

Synthesis, Characterization and Antioxidant Activity of Some New Schiff base Derived from Cytosine

Ali A. Mahmood¹, Farah. Kh. H. Al-Juboory^{2*}, Hala M. Garib Al-Zahwi³

¹Presidency of the University of Kirkuk, Kirkuk, Iraq,

²College of Science, Department of Chemistry, University of Kirkuk, Kirkuk, Iraq,

³College of Science, Department of Chemistry, University of Kirkuk, Kirkuk, Iraq,
farah_aljibory2003@yahoo.com

Abstracts

Cytosine is used to synthesize several derivatives of Schiff base when reacted with some aldehydes which are refluxed by using ethanol as a solvent and non solvent with domestic microwave (Compounds A₁ – A₄). These products of the Schiff base are treated first with chloroacetyl chloride to synthesize four-membered heterocycles (Compounds B₁ – B₄). After that, they are treated with hydroxy acetic acid to synthesize five-membered heterocyclic (Compounds C₁ – C₄). In the third step, the products are used to react with maleic acid anhydride to produce seven-membered heterocyclic (Compound D₁ – D₄). The antioxidant activity of the compounds (A₁ – A₄) and two of the compounds (B₁, B₃), (C₁, C₃) and (D₁, D₃) in different concentrations of (100, 200, 300, 400 and 500 mg/ml) are tested with 1,2 – Diphenyl – 2 – Picryl Hydrazyl (DPPH) for scavenging a radical activity. Finally, these compounds are diagnosed by several technical devices such as IR, proton¹H.N.M. R and ¹³C N.M.R. spectroscopy.

Keywords: Synthesis; Cytosine; Schiff Base; Antioxidant; % DPPH Radical Scavenging Activity.

1 INTRODUCTION

It is well known that the nucleic acids are built from nucleotides. Furthermore, nucleic acid consists of a nitric base, sugar molecule and phosphate. The nitric base includes two types of heterocyclic compound pyrimidines and purines. Additionally, Pyrimidine is similar to diazines six-membered heterocyclic compound which contains two atoms of nitrogen in position 1,3. Cytosine (4 – amino – 2 – oxypyrimidine) is a derivative of pyrimidine and one of the four members of diazines which was discovered in 1894 by Kossel. Moreover, this compound was firstly synthesized in laboratories in the year (1903) [1].

Cytosine was prepared by Peter J. and Leonard Nicholl from treating *B*-ethoxyacrylo nitrile (prepared by reaction of isoxazole with diethyl sulfate in alkaline solution) with urea in a sodium alcoholate solution [2]. The alkylation of the free amino group in some pyrimidine derivatives (6-amino thiouracil) can be successfully synthesized in good yield when it is treated with one equivalent of RCH₂X in DMF. This test is performed by radiating it with microwave – assisted method under solvent-free condition.

Using the same starting material in the similar condition,

as mentioned above, by using of (Na₂CO₃ and Bu₄NI) as catalysts, alkylation occurs on the nitrogen of the heterocyclic ring in excellent yield [3]. Another derivative of (6-amino-4-phenyl-2-thioxo-5 cyano-1,2,3,4-tetrahydro pyrimidine) is refluxed with (ethyl-2-chloroacetate). Then, KOH and ethanol are added as solvents to the thioxo group, and the yield is (87%) [4]. An antioxidant is that material defined as the substance which is capable of stabilization (or deactivation) of free radicals (before they attack living cells) [5]. Francisco J. Martinez and Rodrigo Said Razo – Hernandez synthesized eight derivatives of (3-carboxycoumarin). The derivatives were assayed by quantitative (DPPH) radical scavenging activity methods. Two of the derivatives showed the best radical – scavenging [6], whereas some derivatives of prenylated phenol manifested antioxidant activity scavenging with DPPH radical. Mauricio Osorio, Jacqueline Aravena and Alejandra Vergara synthesized 26 prenylated phenol and their acetylated derivatives in two steps. In addition, all of these compounds were compared with TroloxTM. Furthermore, inactive derivatives of these compounds were the acetylated derivatives. Moreover, other derivatives showed antioxidant similar to TroloxTM. Besides, the dialkyl derivatives compound revealed better

