

Synthesis of Simplified Vancomycin Analogues and Evaluation of Their Antibacterial Properties

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Abstract: The issue of multidrug-resistant bacteria is a significant concern in the global healthcare landscape. While a wide range of medications exists for conventional therapeutic purposes, only a limited number of molecules have the ability to function as ultimate options for treating serious infections. Hence, it is essential to establish strategies for the management of multidrug-resistant microorganisms. In this study, we present a collection of newly synthesized vancomycin derivatives that include thiol- and disulfide-containing functional groups. The newly synthesized compounds demonstrated increased antibacterial efficacy against a diverse array of bacterial strains, including vancomycin-resistant microorganisms as well as Gram-positive bacteria. The synthesized conjugates exhibited significant antibacterial activity against vancomycin-resistant enterococci. In summary, the findings of this study illustrate the capacity of modifying the structure of existing antibiotics to provide strong molecules capable of effectively combating bacteria that have developed resistance to several drugs.

Keywords: vancomycin; antibiotics; disulfide moieties; microbes; multidrug resistance.

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1. Introduction

Vancomycin, a glycopeptide antibiotic, is often used to treat several bacterial infections that have developed resistance to many drugs [1]. The clinical use of vancomycin began in the late 1950s; however, its extensive application was postponed for a considerable period of time owing to the toxicity associated with the original unrefined formulations [2]. Since its discovery in the 1950s, vancomycin has played a crucial role as an essential antibiotic in the treatment of Gram-positive bacterial infections [3-5].

The use of this therapeutic option was regarded as the ultimate course of action for addressing severe infections resulting from methicillin-resistant *Staphylococcus aureus* (MRSA). The first observation of decreased susceptibility of MRSA to glycopeptide antibiotics, referred to as glycopeptide-intermediate *S. aureus* (GISA), occurred in Japan in 1996. This was characterized by a higher vancomycin minimum inhibitory concentration (MIC) range of 4-8 µg/mL, compared to MIC values of < 2 µg/mL for susceptible MRSA strains. The mechanism of action of vancomycin involves the inhibition of bacterial cell wall production by its binding to the D-Ala-D-Ala terminus of peptidoglycan pentapeptide. This binding is facilitated by the formation of five hydrogen bonds, ultimately resulting in the

demise of the bacterial cell. Nevertheless, with the massive use of vancomycin, microorganisms have progressively developed resistance to the drug's mechanism of action. The first instance of vancomycin-resistant *S. aureus* (VRSA) was documented in 2002, with a vancomycin MIC of $> 16 \mu\text{g/mL}$. This resistance mechanism involves the same alteration of Lipid II as vancomycin-resistant enterococci (VRE). However, it is worth noting that there have been rather few cases of VRSA so far. The primary mutation seen is the bacterial alteration of their peptidoglycan pentapeptide terminus, transitioning from D-Ala-D-Ala to D-Ala-D-Lac. The alteration described above reduces the affinity of vancomycin for its target, leading to a significant decrease in antibacterial effectiveness by more than 1000-fold [6,7].

The proliferation of drug-resistant bacteria has engendered significant societal apprehensions, hence necessitating a pressing need for the advancement of novel antibiotics [8,9]. Additional studies have provided estimates indicating that the global annual mortality rate resulting from bacterial illnesses amounts to around 700,000 fatalities [10,11]. In contrast to other medicines, the emergence of resistance to vancomycin exhibited a protracted timeline, with the first instances of VRE infections being documented in the mid-to-late 1980s, some three decades after the introduction of vancomycin. The development of resistance to glycopeptide antibiotics in Enterococci is mostly attributed to the expression of resistance gene clusters, namely *vanA* and *vanB*. The aforementioned clusters are responsible for encoding enzymes that facilitate the production of a modified peptidoglycan precursor known as D-Ala-D-Lac, as opposed to the conventional D-Ala-D-Ala. This modification results in a reduction of the binding affinity of glycopeptides [12].

The existing market of antibiotics does not adequately address the increasing need for novel antibiotic therapies targeting multidrug-resistant bacteria. In order to effectively combat the continuously adapting multidrug-resistant bacteria, it is imperative to discover novel chemicals [13]. Several recent publications have presented several strategies for addressing bacterial infections. The aforementioned strategies include antimicrobial peptides, monoclonal antibodies, bacteriophages, quorum-sensing inhibitors, and polymeric or metallic nanoparticles [14]. Alternative methodologies concentrate on the structural alterations of established antibiotics, like vancomycin, with the aim of restoring their efficacy or modifying their *in vivo* properties. Promising ways for modification include the use of single cationic quaternary ammonium charges and the incorporation of cationic sulfonium moieties in conjunction with saturated fatty acids. Additionally, the inclusion of chlorobiphenyl and dipicolyl extensions has shown potential in this regard [15,16].

The use of semisynthetic methodologies, including structural alterations, has shown considerable potential as an effective solution for addressing the issue of resistance [17,18]. This might potentially give rise to an additional mode of action, hence leading to enhanced antibacterial activity against bacteria that have developed resistance. Recent research has shown that the introduction of positively charged moieties conjugated to vancomycin might impart membrane-disrupting characteristics to the resulting vancomycin derivatives. This, in combination with vancomycin's intrinsic ability to form hydrogen bonds, can lead to an enhanced antibacterial effectiveness [19]. In this study, a collection of new vancomycin derivatives with distinct quaternary ammonium moieties was developed and synthesized (Figure 1). The antibacterial properties against drug-resistant bacteria derived from clinical settings were assessed both *in vitro* and *in vivo*.

2. Materials and Methods

The chemical reagents used in this study were procured from reputable suppliers, namely Sigma-Aldrich and TCI Chemicals, and were employed in their as-received form without undergoing further purification procedures. The reaction solvents were procured from commercial providers, subjected to purification, and then dried using established protocols prior to their use. Specifically, trifluoroacetic acid (TFA) was used as an additive in solvents to enhance peak shapes in HPLC; acetonitrile served as a common solvent in HPLC, particularly in reverse-phase chromatography; and purified water, often HPLC-grade, was essential for preparing mobile phases [20].

2.1. Reverse-phase high-performance liquid chromatography (RP-HPLC).

The analytical data were acquired through the utilization of RP-HPLC on a C18 column (Chromolith® Performance RP-18e, 100 × 3 mm). This was accomplished by coupling the column to an Agilent 1100 series system equipped with UV detection at a wavelength of 214 nm. The substances were dissolved and then separated using a linear gradient of acetonitrile (0.1% trifluoroacetic acid) and water (0.1% trifluoroacetic acid) for a duration of 15 or 25 minutes, respectively. The gradient was modified for each chemical in order to align with their respective features. Subsequently, the purified compounds underwent lyophilization for a duration of 16 to 24 hours using an Alpha 2-4 LD plus system.

2.2. Nuclear magnetic resonance spectra (NMR).

The ¹H nuclear magnetic resonance (NMR) spectra were acquired using Avance III-500 spectrometers operating at a frequency of 500 MHz, equipped with Cryo-BBO or BBO probes. The NMR measurements were performed in DMSO-d₆ solvents. Coupling constants (J) are commonly denoted in hertz (Hz), whereas chemical shifts are often expressed in parts per million (ppm) using the delta (δ) scale.

2.3. High-resolution electrospray mass spectra.

Using a “timsTOF Pro TIMS-QTOF-MS device (Bruker Daltonics GmbH, Bremen, Germany)”, high-resolution electrospray mass spectra (HRMS (ESI)) were captured. The materials were dissolved in a solution (such as MeOH) at a concentration of about 50 µg/mL for analysis using a continuous flow injection technique (2 µL/min) in Experimental Section I-53. The mass spectrometer was run in the positive (or negative) electrospray ionization mode, with a dry gas flow of 4 mL/min at 180°C, a N₂ nebulizer pressure of 0.4 bar, and a capillary voltage of 4000 V and 500 V offset. Mass spectra were obtained at a rate of 1.0 Hz and in a mass range of m/z 50 to 2000, with about 20,000 resolutions (m/z 622).

2.4. Synthesis of lipo-peptide linker.

Using a conventional methodology using the Fmoc-strategy and HBTU (1-[Bis(dimethylamino)methylene]-1H-benzo-triazolium hexafluorophosphate 3-oxide) activation, peptide synthesis was carried out as previously reported [21]. In order to acquire the carboxyl-terminal cysteine residue, the Fmoc-Rink-Amide resin (loading of 0.67 mmol/g) was loaded with Fmoc-cysteine. Each of the following amino acids was linked for a full hour. Following joining, the resin underwent vacuum drying and washing [22].

Eight equivalents of each fatty acid and 7.8 equivalents of HBTU were dissolved in DMSO in order to couple the fatty acids. “N-ethyl-N-(propan-2-yl)propan-2-amine (DIPEA)”, 24 equivalents, was then added. The solution was subjected to an incubation period of 1 hour, subsequent to an initial 5-minute interval, during which it was exposed to the dried resin. The resin was then thoroughly cleaned after the coupling was finished, and it was vacuum-dried. The (lipo-)peptides were finally broken down for two hours using TIS, TFA, and water (2.5/95/2.5 v/v/v, 1 mL/100 mg resin). Diethyl ether was used to precipitate (lipo-)peptides, which were then vacuum-dried. As previously mentioned, analysis and purification were carried out.

