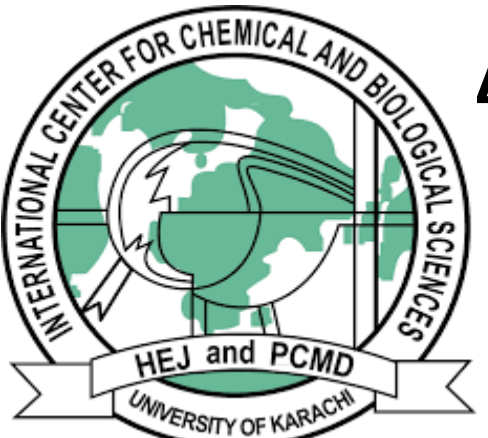




Antimicrobial Peptides of Temporin SHa for Targeting MDR *S. aureus* (NCTC 13277) and *H. Pylori* (ATCC 43504)



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ABSTRACT

Among antimicrobial peptides, temporins, derived from skin secretions of Eurasian and ranid frog, represent one of the largest of antimicrobial peptides. They are linear, hydrophobic and weakly positively charged peptides. This study foregrounds the synthesis of analogs of temporin SHa which were prepared by solid phase synthesis and screened for their biological activities against various pathogenic bacteria and parasites. As the activity of any member of temporins on *H. pylori* had never been addressed so, during antimicrobial screening, temporin-SHa and its analog [G10a] SHa were identified as potential candidates to treat resistant strains of *H. pylori* as well as on methicillin-resistant *Staphylococcus aureus* (MRSA) NCTC (13277). These analogs exhibited significant anti-MRSA activity at physiological salt concentration. Temporin-SHa and analog [G10a] SHa were identified as being active on clinical resistant strains, killing 90–100% of bacteria in less than 1 h and showing low to no toxicity against human gastric cells and tissue and significant anti-biofilm activity against biofilm forming *S. aureus* (ATCC 6538).

INTRODUCTION

❖ Temporin family is one of the largest of antimicrobial peptides with linear, hydrophobic and weakly positively charged peptides

❖ Derived from skin secretions of Eurasian and ranid frog.

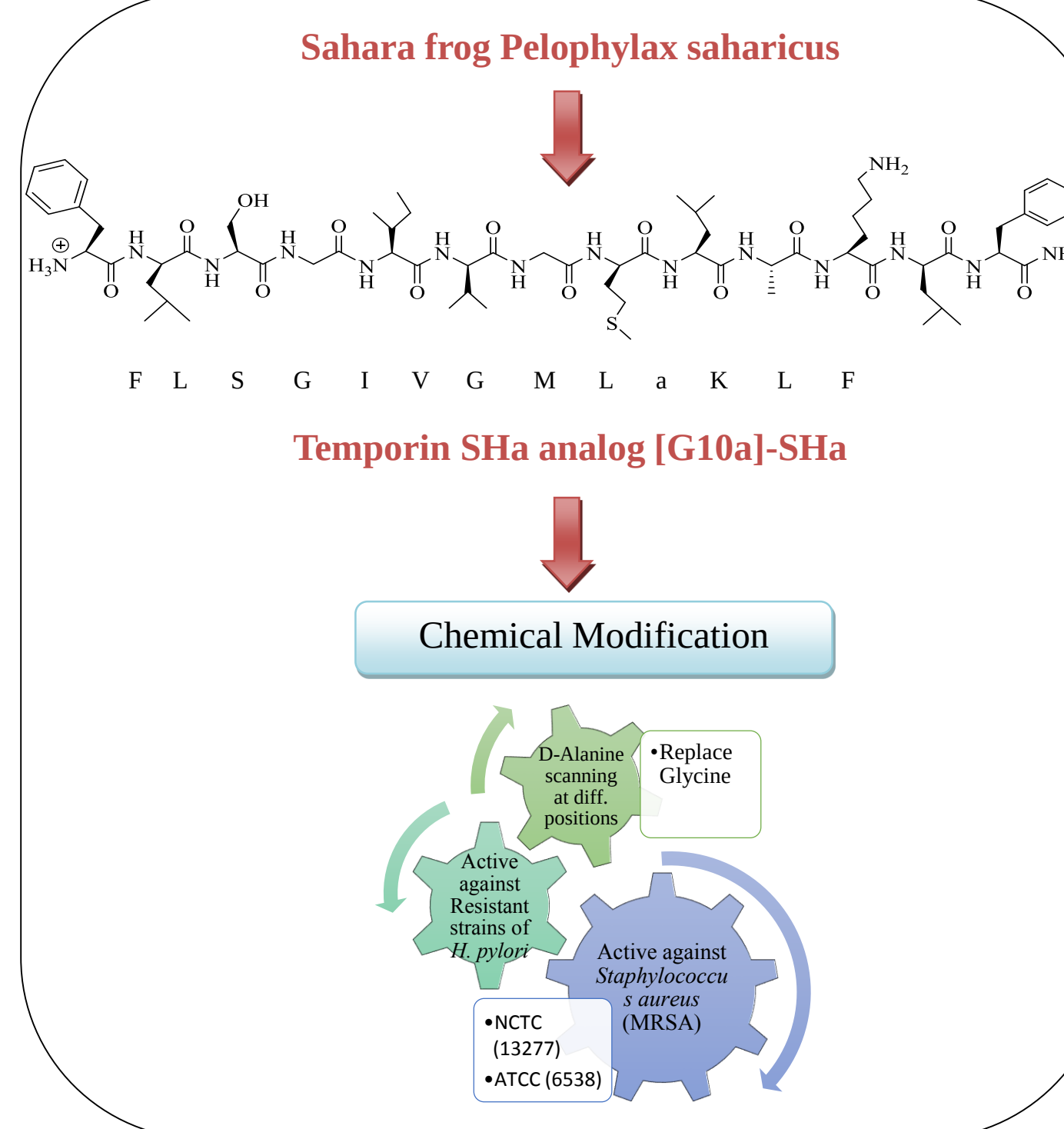
❖ Temporins SHa potent against the *Leishmania* species (*L. infantum*, *L. major*, *L. tropica*, *L. amazonensis* and *L. braziliensis*).

