DMSO-Mediated Induction of Lacthydrazide in *Streptomyces* sp. VITGV100 and its Molecular Docking Evaluation

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Abstract

Streptomyces species are renowned for their ability to produce bioactive secondary metabolites, many of which act as essential antimicrobials against infectious diseases. As multidrug-resistant (MDR) microorganisms pose an increasing global threat, the need for alternative antimicrobial agents has become urgent. In this study, Streptomyces sp. VITGV100 was cultured under static conditions with 0.5% dimethyl sulfoxide (DMSO) as an elicitor to boost secondary metabolite production. The bioactive compounds were extracted using ethyl acetate and were analyzed by liquid chromatography-mass spectrometry (LC-MS). The separation of the active ingredients was performed using thin layer chromatography (TLC). Comparative metabolic profiling and molecular docking studies were conducted to investigate the structural and functional characteristics of these compounds.

The results identified lacthydrazide as a novel antimicrobial agent with notable inhibitory effects against Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa. Fourier Transform Infrared Spectroscopy (FT-IR) was used to identify functional groups, aiding in structural characterization complemented by bioinformatic analyses. Molecular docking against antimicrobial target proteins (PDB IDs: 1KZN, 1GSK, 4RLC and 4URM) showed strong binding affinities and significant protein-ligand interactions, supporting the compound's potential as an effective antimicrobial. This research offers a detailed analysis of lacthydrazide, a metabolite produced by Streptomyces sp. VITGV100 and highlights the importance of DMSO in activating cryptic biosynthetic pathways. These findings advance the ongoing search for new antimicrobial drugs from Streptomyces species.

Keywords: *Streptomyces* sp. VITGV100, TLC, LCMS, FTIR, MIC, Molecular docking, ADME Analysis.

Introduction

The emergence of multidrug-resistant (MDR) pathogens poses a significant threat to global health, highlighting the critical need for the development of new antimicrobial medications[31]. The genus *Streptomyces* is particularly remarkable for its extraordinary capacity to generate a diverse array of secondary metabolites, many of which have revolutionized modern medicine[29]. These bioactive substances, including antibiotics, antifungals and anticancer drugs, highlight *Streptomyces* as a key source of therapeutic agents[3].

About 75% of commercially important antibiotics are derived from *Streptomyces* species, underscoring their critical role in medicine and agriculture[11]. Their genomes contain numerous biosynthetic gene clusters (BGCs), many of which are cryptic, representing a largely untapped reservoir of bioactive compounds[21]. Recent advances in genomics and bioinformatics have significantly enhanced natural product discovery, especially in identifying novel metabolites from *Streptomyces*[15]. Secondary metabolism often takes place in the late stages of growth while genetics determines the timing, environmental conditions can significantly influence its expression[22]. Secondary metabolism can be triggered by nutrient deficiencies, inducers, reduced growth rate, or environmental stimuli from other soil organisms such as Streptomyces and Actinomycetes[42].

Lacthydrazide, a derivative of hydrazide–hydrazone, is considered a promising candidate because of its extensive range of biological activities which include antibacterial, antifungal, antiviral, anticancer and antitubercular properties[28,29]. Among these, lacthydrazide has demonstrated significant potential, although its detailed purification and characterization remain underexplored. Advances in techniques such as LC-MS, FTIR and TLC offer strong tools for investigating the structure and activity of these compounds.

In this study, *Streptomyces* sp. VITGV100, a strain isolated from tomato plants[30], was identified as a promising candidate for metabolite production. Preliminary analyses of its crude extracts demonstrated significant antimicrobial activity, warranting further investigation[30]. This study aims to purify and characterize lacthydrazide from this strain, with a focus on elucidating its chemical structure and bioactive properties. Such efforts are critical for expanding our understanding of *Streptomyces*' metabolic potential and addressing the pressing need for novel therapeutic agents.

Furthermore, this work contributes to ongoing efforts to harness the biosynthetic capabilities of *Streptomyces* species for the development of new drugs. The identification and characterization of lacthydrazide and its derivatives not only

enhance our understanding of secondary metabolite biosynthesis but also open avenues for industrial and pharmaceutical applications. This research delves into the metabolic capabilities of *Streptomyces* sp. VITGV100, offering important insights into its potential as a source of novel bioactive compounds.

Material and Methods

Organisms: A pure culture of *Streptomyces* VITGV100 was sourced from the Microbiology Laboratory at the School of Bioscience and Technology, VIT University, Vellore. To determine the minimum inhibitory concentration (MIC) of the antibiotic, the following test organisms were employed: *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa*.

Extraction: The culture filtrate, totaling 5 liters, underwent three extractions using ethyl acetate as the solvent. This solvent was mixed with the filtrate in equal volumes (1:1 v/v) and then vigorously agitated for 20 minutes. The ethyl acetate layer, which contained the antibiotics, was separated from the water-based layer using a separating funnel. A rotary evaporator (model RE100-Pro) operating at 54°C and 80 rpm was employed to concentrate the organic extracts. GC-MS subsequently analyzed the concentrated crude extract.

Liquid Chromatography–Mass Spectrometry (LC-MS) analysis of isolated compounds: The ethyl acetate extract sourced from the culture filtrate of Streptomyces sp. VITGV100 was analyzed using LC-MS. The analysis was performed with the help of LC-Solution tools from Shimadzu Corporation, Kyoto, Japan. A Waters Micromass Q-Tof micro mass spectrometer facilitated the LC-MS process.

