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Research Article

Antibacterial and Anticancer Properties of L-Cyclic Dipeptides from *Streptomyces sanyensis* ARR153, an Endophyte in *Caulerpa lentillifera* J. Agardh

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Abstract

Background and Objective: Marine algal endophytes remain a highly promising yet underexplored frontier for discovering unique bioactive scaffolds. This study aimed to isolate endophytic actinomycetes from the tissues of the green macroalga *Caulerpa lentillifera* in Phetchaburi Province, Thailand and evaluate their antibacterial and selective anticancer properties. **Materials and Methods:** Actinomycetes were isolated from surface-sterilized *C. lentillifera* tissues using humic acid-vitamins agar. The most antagonistic strain, ARR153, was identified via morphological and 16S rDNA phylogenetic analysis. Following large-scale cultivation on ISP-2 agar, the secondary metabolites were extracted using ethyl acetate and purified via column and thin-layer chromatography. Purified compounds were evaluated using MIC microdilution assays, *in vitro* MTT cytotoxicity tests against human cancer lines (HeLa, HepG2, MDA-MB-231) and normal Vero cells and *in silico* molecular docking/ADMET simulations targeting the EGFR kinase domain. **Results:** Isolate ARR153 was identified as *Streptomyces sanyensis*. Chromatographic purification yielded four L-cyclic dipeptides (diketopiperazines): Cyclo-(L-Leu-L-Val), Cyclo-(L-Leu-L-Thr), Cyclo-(L-Leu-L-Ile) and Cyclo-(L-Leu-L-Leu). These molecules exhibited broad-spectrum bactericidal activity against Gram-positive pathogens, including clinical MRSA (MICs: 16-128 µg/mL). In cytotoxicity assays, the dipeptides induced strong, dose-dependent growth inhibition against MDA-MB-231 and HeLa cells, while showing no toxicity toward normal Vero cells (IC₅₀>400 µg/mL). Notably, Cyclo-(L-Leu-L-Leu) demonstrated a higher selectivity index for breast cancer cells than doxorubicin. *In silico* modeling confirmed that the compounds effectively bind the EGFR active site via essential hydrogen bonds at residue Thr830 with favorable predictive pharmacokinetic profiles. **Conclusion:** This study demonstrates that *C. lentillifera*-associated *S. sanyensis* ARR153 is a valuable source of bioactive L-cyclic dipeptides. These natural products offer potent structural platforms for the design of targeted biopharmaceuticals to counter multidrug-resistant bacterial infections and specific human cancers.

Key words: Antibacterial activity, anticancer activity, *Caulerpa lentillifera* J. Agardh, endophyte, L-cyclic dipeptides, *Streptomyces sanyensis* ARR153

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Endophytes are defined as microorganisms that colonize internal plant tissues without inducing disease symptoms or causing apparent harm to the host¹. Among these, endophytic actinomycetes have garnered substantial global research interest because they remain relatively underexplored compared to their terrestrial counterparts, yet represent a prolific source of novel natural products². These specialized bacteria exhibit extensive metabolic capacity with proven utility in both biomedical and agricultural sectors³. Over the past few decades, a vast array of structurally diverse secondary metabolites possessing various biological activities have been isolated from these organisms⁴, reinforcing their status as a promising reservoir for drug discovery⁵.

While the majority of endophyte research historically focused on terrestrial plants, marine macroalgae have emerged as an equally compelling frontier. *Caulerpa lentillifera* J. Agardh (Ulvothamniales, Bryopsidales), commonly known as sea grape, is a green macroalga widely distributed across the tropical Indo-Pacific region⁶. This species holds considerable promise for the healthcare industry due to an abundance of endogenous bioactive compounds with documented anticancer⁷, antibacterial⁸ and antioxidant properties⁹.

The ecological interactions between macroalgae and their associated endophytes—including bacteria and fungi—are highly complex and fundamental to host vitality. These symbiotic microbes are vital for algal morphogenesis, growth and survival; they actively participate in nitrogen fixation, phytohormone production, nutrient transport, pollutant detoxification and reproductive signaling. Furthermore, they protect the host by releasing antifouling and antimicrobial agents, thereby facilitating algal adaptation to dynamic environmental stressors¹⁰. This reciprocal relationship is so profoundly integrated that the macroalga and its associated microbiota are collectively conceptualized as a single functional entity or holobiont¹¹. Consequently, the secondary metabolites synthesized by the microbial components of this holobiont represent an immense, untapped resource for biotechnological and pharmaceutical applications¹².

To date, marine algal-associated endophytic fungi have been recognized as crucial sources of unique bioactive scaffolds, particularly for oncological therapeutics^{13,14}. Conversely, investigative research into endophytic actinomycetes derived from marine macroalgae remains sparse, leaving a critical knowledge gap regarding their specific antibacterial and anticancer capabilities. Addressing this research deficit is vital, as identifying novel compounds

from these specialized marine actinomycetes may offer potent new strategies to counter multidrug-resistant bacterial infections and various malignancies.

To explore this potential, the present study was designed to isolate and characterize endophytic actinomycetes from *C. lentillifera* specimens collected in Phetchaburi Province, Thailand. Following isolation, the strains were screened for antibacterial efficacy against a panel of human pathogens. The most effective isolate was chosen for detailed subsequent evaluation, which involved isolating, determining the chemical structure and defining the properties of the primary active agents. Ultimately, this study seeks to investigate how these marine-sourced molecules can be used to treat infectious conditions and explore their utility in oncology.

MATERIALS AND METHODS

Study area: The study was conducted between May 2025 and June 2026 within the Departments of Microbiology and Chemistry at Silpakorn University, Nakhon Pathom, Thailand.

Sample collection: Five healthy specimens of *Caulerpa lentillifera* J. Agardh were harvested from Bangkaew subdistrict, Ban Laem District, Phetchaburi Province, Thailand (13.109583 N, 100.060722 E).

Isolation and antibacterial screening of actinomycetes: To isolate endophytic strains, the stolons and individual ramuli ("bunch of grapes") (Fig. 1a) were dissected into 150 fragments measuring approximately 5×5 mm² each. Surface disinfection was achieved via a multi-step regimen: Samples were sequentially rinsed with a Tween 20 solution, sodium hypochlorite and ethanol to eliminate epiphytic contaminants. The surface-sterilized tissue pieces were subsequently pressed onto sterile Whatman filter paper to absorb residual moisture and dried under aseptic conditions in a laminar flow cabinet (Esco Scientific, PA, USA).

The processed algal segments were inoculated onto humic acid-vitamins (HV) agar¹⁵ supplemented with 100 µg/mL of cycloheximide and nystatin to suppress fungal proliferation. Plates were incubated at 32°C for 3 weeks. Colonies displaying characteristic actinomycete morphology (Fig. 1b) were isolated and subcultured onto fresh International *Streptomyces* Project-2 (ISP-2) agar¹⁶ plates for preservation and downstream analysis.

A collection of 25 actinomycete isolates was evaluated for antibacterial efficacy against a panel of indicator strains, including *Staphylococcus aureus* TISTR885, *Staphylococcus*

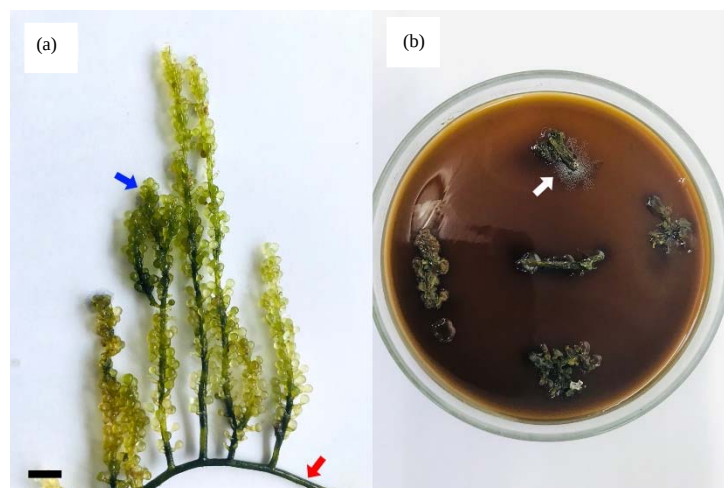


Fig. 1(a-b): Specimens of *Caulerpa lentillifera* J. Agardh utilized for the isolation of endophytic actinomycetes, (a) Morphological features of *C. lentillifera* harvested from the Bangkaew Coast, Phetchaburi, Thailand; blue and red arrows denote the ramuli ("bunch of grapes") and stolon structures, respectively (scale bar = 1 cm) and (b) Development of endophytic actinomycete colonies (indicated by white arrows) emerging from surface-sterilized segments of *C. lentillifera* following a 3-week incubation period on humic acid-vitamins (HV) agar at 32 °C

epidermidis TISTR518, *Bacillus cereus* TISTR687, *Bacillus subtilis* TISTR008, *Escherichia coli* TISTR887, *Salmonella typhimurium* TISTR292, *Pseudomonas aeruginosa* TISTR1287 and a clinical isolate of methicillin-resistant *Staphylococcus aureus* (MRSA) Sp3. Antagonistic activity was determined in triplicate using a modified soft-agar overlay method¹⁷, followed by the measurement of zones of inhibition. Strain ARR153, recovered from the ramulus tissue of *C. lentillifera*, demonstrated the most robust antibacterial profile and was selected for taxonomic identification via morphological, physiological and chemotaxonomic criteria according to protocols established by Cassarini *et al.*¹⁸.