2.5. Synthesis of vancomycin derivatives.

Ethylene diamine had to functionalize the carboxyl function in order to connect the linker moiety to the vancomycin. As a result, 3 eq. of DIPEA and 1 eq. of vancomycin hydrochloride were added to DMSO. Then, ethylene diamine, 3 equivalents of (7-azabenzotriazol-1-yloxy)tripyrrolidino phosphonium hexafluorophosphate (PyAOP), and benzotriazol-1-ol (HOBt), 1 equivalent, were added to this solution. The terminal thiols that would be most accessible to any bacterial homolog were chosen, both with and without internal disulfides (3) and (4). In order to boost lipophilicity, endolipidic disulfides (2,3) were chosen. This should also have a favorable effect on residence duration and may entail other modes of action, such as inhibition of transglycosylases and breakdown of cell-wall integrity. In order to enhance the permeability of the bacterial membrane and enhance the interaction with negatively charged molecules on the cell wall, disulfides with polar end groups, such as amine (7), pyridine (6), and ammonium salts (5), were selected as described in [23]. After one hour of continuous agitating the reaction mixture, the solvent was evaporated at room temperature. The mixture was diluted in a solvent combination of MeCN/H₂O (1:1, with 0.1% HCOOH) and then passed through a solid-phase extraction (SPE) column for filtration. The solvents were subjected to evaporation, and subsequently, the compounds underwent purification using preparative RP-HPLC [24].

2.6. Antimicrobial activity testing.

The determination of the minimum inhibitory concentration (MIC) was conducted using the microdilution method in accordance with the criteria provided by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [25-27]. This was performed using the broth microdilution assay, which included the use of a 96-well plate. The compounds were dissolved in a saline solution with a concentration of 0.9%, which corresponded to an equimolar concentration of 1.28 mg/mL of vancomycin. In order to facilitate dissolution, it is necessary to pre-dissolve certain chemicals in DMSO as their hydrophobicity increases [28,29]. The final concentration of DMSO remained consistently below 1.5% and was compared to the control group treated with DMSO [30]. No antibacterial impact was seen at any dose tested, including the maximum concentration of 10%. To determine the bacterial loading, a culture of the specific strain was prepared by incubating it overnight. The resulting culture was then adjusted to a turbidity level equivalent to 10⁸ colony-forming units per milliliter (CFU/mL). The bacterial solution was diluted to achieve a bacterial concentration of 10⁶ CFU/mL on the 96-well plate [31,32]. The plate was then incubated for 18 hours at a temperature of 35 ± 1°C, following the method

outlined in a previous study [33,34]. The MIC was operationally defined as the concentration at which no observable growth was seen [33-38].

3. Results and Discussion

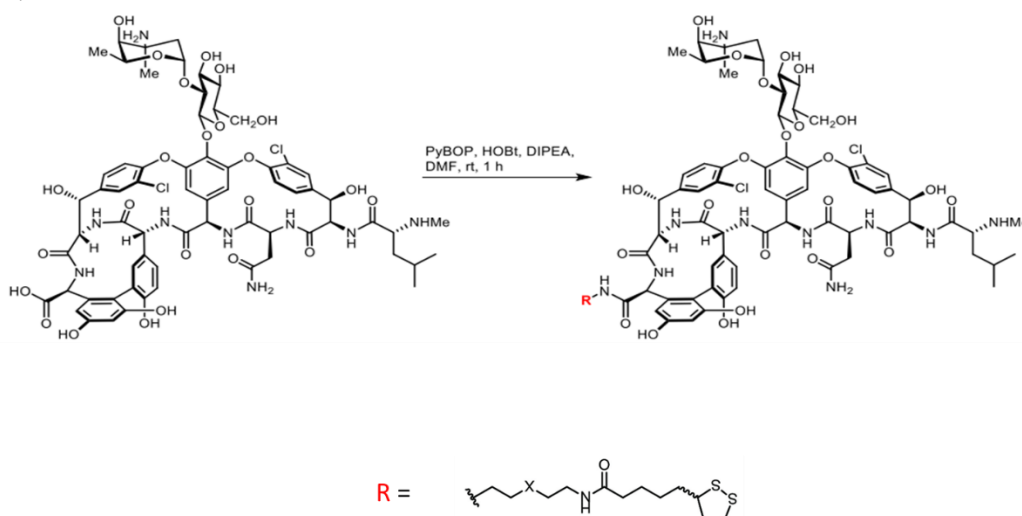
The rising prevalence of bacterial resistance presents several unforeseen challenges to the human population. The need to discover innovative antibiotics with heightened efficacy, particularly in combating multidrug-resistant bacteria, has become more crucial in contemporary times. This work aims to examine the structure-activity connection of synthesized conjugates in relation to their antibacterial activity on vancomycin-resistant enterococci. To do this, we have integrated newly reported techniques of vancomycin modifications.

The objective of this research was to investigate the effects of sulfur and lipoic acid-modified vancomycin on the antimicrobial efficacy of several bacterial species. Consequently, we systematically explored various substitution effects of the sulfur unit on lipoic acid linkers to acquire a deeper understanding of their rational implications. In the first step, a set of vancomycin derivatives was synthesized by the glycopeptide's C-terminal modification using appropriate amines. This process was carried out in the presence of HOBt and PyBOP, which served as coupling reagents (Scheme 1). A derivative of lipoic acid (1) has been selected based on the performance of eukaryotic, thiol-mediated absorption systems that have previously shown varying degrees of effectiveness against bacteria. Two endolipidic disulfides were chosen in order to enhance lipophilicity, which is expected to have a beneficial impact on residence duration and possibly affect other modes of action, such as the inhibition of transglucosylase and the loss of cell-wall integrity [39]. Terminal thiols were chosen, including those with internal disulfides and those without, based on their potential accessibility to bacterial counterparts. In order to improve the permeability of the bacterial membrane and enhance the interaction with negatively charged chemicals on the bacterial cell wall, disulfides containing polar end groups like pyridine, ammonium salts, and amine were ultimately selected.

The conventional broth microdilution susceptibility assays were used to investigate the antibacterial activity of vancomycin derivatives containing sulfide unit against a range of pathogenic Gram-positive bacteria, including clinically derived strains that are resistant, MRSA, vancomycin-intermediate *Staphylococcus aureus* (VISA), and VRE [40,41]. The information is succinctly presented in Table 1. The findings indicate that the inclusion of lipophilic chains plays a crucial role in the antibacterial efficacy against VISA and *S. pneumoniae*. Derivative 2 exhibited significantly increased activity (ranging from 16 to 64-fold) against VanA, VISA, and VanB strains when compared to vancomycin. Compound 5 exhibited notable activity against VISA and *Bacillus subtilis*. Furthermore, these derivatives revealed an eight-fold increase in activity against vancomycin-resistant Enterococcus (VRE) when compared to vancomycin. The potential increase in the antibacterial effectiveness of vancomycin derivatives (2) might be attributed to an additional mode of action that involves the disruption of the integrity of the bacterial cell membrane. Previous studies have shown a similar outcome in relation to the changes of the C-terminus of vancomycin by the addition of aliphatic chains and trimethyl ammonium salt [42,43].

Compound 2 exhibited the highest efficacy as an antibacterial agent against strains that are resistant to vancomycin. The compound 4 showed a significant increase in activity, surpassing 16-fold, against *S. pneumoniae*. Previous research conducted by Matile and

colleagues has shown that including cyclic disulfides or selenides into the structure of vancomycin does not result in any significant enhancement in activity or may even lead to a modest decrease in activity when tested against the Gram-positive bacterium *B. subtilis* [44]. Furthermore, the Gram-negative bacteria exhibited no susceptibility to the antibiotic after its alteration.



Scheme 1. The carboxylic position of vancomycin is subjected to functionalization by the use of a lipo-peptide linker.

One potential explanation for these data might be the occurrence of compound dimerization in the context of the *in vitro* trials. Previous studies conducted by Guffey et al. [45] and Nicolaou et al. [46] examined many instances of dimers that include disulfide, revealing their enhanced efficacy against resistant strains of *Enterococcus*. The introduction of lipoic acid substitutions (1) to vancomycin derivatives did not result in any changes to the antibacterial properties of the original vancomycin against both sensitive and resistant pathogens. However, a notable increase in activity, ranging from 4 to 8-fold, was seen specifically against *VISA* and *S. pneumoniae* for compound 1. The compound 2 demonstrated significant efficacy against *VISA*, exhibiting an 8 to 64-fold increase in activity relative to vancomycin. Additionally, it showed a modest enhancement (4-fold) against *B. subtilis*. Regrettably, the alterations implemented did not have any discernible impact on the antibacterial efficacy against *Escherichia coli*. The findings together show that the inclusion of sulfur-containing linkers significantly enhances the antibacterial characteristics of vancomycin, hence augmenting its efficacy against a wider range of bacterial species. In the current study, most of the analogs were effective against *VISA*, *B. subtilis*, *VanA*, *VanB*, and *S. pneumoniae*.

These findings highlight the importance of investigating the impact of thiol groups on the action of antibiotics, as well as the possible role of thiol-mediated absorption. Compounds 2 and 4 can be further researched to determine their full potential. Compound 2 showed effective antibacterial activity against most of the microbes tested in the current study, indicating its potential as an effective antibiotic. Further investigation is required to explore the characteristics of high activity and potential dimerization in vancomycin derivatives containing free thiol groups. However, the incorporation of disulfide and thiol moieties is anticipated to have the potential to enhance the absorption, distribution, metabolism, and excretion (ADME) characteristics of novel antibiotics. This is due to their conversion into more hydrophilic molecules during metabolization, as shown in previous research conducted by the Judice group.