Fourier Transform Infrared Spectroscopy (FTIR) analysis: Fourier Transform Infrared Spectroscopy was conducted using a KBr pellet (13 mm) made from the pure compound to acquire the FTIR spectra. This was done with a Shimadzu IR Affinity 1 FTIR spectrometer from Japan, covering a scanning range of 400–4000 cm^{-1} with a resolution of 4 cm^{-1}. The FTIR spectra were examined to identify various functional groups.

Thin Layer Chromatography (TLC) Procedure: TLC was used to determine the compound profile of the extract of isolate VITGV100. We utilized silica gel 60 F254 plates (Merck) and added 10 µL of each extract to each plate. The TLC plates were developed with solvent systems such as petroleum ether: ethyl acetate (9:1) and (9:9). The solvent front was allowed to be up to 1 cm from the top edge before the plates were removed, dried and visualized with UV light at 254 nm. Retention factor (Rf) values were derived for the observed spots to identify and to compare the compounds.

Minimum Inhibitory Concentration (MIC) Assay: The VITGV100 strain's minimum inhibitory concentration (MIC) was evaluated in nutrient broth against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, with bacterial cultures adjusted to roughly 10^6 colony-forming units (CFU)/mL. A stock solution of the microbial extract was prepared in 0.5% dimethyl sulfoxide (DMSO) and diluted in broth with serial dilution to obtain final concentrations ranging from 1 to 7 µg/ml. The assay was conducted in sterile 96-well polypropylene microtiter plates, where 100 µL of the bacterial suspension was mixed with 0.5% of the extract dilution. Triplicate wells were prepared for concentration.

Wells containing only the bacterial suspension in the nutrient broth served as negative controls. Following incubation at 37°C for 24 hours, bacterial growth was assessed by measuring turbidity at 600 nm using a microplate reader. The optical density (OD) measurements were taken and the minimum inhibitory concentration (MIC) was identified as the smallest concentration that completely halted growth. The percentage of bacterial growth inhibition was then calculated using the formula[9]:

Percentage inhibition = (OD control – OD sample / OD control) 100

Molecular docking: Molecular docking was conducted to investigate how lacthydrazide interacts with antibacterial target proteins (PDB IDs: 1KZN, 1GSK, 4RLC and 4URM). The binding sites were identified using co-crystallized ligands and the "Define and Edit Binding Site" tool in BIOVIA Discovery Studio to determine the coordinates of the binding pockets. The lacthydrazide ligand was prepared by adding polar hydrogens and Gasteiger charges, followed by conversion to PDBQT format with AutoDock. Protein structures were obtained from the Protein Data Bank (PDB) and refined by removing water molecules and ligands, adding hydrogens and applying Kollmann charges, before being saved in PDBQT format.

A grid box sized 40 × 40 × 40 Å was created around the relevant residues to facilitate docking. Docking was conducted using AutoDock Tools (v1.5.7) to predict binding poses, which were then ranked based on binding energy (ΔG in kcal/mol). The leading poses were evaluated using BIOVIA Discovery Studio to investigate hydrogen bonding and hydrophobic interactions, focusing on crucial residues to confirm potential binding efficacy.

ADME Analysis: To assess the drug-like characteristics of the bioactive compound, an ADME analysis was performed following Lipinski's Rule of 5. This rule evaluates factors such as molecular weight (≤500 Daltons), lipophilicity (logP ≤ 5), the number of hydrogen bond donors (<5), hydrogen bond acceptors (<10) and the molar refractive index (40–130).

Statistical Analysis: The data were presented as the mean ± standard error of the mean, derived from a minimum of three

separate biological experiments. The figures were generated using Origin software.

Results and Discussion

The bioactive compounds found in actinomycetes play an essential role in fighting various diseases. Previous research has shown that actinomycetes are highly effective in controlling pathogen growth[8]. The genus *Streptomyces*, belonging to the *Actinomycetes phylum* was characterized by its filamentous Gram-positive bacteria and has been acknowledged for a long time as a significant source of bioactive compounds, such as antibiotics[8]. The objective of this research is to discover potential bioactive compounds from the culture extracts of *Streptomyces* VITGV100, which was sourced from tomato plants[37]. *Streptomyces* VITGV100 is a promising candidate for discovering antimicrobial compounds due to its ability to produce biologically active secondary metabolites.

A recent genome analysis performed with antiSMASH 6.0 identified 35 gene clusters involved in producing secondary metabolites, some of which showed less similarity to known antimicrobial compounds[30]. Our research intends to stimulate the biosynthetic gene cluster using an elicitor, followed by evaluating its antimicrobial efficacy and identifying the compound. The validation of these antimicrobial properties was carried out through computational analysis of the metabolites collected from the cluster.

In this study, 0.5 % DMSO was employed as an elicitor to produce the unique bioactive compound lacthydrazide which exhibited significant inhibition against both Gram-positive and Gram-negative bacteria. These bacteria are instrumental in evaluating the effectiveness of natural antimicrobial products due to their widespread distribution, unique cell structure and clinical significance.

Extraction and Identification of Bioactive Compounds: The microbiology laboratory at the School of Bioscience and Technology, VIT University, Vellore, supplied a pure culture of *Streptomyces* VITGV100. *Streptomyces* VITGV100 was cultured in 1000 ml Erlenmeyer flasks containing 400 ml of nutrient broth. The flasks were supplemented with a 0.5% concentration of DMSO. The cultures were then incubated at room temperature on a shaker at 150 rpm for 7 days. During incubation, the cultures were centrifuged at 8000 rpm for 20 minutes to separate the cell biomass. The secondary metabolites in the broth were extracted using a two-phase solvent system with ethyl acetate (1:1). A rotary evaporator (model RE100–Pro) at 54°C and 80 rpm was used to concentrate the organic extracts. The resulting crude extract was further analyzed by LCMC, FTIR and MIC assays.