❖ The synthesis and antimicrobial activities of various analogs of SHa, i.e, ([K³] SHa, [A^{2,6,9}] SHa, [A^{2,6,9}, K³] SHa were also reported.

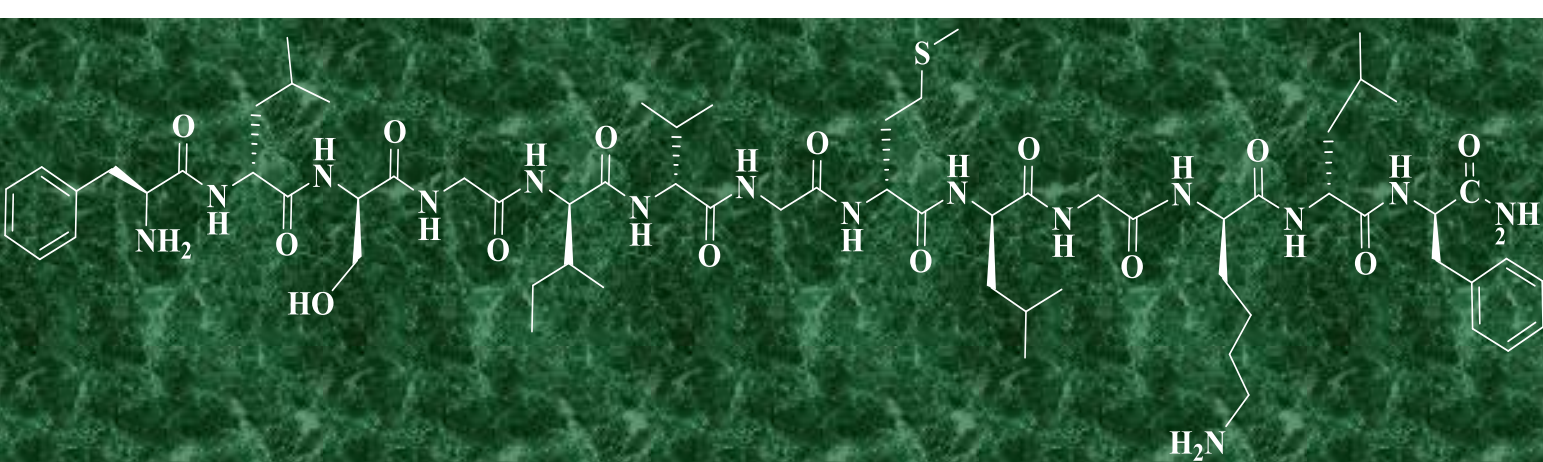
❖ The newly synthesized glycine substituted analogs of Temporin SHa with D-Alanine were found active against *S. aureus* and *H. pylori*.

❖ L-alanine substituted analogs of temporin SHa lack the activities against *S. aureus* and *H. pylori*.

