Article

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Synthesis and Identification of Some New Mono- and Bis-Tetrazole Derivatives with an Evaluation of the Free Radical Scavenging Activity of a Selected Compound

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Abstract. The research involved the preparation of mono- and bis-tetrazole ring derivatives from the 4,4-thio-dianiline compound as a substrate. These derivatives were prepared using two methods. The first method was a multicomponent reaction, where 4,4-thiodianiline reacted with sodium azide and triethyl orthoformate in glacial acetic acid as a solvent with reflux. The second method involved converting 4,4- 4,4-thiodianiline to an organic azide derivative by reacting diazanium salt with inorganic azide, followed by reacting the organic azide with phenyl isothiocyanate. In addition, a chloroacetamide derivative containing a tetrazole ring was prepared using an SN_2 reaction. The characterization of these compounds was conducted by measuring their melting points and using FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopic techniques. Compound C3, which contains a tetrazole ring group with a free aromatic primary amine group, was selected for investigation as an antioxidant compared to Ascorbic acid.

***Keywords*:** Antioxidant, Tetrazole, Azide, Sulfide.

1. Introduction

A multicomponent reaction (MCR), sometimes designated as a "multicomponent assembly process (MCAP)", is a chemical reaction in which three or more compounds

react to form a single product[1]. MCRs have become popular in organic and medicinal chemistry, as they address the diversity and complexity of organic synthesis[2].

Heterocyclic compounds are compounds in which the ring contains at least one different atom of an element other than carbon. These compounds often contain carbon atoms along with one or more atoms of other elements. The most common elements are oxygen, nitrogen, and sulfur; mercury, phosphorus, arsenic, lead, and other elements are present to a lesser extent.[3]

Tetrazole is a heterocyclic compound with four nitrogen atoms and one carbon atom in a five-membered ring[4]. It is an aromatic ring that completely obeys Hückel's law and has six delocalized electrons[5]. Tetrazoles are important nitrogen-rich heterocyclic organic compounds, exhibiting a wide range of applications in various fields such as organic synthesis, materials science, and drug development[6, 7] . Many prepared tetrazoles have anti-hypertensive[8], anti-diabetic properties[9], and anti-inflammatory properties[10].

2. Materials and Methods

2.1 Chemistry

All chemicals utilized were of the utmost quality and procured from Merck and Fluka. The microwave oven and melting points were determined employing the Electro-Thermal 9300 equipment from Melting Point Engineering Ltd., UK.

Thin Layer Chromatography (TLC) was conducted on silica gel plates, with spot visualization achieved through iodine vapors. FT-IR spectra were acquired utilizing a Shimadzu FTIR "Fourier Transform Infrared spectrometer (Model 8400)" with potassium bromide (KBr pellets), and the data are shown in "cm^{-1}." 1H-NMR and 13C NMR spectra, measured in ppm, were obtained in *DMSO-d6* as the solvent using an "Agilent Varian 300 MHz spectrometer" at Tehran University, Iran.

2.2 Preparation Method of Compound

2.2.1 Synthesis of Compound C "(bis(4-(1H-tetrazol-1-yl)phenyl)sulfane)"[11]

0.2 grams (0.00093 mol) of 4,4-thiodianiline was dissolved with 0.12 grams (0.0018 mol) of sodium azide and 0.3 grams (0.002 mol) of triethyl orthoformate in glacial acetic acid in a reaction flask with continuous stirring until dissolved. The mixture was then heated under reflux for nine hours. The reaction was monitored through TLC using the solvent system hexane:ethanol (3:2). After the reaction was complete, ice-cold distilled water was added, then the mixture was filtered and recrystallized with hot absolute ethanol. The percentage of the product was calculated.

2.2.2. Synthesis of Compound C1 "(bis(4-azidophenyl)sulfane)"[12]

One gram (0.0046 mol) of 4,4-thiodianiline was dissolved in 5 ml of HCl in an ice bath to reach a temperature of 0–5 °C, and then 0.63 grams (0.0092 mol) of $NaNO_2$ dissolved in ice-cold distilled water was added to it in another beaker. The beaker was left for half an hour with continuous stirring, then 0.6 grams (0.0092 mol) of NaN_3 dissolved in ice-cold distilled water was added, maintaining the temperature at 0–5 °C in batches until the reaction was complete. The mixture was neutralized using a dilute NaOH solution. A precipitate was observed at the equivalence point, i.e., pH 7. It was then left for 20 minutes, after which the precipitate was filtered and washed with distilled water several times. It was then recrystallized with hot absolute ethanol, and the percentage of the product was calculated.

2.2.3 Synthesis of Compound C2 "(4,4'-(thiobis(4,1-phenylene))bis(1-phenyl-1,4-dihydro-5H-tetrazole-5-thione)"[13]

0.05 grams (0.000186 mol) of C1 was dissolved together with 0.05 grams (0.000372 mol) of phenyl azothiocyanate in DMF (20 ml) with continuous stirring in a reaction flask until completely dissolved. The mixture was then heated under reflux for 10 hours at 110°C. The reaction was monitored by TLC using a solvent system of hexane:ethanol (4:1). It was recrystallized using hot absolute ethanol, and the percentage yield was calculated.

2.2.4 Synthesis of C3 "(4-((4-(1H-tetrazol-1-yl)phenyl)thio)aniline)"[11][11]

0.3 grams (0.0014 mol) of 4,4-thiodianiline was dissolved together with 0.02 grams (0.0014 mol) of triethyl orthoformate and 0.091 grams (0.0014 mol) of sodium azide in glacial acetic acid in a reaction flask with continuous stirring until dissolved. The mixture was then heated under reflux for eight hours, and the reaction was monitored by TLC using a hexane: ethanol (3:2) solvent system. After the reaction was complete, ice-cold distilled water was added, then the mixture was filtered and recrystallized with hot absolute ethanol.

2.2.5 Synthesis of C4 "(4-((4-(1H-tetrazol-1-yl)phenyl)thio)-N-(3-chloroprop-1-en-2-yl)aniline)"[14]

0.008 grams (0.00003 mol) of C3 was dissolved with 0.003 grams (0.00003 mol) of triethylamine and 0.00339 grams (0.00003 mol) of chloroacetyl chloride in 15 ml of DMF in a reaction flask with continuous stirring of the reaction mixture for nine hours.

The reaction was observed by TLC using ethanol: hexane (2:1) solvent system. It was then recrystallized with hot absolute ethanol, and the percentage of the product was calculated.

2.3 Antioxidant Agent

The antioxidant activity of C3 was evaluated to evaluate the antioxidant property. "[2,2-Diphenyl-1-picrylhydrazyl (DPPH)]" was used, which was prepared by dissolving 8 milligrams of DPPH in 100 mL of methanol and leaving it in the dark for 24 hours at a concentration of 80 µM. Then, 100 µL of DPPH was mixed with 100 µL of the prepared C3 compound in a 96-well microplate and incubated for 30 min at ambient temperature. After incubation, the absorbance was measured for different concentrations of 1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg using an ELISA reader at a wavelength of 517 nm, using 100% methanol. The same steps were performed to compare ascorbic acid with standard substances.

3. Results and Discussion

3.1 Chemistry

The tetrazole derivative C was prepared by reacting "4,4-thiodianiline with sodium azide and triethyl orthoformate" in the presence of glacial acetic acid as the solvent (Equation 1).

Compound C was identified by FT-IR by the disappearance of the first two aromatic amine bands (3207-3408 cm^{-1}) (Figure 1) and the appearance of the (C=N) band of the tetrazole ring at 1662 cm^{-1}, the (C-H) tetrazole ring at 3182 cm^{-1}, the (C-H) aromatic at 3051 cm^{-1}, the (C=C) aromatic at 1591 cm^{-1}, the (N=N) tetrazole ring at 1521 cm^{-1}, and the (C-S) at 831 cm^{-1}, as shown in Figure 2. Compound C was identified using ^{1}H NMR, which revealed characteristic signals at the following ppm: (m, 8H, aromatic ring) at 7.0–7.6, (s, 1H, H-C=N tetrazole ring) at 8.3, and DMSO (solvent) (2.5) (Figure 3). The ^{13}C NMR spectrum of (C) showed characteristic signals at the following ppm values: C (for aromatic ring) 118.6–131.4, C(C=N) for tetrazole ring at 157.7, and solvent (DMSO) at 40 (Figure 4).

Organic azide derivative C was prepared by reacting 4,4-thiodianiline with sodium nitrite and sodium azide, adding 5 ml of HCl. FT-IR identified compound C1 through the disappearance of the two primary aromatic amine bands at 3207 cm^{-1}, the appearance of the two azide bands at 2139–2098 cm^{-1}, and the aromatic (C=C) band at 1643 cm^{-1}, as shown in Figure 5. Compound C1 was identified by ^{1}H-NMR, which revealed characteristic signals at the following ppm values: (m, 8H, aromatic ring) at 7.7–7.3, and solvent (DMSO) at 2.5, as shown in Figure 6. The ^{13}C NMR spectrum of

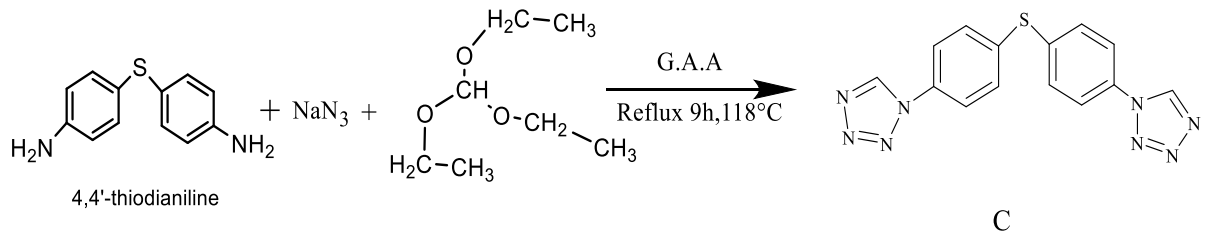
(C1) showed characteristic signals at the following ppm values: C (aromatic ring) 116.6–139, and solvent (DMSO) at 40, as shown in Figure 7.

