Mathematical Statistician and Engineering Applications
ISSN:2094-0343
2326-9865

Synthesis, Characterization and antioxidant activity study of Pyridine derivatives: A Comprehensive Study

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Article Info
Page Number: 481 - 492
Publication Issue:
Vol 72 No. 2 (2023)

Article History:
Article Received: *15 October 2023*
Revised: *24 November 2023*
Accepted: *18 December 2023*

Abstract
A preliminary investigation was conducted on 2-amino-4-aryl-3,5-dicarbonitrile-6-thiopyridines as a novel candidate as antioxidant research. A particular shape of the chosen pyridine derivatives having higher biological activity was taken with particular attention. Various structures were employed here. Yields for the target molecules under study ranged from 53% to 91%. The work that was presented demonstrated that the structure of the pyridine compounds under study affects their antioxidant activity.

Keywords: -research, molecules, compounds

1.2 Introduction

It has been difficult in recent decades to develop an antioxidant chemical that is both safe and efficient. The significance of (ROS) well known as reactive oxygen species in food, medications, even in alive systems has drawn more attention lately (Riley 1994). The regular, spontaneous metabolism of aerobic cells is linked to produce free radicals. ROS, which results as oxygen metabolites, are unavoidably exposed to them. Numerous degenerative and chronic diseases, including cancer, atherosclerosis, wound healing, cirrhosis, diabetes, and aging, are linked to oxidative stress, they are primarily characterized by reactive nitrogen and oxygen species. Because so reactive free radicals, they produce carbon and peroxy radicals and damage lipids membrane. As a result, researchers across a wide range of fields are becoming increasingly interested in both naturally occurring antioxidants and their synthetic counterparts, which may offer active ingredients that either prevent or lessen the effects of oxidative stress [1].

A variety of pharmacologically significant chemicals as well as some natural items have the pyridine ring structure. Due to their diverse range of biological activity, 2-amino-4-aryl-3,5-dicarbonitrile-6-thiopyridines, among others, possess much attention [1].

The molecules of pyridine derivatives were discovered to alter the androgen receptor role [5], serve as an agonist of the A1 receptor for adenosine long-term therapy of cardiac conditions [3, 4], prevent the accumulation of PrPSc in mouse neuroblastoma cells (ScN2a) infected with scrapie [2], and serve as IKK-2 in the treatment of HBV disesase [6]. Furthermore, those substances were frequently employed as potassium channel openers to help treat incontinence in the urine [12], as well as anti-bacterial [10], anti-prion [7, 8], anti-cancer [11] agents, anti-hepatitis B virus [9]. The substances recently have been identified as possible targeting agents

for the creation of novel medications to treat conditions like Parkinson's, Alzheimer's, renal, Creutzfeldt-Jacob disorders, hypoxia, epilepsy, and asthma. The professional scientific literature currently lacks sufficient information on the antioxidant analysis of basic synthetic pyridine derivatives. Examining pyridine derivatives and their possible antioxidant activity may show that they are useful as antiantioxidant agents in many novel drug resistance scenarios. The study's objective is to examine the structural characteristics of 2-amino-4-aryl-3,5-dicarbonitrile-6-sulfanylpyridines in order to determine its potential antioxidant activity.

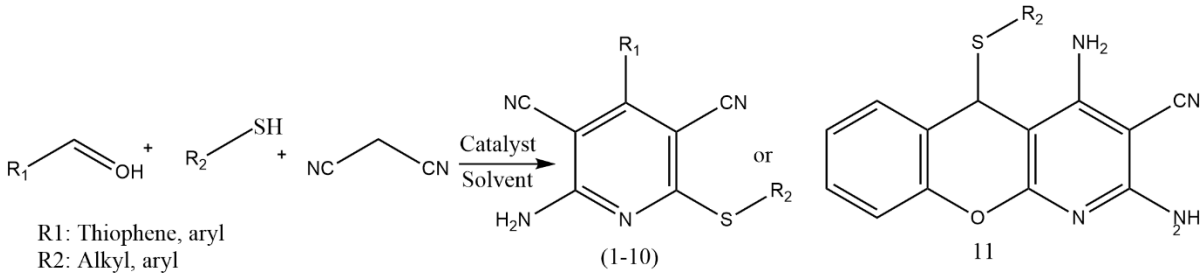
1.3 Results and Discussion

1.3.1 Procedures for the Preparation of Pyridine Derivatives

All of the chemicals were analytical grade, and the solvents were bought from Sigma-Aldrich. The uncorrected melting temperatures of each and every produced compound were measured in capillary tubes. Benzophenone was present during the distillation of toluene over Na/K-alloy. CDCl3 or DMSO-d6 solution were used to record 1H NMR spectra using an NMR spectrometer Varian Gemini 400 (400 MHz). Applied Biosystems' API 3000 mass spectrometer MS spectra were rearranged. Aluminum sheets made of Kieselgel 60 F254 underwent Thin Layer Chromatography (TLC) analysis. In Potassium Permanganate (KMnO4) solution with Potassium Carbonate (K2CO3) and Sodium Hydroxide (NaOH), TLC was formed.