Large-scale cultivation and extraction of strain ARR153: For large-scale secondary metabolite production, strain ARR153 was cultured on 600 Petri dishes containing ISP-2 agar (equivalent to ~12 L of medium) and incubated at 32°C for 21 days. The culture media and biomass were subjected to exhaustive extraction using Ethyl Acetate (EtOAc) as the organic solvent¹⁹. The combined organic phases were evaporated to dryness under reduced pressure using a rotary evaporator R-300 (BUCHI Labortechnik AG, Switzerland), yielding 17.55 g of a dark brown crude extract. This residue was partitioned into two portions: One aliquot was reconstituted in Dimethyl Sulfoxide (DMSO) for bioactivity assays, while the remainder was dissolved in dichloromethane (CH₂Cl₂) for chromatographic purification.

Scanning electron microscopy (SEM): The cellular and spore micromorphology of strain ARR153 was analyzed via scanning electron microscopy (SEM) following sample preparation techniques described by Castillo *et al.*²⁰ Prepared specimens were dehydrated to the critical point using liquid CO₂ (Quorum K850, United Kingdom), coated with gold by a sputter coater (Safematic CCU-010HV, Switzerland) and viewed under a TESCAN Mira3 scanning electron microscope (Czech Republic). Spore chain architecture and spore surface ornamentations were recorded.

16S rDNA sequencing and phylogenetic analysis: To establish the taxonomic identity of strain ARR153, the isolate was inoculated into ISP-2 broth and incubated at 32°C for 7 days with continuous agitation at 150 rpm. Biomass was harvested via centrifugation, rinsed with phosphate-buffered saline (PBS) and subjected to genomic DNA extraction. Amplification of the 16S rDNA gene was executed using primers A20F (5'-AGTTTGATCCTGGCTC-3') and B1540R (5'-AAGGAGGTGATCCAGCC-3') according to the methodology of Taechowisan *et al.*²¹

Amplicons were purified with a QIAquick gel extraction kit (Qiagen, Germany) per the manufacturer's instructions and sequenced via Sanger sequencing at 1st BASE, Singapore. Sequence alignment and homology searches were performed using the NCBI BLAST tool against valid type strains. Related sequences retrieved from the GenBank database were aligned

utilizing Clustal W and a phylogenetic tree was generated through the neighbor-joining method in MEGA 11 software²². *Microbispora corallina* DF-32 (JCM 10267) was employed as the outgroup.

Determination of MIC and MBC: The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of both the crude extract and purified fractions were determined against the designated pathogens using standardized microdilution procedures adapted from Pfaller *et al.*²³ Chloramphenicol (Thermo Fisher Scientific, USA) was maintained as the reference positive control.

In vitro cytotoxicity assay: The cytotoxic potential of the crude extract and its isolated constituents was evaluated using the standard MTT colorimetric assay²⁴ against three human cancer cell lines: cervical carcinoma (HeLa), hepatocellular carcinoma (HepG2) and breast adenocarcinoma (MDA-MB-231). Test concentrations ranged from 10 to 160 µg/mL. To determine the selectivity of the compounds toward malignant cells over healthy tissue, normal African green monkey kidney cells (Vero) were integrated as a non-cancerous control. The Selectivity Index (SI) was calculated using the following formula:

$$SI = \frac{IC_{50} \text{ of non-cancerous cells (Vero)}}{IC_{50} \text{ of cancer cells}}$$

Doxorubicin hydrochloride (Thermo Fisher Scientific, USA) was utilized as the positive reference standard.

Purification and structural elucidation of compounds: The crude extract (17.00 g) was fractionated by gravity column chromatography over silica gel 60 (Merck, 0.040-0.063 mm), employing a stepwise gradient elution of CH₂Cl₂ and methanol (MeOH). Fractions eluted with 7-8% MeOH in CH₂Cl₂ were collected and further resolved by preparative thin-layer chromatography (TLC) using a CH₂Cl₂: EtOAc (3:5, v/v) mobile phase, yielding compound **1** (11.58 mg) and compound **2** (12.64 mg). Fractions recovered from the 8-9% MeOH in CH₂Cl₂ eluate were similarly purified by TLC to afford compound **3** (10.17 mg) and compound **4** (9.86 mg).

Structural characterization of compounds **1-4** was accomplished using multiple spectroscopic platforms. Melting points were determined using a Stuart SMP20 apparatus (Cole-Parmer, UK). Ultraviolet (UV) spectra were acquired on a PerkinElmer Lambda 35 spectrophotometer (USA). Nuclear Magnetic Resonance (¹H-NMR at 400 MHz;¹³

C-NMR at 100 MHz) profiles were recorded on a Bruker Avance III NMR Spectrometer (Bruker, Germany). High-resolution mass spectra were gathered utilizing a POLARIS Q mass spectrometer (Thermo Fisher Scientific, USA).

Molecular docking and ADMET prediction: Three-dimensional coordinates for the ligands Cyclo-(L-Leu-L-Val), Cyclo-(L-Leu-L-Thr), Cyclo-(L-Leu-L-Ile) and Cyclo-(L-Leu-L-Leu) were generated and energy-minimized using UCSF Chimera. The crystal structure of the target protein, Epidermal Growth Factor Receptor (EGFR; PDB ID: 1M17), was obtained from the RCSB Protein Data Bank. *In silico* docking simulations were run via AutoDock Vina embedded within the UCSF Chimera²⁵. A 20x20x20 Å³ cubic grid box was centered around the binding pocket defined by the co-crystallized antagonist [6,7-bis(2-methoxy-ethoxy)quinazoline-4-yl]-(3-ethynylphenyl)amine (AQ4, DrugBank ID: DB00530). Binding scores were documented as binding affinities (kcal/mol), selecting the lowest-energy conformer as the primary pose. Visualizations of receptor-ligand interaction dynamics (including hydrogen bonding) were mapped using Discovery Studio Visualizer. Doxorubicin and AQ4 served as comparative reference controls. Computational Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) parameters for all ligands were modeled using the online pkCSM platform²⁶, with Doxorubicin and AQ4 establishing baseline profiles.

Statistical analysis: All data are derived from experiments conducted in triplicate and are displayed as Mean ± Standard Deviation (SD). Statistical evaluation was carried out via SPSS for Windows version 11.01 (SPSS Inc., Chicago, IL, USA). Variations among treatment means were analyzed by One-way Analysis of Variance (ANOVA), followed by Tukey's *post hoc* multiple comparison test. Differences were considered statistically significant at values of p < 0.05.

RESULTS

The stolons and ramuli ("bunch of grapes") of *Caulerpa lentillifera* were processed to isolate endophytic actinomycetes (Fig. 1a). After a 3-week incubation on HV agar, dry, powdery, white colonies of endophytic actinomycetes grew around the sample tissues (Fig. 1b). A total of 300 tissue segments of *C. lentillifera* yielded 25 distinct isolates, with 15 strains recovered from stolon tissues and 10 from ramulus tissues, representing tissue-specific isolation prevalences of 10.0% and 6.6%, respectively.

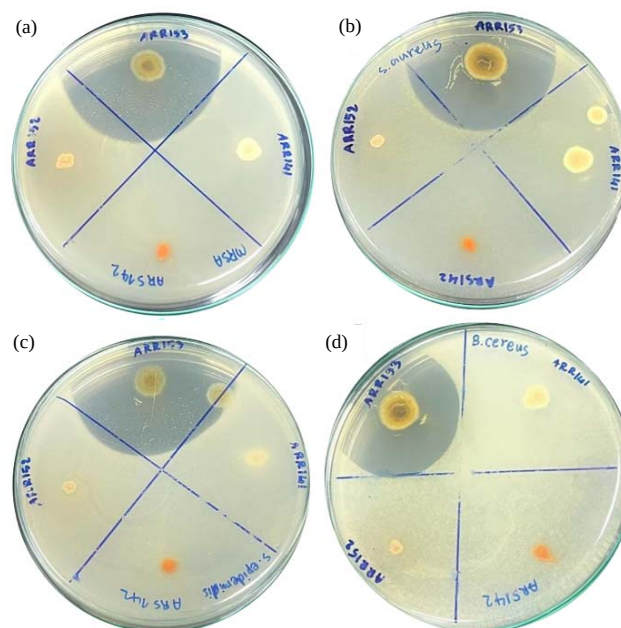


Fig. 2(a-d): Evaluation of antibacterial efficacy via the soft-agar overlay method. Indicator pathogenic strains suspended in soft agar were overlaid across 7-day-old colonies of *Streptomyces sanyensis* ARR153 precultured on International *Streptomyces* Project-2 (ISP-2) agar. Clear zones of inhibition surrounding the actinomycete colonies were quantified following a 24 hrs incubation period at 37°C against: (a) Methicillin-resistant *Staphylococcus aureus* Sp3, (b) *Staphylococcus aureus* TISTR885, (c) *Staphylococcus epidermidis* TISTR518 and (d) *Bacillus cereus* TISTR687