Compound C2 was prepared by reacting the bis-tetrazole derivative, the organic azide derivative C1, with phenyl azothiocyanate using DMF as a solvent. FT-IR identified compound C2 through the disappearance of the azide band at 2139–2098 cm^{-1}, and also the disappearance of the isothiocyanate band at 2240 cm^{-1} and the appearance of the (N=N) tetrazole ring band at 1639 cm^{-1}, the (C=S) at 1527[13] cm^{-1}, the (C-H) aromatic band at 3062 cm^{-1}, and the (C=C) aromatic band at 1527 cm^{-1}, as shown in Figure 8. Compound C2 was identified using ^{1}H-NMR, which revealed characteristic signals at the following ppm values: (m, 8H, aromatic rings) at 7.9–7.5, and solvent (DMSO) at 2.5 ppm (Figure 9). The ^{13}C NMR spectrum of C2 showed characteristic signals at the following ppm values: C (aromatic ring) at 116.6–131.4, C (C=S) at 176.2, and solvent (DMSO) at 40 (Figure 10).

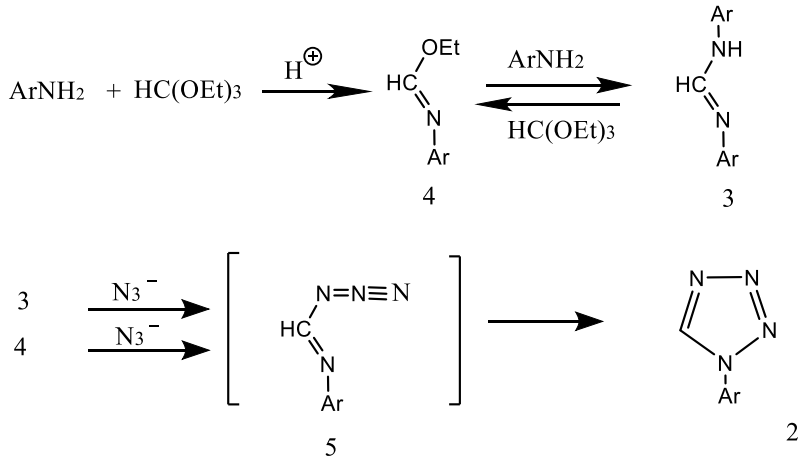
The C3 mona-tetrazole derivative was prepared by reacting 4,4-thiodianiline with sodium azide and triethyl orthoformate in the presence of glacial acetic acid (Scheme 2). Compound C3 was identified by FT-IR through the disappearance of the first aromatic amine band at 3207 cm^{-1} with the remaining of the second amine band at 3446 cm^{-1}, and the appearance of the (C-H) band of the tetrazole ring and the aromatic overlap with the amine group at 3446 cm^{-1}, (C=N) of the tetrazole ring at 1653 cm^{-1}, (C=C) aromatic at 1631 cm^{-1}, (N=N) at 1519-1539 cm^{-1}, and (C-S) at 835 cm^{-1} (Figure 11). Compound C3 was identified using ^{1}H-NMR. which revealed characteristic signals at the following ppm values: (m, 8H, aromatic rings) at 7.9-7.5, (s, H, NH_2) at 5.3, (s, 1H, H-C=N) tetrazole ring at 8.9, and solvent (DMSO) at 2.5 (Figure 12). The ^{13}C NMR spectrum of C3 showed characteristic signals at the following ppm values: C (aromatic ring) at 106.1-127, C(C=N) of the tetrazole ring at 150, and solvent (DMSO) at 40, as displayed in Figure 13.

Chloroacetamide derivative C4 was prepared by reacting compound C3 with triethylamine and chloroacetyl chloride using DMF as a solvent under continuous stirring for 9 hours. Triethylamine acts as a basic catalytic agent by abstracting a proton from the amine group, thereby increasing the nucleophilicity of the nitrogen atom. At the same time, chlorine serves as a good leaving group, enhancing the carbonyl carbon's electrophilicity and facilitating its reaction with nucleophiles as the nucleophilic attack proceeds via an SN_2 mechanism, as seen in Scheme 4. Compound C4 was identified by the disappearance of the second amine group and the appearance of the carbonyl amide group at 1730 cm^{-1} [12]. The reason for the higher carbonyl (C=O) stretching frequency (1730 cm^{-1}) is the presence of a chlorine atom (Cl) near the amide group. Chlorine acts as an electron-withdrawing group through an inductive effect, which reduces the resonance between the nitrogen and the carbonyl group. This increases the double-bond character of the C=O bond, making it stronger and causing it to absorb at a higher frequency in the FT-IR spectrum: NH at 3435 cm^{-1}, aromatic (C-H) at 3014 cm^{-1},

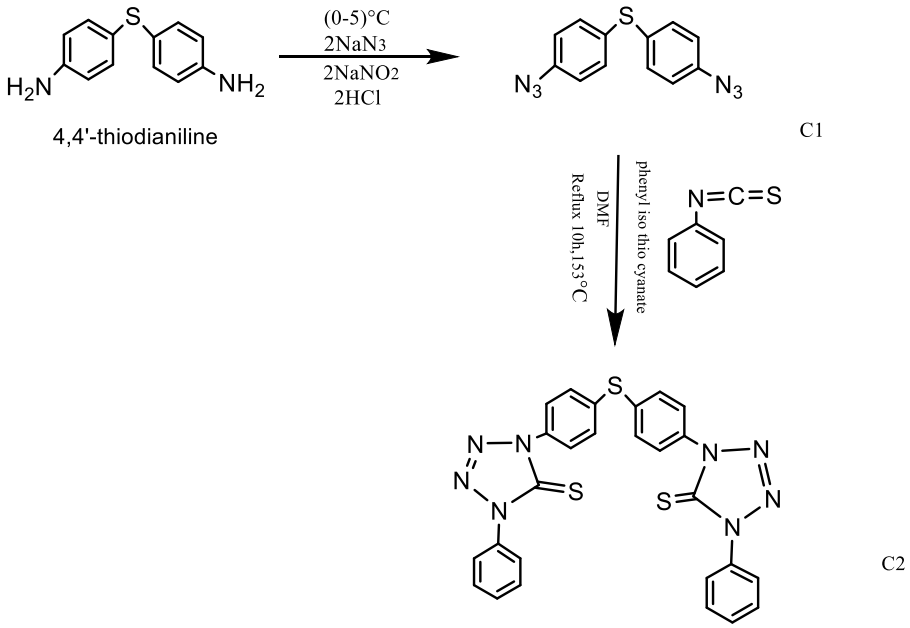
aliphatic (CH_2) at 2850-2779 cm^{-1}, (N=N) tetrazole ring at 1463 cm^{-1}, aromatic (C=C) at 1622 cm^{-1}, (C=N) tetrazole ring at 1651 cm^{-1}, and (C-Cl) at 671 cm^{-1} (see Figure 14). The compound C4 was identified using ^{1}H-NMR, which revealed characteristic signals at the following ppm values: (s, 2H, CH_2 -Cl) at 4.1, (m, H, aromatic ring) at 7.0-7.9, (s, H, H-C=N) tetrazole ring at 9.7, (s, H, NH) at 10.6, and solvent (DMSO) at 2.5, as displayed in Figure 15. The ^{13}C NMR spectrum of C4 showed characteristic signals at the following ppm values: C (aromatic rings) at 103-130, C(C=N) tetrazole ring at 150.4, C(C=O) carbonyl amide at 169.4,) C(CH_2 -Cl) at 48.4, and solvent (DMSO) at 40, as displayed in Figure 16.



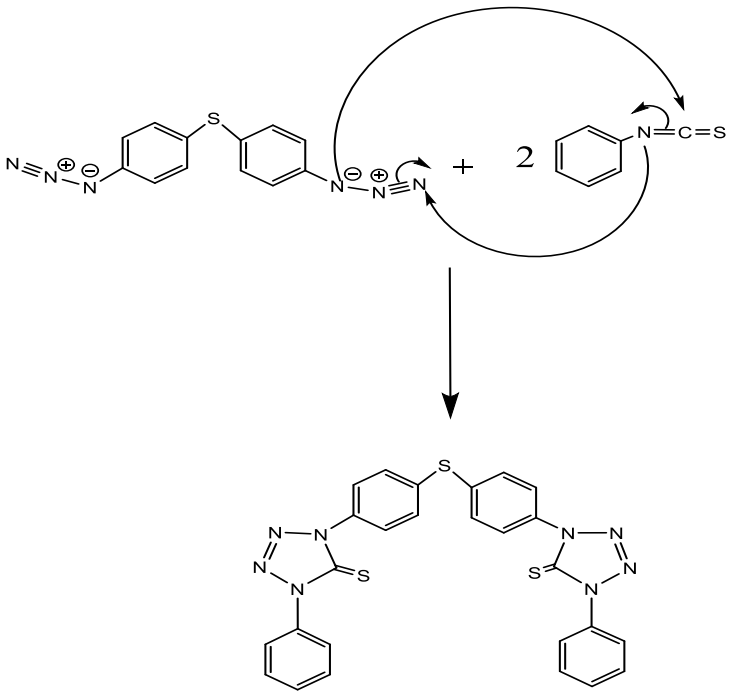
Equation 1. Synthesis of ring derivatives of tetrazoles.



