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Antimicrobial peptidomimetics against methycillin-resistant *Staphylococcus aureus* (MRSA)

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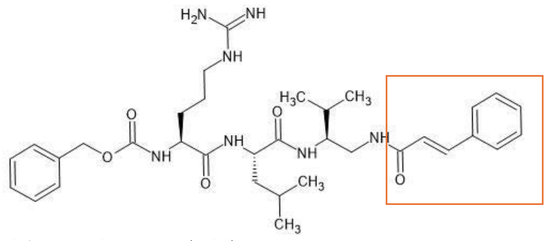
INTRODUCTION

Staphylococcus aureus is considered the leading cause of human diseases not only in hospitalized individuals, but also in individuals living in the community. *Staphylococcus aureus* mainly causes skin infections, such as purulent wounds, soft tissue inflammation, impetigo, and may also cause inflammation of the endocardium, lungs, and bone marrow. The number of infections caused by MRSA strains is increasing around the world. WHO predicts that by 2050, mortality caused by AMR (antimicrobial resistance) will be one of the main causes of death. [1]
In this work, we report the development of novel peptidyl derivatives based upon the structure of the *N*-terminal fragment ($R^8L^9V^{10}G^{11}$) of the human cystatin C protein. A derivative called Cystapep 1 (A-20) has a strong activity against Gram-positive bacteria, including MRSA strains. [2] We synthesized analogues of Cystapep 1 with modifications at the highlighted site on Scheme 1.



Scheme 1. Cystapep 1 (A-20) structure.

QSAR STUDIES

The QSAR analysis of Cystapep 1 and its derivatives was essential to establish which elements of the molecule are crucial for its antibacterial activity. Analysis was carried out based on 80 Cystapep 1 derivatives with established antibacterial activity for *Staphylococcus aureus* and computed descriptors. The calculated descriptors represented different levels of chemical structures/properties and therefore different theoretical dimensions (0-3D).
The simplest, constitutional and topological descriptors (ON1V, GNar, ZM1V), which encode information about molecular weight, size and shape, were obtained using the Dragon-7 software. More complex electronic descriptors related to the 3D conformation of the molecule, were calculated using the MOPAC2016 package. These descriptors were used for principal component analysis (PCA) (Figure 2). This graph shows that ON1V, volume, ZM1V, LogS and Molecular weight are the most influential variables in PC1, while MLOGP, TPSA, LogP, and LogKp form PC2. The classification tree shown in Figure 1 reveals four descriptors as being the most effective in determining the high activity against *Staphylococcus aureus*. These descriptors are lipophilicity, TPSA (topological polar surface area), Hy (hydrophilic factor) and ZM1V (topological/structure descriptor). On this basis, we designed new derivatives with potentially high antibacterial activity. By using a classification tree, we were able to predict, which peptidomimetic has high (Klasa-3) or low (Klasa 1) activity against *Staphylococcus aureus.*

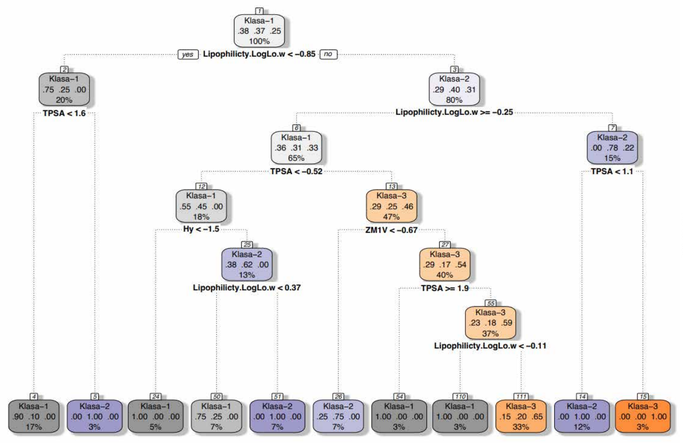


Figure 1. Classification tree.