AIMS AND OBJECTIVES OF CURRENT STUDY



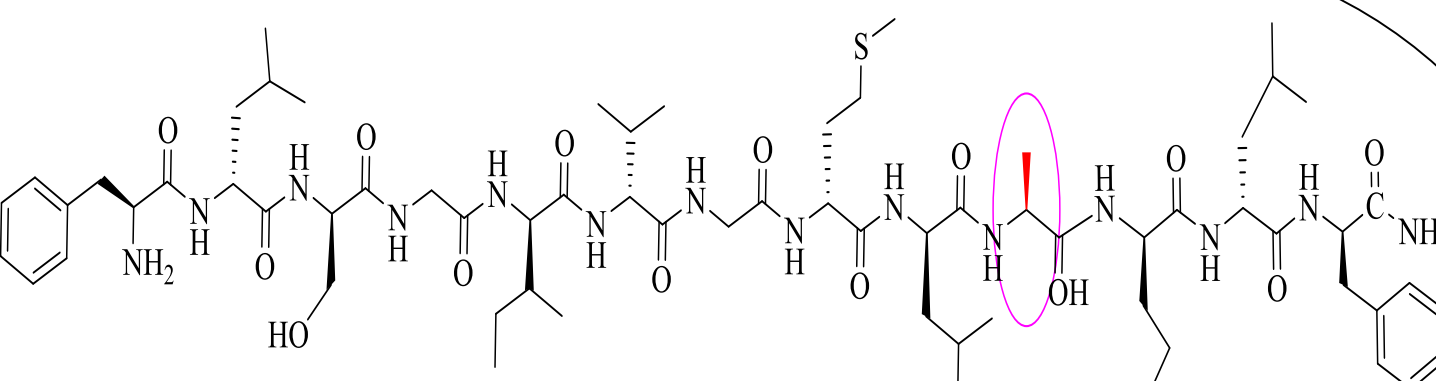
TEMPORIN SHa



CURRENT WORK

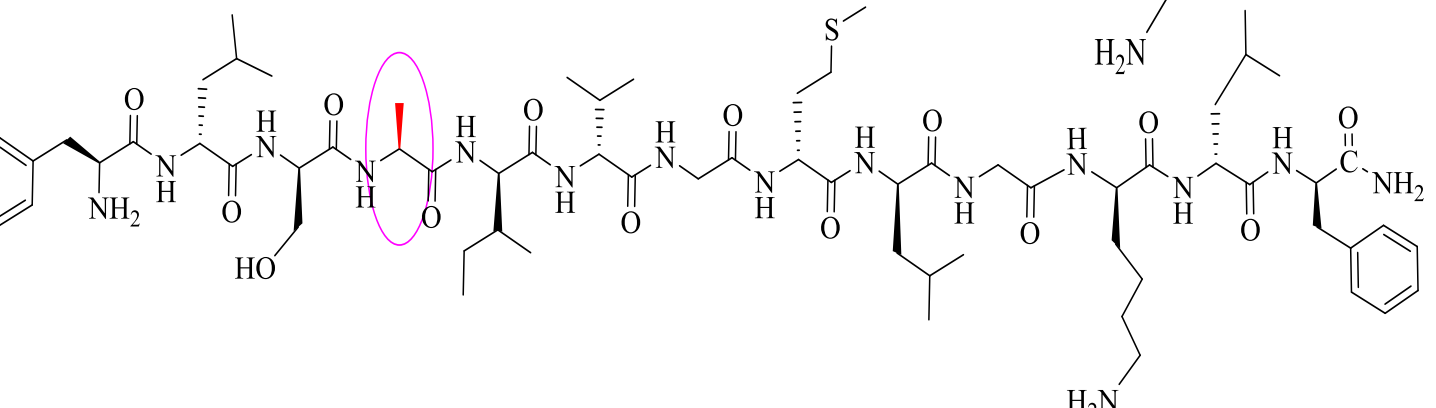
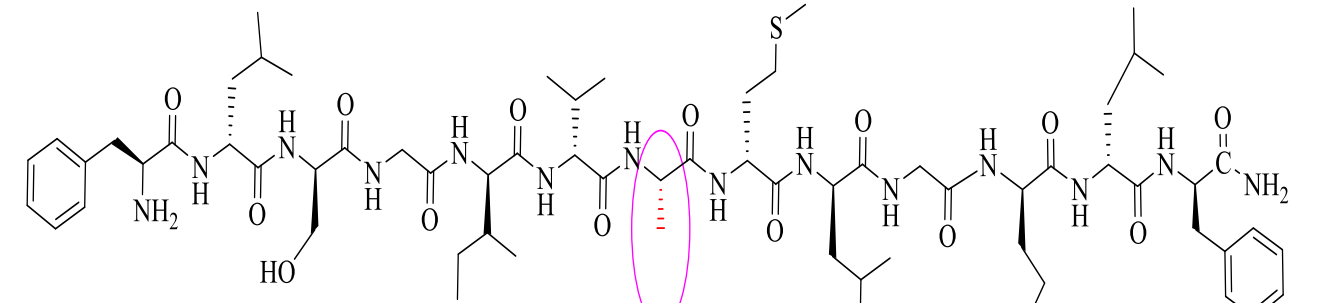
[G10a]-SHa
C₆₈H₁₁₁N₁₅O₁₄S
MW= 1394.77

