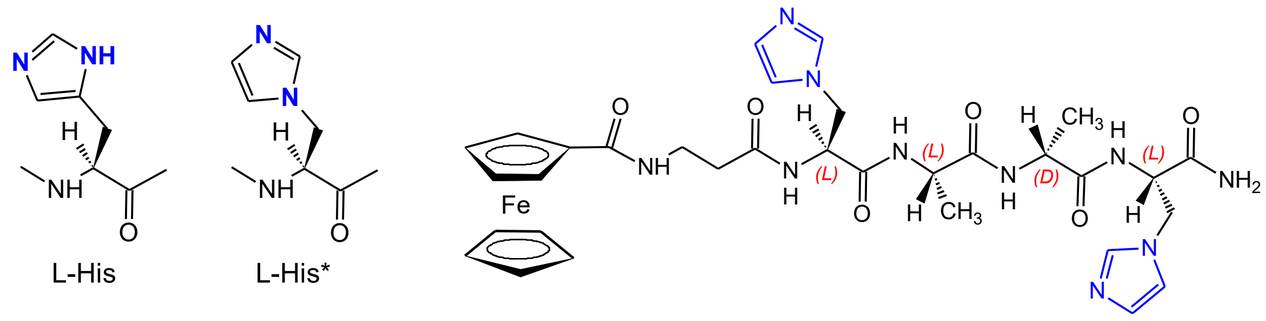


Figure 1. Molecular structures of His and its structural isomer His* and of Fc-βAla-His*-Ala-D-Ala-His*-NH_2.

Table 1. Amino acid sequences of the peptides discussed. Ahx, 6-aminohexanoyl; Laur, Lauroyl (dodecanoyl).

Code	Peptide sequence	Reference
1	Fc-CO-βAla-^{L}His*-Ala-^{D}Ala-^{L}His*-NH_2	This work
2	Fc-CO-βAla-^{D}His*-Ala-^{D}Ala-^{D}His*-NH_2	This work
3	Fc-CO-Ahx-^{L}His*-Ala-^{D}Ala-^{L}His*-NH_2	This work
4	Fc-CO-Ahx-^{D}His*-Ala-^{D}Ala-^{D}His*-NH_2	This work
5	Laur-^{L}His*-Ala-^{D}Ala-^{D}His*-NH_2	This work
6	Laur-^{D}His*-Ala-^{D}Ala-^{L}His*-NH_2	This work
7	Laur-^{L}His*-Ala-^{D}Ala-^{L}His*-NH_2	Ref. 3
8	Laur-^{D}His*-Ala-^{D}Ala-^{D}His*-NH_2	Ref. 3

Table 2. Bacteria growth inhibition after 20 hours from peptide incubation at 37 °C. The values are the diameters of inhibition in millimeters, the higher the better.

	Microorganism strain	Peptide							
		1	2	3	4	5	6	7[a]	8[a]
Gram-positive	Staphylococcus aureus ATCC 25923	-	-	-	-	-	-	9	12
	Streptococcus pyogenes ATCC 19615	-	-	-	-	-	-	-	17
	Enterococcus faecalis ATCC 29212	-	-	-	-	-	-	12	9
	Bacillus subtilis ATCC 6633	-	-	-	-	8	12	11	12
	Vancomycin-resistant enterococcus ATCC 51299	-	-	-	-	-	-	b	b
Gram-negative	Escherichia coli ATCC 25922	-	-	-	-	-	-	9	7
	Pseudomonas aeruginosa ATCC 27853	-	-	-	-	-	-	8	8
	Acinetobacter Baumannii ATCC 19606	-	-	-	-	-	-	-	-
	Proteus mirabilis ATCC 25933	-	-	-	-	-	-	b	b
	Klebsiella pneumoniae ATCC 13883	-	-	-	-	-	-	b	b

[a]Data from ref. 2. [b] Strain not investigated in ref. 2

DATA INTERPRETATION

The action of the six new peptides (Table 2) does not correspond to expectations, contrary to what happened with literature Lys-rich hexapeptides.[4] Insertion of Fc did not enhance the antibacterial activity of our lead tetrapeptide, but actually eliminated it. Perhaps, the too bulky Fc prevents the peptide from interacting properly with the membrane. We are therefore planning the synthesis of an analog with a longer spacer, so that the peptide can first bind to the membrane and then possibly drag Fc inside. Compounds **5** and **6** with the two His* of opposite configurations are active only against *bacillus subtilis*. The mechanism of action might involve membrane insertion of the lauroyl group and a subsequent electrostatic interaction of the positively charged His* with the negatively charged bacterial membranes. Then, the different behavior could result from a less productive alignment of the positive charges that disadvantages interaction with membranes.

CONCLUSIONS

- Replacement of lauroyl with Fc in an antimicrobial lipopeptide abolished the activity. This outcome is ascribable to the too close proximity of Fc to the peptide. We are now planning the synthesis of analogsa with a longer spacer between Fc and the peptide.
- Chirality plays an important role in the potency of our lead, antibacterial lipopeptide.3
- Chirality could, however, be exploited to design antimicrobial peptides capable of selectively targeting specific bacterial strains.

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