

Molecular Docking of Cyclic Peptides Against the Quorum-Sensing LasR Protein of *Pseudomonas aeruginosa*

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Abstract

The emerging resistance of microorganisms to commonly used antibiotics and the emergence of *P. aeruginosa* as one of the ES-KAPE pathogens necessitate the search for and development of newer drugs. So far, only a limited number of synthetic compounds have been reported to disrupt quorum sensing (QS). Therefore, the identification and development of novel compounds targeting quorum sensing may provide an effective strategy for controlling *P. aeruginosa* infections. Hence, in this study, peptide-protein docking was used to target the LasR protein with a small peptide library, and the resulting peptides' pharmacokinetic properties were evaluated. Out of the 101 peptides, Gramicidin C (-101.5 ± 4.4), Epilancin (-99.8 ± 9.1), Cathelicidin-DM (-91.4 ± 6.3), Gassericin A (-86.4 ± 17.6), PO4 (-83.9 ± 5.7), Gramicidin B (-81.1 ± 1.0), and Salivaricin P (-79.7 ± 3.6) exhibited the highest Haddock score by extending the hydrogen bond interaction with the key Tyr 56 and Ser 129 amino acid residues of the LasR protein. These in silico findings suggest that the identified peptides exhibited high binding affinity toward the LasR protein of *P. aeruginosa*

and may interfere with LasR-mediated quorum sensing, warranting further in vitro and in vivo studies to validate their effects on biofilm formation. A few identified peptides exhibited potential pharmacokinetic challenges; therefore, further formulation strategies would be required to overcome these limitations.

Keywords: Antimicrobial peptides, Virtual screening, HADDOCK, Molecular docking, AD-MET

Introduction

Pseudomonas aeruginosa is an opportunistic pathogen that causes urinary tract infections, bloodstream infections, and hospital-acquired pneumonia, primarily affecting immunocompromised individuals and patients with implanted medical devices, such as intubation tubes. It is a leading cause of mortality among individuals with cystic fibrosis, AIDS, severe burns, and neutropenic cancer patients. It has also been reported to cause ventilator-associated pneumonia with a mortality rate of 13.5% (1). These infections are difficult to treat, in part, because *P. aeruginosa* forms resilient biofilms and tightly regulates its virulence through quorum sensing (QS).

P. aeruginosa, through the QS process, performs coordinated action and forms complex communities. This promotes the optimization of nutrients and the acquisition and regulation of virulence factors, leading to sustained pathogenicity and survival in the host cells (2).

QS is a cell-density-dependent communication process in which bacteria use secreted autoinducers to coordinate gene expression and act collectively. In *P. aeruginosa*, this regulation is governed primarily by two acyl-homoserine lactone (AHL)-based systems: LasI/LasR and RhII/RhlR. LasI produces the autoinducer 3-oxo-C12-HSL, which binds its cognate Lux-type receptor LasR; similarly, RhII synthesizes the signal recognized by RhlR. Upon binding their respective autoinducers, LasR (transcription factor) and RhlR dimerize and bind to DNA, regulating the expression of multiple genes responsible for biofilm formation, virulence factor production, and broader QS regulation (3,4). Because these QS pathways enable bacteria to achieve high cell densities and coordinate group-beneficial behaviors, disrupting them represents a promising antivirulence strategy that may reduce pathogenicity without imposing the selective pressure typical of antibiotics.

Most QS inhibitor efforts have centered on synthetic AHL mimics, but these often lack potency or specificity and closely resemble native signals. Natural, plant-derived inhibitors have also been explored, though many exhibit weak activity or poor stability. Emerging peptide-based inhibitors, including Trp-rich peptides, can reduce *P. aeruginosa* virulence and biofilms. However, systematic computational screening of cyclic peptides targeting LasR remains largely underexplored.

Only a limited number of screened synthetic compounds have been shown to inhibit QS in *P. aeruginosa*, yet these molecules generally exhibit low potency or share structural features with native N-acyl homoserine lactone signals, thereby imposing structural limitations that limit their therapeutic utility. Compounding

this issue, *P. aeruginosa* has developed significant resistance to commonly used antibiotics and is now classified as a priority pathogen on the CDC's ESKAPE list (5,6).

These challenges underscore the need for alternative strategies to inhibit QS pathways and control the organism's pathogenesis and associated infections. Cyclic peptides were selected and would typically be ideal as they exhibit higher structural stability, enhanced target affinity, and improved resistance to proteolytic degradation compared with linear peptides or small molecules, and also the virtual peptide-protein docking, a suitable first-line screening strategy, helps to rapidly narrow large molecular libraries and predict binding feasibility before further *in vitro* analysis. Based on this rationale, we hypothesize that specific screened peptides with strong predicted binding to LasR will serve as effective antagonists, inhibiting quorum-sensing-mediated virulence and thereby providing promising candidates for further *in vitro* and *in vivo* validation. To address this, the present study employed peptide-protein docking to evaluate the interaction of screened peptides with the LasR protein and assess their pharmacokinetic properties as potential quorum-sensing inhibitors.

Materials and Methods

Protein preparation and peptide screening

101 antibacterial peptides were sourced from the Antimicrobial Peptide Database (<https://aps.unmc.edu/>), and their structures were retrieved from the Protein Data Bank (<https://www.rcsb.org/>). The Swiss Model and the PEPstr Model were utilized to predict the unavailable structures (7). The LasR structure (PDB ID: 4NG2) with a resolution of 2.41 Å was retrieved from the Protein Data Bank.

Peptides were selected from a curated library based on physicochemical suitability (length < 40 residues, cyclic or semi-cyclic stability profiles) and reported drug-likeness filters (solubility, toxicity, and stability).

Molecular docking

Peptide-protein docking analysis was performed on the HADDOCK webserver, using LasR as the receptor and the peptide as the ligand, with flexible docking (8). Hydrogen bond

interactions were analysed using PDBsum (<https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/>) (9). All peptides included in this study were cyclic, selected based on their length (< 40 amino acids), stability, and Protein Data Bank (PDB) ID, which are provided in Table 1.

Table 1: Structural features of selected antimicrobial peptides retrieved from the Antimicrobial Database for Molecular Docking

S. No	Peptide	Length (aa)	Cyclicity / Topology	Stability	Database ID (APD3/UniProtKB)
1	ABP118	~58 ($\alpha+\beta$ peptides)	Linear (two-component bacteriocin)	Moderate	APD3
2	Amoebapore A	77	Linear	Moderate	UniProt: P83428
3	Amylocyclacin	64	Head-to-tail cyclic	High	APD3
4	Baceridin	13	Macrocyclic (NRP)	High	APD3
5	Brevicidine	12	Macrocyclic lipopeptide	High	UniProt: A0A1V0X1A0
6	BrevicidineB	12	Macrocyclic lipopeptide	High	APD3
7	Brevinin-1	24	Disulfide-looped	Moderate	APD3
8	Brevinin-1DYa	24	Disulfide-looped	Moderate	APD3
9	Brevinin-1DYb	24	Disulfide-looped	Moderate	APD3
10	Brevinin-1TSa	24	Disulfide-looped	Moderate	APD3
11	Brevinin-2DYb	33	Linear	Low-Moderate	APD3
12	Brevinin-2DYd	33	Linear	Low-Moderate	APD3
13	Brevinin-2DYe	33	Linear	Low-Moderate	APD3
14	Brevinin-2GHb	33	Linear	Low-Moderate	APD3
15	Brevinin-2GHc	33	Linear	Low-Moderate	APD3
16	Brevinin-2TSa	33	Linear	Low-Moderate	APD3
17	BTD1	~15	Linear	Low	APD3
18	Carnocyclin A	60	Head-to-tail cyclic	High	UniProt: Q8ZPB8
19	Cathelicidin-DM	36	Linear amphipathic	Moderate	APD3
20	Centrocin-2	28	Disulfide-bridged	Moderate	APD3
21	Ceratotoxin A	36	Linear	Low	UniProt: P83619
22	Ceratotoxin B	35	Linear	Low	UniProt: P83620
23	Ceratotoxin C	35	Linear	Low	UniProt: P83621
24	Champacyclin	~59	Head-to-tail cyclic	High	APD3
25	Chrombacin	~33	Linear	Low	APD3
26	Cinnamycin	19	Lantibiotic	High	UniProt: P08535
27	Circulin A	31	Head-to-tail cyclic	High	UniProt: P83409
28	Circulin B	31	Head-to-tail cyclic	High	UniProt: P83410
29	Colistin A	10	Cyclic lipopeptide	High	UniProt: P0C1S3