Furthermore, the potential for enhancing the efficacy of vancomycin against vancomycin-resistant bacteria exists via the alteration of the glucosamine component by introducing a CBP group at the C-terminal position of the vancomycin molecule. Further modifications are needed in the current analogs to make them effective against MRSA and VRE as they were found to be more resistant to the compounds than the other bacterial species.

Table 1. MIC values of vancomycin derivatives against various bacterial species.

Derivatives	MIC ($\mu\text{g/ml}$)							
	<i>Bacillus subtilis</i>	VISA	MRSA	VRE	VanA	VanB	<i>S. pneumoniae</i>	<i>E. coli</i>
1	>2	4	2	>4	2	>2	8	<1
2	>8	>64	2	>4	>128	>128	>8	1
3	4	8	4	>2	2	2	4	1
4	>4	4	8	8	>4	>16	>16	<1
5	>8	4	>4	8	4	4	>2	<1
6	>4	>2	3	2	2	>2	4	1
7	>8	>4	2	4	1	>2	4	<1
Vancomycin	8	8	>1	>8	>128	16	0.5	1

3.1. Derivatives.

3.1.1. Derivative 1.

The product, which eluted at a retention time of 37 minutes, was collected and then subjected to lyophilization. This process yielded a white solid product weighing 3.9 mg, corresponding to a molar quantity of 0.0021 mmol (Figure 1). The overall yield of the product was determined to be 7%. The compound's purity was assessed using analytical RP-HPLC using a Gemini-NX column. The elution of the product occurred at a retention time of 15.1 minutes, and its detection was achieved at a wavelength of 280 nm.

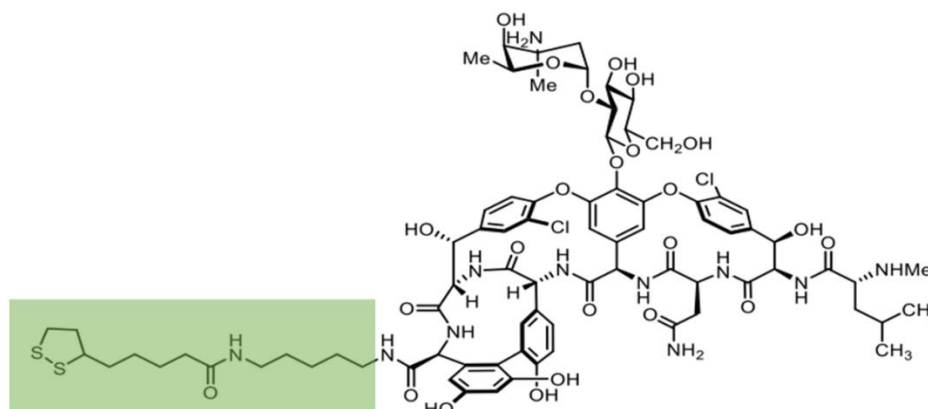


Figure 1. Chemical structure of derivative 1.

^1H NMR (Figure 2) (500 MHz, DMSO- d_6) δ 0.87, 0.91, 1.06 (d, $J = 6.5$ Hz, 3H), 1.31 – 1.24 (m, 5H), 1.37 – 1.32 (m, 2H), 1.42 – 1.37 (m, 3H), 1.57 – 1.42 (m, 6H), 1.78 – 1.59 (m, 3H), 1.89 – 1.80 (m, 2H), 2.07 – 2.02 (t, $J = 7.3$ Hz, 2H), 2.13 (dd, $J = 15.7, 5.1$ Hz, 1H), 2.29 (s, 3H), 2.42 – 2.35 (m, 1H), 3.02 – 2.98 (m, 3H), 3.17 – 3.10 (m, 5H), 3.24 – 3.18 (m, 3H), 3.46 (t, $J = 8.7$ Hz, 2H), 3.62 – 3.50 (m, 4H), 3.67 (d, $J = 10.9$ Hz, 1H), 4.20 (d, $J = 10.9$ Hz, 1H), 4.37 (d, $J = 5.7$ Hz, 1H), 4.41 (d, $J = 4.7$ Hz, 1H), 4.65 (q, $J = 6.4$ Hz, 1H), 4.89 - 4.83 (m, 1H), 5.13 (d, $J = 3.4$ Hz, 1H), 5.23 – 5.17 (m, 3H), 5.26 (d, $J = 7.7$ Hz, 1H), 5.53 – 5.49 (m, 1H), 5.72 (d, $J = 7.9$ Hz, 1H), 6.23 (d, $J = 2.2$ Hz, 1H), 6.33 (d, $J = 2.2$ Hz, 1H), 6.69 – 6.63 (m, 2H), 6.73 (dd, $J = 8.4, 1.6$ Hz, 1H), 6.89 – 6.82 (m, 1H), 7.23 – 7.15 (m, 1H), 7.27 (dd, $J = 25.3, 8.3$ Hz, 2H), 7.35 – 7.29 (s, 1H), 7.45 (ddd, $J = 23.1, 0.6$ Hz, 2H), 7.88 - 7.80 (m, 2H),

8.27 (s, 1H), 8.58 (d, $J = 2.1$ Hz, 1H), 9.00 (s, 1H). HRMS (ESI): calcd for $C_{78}H_{99}O_{24}N_{11}Cl_2S_4$ $[M+2H]^{2+}$, $m/z = 885.75567$, found 885.75529.”

Figure 3 displays the HPLC chromatogram of the derived compound.

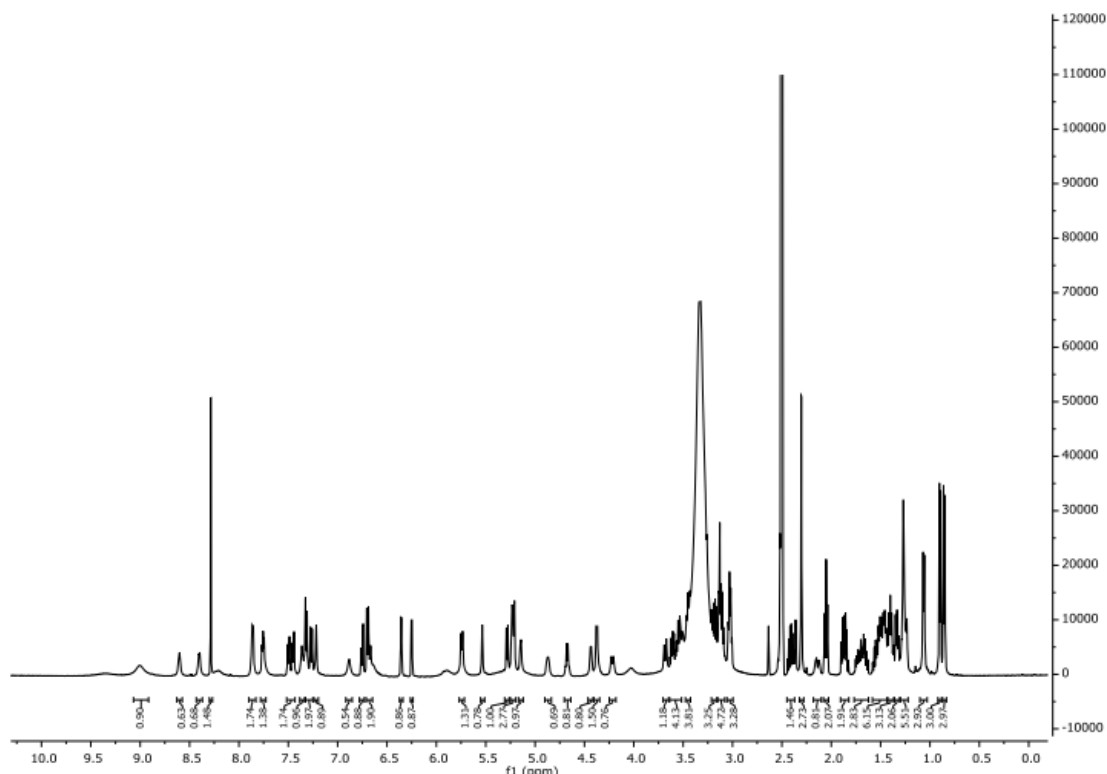


Figure 2. NMR spectra of derivative 1.

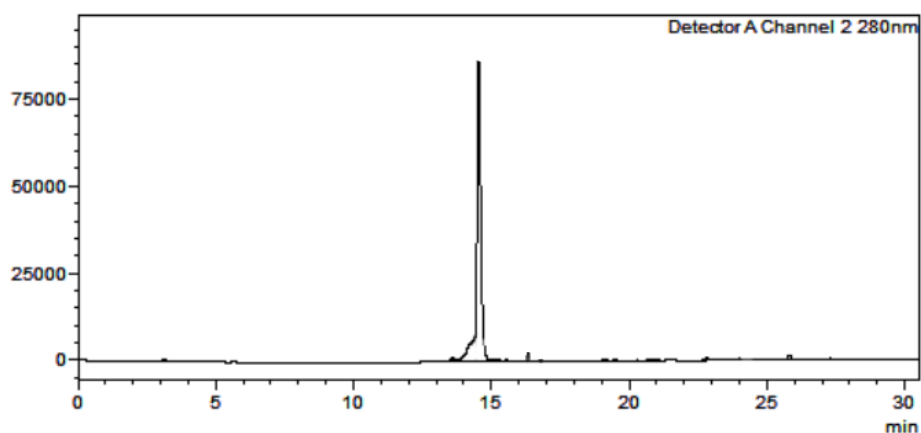


Figure 3. HPLC chromatogram of derivative 1.