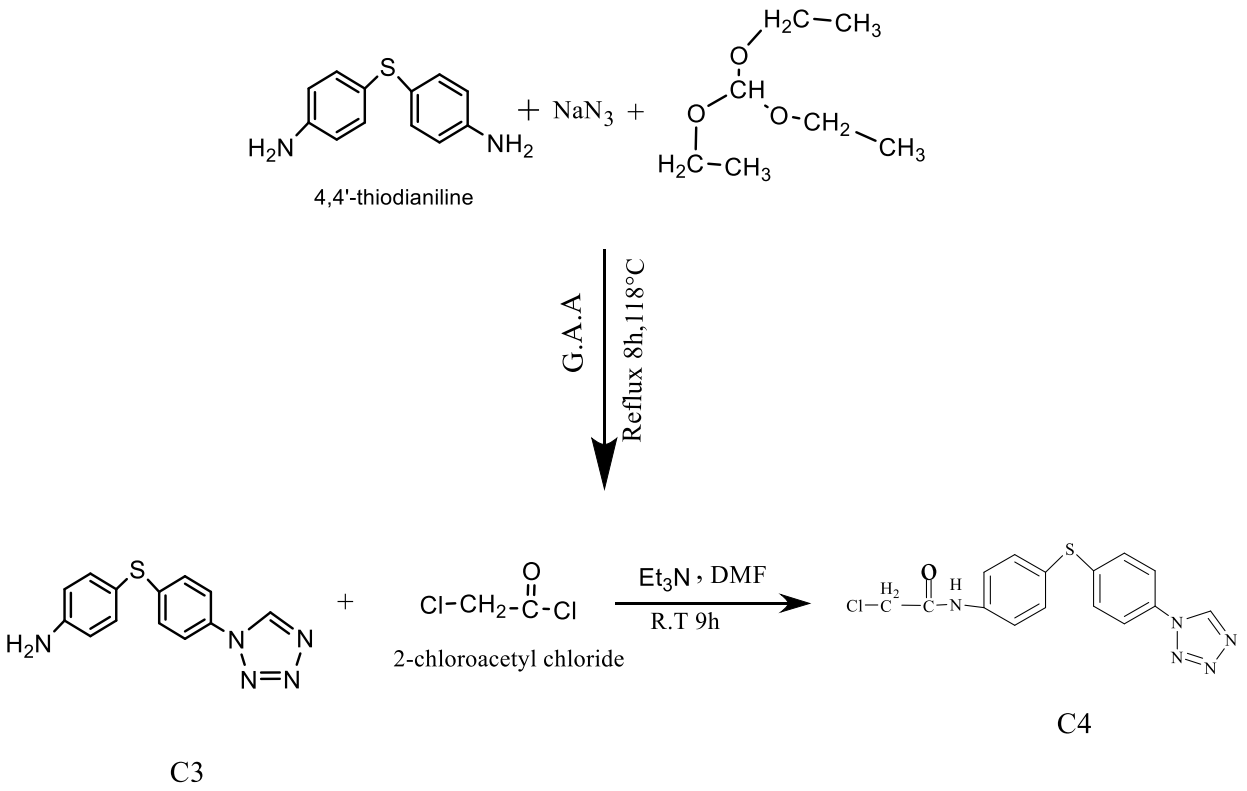
Scheme 1. Mechanism for the preparation of tetrazole ring derivative by Multicomponent reaction[11]



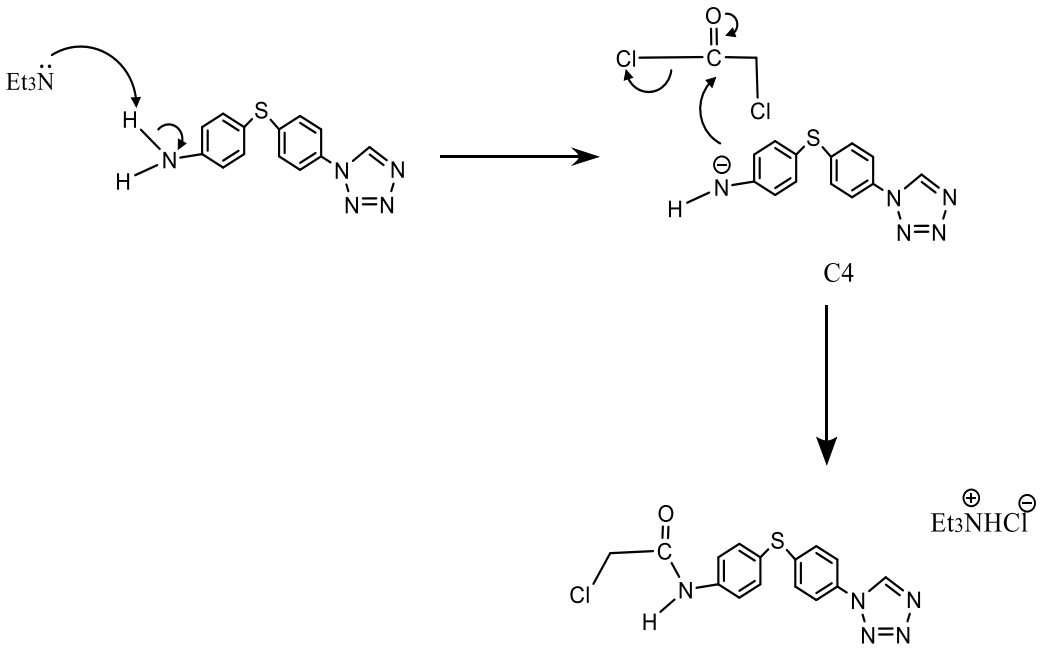
Scheme 2. Synthesis of organic azide derivatives and tetrazole ring derivatives.



Equation 2. Mechanics Synthesis of tetrazole ring derivatives.



Scheme 3: Synthesis of ring derivatives of tetrazole.



Scheme 4. Mechanism for the preparation of the tetrazole ring and the chloroacid amide derivative[15]