30	CRS4C1A	~42	Disulfide-rich	High	UniProt: P0DMV9
31	CXCL12	68	Chemokine (folded)	High	UniProt: P48061
32	Cyclopeptide 2	~9	Cyclic	High	APD3
33	Cyclopsychotride A	8	Cyclic	High	APD3
34	Daptomycin	13	Cyclic lipopeptide	High	UniProt: Q9R9R2
35	Darobactin	7	Macrocyclic	High	UniProt: A0A1L5ZQW7
36	Duramycin	19	Lantibiotic	High	UniProt: P08536
37	Elafin	57	Disulfide-stabilized	High	UniProt: P19957
38	Enterocin RM6	62	Head-to-tail cyclic	High	APD3
39	Esculentin-1 SEB	46	Linear	Low	APD3
40	Gallidermin	21	Lantibiotic	High	UniProt: P19945
41	Garvicin ML	~60	Linear bacteriocin	Moderate	APD3
42	Gassericin A	58	Head-to-tail cyclic	High	APD3
43	Gramicidin S	10	Cyclic	High	UniProt: P0C1S4
44	Guentherin	~47	Linear	Moderate	APD3
45	Human KR20	20	Linear	Low	APD3
46	Human salvic	46	Linear	Moderate	APD3
47	Japonicin-1	~52	Linear bacteriocin	Moderate	APD3
48	Japonicin-1 NPB	~52	Linear bacteriocin	Moderate	APD3
49	Japonicin-2	~52	Linear bacteriocin	Moderate	APD3
50	KR12	12	Linear	Low	APD3
51	Labaditin	19	Disulfide-cyclized	High	APD3
52	Lactococcin	~55	Linear bacteriocin	Moderate	APD3
53	Lactocyclicin Q	61	Head-to-tail cyclic	High	UniProt: Q83VS8
54	Lassomycin	9	Macrocyclic	High	UniProt: A0A0H3HJK6
55	Lugdunin	6	Macrocyclic	High	UniProt: A0A1B0GTZ3
56	Lysocin E	37	Macrocyclic	High	APD3
57	MDN0666	~12	Cyclic	High	APD3
58	MDN0666 Beta	~12	Cyclic	High	APD3
59	Microcin J25	21	Lasso peptide	Very high	UniProt: P69738
60	Micrococcin P1	14	Thiopeptide	Very high	UniProt: P0A3J2
61	Mundticin ATO6	43	Linear bacteriocin	Moderate	APD3
62	Myxinidin	12	Linear	Low	APD3
63	Nisin E	34	Lantibiotic	High	UniProt: Q9KZQ8
64	Nisin P	34	Lantibiotic	High	UniProt: A0A2X0QF41
65	Nisin Z	34	Lantibiotic	High	UniProt: P29598
66	PO3	~11	Cyclic	Moderate-High	APD3
67	PO5	~11	Cyclic	Moderate-High	APD3
68	PO4	~12	Cyclic	Moderate-High	APD3

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69	P16	16	Linear	Low	APD3
70	Paenibacterin	13	Cyclic lipopeptide	High	UniProt: A0A0D6H9M8
71	Palustrin-1C	31	Linear	Low	APD3
72	Palustrin-1d	31	Linear	Low	APD3
73	Palustrin-2b	29	Linear	Low	APD3
74	Palustrin-2c	29	Linear	Low	APD3
75	Palustrin-3a	29	Linear	Low	APD3
76	Palustrin-3b	29	Linear	Low	APD3
77	Palustrinca	30	Linear	Low	APD3
78	Pep5	34	Lantibiotic	High	UniProt: P21538
79	Polymyxin B	10	Cyclic lipopeptide	High	UniProt: P0C1S2
80	Ponericin W3	24	Linear	Low	APD3
81	Ponericin W5	24	Linear	Low	APD3
82	PSDEF1	~15	Disulfide-rich	Moderate	APD3
83	Ranacyclin E	17	Disulfide-cyclized	High	APD3
84	Ranacyclin T	17	Disulfide-cyclized	High	APD3
85	Ranaturin-2csa	25	Linear	Low	APD3
86	Salivaricin B	~54	Head-to-tail cyclic	High	UniProt: Q6S6H4
87	Salivaricin P	~44	Linear bacteriocin	Moderate	APD3
88	SOD7	~17	Linear	Low	APD3
89	Subtilin	32	Lantibiotic	High	UniProt: P04170
90	Subtilisin A1	35	Disulfide-cyclized	High	UniProt: P54531
91	Taromycin A	13	Cyclic lipopeptide	High	UniProt: A0A0F7U6K6
92	Teixobactin	11	Depsipeptide	Very high	UniProt: A0A0H3HJK7
93	Temporin-1 CEB	17	Linear	Low	APD3
94	Temporin-1 csb	17	Linear	Low	APD3
95	Wollamide A	8	Cyclic	High	UniProt: A0A1B2E4R7
96	Wollamide B	8	Cyclic	High	UniProt: A0A1B2E4R8
97	TCP	~30	Linear	Low	APD3
98	Actagardine	19	Lantibiotic	High	UniProt: P51818
99	Epilancin	21	Lantibiotic	High	UniProt: Q9Z4J9
100	Gramicidin B	15	Linear	Moderate	UniProt: P0C1S6
101	Gramicidin C	15	Linear	Moderate	UniProt: P0C1S7

Docking was performed with 100 independent runs for each peptide; the resulting poses were clustered with an RMSD tolerance of 2.0 Å, and ranking was based on binding energy using default scoring weights without additional restraints.

Docking protocol validation was carried out by re-docking the native co-crystallized autoinducer ligand (AHL) of LasR using the same three-stage HADDOCK workflow (rigid-body docking, semi-flexible refinement, and water

refinement). A successful re-docking was defined by a heavy-atom RMSD ≤ 2.0 Å between the best-scoring pose and the crystallographic ligand, confirming the reliability of the docking setup for peptide screening.

Pharmacokinetic properties

Pharmacokinetic properties, including water solubility, Caco-2 permeability, intestinal absorption (human), skin permeability, BBB permeability, CYP2D6 inhibition, AMES toxicity, oral rat acute toxicity (LD50), and hepatotoxicity, were derived from the PKCSM-Pharmacokinetics (<https://biosig.lab.uq.edu.au/pkcsm/>) (10). The scatterplot was generated using Python with the packages pandas for data handling, NumPy for numerical operations, matplotlib.pyplot for plot visualization, and seaborn for graphical representation.