1.3.2 General Protocol for the preparation of (1-11)

One millimol of aldehyde combined with, 100 mg of PPL, 2 mmol of malononitrile CH2(CN)2, 1 milimole of thiol in 2 mililiter of (EtOH) ethanol was shaken for eighteen hours at 40 °C at 200 rpm. By removing the catalyst passing via the bed of celite, reaction was stopped. It is kept track of the derivatives' melting points and yields. The synthesized compounds' spectral data and melting points were consistent utilizing the information from the literature. Using NMR and mass spectroscopy, the structures of all the compounds were verified, and for certain compounds, the accurate Each synthesis process included documentation of the elemental analysis data.



Scheme: Synthesis of compounds 1-11.

Compound 1: 2-Amino-4-(4-cyanophenyl)-6-(4-methylphenylsulfanyl)-3,5-pyridinedicarbonitrile. It was produced using a conventional procedure, yielding 84% of the sample as a white crystal with mp 273 °Celcius, ^{1}H Spectrum ((Dimethyl Sulfoxide) -d6), NH2-6.89; CH (aromatic protons)7.47, 7.84, 7.29, 7.47, 7.84, 7.29, 7.84, CH3-2.32. ^{13}C NMR, 165.9,

160.2, 142.3, 92.8, 87.3, 113.1, 137.9, 129.3, 129.8, 132.7, 132.7, 112.5, 113.7, 118.6, 21.3. Chemical Formula: C21H13N5S, Exact Mass: 367.09, Molecular Weight: 367.43, m/z: 367.09, Elemental Analysis: Carbon, 68.65; Hydrogen, 3.57; Nitrogen, 19.06; Sulphur, 8.73.

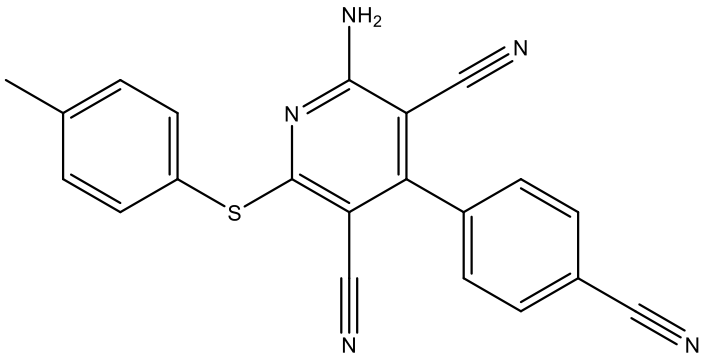


Figure 1: Structure of Compound 1.

Compound 2: 2-Amino-4-(thiophen-2-yl)-6-(4-methylphenylsulfanyl)-3,5-pyridinedicarbonitrile. A yellow-coloured crystalline compound with a yield of 80% was obtained as Compound 2 using the general method; its melting point was recorded at 196 °C [2]. ^{1}H Spectrum (Dimethyl Sulfoxide), NH2-6.89, CH (aromatic protons) 7.70, 7.40, 7.13, 7.47, 7.29, 7.47, 7.29, CH3-2.32. ^{13}C NMR, 138.2, 128.6, 127.6, 128.0, 187.2, 128.6, 166.0, 155.8, 93.4, 87.9, 137.9, 129.3, 112.5, 113.7, 21.3. Chemical Formula: C18H12N4S2; Exact Mass: 348.05, Molecular Weight: 348.44, m/z: 348.05. Elemental analysis: Carbon, 62.05; Hydrogen, 3.47; Nitrogen, 16.08; Sulphur, 18.40.

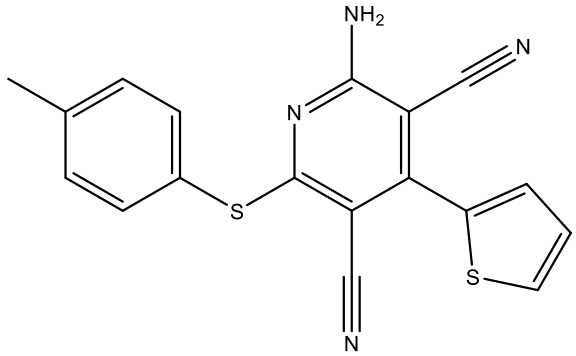


Figure 2: Structure of Compound 2.