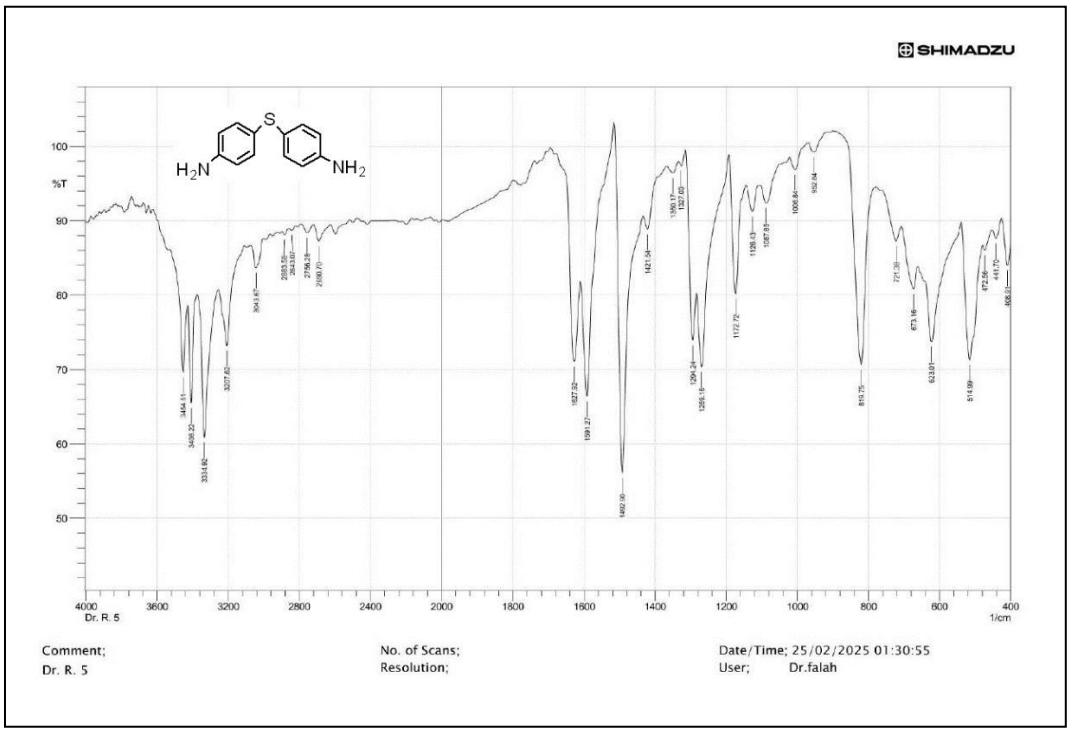


Figure 1. FT-IR Spectrum for 4,4-thiodianiline

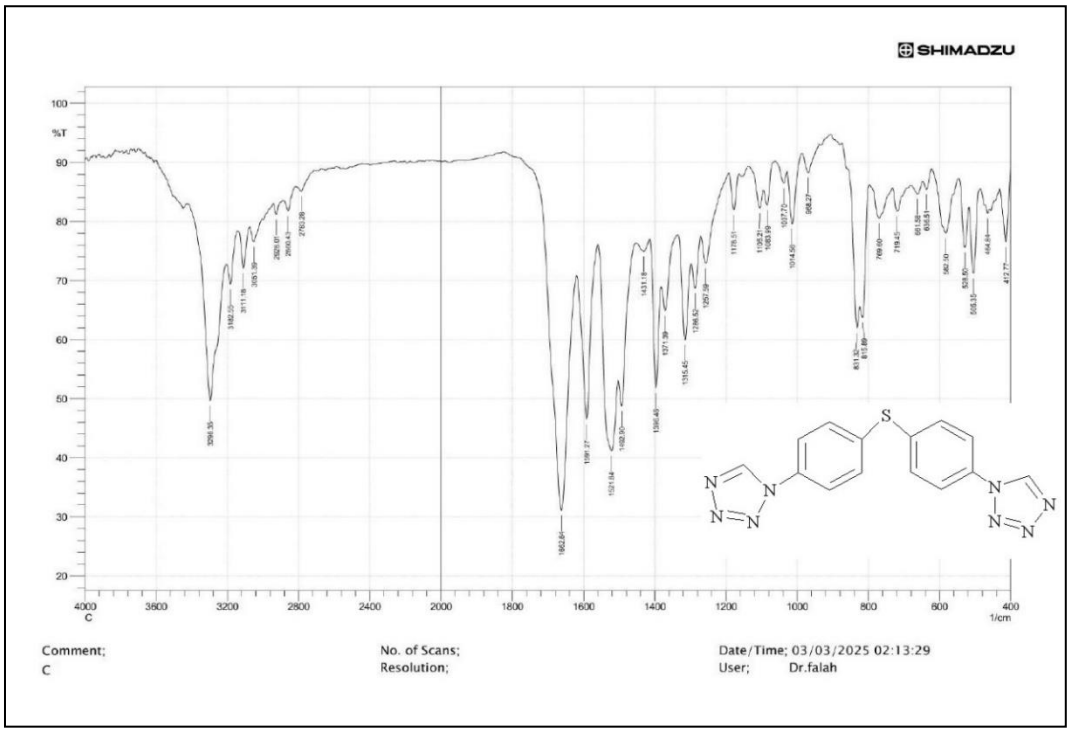


Figure 2. FTIR Spectral for C

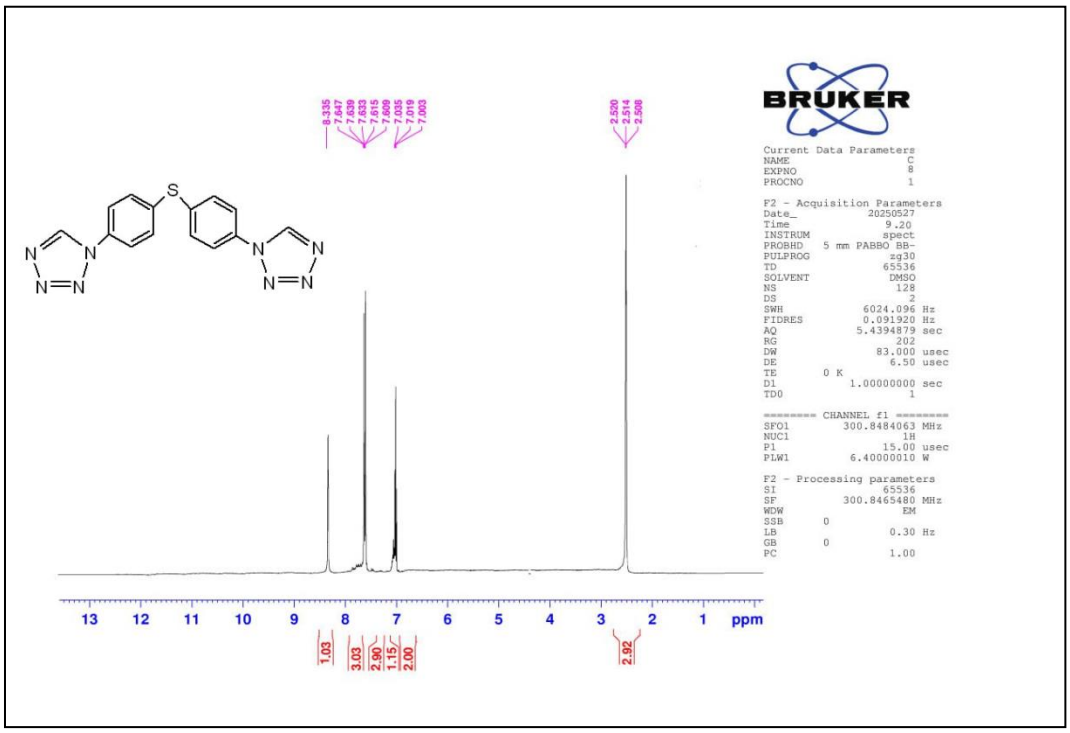


Figure 3. ^{1}H-NMR Spectral for C

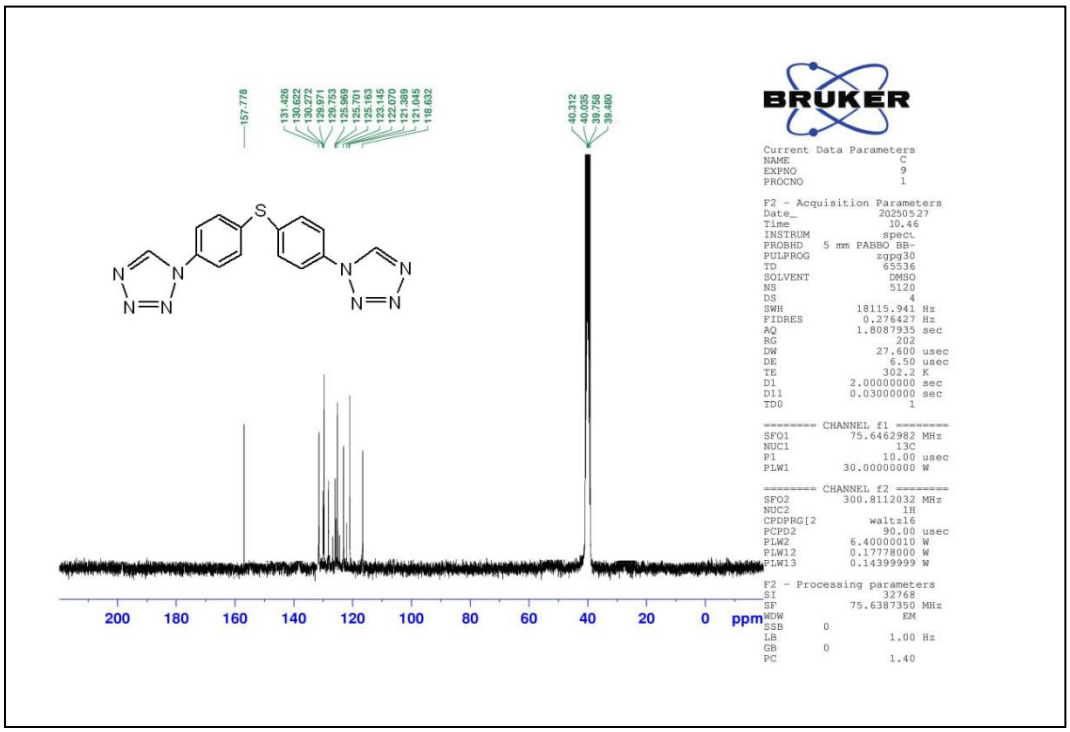


Figure 4. ^{13}C NMR Spectra of C

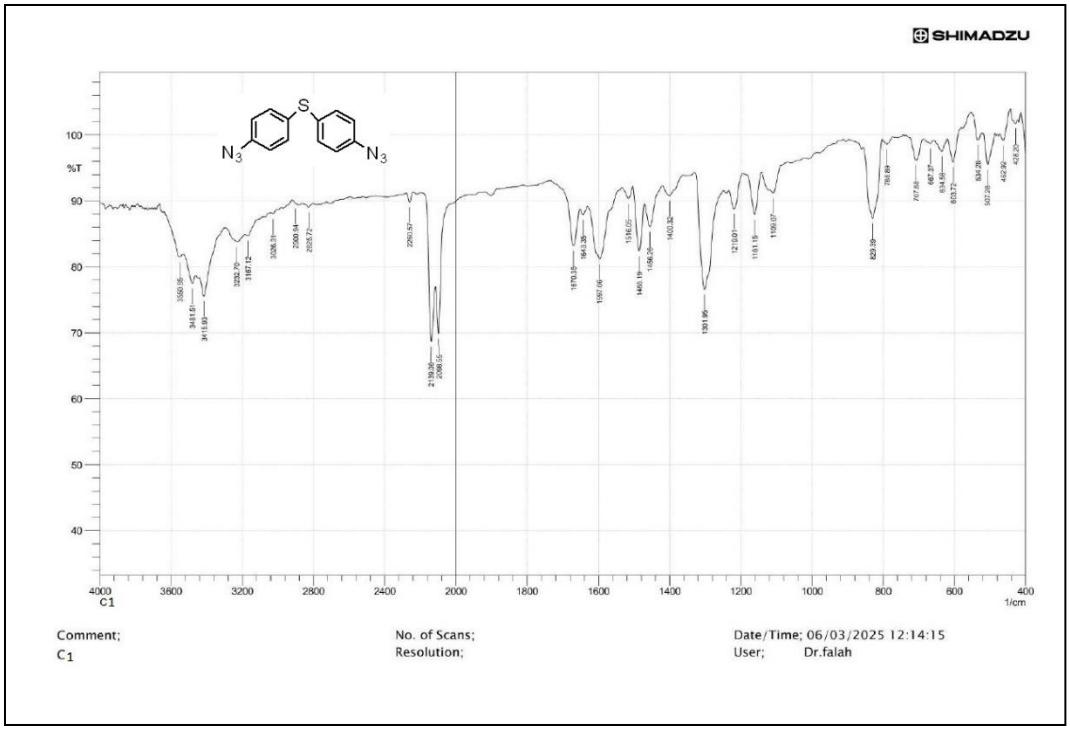


Figure 5. FT-IR Spectra of C1

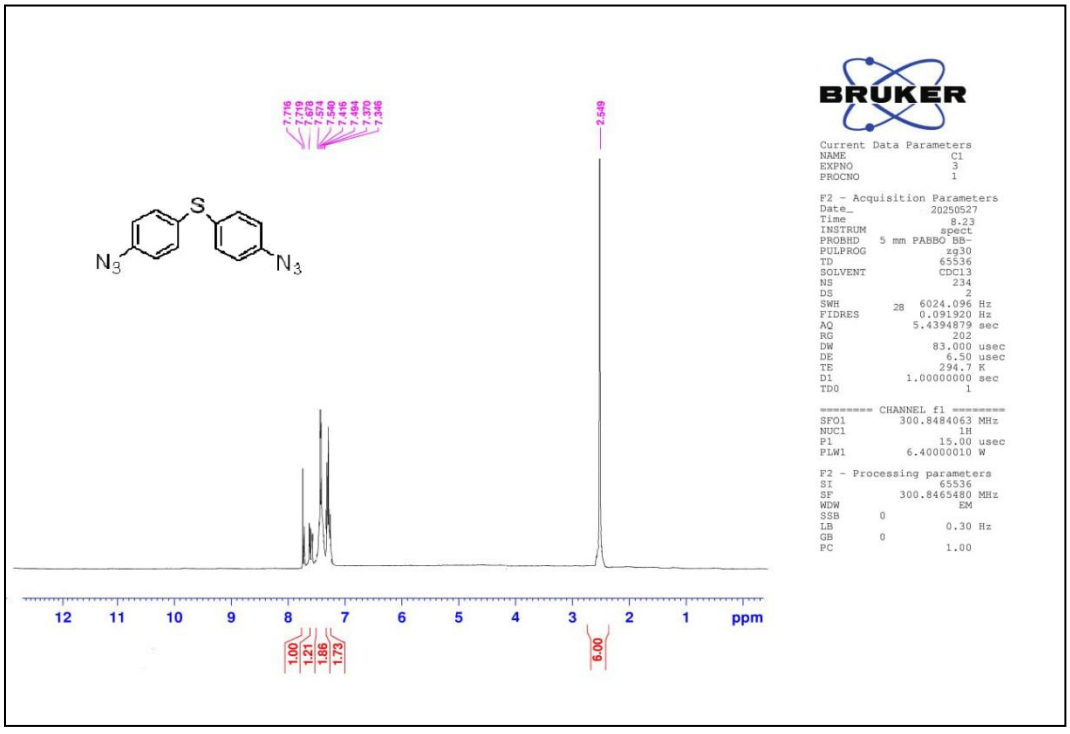


FIGURE 6. ^{1}H NMR Spectra of C1

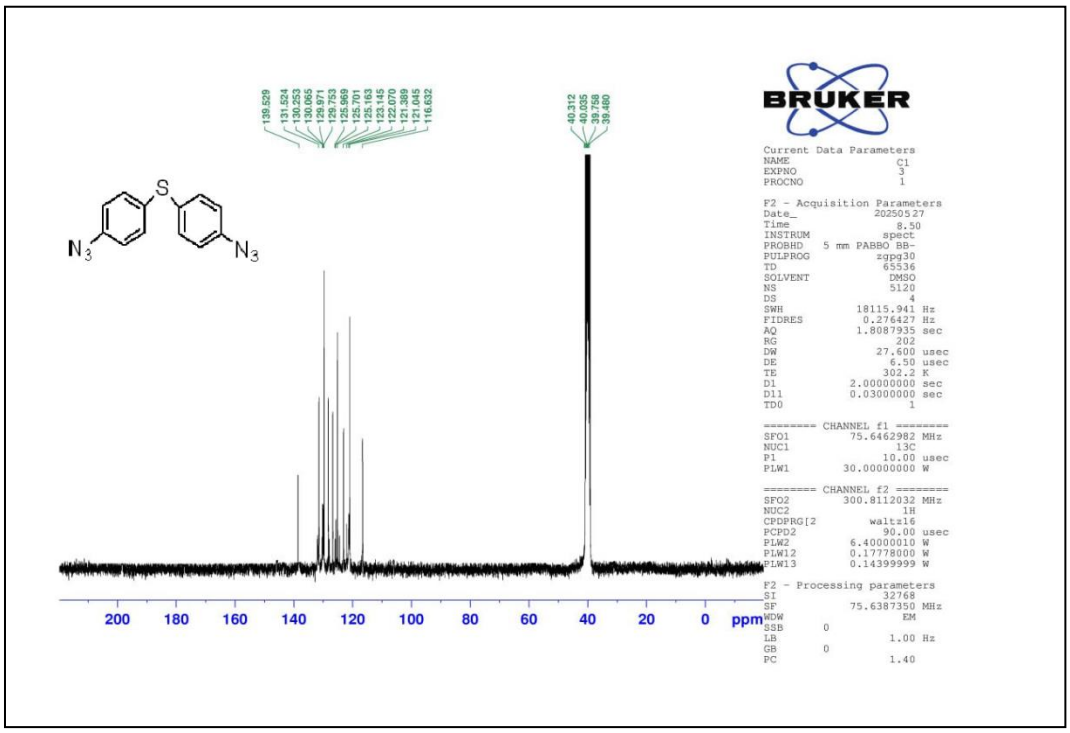


FIGURE 7. ^{13}C NMR Spectra of C1

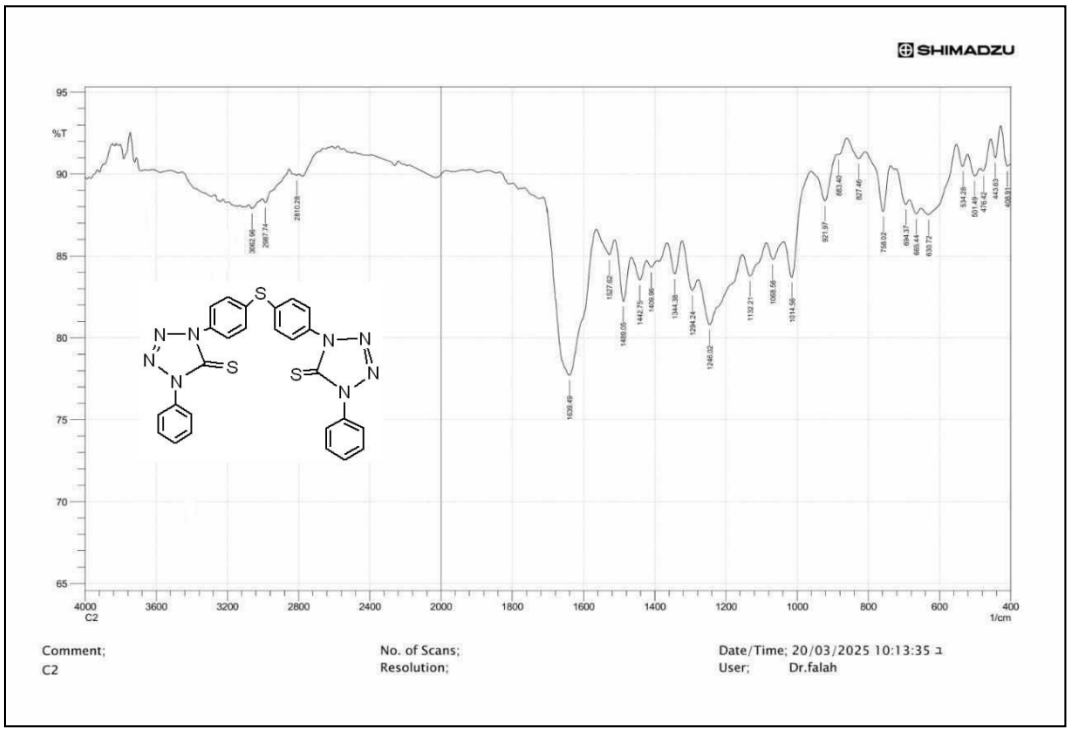


FIGURE 8. FT-IR Spectra of C2

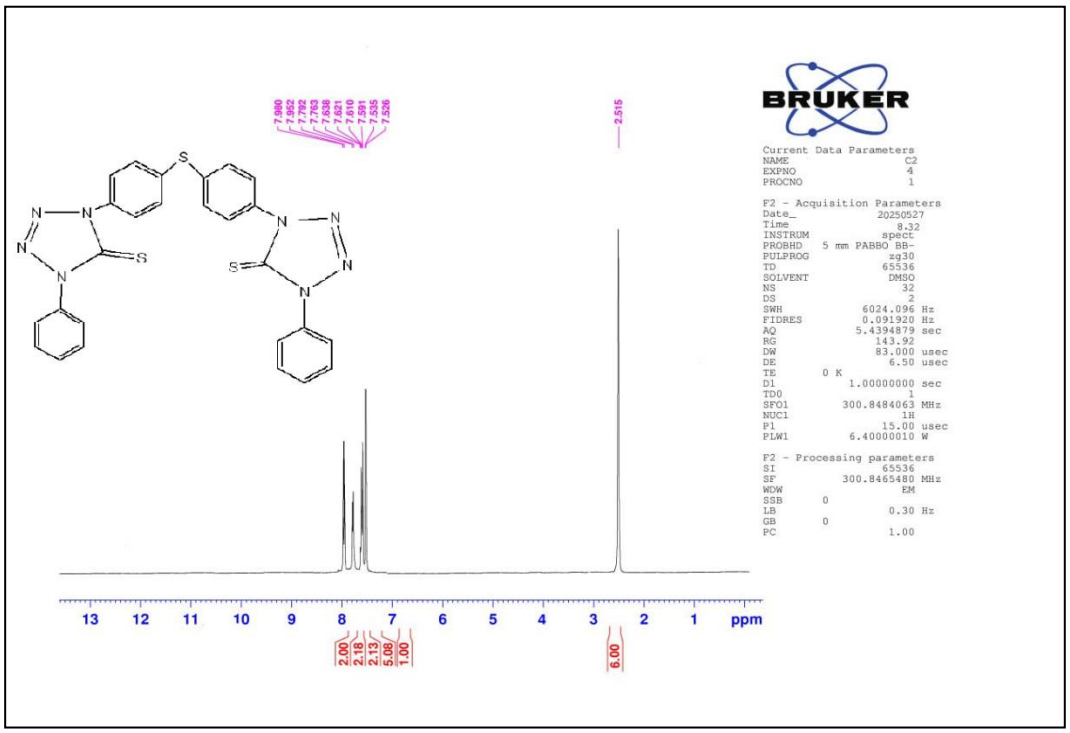


FIGURE 9. ^{1}H-NMR Spectra of C2

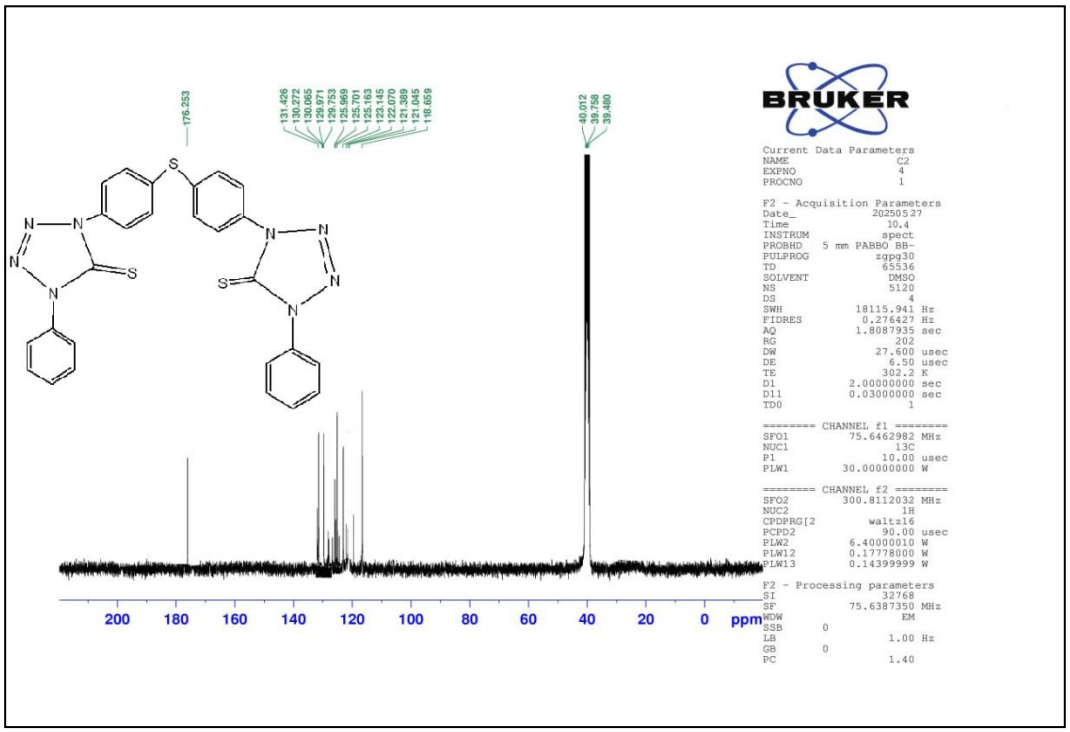


FIGURE 10. ^{13}C NMR Spectra of C2