Results and Discussion

Peptide-protein docking was performed to estimate the binding affinities of the peptides for LasR. Out of the 101 peptides, Gramicidin C (-101.5 ± 4.4), Epilancin (-99.8 ± 9.1), Cathelicidin-DM (-91.4 ± 6.3), Gassericin A (-86.4 ± 17.6), PO4 (-83.9 ± 5.7), Gramicidin B (-81.1 ± 1.0), Salivaricin P (-79.7 ± 3.6), Mundtacin ATO6 (-77.4 ± 5.1), SOD7 (-76.4 ± 6.8), and Japonicin-1 (-75.2 ± 1.9) exhibited the highest binding energies. The binding energies of the remaining peptides are represented in Table 2 and Supplementary Table S1.

Gramicidin C interacted with LasR, forming eight hydrogen bonds, in which Lys 16 interacted with Leu 14 at a distance of 2.55 Å. The pharmacokinetic properties of Gramicidin C exhibited the water solubility of -2.892, Caco-2 permeability of 1.672, and no predicted human intestinal absorption. Additionally, the peptide exhibited a skin permeability of -2.735 and a Blood-Brain Barrier (BBB) permeability of -3.516. Gramicidin C was identified as a CYP2D6 inhibitor. Toxicity assessments indicated that it is AMES non-toxic but exhibited hepatotoxicity, with an oral rat acute toxicity (LD50) value of 2.482.

Epilancin formed 12 hydrogen bonds, in which Lys16 interacted with Leu21 at a distance of 2.89 Å. Likewise, the evaluated Epilancin showed a water solubility of -2.892, a Caco-2 permeability of 0.657, and no predicted human intestinal absorption. The peptide exhibited a skin permeability of -2.735 and a BBB permeability of -4.956. Metabolic analysis indicated that Epilancin is not a CYP2D6 inhibitor. Toxicity assessments showed that it is also AMES-toxic and does not exhibit hepatotoxicity, with an oral rat acute toxicity (LD50) value of 2.482.

Cathelicidin-DM formed 13 hydrogen bonds, in which Asp46 interacted with Lys8 at a distance of 2.54 Å. Cathelicidin-DM demonstrated a water solubility of -2.892, a Caco-2 permeability of 0.428, and no human intestinal absorption. Its skin permeability is -2.735, and the BBB permeability is -7.009. Neither was CYP2D6 metabolism inhibited, nor did it exhibit AMES toxicity. The oral rat acute toxicity (LD50) is 2.482, and it is found to be hepatotoxic.

Gassericin A formed 9 hydrogen bonds in which Glu48 interacted with Arg18 at a distance of 3.03 Å. Gassericin A exhibited water solubility of -2.892 and moderate Caco-2 permeability of 0.881, yet showed no human intestinal absorption and skin permeability of -2.735. Its Blood-Brain Barrier permeability was estimated to be -8.683, and it was a non-CYP2D6 inhibitor and nontoxic by *in silico* AMEs test. However, it exhibited the oral acute toxicity (LD50) of 2.482 and hepatotoxicity. PO4 exhibits a water solubility of -2.892, Caco-2 permeability of 0.708, skin permeability of -2.735, and BBB permeability of -3.137. Neither inhibits CYP2D6 metabolism nor exhibits AMES toxicity, but it is hepatotoxic.

PO4 formed 10 hydrogen bonds, in which Lys16 interacted with Leu3 at a distance of 2.79 Å. Gramicidin B formed 3 hydrogen bonds in which Glu 48 interacted with Val7 at a distance of 2.79 Å. Mundtacin ATO6 formed 9 hydrogen bonds, in which Lys42 interacted with Lys12 at a distance of 2.85 Å.

Salivaricin P formed 6 hydrogen bonds, in which Glu48 interacted with Gly6 at a dis-

tance of 3.27 Å. Salivaricin P exhibited the water solubility of -2.892, and the Caco-2 permeability of -3.341. Its skin permeability is -2.735, and BBB permeability is -13.394. It does not inhibit CYP2D6 metabolism, nor does it exhibit AMES toxicity. The oral rat acute toxicity (LD50) is 2.482, and it is also found to be hepatotoxic.

The docking results revealed that the top-ranking peptides formed multiple hydrogen bonds and hydrophobic contacts within the LasR ligand-binding domain (LBD), representing functional implications. Key residues such as Tyr56 and Ser129, which are integral components of the AHL-binding pocket, participate in stabilizing the native N-acyl homoserine lactone through hydrogen bonding and orientation of the lactone ring. Peptides that extended interactions with these residues likely interfered with the conformational changes required for

LasR activation, particularly the stabilization of the activation helix ($\alpha 9$) that is essential for downstream DNA binding. Additional contacts observed with residues in the hydrophobic core of the LBD (e.g., Leu36, Ile52, Val76) further support the potential of these peptides to disrupt ligand accommodation and prevent formation of the active LasR homodimer. Thus, the interaction implicates the blocking of LasR activation and transcriptional regulation.

Re-docking validation of the LasR co-crystallized ligand (PDB ID: 4NG2) was performed to confirm the reliability of the docking protocol. The re-docked pose reproduced the experimental conformation, and the RMSD indicated that the docking parameters accurately replicated the native binding mode. The RMSD values of the docked peptides are given in Table 2.

Table 2: Predicted Haddock score of cyclic peptides docked against the *Pseudomonas aeruginosa* LasR protein.

S. No	Peptide	Haddock score	RMSD
1	GramicidinC	-101.5 $\pm$ 4.4	51.3 $\pm$ 49.4
2	Epilancin	-99.8 $\pm$ 9.1	9.4 $\pm$ 0.3
3	Cathelicidin-DM	-91.4 $\pm$ 6.3	16.1 $\pm$ 0.8
4	GassericinA	-86.4 $\pm$ 17.6	0.9 $\pm$ 0.5
5	PO4	-83.9 $\pm$ 5.7	5.6 $\pm$ 0.1
6	GramicidinB	-81.1 $\pm$ 1.0	34.1 $\pm$ 19.0
7	Salivaricin P	-79.7 $\pm$ 3.6	0.5 $\pm$ 0.3
8	Mundticin ATO6	-77.4 $\pm$ 5.1	3.8 $\pm$ 0.4
9	SOD7	-76.4 $\pm$ 6.8	0.6 $\pm$ 0.3
10	Japonicin-1	-75.2 $\pm$ 1.9	3.4 $\pm$ 0.1
11	Humansalvic	-73.4 $\pm$ 2.2	15.0 $\pm$ 1.1
12	Brevicidine	-73.2 $\pm$ 4.2	1.7 $\pm$ 0.1
13	Esculentin1SEB	-72.3 $\pm$ 12.0	0.9 $\pm$ 0.6
14	Chrombacin	-68.6 $\pm$ 1.3	0.6 $\pm$ 0.6
15	Subtilin	-68.1 $\pm$ 4.4	0.7 $\pm$ 0.7
16	Lactococcin	-66.6 $\pm$ 12.4	1.1 $\pm$ 1.1
17	Brevinin-2GHb	-65.7 $\pm$ 10.4	7.8 $\pm$ 0.1
18	NisinE	-47.5 $\pm$ 10.2	0.7 $\pm$ 0.5
19	CirculinA	-63.4 $\pm$ 13.3	0.7 $\pm$ 0.5
20	Centrocin2	-63.1 $\pm$ 6.6	1.0 $\pm$ 0.8

21	Gallidermin	-62.8 ± 7.0	6.9 ± 0.1
22	Elafin	-62.5 ± 8.8	10.7 ± 0.1
23	CXCL12	-62.1 ± 7.2	12.0 ± 0.3
24	PolymyxinB	-61.8 ± 9.1	0.4 ± 0.3
25	GarvicinML	-61.5 ± 9.4	0.6 ± 0.4

The hydrogen-bond interactions of the other compounds are presented in Table 3 and Figure 1. Cyclic peptides were tested for intestinal absorption and permeability across the Blood-Brain Barrier using the scatter plot. The white region represents the GI absorption zone,

while the BBB permeability zone is represented by the yellow region. The Y-axis shows Caco-2 permeability, a stand-in for intestinal absorption, and the X-axis shows BBB permeability. The scattered plot is represented in Figure 2.