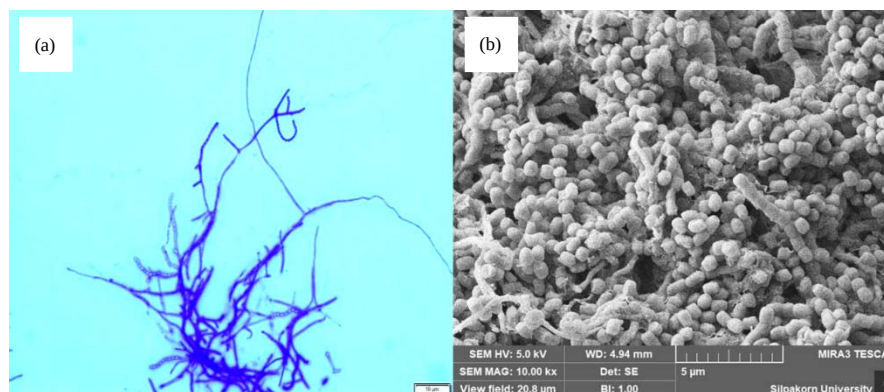


Fig. 3(a-b): Morphological and microscopic characterization of *Streptomyces sanyensis* ARR153. Diagnostic features recorded for preliminary taxonomic identification after cultivation on ISP-2 medium at 32°C for 21 days, (a) Light micrograph detailing the characteristic rectiflexuous arrangement of the spore chains and (b) Scanning electron micrograph capturing the fine spore surface ornamentation

Primary antimicrobial screening using the soft-agar overlay method identified a single highly antagonistic strain, designated as isolate ARR153, which originated from the ramulus tissue of *C. lentillifera*. This isolate exhibited robust, broad-spectrum inhibitory zones measuring between 4.8 and 6.9 cm in diameter against several pathogenic test bacteria, including Methicillin-Resistant

Staphylococcus aureus (MRSA) Sp3 (Fig. 2a), *S. aureus* TISTR885 (Fig. 2b), *S. epidermidis* TISTR518 (Fig. 2c) and *Bacillus cereus* TISTR687 (Fig. 2d).

Microscopic evaluation of strain ARR153 revealed well-developed, non-fragmented aerial and substrate mycelia. Morphological profiling showed monopodially branched sporophores bearing rectiflexuous spore chains (Fig. 3a) with

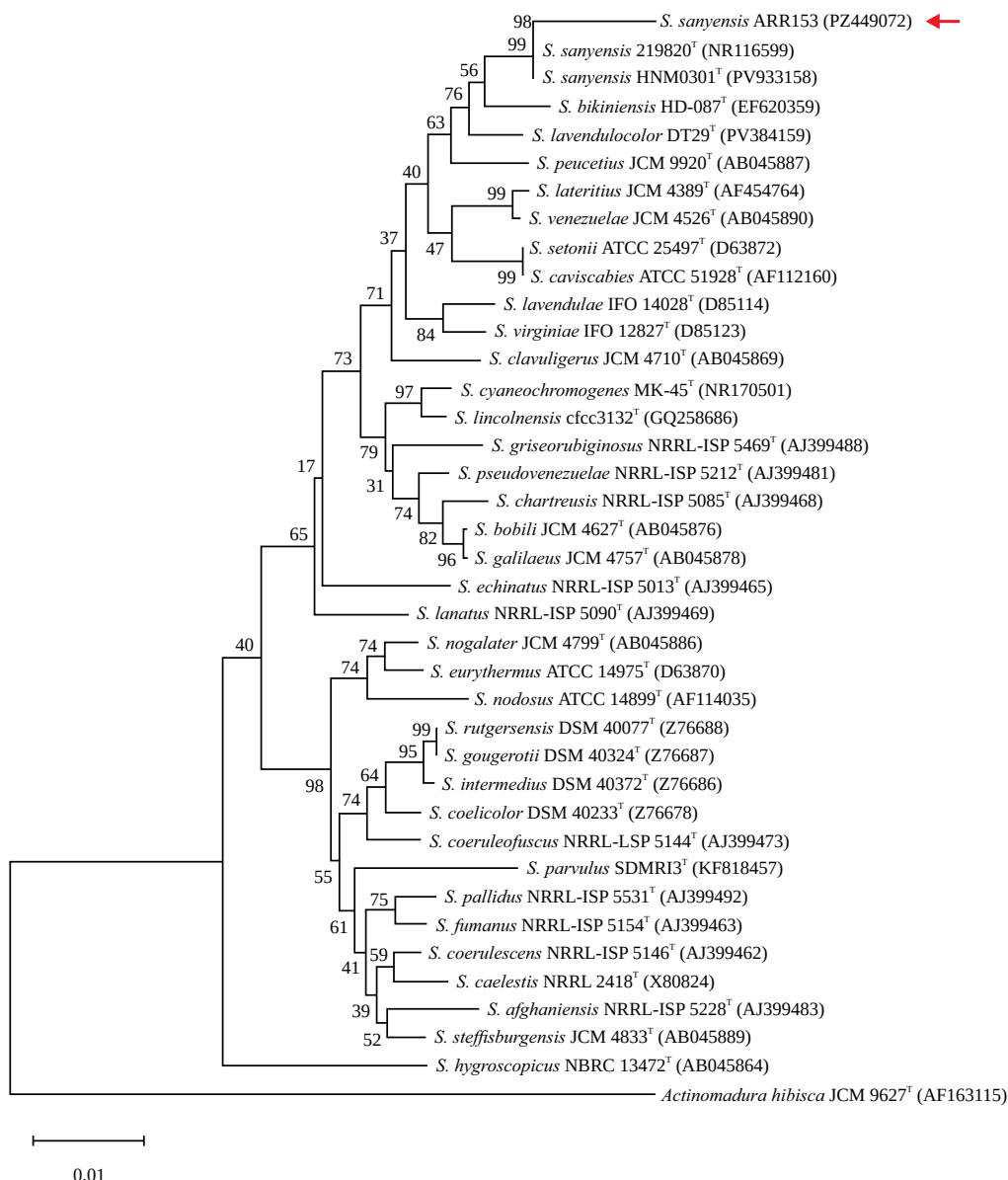


Fig. 4: Evolutionary relationships of *Streptomyces sanyensis* ARR153

The phylogenetic tree was constructed using the neighbor-joining method in MEGA11 based on 16S rRNA gene sequences of strain ARR153 (highlighted by the red arrow) and structurally related reference strains retrieved from GenBank (corresponding accession numbers are enclosed in parentheses). Node values represent bootstrap percentages calculated from 1,000 replicates to evaluate topology robustness. The scale bar corresponds to 0.01 substitutions per nucleotide site

smooth-surfaced, flexible, short rod-shaped spores (Fig. 3b). Vegetative growth on agar plates initially appeared creamy white, transitioning to a creamy yellow coloration after 7 days of incubation; production of diffusible or soluble pigments was not observed. Chemotaxonomic analysis confirmed the presence of LL-diaminopimelic acid within whole-cell hydrolysates, aligning the isolate with the genus *Streptomyces*. Subsequent 16S rRNA gene sequencing and

BLAST analysis revealed that strain ARR153 shared 98.92% sequence identity with *Streptomyces sanyensis* strain 219820^T. Neighbor-joining phylogenetic tree topology (Fig. 4) corroborated this taxonomic assignment, with strain ARR153 clustering tightly within the *S. sanyensis* clade. The complete 16S rDNA sequence was deposited in the GenBank database under accession number PZ449072.

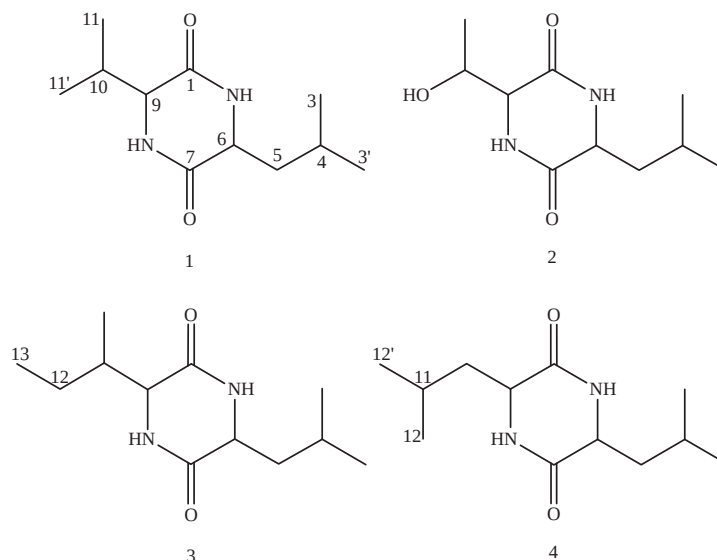


Fig.5(a-d): Chemical structures of the isolated secondary metabolites, (a) Compound **1**: Cyclo-(L-Leu-L-Val), (b) Compound **2**: Cyclo-(L-Leu-L-Thr), (c) Compound **3**: Cyclo-(L-Leu-L-Ile) and (d) Compound **4**: Cyclo-(L-Leu-L-Leu)

Chromatographic purification of the organic crude extract from *S. sanyensis* ARR153 yielded four pure compounds. Information about the chemical structure of these compounds, determined using various spectroscopic techniques, is presented in the following sections.