activity than monoalkyl of the phenol derivatives [7]. In this work, the derivative of pyrimidine (cytosine) was used as starting material and treated with four types of aldehyde. There of these types are a derivative of benzaldehyde, and the latter is furfural (five-membered heterocyclic aldehyde). Clearly, results revealed a good yield of four Schiff base derivatives ($A_1 - A_4$). Then, these products were treated with three types of reagents to synthesize the four-, five- and seven-membered heterocyclic compounds. The steps we used as follows; firstly, the products of Schiff base were treated with the reagent chloro acetyl chloride to synthesize four-membered heterocyclic compounds ($B_1 - B_4$). Secondly, the Schiff base was treated with hydroxyl acetic acid, and the products were five-membered heterocyclic compounds ($C_1 - C_4$). Thirdly, treating malic acid anhydride with Schiff base was seven - membered heterocyclic compound ($D_1 - D_4$). Lastly, the antioxidant of some of these compounds was tested with *Antioxidant Assay by Scavenging activity of 1,2-Diphenyl-2-Picrylhydrazyl (DPPH) Radical*.

2 EXPERIMENTAL RESULTS AND INTERPRETATION

2.1 Materials and Analysis

All materials are of high purity and were purchased from Sigma Aldrich which used without any further purification. The compounds were synthesized and characterized by using UV, IR, ^1H NMR and ^{13}C NMR spectroscopy analysis. The IR spectra were recorded on Perkin - Elmer spectrum in the form of KBr pellet. ^1H NMR was recorded in DMSO using TMS as internal standard from Ahl-Albait University 300MHz. The melting point of all compound was incorrect and measured by Electrothermal 9300 melting point apparatus.

2.2 Synthesis

2.2.1 Preparation of Schiff Base

The derivatives were prepared through the reaction between cytosine and a set of aldehydes with proportions of mole (1: 1) of each cytosine and aldehyde, respectively. In the (100 ml) round flask, we added (1 ml, 0.1 mole) of the aldehyde with few drops of ethanol absolute and two drops of glacial acetic acid. Then, (1.08 gm) of cytosine added after mixing and stirring of the solutions. After that, the mixing is radiated with domestic (*economic*) microwave with monitoring the changes of the color every (10 secs). Besides, the reaction followed up using (T. L. C.) technique to complete the reaction. At the end of

the reaction, the solution is dried for one day. A color crystal of the compound (A_1) is formed, washed and filtered with cold ethanol to remove non-reacted aldehydes; additionally, in order to separate the solid precipitate by filtrate and re-crystallize with absolute ethanol. By the same methods, we can synthesize the derivative compounds from ($A_2 - A_4$). The obtained yield of the products is in the range (73 - 93%). Accordingly, the physical properties of the compounds are shown in Table 1 [8].

2.2.2 Preparation of Azetidine

Firstly, dissolving (0.005 mmole) of prepared Schiff base ($A_1 - A_4$) in (40 ml) of dioxane. Secondly, adding (1.4 ml, 0.01 mole) tri-ethylamine and put the mixture in a ice bath with a stirrer. After that, gradually add (0.8 ml, 0.01 mole) chloroacetyl chloride for (20 min). Then, stir the solution for 3 hours and leave it at room temperature for two days. In order to complete the precipitate of the solid product, the product is added to crushed ice, filtered, dried, and re-crystallized from rectified spirit. The yield of the products ($B_1 - B_4$) is between (60 - 75) %. Consequently, the physical properties are listed in Table 2 [9].

