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In silico Molecular Docking of Cyclic Peptides against TEM-1 Beta-Lactamases for Effective Antimicrobial Drug Development

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Abstract

Targeting the class A Beta lactamases Omega loop is an ideal way to combat drug resistance because of its significant role in the catalytic activity and deacylation process inhibition. Therefore, the molecular docking approach with computerized peptide-based *in silico* screening has been applied for the identification of inhibitors of TEM-type βLs. Among the subjected 105 peptides, Chrombacin (-47.8 KJ/mol), Gassericin A (-35.7 KJ/mol), Duramycin (-34.1 KJ/mol), Brevinin-1DYa (-34.0 KJ/mol), Amoebapore A (-31.2 KJ/mol), Mundticin ATO6 (-29.0 KJ/mol), Lactocyclicin Q (-26.3 KJ/mol), Cinnamycin (-25.9 KJ/mol showed highest binding energy. Among the peptides that showed the highest docking score Elafin, Cinnamycin, Duramycin interacted with Lys 73 of the α domain of catalytic residues of TEM-1 Beta lactamases, whereas Taromycin A, Gassericin A interacted with Lys 234 of the β domain, depicting a strong inhibition and also exhibited desirable physicochemical properties. Hence further *in vitro* examination of these cyclic peptides against the resistant strains is warranted to help design further novel inhibitors based on their scaffolds and also for the development of an effective drug combination regime.

Keywords: Cyclic Peptides, Protein-Peptide Docking, Allergenicity, Toxicity, TEM-1 Beta-lactamases

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INTRODUCTION

Beta-lactamases are responsible for catalyzing β-lactam antibiotic hydrolysis, which is the most common factor for drug resistance in bacteria.[1] Depending on fundamental sequence homogeneity, β-lactamases are classified into distinct four classes such as A-D, and among them, catalytic serine is the principal nucleophile in A, C and D of β-lactamases classes. β-lactamases of class B are metalloenzymes that hydrolyze the β-lactam ring using zinc ions.[2]

Class A β-lactamases show a wide spectrum of hydrolysis of substrate for penicillin, cephalosporins, and carbapenems.[3,4] By acylating the serine residue in the active-site in key PBPs, Beta-lactam antibiotics impede the proliferation of replicating microorganisms.[5] Thus, these enzymes are unable to affect the cross-linking of peptide chains to form peptidoglycan through the final phases of cell wall structure.

TEM-1 Beta-lactamases, class A enzymes are encoded by plasmids in Gram-negative bacteria.[6] The amino acid sequence of the blaTEM-1 gene is altered by mutations, which gives the enzyme a wider range of cephalosporin hydrolysis capabilities and protects it against mechanism-based inhibitors, which can lead to the establishment of resistance by TEM-1 β-lactamases.[7,8]

TEM-1, TEM-2 or SHV-1 genes give rise to ESBLs by modifying the amino acids near the active site of the enzyme. *Escherichia coli* and *Klebsiella* of Enterobacteriaceae encode the plasmids that are easily interchanged between bacterial species.[9] TEM-1-β-lactamase is comprised of 286 amino acid residues of MW 28,500 and lacks the first 23 (3-25) amino acid region, which is a signal peptide.[10] Two Cys residues (C77, C123) in mature TEM-1-β-lactamase form a disulfide bond, making it distinct from other class A enzymes.[11] The conformations of the catalytic cleft areas, found at the two-domain contact, are extremely similar. The amino acid residues Ser-70, Ser-130, Lys -73, Lys-234 and Glu-166 of class A β-lactamases are found to be catalytic and are bound to the substrate by either hydrophobic or ionic interactions.[12]

The two steps of acylation and deacylation includes the removal of proton from the catalytic residue Ser70 in class A β-lactamases.[13,14] The amide bond 456 of β-lactam is broken by oxygen present in Ser70 through a carbonyl group and acyl-intermediate formation occurs after the acylation step. Ser70, Glu166, Asn170 which coordinated water moleculess during acylation become active and attack the covalent bond within the acyl-intermediate structure. This enables the β-lactam antibiotic to hydrolyse and the enzyme to regenerate.[15,16] Similar to this, it has been discovered that the residues Glu166 and Asn170 present in the TEM-1 Ω-loop active site coordinate the hydrolytic water and are essential for diacylation and also the change in Glu166 residue results in the stable acyl-enzyme intermediates formation. Further, mutation increased the catalytic activity of the enzymes resulting in the faster inactivation of the existing drugs.[17]

Hence, to combat the present resistance pattern, a deeper understanding of the interacting molecules at the active site of TEM is required, and thus through *in silico* screening, cyclic plant and animal peptides were explored for their molecular interaction with TEM-1 Beta-lactamases.