Compound 1: It was a white amorphous powder, MP 145-147°C, HRMS-FAB: m/z 213.1601 $[M+H]^+$, Calcd for $C_{11}H_{21}N_2O_2$ 213.1603; FAB-MS (positive): 213 $[M+H]^+$; IR $_{\text{vmax}}$ (KBr) cm^{-1} : 3110, 3050, 2960, 1665; $^1\text{H-NMR}$ (400 MHz, CDCl_3), δ_{H} : 0.94 (6H, d, $J=6.8$, H-3, H-11), 0.97 (3H, d, $J=7.1$, H-3'), 1.04 (3H, d, $J=6.3$, H-11'), 1.61 (1H, m, H-5a), 1.77 (1H, m, H-4), 1.88 (1H, m, H-5b), 2.41 (1H, m, H-10), 3.89 (1H, m, H-9), 4.01 (1H, m, H-6), 6.46 (1H, brs, N-H) and 6.68 (1H, brs, N-H). $^{13}\text{C-NMR}$ (125 MHz, CDCl_3), δ_{C} : 168.96 (s, C-7), 167.42 (s, C-1), 60.29 (d, C-9), 53.15 (d, C-6), 43.85 (t, C-5), 31.5 (d, C-10), 23.5 (d, C-4), 23.1 (q, C-3'), 21.7 (q, C-3), 18.7 (q, C-11) and 17.3 (q, C-11').

Compound 2: It was a white amorphous powder, MP 150-152°C, HRMS-FAB: m/z 215.1392 $[M+H]^+$, Calcd for $C_{10}H_{19}N_2O_3$ 215.1396; FAB-MS (positive): 215 $[M+H]^+$; IR $_{\text{vmax}}$ (KBr) cm^{-1} : 3325, 3204, 3060, 2965, 1670; $^1\text{H-NMR}$ (400 MHz, CD_3OD), δ_{H} : 0.95 (3H, d, $J=6.8$, H-3), 0.97 (3H, d, $J=6.8$, H-3'), 1.23 (3H, d, $J=6.8$, H-11), 1.73 (1H, m, H-5a), 1.86 (1H, m, H-5b), 1.84 (1H, m, H-4), 3.71 (1H, m, H-9), 3.88 (1H, m, H-6), 4.17 (1H, m, H-10), 6.47 (1H, brs, N-H), 6.69 (1H, brs, N-H) and 12.17 (1H, brs, OH-10). $^{13}\text{C-NMR}$ (125 MHz, CD_3OD), δ_{C} : 171.88 (s, C-7), 169.46 (s, C-1), 68.76 (d, C-10), 62.24 (d, C-9), 54.63 (d, C-6), 46.47 (t, C-5), 25.10 (d, C-4), 23.66 (q, C-3'), 21.65 (q, C-3) and 19.98 (q, C-11).

Compound 3: It was a white amorphous powder, MP 148-150°C, HRMS-FAB: m/z 227.1758 $[M+H]^+$, Calcd for $C_{12}H_{23}N_2O_2$ 227.1760; FAB-MS (positive): 227 $[M+H]^+$; IR $_{\text{vmax}}$ (KBr) cm^{-1} : 3210, 3055, 2960, 1668; $^1\text{H-NMR}$ (400 MHz, CD_3OD), δ_{H} : 0.96 (6H, m, $J=6.6$, H-3', H-13), 0.98 (3H, m, $J=6.8$, H-3), 1.02 (3H, m, $J=6.7$, H-11), 1.25 (1H, m, H-12a), 1.54 (1H, m, H-12b), 1.63 (1H, m, H-5a), 1.77 (1H, m, H-5b), 1.86 (1H, m, H-4), 1.93 (1H, m, H-10), 3.84 (1H, m, H-9), 3.95 (1H, m, H-6), 6.65 (1H, brs, N-H) and 6.82 (1H, brs, N-H). $^{13}\text{C-NMR}$ (125 MHz, CD_3OD), δ_{C} : 171.17 (s, C-1), 169.54 (s, C-7), 60.94 (d, C-6), 54.33 (d, C-9), 40.54 (d, C-10), 45.78 (t, C-5), 25.30 (d, C-4), 23.61 (q, C-3'), 21.86 (q, C-3), 15.70 (q, C-11), 25.88 (t, C-12) and 12.06 (q, C-13).

Compound 4: It was a white amorphous powder, MP 146-148°C, HRMS-FAB: m/z 227.1797 $[M+H]^+$, Calcd for $C_{12}H_{23}N_2O_2$ 227.1760; FAB-MS (positive): 227 $[M+H]^+$; IR $_{\text{vmax}}$ (KBr) cm^{-1} : 3195, 3055, 2960, 1670; $^1\text{H-NMR}$ (400 MHz, CD_3OD), δ_{H} : 0.96 (6H, d, $J=6.6$, H-3, H-12), 0.98 (6H, d, $J=6.6$, H-3', H-12'), 1.62 (2H, m, $J=5.1$, H-5a, H-10a), 1.72 (2H, m, $J=4.8$, H-15b, H-10b), 1.85 (2H, d, H-4, H-11), 3.90 (2H, d, $J=4.8$, H-6, H-9) and 6.84 (2H, brs, N-H). $^{13}\text{C-NMR}$ (125 MHz, CD_3OD), δ_{C} : 171.22 (s, C-1, C-7), 54.71 (d, C-6, C-9), 45.88 (t, C-5, C-10), 25.34 (d, C-4, C-11), 23.58 (q, C-3', C-12'), 21.92 (q, C-3, C-12).

Comprehensive spectroscopic analyses identified these structures as L-cyclic dipeptides (diketopiperazines): compound **1**: Cyclo-(L-Leu-L-Val) (Fig. 5a), compound **2**: Cyclo-(L-Leu-L-Thr) (Fig. 5b), compound **3**: Cyclo-(L-Leu-L-Ile) (Fig. 5c) and compound **4**: Cyclo-(L-Leu-L-Leu) (Fig. 5d). Quantitative production metrics revealed that the isolated

Table 1: MIC of the purified compounds and crude extract against tested bacteria

Test substances	MIC (µg/mL)							
	S.a. ^a	S.e.	B.c.	B.s.	MRSA Sp3	E.c.	S.t.	P.a.
Crude extract	64	64	128	128	256	>512	>512	>512
Compound 1	16	16	32	32	128	>512	>512	>512
Compound 2	16	16	32	64	128	>512	>512	>512
Compound 3	32	32	32	64	128	>512	>512	>512
Compound 4	32	32	32	64	128	>512	>512	>512
Chloramphenicol	2	2	2	2	8	16	16	32

^aS.a.: *Staphylococcus aureus* TISTR885, S.e.: *Staphylococcus epidermidis* TISTR518, B.c.: *Bacillus cereus* TISTR687, B.s.: *Bacillus subtilis* TISTR008, MRSA Sp3: Methicillin-resistant *Staphylococcus aureus* Sp3, E.c.: *Escherichia coli* TISTR887, S.t.: *Salmonella typhimurium* TISTR292 and P.a.: *Pseudomonas aeruginosa* TISTR1287

Table 2: MBC of the purified compounds and crude extract against tested bacteria

Test substances	MBC (µg/mL)							
	S.a. ^a	S.e.	B.c.	B.s.	MRSA Sp3	E.c.	S.t.	P.a.
Crude extract	256	256	512	512	512	>512	>512	>512
Compound 1	64	64	128	128	512	>512	>512	>512
Compound 2	64	64	128	256	512	>512	>512	>512
Compound 3	128	128	128	256	512	>512	>512	>512
Compound 4	128	128	128	256	512	>512	>512	>512
Chloramphenicol	16	16	16	16	32	32	32	64

^aS.a.: *Staphylococcus aureus* TISTR885, S.e.: *Staphylococcus epidermidis* TISTR518, B.c.: *Bacillus cereus* TISTR687, B.s.: *Bacillus subtilis* TISTR008, MRSA Sp3: Methicillin-resistant *Staphylococcus aureus* Sp3, E.c.: *Escherichia coli* TISTR887, S.t.: *Salmonella typhimurium* TISTR292, P.a.: *Pseudomonas aeruginosa* TISTR1287

Table 3: IC₅₀ values and selectivity indices (SI) of crude extract and purified compounds against cancer cell lines

Test substances	Vero cells ^a	MDA-MB-231 cells		HeLa cells		HepG2 cells	
	IC ₅₀ (µg/mL)**	IC ₅₀ (µg/mL)	SI**	IC ₅₀ (µg/mL)	SI	IC ₅₀ (µg/mL)	SI
Crude extract	1142.78±112.43 ^a	395.01±41.16 ^a	2.89	508.58±72.38 ^a	2.25	904.54±96.64 ^a	1.26
Compound 1	487.95±67.51 ^b	59.86±18.01 ^b	8.15	68.16±21.54 ^b	7.16	382.41±52.08 ^b	1.28
Compound 2	460.70±70.25 ^b	47.85±15.22 ^b	9.63	53.69±16.67 ^b	8.58	432.34±62.25 ^b	1.07
Compound 3	611.20±53.87 ^c	49.04±18.59 ^b	12.46	55.94±18.03 ^b	10.93	527.23±55.74 ^b	1.16
Compound 4	688.85±58.04 ^c	30.13±12.43 ^b	22.86	44.70±13.15 ^b	15.41	411.35±48.16 ^b	1.67
Doxorubicin hydrochloride	135.45±24.63 ^d	9.85±4.62 ^b	13.75	7.56±3.41 ^b	17.92	115.57±28.62 ^c	1.17

^aVero cells: African green monkey kidney cell line, MDA-MB-231 cells: Human breast cancer cell line, HeLa cells: Human cervical carcinoma cell line, HepG2: human hepatocellular carcinoma cell line, **IC₅₀ values represent the concentration causing 50% growth inhibition. The values are expressed as Mean±Standard Deviation of the three replicates, **SI: Selectivity indices (SI) were calculated as the ratio of the IC₅₀ in the Vero cell line to the IC₅₀ in the cancer cell lines and ^{a,b,c,d}: Different letters indicated statistically significant differences within the same category (p<0.05)

strain produced these diketopiperazines at concentrations of 0.68, 0.74, 0.60 and 0.58 mg/g of dry crude extract, corresponding to volumetric yields of 1.00, 1.09, 0.88 and 0.85 mg/L of liquid culture medium, respectively.

