https://doi.org/10.17952/37EPS.2024.P1062

37th European Peptide Symposium and
14th International Peptide Symposium

# Antimicrobial activity of Histatin 5 based metallopeptides

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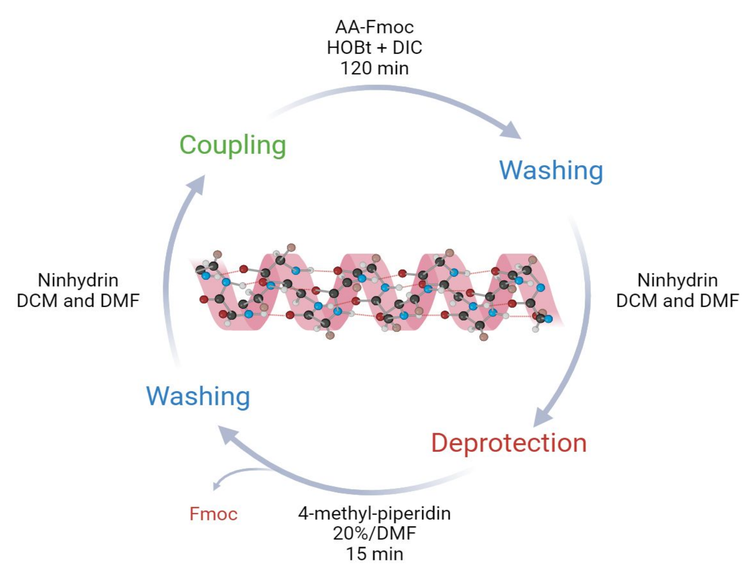
## PURPOSE

With the aim of developing new antifungal agents to combat *Candida albicans* infections, whose resistance is increasing worldwide[1], proteolytic fragments of Histatin 5[2], a salivary peptide, were complexed with silver ions. Studies have reported that Hst5 activity is enhanced when arginine residues replace some lysine residues[3]. Therefore, this amino acid exchange was applied to the developed fragments, which were also complexed with silver ions.

## METHODOLOGY

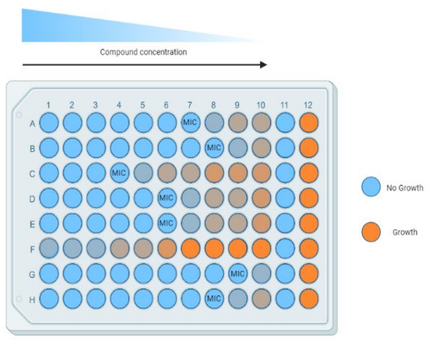



MALDI-TOF
Mass spectrometry

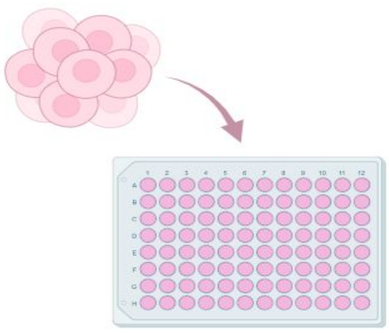
Circular Dichroism

14 Hst5
14 Hst5 K11,17R

+ 47 Ag




*C. albicans*
ATCC 10231

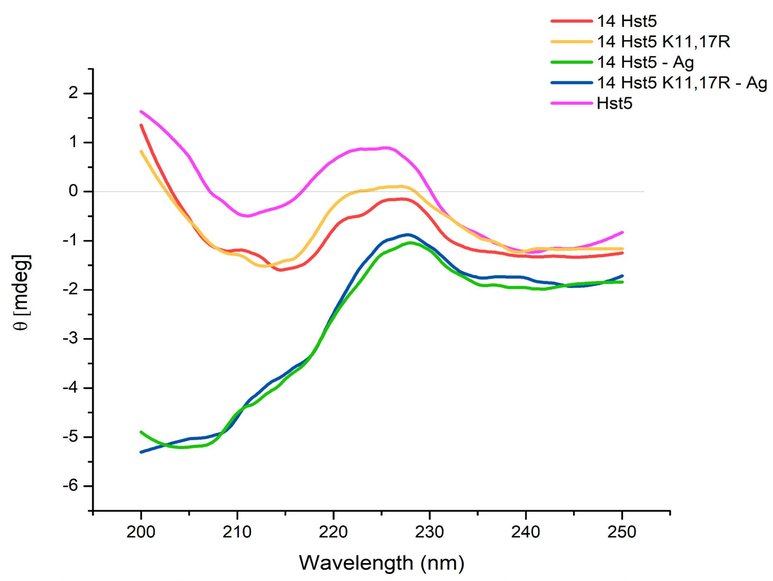


*Oral squamous cell carcinoma*

## RESULTS

| Metallopeptide | Theoretical molecular mass (g mol $^{-1}$) | Experimental molecular mass (g mol$^{-1}$) |
|---|---|---|
| *14 Hst5 – Ag* | 2141,13 | 2129,92 |
| *14 Hst5 K11,17R – Ag* | 2197,16 | 2195,98 |