Compound 3: 2-Amino-6-((4-aminophenyl)thio)-4-phenylpyridine-3,5-dicarbonitrile. It was produced using a standard procedure and yielded 54% as a yellow crystal with a melting point of 224 ° Celsius, ^{1}H Spectrum (Dimethyl Sulfoxide), NH2-6.89, NH2-5.50, CH (aromatic protons) 7.13, 6.61, 7.51, 7.13, 6.61, 7.51, 7.46, 7.41. 13C NMR,187.1, 121.6, 165.9, 142.5, 160.2, 138.0, 92.8, 87.3, 129.0, 115.5, 127.4, 127.0, 115.5, 127.4, 127.0, 115.5, 127.4, 129.2, 112.5, 113.7. Chemical Formula: C19H13N5S, Exact Mass: 343.09, Molecular Weight: 343.41, m/z: 343.09, Elemental Analysis: Carbon, 66.45; Hydrogen, 3.82; Nitrogen, 20.39; Sulphur, 9.34.

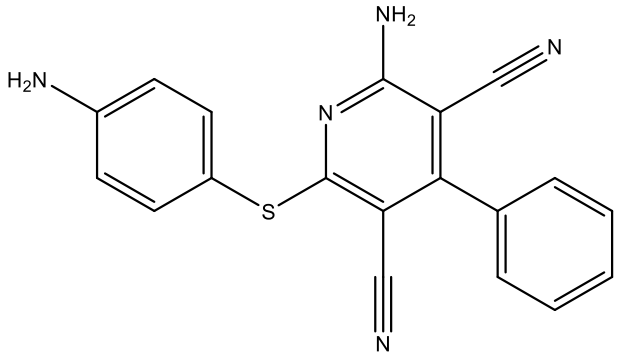


Figure 3: Structure of 2 Compound 3.

Compound 4: 2-Amino-4-phenyl-6-(p-tolylthio)pyridine-3,5-dicarbonitrile. It was produced using a conventional procedure and yielded 90% as a white crystal at 249 ° Celsius, ^{1}H Spectrum (Dimethyl Sulfoxide), NH2- 6.89, CH (aromatic protons) 7.47, 7.51, 7.29, 7.47, 7.29, 7.46, 7.41, CH3-2.32, 13C NMR, 187.1, 128.6, 165.9, 160.2, 138.0, 92.8, 87.3, 137.9, 129.3, 127.4, 129.3, 129.2, 112.5, 113.7, 21.3.Chemical Formula: C20H14N4S, Exact Mass: 342.09, Molecular Weight: 342.42, m/z: 342.09, Elemental Analysis: Carbon, 70.15; Hydrogen, 4.12; Nitrogen, 16.36; Sulphur, 9.36.

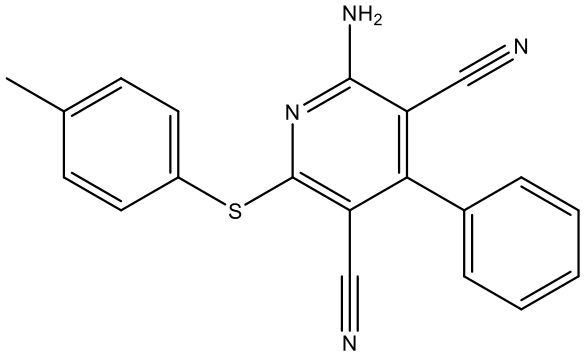


Figure 4: Structure of Compound 4.

Compound 5: 2-Amino-6-(octylthio)-4-phenylpyridine-3,5-dicarbonitrile. It was produced using the standard procedure in a white crystal with a 78% yield and an mp of 148 ° Celcius, ^{1}H Spectrum (Dimethyl Sulfoxide), NH2-6.89, CH (aromatic protons) 7.51, 7.46, 7.41, CH2-3.35, Protons present in allyl region CH2 (1.78, 1.47, 1.33, 1.25, 1.26, (CH3) methyl -0.88. 13C NMR 167.2, 165.4, 159.2, 138.0, 95.6, 127.4, 129.2, 36.2, 112.5, 113.7, 30.2, 28.5, 28.9, 29.3, 31.9, 22.7, 14.1. Chemical Formula: C21H24N4S, Mass of the product: 364.17, Formula Mass: 364.51, m/z: 364.17, Elemental Analysis: Carbon, 69.20; Hydrogen, 6.64; Nitrogen, 15.37; Sulphur, 8.80.