Table 1 and 2 display the antibacterial efficacy of both the raw crude extract and the separated cyclic dipeptides, quantified by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) metrics. The refined compounds demonstrated wide-ranging bactericidal effects against the tested Gram-positive bacteria, successfully restricting the growth of *S. aureus* TISTR885, *S. epidermidis* TISTR518, *B. cereus* TISTR687, *B. subtilis* TISTR008 and MRSA Sp3. For these pure substances, MIC values varied between 16 and 128 µg/mL, whereas MBC levels ranged from 64 to 512 µg/mL. The cytotoxic activities of the crude extract and its constituent compounds were assessed using an MTT assay against normal Vero cells and three human cancer cell lines

(HeLa, HepG2 and MDA-MB-231). The crude extract exhibited lower baseline cytotoxicity than the isolated fractions across all test concentrations (Fig. 6a). Treatment with the purified compounds elicited a dose-dependent decrease in malignant cell viability (Fig. 6b-e). While the positive standard, doxorubicin hydrochloride, displayed superior cytotoxic effects at maximum concentrations (Fig. 6f), no such reductions in viability were observed in the untreated control cohorts.

The purified diketopiperazines demonstrated strong, targeted cytotoxicity against the breast cancer line MDA-MB-231 IC₅₀ range: 30.13-59.86 µg/mL) and the cervical cancer line HeLa IC₅₀ range: 44.70-68.16 µg/mL). Conversely, these molecules exhibited weak antiproliferative effects against hepatocellular carcinoma HepG2 cells, with IC₅₀ values ranging between 382.41 and 527.23 µg/mL. Importantly, neither the crude extract nor the isolated compounds

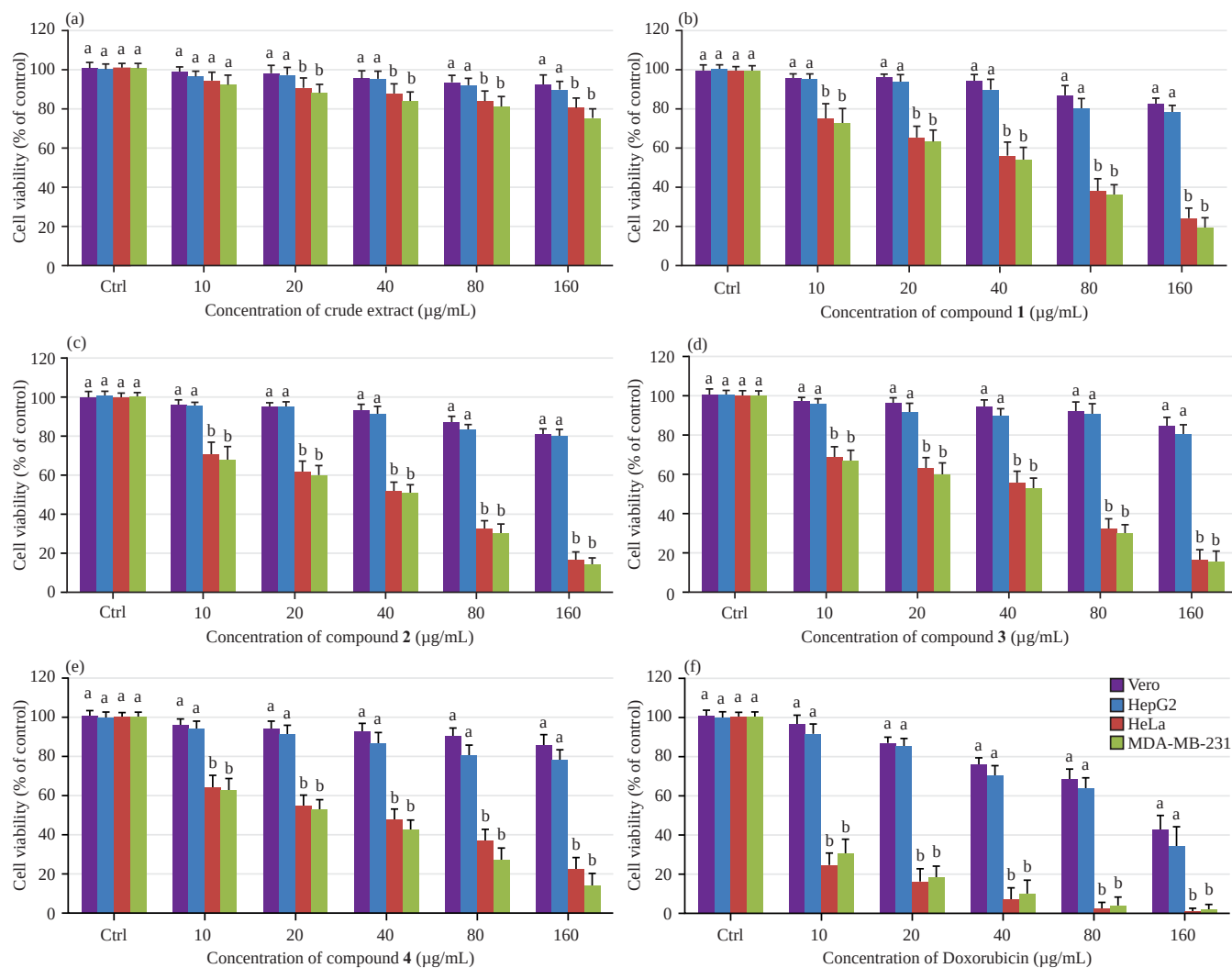


Fig. 6(a-f): Dose-dependent antiproliferative profiles determined by MTT assay, cell viability percentages were tracked after a 24 hrs exposure to varying concentrations of the total crude extract, (a) Purified cyclic dipeptides **1-4** (b-e): Reference standard doxorubicin hydrochloride and (f) Assays were conducted against human malignancies (HeLa, HepG2 and MDA-MB-231) alongside a non-cancerous control line (Vero)

Data represent the Mean $\pm$ SD of triplicate trials expressed relative to untreated vehicle controls. Statistical variance was assessed via one-way ANOVA followed by Tukey's *post hoc* test using SPSS version 11.01, lowercase letters denote significant differences ($p < 0.05$) among treatments within the same cell type at identical concentrations

demonstrated discernible cytotoxicity toward the non-cancerous Vero line, with all calculated IC_{50} values exceeding 400 µg/mL.

Among the isolated agents, compound **4** emerged as the most potent kinetic inhibitor following a 24 hrs treatment window, registering IC_{50} values of 30.13 ± 12.43 µg/mL and 44.70 ± 13.15 µg/mL against MDA-MB-231 and HeLa cells, respectively. The anti-cancer IC_{50} concentrations calculated for these compounds were markedly lower than their corresponding toxic thresholds in Vero cells.

As detailed in Table 3, the Selectivity Indices (SI) of the crude extract and compounds **1-3** were lower than those recorded for doxorubicin hydrochloride. Notably, however, compound **4** exhibited a higher SI value against MDA-MB-231 cells than the reference chemotherapeutic agent, signifying a highly precise and targeted mechanism of toxicity toward this specific breast cancer cell line.

To explore the putative biochemical mechanisms underlying the observed anticancer profiles, molecular docking simulations were conducted to evaluate the binding

dynamics of compounds **1-4** within the active site of the Epidermal Growth Factor Receptor (EGFR; PDB ID: 1M17), alongside the reference inhibitor AQ4 and doxorubicin. All six test ligands successfully accommodated the internal geometry of the EGFR kinase domain.