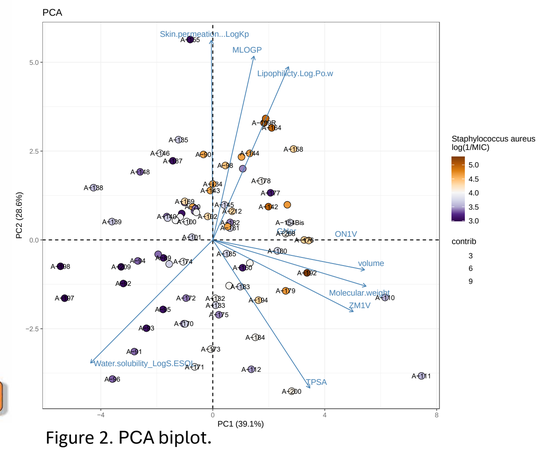


Figure 2. PCA biplot.

The most promising molecules A-75, A-76, A-238, A-241, A-243, and the leading structure A-20 were examined on MRSA isolates: RA636, O2449, 342, 124, 115, 44, 343, O2229, RA532, RA604, 352 (clinical and laboratory strains) by measuring the growth of bacterial cells for 20h (Figure 3). The peptidomimetics have strong inhibitory effect on MRSA growth. No differences were observed between concentrations inhibiting bacterial cells growth, only one strain MRSA 342 was more resistant, and inhibition concentration was one fold higher.

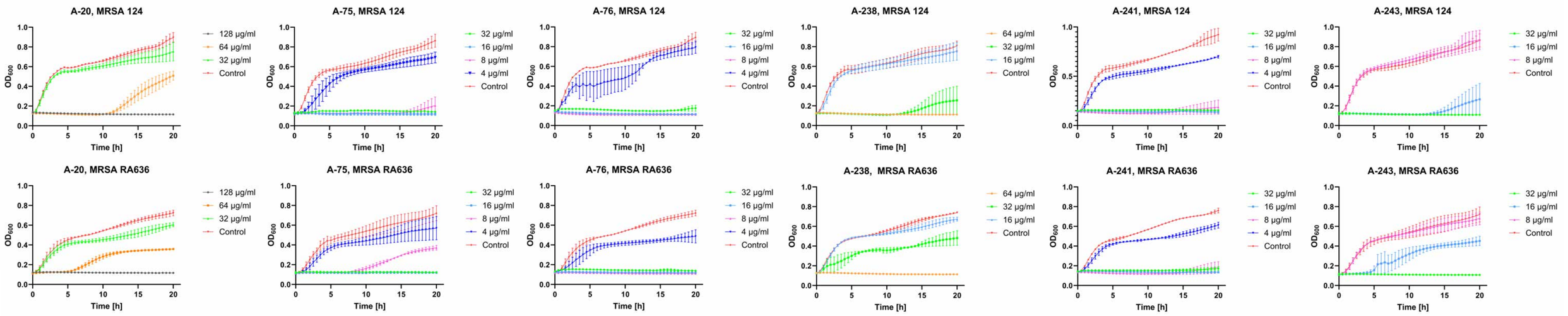


Figure 3. A-20, A-75, A-76, A-238, A-241, A-243 effect on MRSA 124 (clinical strain) and MRSA RA636 (laboratory strain) growth in different concentrations.

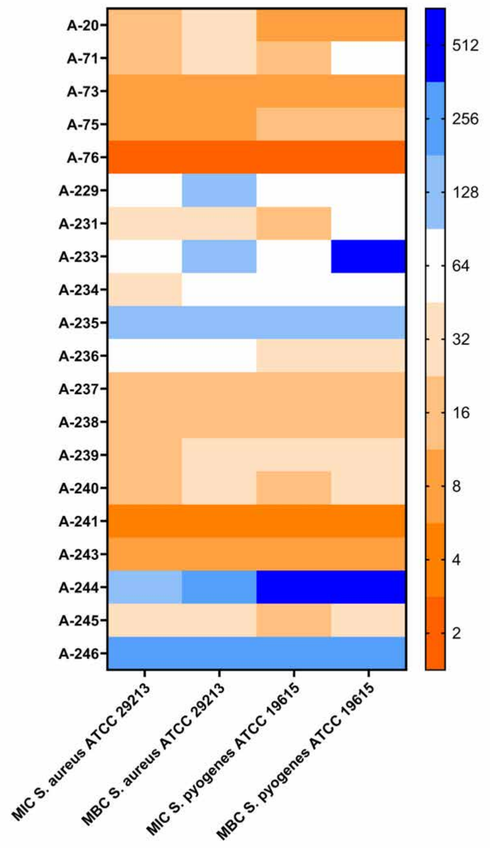


Figure 4. A-20 and its analogues antimicrobial activity against *Staphylococcus aureus* and *Streptococcus pyogenes.*