MATERIALS AND METHODS

Protein preparation and peptide screening

Potential 105 cyclic peptides of both plant and animal origin were collected from the APD data repository (https://aps.unmc.edu/). The structure of peptides directly available in the Protein Data Bank (PDB) (https://www.rcsb.org/) was retrieved and the Pepstor and Swiss models were used to model the unavailable structures.[18] The structure of the TEM-1 beta-lactamase protein (PDB ID: 5HVI) with a resolution of 1.64 Å was retrieved from the protein data bank.

Molecular docking

TEM-1 Beta-lactamase as the receptor molecule and peptide as the ligand, protein-protein docking analysis was performed using flexible docking in the HADDOCK webserver[19] and ClusPro.[20]

Physicochemical properties

Physicochemical properties were assessed using the Expasy protparam tool (https://web.expasy.org/protparam/).[21] Extinction

coefficient, molecular weight, predicted half-life, aliphatic index, instability index, theoretical PI and grand average of hydropathicity (GRAVY) are among the pertinent properties provided by this tool and for which the peptides were evaluated.

Predicting toxicity and allergenicity

An allergenicity prediction web server was used to predict the allergenic characteristics of the peptides (https://ddg-pharmfac.net/AllergenFP/).[22] ToxinPred webserver was used to predict the peptide toxicity (https://webs.iiitd.edu.in/raghava/toxinpred/).[23]

RESULTS

Protein-protein docking was employed to predict the binding affinities of the peptides against TEM-1 Beta-lactamase. Among the subjected 105 peptides, Chrombacin (-47.8 KJ/mol), Gassericin A (-35.7 KJ/mol), Duramycin (-34.1 KJ/mol), Brevinin-1DYa (-34.0 KJ/mol), Amoebapore A (-31.2KJ/mol), Mundticin ATO6 (-29.0 KJ/mol), Lactocyclicin Q (-26.3 KJ/mol), Cinnamycin (-25.9KJ/mol), Amylocyclicin (-25.3 KJ/mol), Brevinin-1DYb (-24.1 Kj/mol), Palustrin-2c (-23.9KJ/mol), P-04 (-23.6 KJ/mol), Taromycin A (-22.9 KJ/mol), Japonicin-1(-22.7 KJ/mol), Guentherin (-20.7 KJ/mol), Elafin (-20.4KJ/mol) revealed the maximum binding energy and the binding energies of remaining peptides are exhibited in Table 1.

Chrombacin interacted with the amino acid residues Tyr 105, Ala 170, and Arg 241 of TEM beta-lactamases and formed 11 hydrogen bonds. Likewise, Lactocyclicin Q formed five hydrogen bonds in which Val 21 interacted with Gln 99 at a distance of 2.79. Mundicitin ATO6 formed 3 hydrogen bonds and 1 salt bridge in which Lys 43 interacted with Asn 100 at a distance of 2.72. Amylocyclicin formed eleven hydrogen bonds in which Lys 59 interacted with Asp 101 at a distance of 2.81. Brevenin 1 DYB formed three hydrogen bonds in which Lys 19 interacted with Val 103 at a distance of 2.81. Pro 3 and Arg 244 interacted to establish one hydrogen bond with Japonicin-1 at a distance of 3.11. Elafin formed twelve hydrogen bonds and 2 salt bridges in which Glu 3 interacted with Lys 73 of the TEM-1 Ω-loop at a distance of 2.66. Cinnamycin formed five hydrogen bonds in which Asn 17 interacted with Lys 73 at a distance of 2.69. Duramycin formed five hydrogen bonds in which Asn 17 interacted with Lys 73 at a distance of 2.69. The hydrogen bond interactions of other peptides with the hydrogen bond distance are depicted in Table 2 and Figure.