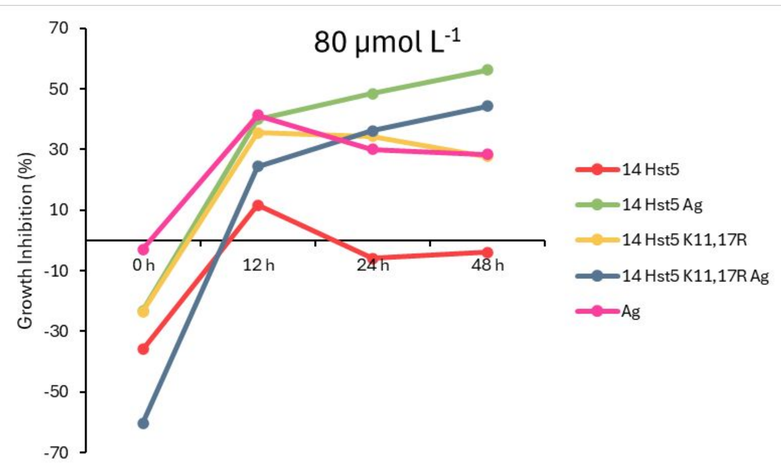
**MALDI-TOF mass spectrometry** comparing the experimental molecular mass obtained for the two metallopeptides and the predicted theoretical molecular mass. The coordination compounds were prepared in a 1:1 (v/v) ratio with a 10 mg/mL 2,5-dihydroxybenzoic acid (DHB) matrix, analyzed by a Bruker Daltonics Ultraflextreme MALDI spectrometer, with TOF/TOF analyzer, and MS and MS/MS data acquisition (reflector mode).




**Circular dichroism** showing the secondary structure of the peptides, which is similar to the Hst5 alpha-helix, and the random structure adopted by the peptides when coordinated with Ag(I). The peptide and metallopeptide solutions were prepared in water at a concentration of 10 µmol L$^{-1}$, for a final volume of 500 µL. The spectra were obtained with an accumulation of 12 scans, from 200 to 250 nm, with measurements every 0.1 nm, at a speed of 100 nm min$^{-1}$.

| Concentration (µmol L$^{-1}$) | Compound | % Inhibition (planktonic cells)[1] | % Inhbition (biofilm)[2] | % Cell viability (OSCC) |
|---|---|---|---|---|
| 160 | *14 Hst5* | 96,82 | 97,61 | 47,08 |
| | *14 Hst5 – Ag* | 93,47 | 95,97 | 0 |
| | *14 Hst5 K11,17R* | 98,44 | 71,15 | 44,94 |
| | *14 Hst5 K11,17R - Ag* | 93,58 | 96,89 | 0 |
| 80 | *14 Hst5* | 96,91 | 81,75 | 55,25 |
| | *14 Hst5 – Ag* | 97,06 | 94,68 | 0 |
| | *14 Hst5 K11,17R* | 96,94 | 0,0 | 50,33 |
| | *14 Hst5 K11,17R - Ag* | 97,35 | 96,39 | 20,46 |
| 40 | *14 Hst5* | 6,51 | 0,55 | 51,35 |
| | *14 Hst5 – Ag* | 97,54 | 87,52 | 25,12 |
| | *14 Hst5 K11,17R* | 94,27 | 0,41 | 49,43 |
| | *14 Hst5 K11,17R - Ag* | 97,19 | 95,22 | 45,32 |
| 20 | *14 Hst5* | 6,03 | 0,76 | 61,86 |
| | *14 Hst5 – Ag* | 97,19 | 1,41 | 73,90 |
| | *14 Hst5 K11,17R* | 6,31 | 0,16 | 51,51 |
| | *14 Hst5 K11,17R - Ag* | 97,33 | 15,30 | 76,46 |

**Inhibition of *Candida albicans* growth in planktonic and biofilm conformation by peptides and metallopeptides and cell viability of OSCC.** The concentrations of peptides and metallopeptides in the inhibition assays were 160 to 5 µmol L$^{-1}$ for planktonic cells and 320 to 10 µmol L$^{-1}$ for biofilm. The peptides and metallopeptides were incubated with the sponsored cells for 48 h before reading the OD at 595 nm. The peptides and metallopeptides were incubated with OSCC cells at the same concentration as in the planktonic cell assay for 48 h, and the resazurin method was used to evaluate cell viability by reading the OD at 570 and 600 nm.




**Inhibition of mature biofilm growth by peptides and metallopeptides at 80 µmol L$^{-1}$.** Standardized *C. albicans* (ATCC 10231) were incubated for 48 h in a 96-well cell plate for maturation. The plate was washed with PBS solution, and peptides and metallopeptides were added at a concentration of 360 to 10 µmol L$^{-1}$. OD reading at 595 nm was taken 0, 12, 24, and 48 h after peptides and metallopeptides were added. The percentage of growth inhibition was calculated according to the positive and negative growth controls.

## CONCLUSION

The coordination between the proposed peptides and Ag(I) occurred effectively, in the molar ratio 1:1. The antifungal activity against biofilms was optimized in both peptides when compared with Hst5, and the coordination with Ag(I) further improved the inhibition of growth of resistant *C. albicans*. The metal without the presence of the peptide did not show the same inhibition profile against the same strains, reinforcing the synergism. The cytotoxicity presented by the compounds were below 50% in most of the cases.

## FUNDING AND ACKNOWLEDGMENTS

CAPES
FAPESP
Instituto de Química

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