Table 3: Hydrogen bond interactions of cyclic peptide with LasR protein of *Pseudomonas aeruginosa*

S. No	Peptide	Hydrogen bond interaction	Distance Å
1	GramicidinC	LYS16 - DLE14	2.55
		SER44 - TRP13	2.87
		GLU48 - TRP15	2.87
		ILE52 - ALA5	3.04
		TYR56 - TRP9	2.89
		THR75 - TRP9	3.23
		ALA127 - TRP9	2.75
		SER129 - TRP9	2.76
2	Epilancin	LYS16 - LEU21	2.89
		GLU48 - DAL12	2.76
		GLU48 - ILE9	2.77
		ASN49 - DAL12	2.36
		ALA50 - LEU21	3.08
		ARG61 - ARG17	3.23
		ASP65 - LYS13	2.66
		ALA67 - DBU8	3.19
		ARG71 - ILE4	2.75
		VAL72 - LYS6	2.87
		LYS97 - ALA2	2.87
		LYS97 - LYS6	2.73
3	Cathellicidin-DM	ASP46 - LYS8	2.54
		ARG66 - SER2	2.79
		TYR69 - ARG4	2.73
		ARG71 - GLY9	2.92
		SER77 - LYS15	2.81
		THR80 - LYS13	2.80
		GLN94 - GLY18	2.84
		GLN94 - ARG17	2.93
		GLN94 - ARG17	2.74
		ARG96 - LEU16	3.14
		LYS97 - LEU14	2.71
		HIS99 - THR21	3.02
		GLU100 - ARG4	3.08

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4	GassericinA	GLU48 - ARG18 ALA50 - ARG18 PHE51 - ASP6 ILE52 - ARG18 TYR56 - ASP22 ARG60 - ASP22 GLU62 - LYS19 ASP65 - ARG18 ASP65 - ARG18	3.03 3.28 2.78 3.14 2.95 2.67 2.56 2.58 3.15
5	PO4	LYS16 - LEU3 TYR47 - LYS11 GLU48 - LYS15 ILE52 - TYR7 ALA58 - LYS18 GLU62 - ARG14 ASP65 - LYS15 ASP65 - LYS11 ARG66 - LYS15 ARG66 - LYS18	2.79 2.88 3.05 2.97 2.78 2.57 2.89 2.68 2.90 2.79
6	GramicidinB	GLU48 - VAL7 ARG71 - VAL7 ALA127 - TRP15	2.79 3.08 3.24
7	SalivaricinP	GLU48 - GLY6 ASN49 - VAL12 GLU62 - GLY21 ARG66 - GLY5 TYR69 - LYS1 GLU104 - LYS1	3.27 3.19 2.97 3.12 2.67 2.59
8	Mundtacin ATO6	LYS42 - LYS12 ASP43 - LYS12 ASP46 - ASN10 ASN49 - SER15 ASN49 - LYS1 ARG61 - ASP17 ARG61 - ASP17 GLU62 - TYR2 ASP65 - LYS20	2.85 2.69 2.72 2.80 2.84 2.61 2.92 2.75 2.58
9	SOD7	LYS42 - SER4 ASP43 - LYS7 SER44 - LYS9 GLN45 - ASP38 GLU48 - CYS19 ASN49 - CYS19 ASP65 - TYR18 THR80 - CYS8 THR80 - LYS9 LYS97 - LYS13	3.25 2.62 2.88 2.87 2.89 2.91 2.58 3.31 2.84 2.64
10	Japonicin-1	ARG61 - PHE1 ALA67 - LYS12	3.08 2.71

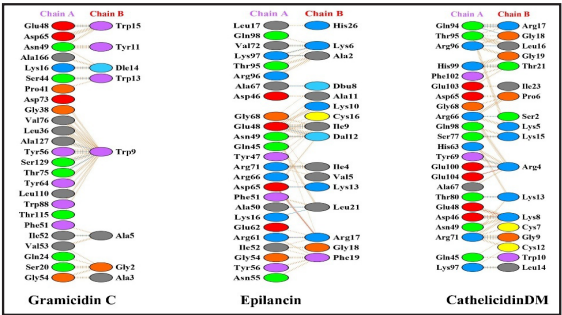


Fig. 1: Hydrogen bond interactions of cyclic peptides Gramicidin C, Epilancin, Cathelcidin-DM with the LasR protein of *Pseudomonas aeruginosa*

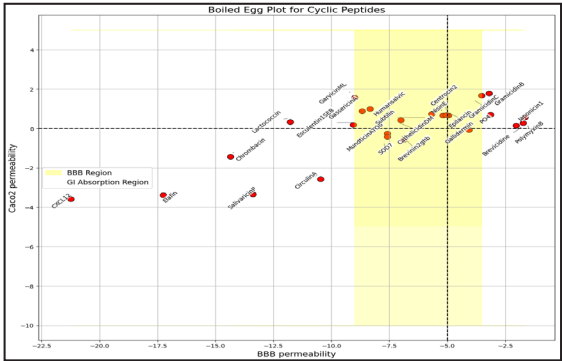


Fig. 2: The scatter plot for cyclic peptides illustrates their permeability across the Blood-Brain Barrier and intestinal absorption.

However, despite promising docking affinities, these peptides face major pharmacokinetic limitations; many showed poor intestinal absorption and negligible BBB permeability. Hence, these could be considered as preliminary lead structures requiring further optimisation through cyclisation refinement or the incorporation of non-natural amino acids. These modifications can enhance their stability, permeability, and bioavailability. Ultimately, experimental validation is needed before considering them for therapeutic use.

The pharmacokinetic properties of the other peptides are presented in Table 4, while additional peptides are listed in Supplementary Table S2, and the metabolism of other CYP2D6 inhibitors and substrates is presented in Supplementary Table S3.