3.1.2. Derivative 2.

The product, which had an elution time of 35.4 minutes, was collected and subjected to lyophilization. This process yielded a white solid product weighing 2.45 mg, corresponding to 0.027 mmol and a yield of 6.1% (Figure 4). The compound's purity was assessed using analytical RP-HPLC using a Gemini-NX column. The elution of the product occurred at a retention time of 13 minutes, and its detection was achieved at a wavelength of 280 nm.

¹H NMR (Figure 5) (500 MHz, DMSO-*d*₆) δ 0.90 - 0.85 (dd, $J = 6.6, 20.6$ Hz, 6H), 1.06 (d, $J = 6.4$ Hz, 2H), 1.24 (m, 3H), 1.28 (t, $J = 7.3$ Hz, 3H), 1.40 (m, 1H), 1.47 (m, 1H), 1.61 - 1.52 (m, 1H), 1.66 (m, 1H), 1.73 (p, $J = 6.8$, 1H), 1.84 (m, 1H), 2.14 (m, 1H), 2.30 (s, 2H), 2.52 (m, 1H), 2.54 (s, 1H), 2.76 (q, $J = 7.3$ Hz, 2H), 2.83 (m, 2H), 2.93 (m, 1H), 3.02 (t,

J = 6.8 Hz, 1H), 3.43 (m, 2H), 3.54 (m, 2H), 3.68 (d, J = 10.6 Hz, 1H), 4.04 (m, 1H), 4.22 (d, J = 11.4 Hz, 1H), 4.37 (d, J = 5.6 Hz, 1H), 4.43 (m, 1H), 5.14 (m, 1H), 5.21 (m, 2H), 5.28 (d, J = 7.8 Hz, 1H), 5.53 (s, 1H), 5.75 (d, J = 7.6 Hz, 1H), 5.88 (m, 1H), 6.24 (d, J = 2.0 Hz, 1H), 6.35 (d, J = 1.9 Hz, 1H), 6.64 (m, 1H), 6.70 (d, J = 8.5 Hz, 1H), 6.78 – 6.73 (dd, J = 1.6, 8.3 Hz, 1H), 6.90 (m, 1H), 7.21 (s, 1H), 7.30 (dd, J = 24.6, 8.3 Hz, 2H), 7.39 – 7.34 (m, 1H), 7.51 – 7.43 (m, 2H), 7.73 (m, 1H), 7.86 (d, J = 1.8 Hz, 1H), 8.13 (m, 1H), 8.33 (s, 1H), 8.42 (m, 1H), 8.62 (m, 1H), 9.04 (s, 1H), 9.34 (s, 1H). HRMS (ESI): calcd for C₇₀H₈₆Cl₂N₁₀O₂₃ [M+2H]²⁺, m/z = 784.23444, found 784.23374”.

Figure 6 displays the HPLC chromatogram of the derived compound.

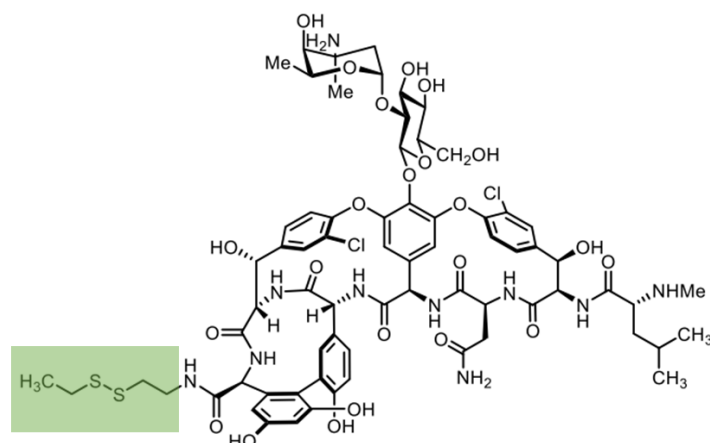


Figure 4. Chemical structure of derivative 2.

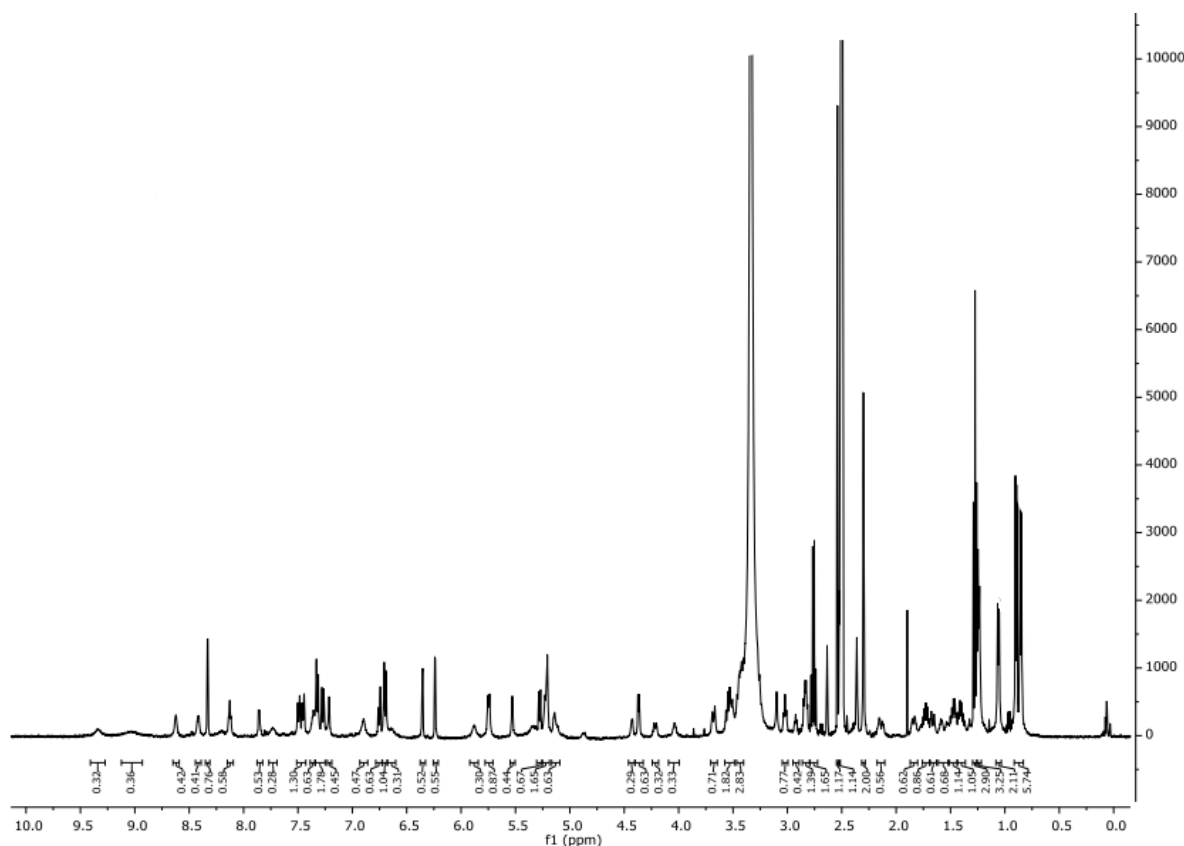


Figure 5. NMR spectra of derivative 2.

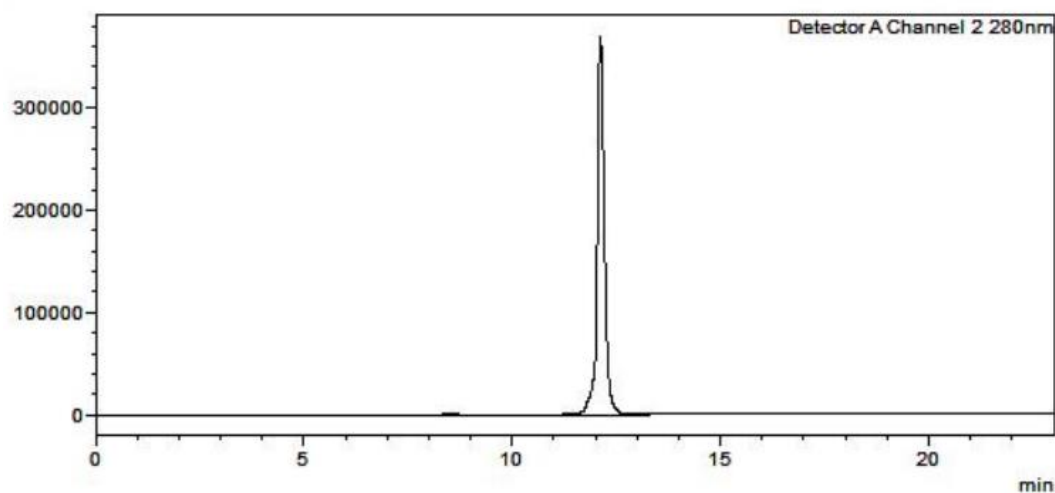


Figure 6. HPLC chromatogram of derivative 2.