Table 1. Binding Energy of cyclic peptides against the TEM-1 beta-lactamases

No.	Name	Haddock Binding Affinity Kj/mol	ClusPro Binding Affinity Kj/mol
1	Amoebapore A	-31.2	-940.5
2	Amylocyclicin	-25.3	-771.0
3	Brevenin-1DYa	-34.0	-1228.2
4	Brevenin-1Dyb	-24.1	-1142.2
5	Chrombacin	-47.8	-1165.3
6	Cinnamycin	-25.9	-1015.2
7	Duramycin	-34.1	-1001.8
8	Elafin	-20.4	-1080.0
9	Gassericin A	-35.7	-1341.8
10	Guentherin	-20.7	-903.3
11	Japonicin-1	-22.7	-1100.0
12	Lactocyclicin Q	-26.3	-1012.4
13	Mundicitin ATO6	-29.0	-949.9
14	P-04	-23.6	-1123.1
15	Palustin-2c	-23.9	-1106.0
16	Taromycin A	-22.9	-802.0

The binding energies of the various cyclic peptides are expressed in kJ/mol

In the hydrogen bond interaction the left side amino acid residues represents TEM-1 Beta-lactamases and the right side residues represents different cyclic peptides.

Hydrogen bond interaction are represented by blue lines between TEM-1 Beta-lactamases and the different cyclic peptides. Elafin formed twelve hydrogen bonds, whereas Cinnamycin and Durmaycin formed five hydrogen bonds representing the stabilisation and interaction with key residues.

The tested peptides were non-toxic, and by way of the AllergenFp web server, the non-allergenic peptides were screened. 16 peptides out of 105 peptides are non-allergenic and 13 peptides were non-toxic. Table 3 represents allergenic and toxic profile of the subjected peptides

Hence in the subjected analysis, Chrombacin, Cinnamycin, Duramycin, Guentherin,

Table 2. Molecular Interaction profile of the compounds against the target TEM -1 beta-lactamases

No.	Peptide	Peptide sequence	Hydrogen bond interaction	Distance (Å)
1	Amoebapore A	GEILCNLCTGLINTLENLLTTK	LYS111-ASP66	2.59
		GADKVKDYISSLCNKASGFIA	GLY218-GLU2	2.93
		TLCTKVLDFGIDKLIQLIEDKV	ASN276-GLU2	2.94
		DANAICAKIHAC		
2	Amylocyclicin	LASTLGISTAAAKKAIDIIDAA	ASP101-LYS59	2.81
		STIASIISLIGIVTGAGAISYAIV	ASN132-THR9	3.13
		ATAKTMIKKYGKKYAAAW	GLY236-ILE7	2.77
			ALA237-ILE7	3.07
			GLY242-SER8	2.92
			ARG244-SER3	2.83
			GLN269-GLY37	2.80
			GLN269-ILE34	2.75
			ARG275-THR36	3.11
			ARG275-THR36	2.77
			ASN276-THR4	2.84
3	Brevenin-1DYa	FLSLALAALPKFLCLVFKKC	-	-
4	Brevenin-1Dyb	FLSLALAALPKLFCLIFKKC	VAL103-LYS19	2.81
			PRO219-LEU6	2.81
			ASN276-LEU6	3.28
5	Chrombacin	AAEFPDFYDSEEQMGPHQEA	TYR105-SER10	3.20
		EDEKDRADQRVLTEEEKKELE	ASN170-MET14	2.88
		NLAAMDLELQKIAEKFSQR	ASN170-GLU19	3.11
			ALA172-MET14	2.70
			ARG241-GLU12	2.77
			ARG241-ASP9	2.69
			ARG241-ASP9	2.72
			ARG241-GLU12	2.66
			ARG244-TYR8	2.88
			ARG244-TYR8	3.16
			ARG275-GLU12	2.63
			ASN276-GLU3	3.10
6	Cinnamycin	CRQSCSFGPFTFVCDGNTK	LYS73-ASN17	2.69
			ASN132-VAL13	3.04
			ASN136-GLY16	2.85
			ASN170-ASN17	2.89
			ARG275-THR11	2.68
7	Duramycin	CKQSCSFGPFTFVCDGNTK	LYS73-ASN17	2.69
			TYR105-ASP15	2.64
			ASN132-GLY16	3.03
			ASN132-ASN17	2.97
			ASN13-LYS19	2.68
8	Elafin	AQEPVKGPVSTKPGSCPIILIR	LYS73-GLU3	2.66
		CAMLNPPNRCLKDTDCPGIK	TYR105-GLU3	2.85
		KCCEGSCGMACFVPQ	TYR105-LYS6	2.74
			ALA126-LYS12	3.21
			ASP131-LYS12	3.09
			ASP214-LYS12	2.63
			LYS215-SER10	2.64
			LYS215-LYS12	2.77
			LYS215-PRO13	3.19