Table 4: Pharmacokinetic properties of the selected Cyclic peptides

S. No	Cyclic peptide	Water solubility	Caco-2 permeability	Intestinal absorption (human)	Skin Permeability	BBB permeability	CYP 2D6 inhibitor	AMES toxicity	Oral Rat Acute Toxicity (LD50)	Hepato-toxicity
1	GramicidinC	-2.892	1.672	0	-2.735	-3.516	Yes	No	2.482	Yes
2	Epilancin	-2.892	0.657	0	-2.735	-4.956	No	No	2.482	No
3	Cathelcidin-DM	-2.892	0.428	0	-2.735	-7.009	No	No	2.482	Yes
4	GassericinA	-2.892	0.881	0	-2.735	-8.683	No	No	2.482	Yes
5	PO4	-2.892	0.708	0	-2.735	-3.137	No	No	2.482	Yes
6	GramicidinB	-2.892	1.788	0	-2.735	-3.192	No	No	2.482	Yes
7	SalivaricinP	-2.892	-3.341	0	-2.735	-13.394	No	No	2.482	Yes
8	Mundticin ATO6	-2.892	-0.258	0	-2.735	-7.582	No	No	2.482	Yes
9	SOD7	-2.892	-0.421	0	-2.735	-7.6	No	No	2.482	No
10	Japonicin-1	-2.892	0.566	0	-2.735	-1.646	No	No	2.482	Yes
11	Humansalvic	-2.892	0.985	0	-2.735	-8.334	No	No	2.482	Yes

12	Brevicidine	-2.892	0.141	0	-2.735	-2.031	No	No	2.482	Yes
13	Esculentin1SEB	-2.892	0.193	0	-2.735	-9.056	No	No	2.482	No
14	Chrombacin	-2.892	-1.425	0	-2.735	-14.359	No	No	2.482	No
15	Subtilin	-2.892	0.729	0	-2.735	-5.681	No	No	2.482	Yes
16	Lactococcin	-2.892	0.33	0	-2.735	-11.784	No	No	2.482	No
17	Brevinin-2GHb	-2.892	-0.583	0	-2.735	-6.905	No	No	2.482	No
18	NisinE	-2.892	0.666	0	-2.735	-5.202	No	No	2.482	Yes
19	CirculinA	-2.892	-2.569	0	-2.735	-10.468	No	No	2.482	Yes
20	Centrocin2	-2.892	0.684	0	-2.735	-5.058	No	No	2.482	Yes
21	Gallidermin	-2.892	-0.068	0	-2.735	-4.069	No	No	2.482	Yes
22	Elafin	-2.892	-3.373	0	-2.735	-17.264	No	No	2.482	No
23	CXCL12	-2.892	-3.586	0	-2.735	-21.243	No	No	2.482	Yes
24	PolymyxinB	-2.892	0.28	0	-2.735	-1.722	No	No	2.482	No
25	GarvicinML	-2.892	1.567	0	-2.735	-8.991	No	No	2.482	Yes

Discussion

Targeting the LasR protein with cyclic peptides offers a promising strategy for developing quorum-sensing inhibitors. LasR, a multifunctional regulator with ligand-binding, N-terminal, and C-terminal domains, activates the elastase gene (LasB) upon binding its autoinducer (3-oxo-C12-HSL) and undergoing dimerisation. Inhibiting this dimerisation by blocking key active-site residues can disrupt LasR's transcriptional activity and prevent downstream gene activation (11-13).

At the structural level, the LasR ligand-binding domain (LBD) is known to adopt a flexible conformation upon ligand binding, as highlighted by crystallographic studies of LasR in complex with its native autoinducer (3O-C12-HSL). This flexibility can make it challenging to design ligands that reliably enforce an antagonist conformation, especially given that LasR activation depends on stabilising a multimeric form (dimer or higher). Indeed, multimerisation of LasR has been shown to rely heavily on its N-terminal region. Thus, a small library of diverse cyclic peptides would be an ideal ligand for targeting LasR's binding pocket, thereby further inhibiting biofilm formation (14).

In this study, Gramicidin C and Gassericin A extended their interactions with the key residues Tyr 56 and Ser 129, while the other compounds interacted with other amino acid residues, both via hydrogen bonds and non-covalent interactions. The identified peptides were confirmed to be antagonists because none of them extended hydrophobic interactions with Val-76, Cys-79, and Leu-125 of the LasR protein to function as activators. By extending interactions across these critical sites, the peptides likely disrupted the conformational changes and activation-helix stabilisation required for LasR activation and homodimer formation. Overall, these interactions indicate a strong potential to block LasR function and its downstream transcriptional regulation.

These interactions were previously shown to be crucial by the association-dissoci-

ation model, which serves as an autoinducer or agonist molecule.

Peptide-based modulation of LasR: for instance, QslA, a natural antiactivator, has been shown to bind the LasR LBD and disrupt dimerisation by occupying its dimer interface. This suggests that protein–protein interactions (PPIs) can indeed modulate LasR functionality, but also underscores the importance of targeting the dimer interface rather than just the AHL binding pocket.

Docking scores in our study provide a relative ranking of peptide–LasR interactions but don't directly translate into experimentally measured binding affinity (K_n). Scoring functions are approximate and often fail to fully capture the complexity of real binding thermodynamics, which emphasizes further *in vitro* investigations (15).

GS is a cyclic peptide naturally produced by *Aneurinibacillus migulanus*, which exerts antimicrobial activity by creating pores in the outer membrane (16). Native GS serves as a natural scaffold for modifying amino acids to maintain antimicrobial activity while reducing toxicity. Epilancin 15X, a lantibiotic produced by *Staphylococcus epidermidis*, exhibits submicromolar antimicrobial activity against certain pathogenic Gram-positive bacteria; however, its effectiveness against *Pseudomonas aeruginosa* has not yet been evaluated (17). Likewise, Gassericin A is a cyclic peptide consisting of 58 amino acid residues (5,654 Da) and exhibits antibacterial activity against multiple foodborne Gram-positive pathogens, including *B. cereus*, *L. monocytogenes*, and *S. aureus*. However, its efficacy against *Pseudomonas aeruginosa* has not yet been reported. Salivaricins are lantibiotics produced by the oral bacterium *S. salivarius*, known for their selective antimicrobial activity against bacteria in the oral cavity and upper respiratory tract. However, their antimicrobial activity against *P. aeruginosa* has not yet been documented (18).

Among the screened peptides, Epilancin, SOD7, Esculentin, and Chrombacin were

non-hepatotoxic and were not CYP2D6 inhibitors; however, all lacked intestinal absorption. Thus, the addition of absorption enhancers and protease inhibitors could increase drug absorption. Also, the alternative method of administration of the drug by either oral or parenteral administration could be considered.

Bakuchiol, V-06-018, 4-Br-PHL, and their derivatives are among the few well-studied LasR inhibitors and quorum-sensing antagonists reported against *P. aeruginosa* (19,20). Bakuchiol has been shown to inhibit *P. aeruginosa* biofilm formation by 75.5%, while reducing pyocyanin and rhamnolipid production by 71.5% and 66.9%, respectively (21). The naturally derived phenolic compound Norlobaridone, isolated from *Parmotrema*, reduced the *P. aeruginosa* biofilm formation by 64.6% and its comparative virulence factors, such as 61.1% pyocyanin and 55% rhamnolipids, in a dose-dependent manner. V-06-018 effectively regulates the virulence factors such as elastase and pyocyanin by disrupting the interaction of the autoinducer signal molecule with the LasR and thus reducing its pathogenicity (22). Senno-side, an anthraquinone compound, suppresses quorum-sensing and QS-regulated processes, including virulence factor production, biofilm formation, motility, and the pathogenicity of *P. aeruginosa*, even at subinhibitory concentrations (23). Likewise, mBTL, a synthetic analogue of native *P. aeruginosa* autoinducers, has been shown to inhibit LasR expression. Thus, the earlier reported compounds, both synthetic and natural, reduced biofilm formation by 75%, leading to the exploration of cyclic peptides in this study.

Despite many efforts to inhibit *P. aeruginosa* quorum sensing with small molecules, most of them mimic AHLs and suffer from limited potency, rapid degradation, or low specificity. In contrast, cyclic peptide-based LasR inhibitors have been little explored, and no comprehensive screening has targeted the LasR binding domain. Our study bridges this gap by using *in silico* peptide–peptide docking, coupled

with structure-based docking and pharmacokinetic (ADMET) predictions, on a curated library of cyclic and semicyclic peptides and molecules with greater stability, tunability, and protease resistance than linear peptides or small-molecule AHL mimics for the development of non-AHL-mimetic LasR antagonists.