[FLSGIVGMLaKL
F]



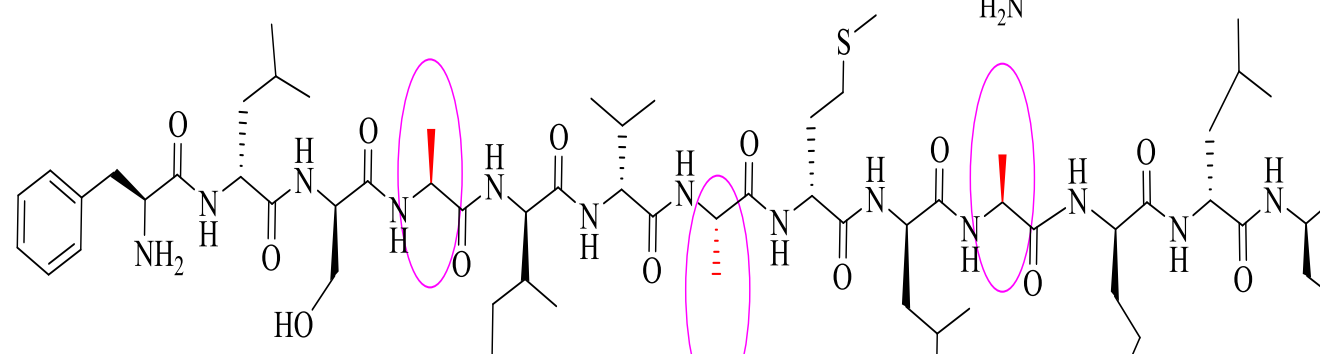
[G4a]-SHa
C₆₈H₁₁₁N₁₅O₁₄S
MW= 1394.77

[FLSaIVGMLGKLF]



[G7a]-SHa
C₆₈H₁₁₁N₁₅O₁₄S
MW= 1394.77

[FLSGIVaMLGKLF]



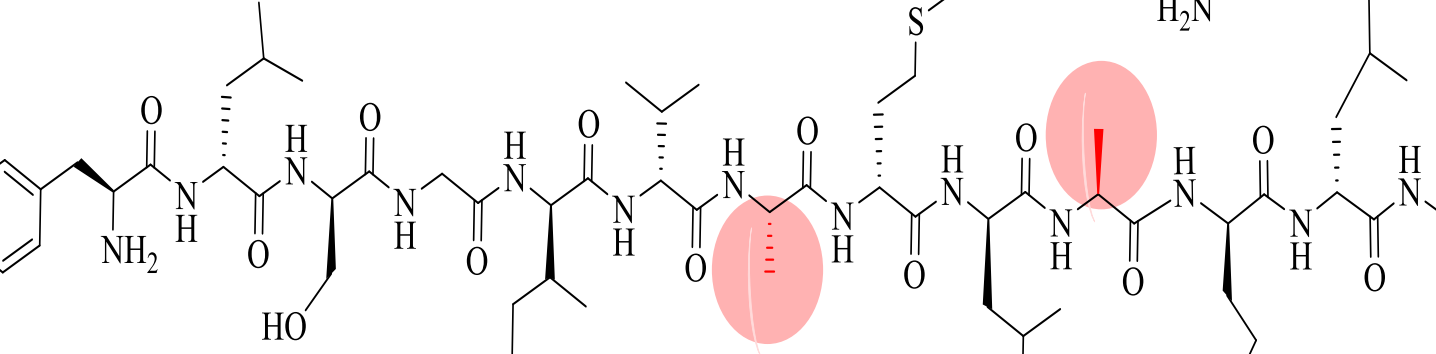
[G4,7,10a]-SHa
C₆₈H₁₁₁N₁₅O₁₄S
MW= 1422.82

[FLSaIVaMLaKLF]