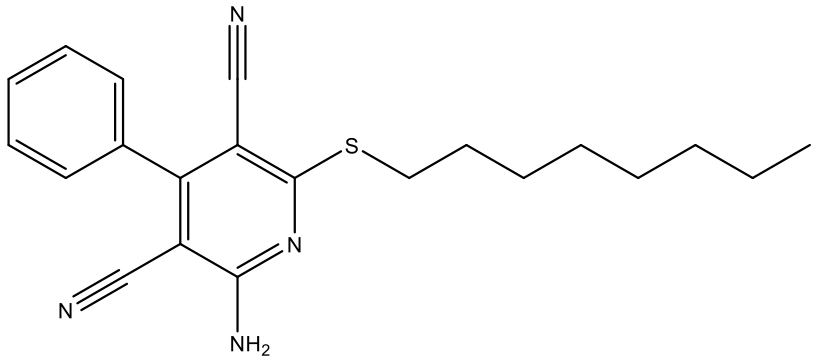


Figure 5: Structure of Compound 5.

Compound 6: 2-Amino-4-(p-tolyl)-6-(p-tolylthio)pyridine-3,5-dicarbonitrile. It was prepared using a conventional technique and produced a white crystal with a 93% yield at 224 ° Celcius, ^{1}H Spectrum (Dimethyl Sulfoxide), NH2-6.89, CH (aromatic protons) 7.47, 7.33, 7.29, 7.15, 7.29, 7.15, CH2-2.32, CH2-2.34. 13C NMR 187.1, 128.6, 165.9, 160.2, 135.0, 92.8, 87.3, 137.9, 132.2, 129.3, 127.3, 129.5, 112.5, 113.7, 21.3. Chemical Formula: C21H16N4S, Exact Mass: 356.11, Molecular Weight: 356.45, m/z: 356.11, Elemental Analysis: Carbon, 70.76; Hydrogen, 4.52; Nitrogen, 15.72; Sulphur, 8.99.

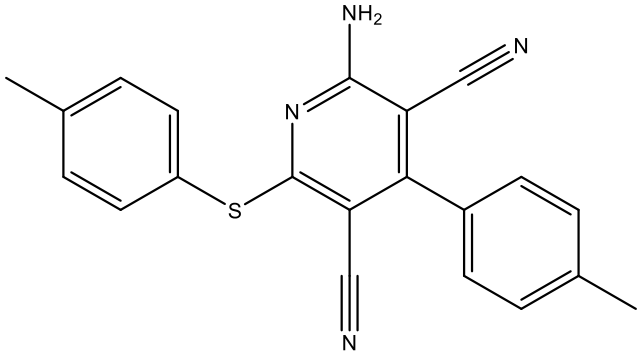


Figure 6: Structure of Compound 6.

Compound 7: 2-Amino-6-((4-chlorophenyl)thio)-4-phenylpyridine-3,5-dicarbonitrile. It was produced using a standard procedure and yielded 91% as a white crystal with a melting point of 245 ° Celsius, ^{1}H Spectrum (Dimethyl Sulfoxide), NH2-6.89, CH (aromatic protons) 7.30, 7.51, 7.30, 7.74, 7.51, 7.46, 7.41. 13C NMR 131.1, 187.1, 129.7, 165.9, 160.2, 138.0, 92.8, 87.3, 129.1, 136.7, 127.4, 129.1, 136.7, 127.4, 129.2, 112.5, 113.7. Chemical Formula: C19H11ClN4S, Exact Mass: 362.04, Molecular Weight: 362.84, m/z: 362.04, Elemental Analysis: Carbon, 62.90; Hydrogen, 3.06; Chlorine, 9.77; Nitrogen, 15.44; Sulphur, 8.84.

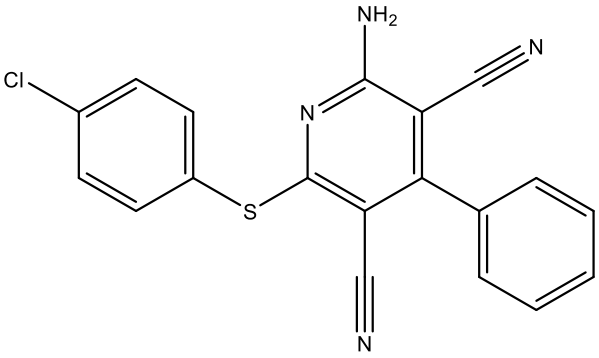


Figure 7: Structure of Compound 7.