3.1.3. Derivative 3.

The product, which had a retention time of 31.6-34.7 minutes, was isolated and subjected to lyophilization. This process yielded a white solid product weighing 15 mg (0.029 mmol), corresponding to a yield of 33% (Figure 7). The compound's purity was assessed using analytical RP-HPLC using a Gemini-NX column. The elution of the product occurred at a retention time of 14.2 minutes, and its detection was achieved at a wavelength of 280 nm.

¹H NMR (Figure 8) (500 MHz, MeOD) δ 0.90 (t, J = 6.8 Hz, 3H), 0.93 (d, J = 6.3 Hz, 3H), 0.96 (d, J = 6.3 Hz, 3H), 1.20 (d, J = 6.3 Hz, 3H), 1.37 – 1.25 (m, 18H), 1.45 – 1.39 (m, 3H), 1.48 (s, 3H), 1.65 – 1.56 (m, 1H), 1.70 (p, J = 7.2 Hz, 3H), 1.81 – 1.76 (m, 1H), 1.88 – 1.84 (m, 1H), 2.00 – 1.92 (m, 1H), 2.10 – 2.02 (m, 1H), 2.38 – 2.15 (m, 1H), 2.66 (s, 3H), 2.74 (t, J = 7.3 Hz, 2H), 2.90 – 2.78 (m, 2H), 3.20 – 3.11 (m, 1H), 3.69 – 3.54 (m, 3H), 3.80 – 3.74 (m, 1H), 3.94 – 3.81 (m, 2H), 5.40 – 5.30 (m, 1H), 5.46 – 5.40 (m, 1H), 5.55 – 5.48 (m, 1H), 6.18 – 5.79 (m, 1H), 6.39 (d, J = 2.2 Hz, 1H), 6.43 (d, J = 2.2 Hz, 1H), 6.91 – 6.75 (m, 1H), 7.06 (s, 1H), 7.28 – 7.16 (m, 1H), 7.68 – 7.56 (m, 2H), 8.45 (s, 2H). HRMS (ESI): calcd for C₇₉H₁₀₄Cl₂N₁₀O₂₃S₂ [M+2H]⁺, m/z = 847.30416, found 847.30358”.

Figure 9 displays the HPLC chromatogram of the derived compound.

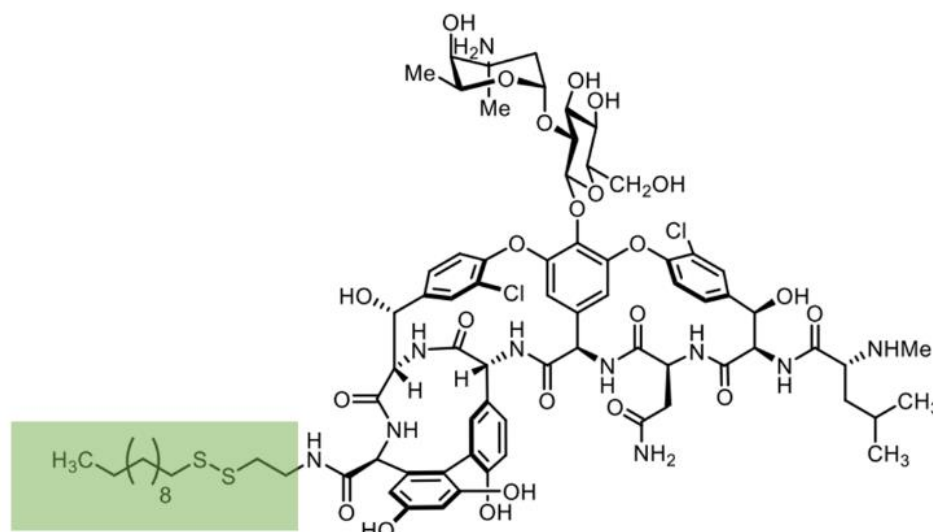


Figure 7. Chemical structure of derivative 3.

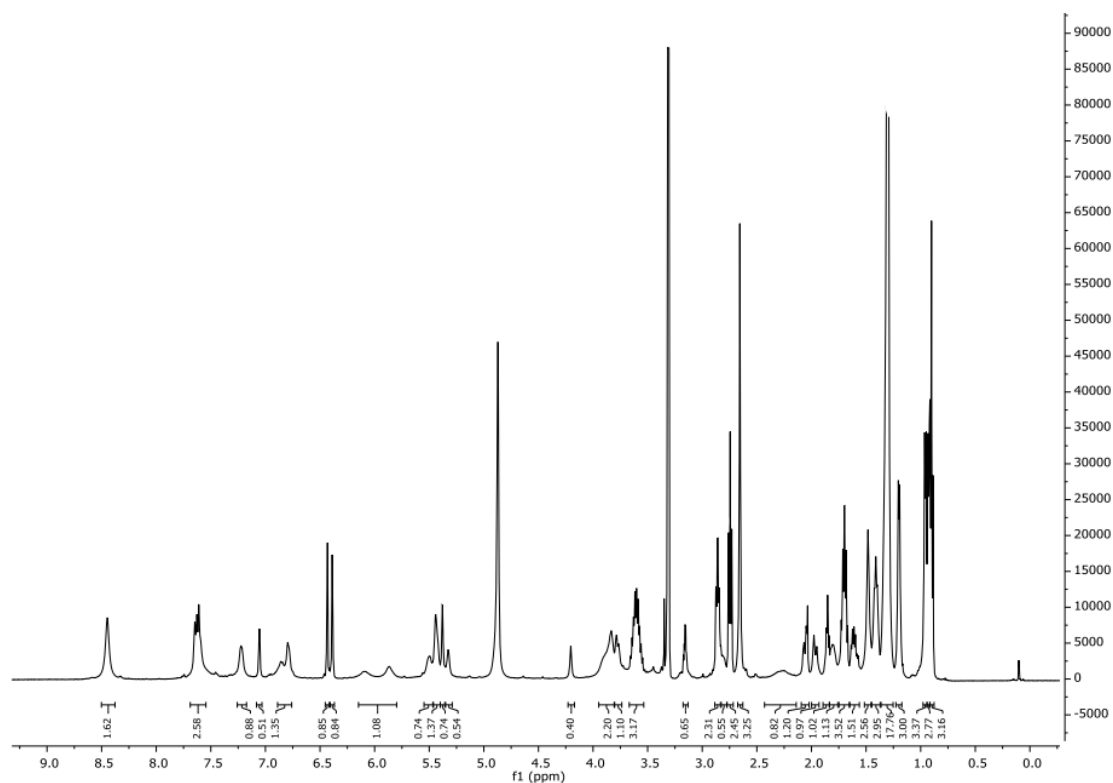


Figure 8. NMR spectra of derivative 3.

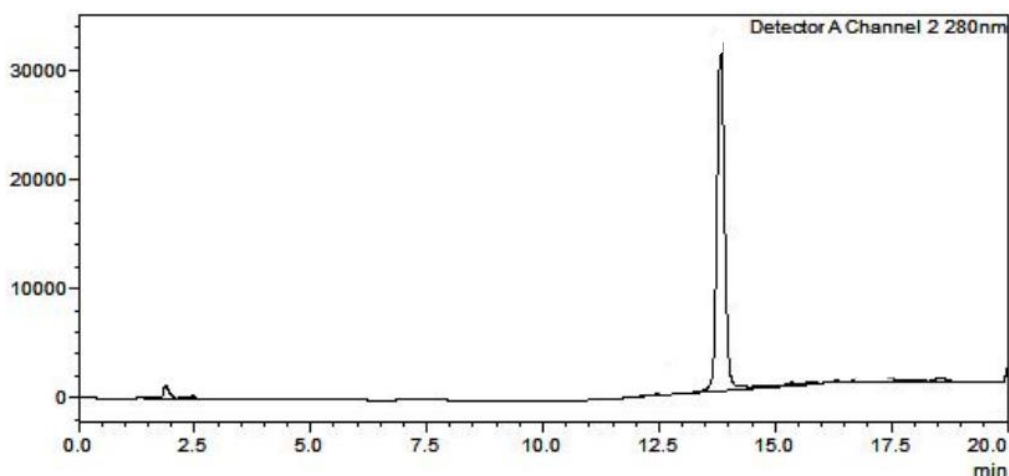


Figure 9. HPLC chromatogram of derivative 3.

3.1.4. Derivative 4.

The product, which eluted within the time range of 29.3-31.2 minutes, was isolated and subjected to lyophilization. This process yielded a white solid product weighing 4.98 mg, corresponding to 0.053 mmol, and a yield of 5.7% (Figure 10). The compound's purity was assessed using analytical RP-HPLC using a Gemini-NX column. The elution of the product occurred with a retention time of 13.1 minutes, and its detection was achieved at a wavelength of 280 nm.