The calculated binding free energies, listed in Table 4, demonstrated a graded hierarchy of affinity: doxorubicin: -7.631 kcal/mol, AQ4 (Reference Inhibitor): -6.285 kcal/mol, compound **4**: -5.993 kcal/mol, compound **3**: -5.881 kcal/mol, compound **1**: -5.680 kcal/mol

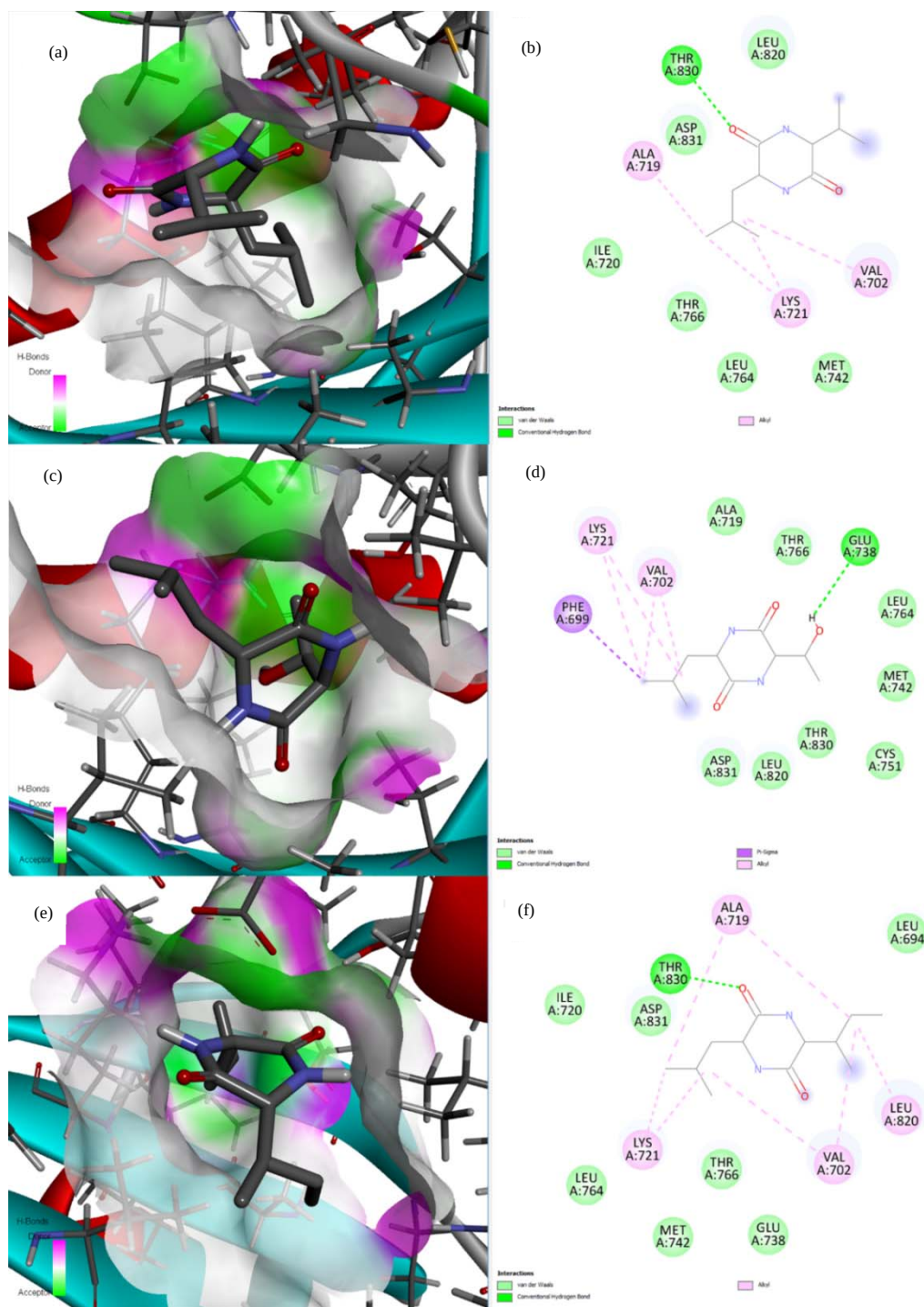


Fig. 7(a-l): Continue

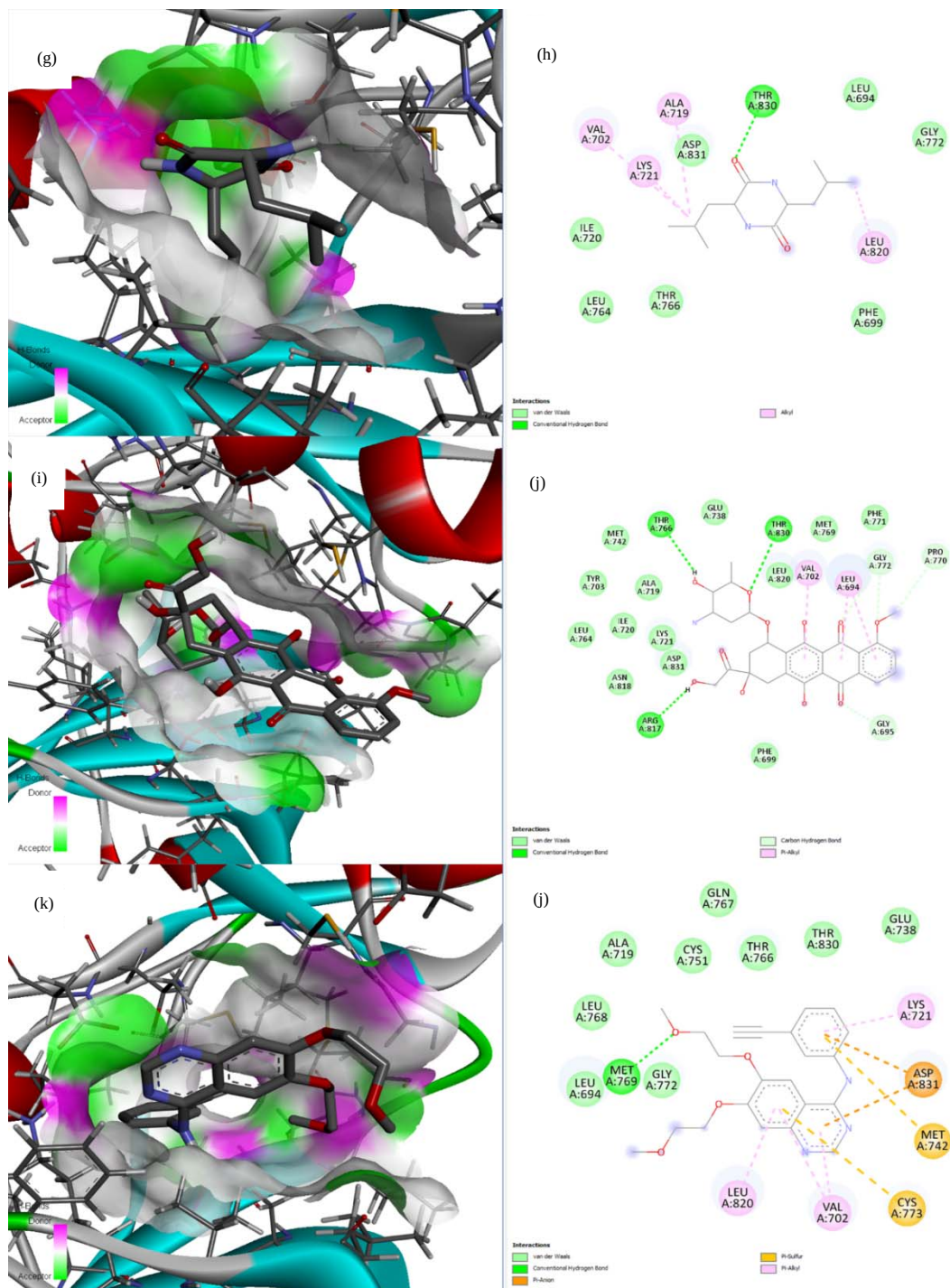


Fig. 7(a-l): *In silico* molecular docking profiles within the active domain of EGFR, Three-dimensional spatial orientations and corresponding two-dimensional (2D) interaction loops are illustrated between the binding pocket of EGFR and (a, b) Compound **1**, (c, d) Compound **2**, (e, f) Compound **3**, (g, h) Compound **4**, (i, j) Doxorubicin and (k, l) Co-crystallized small-molecule inhibitor AQ4

Pink and green shading mapping the binding cleft boundaries highlights hydrogen bond donor and acceptor regions, respectively. Intermolecular force networks are color-coded as follows: Dark green dashes (conventional hydrogen bonds), light green dashes (carbon-hydrogen bonds) and pale purple, pink, pale pink, brown or pale brown dashes indicating pi-sigma, alkyl, pi-alkyl, pi-anion and pi-sulfur contacts, respectively. Light green residues represent neighboring amino acids participating in classic van der Waals interactions

Table 4: Results of predictions of molecular interaction between the EGFR protein* and the test compounds