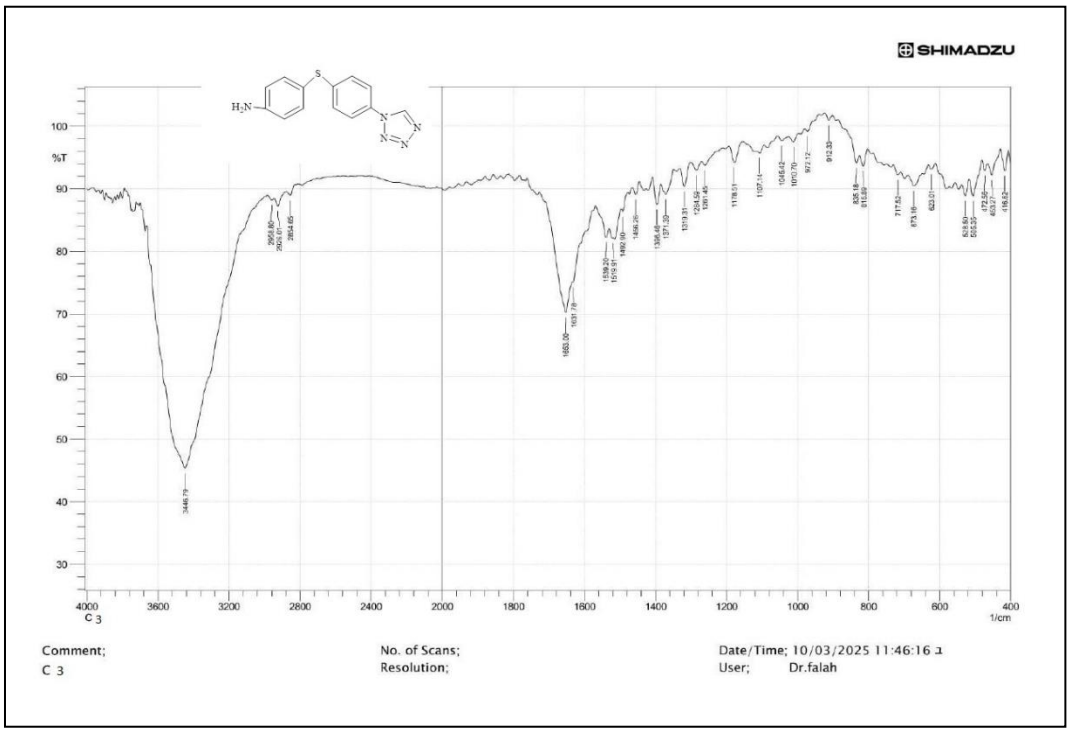


FIGURE 11. FT-IR Spectra of C3

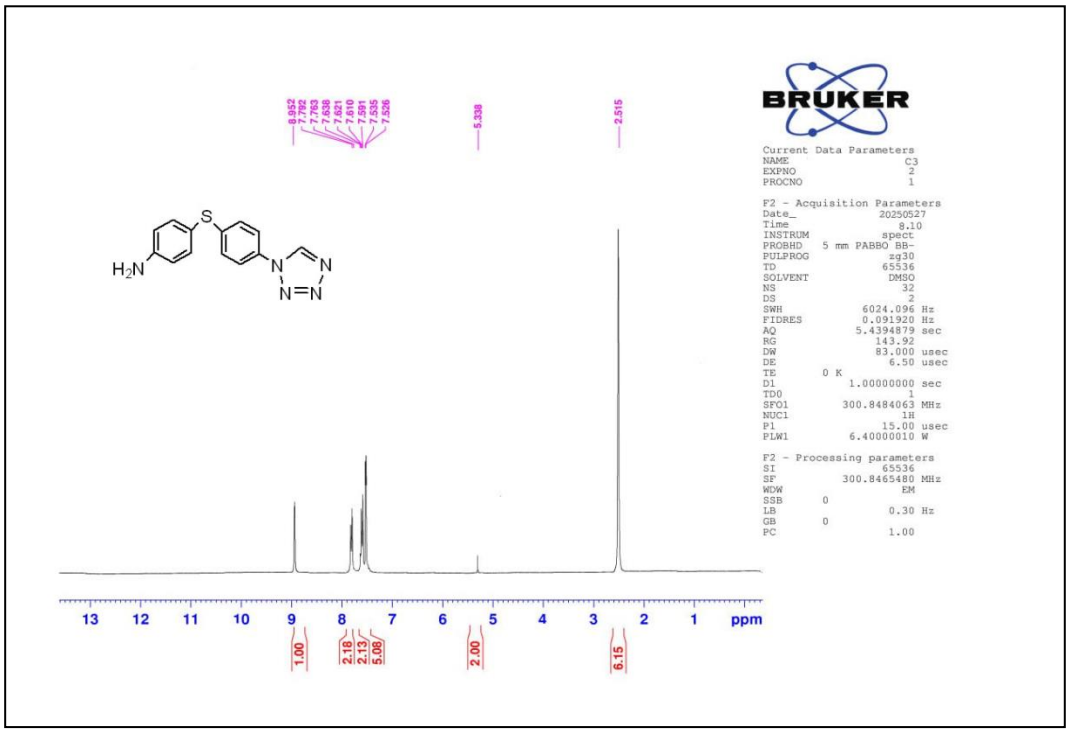


FIGURE 12. ^{1}H-NMR Spectra of C3

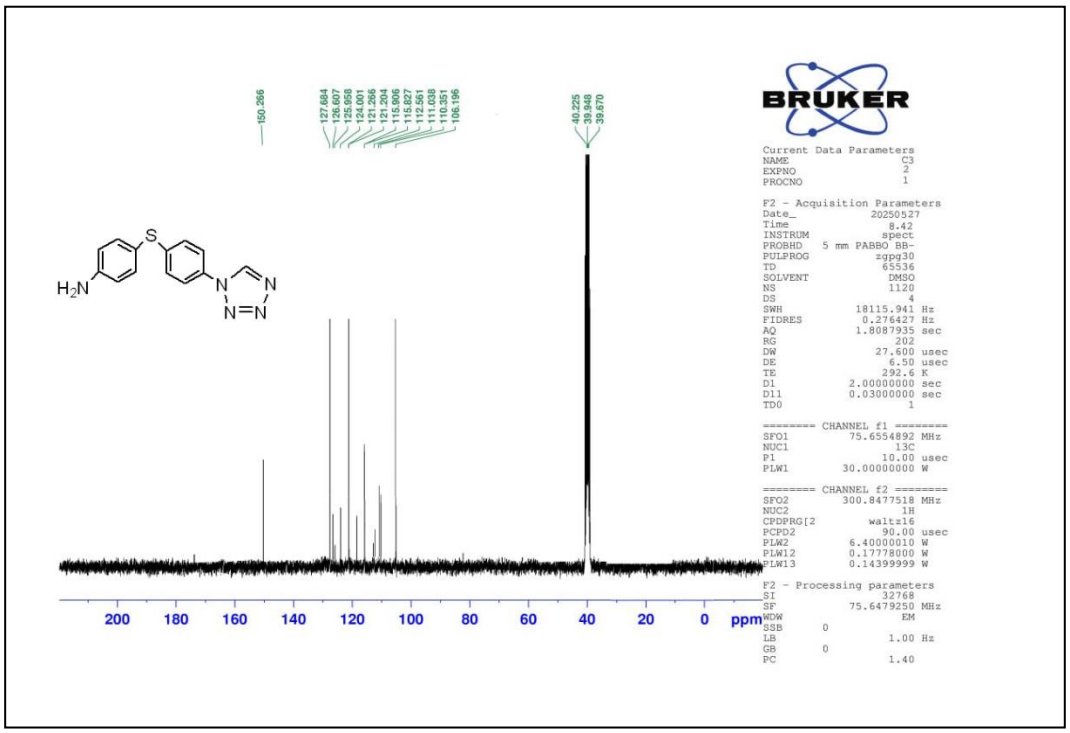


FIGURE 13. ^{13}C NMR Spectra of C3

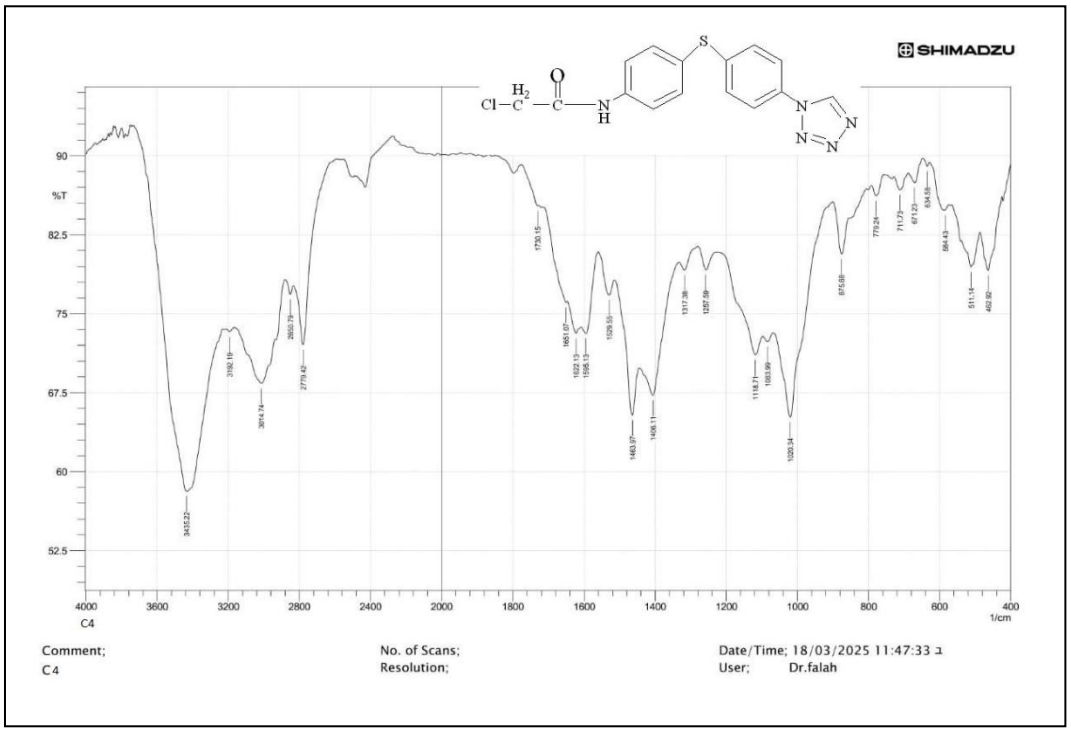


FIGURE 14. FT-IR Spectra of C4

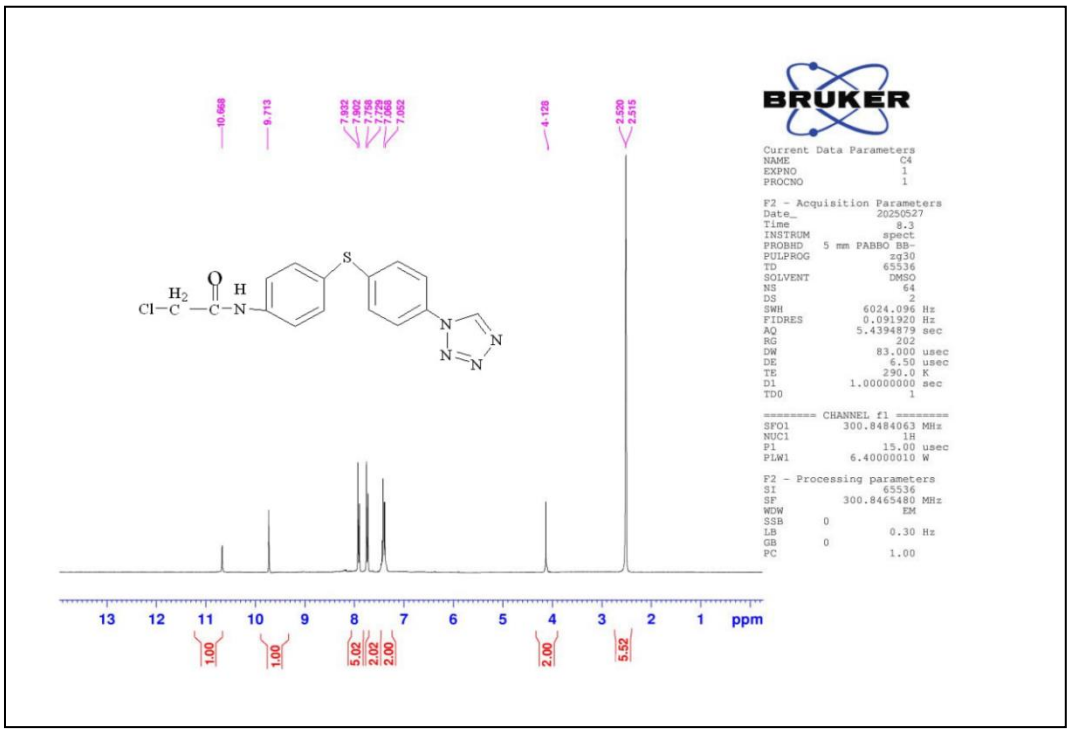


FIGURE 15. ^{1}H-NMR Spectra of C4

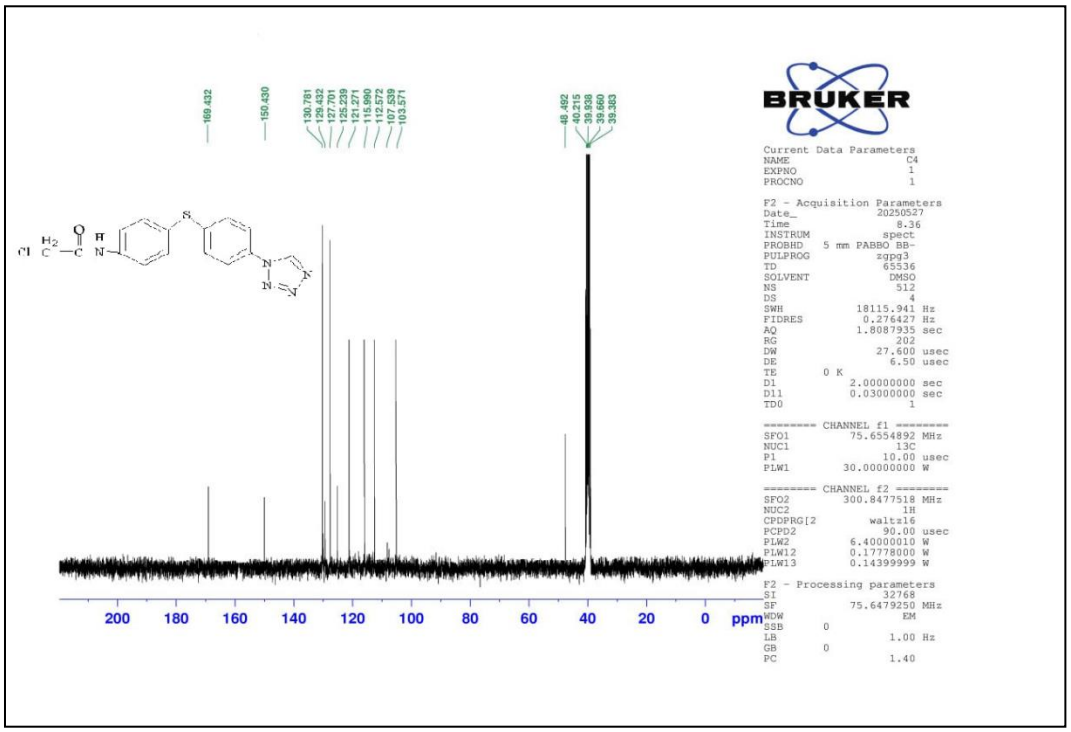


Figure 16. ^{13}C NMR Spectra of C4

Table 1. The physical characteristics of the prepared derivatives

Component No.	Melting Point (°C)	% Yield	Colour	M.F.	M.Wt	Rf
C	84-86	65	Light yellow	$C_{14}H_{10}N_8S$	268	0.93 3Hexane:2Ethanol
C1	179-181	70	Light brown	$C_{12}H_8N_6S$	322	___
C2	Oil	55	Dark brown	$C_{26}H_{16}N_8S_3$	539	0.6 4Hexane:1Ethanol