Despite exhibiting strong *in silico* binding energies, the selected peptides present significant limitations in intracellular target engagement that are rooted in their structural chemistry. Linear hydrophobic peptides such as Gramicidin B and C preferentially partition into lipid bilayers and form membrane-associated channels, a property driven by their alternating hydrophobic residues, which inherently limit cytosolic penetration and favour membrane sequestration (24). Epilancin, an antibiotic stabilised by multiple lanthionine bridges, displays enhanced conformational rigidity and proteolytic stability; however, this same rigidity reduces the entropic adaptability required for membrane translocation, thereby constraining intracellular delivery (25). Cationic amphipathic peptides, including Cathelicidin-DM, SOD7, and Japonicin-1, exhibit strong electrostatic interactions with negatively charged membranes, promoting surface accumulation and membrane disruption rather than productive intracellular uptake, while remaining susceptible to intracellular proteases (26,27). Similarly, structured bacteriocins such as Gassericin A, Salivaricin P, Mundticin ATO6, and PO4 possess compact folds and high charge density, which restrict passive diffusion across lipid bilayers and, in the absence of dedicated transport mechanisms, result in limited cytosolic access (28). Collectively, these observations underscore that binding affinity alone is insufficient to predict intracellular efficacy and that peptide size, rigidity, charge distribution, and secondary structure critically govern permeability and bioavailability, which require further chemical modification or other delivery strategies to improve.

While cyclic peptides are more stable than linear ones, their large size, high polarity,

and flexibility limit oral bioavailability and expose them to proteolytic degradation. Further chemical modification or advanced delivery strategies, such as N-methylation, incorporation of non-natural amino acids, cyclisation, PEGylation, lipidation, prodrug derivatisation, nano-encapsulation, and the use of absorption enhancers (e.g., medium-chain fatty acids), may improve stability and uptake, making them more suitable as therapeutic candidates. (29,30).

Intracellular delivery is also a major concern as LasR is cytoplasmic, and the *P. aeruginosa* outer membrane is highly restrictive — generally allowing only small (< 200 Da) molecules to pass. Many of our peptides are far larger, so their uptake is unlikely without chemical modifications (e.g., lipidation, cyclisation, cell-penetrating tags, or nanoparticle-based delivery) (31).

Hence, these *in silico* findings suggest that the identified peptides, which exhibit higher binding energies, may effectively bind to the LasR protein of *P. aeruginosa* and, consequently, inhibit biofilm formation and associated virulence factors. However, further *in vitro* and *in vivo* studies are required to validate their potential as candidate drug molecules against *P. aeruginosa*.

Conclusion

Gramicidin C and Gassericin A extended their interactions with the key residues Tyr 56 and Ser 129 of the LasR protein. The identified high-affinity compounds may potentially act as LasR antagonists, as suggested by their predicted binding modes; however, this inference is based solely on docking analyses. Consequently, their ability to suppress biofilm formation and downstream virulence factors remains hypothetical and requires further validation through *in vitro* and *in vivo* studies. Both compounds have limited intestinal absorption and permeability, highlighting potential pharmacokinetic challenges and further formulation strategies would be required to overcome these limitations.

Acknowledgements

The management of Saveetha Institute of Medical and Technical Sciences, Chennai, is deeply appreciated by the authors for their ongoing assistance and provision of the facilities required to conduct this study.

Author Contribution

Dr. K. Sangeetha: Conceived and designed the study, supervised the research work, and finalised the manuscript for submission.

A. Sowmiya: Performed the experimental work, data collection, and initial manuscript drafting.

Dr. K. A. Selvam: Contributed to data analysis, interpretation of results, and manuscript refinement.

Dr. Indu Purushothaman: Assisted in literature review, visualisation, and validation of experimental results.

All authors have read and approved the final version of the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

All data generated or analysed during this study are included in this published article.

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Supplementary Information

Supplementary Table 1: Binding energy of cyclic peptide against LasR

S. No	Cyclic peptide	Haddock score
1	ABP118	-47.4 ± 7.0
2	Amoebapore A	-51.2 ± 8.0
3	Amylocyclin	-50.9 ± 3.6
4	Baceridin	-39.7 ± 5.4
5	BrevicidineB	-58.4 ± 5.8
6	Brevinin-1	-42.9 ± 5.3
7	Brevinin-1DYa	-46.4 ± 10.2
8	Brevinin-1DYb	-64.5 ± 6.9
9	Brevinin-1TSa	-45.0 ± 2.5
10	Brevinin-2DYb	-55.2 ± 3.5
11	Brevinin-2DYd	-59.8 ± 8.9
12	Brevinin-2DYe	-49.0 ± 4.0
13	Brevinin-2GHc	-46.6 ± 2.4

14	Brevinin-2TSa	-43.1 ± 4.4
15	BTD1	-53.8 ± 7.6
16	CarnocyclinA	-51.0 ± 13.5
17	CeratotoxinA	-40.6 ± 2.0
18	CeratotoxinB	-47.8 ± 7.3
19	CeratotoxinC	-57.4 ± 6.2
20	Champacyclin	-54.5 ± 5.5
21	Cinnamycin	-53.4 ± 7.5
22	CirculinB	-38.4 ± 6.5
23	ColistinA	-49.8 ± 5.4
24	CRS4C1A	-56.1 ± 3.6
25	Cyclopeptide2	-17.3 ± 0.8
26	CyclopsychotrideA	-40.8 ± 4.1
27	Daptomycin	-53.3 ± 5.7
28	Darobactin	-58.6 ± 2.5
29	Duramycin	-56.7 ± 4.1
30	EnterocinRM6	-52.8 ± 4.4
31	GramicidinS	-57.0 ± 6.5

Molecular docking of cyclic peptides against the quorum sensing LasR protein of
Pseudomonas aeruginosa

32	Guentherin	-60.7 ± 9.4
33	HumanKR20	-47.3 ± 9.2
34	Japonicin-1NPB	-54.2 ± 5.1
35	Japonicin-2	-44.7 ± 10.8
36	KR12	-43.4 ± 7.1
37	Labaditin	-44.9 ± 4.5
38	LactocyclinQ	-52.4 ± 3.1
39	Lassomycin	-41.4 ± 1.8
40	Lugdunin	-42.5 ± 2.4
41	LysocinE	-60.1 ± 3.7
42	MDN0666	-40.6 ± 6.2
43	MDN0666Beta	-33.9 ± 0.9
44	MicrocinJ25	-48.6 ± 8.7
45	MicrococcinP1	-38.3 ± 1.9
46	Myxinidin	-51.5 ± 6.5
47	NisinP	-40.0 ± 1.4
48	NisinZ	-50.2 ± 8.0
49	PO3	-58.9 ± 3.9
50	PO5	-47.7 ± 10.6
51	P16	-44.1 ± 5.1
52	Paenibacterin	-52.8 ± 5.6
53	Palustrin1C	-55.0 ± 3.7
54	Palustrin1d	-42.8 ± 8.5
55	Palustrin2b	-44.0 ± 8.4
56	Palustrin2c	-46.8 ± 7.7
57	Palustrin3a	-53.1 ± 16.4
58	Palustrin3b	-46.9 ± 19.0
59	Palustrinca	-53.8 ± 2.0
60	Pep5	-45.0 ± 4.2
61	PonericinW3	-51.3 ± 3.3
62	PonericinW5	-51.2 ± 2.1
63	PSDEF1	-49.6 ± 8.2
64	RanacyclinE	-55.4 ± 0.5
65	RanacyclinT	-58.5 ± 13.3
66	Ranaturerin-2csa	-52.2 ± 4.7
67	SalivaricinB	-42.6 ± 7.3
68	SubtilosinA1	-53.4 ± 6.9
69	TaromycinA	-56.5 ± 4.3
70	Teixobactin	-54.3 ± 9.0
71	Temporin1CEB	-49.0 ± 3.2
72	Temporin1csb	-50.6 ± 2.0
73	WollamideA	-51.4 ± 3.8
74	WollamideB	-48.8 ± 1.7
75	TCP	-58.9 ± 12.8
76	Actagardine	-59.9 ± 0.7

