Optimizing biological activity of Lactoferricin derived peptides: Conjugation with non peptidic motifs as a strategy

Daniel Castellar [1] , Javier García [1] , Zuly Rivera [2] , Ricardo Fierrro [2]

[1] Pharmacy Department, Universidad Nacional de Colombia– Bogotá D.C, Colombia

[2] Chemistry Department, Universidad Nacional de Colombia– Bogotá D.C, Colombia

https://doi.org/10.17952/37EPS.2024.P2213

37th EPS European Peptide Symposium

14th IPS International Peptide Symposium

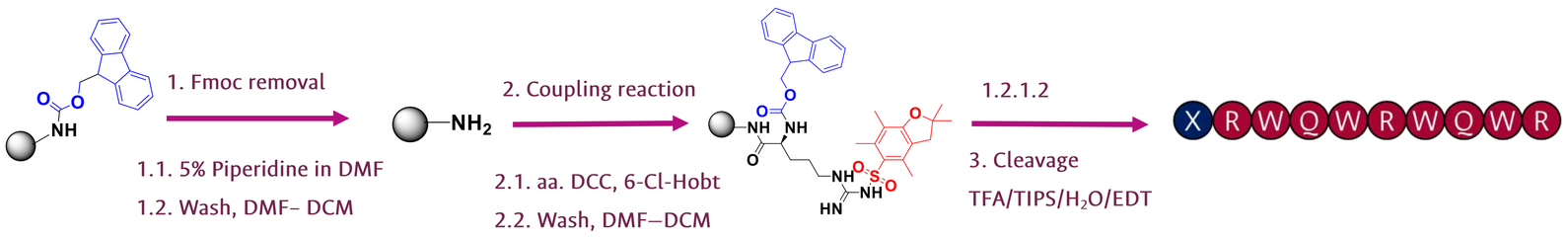
Florence, Italy 25 - 29 August 2024

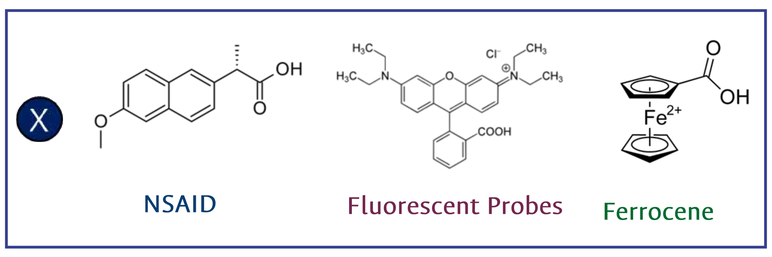
INTRODUCTION

Synthetic peptides derived from Bovine Lactoferricin have been established as promising molecules against multiple cancers. The palindromic sequence RWQWRWQWR has demonstrated selective cytotoxic activity against breast and cervical cancers, establishing this peptide as a potential anticancer agent. To broaden the biological applications of this sequence, we utilized three different functional moieties to enhance the peptide's biological activities: i) non-steroidal anti-inflammatory Drugs (NSAID) , which could optimize the anticancer activity of the sequence by modulating anti-inflammatory signals; ii) fluorescent probes, enabling the peptide to be used as a bioanalytical tool in cancer cell imaging; and iii) ferrocene, which could enhance redox reactions within cancer cells. Some of these motifs proved useful for optimizing the cytotoxic activity of the core sequence and for enabling the peptide to be used in other applications such as cancer cell imaging.

