SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NEW ANALOGUES OF AUREIN 1.2

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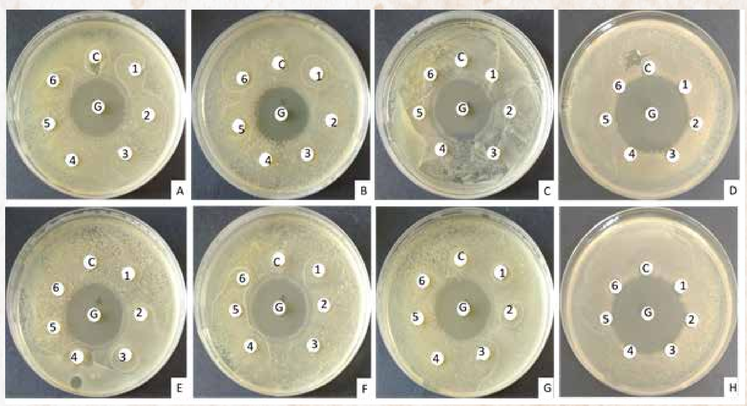
INTRODUCTION

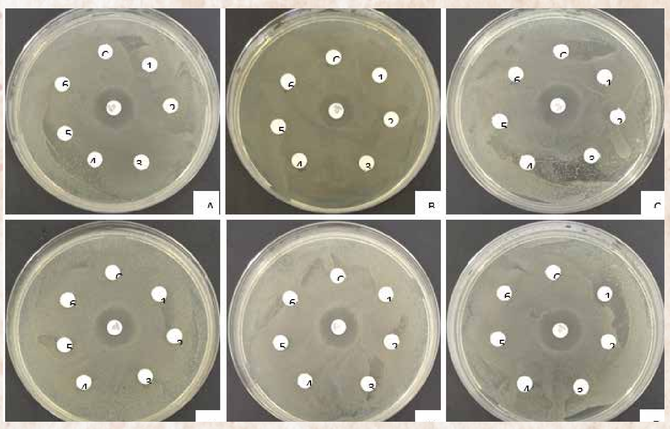
Antimicrobial peptides are identified as the most promising option to the conventional molecules used nowadays against infections because they mimic the natural metabolic effects within the body with low toxicity and better selectivity which proves they're one of the alternatives for therapeutic intervention. Aurein 1.2 is a short multi-functional antimicrobial peptide isolated from Litoria aurea and Litoria raniformis skin secretion. Aurein peptides can be classified into five distinct groups (Aureins -1 to -5), among which Aurein-1 peptides are considered to be one of the shortest α-helical peptides with antimicrobial and anticancer activity. Many antimicrobial peptides have the ability to cross cell membranes and destroy bacteria. Biological activity of Aurein has been widely investigated however, its lack of a potent action failed to provide Aurein 1.2 with a competitive edge for further development as a therapeutic agent for clinical use.

METHODS

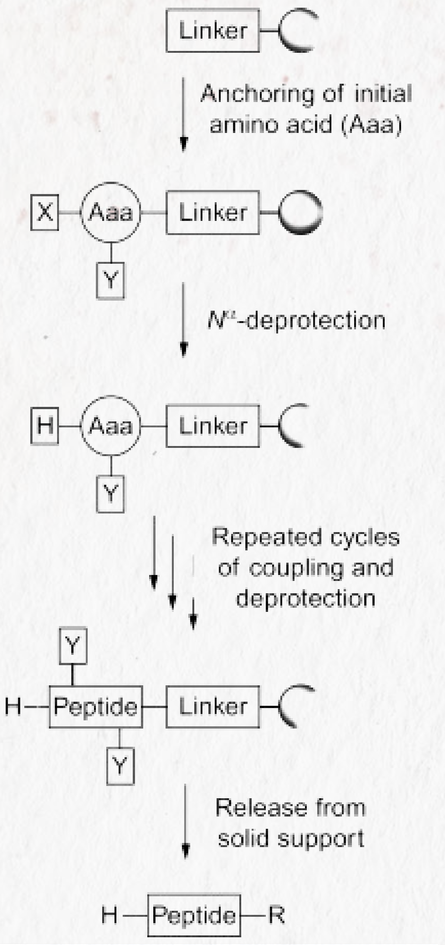
The conventional solid-phase peptide synthesis based on Fmoc (9-fluorenylmethoxycarbonyl) chemistry was employed to synthesize a series of new analogs of Aurein 1.2. Rink-amide MBHA resin was used as a solid-phase carrier to obtain C-terminal amides. TBTU (2-(1Hbenzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate) or DIC (N,N′-Diisopropylcarbodiimide) were used as a coupling reagents. The peptide purity was monitored on a RP-HPLC. The LC/MC spectra were recorded on a LTQ XL OrbitrapDiscovery instrument. The optical rotation was also measured. The obtained new analogues were estimated for their antibacterial activity against Gram positive Bacillus subtilis strain 3562 and Gram-negative E. coli NBIMCC 8785 using broth microdilution method in concentrations from 0 to 320 μg/mL to determine the minimal inhibitory concentration (MIC).