Supplementary Table 2: Pharmacokinetic properties of Cyclic peptides

S. No	Cyclic peptide	Water solubility	Caco-2 permeability	Intestinal absorption (human)	Skin Permeability	BBB permeability	CYP2D6 inhibitor	AMES toxicity	Oral Rat Acute Toxicity (LD50)	Hepato-toxicity
1	ABP118	-2.892	0.699	0	-2.735	-3.537	No	No	2.482	Yes
2	Amoebapore A	-2.892	-8.885	0	-2.735	-31.501	No	No	2.482	No
3	Amylocyclin	-2.892	0.932	0	-2.735	-10.354	No	No	2.482	No
4	Bacerein	-3.071	0.2	15.145	-2.735	-1.113	No	No	2.592	Yes
5	BrevicidineB	-2.892	0.141	0	-2.735	-2.031	No	No	2.482	Yes
6	Brevinin-1	-2.892	0.839	0	-2.735	-3.652	No	No	2.482	No
7	Brevinin-1DYa	-2.892	0.824	0	-2.735	-3.051	No	No	2.482	No
8	Brevinin-1DYb	-2.892	0.802	0	-2.735	-3.124	No	No	2.482	No
9	Brevinin-1TSa	-2.892	0.365	0	-2.735	-4.139	No	No	2.482	No
10	Brevinin-2DYb	-2.892	-0.132	0	-2.735	-6.365	No	No	2.482	No
11	Brevinin-2DYd	-2.892	-0.103	0	-2.735	-6.405	No	No	2.482	No

12	Brevinin-2Dye	-2.892	0.174	0	-2.735	-6.881	No	No	2.482	No
13	Brevinin-2Ghc	-2.892	-0.326	0	-2.735	-6.464	No	No	2.482	Yes
14	Brevinin-2TSa	-2.892	0.814	0	-2.735	-5.043	No	No	2.482	No
15	BTD1	-2.892	0.412	0	-2.735	-4.08	No	No	2.482	No
16	CarnocyclinA	-2.892	0.981	0	-2.735	-6.612	No	No	2.482	Yes
17	CeratotoxinA	-2.892	0.433	0	-2.735	-5.654	No	No	2.482	No
18	CeratotoxinB	-2.892	0.484	0	-2.735	-5.472	No	No	2.482	No
19	CeratotoxinC	-2.892	1.008	0	-2.735	-3.184	No	No	2.482	Yes
20	Champacyclin	-2.903	0.335	0	-2.735	-1.402	No	No	2.507	Yes
21	Cinnamycin	-2.892	0.171	0	-2.735	-3.662	No	No	2.482	Yes
22	CirculinB	-2.892	-2.504	0	-2.735	-10.641	No	No	2.482	Yes
23	ColistinA	-2.892	0.235	0	-2.735	-1.909	No	No	2.482	Yes
24	CRS4C1A	-2.892	0.755	0	-2.735	-7.312	No	No	2.482	No
25	Cyclopeptide2	-2.001	-0.342	12.898	-2.735	-0.87	No	No	1.731	Yes
26	CyclopsychotrideA	-2.892	-2.825	0	-2.735	-11.035	No	No	2.482	Yes
27	Daptomycin	-2.892	-0.716	0	-2.735	-3.073	No	No	2.482	Yes
28	Darobactin	-2.89	0.155	0	-2.735	-1.372	No	No	2.541	Yes
29	Duramycin	-2.892	0.193	0	-2.735	-3.513	No	No	2.482	Yes
30	EnterocinRM6	-2.892	1.733	0	-2.735	-11.3	No	No	2.482	No
31	GramicidinS	-2.892	0.103	17.346	-2.735	-2.225	No	No	2.482	Yes
32	Guentherin	-2.892	0.62	0	-2.735	-5.292	No	No	2.482	No
33	HumanKR20	-2.892	-0.098	0	-2.735	-5.196	No	No	2.482	Yes
34	Japonicin-1NPB	-2.892	0.71	0	-2.735	-2.442	No	No	2.482	No
35	Japonicin-2	-2.892	0.733	0	-2.735	-3.926	No	No	2.482	No
36	KR12	-2.892	0.054	0	-2.735	-2.981	No	No	2.482	Yes
37	Labaditin	-2.893	0.172	0	-2.735	-1.607	No	No	2.557	Yes
38	LactocyclinQ	-2.892	1.669	0	-2.735	-8.715	No	No	2.482	No
39	Lassomycin	-2.892	0.036	0	-2.735	-3.627	No	No	2.482	Yes
40	Lugdunin	-2.912	0.224	0	-2.735	-1.255	No	No	2.525	Yes
41	LysocinE	-2.892	-0.029	0	-2.735	-2.841	No	No	2.482	Yes
42	MDN0666	-2.892	0.018	0	-2.735	-1.766	No	No	2.483	Yes
43	MDN0666Beta	-2.892	0.05	0	-2.735	-1.7	No	No	2.483	Yes
44	MicrocinJ25	-2.892	-0.164	0	-2.735	-5.352	No	No	2.482	Yes

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45	MicrococinP1	-2.892	0.037	0	-2.735	-2.691	No	No	2.482	Yes
46	Myxinidin	-2.892	-0.274	0	-2.735	-2.697	No	No	2.482	Yes
47	NisinP	-2.892	0.686	0	-2.735	-5.229	No	No	2.482	Yes
48	NisinZ	-2.892	-2.161	0	-2.735	-11.369	No	No	2.482	No
49	PO3	-2.892	0.439	0	-2.735	-3.117	No	No	2.482	Yes
50	PO5	-2.892	0.18	0	-2.735	-2.448	No	No	2.482	No
51	P16	-2.892	0.318	0	-2.735	-3.176	No	No	2.482	No
52	Paenibacterin	-2.892	0.171	0	-2.735	-2.968	No	No	2.482	No
53	Palustrin1C	-2.892	0.802	0	-2.735	-4.661	No	No	2.482	Yes
54	Palustrin1d	-2.892	0.796	0	-2.735	-4.51	No	No	2.482	Yes
55	Palustrin2b	-2.892	-0.398	0	-2.735	-6.522	No	No	2.482	No
56	Palustrin2c	-2.892	-0.398	0	-2.735	-6.522	No	No	2.482	No
57	Palustrin3a	-2.892	1.059	0	-2.735	-7.535	No	No	2.482	No
58	Palustrin3b	-2.892	1.045	0	-2.735	-7.554	No	No	2.482	No
59	Palustrinca	-2.892	-0.237	0	-2.735	-6.214	No	No	2.482	No
60	Pep5	-2.892	0.636	0	-2.735	-5.915	No	No	2.482	No
61	PonericinW3	-2.892	0.822	0	-2.735	-4.758	No	No	2.482	No
62	PonericinW5	-2.892	0.723	0	-2.735	-3.72	No	No	2.482	No
63	PSDEF1	-2.892	-5.101	0	-2.735	-18.843	No	No	2.482	Yes
64	RanacyclinE	-2.892	-0.102	0	-2.735	-3.533	No	No	2.482	No
65	RanacyclinT	-2.892	0.019	0	-2.735	-3.411	No	No	2.482	No
66	Ranaturin-2csa	-2.892	0.017	0	-2.735	-6.327	No	No	2.482	No
67	SalivaricinB	-2.892	0.474	0	-2.735	-4.603	No	No	2.482	Yes
68	SubtilisinA1	-2.892	0.343	0	-2.735	-5.324	No	No	2.482	Yes
69	TaromycinA	-2.892	-0.737	0	-2.735	-3.062	No	No	2.482	Yes
70	Teixobactin	-2.892	0.07	0	-2.735	-2.383	No	No	2.482	No
71	Temporin1CEB	-2.892	0.466	0	-2.735	-2.132	No	No	2.482	Yes
72	Temporin1csb	-2.892	0.544	0	-2.735	-1.916	No	No	2.482	Yes
73	WollamideA	-2.898	0.17	0	-2.735	-1.21	No	No	2.521	Yes
74	WollamideB	-2.896	0.147	0	-2.735	-1.135	No	No	2.512	Yes
75	TCP	-2.892	-4.933	0	-2.735	-28.307	No	No	2.482	Yes
76	Actagardine	-2.892	0.455	0	-2.735	-2.26	No	No	2.482	Yes