			ARG244-PRO4	2.75
			ARG244-LYS6	2.64
			ARG244-LYS6	2.83
9	Gassericin A	IYWIADQFGIHLATGTARKLL	PRO167-ARG18	2.84
			LYS215-GLN7	2.78
		DAMASGASLGTAFAAILGVTL	LYS234-ASP6	2.68
		PAWALAAAGALGATAA	GLY236-ASP6	3.11
			GLY238-ALA13	2.82
			ARG244-ASP6	2.76
			ARG244-ASP6	2.87
			ARG244-ASP6	2.90
			ARG275-PHE8	2.71
			ARG275-ALA5	3.05
10	Guentherin	VIDDLKKVAKKVRRELLCKK	TYR105-ILE2	3.17
			ASP131-LYS6	2.53
		HHKKLN	ASN170-ASP4	2.75
			GLY218-LYS19	2.77
			ASP273-GLU15	2.69
			ASP273-LYS11	2.68
			ASP273-ARG14	312
			ASN276-GLU15	2.67
11	Japonicin-1	FFPIGVFCKIFKTC	ARG244-PRO3	3.11
12	Lactocyclicin Q	LIDHLGAPRWAVDTILGAIAVG	GLN99-VAL21	2.79
			TYR105-ASP13	2.92
		NLASWVLALVPGPGWAVKAG	ASP273-ASP3	2.71
		LATAAAIVKHQGKAAAAAW	ARG275-ILE2	2.88
			ARG275-ASP3	2.79
13	Mundicitin ATO6	KYYGNGVSCNKKGCSVDWG	ASN100-LYS43	2.72
			ASP101-LYS43	2.59
		KAIGIIGNNSAANLATGGAAG WSK	ARG275-SER15	2.87
14	P-04	FSLFFPYAALKWLRKLLKK	LYS215-TRP12	2.71
			GLY218-LYS15	2.69
			ARG275-TYR7	3.33
15	Palustrin-2c	GFLSTVKNLATNVAGTVIDTL KCKVTGGCRS	-	-
16	Taromycin A	WNDTGKDADGAEY	LYS234-GLU12	2.63
			GLY236-GLU12	2.78
			ARG244-GLU12	2.59

Elafin, Mundicitin ATO6, and Taromycin A were found to be non-polar and exhibited a positive Gravy while the remaining compounds were found to be polar. Brevenin 1 DYA, Brevenin 1DYB, Japonicin 1, and P-04 displayed a relatively low half-life of 1.1 hours and the remaining compounds exhibited an increased half-life of 30 hours. Table 4 displays the physicochemical features of the tested peptides.

DISCUSSION

Treatment of infectious diseases is difficult due to increased bacterial resistance and also is a significant global health concern.[24] Beta-lactamases have developed mutations that could exhibit resistance to inhibitors.[8] Despite the effectiveness of some combination regimens, beta-lactamase enzymes mutate exhibiting resistance to the inhibitors and consequently, emphasizing the requirement of newer drugs or a newer combination regime.