The disk diffusion assay - B. subtilis 3562
Legend: A – Aurein 1,2; B - EH [Orn]7; C - EH [Orn]7,8; D – EH[Dap]7,8; E - EH[Dab]8; F - EH[Dab]7; G - EH[Dab]7,8; H – EH[Orn]8
1 – 10 μg/ml peptide; 2 - 20 μg/ml peptide; 3 – 40 μg/ml peptide; 4 – 80 μg/ml peptide; 5 – 160 μg/ml peptide; 6 – 320 μg/ml peptide; G –10 μg/disc Gentamicin;



The disk diffusion assay - E. coli K12



PURPOSE

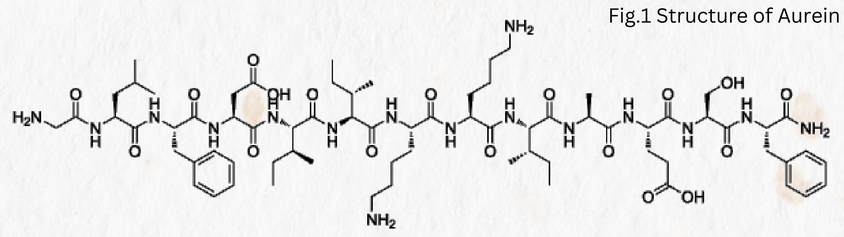
Aurein 1.2 was chosen as a template for rational modification to achieve a higher bio-functionality. The modifications of the six new analogues were made in the primary structure by incorporating unnatural amino acids in order to improve the pharmacodynamics of the new peptides. Aurein 1.2 was modified at position 7 and 8.

The six new modified analogs followed the structure:

Gly-Leu-Phe-Asp-Ile-Ile-**Xxx**^{7}-**Xxx**^{8}-Ile-Ala-Glu-Ser-Phe-NH_2

where Xxx is Orn or Dab (diaminobutyric acid). Lysine residues were replaced sequentially and simultaneously with the unnatural amino acids.

Fig.1 Structure of Aurein 1.2



RESULTS

The MIC of the original peptide Aurein 1,2 is 160 μg/ml, and the MIC of the derivatives decreases to 80 μg/ml, which indicates better antibacterial activity of the newly synthesized peptides. Only in EH [Dab] 7,8 the value of MIC is the same as in the starting compound (Table 1).

The values of the minimum bactericidal concentration are higher than the MIC - 320 μg/ml for the newly obtained compounds, and for EH [Dab] 7,8 and the starting peptide are not reported, which means that the derived compounds have a bacteriostatic effect against B. subtilis 3562. The replacement of lysine with the unnatural amino acids Orn and Dab leads to a increase in the antibacterial activity of the obtained peptides compared to Aurein 1,2 against the B. subtilis 3562.

The test results with Gram negative strain E. coli K12, showed that the MIC of the newly synthesized peptides increased - 80 μg/ml, even for EH [Dab] 7,8 the MIC was 160 μg/ml, compared to the starting peptide Aurein 1,2, in which MIC is 40 μg/ml. This means that in the case of derivative compounds, the antibacterial effect is significantly reduced. A minimum bactericidal concentration was not reported against E. coli K12, which indicates a very weak bacteriostatic effect of the studied peptides against this strain.

Name	*Bacillus subtilis* 3562		*Escherichia coli* K12	
	MIC μg/ml	MBC μg/ml	MIC μg/ml	MBC μg/ml
1. Aurein 1,2	160	NA	40	NA
2. EH [Orn]^{7}	80	320	80	NA
3. EH [Orn]^{8}	80	320	40	NA
4. EH [Orn]^{7,8}	80	320	80	NA
5. EH [Dab]^{7}	80	320	80	NA
6. EH [Dab]^{8}	80	320	80	NA
7. EH [Dab]^{7,8}	160	NA	160	NA

Table 1. Minimal inhibitory concentration and minimal bactericidal concentration of tested peptides against B. subtilis 3562 and E. coli K12

CONCLUSIONS

The replacement of Lysine with the unnatural amino acids Orn and Dab in the primary structure of Aurein 1.2 leads to a significant increase in the antibacterial activity of the obtained peptides against the investigated microorganisms

[1] Roudi, R.; Syn, N.L.; Roudbary, M. Antimicrobial peptides as biologic and immunotherapeutic agents against cancer: A comprehensive overview. Front. Immunol. 2017, 8, 1320

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