Supplementary Table 3: Metabolism of various inhibitors and substrates

S. No	Cyclic peptide	CYP2D6 substrate	CYP3A4 substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP3A4 inhibitor
1	ABP118	No	Yes	No	No	No	No
2	Amoebapore A	No	Yes	No	No	No	No
3	Amylocyclin	No	No	No	No	No	No
4	Baceridin	No	Yes	No	No	No	No
5	Brevicidine	No	No	No	No	No	No
6	BrevicidineB	No	No	No	No	No	No
7	Brevinin-1	No	Yes	No	No	No	No
8	Brevinin-1DYa	No	Yes	No	No	No	No
9	Brevinin-1DYb	No	Yes	No	No	No	No
10	Brevinin-1TSa	No	Yes	No	No	No	No
11	Brevinin-2DYb	No	Yes	No	No	No	No
12	Brevinin-2DYd	No	Yes	No	No	No	No
13	Brevinin-2DYe	No	Yes	No	No	No	No
14	Brevinin-2GHb	No	No	No	No	No	No
15	Brevinin-2GHc	No	Yes	No	No	No	No
16	Brevinin-2TSa	No	No	No	No	No	No
17	BTD1	No	No	No	No	No	No
18	CarnocyclinA	No	No	No	No	No	No
19	Cathelicidin-DM	No	Yes	No	No	No	No
20	Centrocin2	No	Yes	No	No	No	No
21	CeratotoxinA	No	Yes	No	No	No	No
22	CeratotoxinB	No	No	No	No	No	No
23	CeratotoxinC	No	Yes	No	No	No	No
24	Champacyclin	No	Yes	No	No	No	No
25	Chrombacin	No	Yes	No	No	No	No
26	Cinnamycin	No	No	No	No	No	No
27	CirculinA	No	Yes	No	No	No	No
28	CirculinB	No	Yes	No	No	No	No
29	ColistinA	No	No	No	No	No	No
30	CRS4C1A	No	No	No	No	No	No
31	CXCL12	No	Yes	No	No	No	No
32	Cyclopeptide2	No	No	No	No	No	No
33	CyclopsychotrideA	No	Yes	No	No	No	No
34	Daptomycin	No	No	No	No	No	No
35	Darobactin	No	No	No	No	No	No
36	Duramycin	No	No	No	No	No	No
37	Elafin	No	Yes	No	No	No	No
38	EnterocinRM6	No	Yes	No	No	No	No
39	Esculentin1SEB	No	Yes	No	No	No	No
40	Gallidermin	No	No	No	No	No	No
41	GarvicinML	No	Yes	No	No	No	No
42	GassericinA	No	Yes	No	No	No	No

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43	GramicidinS	No	Yes	No	No	No	No
44	Guentherin	No	Yes	No	No	No	No
45	HumanKR20	No	Yes	No	No	No	No
46	Humansalvic	No	Yes	No	No	No	No
47	Japonicin-1	No	Yes	No	No	No	No
48	Japonicin-1NPB	No	Yes	No	No	No	No
49	Japonicin-2	No	Yes	No	No	No	No
50	KR12	No	Yes	No	No	No	No
51	Labaditin	No	No	No	No	No	No
52	Lactococcin	No	No	No	No	No	No
53	LactocyclinQ	No	Yes	No	No	No	No
54	Lassomycin	No	Yes	No	No	No	No
55	Lugdunin	No	Yes	No	No	No	No
56	LysocinE	No	No	No	No	No	No
57	MDN0666	No	No	No	No	No	No
58	MDN0666Beta	No	No	No	No	No	No
59	MicrocinJ25	No	No	No	No	No	No
60	MicrococcinP1	No	No	No	No	No	No
61	Mundticin ATO6	No	No	No	No	No	No
62	Myxinidin	No	Yes	No	No	No	No
63	NisinE	No	No	No	No	No	No
64	NisinP	No	No	No	No	No	No
65	NisinZ	No	No	No	No	No	No
66	PO3	No	Yes	No	No	No	No
67	PO5	No	No	No	No	No	No
68	PO4	No	Yes	No	No	No	No
69	P16	No	No	No	No	No	No
70	Paenibacterin	No	No	No	No	No	No
71	Palustrin1C	No	No	No	No	No	No
72	Palustrin1d	No	No	No	No	No	No
73	Palustrin2b	No	No	No	No	No	No
74	Palustrin2c	No	No	No	No	No	No
75	Palustrin3a	No	No	No	No	No	No
76	Palustrin3b	No	No	No	No	No	No
77	Palustrinca	No	Yes	No	No	No	No
78	Pep5	No	No	No	No	No	No
79	PolymyxinB	No	No	No	No	No	No
80	PoneracinW3	No	Yes	No	No	No	No
81	PoneracinW5	No	Yes	No	No	No	No
82	PSDEF1	No	Yes	No	No	No	No
83	RanacyclinE	No	No	No	No	No	No
84	RanacyclinT	No	No	No	No	No	No
85	Ranatuerin-2csa	No	Yes	No	No	No	No
86	SalivaricinB	No	No	No	No	No	No
87	SalivaricinP	No	Yes	No	No	No	No

88	SOD7	No	No	No	No	No	No
89	Subtilin	No	No	No	No	No	No
90	SubtilosinA1	No	Yes	No	No	No	No
91	TaromycinA	No	No	No	No	No	No
92	Teixobactin	No	No	No	No	No	No
93	Temporin1CEB	No	Yes	No	No	No	No
94	Temporin1csb	No	Yes	No	No	No	No
95	WollamideA	No	Yes	No	No	No	No
96	WollamideB	No	Yes	No	No	No	No
97	TCP	No	Yes	No	No	No	No
98	Actagardine	No	Yes	No	No	No	No
99	Epilancin	No	No	No	No	No	No
100	GramicidinB	No	Yes	No	No	No	No
101	GramicidinC	No	Yes	No	No	No	No