LC–MS Analysis: LC-MS analysis of crude extracts from *Streptomyces* VITGV100 confirmed the identity of the bioactive compound. The LC-MS data were examined based on m/z and retention time. The ESI–MS spectra revealed a prominent peak at m/z 105.0421 [M + H] +, which corresponds to a monoisotopic mass of 104.11 g/mol (Fig. 1). This indicates that the bioactive compound is prevalent in the extract. After assessment and interpretation, the compound identified at a retention time (RT) of 1.27 was determined to be lacthydrazide (Fig. 2). The molecular mass of this identified compound is 105.0421 (Fig. 1). The compound lacthydrazide has been previously reported from plasma exosome metabolites in ovarian cancer (OC) patients using machine learning algorithms[20].

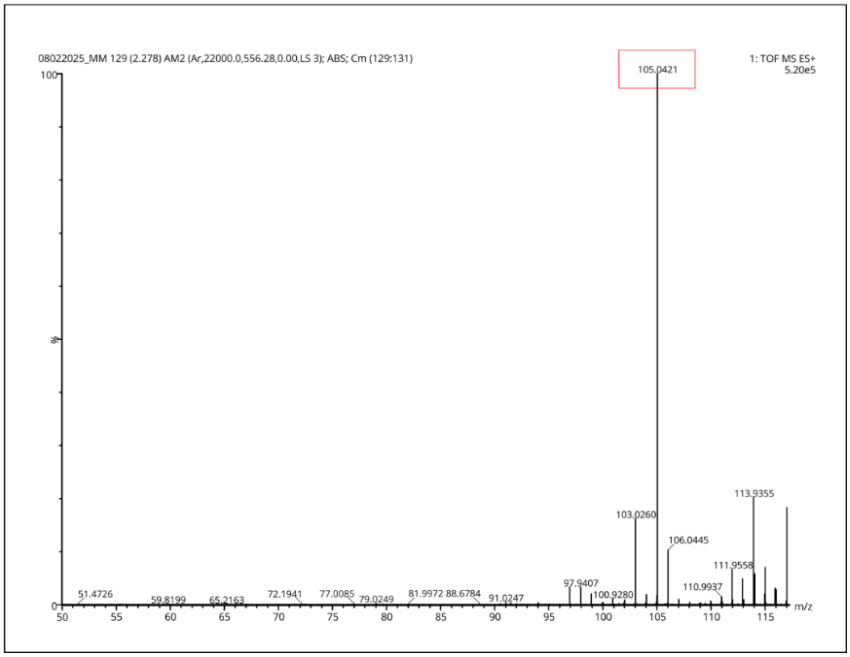


Fig. 1: LC-MS-ES spectrum of the compound lacthydrazide. The retention time was 1.27 min and the mass of the spectrum was 105.0421

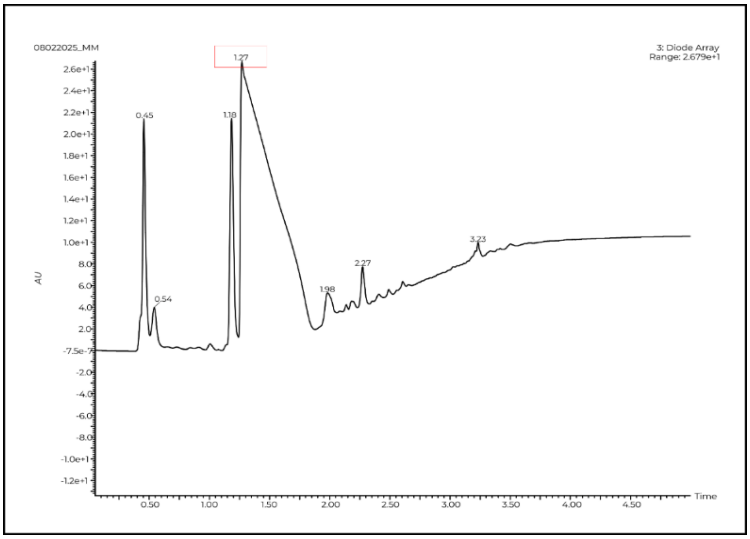


Fig. 2: LC–MS chromatogram of the secondary metabolite of *Streptomyces* sp. VITGV100. The peak corresponds to the bioactive molecule lacthydrazide (peak at 1.27, m/z = 105.0421).

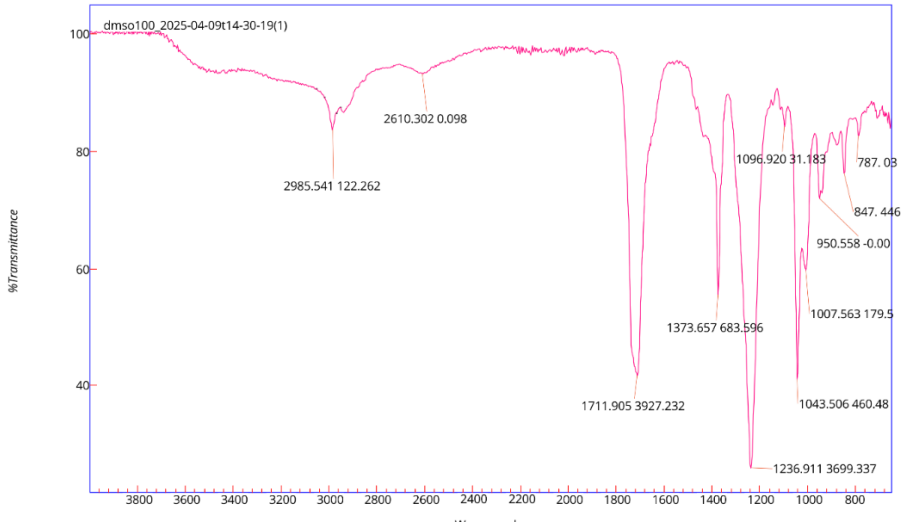


Fig. 3: FT-IR spectrum of the bioactive metabolite in the crude ethyl acetate extract from the fermentation broth of *Streptomyces* VITGV100.