Temporin SHa PEPTIDES

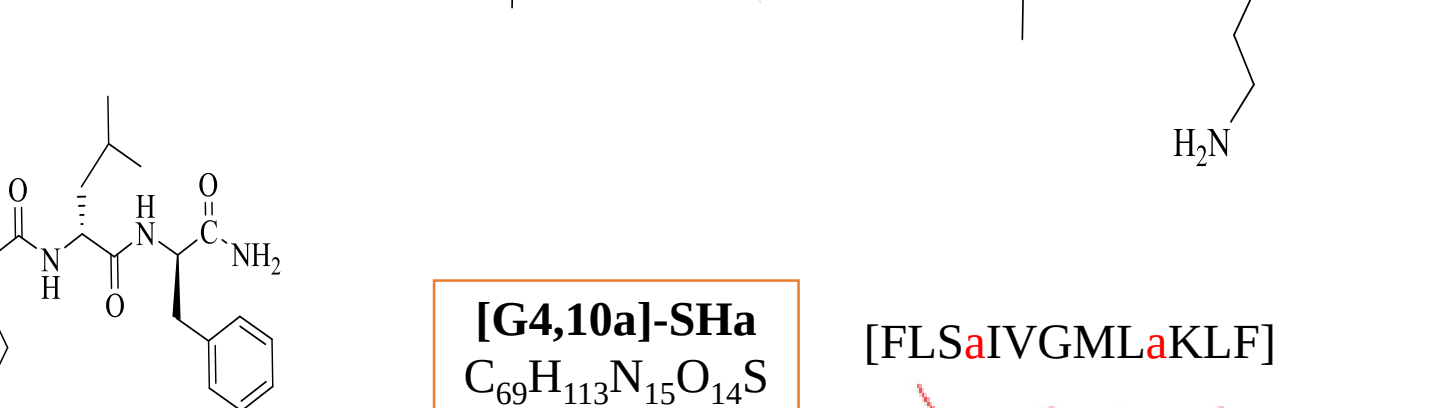
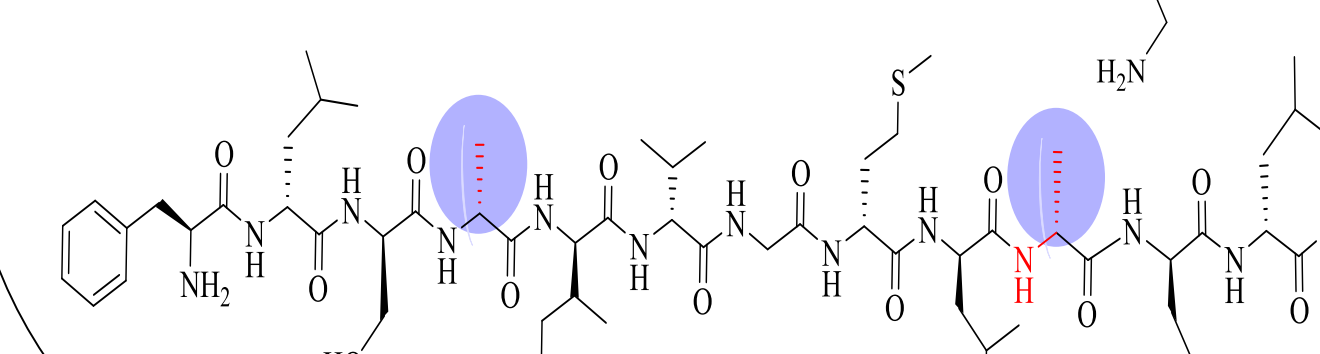
[G4,7a]-SHa
C₆₉H₁₁₃N₁₅O₁₄S
MW= 1408.79

[FLSaIVaMLGKLF]



[G7,10a]-SHa
C₆₈H₁₁₁N₁₅O₁₄S
MW= 1408.79

[FLSGIVaMLaKLF]