C3	197-199	50	Purple	$C_{13}H_{11}N_5S$	296	0.853 3Hexane:2Ethanol
C4	Oil	67	Dark brown	$C_{15}H_{12}N_5OSCl$	345	0.63 1Hexane:2Ethanol

3.2 Biological Activity

The in vitro antioxidant activity of the C3 derivative was evaluated using reducing power and DPPH radical scavenging activity. Reducing power was observed at all concentrations (1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg). C3 outperformed ascorbic acid in a concentration-dependent manner. It reached 0.614 at 0.03125 mg, which significantly decreased to 0.49 at 1 mg. The table below indicates the reducing capacity of C3 compared to ascorbic acid.

Table 2. Reducing the power of ascorbic acid and its ability to scavenge free radicals

Sample Name	Concentration (mg/ml)	Absorbency	Scavenging Ratio %
Vit C	1	0.029	96.38
Vit C	0.5	0.04	95.01
Vit C	0.25	0.045	94.38
Vit C	0.125	0.049	93.88
Vit C	0.0625	0.055	93.13
Vit C	0.03125	0.0612	92.36

Table 3. Reducing the power of C3 and free radical scavenging

Sample Name	Concentration (mg/ml)	Absorbency	Scavenging Ratio %
C3	1	0.49	38.83
C3	0.5	0.574	28.34
C3	0.25	0.591	26.22
C3	0.125	0.596	25.59
C3	0.0625	0.603	24.72
C3	0.03125	0.614	23.35
Standard		0.801	

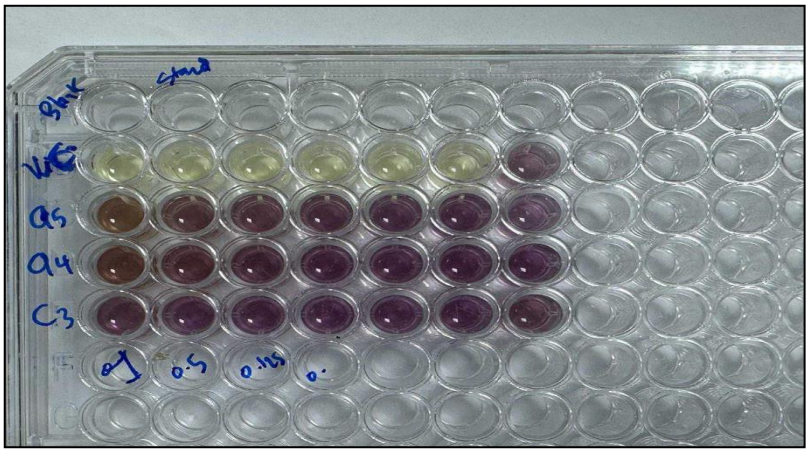


Figure 17. The microplate containing Blank, Control, and six concentrations of C3 compound.

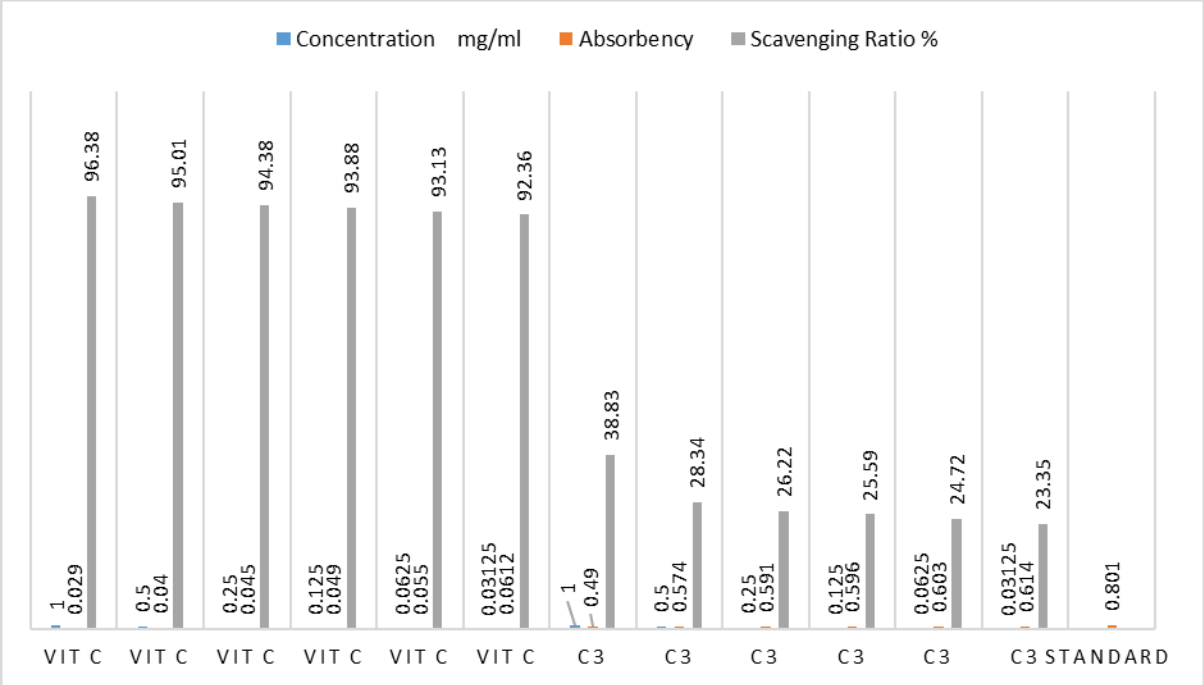


Figure 18. Free radical scavenging activity of C3 with ascorbic acid.

4. Conclusions

The study successfully synthesized a series of cyclic and heterocyclic analogues, including an organic azide derivative and tetrazole derivatives, from 4,4'-thiodianiline. The characterization of these compounds was confirmed using FT-IR and ^{1}H NMR techniques. The FT-IR spectra provided clear evidence of structural changes, such as the disappearance of aromatic primary amine groups and the appearance of distinct functional groups, including the azide (N_3) group, the amide (C=O), and the (N=N) tetrazole ring. The ^{1}H NMR and ^{13}C NMR spectra also revealed distinctive chemical shifts corresponding to the synthesized derivatives. The antioxidant activity of one of these chemical compounds was compared with that of ascorbic acid. These results indicate that the synthesized derivative has excellent potential as an antioxidant agent.

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