Table 3. Toxicity and allergenicity profile of cyclic peptides

No.	Compounds	Allergenic	Toxic
1	AmoebaporeA	Non-allergenic	Toxic
2	Amylocyclicin	Non-allergenic	Non-toxic
3	Brevenin-1DYa	Non-allergenic	Non-toxic
4	Brevenin-1Dyb	Non-allergenic	Non-toxic
5	Chrombacin	Non-allergenic	Non-toxic
6	Cinnamycin	Non-allergenic	Non-toxic
7	Duramycin	Non-allergenic	Non-toxic
8	Elafin	Non-allergenic	Toxic
9	Gassericin A	Non-allergenic	Non-toxic
10	Guentherin	Non-allergenic	Non-toxic
11	Japonicin-1	Non-allergenic	Non-toxic
12	Lactocyclicin Q	Non-allergenic	Toxic
13	Mundicitin ATO6	Non-allergenic	Non-toxic
14	P-04	Non-allergenic	Non-toxic
15	Palustrin-2c	Non-allergenic	Non-toxic
16	Taromycin A	Non-allergenic	Non-toxic

The unique loop structure known as the omega loop is a non-regular structural motif resembling the upper-case Greek letter omega (Ω), is present in all serine beta-lactamases and is crucial for maintaining the stability between the enzyme and substrate. Specifically, it has been demonstrated that the conserved Ω-loop of class A beta-lactamases is essential to the hydrolytic process during beta-lactam hydrolysis. The omega loop (Ω-loop), a conserved non-active site structural domain seen in class A serine β-lactamases (SBLs), is thought to contain a glutamic acid residue that directly contributes to the hydrolysis of β-lactam antibiotics by supplying a water molecule during catalysis. Hence, targeting the β-lactamases' Ω-loop could increase the potency of β-lactam antibiotics and ultimately decrease β-lactam resistance. Thus, In this study, the cyclic peptides were screened to target against the conserved Ω-loop of class A beta-lactamases and thus subsequently the identified hit compounds may function as inhibitors of β-lactamase, lowering the minimum inhibitory concentrations and profoundly altering the resistance profile of β-lactams in bacteria.

Among the peptides which showed the highest docking score Elafin, Cinnamycin, Duramycin interacted with Lys 73 of the α domain of catalytic residues of TEM-1 Beta-lactamases whereas Taromycin A, Gassericin A interacted with Lys 234 of the β domain. However, none of the peptides showed any interaction with the other residues of the catalytic site of the SDN-loop amino acids (Asn132 & Ser130) and Ω-loop residues (Glu166 & Asn170).

Lys 73 residue in the Ω-loop of TEM-type beta-lactamase in its apo form interacts with Glu166. The residues Asn170 and Lys 73 of the Ω-loop formed hydrogen bond in the active site when Glu166 loses a proton and the distributed charged residues of active sites have been altered during the binding of antibiotics to the enzyme. Specifically, Lys73 residue deprotonates and attacks Ser70 nucleophilically, causing it to deprotonate as well. An acyl-enzyme covalent complex is produced as a result of this interaction of the Beta-lactam ring with antibiotic's carbonyl carbon atom. Hence the compounds Elafin, Cinnamycin, and Duramycin interacting with Lys 73 of TEM-1 beta-lactamases may aid in preventing resistance and the hydrolysis process.[17]

All serine beta-lactamases of classes A, C, and D have an expanded second loop referred to as the Ω-loop. The Ω-loop of TEM-type β-Lactamases is situated at the base of the active region of the enzyme and consists of 16 residues (Arg164–Asp179). Glu166 is an essential component in the hydrolysis of β-lactams and is substantially conserved in class A β-Lactamases. However, none of the peptides interacted with Glu 166 in our study.[17]

Asp 4 of Guentherin A interacted with the Asn 170 residue located at the bottom of the enzymatic active site of TEM-type Ω-loop βLs which is comprised of 16 aminoacid residues from Arg164 to Asp179. Met 14 of the peptide interacted with the Chrombacin Ala 172 at the hydrogen bond distance of 2.70, and Agr 18 of Gassericin A interacted with the Pro 167 at 2.83. Notably, none of the compounds tested reacted with the highly prevalent residue Glu166 of class A, which is important for the hydrolysis of β-lactams.[25]

The N-terminal portion for the omega loop (Arg164–Asn170 residues) in TEM-type βLs has a stiff conformation, while Arg 164 is involved in maintaining bottleneck loop structure. The proximity of these residues to one another can be attributed to α-helix formation by the Pro167–Asn170 residues.