Compound 8: 2-Amino-4-(4-methoxyphenyl)-6-(p-tolylthio)pyridine-3,5-dicarbonitrile. It was produced using a standard procedure and yielded 84% as a white crystal with a melting point of 230 ° Celsius, ^{1}H Spectrum (Dimethyl Sulfoxide), NH2-6.89, CH (aromatic protons) 7.47, 7.02, 7.71, 7.29, 7.47, 7.02, 7.71, 7.29, 3.81, 2.32. 13C NMR 187.1, 128.6, 161.1, 165.9, 160.2, 130.3, 92.8, 87.3, 137.9, 129.3, 114.8, 129.6, 114.8, 55.8, 112.5, 113.7, 21.3. Chemical Formula: C21H16N4OS, Exact Mass: 372.10, Molecular Weight: 372.45, m/z: 372.10, Elemental Analysis: Carbon, 67.72; Hyrogen, 4.33; Nitrogen, 15.04; Oxygen, 4.30; Sulphur, 8.61

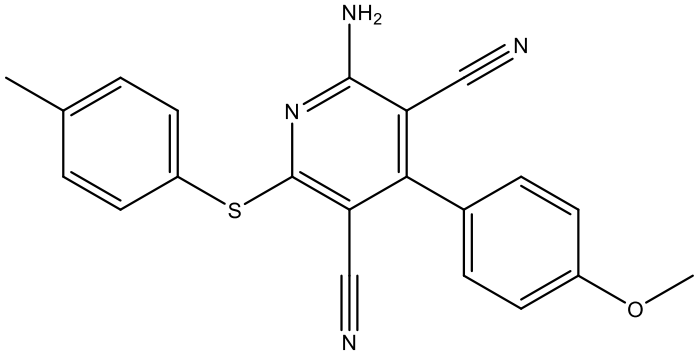


Figure 8: Structure of Compound 8.

Compound 9: 2-Amino-4-(4-nitrophenyl)-6-(p-tolylthio)pyridine-3,5-dicarbonitrile. It was created using a conventional procedure and produced a white crystal with a 90% yield, mp of 299 ° Celsius, ^{1}H Spectrum (Dimethyl Sulfoxide), NH2- 6.89, CH (aromatic protons) 7.47, 8.23, 7.79, 7.29, 7.47, 8.23, 7.79, 7.29, CH2-2.32. 13C NMR 187.1, 128.6, 165.9, 148.4, 160.2, 144.1, 92.8, 87.3, 137.9, 129.3, 124.4, 128.3, 129.3, 124.4, 128.3, 129.3, 112.5, 113.7, 21.3. Chemical Formula: C20H13N5O2S, Exact Mass: 387.08, Molecular Weight: 387.42, m/z: 387.08, Elemental Analysis: Carbon, 62.01; Hydrogen, 3.38; Nitrogen, 18.08; Oxygen, 8.26; Sulphur, 8.28

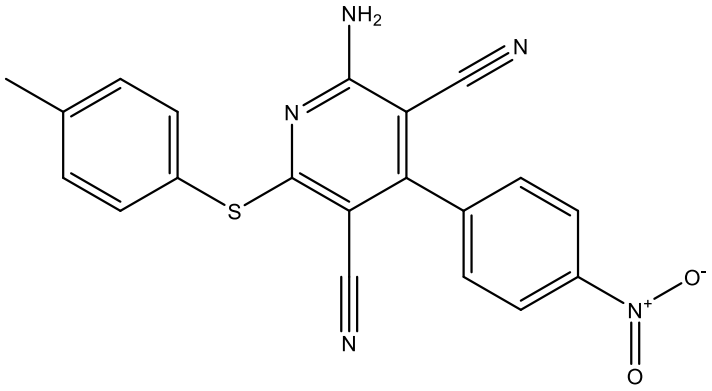


Figure 9: Structure of Compound 9.

Compound 10: 2-Amino-6-((4-bromophenyl)thio)-4-phenylpyridine-3,5-dicarbonitrile. It was prepared using a conventional approach and produced a white crystal with a 76% yield, mp 256 Celsius, ^{1}H Spectrum (Dimethyl Sulfoxide), NH2- 6.89, CH (aromatic protons) 7.76 7.53, 7.51, 7.76, 7.53, 7.51, 7.46, 7.46, 7.41. 13C NMR 119.9, 187.1, 130.6, 165.9, 160.2, 138.0, 92.8, 87.3, 131.9, 128.7, 129.2, 112.5, 113.7, Chemical Formula: C19H11BrN4S, Exact Mass: 405.99, Molecular Weight: 407.29, m/z: 405.99, Elemental Analysis: Carbon, 56.03; Hydrogen, 2.72; Bromine, 19.62; Nitrogen, 13.76; Sulphur, 7.87

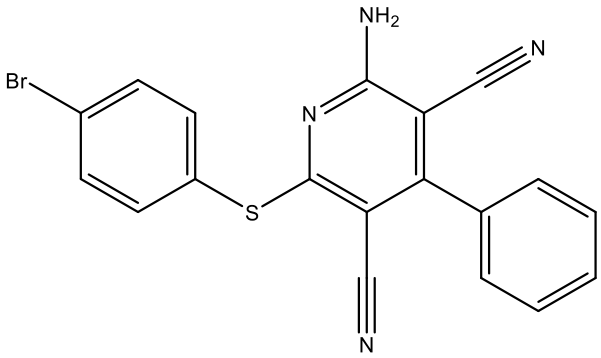


Figure 10: Structure of Compound 10.