¹H NMR (Figure 11) (500 MHz, DMSO-d₆) δ 0.86 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 1.06 (d, J = 6.3 Hz, 2H), 1.17 (t, J = 7.1 Hz, 1H), 1.28 - 1.22 (m, 3H), 1.44 - 1.38 (m, 3H), 1.52 - 1.46 (m, 3H), 1.69 - 1.61 (m, 2H), 1.77 - 1.69 (m, 1H), 1.87 - 1.80 (m, 1H), 2.18 - 2.12 (m, 1H), 2.30 (s, 2H), 2.71 (t, J = 7.4 Hz, 2H), 3.05 - 2.99 (m, 1H), 3.48 - 3.42 (m, 2H), 3.59 - 3.50 (m, 2H), 3.68 (d, J = 10.0 Hz, 1H), 4.23 - 3.97 (m, 5H), 4.37 (d, J = 5.6 Hz,

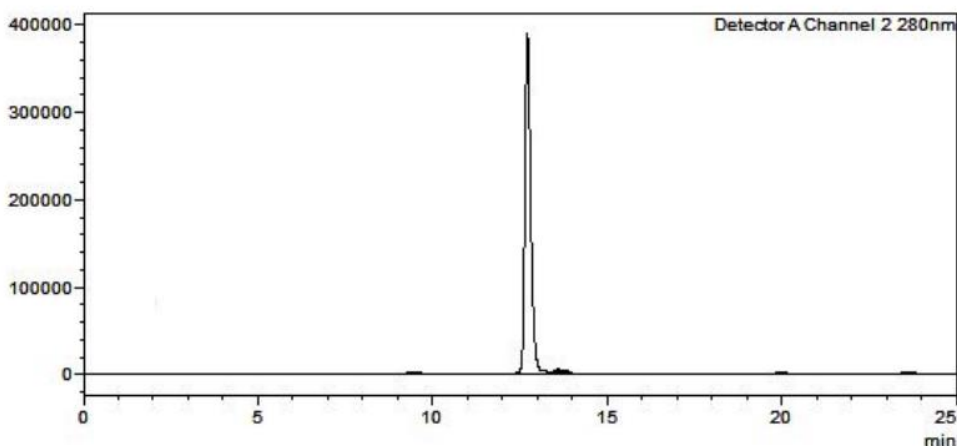


Figure 12. HPLC chromatogram of derivative 4.

3.1.5. Derivative 5.

The product, which eluted at a retention time of 19 minutes, was collected and subjected to lyophilization. This process yielded a white solid product weighing 9.8 mg, corresponding to 0.099 mmol, and a yield of 5.7% (Figure 13). The compound's purity was assessed using analytical RP-HPLC on a Synergy Hydro column. The elution of the product occurred at a retention time of 12.9 minutes, and its detection was achieved at a wavelength of 280 nm.

¹H NMR (Figure 14) (500 MHz, MeOD) δ 0.96 (d, J = 6.0 Hz, 3H), 0.99 (d, J = 6.0 Hz, 3H), 1.20 (d, J = 6.4 Hz, 3H), 1.41 – 1.36 (m, 1H), 1.54 – 1.44 (m, 4H), 1.65 – 1.59 (m, 1H), 1.86 – 1.72 (m, 5H), 1.97 – 1.92 (m, 1H), 2.06 (dd, J = 13.6, 4.4 Hz, 1H), 2.34 – 2.20 (m, 1H), 2.66 (s, 3H), 2.80 (t, J = 7.0 Hz, 2H), 2.89 (t, J = 7.0 Hz, 2H), 3.59 – 3.49 (m, 2H), 3.73 – 3.60 (m, 2H), 3.79 – 3.73 (m, 1H), 3.90 – 3.79 (m, 2H), 4.21 (s, 1H), 5.31 (d, J = 3.5 Hz, 1H), 5.39 -5.35 (m, 1H), 5.43 (d, J = 3.5 Hz, 1H), 5.47 (d, J = 6.7 Hz, 1H), 5.90 – 5.76 (m, 1H), 6.41 (d, J = 2.3 Hz, 1H), 6.44 (d, J = 2.3 Hz, 1H), 6.82 (d, J = 8.2 Hz, 1H), 6.99 – 6.89 (m, 1H), 7.06 (d, J = 2.1 Hz, 1H), 7.25 (d, J = 8.5 Hz, 1H), 7.37 – 7.28 (m, 1H), 7.59 – 7.54 (m, 1H), 7.63 (d, J = 6.6 Hz, 2H), 7.72 – 7.67 (m, 1H), 8.36 (s, 1H). HRMS (ESI): calcd for C₇₃H₉₃O₂₃N₁₁Cl₂S₂ [M+2H]²⁺, m/z = 812.76266, found 812.76277". Figure 15 displays the HPLC chromatogram of the derived compound.

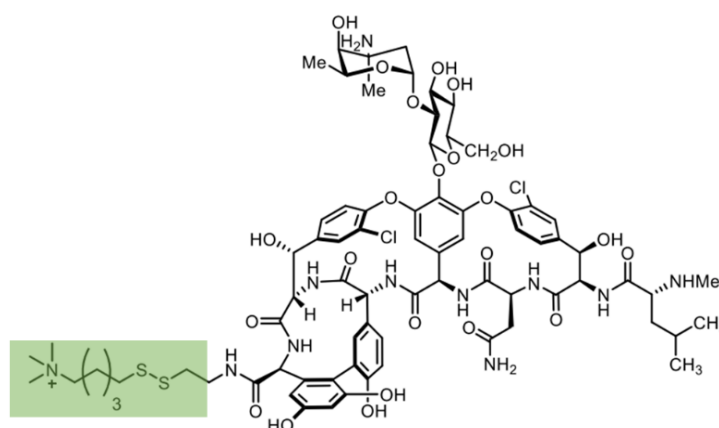


Figure 13. Chemical structure of derivative 5.

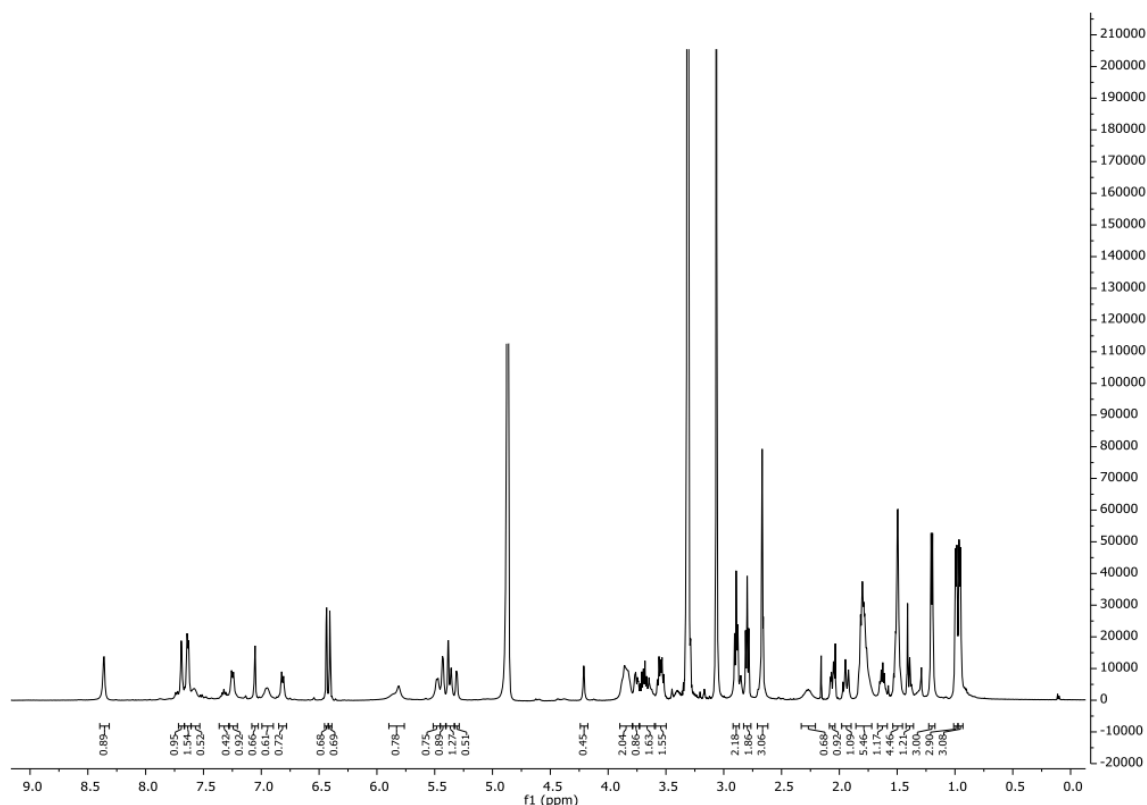


Figure 14. NMR spectra of derivative 5.

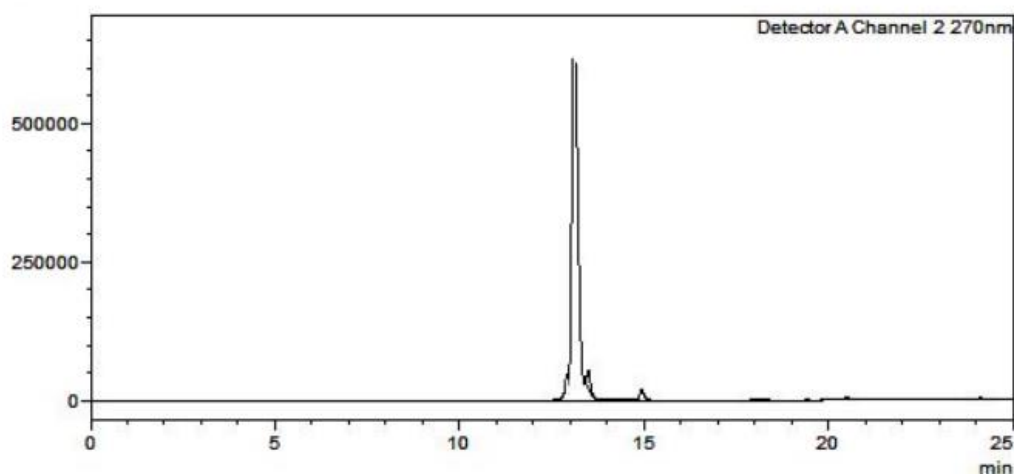


Figure 15. HPLC chromatogram of derivative 5.