FTIR Analysis: The spectra of the purified molecule exhibited peaks matching those in the standard library spectra (Fig. 3). Notable peaks indicating its functional groups align with previously reported hydrazide derivatives. Peaks at 2985, 2610 and 1373 cm^{-1} signify C-H stretching, typical for aliphatic hydrocarbons and consistent with findings for comparable hydrazide compounds[26,35]. A significant peak at 1711 cm^{-1} implies C=O stretching, a defining characteristic of hydrazide compounds[2], especially in lacthydrazide and similar derivatives, while a peak at 1236 cm^{-1} indicates alkyl aryl ether C-O stretching, which corresponds to the structure of specific hydrazide derivatives that include ether linkages[22]. The signal at 1043 cm^{-1} is suggestive of anhydride CO-O-CO stretching, potentially indicative of a lactone-like structure often seen in lacthydrazide derivatives.

The dual peaks at 1007 cm^{-1} and 1096 cm^{-1} correspond to C-F stretching in the fluoro compound. This distinctive feature may suggest the fluorination of the hydrazide compound, which has been reported to enhance the biological activity of hydrazide derivatives[7,19]. The peaks at 950 and 787 cm^{-1} relate to alkene C=C stretching whereas the peak at 847 cm^{-1} pertains to halo complex C-Cl stretching[4]. The spectra confirm the presence of characteristic functional groups consistent with lacthydrazide derivatives.

TLC assay: Thin-layer chromatography (TLC) was employed to separate and analyze the components of the secondary metabolite extracts. Crude extracts from the strain *Streptomyces* sp. VITGV100, obtained using ethyl acetate, was resolved on TLC plates with two different solvent systems utilizing varying ratios of petroleum ether and methanol (9:1 and 9:9, v/v). The extract treated with the 9:1 (v/v) solvent system produced two distinct bands, exhibiting retention factors (Rf) of 0.96 and 0.90 (Fig. 4A). Similarly, the 9:9 (v/v) solvent system yielded two bands with Rf values of 0.91 and 0.83 (Fig. 4B), indicating the presence of

non-polar to moderately polar compounds[39]. These Rf values imply a strong affinity of the metabolites for the non-polar solvent, which aligns with the characteristics of hydrazide derivatives, as detailed in studies utilizing similar solvent conditions[13].

These TLC results demonstrate the separation and identification of the chemical compounds present in the extracts and reflect the differences in secondary metabolite composition influenced by the solvent ratio used. This emphasizes the potential of different retention factors to reveal the chemical diversity within the extracts of *Streptomyces* sp. VITGV100 under different extraction conditions.

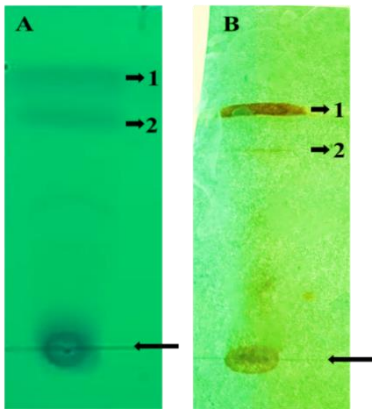


Fig. 4 (A and B): Silica thin-layer chromatographic (TLC) separations of extract of VITGV100, developed in (A) and (B) Petroleum ether and methanol (9:1 and 9:9) v/v

MIC Assay: A 96-well microtiter assay[6,40] was employed to measure the MIC of the crude extract from *Streptomyces* sp. VITGV100. This extract's antibacterial properties were evaluated by measuring the inhibition percentages against *E. coli, B. subtilis, P. aeruginosa* and *S. aureus*. Inhibition rates for 0.5% DMSO in these pathogens were calculated in triplicate, revealing significantly higher inhibition rates due to elicitors. This increased the efficacy of DMSO in promoting *Streptomyces* as an elicitor by enhancing the solubility and bioavailability of antimicrobial compounds.

Notably, 0.5 % DMSO achieved the highest inhibition rate of 74.32±0.94 µg/ml against *B. subtilis* at a concentration of 7 µg/ml (Fig. 5 and Table 1) and the IC_{50} values were calculated against the pathogens, 3.31, 0.67, 2.23 and 0.35 mg/ml. The IC50 result shows that *S. aureus* has a stronger inhibition activity (0.35 mg/ml) than other pathogens, highlighting its strong antimicrobial capabilities. The differences in cell wall composition between the two bacterial groups may explain the variation in bacterial activity, with Gram-positive bacteria being more resistant to antimicrobial substances[33]. Previous studies on *Streptomyces lavendulae* SCA5 further substantiate the role of DMSO as a neutral solvent in MIC assays[18].

Molecular docking: Molecular docking refines the shape and orientation of receptors and ligands to reduce binding energy, offering a computational method for forecasting receptor-ligand interactions[25]. While experimental validation is vital for affirming ligand efficacy, docking is a reliable tool for hypothesizing possible binding affinities and interaction sites[14]. This study employed molecular docking to pinpoint potential inhibitors for target proteins by predicting their binding conformations and corresponding interaction compounds[16]. The findings were assessed through analysis of docking configurations and an in-depth evaluation of protein-ligand interactions[12].