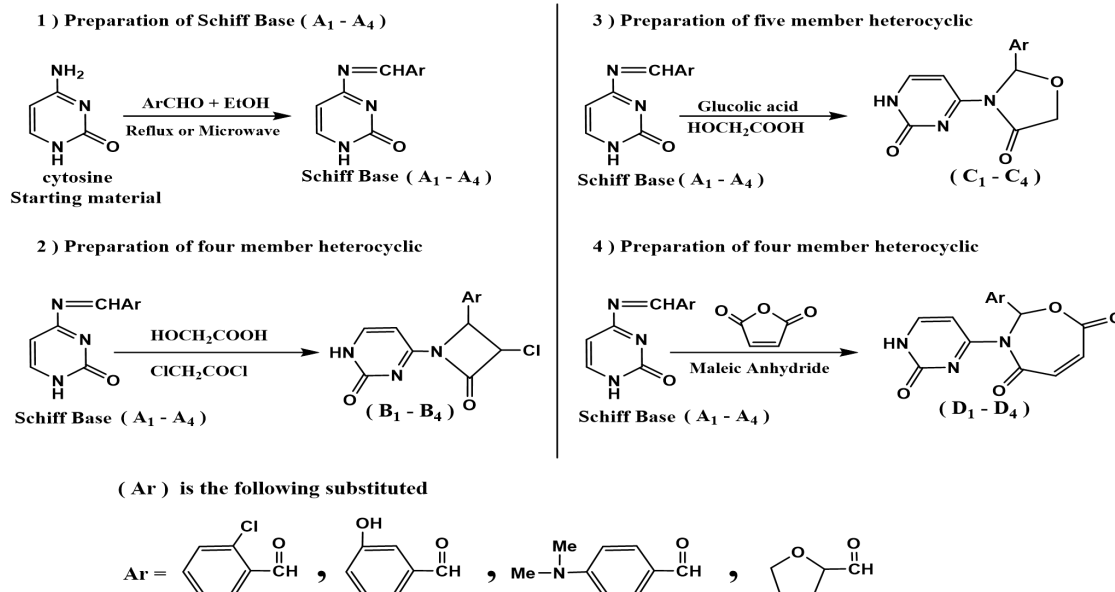
2.2.3 Preparation of Oxazolidine

The first step is mixing (0.05 mmole) of prepared Schiff base ($A_1 - A_4$) with (25 ml) of the solvent dioxane with (0.01 mole) hydroxyl acetic acid dissolved in the same solvent. Later, putting the mixture in (100 ml) round flask and reflux for 5 hours to cool to room temperature. After that, the colored solid product is precipitated, filtered, dried, and re-crystallized from ethanol absolute. Accordingly, the yield of the product ($C_1 - C_4$) is between (65-83) %. Finally, Table 3 shows the physical properties of the compounds [10].

2.2.4 Preparation of Oxazepene

Putting (0.05 mmole) of Schiff base ($A_1 - A_4$) with (0.01 mmole) Malic acid anhydride in mortar casserole and grinding it well until the mixed material become homogenous. After that, the mixture is radiated in a domestic microwave for about 1 to 2 minutes. Then, the product will be cooled to room temperature at the end of the radiation. The final product appears as a color solid which is washed with cold benzene and filtrate. Lastly, the product re-crystallized with ethanol absolute [11]. The yield of the product ($D_1 - D_4$) is between (63- 89) %. Finally, the physical properties of the products are shown in Table 4.

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Scheme Show the Route of Synthesis of Compound (A₁) to (D₄)

3 Antioxidant Assay by Scavenging activity of (1,2-Diphenyl-2-Picrylhydrazyl) (DPPH) Radical

The study of DPPH (1,2-Diphenyl-2-Picrylhydrazyl) radical scavenging activity is a standard examination in antioxidant activity. This technique is a fast method used to investigate radical scavenging activity of specific compounds [13].