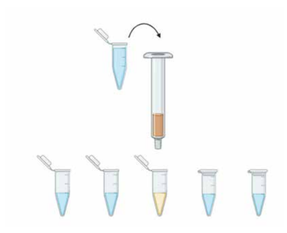
METHODS

1). Solid Phase Peptide Synthesis

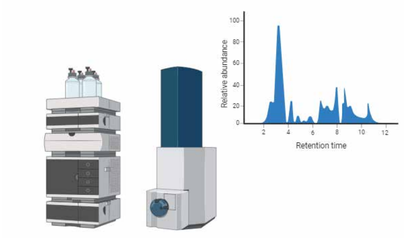




2). Peptide purification and Characterization

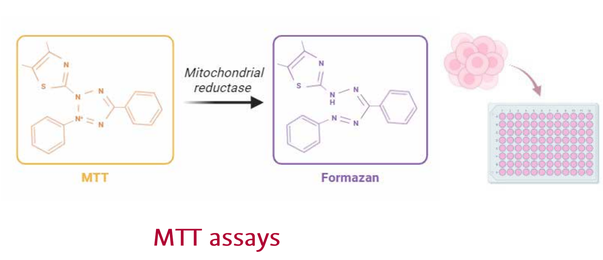


RP– SPE Purification



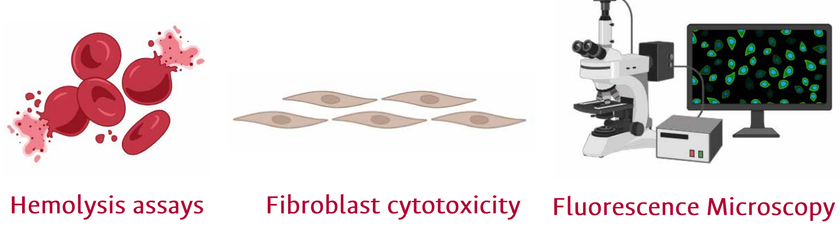
LC– ESI –QTOF

3). Cytotoxic Activity in Cancer Cells



MTT assays

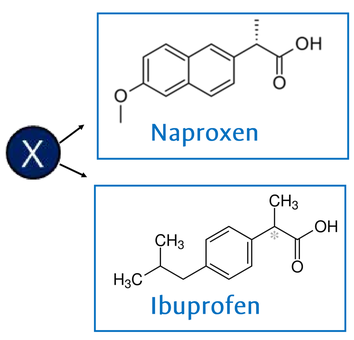
4). Other biological assays



Hemolysis assays | Fibroblast cytotoxicity | Fluorescence Microscopy

RESULTS AND DISCUSSION

NSAID— LfcinB conjugates



Ornithine Moiety Spacer

Enhance Solubility (Direct conjugation of NSAID produce water insoluble peptides)

Palindromic sequence

Lactoferricin derived peptide with in vitro anticancer activity

Code	Sequence	t_R (min)	Purity (%)	Expected mass (amu)	Experimental mass (amu)
$(Orn)_3$-$(LfcinB)_{Pal}$	OOORWQWRWQWR	5,7	99,9	1828,0000	1828,0125
NAP $(Orn)_3$-$(LfcinB)_{Pal}$	NAP-OOORWQWRWQWR	7,0	94,3	2040,0900	2040,1200
IBU $(Orn)_3$-$(LfcinB)_{Pal}$	IBU-OOORWQWRWQWR	7,5	97,7	2016,1200	2016,1340

Table 1. Characterization of NSAID conjugated peptides optimized with a polar Ornithine moiety spacer

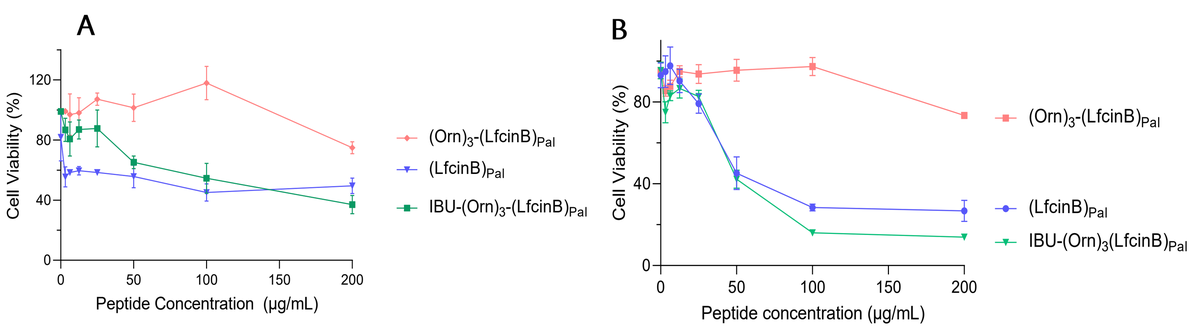


Figure 1. Cytotoxic activity of the Ibuprofen conjugated peptide IBU $(Orn)_3$-$(LfcinB)_{Pal}$ compared to the activity of the palindromic sequence and its non conjugated ornithine derivate against **A)** MCF-7 (breast cancer) and **B)** HeLa (cervical cancer) cells. Experiments were conducted in triplicates (n=3)