Purified compounds	Binding energy (kcal/mol)	Interaction types**	Hydrogen bonds		Bond length (Å ^o)	Docking site
			H-bond donors	H-bond acceptors		
Compound 1	-5.680	CoH	Thr830: HG1	LIG***: O	2.24195	Active pocket site
		A	Val702 (Alkyl)	LIG: (Alkyl)	5.27032	
		A	Ala719 (Alkyl)	LIG: C (Alkyl)	3.95149	
		A	Lys721 (Alkyl)	LIG: (Alkyl)	3.65736	
		A	LIG: C (Alkyl)	Lys721 (Alkyl)	4.02124	
Compound 2	-5.431	CoH	LIG: H	Glu738: OE2	2.64433	Active pocket site
		Pi-S	LIG: C	Phe699 (Pi-orbitals)	3.93583	
		A	Val702 (Alkyl)	LIG: (Alkyl)	4.29267	
		A	Lys721 (Alkyl)	LIG: (Alkyl)	5.01844	
		A	LIG: C (Alkyl)	Val702 (Alkyl)	5.16288	
		A	LIG: C (Alkyl)	Lys721 (Alkyl)	4.97381	
Compound 3	-5.881	CoH	Thr830: HG1	LIG: O	2.25259	Active pocket site
		A	Val702 (Alkyl)	LIG: (Alkyl)	4.30231	
		A	Val702 (Alkyl)	LIG: (Alkyl)	5.31632	
		A	Ala719 (Alkyl)	LIG: (Alkyl)	4.94078	
		A	Ala719 (Alkyl)	LIG: C (Alkyl)	4.06808	
		A	Lys721 (Alkyl)	LIG: (Alkyl)	3.64895	
		A	LIG: (Alkyl)	Leu820 (Alkyl)	4.91132	
		A	LIG: C (Alkyl)	Lys721 (Alkyl)	3.98214	
Compound 4	-5.993	CoH	Thr830: HG1	LIG: O	2.12066	Active pocket site
		A	Val702 (Alkyl)	LIG: (Alkyl)	4.25248	
		A	Ala719 (Alkyl)	LIG: (Alkyl)	4.13737	
		A	Lys721 (Alkyl)	LIG: (Alkyl)	3.78661	
		A	LIG: C (Alkyl)	Leu820 (Alkyl)	5.02793	
AQ4	-6.285	CoH	Met769: HN	LIG: O	2.01316	Active pocket site
		Pi-An	Asp831: OD2 (negative)	LIG: (Pi-orbitals)	3.80199	
		Pi-An	Asp831: OD2 (negative)	LIG: (Pi-orbitals)	4.21580	
		Pi-Su	Met742: SD (sulfur)	LIG: (Pi-orbitals)	5.34340	
		Pi-Su	Cyc773: SG (sulfur)	LIG: (Pi-orbitals)	5.99187	
		Pi-A	LIG: (Pi-orbitals)	Val702 (Alkyl)	5.44382	
		Pi-A	LIG: (Pi-orbitals)	Leu820 (Alkyl)	5.01507	
		Pi-A	LIG: (Pi-orbitals)	Val702 (Alkyl)	5.01524	
		Pi-A	LIG: (Pi-orbitals)	Lys721 (Alkyl)	4.96839	
Doxorubicin	-7.631	CoH	Thr830: HG1	LIG: O	2.87506	Active pocket site
		CoH	LIG: H	Thr766: OG1	2.66515	
		CoH	LIG: H	Arg817: O	2.21290	
		CaH	Gly685: HA2	LIG: O	2.82660	
		CaH	Gly772: HA1	LIG: O	2.39844	
		CaH	Gly772: HA2	LIG: O	2.62169	
		CaH	LIG: C	Pro770: O	3.13372	
		Pi-A	LIG: (Pi-orbitals)	Val702 (Alkyl)	4.79940	
		Pi-A	LIG: (Pi-orbitals)	Leu694 (Alkyl)	4.34521	
		Pi-A	LIG: (Pi-orbitals)	Leu694 (Alkyl)	5.30959	

*The EGFR protein, PDB ID: 1M17, **Interaction types, CoH: Conventional hydrogen bond, CaH: Carbon hydrogen bond, A: Alkyl interaction, Pi-A: Pi-Alkyl interaction, Pi-An: Pi-Anion interaction, Pi-S: Pi-Sigma interaction and Pi-Su: Pi-Sulfur interaction and ***LIG; Ligand: Compounds **1, 2, 3, 4**, ([6,7-bis(2-methoxy-ethoxy)quinazoline-4-yl]-(3-ethynylphenyl)amine (AQ4) and doxorubicin

and compound **2**: -5.431 kcal/mol. The theoretical binding affinity of doxorubicin surpassed both the small-molecule control AQ4 and all four isolated diketopiperazines, suggesting a more thermodynamically stable receptor-ligand complex.

Hydrogen bonding constituted the primary stabilizing force within the protein-ligand binding pockets. The isolated compounds established distinct residue-level interaction

networks: Compound **1** formed a key hydrogen bond with Thr830 (Fig. 7a-b), whereas compound **2** bound selectively to Glu738 (Fig. 7c-d). Compounds **3** and **4** shared an identical hydrogen-bonding footprint with compound **1**, anchoring tightly to the Thr830 residue (Fig. 7e-f and Fig. 7g-h, respectively). In comparison, doxorubicin established multiple hydrogen bonds spanning Thr766, Arg817 and Thr830 (Fig. 7i-j), while AQ4 interacted predominantly with

Table 5: Predicted absorption, distribution, metabolism, excretion and toxicity properties of the compound agents

Properties ^a	Compound agents					
	Compound 1	Compound 2	Compound 3	Compound 4	AQ4 ^b	Doxorubicin
TPSA	90.806	89.235	97.171	97.171	169.532	222.081
Consensus log P_{ow}	0.6717	-0.6035	1.0618	1.0618	3.4051	0.0013
Absorption						
Water solubility (log mol/L)	-1.58	-1.20	-1.76	-1.93	-5.09	-2.92
Caco2 cell permeability (log Papp in 10 ⁶ cm/s)	1.17	0.68	1.211	1.216	1.222	0.457
Human intestinal absorption (% absorbed)	76.35	69.16	78.14	74.68	95.93	62.37
Skin permeability (log Kp cm/s)	-3.237	-4.345	-3.219	-3.109	-2.746	-2.735
P-Glycoprotein substrate	No	No	No	No	No	Yes
P-Glycoprotein inhibitor	No	No	No	No	Yes	No
Distribution						
BBB permeability (log BB)	-0.138	-0.439	-0.113	-0.102	-0.509	-1.379
CNS permeability (log PS)	-2.932	-3.458	-2.935	-2.916	-3.402	-4.307
Metabolism						
CYP2D6 substrate	No	No	No	No	No	No
CYP3A4 substrate	No	No	No	No	Yes	No
CYP1A2 inhibitor	No	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	Yes	No
CYP2C9 inhibitor	No	No	No	No	Yes	No
CYP2D6 inhibitor	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	Yes	No
Excretion						
Total clearance (log mL/min/kg)	1.255	1.349	1.362	1.275	0.628	0.987
Renal OCT2 substrate	No	No	No	No	No	No
Toxicity						
Ames_test	No	No	No	No	No	No
Human Max. tolerated dose (Log mg/kg/day)	0.585	1.050	0.481	0.443	0.674	0.081
Oral rat acute toxicity (LD ₅₀) (mol/kg)	1.994	1.612	2.189	2.055	2.607	2.408
<i>Tetrahymena pyriformis</i> toxicity	0.513	-0.364	0.579	0.781	0.319	0.285
Minnow toxicity	1.986	3.159	1.972	1.730	-1.501	4.412
hERG I inhibitor	No	No	No	No	No	No
hERG II inhibitor	No	No	No	No	Yes	Yes
Hepatotoxicity	No	No	No	No	Yes	Yes
Skin sensitization	No	No	No	No	No	No

^aBBB: Blood-brain barrier, CNS: Central Nervous System, CYP: Cytochrome p450, Caco2: Caucasian colon adenocarcinoma cell line, hERG: human ether-a-go-go-related gene, Kp: skin permeability constant, LD: lethal dose; log P_{ow} : log of relative solubility of the drug in n-octanol, OCT2: organic cation transporter 2, TPSA: topological polar surface area and ^bAQ4: [6,7-bis(2-methoxy-ethoxy)quinazoline-4-yl]-(3-ethynylphenyl)amine

Met769 (Fig. 7k-l). These structural models confirm that the marine-derived diketopiperazines successfully occupy and interact with the EGFR catalytic site in a manner analogous to established clinical inhibitors, reinforcing the observed in vitro cytotoxicity profiles. Detailed bond characteristics, including specific spatial lengths and corresponding contact residues, are compiled in Table 4.

The pharmacokinetic profiles of compounds **1-4** were virtually screened utilizing the pkCSM platform and contrasted against reference values for AQ4 and doxorubicin (Table 5). Absorption: The purified diketopiperazines displayed exceptional human intestinal absorption (HIA) profiles. These predicted HIA percentages were significantly superior to those calculated for doxorubicin (62.37%), though they remained lower than the value obtained for AQ4 (95.93%). Additionally, the isolated compounds demonstrated high predicted Caco-2 monolayer permeability. Crucially, none of the four

compounds were classified as substrates or inhibitors of the P-glycoprotein (P-gp) efflux pump. Distribution: Computational modeling predicted moderate blood-brain barrier penetration ranges ($-1 > \log BB > -0.3$) along with moderate central nervous system accumulation profiles ($-3.5 > \log PS > -2.0$). Metabolism: The isolated cyclic dipeptides were not predicted to serve as substrates or function as inhibitors for any of the major cytochrome P450 (CYP450) enzymatic isoforms. Excretion: None of the compounds were predicted to act as organic cation transporter 2 (OCT2) substrates. Moreover, their total clearance rates suggested a longer physiological persistence within the host model compared to both AQ4 and doxorubicin. Toxicity: Safety profiling indicated a favorable toxicological outlook, with none of the four purified compounds showing predictive liabilities for Ames mutagenicity, cardiotoxicity, hepatotoxicity or skin sensitization.