The compound lacthydrazide was identified and docked to the target proteins using AutoDock tools. 3D and 2D visualisation of protein-ligand interaction shown in (Fig. 6). Binding energies, interacting residues and bond lengths between the docked compounds and the proteins (Table 2). Results from the docking analysis show that lacthydrazide inhibits the target proteins of several antimicrobial agents. These compounds exhibited favourable binding energy and bond length, featuring hydrogen bonds, carbon-hydrogen bonds, Pi-Alkyl, Pi-Anion and some are unfavourable acceptors with the binding residues of the proteins[38]. Significantly, most interacting residues were found within the binding sites of co-crystallized ligands on the target proteins.

The standard compound tetracycline was also docked with the antimicrobial proteins and the result of this docking analysis was a moderate binding energy of both the compound lacthydrazide and the tetracycline. The compound lacthydrazide has comparatively lower binding energies with the target proteins than tetracycline. Furthermore, the lower is the binding energy, the higher is the stability of the complex[10]. The present study suggests lacthydrazide as a potent inhibitor for target proteins with significant binding energy and appropriate interactions.

Table 1
Percent inhibition of bacterial pathogen at different concentrations of 0.5 % DMSO in stimulating *Streptomyces* VITGV100

0.5% of DMSO to stimulate VITGV100	Percent inhibition			
	E. coli	***B. subtilis***	***P. aeruginosa***	***S. aureus***
1 µg/ml	42.93±2.05	53.04±2.01	44.38±4.3	53.6±2.06
3 µg/ml	46.17±1.98	59.15±1.81	46.61±4.4	61.17±3.7
5 µg/ml	50.42±1.31	64.63±2.92	54.25±3.2	65.34±1.4
7 µg/ml	51.17±1.36	74.32±0.94	66.75±2.7	72.4±2.3

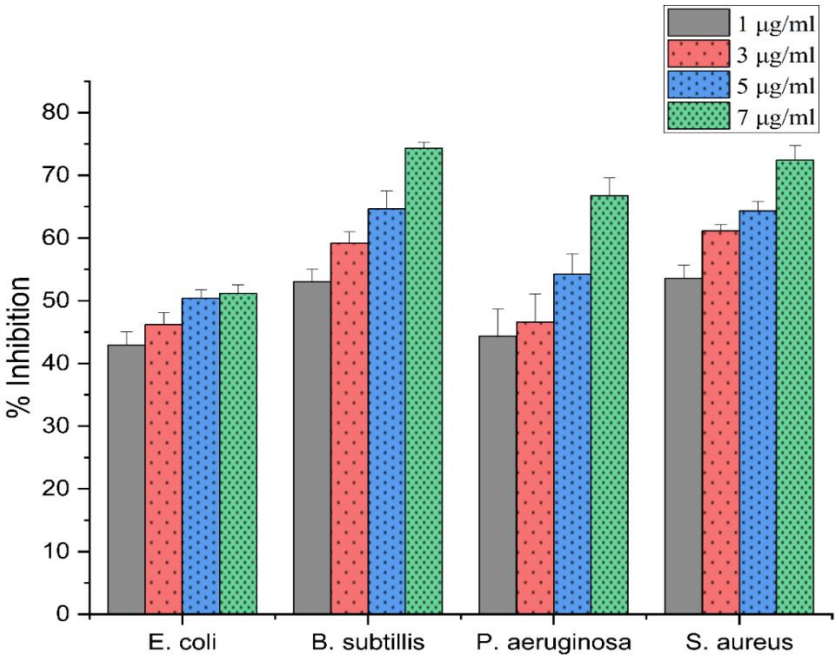


Fig. 5: The extract of 0.5% of DMSO, when used as an elicitor in *Streptomyces* VITGV100 as trigger for inhibiting the growth of Gram-positive and Gram-negative bacteria, was evaluated using OD_{600} measurements. Inhibition rate (%) was calculated for the hardelicitor: 0.5% of DMSO, where triplicate samples were held at volumes of 1, 3, 5, 7 µg/ml against the *E. coli* (Ec), *B. subtilis* (Bs), *P. aeruginosa* (Pa) and *S. aureus* (Sa).