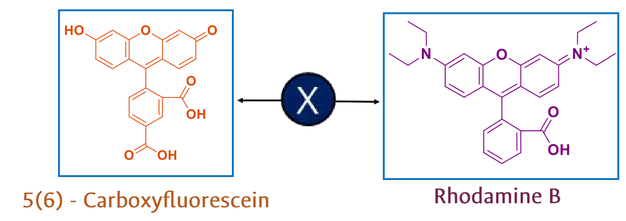
Code	Sequence	IC 50 µM (HeLa)	IC50 µM (MFC-7)	IC50 µM Fibroblast (L929)	Hemolysis (%) *	Selectivity Index
$(Orn)_3$-$(LfcinB)_{Pal}$	OOORWQWRWQWR	>109	> 109	>109	< 10 %	N.A
NAP $(Orn)_3$-$(LfcinB)_{Pal}$	NAP-OOORWQWRWQWR	85	> 98	> 98	<10 %	>1
IBU $(Orn)_3$-$(LfcinB)_{Pal}$	IBU-OOORWQWRWQWR	29	55	> 81	<10 %	>2

Table 2. Summary of the cytotoxic activity of the NSAID conjugated peptides against cancer cells, fibroblast and erythrocytes. *Percentage of hemolysis is reported at a maximum concentration of 200 µg/mL

Ferrocene— Lfcin B conjugates

Figure 4. Representative RP-HPLC-chromatogram depicting the degradation process of the Ferrocene conjugated peptide optimized with an Ornithine moiety. A) Crude peptide B) Peptide after RP-SPE purification process

Fluorescent probes— Lfcin B conjugates



Palindromic sequence

Code	Sequence	t_R (min)	Purity (%)	Expected mass (amu)	Experimental mass (amu)
(5(6) FAM)-$(LfcinB)_{Pal}$	(5(6) FAM)-RWQWRWQWR	8,2	96,9	1843,8100	1843,8088
(RhodB)-$(LfcinB)_{Pal}$	(RhodB)-RWQWRWQWR	*9,8 and 10,1	30,2 and 65,0	1910,9900	1910,9800

Table 3. Characterization of the fluorescent peptides obtained.* Rhodamine B peptide is a mixture of two isomers

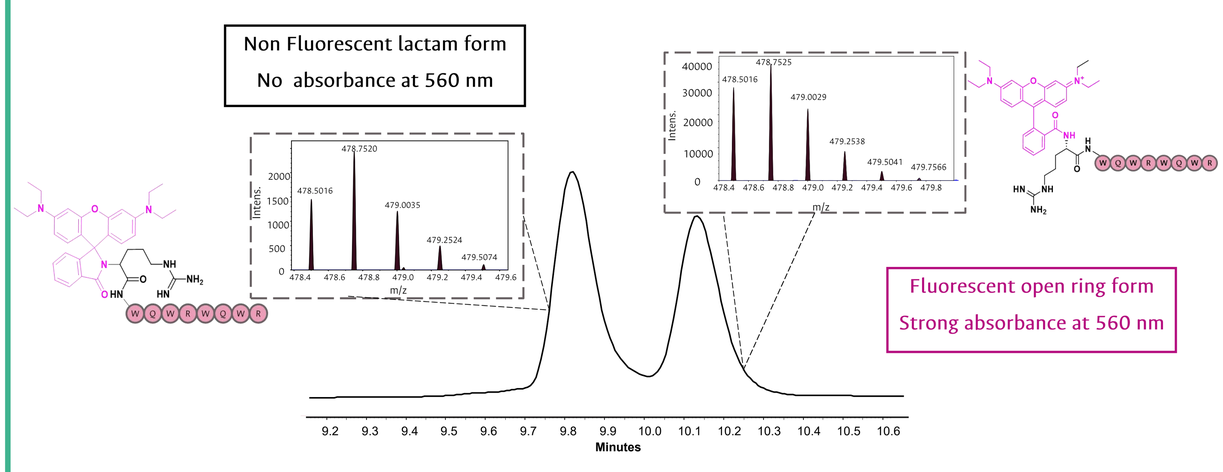


Figure 2. Representative RP-HPLC-chromatogram of the Rhodamine B conjugated peptide presenting the pair of isomers detected by LC-ESI-QTOF (isotopic distribution showed) which correspond to an open ring—lactam equilibrium