Compound 11: 2,4-Diamino-5-[(4-methylphenyl)thio]-5H-[1]benzopyrano[2,3-b]pyridine-3-carbonitrile. It was produced using a standard procedure, yielding an 80% white crystal at a melting point of 224 ° Celsius, ^{1}H Spectrum (Dimethyl Sulfoxide), NH2- 6.89, NH2-6.35, CH-5.45, CH (aromatic protons) 7.23, 6.98, 7.01, 7.09, 7.23, 7.09, 7.18, 6.85, CH3-2.32. 13C NMR 133.4, 41.9, 169.2, 148.7, 160.1,165.8, 96.6, 133.6, 71.4, 137.5, 128.6, 119.8, 128.7, 129.2, 128.1, 124.6, 114.5, 21.3. Chemical Formula: C19H11BrN4S, Exact Mass: 405.99, Molecular Weight: 407.29, m/z: 405.99, Elemental Analysis: Carbon, 56.03; Hydrogen, 2.72; Bromine, 19.62; Nitrogen, 13.76; Sulphur, 7.87.

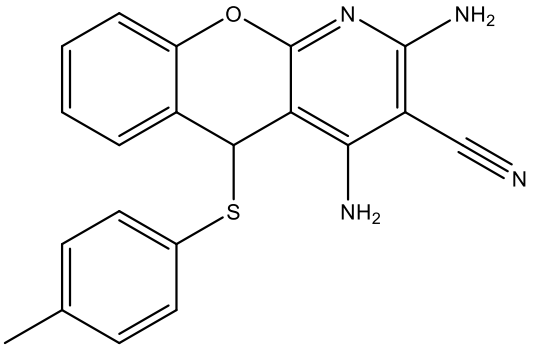


Figure 11: Structure of Compound 11.

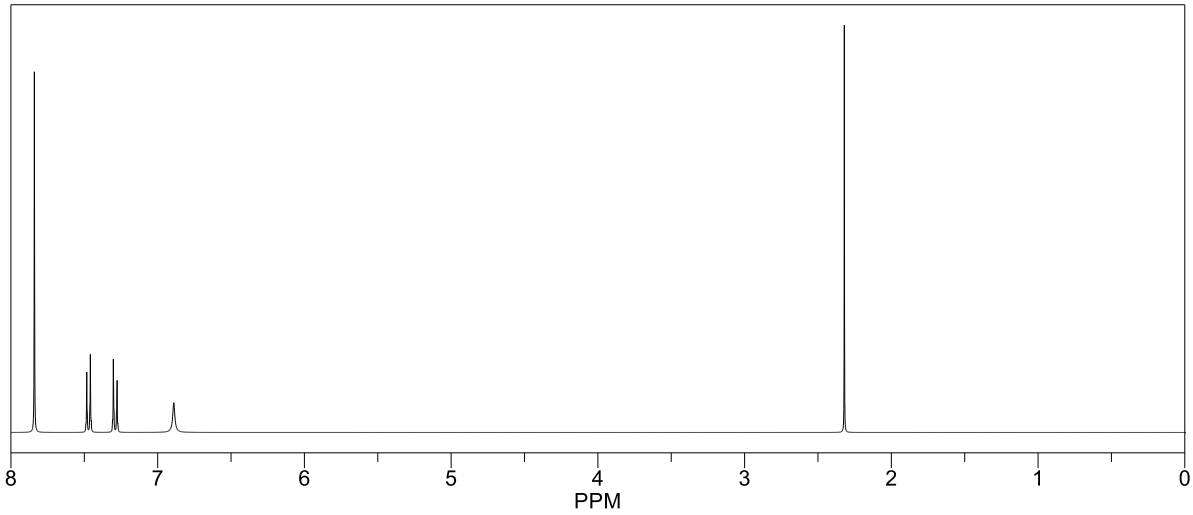


Figure 12: 1H-Nuclear Magnetic Resonance spectrum of First Compound.