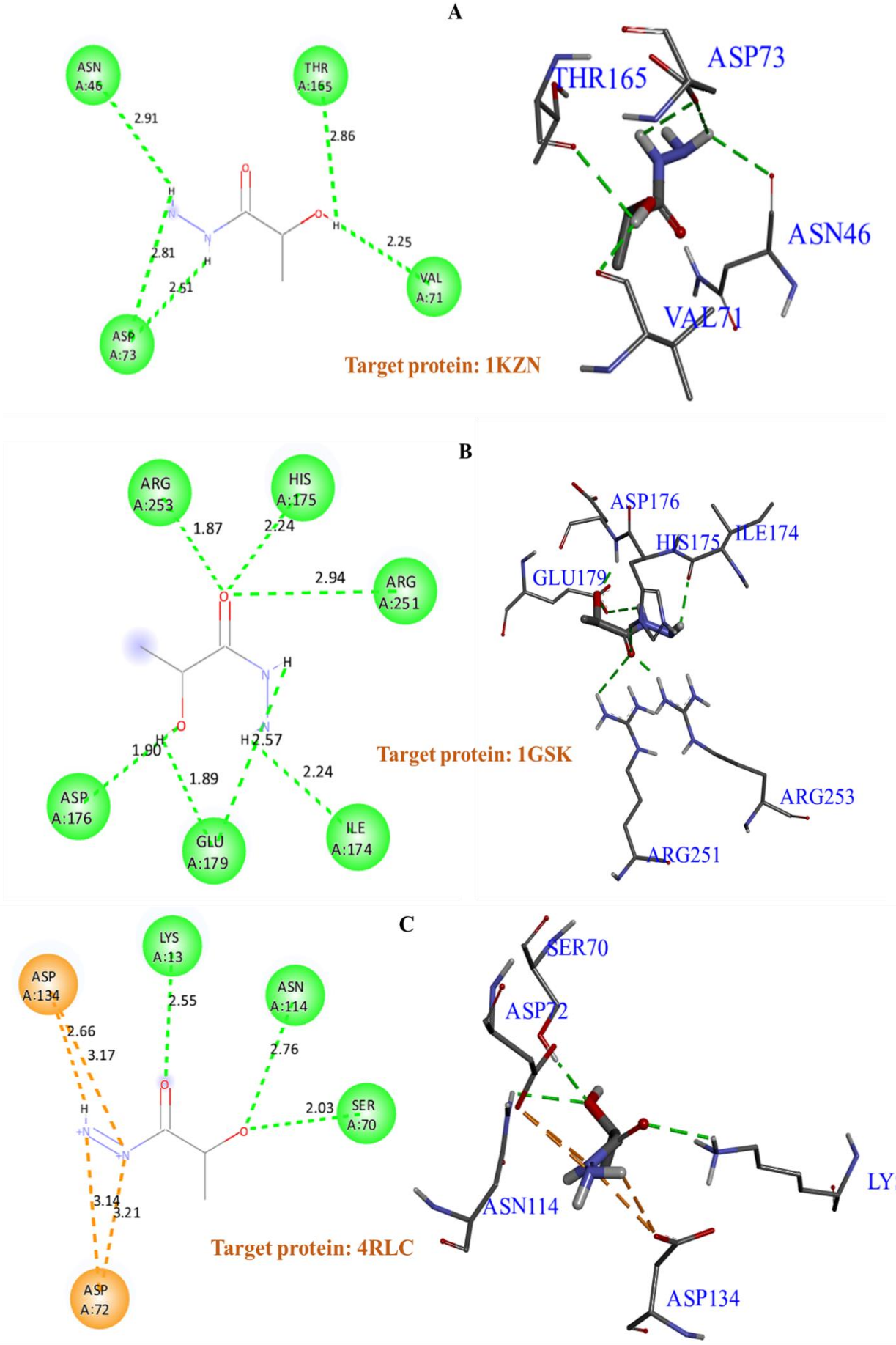
Table 2
The binding energies, interacting residues and bond length of target proteins with potent compounds

Target Proteins (PDBID)	Binding Energy (kcal/mol)		Interacting Residues		Bond length	
	Lacthydrazide	Tetracycline	Lacthydrazide	Tetracycline	Lacthydrazide	Tetracycline
1KZN	-4.5	-6.9	THR165, ASP73, VAL71, ASN46	GLN72, GLU58, ASP74, HR163	2.91, 2.86, 2.81, 2.51, 2.25	5.25, 3.77, 3.64, 2.58, 2.55
1GSK	-5.1	-7.2	ARG251, ARG253, GLU179, ASP176, ILE174, HIS175	THR260, THR418	2.94, 2.57, 2.24, 1.90, 1.89, 01.87,	2.73, 2.67, 1.97
4RLC	-4.8	-6.3	ASP72, ASN114, ASP134, LYS13, SER70	GLY31, LEU45	3.21, 3.17, 3.14, 2.76, 2.66, 2.55, 2.03	4.83, 2.99, 2.52
4URM	-4.9	-8.1	THR173, GLU58, ASP81	ARG200, GLU220, ASN221, PRO87	3.20, 3.08, 2.34, 1.99, 1.87	5.22, 5.17, 4.13, 3.21, 2.55, 2.47, 2.45

In a prior study, benzamide, benzo hydrazide, carbon hydrazide and methyl (4-(5,6,7,8-tetrahydrocarbazol-9-yl) benzoyl)-2-hydrazine carbodithioic acid were recognized as promising candidates against *E. coli* due to their ability to inhibit the target protein 1KZN[23].

Furthermore, the antimicrobial molecules napin and procruciferin exhibit strong binding affinity with known bacterial receptors 4URM from *S. aureus*, indicating their potential to inhibit bacterial activity[32] . Therefore, molecular docking methods should be considered to confirm the efficacy of these potent metabolites.

ADME analysis: Lipinski's rule of five stated that an active molecule must have a molecular weight of less than 500 to be deemed a drug candidate, as larger molecules have difficulty in passing through cell membranes[1]. In addition, a LogP value below 5 is preferred, as higher lipophilicity can reduce the water solubility of the active compound and make it more difficult to pass through biological barriers [17].