3.1.6. Derivative 6.

The product, which eluted between 24.5 and 25.8 minutes, was collected and subjected to lyophilization. This process yielded a white solid product weighing 18 mg, corresponding to 0.051 mmol, and a yield of 17% (Figure 16). The compound's purity was assessed using analytical RP-HPLC using a Gemini-NX column. The elution of the product occurred at a retention time of 12 minutes, and its detection was achieved at a wavelength of 280 nanometers.

¹H NMR (Figure 17) (500 MHz, MeOD) δ 0.97 (dd, $J = 14.3, 6.3$ Hz, 6H), 1.20 (d, $J = 6.5$ Hz, 3H), 1.49 (s, 2H), 1.63 – 1.55 (m, 2H), 1.80 – 1.69 (m, 2H), 1.96 – 1.90 (m, 1H), 2.05 (dd, $J = 13.6, 4.6$ Hz, 1H), 2.33 – 2.24 (m, 1H), 2.58 (s, 3H), 2.84 (dd, $J = 1.5$ Hz, $J = 15.1$ Hz, 1H), 3.00 (t, $J = 6.8$ Hz, 2H), 3.42 – 3.36 (m, 1H), 3.60 – 3.50 (m, 2H), 3.68 – 3.60 (m,

2H), 3.75 (dd, $J = 11.6, 4.5$ Hz, 1H), 3.90 – 3.80 (m, 2H), 4.20 – 4.17 (m, 1H), 5.30 (d, $J = 3.5$ Hz, 1H), 5.38 – 5.34 (m, 1H), 5.43 (d, $J = 4.2$ Hz, 1H), 5.48 – 5.44 (m, 1H), 5.84 – 5.76 (m, 1H), 6.39 (d, $J = 2.3$ Hz, 1H), 6.43 (d, $J = 2.3$ Hz, 1H), 6.81 (d, $J = 8.5$ Hz, 1H), 6.98 – 6.91 (m, 1H), 7.05 (d, $J = 2.4$ Hz, 1H), 7.25 – 7.19 (m, 2H), 7.59 – 7.55 (m, 1H), 7.64 – 7.60 (m, 2H), 7.70 – 7.66 (m, 1H), 7.84 – 7.77 (m, 2H), 8.38 – 8.35 (m, 1H), 8.44 (s, 2H). HRMS (ESI): calcd for $C_{73}H_{85}Cl_2N_{11}O_{23}S_2$ $[M+2H]^{2+}$, $m/z = 808.73136$, found 808.73176 ". Figure 18 displays the HPLC chromatogram of the derived compound.

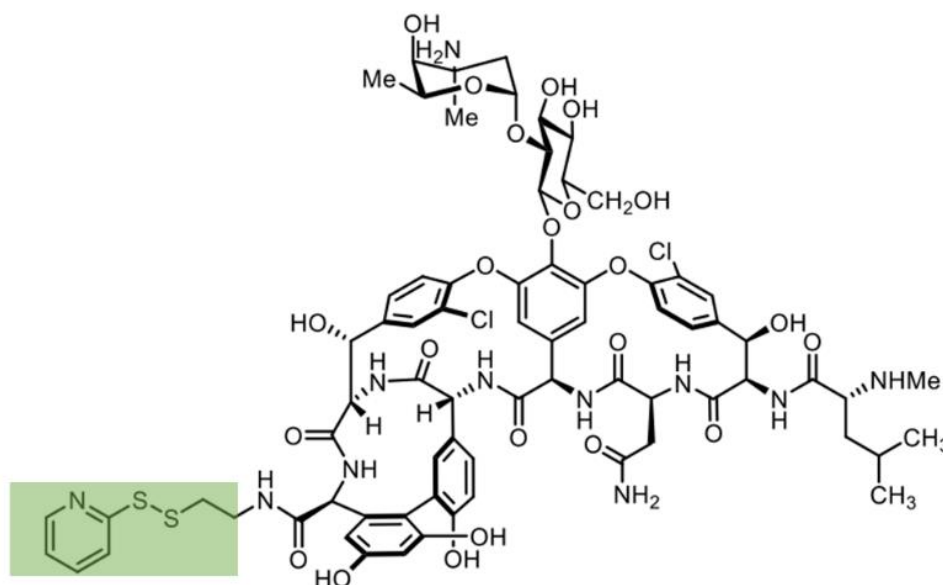


Figure 16. Chemical structure of derivative 6.

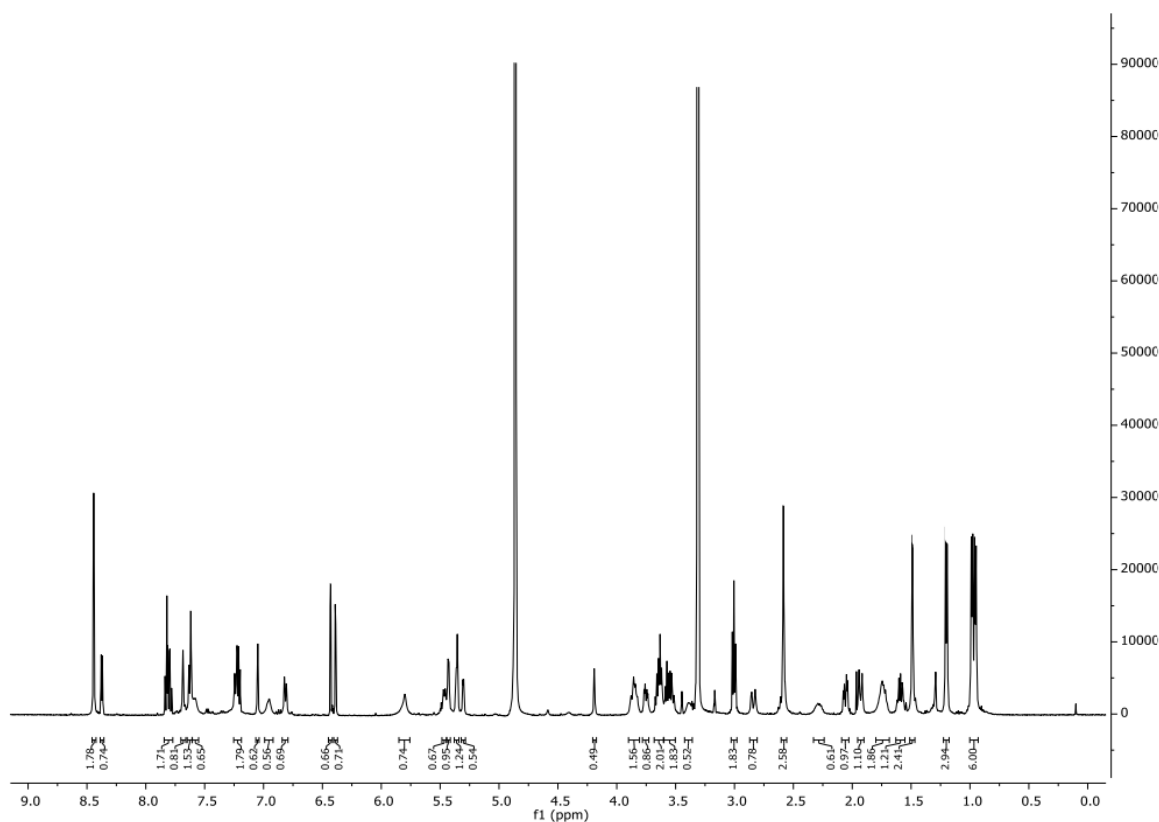


Figure 17. NMR spectra of derivative 6.

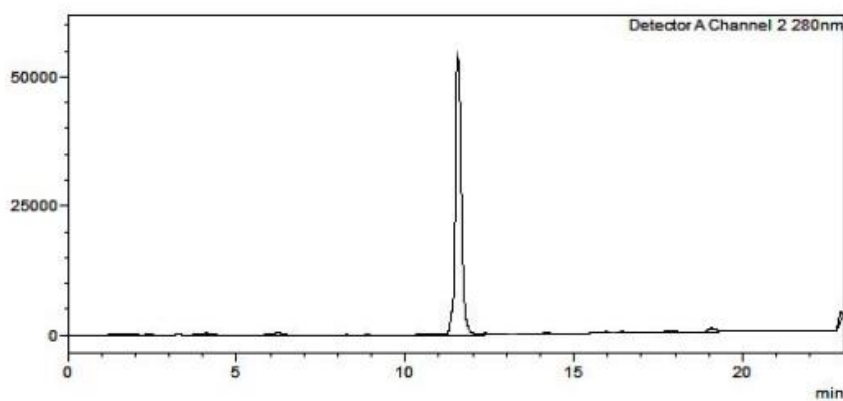


Figure 18. HPLC chromatogram of derivative 6.

3.1.7. Derivative 7.

The product, which had a retention time of 23-24.7 minutes, was isolated and subjected to lyophilization. This process yielded a white solid product weighing 38 mg, corresponding to 0.067 mmol, and a yield of 36% (Figure 19). The compound's purity was assessed using analytical RP-HPLC using a Gemini-NX column. The elution of the product occurred at a retention time of 2.7 minutes, and its detection was achieved at a wavelength of 280 nm.