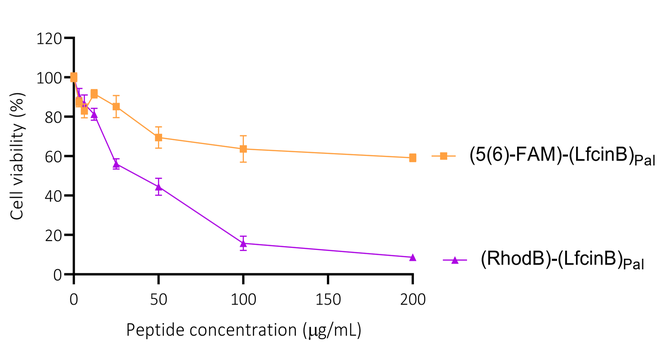


Figure 3. Cytotoxic activity of the fluorescein and Rhodamine B conjugated peptides against MCF-7. Experiments were conducted in triplicates (n=3)

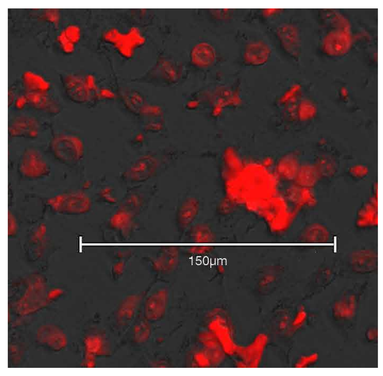


Figure 4. Fluorescence Microscopy images of MCF-7 cells incubated with 200 µg/mL of the RhodB conjugated peptide

CONCLUSION

The fluorescent probe Rhodamine B was successfully conjugated to the amino terminal region of the palindromic peptide, and the resulting fluorescent product retained the cytotoxic activity of the core sequence. Fluorescence microscopy revealed colocalization of the fluorescent peptide with MCF-7 cells, demonstrating its potential for cancer cell imaging.

Moreover, the conjugation of NSAID to the palindromic sequence was effectively achieved using an ornithine moiety, which enhanced the solubility profile. The ibuprofen-conjugated peptide exhibited enhanced selective cytotoxic activity against MCF-7 and HeLa cells, in contrast to the non-conjugated ornithine counterpart, which lost its cytotoxic activity.

For the case of the organometallic—LfcinB conjgutates, a degradation process was observed in solution for the ferrocene-conjugated peptide with the same ornithine spacer, preventing this molecule from being evaluated in biological assays. Further experiments are required to study the stability of this organometallic conjugate.

Overall we succesfully obtained novel Ibuprofen-LfincB conjugates with enhanced selective cytotoxic activity againts cervical and breast cancer cells. Additionally, we demonstrated the effect of conjugating the fluorescent probe Rhodamine B on the cytotoxic activity of the palindromic peptide and its utility for future experiments involving cell distribution and imaging.

ACKNOWLEDGEMENTS

We thank PhD students Karla Rodriguez , Natalia Ardila and PhD Andrea Barragan for their support with cytotoxic activity assays. We also thank Fernando Chavez and Dennis Salazar for their support with peptide synthesis and the research group leaders for their assessment

REFERENCES

Ardila-Chantré, N., Hernández-Cardona, A. K., Pineda-Castañeda, H. M., Estupiñan- Torres, S. M., Leal-Castro, A. L., Fierro-Medina, R., ... & García-Castañeda, J. E. (2020).. RSC advances, 10(49), 29580-29586.

Barragán Cárdenas, A., Insuasty Cepeda, D. S., Niño Ramírez, V. A., Umaña Pérez, A, Ochoa Zarzosa, A., López Meza, J. E., ... & García Castañeda, J. E. (2020). ChemistrySelect, 5(31), 9691-9700.