A
ASN A:46
THR A:165
2.91
2.86
2.81
2.51
2.25
ASP A:73
VAL A:71
Target protein: 1KZN
ASP73
THR165
ASN46
VAL71
B
ARG A:253
HIS A:175
1.87
2.24
2.94
ARG A:251
1.90
1.89
2.57
2.24
ASP A:176
GLU A:179
ILE A:174
Target protein: 1GSK
ASP176
HIS175
ILE174
GLU179
ARG253
ARG251
C
LYS A:13
ASP A:134
ASN A:114
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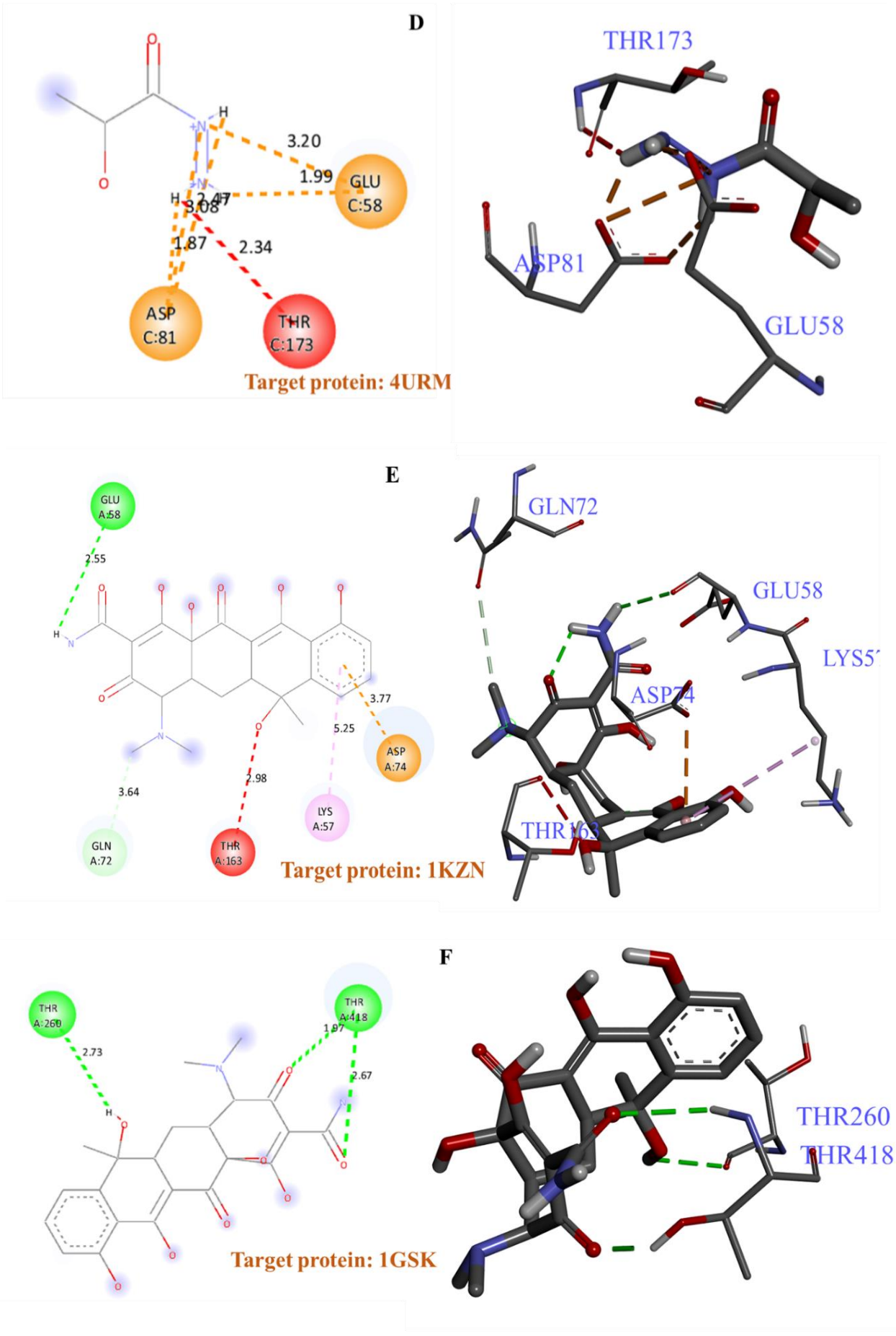
D
3.20
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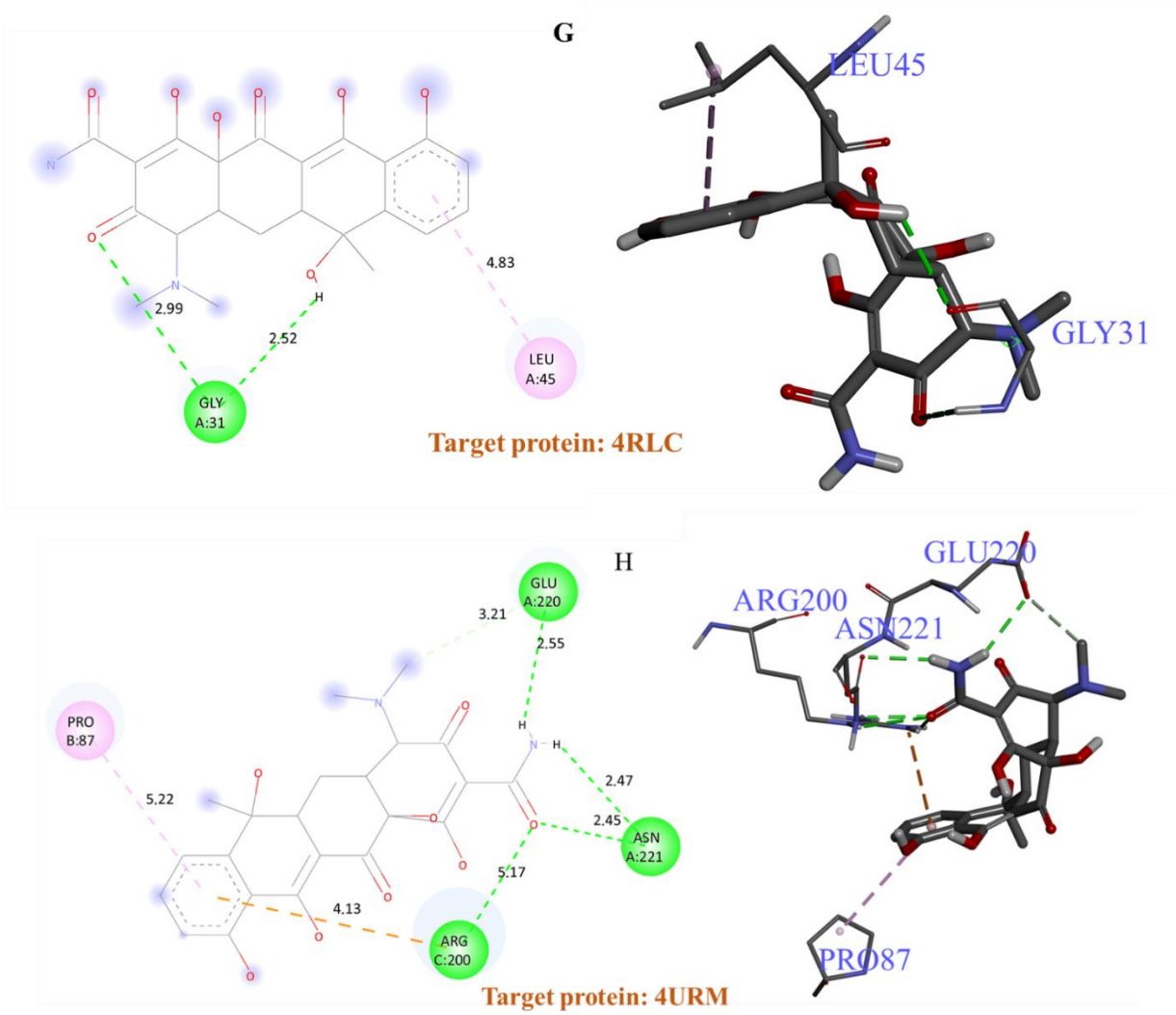