The effects of free radical scavenging on Schiff base derivatives with (DPPH) radical were assessed with different concentrations of (100, 200, 300, 400 and 500 mg/ml) of the test compound in (1 ml) DMF. After that, we added (1 ml) of 0.4 mM of methanol solution of (DPPH). The solution must then stirred carefully for half of hour in the time of incubation period at room temperature. The antiradical power of an antioxidant of the scavenging ability is determined by measuring the decrease in the absorbance of DPPH at 517 nm. The standard drugs were used in this work is ascorbic acid. The following equation calculates the percentage inhibition (I %) of free radical production from (DPPH).

$$\text{DPPH Scavenging Ability (\%)} = \frac{\text{Abscontrol} - \text{Abssample}}{\text{Abscontrol}} \times 100$$

4 Results and Discussion

Schiff base (A₁ - A₄) was prepared by reaction of cytosine with three substitute of benzaldehyde (ortho chloro, meta hydroxyl), (para N,N-dimethylamino benzaldehyde) and the fourth aldehyde is furfural. These products were used **first time** to prepare four ring heterocyclic compound by treating it with substituting acid chloride (B₁ - B₄). In the **second time** we prepared from it five ring heterocyclic compound by reaction with aglyc heterocyclic ring₄. Lastly, we prepared seven rings heterocyclic compound by reaction with maleic anhydride (D₁ - D₄). These compounds were identified by some technical instrument like IR, ¹H NMR, and ¹³C NMR spectroscopic. Firstly we use IR techniques for the compounds (B₁), (B₂), (B₃), (B₄), (C₁), (C₂), (C₃), (C₄), (D₁), (D₂), (D₃) and (D₄), the value of IR is appear in below table (5):

The second techniques that be used in this work is (¹³ C NMR) spectrometric for the compounds (B₁), (B₃), (C₂) and (D₄) as show in table (6) [12].

Finally, the third techniques that be used in this research is (¹H NMR) spectroscopic for the compounds (B₁), (B₃),

(C₂) and (D₄) as show in below table 7.

According to Figure 1, the compounds (A₁ - A₄) show the same activity of scavenging of free radical except the compound A₃ where the activity is lower than the others. It was because of some hindrance effect of two methyl group on (N) comparing with other group which is alone and less hindrance. On the other hand, as showing in Figure 2, the activity of compound B₃ is higher than doublets in comparing with compound B₁ because of the different functional group on four ring heterocyclic. Furthermore, Figure 3 shows that the %DPPH radical activity in the compound (C₁, C₃) is similar approximately. Moreover, Figure 4 illustrates that the activity of the compound D₁ is higher than D₃.

5 CONCLUSION

In the first step of our research we prepare Schiff base (A₁ - A₄), and from it in the second step we synthesis three type of compounds four, five and seven heterocyclic compounds (B₁ - D₄). In the final step of this study after the analysis and identification of some compounds with (IR, ¹H NMR, ¹³C NMR) spectroscopic. The IR ν (cm⁻¹) in the compound (B₁, B₂, B₃, B₄, C₁, C₂, C₃, C₄, D₁, D₂, D₃, D₄) of (C=N) bond show value between (1560-1672), while the (C=C) bond show (1425-1506), but the (C-H_{Aromatic}) show value between (3010-3093), the Bond (O=C-O_{Cytosine}) show approximate value between (1630-1641). The ¹H NMR for the compounds (B₁), (B₃), (C₂) and (D₄) appear that a signal for (1H,d) at (4-4.7) ppm, and another signal for (1H,d) show at (4.3-4.5) ppm, the signal of (1H,s)_{of cytosine} show tow value at (3.8) and (4.1)ppm, the signal of (1H,d)_{quartet ring} appear at (3.7) and (3.9) ppm, while the signal for (2H,s)_{azole and seven ring} appear at (2.8) ppm only. The proton NMR for (B₁, B₃, C₂) show signal of (1H, dd)_{benzene} at (7.1-7.5), (6.5-6.9) and (7.1-7.6) ppm. The ¹³C NMR for the compounds (B₁), (B₃), (C₂) and (D₄) show these value ppm: the bond (C-N) in these compound show signal at (65,66,42,51) ppm, (C=O)_{cytosine} show the signal at (195) ppm for (B₁), (B₃), (C₂) and (197) ppm for (D₄).