Before the catalytically active orientations form, the Peptidyl-proline bond (Glu166–Pro167) must be formed in trans configuration and the hydrogen bonding complex occurs with Glu166, Asn170 and water molecule.[17] However, the disturbances at these specific sites were not observed in our interaction analysis.

Mutations in the loop destabilize the conformation due to the rapid exchange of water

Table 4. Physicochemical features of different cyclic peptides

No.	Name	Molecular weight	Theoretical PI	Estimated half-life	Instability index	Grand average of hydropathicity
1	Amoebapore A	8244.70	5.65	30 hours	24.71	0.406
2	Amylocyclicin	6399.62	9.82	5.5 hours	2.65	0.850
3	Brevenin-1DYa	2225.87	9.39	1.1 hours	21.25	1.585
4	Brevenin-1Dyb	2239.89	9.39	1.1 hours	17.00	1.600
5	Chrombacin	7057.66	4.29	4.4 hours	60.03	-1.307
6	Cinnamycin	2097.37	7.96	1.2 hours	77.73	-0.221
7	Duamycin	2069.35	7.95	1.2 hours	80.05	-0.189
8	Elafin	6007.20	8.51	4.4 hours	57.38	-0.019
9	Gassericin A	5671.60	6.75	20 hours	3.73	0.997
10	Guentherin	3140.87	10.23	100 hours	27.69	-0.923
11	Japonicin-1	1650.07	8.90	1.1 hours	6.24	1.350
12	Lactocyclicin Q	6078.16	9.70	5.5 hours	8.94	0.826
13	Mundicitin ATO6	4289.81	9.45	1.3 hours	6.27	-0.253
14	P-04	2369.97	10.58	1.1 hours	35.30	0.332
15	Palustrin 2c	3154.69	9.39	30 hours	3.51	0.345
16	Taromycin A	1441.43	3.84	2.8 hours	-18.96	-1.654

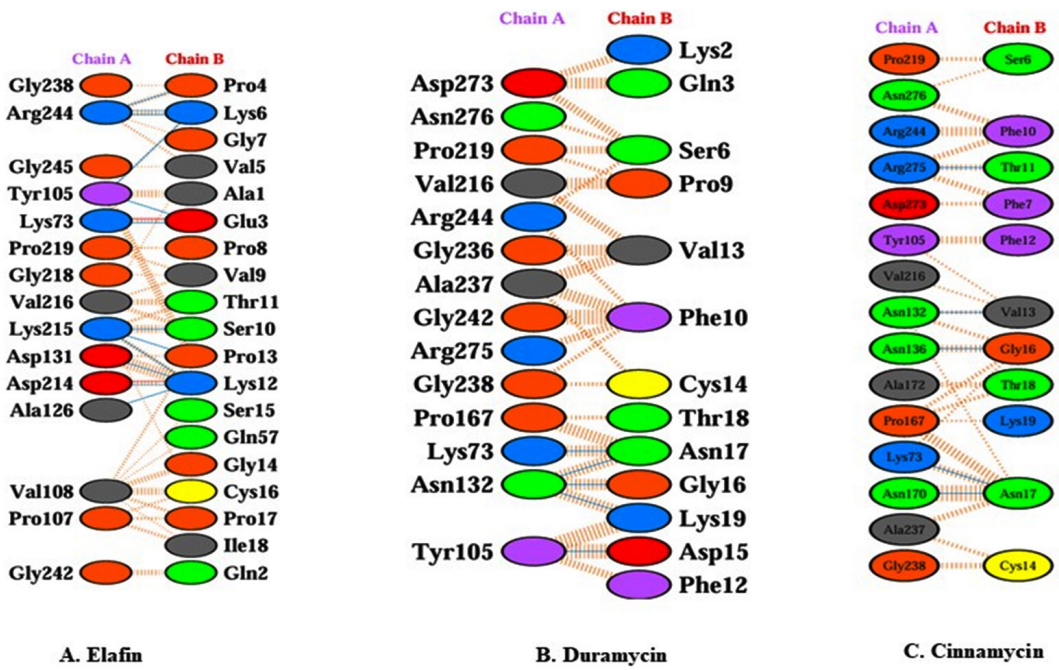

Figure. Molecular interactions of the cyclic peptides A. Elafin, B. Duramycin, C. Cinnamycin against the TEM-1 beta-lactamases