[G4,10a]-SHa
C₆₉H₁₁₃N₁₅O₁₄S
MW= 1408.79

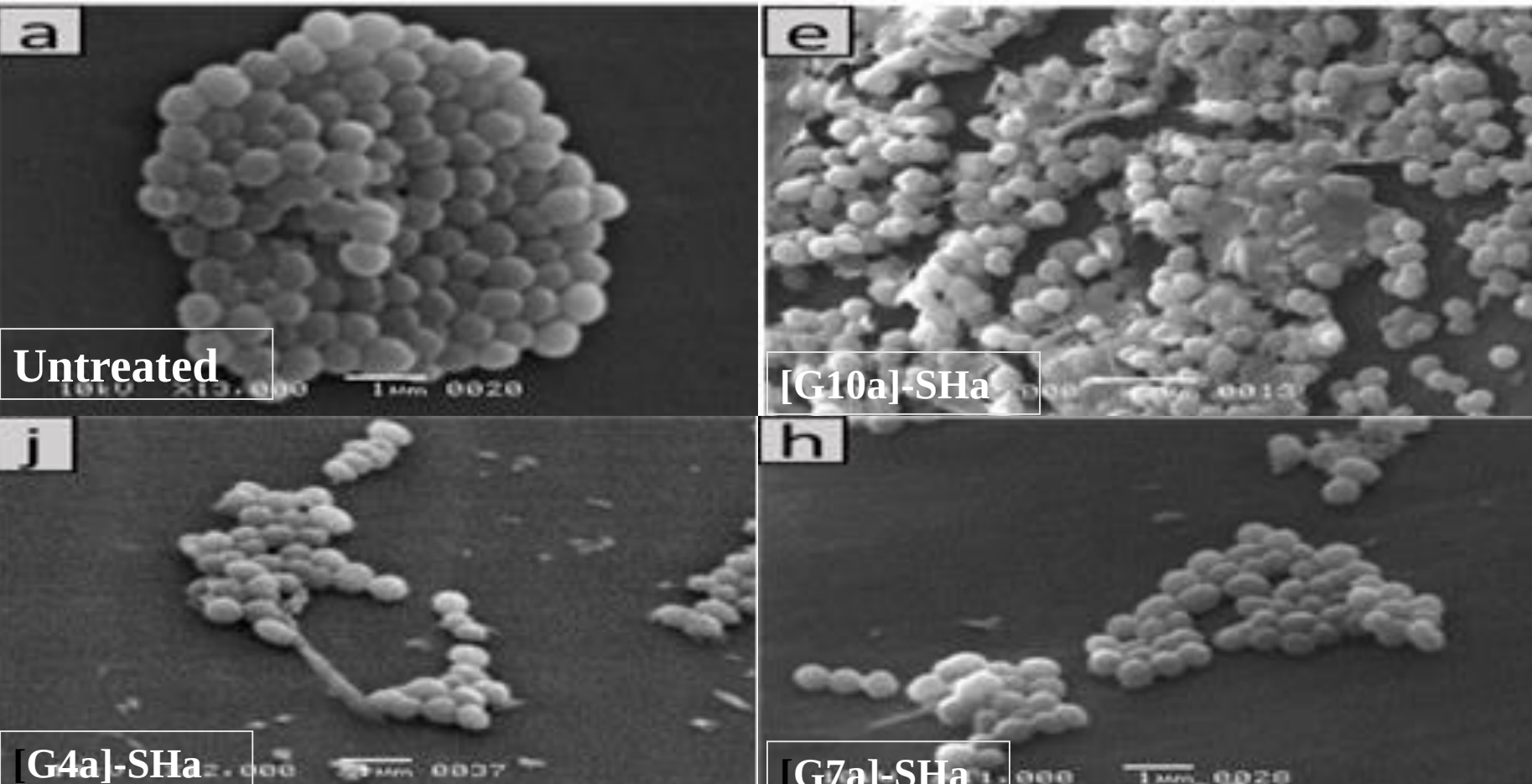
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