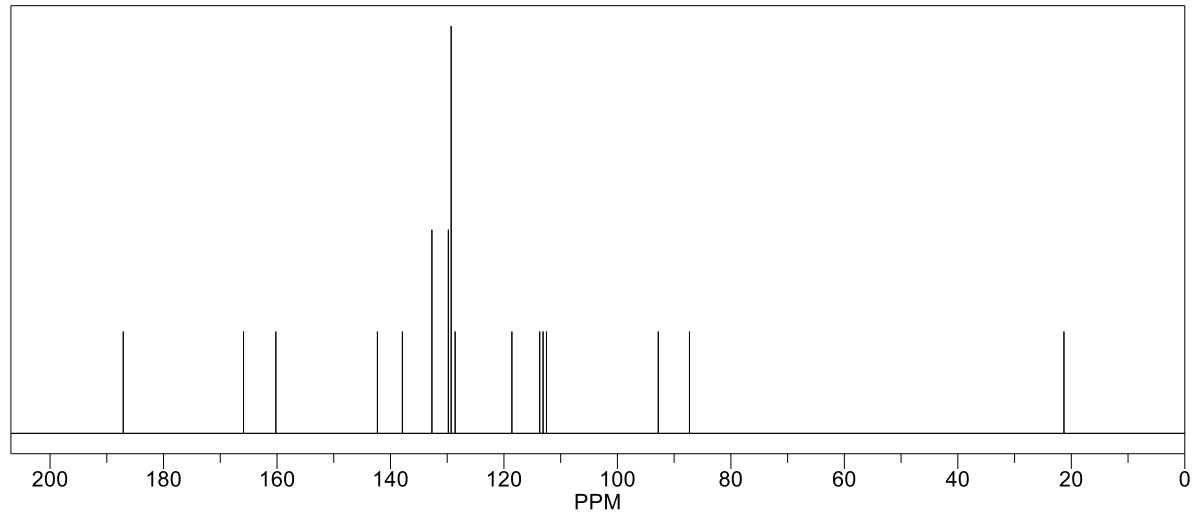


Figure 13: 13C-NMR spectrum of First Compound.

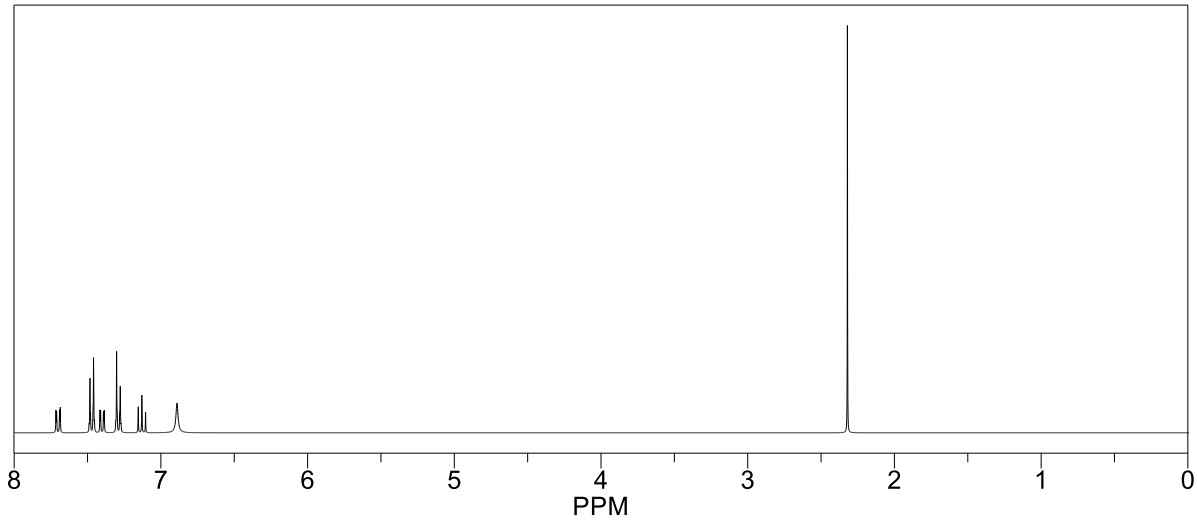


Figure 14: 1H-NMR spectrum of Second Compound.