DISCUSSION

In this study, four known bioactive diketopiperazines (DKPs), specifically categorized as L-cyclic dipeptides, were successfully isolated from the marine-derived endophyte *Streptomyces sanyensis* ARR153. Their structural identities were established via comprehensive comparative analysis of their spectroscopic profiles against historical literature data^{27,28}. Diketopiperazines represent a structurally diverse class of secondary metabolites synthesized ubiquitously by various terrestrial and marine taxonomies²⁹, including bacteria, fungi, plants and animals³⁰. Natural products incorporating the core diketopiperazine scaffold are of significant pharmacological interest due to their broad spectrum of biological activities, encompassing antiviral, antifungal, antibacterial and antineoplastic properties³¹. This investigation integrated the isolation and taxonomic validation of the producing symbiont, quantification of metabolic yields and the subsequent evaluation of the antibacterial and targeted oncological efficacy of its purified dipeptides.

The volumetric yield of individual diketopiperazines is highly variable across different *Streptomyces* species. For context, unoptimized fermentation yields reported for *Streptomyces fungicidicus* (0.23-0.33 mg/L)³², *Streptomyces* sp. RK44 (0.20-0.38 mg/L)³³ and *Streptomyces* sp. SUK 25 (0.23-0.36 mg/L)³⁴ remain relatively low, whereas *Streptomyces antimicrobicus* BN122 demonstrates a higher production capacity of 1.04-1.10 mg/L³⁵. Notably, *S. sanyensis* ARR153, isolated here from the ramulus tissues of the macroalga *Caulerpa lentillifera*, synthesized a substantial baseline concentration of these target cyclic dipeptides (0.85-1.09 mg/L) under completely unoptimized wild-type batch fermentation. This robust metabolic baseline suggests that implementing strategic bioprocess and media optimization frameworks in future investigations could substantially maximize the industrial and pharmaceutical scalability of these metabolites.

Phylogenetic topology positioned isolate ARR153 within a tight evolutionary cluster containing *Streptomyces sanyensis* strain 219820^T, supported by a high 16S rDNA sequence identity of 98.92%. Interestingly, the type strain 219820^T was originally recovered from marine mangrove sediments in Sanya, Hainan Province, China and was documented to possess crude cytotoxic properties against the human colon carcinoma line HCT-116³⁶. However, prior to this investigation, the specific purified secondary metabolites responsible for the bioactivity of *S. sanyensis* had not been characterized.

When evaluated for antimicrobial efficacy, the isolated L-cyclic dipeptides: Cyclo-(L-Leu-L-Val), Cyclo-(L-Leu-L-Thr), Cyclo-(L-Leu-L-Ile) and Cyclo-(L-Leu-L-Leu) demonstrated selective bactericidal activity restricted to Gram-positive pathogens and clinical MRSA strains. The lack of inhibitory action against Gram-negative bacteria observed in this study aligns with historical literature profiles for these specific peptide scaffolds^{33,35}, a limitation typically attributed to the restrictive outer membrane permeability barriers characteristic of Gram-negative cell walls.

In preclinical oncological research, diketopiperazines have attracted attention due to their ability to arrest malignant cell proliferation and induce apoptotic pathways³⁷. Here, the purified molecules demonstrated pronounced, dose-dependent cytotoxicity against human breast carcinoma (MDA-MB-231) and cervical carcinoma (HeLa) lines, alongside weak activity against hepatocellular carcinoma (HepG2) cells. Importantly, the calculated IC₅₀ thresholds against the malignant panels were significantly lower than the values recorded against normal, non-cancerous Vero cells IC₅₀>400 µg/mL. This differential cytotoxicity underlines a favorable safety margin, which is a crucial benchmark in minimizing off-target tissue damage during therapeutic translations. Most notably, compound **4** (Cyclo-(L-Leu-L-Leu)) achieved an exceptionally high Selectivity Index (SI) against the MDA-MB-231 cell line, outperforming the clinical standard doxorubicin hydrochloride. This finding highlights the clinical potential of compound **4** as a highly targeted lead scaffold for triple-negative breast cancer therapies.

To elucidate the structural mechanisms driving this observed antiproliferative profile, molecular docking simulations targeted the Epidermal Growth Factor Receptor (EGFR; PDB ID: 1M17), a receptor tyrosine kinase frequently deregulated and overexpressed in aggressive malignancies³⁸. This computational approach builds upon existing medicinal chemistry strategies that leverage cyclic dipeptide configurations to engineer novel EGFR kinase inhibitors³⁹. Although the isolated ligands exhibited favorable, negative binding free energies (ranging from -5.431 to -5.993 kcal/mol), their theoretical affinities were lower than those calculated for the co-crystallized inhibitor AQ4 (-6.285 kcal/mol) and doxorubicin (-7.631 kcal/mol).

Crucially, structural interaction mapping revealed that the O-keto moiety of compounds **1**, **3** and **4** serves as a critical pharmacophore, establishing an essential stabilizing hydrogen bond with the Thr830 residue within the EGFR catalytic pocket. This specific interaction directly correlates with the enhanced binding energies observed for these configurations. Furthermore, this binding footprint mimics the key residue

anchoring patterns displayed by doxorubicin, providing initial structural and computational validation that these marine-derived diketopiperazines can effectively occupy the EGFR active site to mediate competitive enzyme inhibition.

In silico ADMET profiling is an indispensable tool for early-stage drug candidate screening to minimize downstream attrition rates⁴⁰. The virtual pharmacokinetic evaluation demonstrated that the purified diketopiperazines possess highly favorable drug-like properties. Regarding absorption parameters, the isolated peptides displayed excellent human intestinal absorption (HIA) percentages alongside superior Caco-2 monolayer permeability. The calculated HIA values were significantly higher than those modeled for doxorubicin (62.37%), highlighting a distinct oral bioavailability advantage⁴¹.

Furthermore, the compounds were predicted to act as neither substrates nor inhibitors of the P-glycoprotein (P-gp) efflux pump. This indicates that these molecules may evade active multi-drug resistance efflux mechanisms while avoiding harmful drug-drug interactions, thereby enhancing systemic bioavailability⁴².

Regarding physiological distribution, both blood-brain barrier (log BB) and central nervous system (log PS) penetration indices fell squarely within established acceptable therapeutic ranges⁴³. From a metabolic standpoint, the molecules did not interact with any major cytochrome P450 (CYP450) biotransformation isoforms, suggesting a low risk of metabolic interference or toxicity. Excretion modeling indicated low clearance rates, resulting in extended physiological persistence and a prolonged therapeutic half-life compared to both AQ4 and doxorubicin⁴⁴. Finally, predictive toxicological profiling confirmed an excellent safety outlook, with zero liability for Ames mutagenicity, cardiotoxicity, hepatotoxicity or skin sensitization.

CONCLUSION

The inner tissue niches of the marine macroalga *C. lentillifera* represent a highly valuable and underexplored microenvironment for the isolation of unique actinomycetes. *Streptomyces sanyensis* ARR153 emerges as a highly efficient biological factory for the production of functional L-cyclic dipeptides that possess robust antibacterial properties and precise, selective anticancer activity.

In summary, this investigation successfully isolated and characterized four bioactive L-cyclic dipeptides from the endophytic marine bacterium *Streptomyces sanyensis* ARR153, derived from the tissues of the macroalga *Caulerpa lentillifera*. These purified diketopiperazines demonstrated robust, selective antibacterial efficacy against a panel of Gram-

positive pathogens, including clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA). Furthermore, *in vitro* cytotoxicity assays revealed that these metabolites possess distinct antiproliferative properties against multiple human cancer cell lines. Notably, compound **4** (Cyclo-(L-Leu-L-Leu)) exhibited profound, targeted cytotoxic effects against MDA-MB-231 triple-negative breast cancer cells, displaying a superior selectivity index relative to the conventional chemotherapeutic agent doxorubicin hydrochloride. While these findings underscore the therapeutic potential of marine-derived diketopiperazines, further comprehensive research is warranted to optimize their structural configuration for enhanced cellular selectivity and to definitively elucidate their precise molecular mechanisms of action. Consequently, subsequent *in vivo* evaluations, combined with the development of targeted drug delivery strategies, will be essential steps toward advancing these naturally occurring L-cyclic dipeptides into viable, next-generation therapeutic candidates for oncological applications.

SIGNIFICANCE STATEMENT

This study establishes the marine macroalga-associated endophyte *Streptomyces sanyensis* ARR153 as a prolific and novel biological source of therapeutic L-cyclic dipeptides. By demonstrating that these purified diketopiperazines possess robust, broad-spectrum antibacterial activity against Gram-positive pathogens (including MRSA) alongside highly selective cytotoxicity against human cancer cell lines, this work highlights a favorable safety profile that minimizes damage to non-cancerous cells. These findings offer a compelling alternative to traditional drug discovery pipelines, suggesting that algal-derived microbial symbionts harbor immense, untapped chemical scaffolds. Ultimately, this research provides a foundation for the development of next-generation, targeted biopharmaceuticals capable of countering multidrug-resistant bacterial infections and aggressive malignancies.

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