Figure 6: (A, B, C and D) represent binding interactions of lacthydrazide with target protein crystal Structure of *E. coli* 24kDa Domain in Complex with Clorobiocin (1KZN), crystal structure of CotA, an endospore coat protein from Bacillus subtilis (1GSK), crystal structure of the N-terminal beta-barrel domain of *Pseudomonas aeruginosa* OprF (4RLC) and (Crystal Structure of Staph Gyrase B 24kDa in Complex with Kibdelomycin (4URM) respectively; (E, F, G and H) represent binding interactions of tetracycline with target protein crystal Structure of *E. coli* 24kDa Domain in Complex with Clorobiocin (1KZN), crystal structure of CotA, an endospore coat protein from *Bacillus subtilis* (1GSK), crystal structure of the N-terminal beta-barrel domain of Pseudomonas aeruginosa OprF (4RLC) and (Crystal Structure of Staph Gyrase B 24kDa in Complex with Kibdelomycin (4URM).

Table 3
***In silico* analysis of ADME properties and Lipinski's rule of 5 compliances for selected compounds**

Compounds	Molecular weight	No. of Rotatable Bonds	H-bond acceptor	H-bond donor	LogP	Molecular refractivity	Topological Polar Surface Area	Solubility (mg/ml)	Lipinski's rule of 5
Lacthydrazide	104.11 g/mol	2	4	3	0.41	23.41	75.35	3.84e+02	Yes, 0 violations

The compound should have fewer than five hydrogen bond donors to avoid impeding membrane penetration. Similarly, more than 10 hydrogen bond acceptors might cause a slowdown or prevent the compound from entering cells effectively[42]. Furthermore, their polar surface area (PSA) and solubility values back up their favourable ADME profiles[5]. PSA is an important metric for determining a compound's capacity to penetrate membranes and be absorbed in the gastrointestinal tract[27]. Lacthydrazide has a PSA of 75.35 Å² and demonstrates very high solubility (3.84e+02 mg/mL). Veber et al[36] in their study defined good bioavailability as <140 Å² for oral medicines[36]. These findings highlight their potential for efficient absorption and distribution in biological systems and indicate that both drugs have the physicochemical qualities required for efficient bioavailability. Lacthydrazide has no deviations from Lipinski's rule of 5, suggesting that they are promising drug. The details of the bioactive compound concerning adherence to Lipinski's rule are given in table 3.

Conclusion

This study emphasizes the remarkable potential of *Streptomyces* sp. VITGV100 as a novel producer of bioactive lacthydrazide compounds, marking the report of such metabolites within the *Streptomyces* genus. The strain exhibited strong antimicrobial activity against both Gram-positive and Gram-negative bacteria, highlighting its therapeutic significance. Lacthydrazide derivatives were effectively extracted and purified from the culture supernatant, with their structures and bioactivity validated using advanced spectroscopic techniques like LC-MS, FTIR and TLC. Additionally, *in silico* docking studies indicated favorable binding interactions between lacthydrazide and tetracycline, along with their respective antibacterial targets, suggesting their potential bioactivity.

Furthermore, ADME analysis confirmed the safety of these compounds, showing compliance with Lipinski's rule of 5, which positions them as promising candidates for future pharmaceutical applications. Overall, the findings position *Streptomyces* sp. VITGV100 as a valuable microorganism for generating bioactive compounds with significant antimicrobial activity. These results not only broaden the known metabolic capabilities of *Streptomyces* but also set the stage for further investigation into lacthydrazide derivatives as potential drug development candidates. Future research focusing on the structural optimization of these compounds will unveil new avenues for addressing multidrug-resistant pathogens.

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