molecules close to the Ω-loop, thus increasing the catalytic effectiveness of ceftazidime and cefotaxime hydrolysis, in specific, the substitution Arg164Ser maintains a hydrogen bond between Ser164 and Asp179, which further stabilize the omega loop conformation and has been observed commonly amongst clinical strains producing TEM-type β-Lactamases.[26]

Hence, the design and identification of new molecules that could inhibit this process of inactivation are required to overcome this resistance, and hence the screened peptides in this study provide a clear insight into the interaction with the active site and also on their conformation. According to CAPRI (Critical Assessment of Predicted Interactions) experiments, HADDOCK and ClusPro are found to be the best automated protein docking systems. HADDOCK uses Fast Fourier Transforms (FFTs)-based rigid-body docking techniques and as a part of the preliminary investigation in this study, the cyclic peptides were subjected to the class A Beta-lactamases Omega loop, and hence in futuristic studies , the lead compound could be further validated by *in vitro* antibacterial studies against the resistant strains.[27-29]

Substantial reports exist on the antimicrobial activity of reported peptides. Gassericin A bacteriocin produced by *Lactobacillus gasseri* LA39 has shown antibacterial activity against *Staphylococcus aureus, Listeria monocytogenes, and Bacillus cereus*.[30] Likewise, the 19-amino acid tetracyclic lanthipeptide Duramycin by streptomycetes and Brevinin-1 ,skin peptide from *H. rugulosus* exhibited antimicrobial activities.

Cinnamycin (19-residue tetracyclic peptide) of the lantibiotic family possesses the thioether amino acids meso-lanthionine and (2S,3S,6R)-3-methyllanthionine and are formed by cross-linking serine or threonine with cysteine, and has the potential to locate and rupture PE-containing membranes, including those found in bacteria and cancer cells, due to its specific binding to PE lipids.[31]

Elafin is an antimicrobial peptide that has exhibited antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa,* and likewise, Japonicin-1 has demonstrated effective antimicrobial activity against *Staphylococcus aureus* and MRSA. Lactocyclicin Q has a cyclic structure with a 19-residue tetracyclic peptide bound at N and C termini and showed antimicrobial activity against *Lactococcus* and *Enterococcus*. Taromycins A and B showed calcium ion-dependent broad-spectrum antibacterial activity against MRSA and *E. faecium*.[32]

Therefore, our research provides more details on how these specific peptides interact with TEM-Bl, which could aid in the development of efficient medications to combat bacterial strains that are resistant to current beta-lactam antibiotics and also for a combinational regime.

CONCLUSION

Elafin, Cinnamycin, and Duramycin interacted with Lys 73 of the α domain of the catalytic residues in TEM-1 beta-lactamases depicting their unique mechanism of preventing the hydrolysis process. On the other hand, Taromycin A and Gassericin A exhibited a different yet equally significant interaction by binding with Lys 234 of the β domain. These distinct binding sites underscore their potential as robust inhibitors of TEM-type β-lactamases, a novel aspect of their inhibitory action. Also, favorable physicochemical characteristics were displayed by all five compounds. These characteristics enhance their potential efficacy and stability as antibiotic agents. The unique binding interactions suggest that these compounds could be developed into highly effective inhibitors capable of overcoming the mechanisms of resistance exhibited by TEM-type β-lactamases.

Hence, it is imperative to conduct further *in vitro* testing of Elafin, Cinnamycin, Duramycin, Taromycin A, and Gassericin A against resistant bacterial strains to validate their inhibitory efficacy, which could lead to breakthroughs in the fight against antibiotic-resistant bacteria, paving the way for novel therapeutic strategies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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