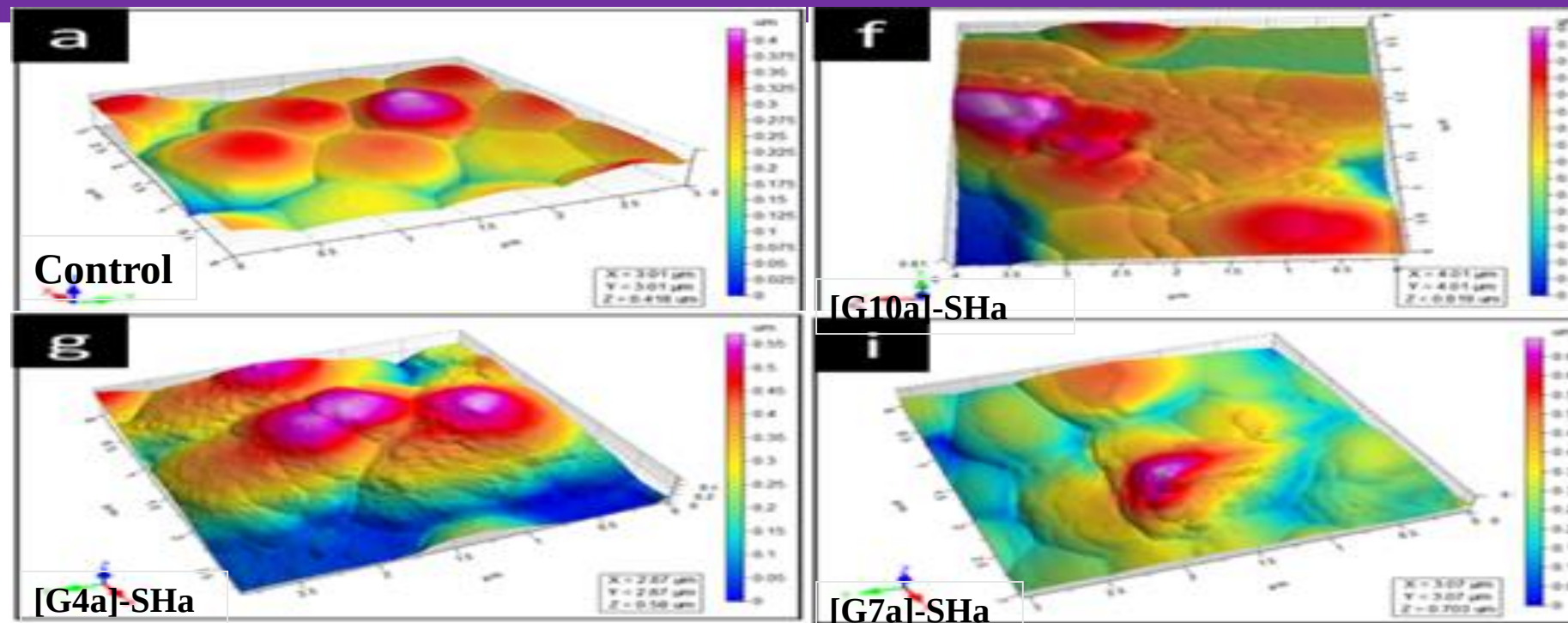
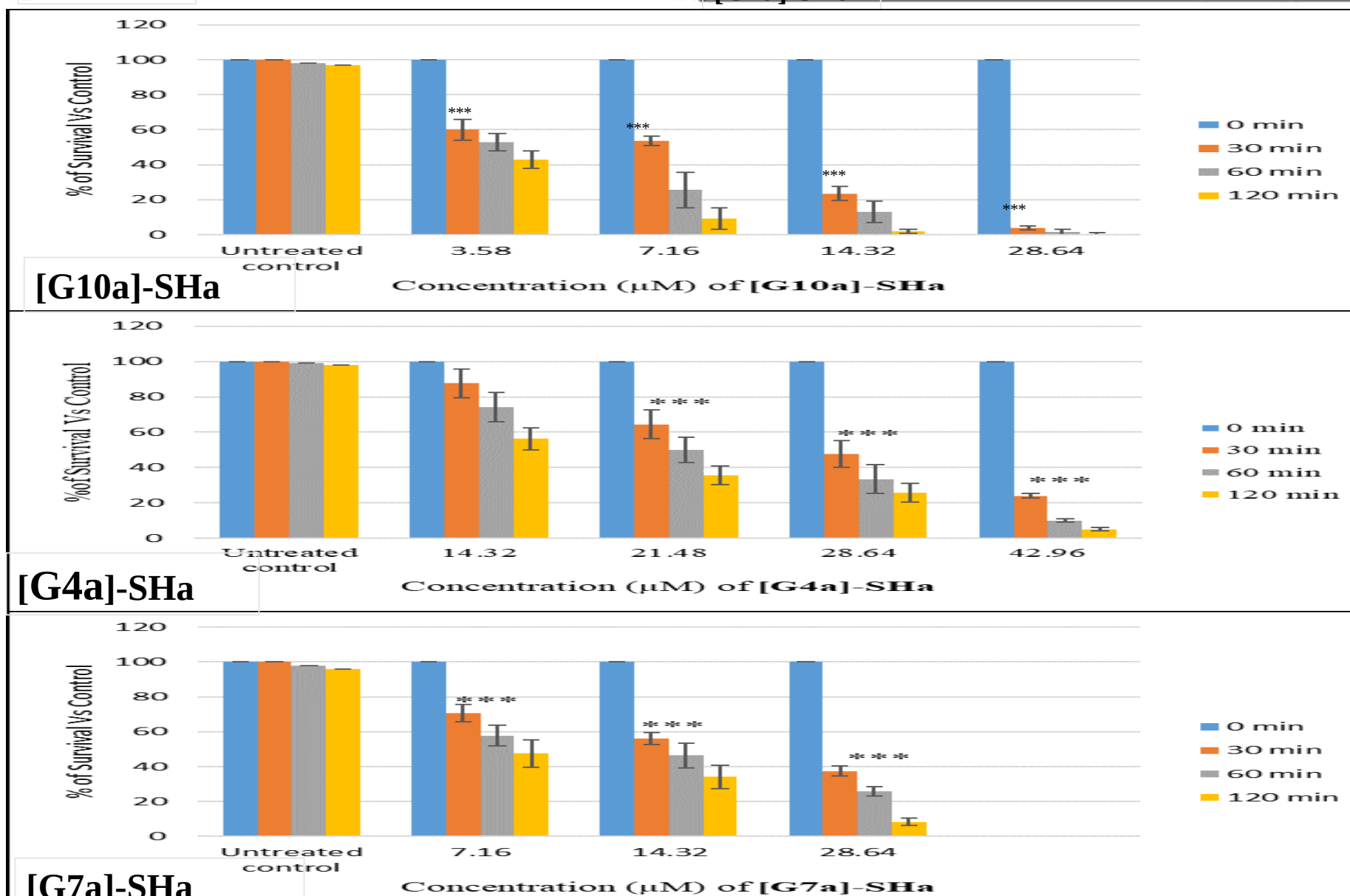
[G4,10a]-SHa
C₆₈H₁₁₁N₁₅O₁₄S
MW= 1408.79