The bond (C=C) show signal between (118-146) ppm, the bond (C-N)_{quartet ring} show signal at (72,71), while the bond (C-N)_{azole and seven ring} show signal at (55,58) ppm. We study the Antioxidant Assay by Scavenging activity of (DPPH) Radical with the compound (A₁ - A₄), (B₁, B₃),

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(C₁ , C₃) , (D₁ , D₃) in several concentration (100, 200, 300, 400 and 500 mg/ml) . According to the figure (1 – 5) show that all these compounds have a good Scavenging activity with (DPPH).

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Significance of Research

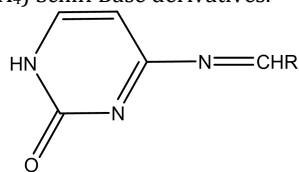
Cytosine is one of four nitrogen base who has important role in a variety range of application. The goal of this study is synthesis of Schiff base derived from cytosine in good yield and then from it we synthesis (4,5 and 7) heterocyclic ring, after identification of some new Schiff base and heterocyclic ring with the spectroscopic methods like IR, H¹ NMR and C¹³ NMR, then we go to with some of these compounds and applied it to show the activity of Radical Scavenging with the compound DPPH (of 1,2-Diphenyl-2-Picrylhydrazyl). These compounds appear that they have a good Scavenging activity with (DPPH).

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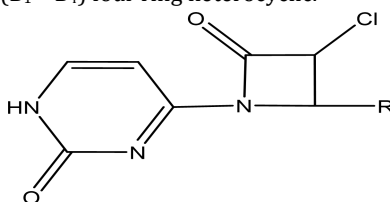
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Table 1. The properties of the compound (A₁ – A₄) Schiff Base derivatives.



Comp. No.	R	Name of Compound	Color	Reaction Time	M.P. °C	Yield
A ₁		4-((2-chlorobenzylidene) amino) pyrimidin-2(1H)-one	Milky	4 min	110 – 113	73
A ₂		4-((3-hydroxybenzylidene) amino) pyrimidin-2(1H)-one	White Green	3.5 min	97 -100	90
A ₃		4-((4-(dimethylamino) benzylidene)) (amino) pyrimidin-2(1H)-one	Yellow	3 min	150 – 152	82
A ₄		4-((furan-2-ylmethylene) amino) pyrimidin-2(1H)-one	White	4 min	176 – 178	93

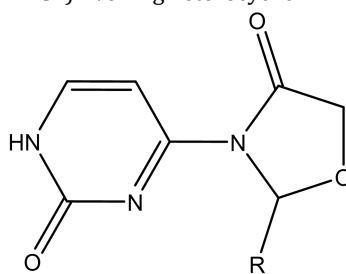
Table 2. The properties of the compounds (B₁ – B₄) four ring heterocyclic.



Comp. No.	R	Name of Compound	Color	M.P. °C	Yield
B ₁		4-(3-chloro-2-(2-chlorophenyl)-4-oxoazetidin-1-yl) pyrimidin-2(1H)-one	White	250 - 253	68
B ₂		4-(3-chloro-2-(3-hydroxyphenyl)-4-oxoazetidin-1-yl) pyrimidin-2(1H)-one	White Yellow	140 – 143	65
B ₃		4-(3-chloro-2-(4-((dimethylamino)phenyl)-4-oxoazetidin-1-yl) pyrimidin-2(1H)-one	Orange Yellowish	165 – 168	75
B ₄		4-(3-chloro-2-(furan-2-yl)-4-oxoazetidin-1-yl) pyrimidin-2(1H)-one	Yellow	260 dec.	60

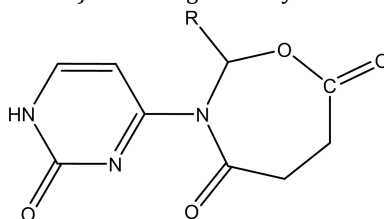
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Table 3. The properties of the compounds (C1 – C4) five ring heterocyclic.