The susceptibility of bacterial strains of *S. aureus* and *S. pyogenes* has been examined by broth microdilution tests. The most active peptidomimetics are A-73, A-75, A-76, A-241, A-243 (Figure 4). Most of these compounds have similar activity on both tested bacterial strains. There was no difference between MIC (minimal inhibitory concentration) and MBC (minimal bactericidal concentration) value is two fold higher than MIC value.

CYTOTOXICITY

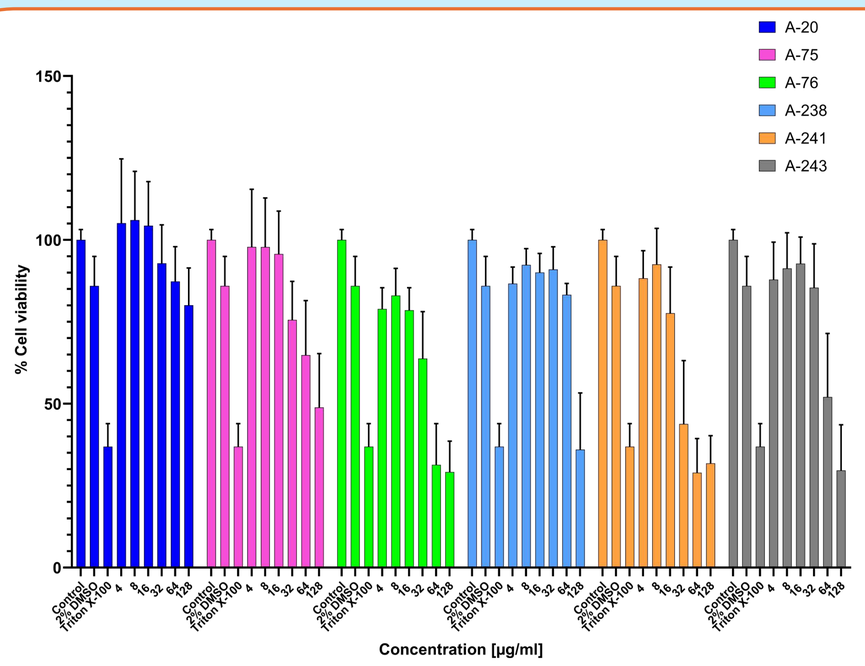


Figure 5. A-20, A-75, A-76, A-238, A-241, A-243 cytotoxicity effect on HaCaT cells.

To investigate the cytotoxicity of the peptidomimetics (Figure 5), a Neutral Red assay was performed on human keratinocytes (HaCaT cells). Cells were cultured with compounds at different concentrations for 24h. Maximum cytotoxicity was determined as 0.1% Triton X-100 solution, and a control for the 2% DMSO solution was performed, in which the compounds were dissolved. A-20 show no negative effect on HaCaT cells. The analogues A-75, A-238, A-243 do not show cytotoxicity at concentrations below 32 µg/ml, A-76, A-241 at concentrations below 16 µg/ml.

CONCLUSIONS

- Principal component analysis (PCA) showed that the more lipophilic molecule is, the more antimicrobial activity it has. Water-soluble molecules are characterized by low activity.
- A-20 and its analogues have a strong inhibitory effect on the growth of MRSA (both clinical and laboratory strains). There is no difference in MRSA growth inhibition between laboratory and clinical strains.
- The tested peptidomimetics do not show cytotoxicity concentrations at which they inhibit the growth of MRSA.

[1] J. O'Neill, Tackling drug-resistant infections globally : final report and recommendations, The Review on Antimicrobial Resistance, 2016
[2] [2] Dzierżyńska M., Sikorska E., Pogorzelska A., Mulkiewicz E., Sawicka J., Wyrzykowski D., Małuch I., Grubb A., Kasprzykowski F., Rodziewicz-Motowidło S., *Prot Pep Letters*, 2019, *26*, 1-12.

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