[FLSaIVGMLaKLF]

SEM RESULTS OF TEMPORIN PEPTIDES AGAINST *S. AUREUS*



AFM IMAGES & CONCENTRATION-TIME KILLING KINETICS ASSAY AGAINST *S. AUREUS*



SUSCEPTIBILITY TEST AT DIFFERENT PHYSIOLOGICAL CONCENTRATIONS.

Peptides	NaCl (mM)			MgCl ₂ (mM)			CaCl ₂ (mM)		
	50	150	300	1	3	5	1	3	5
[G10a]-SHa	7.16	7.16	7.16	7.16	7.16	7.16	7.16	7.16	7.16
[G4a]-SHa	14.34	14.34	14.34	14.34	14.34	14.34	14.34	14.34	14.34
[G7a]-SHa	7.16	7.16	7.16	7.16	7.16	7.16	7.16	7.16	7.16

ACTIVITY RESULTS OF TEMPORIN PEPTIDES

Compou nds	MIC (µM)	MBC (µM)	% Inhibition at 20 µM (Mean ± S.D)
		MHB 30% FBS 30% human serum 10% human blood	
SHa	3.62	29 159	>220 >220 89.198 ± 0.413
[G10a]-SHa	3.58	17.9 36	58 >72 88.213 ± 0.253
[G4a]-SHa	14.34	57.3 143.38	143.38 186 83.243 ± 2.124
[G7a]-SHa	7.16	35.8 143.38	180 186 85.125 ± 1.254

ACTIVITY OF TEMPORIN SHa ANALOGS ON *H. PYLORI* (ATCC 43504) AND IN-VITRO TOXICITY AGAINST HUMAN N87 CELLS

Peptides	MIC ion <i>H. Pylori</i> (ATCC 43504) (µM)	IC50 on N87 cells (uM)
SHa	3.12	1558±324
[G10a]-SHa	1.5	90.41±10.4
[G4a]-SHa	12.5	1247±240
[G7a]-SHa	3.12	520.1±148

IMPACT OF GASTRIC FACTORS ON THE ACTIVITY OF TEMPORIN SHa ANALOGS ON *H. PYLORI* (ATCC 43504)

Peptides	MIC in the presence of mucins (µM)	MIC after Digestion by Pepsin (uM)
SHa	6.25	>50
[G10a]-SHa	3.12	25
[G4a]-SHa	25	>50
[G7a]-SHa	6.25	>50

CONCLUSION

❖ *H. Pylori* had never been explored in past by any member of temporin class.
❖ [G10a]-SHa was active against *H. Pylori*
❖ [G10a]-SHa was active on clinical strains and showed no toxicity to human gastric cells and tissues.
❖ Gastric mucins did not prevent the antimicrobial effect of [G10a]-SHa

PUBLICATIONS

➤ "Temporin-SHa and its analogs as potential candidates for the treatment of helicobacter pylori." Biomolecules 9(10): 598, 2019.
➤ "Serum Stable and Low Hemolytic Temporin-SHa Peptide Analogs Disrupt Cell Membrane of Methicillin-Resistant Staphylococcus aureus (MRSA)." Probiotics and Antimicrobial Proteins: 1-15, 2022.