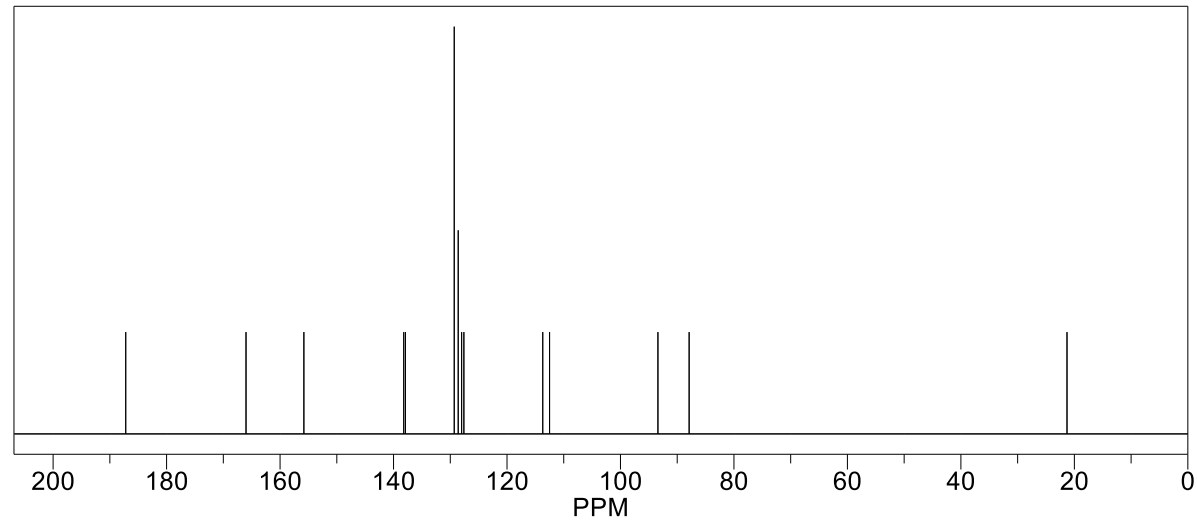


Figure 15: 13C-Nuclear Magnetic Resonance spectrum of Second Compound.

1.4 Biological Assessment:

1.4.1 Free radical scavenging assays (13, 14)

The stability of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) allowed for the scavenging activity to be used in determining the antioxidant activity. The effect of chemicals under study on DPPH was calculated using, with some slight modifications, the Blois technique (13, 14). The control solution or drug in the ethanol, 250 µmoles/l (0.2 ml) concentration value, was combined with DPPH at a 150 µmoles/L (4 ml) concentration value. After carefully mixing the obtained reaction mixture with a vortex, it was left to sit for 60 minutes under dark condition at room temperature. Concurrently, the control was made out of a combination of 4 ml of DPPH in ethanol and 0.2 ml of ascorbic acid in ethanol, both without any sample fraction. Using ethanol as a blank, the mixture's absorbance reduction was measured at 540 nm. The standard utilized was ascorbic acid. Measurements were also made of the DPPH solution's absorbance. The

following formula was used to compute the proportion of DPPH scavenging activity, that cam be expressed as a percent inhibition:

% Inhibition =ADPPH−Ac/ADPPHX100%

Where Ac is the sample's absorbance

ADPPH - absorbance of solution DPPH free radicals.

Every experiment was run three times, and average results were noted. The means ± S.D. are used to express the results.

1.4.2 Application Part

DPPH free radical action was used to calculate the antioxidant assay. Because of its strong purple color and great stability in a methanolic solution, DPPH radical has found several uses. The greatest absorbance of the oxidized DPPH radical is determined at a wavelength of roughly 540 nm. When antioxidants are used to reduce radicals, absorbance falls. The equivalent anion (DPPH) in basic medium is obtained upon its reduction. Other odd number of electron species that produce para substituted products at phenyl rings position were scavenged by the DPPH radical.

The DPPH technique is characterized an easy, quick, and practical way to screen several samples for DPPH scavenging activity. Because of such benefits, this technique is worth investigating for scavenging radicals in newly synthesized compounds and identifying potential antioxidant medication candidates.

In this study, we describe a modified spectrophotometric approach that makes use of the particular absorbance features of the DPPH radical. Using a reliable DPPH assay, The compounds' respective capacities to scavenge free radicals were measured by measuring the At 540 nm, the solution being combined with DHHP begins to lose color. At 150 µmoles/L of ethanol, It was discovered that the DPPH solution's absorbance was 0.770. The table below lists the absorbances and percentage inhibitions of activity (ascorbic acid) for scavenging free radicals for each chemical and the standard.

compound	**A_s sample absorbance value**	**Inhibition activity in (%)**
Control	*0.870±0.0249*	–
1	*0.592±0.025*	31.95
2	*0.807±0.020*	7.24
3	*0.606±0.015*	30.34
4	*0.784±0.020*	9.89
5	*0.823±0.025*	5.40

6	*0.830±0.025*	4.59
7	*0.832±0.025*	4.37
8	*0.806±0.020*	7.35
9	*0.800±0.020*	8.04
10	*0.790±0.020*	9.19
Ascorbic acid	*0.680±0.015*	21.8

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