¹H NMR (Figure 20) (500 MHz, DMSO-d₆) δ 0.86 (d, J = 6.6 Hz, 3H), 0.90 (d, J = 6.6 Hz, 3H), 1.06 (d, J = 6.3 Hz, 3H), 1.25 (s, 3H), 1.44 - 1.36 (m, 1H), 1.51 - 1.45 (m, 1H), 1.67 (d, J = 12.8 Hz, 1H), 1.76 - 1.69 (m, 1H), 1.88 - 1.81 (m, 1H), 2.14 (dd, J = 5.6, 16.1 Hz, 1H), 2.30 (s, 3H), 2.80 - 2.74 (m, 1H), 2.90 - 2.81 (m, 4H), 3.03 (t, J = 7.0 Hz, 1H), 3.11 (s, 1H), 3.56 - 3.43 (m, 3H), 3.68 (d, J = 10.0 Hz, 1H), 4.23 - 4.17 (m, 1H), 4.37 - 4.32 (m, 1H), 4.38 (d, J = 5.8 Hz, 1H), 4.44 (d, J = 4.8 Hz, 1H), 4.67 (q, J = 6.4 Hz, 1H), 4.90 - 4.83 (br, 1H), 5.14 (d, J = 3.7 Hz, 1H), 5.24 - 5.21 (m, 3H), 5.29 (d, J = 7.8 Hz, 1H), 5.54 (s, 1H), 5.74 (d, J = 7.8 Hz, 1H), 6.25 (d, J = 2.1 Hz, 1H), 6.36 (d, J = 2.1 Hz, 1H), 6.68 - 6.57 (m, 1H), 6.70 (d, J = 8.4 Hz, 1H), 6.76 (dd, J = 8.4, 1.8 Hz, 1H), 6.90 - 6.86 (m, 1H), 7.24 - 7.21 (br, 1H), 7.27 (d, J = 8.4 Hz, 1H), 7.32 (d, J = 8.3 Hz, 1H), 7.38 - 7.34 (br, 1H), 7.45 (d, J = 8.5, 2H), 7.50 (d, J = 8.5, 2H), 7.79 - 7.70 (m, 1H), 7.85 (s, 1H), 8.17 - 8.12 (m, 1H), 8.29 (s, 3H), 8.44 (d, J = 6.3, 1H), 8.62 - 8.59 (br, 1H). HRMS (ESI): calcd for C₇₀H₈₈Cl₂N₁₁O₂₃S₂ [M+2H]²⁺, m/z = 528.16188, found 528.16217". Figure 21 displays the HPLC chromatogram of the derived compound.

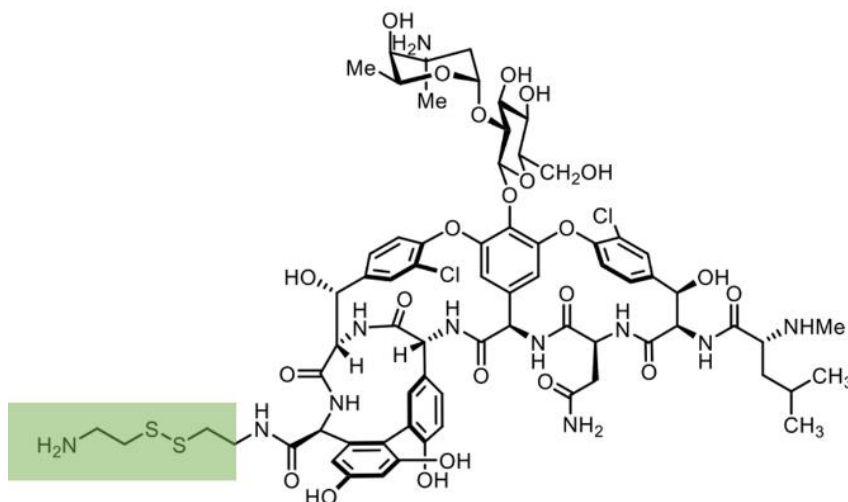


Figure 19. Chemical structure of derivative 7.

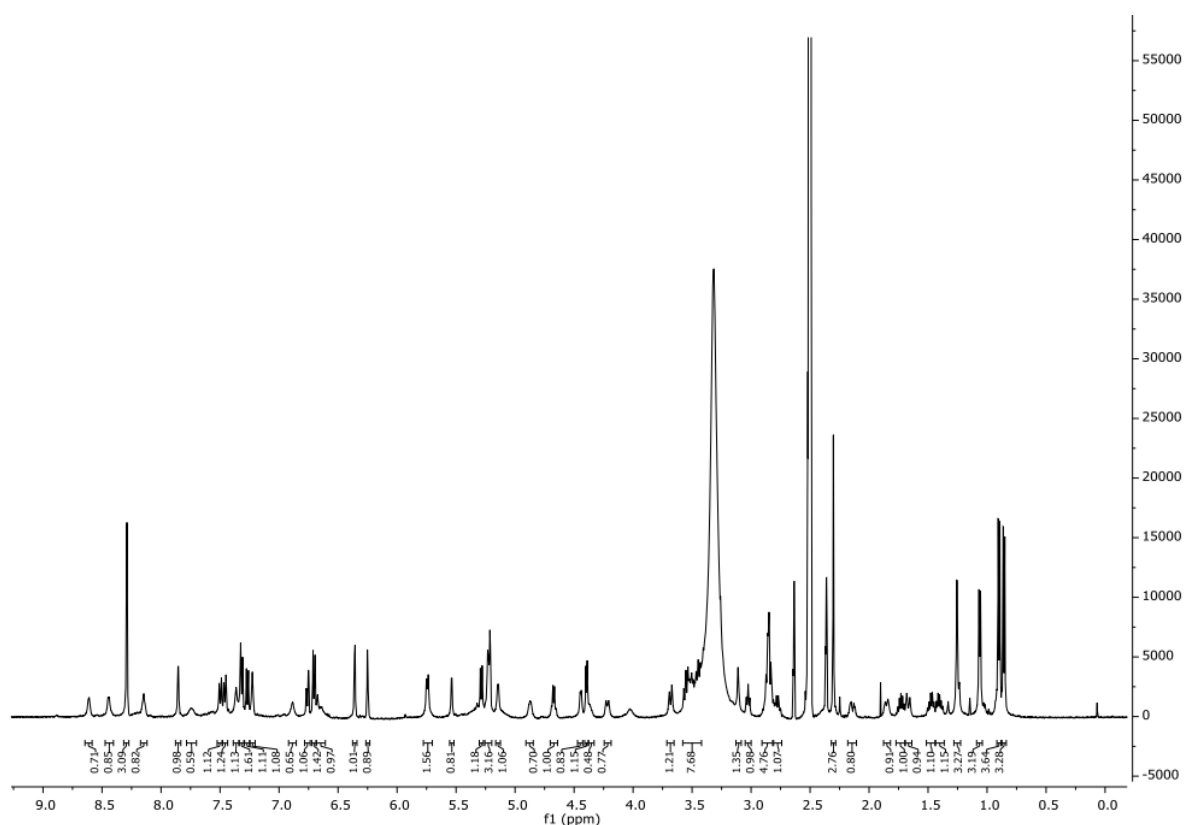


Figure 20. NMR spectra of derivative 7.

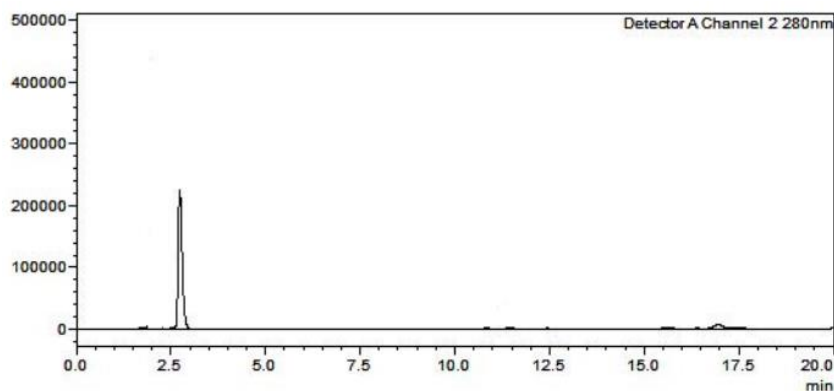


Figure 21. HPLC chromatogram of derivative 7.

4. Conclusions

With this study, we proposed that the antibacterial efficacy of vancomycin may be enhanced by structural optimization, namely by including sulfur-containing linkers. In the current study, vancomycin analogs 2 and 4 were found to be the most effective antibiotic. This approach facilitates the development of novel semisynthetic derivatives of vancomycin that may be used in the management of bacterial infections caused by vancomycin-resistant strains, including Gram-negative bacteria. Additionally, it enables the suppression of VanB-resistant enterococcus. In general, this study offers potential avenues for further exploration of disulfide and thiol-containing molecules that have been traditionally undervalued in the field of medicinal chemistry.

Author Contributions

Conceptualization, P.P.; writing—original draft preparation, S.K.S.; validation, S.K.S.; data curation, S.K.S.; writing—review and editing, S.K.S.

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Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflict of Interest

The authors have declared that no conflict of interest exists.

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