Comp. No.	R	Name of Compound	Color	M.P. ° C	Yield
C ₁		2-(2-chlorophenyl)-3-(2-oxo-1,2- dihydropyrimidin-4-yl) oxazolidine-4-one	White	170 – 172	68
C ₂		2-(2-hydroxyphenyl)-3-(2-oxo-1,2- dihydropyrimidin-4-yl) oxazolidine-4-one	Light Yellow	178 – 180	79
C ₃		2-(4-(dimethylamino) phenyl)-3-(2-oxo-1,2- dihydropyrimidin-4-yl) oxazolidine-4-one	Milky	Oily	65
C ₄		2-(furan-2-yl)-3-(2-oxo-1,2- dihydropyrimidin-4-yl) oxazolidine-4-one	Light Brown	185 dec.	83

Table 4. The properties of the compound (D1 – D4) seven ring heterocyclic.



Comp. No.	R	Name of Compound	Color	Reaction Time	M.P. ° C	Yield
D ₁		2-(2-chlorophenyl)-3-(2-oxo-1,2-dihydro pyrimidin-4-yl)-1,3-oxazepane-4,7-doine	Milky	3 min	96 – 99	63
D ₂		2-(2-hydroxyphenyl)-3-(2-oxo-1,2-dihydro pyrimidin-4-yl)-1,3-oxazepane-4,7-doine	Brown	4 min	150 – 153	85
D ₃		2-(4-(dimethylamino) phenyl)-3-(2-oxo-1,2- dihydro pyrimidin-4-yl)-1,3-oxazepane-4,7- doine	Orange	3.5 min	108 – 11	76
D ₄		2-(furan-2-yl)-3-(2-oxo-1,2-dihydro pyrimidin-4-yl)-1,3-oxazepane-4,7-doine	Light Yalow	3 min	165 – 168	89

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Table 5.

The Bond	(C=N)	(C=C)	(C - H _{Aro.})	(O=C - N _{of cytosine})	(C - Cl)	Compound
IR ν (cm ⁻¹)	1588	1506	3045	1630	760	B ₁
The Bond	(C=N)	(C=C)	(C - H _{Aro.})	(O=C - N _{of cytosine})	(C - OH)	Compound
IR ν (cm ⁻¹)	1615	1472	3065	1640	3256	B ₂
The Bond	(C=N)	(C=C)	(C - H _{Aro.})	(O=C - N _{of cytosine})	[C - N (CH ₃) ₂]	Compound
IR ν (cm ⁻¹)	1579	1425	3025	1641	845	B ₃
The Bond	(C=N)	(C=C)	(C - H _{Aro.})	(O=C - N _{of cytosine})	- C - O - C - furan	Compound
IR ν (cm ⁻¹)	1590	1462	3019	1638	1250	B ₄
The Bond	(C=N)	(C=C)	(C - H _{Aro.})	(O=C - N _{of cytosine})	(C - Cl)	Compound
IR ν (cm ⁻¹)	1560	1471	3010	1632	780	C ₁
The Bond	(C=N)	(C=C)	(C - H _{Aro.})	(O=C - N _{of cytosine})	(C - OH)	Compound
IR ν (cm ⁻¹)	1609	1458	3093	1635	3230	C ₂
The Bond	(C=N)	(C=C)	(C - H _{Aro.})	(O=C - N _{of cytosine})	[C - N (CH ₃) ₂]	Compound
IR ν (cm ⁻¹)	1603	1490	3040	1641	860	C ₃
The Bond	(C=N)	(C=C)	(C - H _{Aro.})	(O=C - N _{of cytosine})	- C - O - C - furan	Compound
IR ν (cm ⁻¹)	1672	1450	3060	1637	1256	C ₄
The Bond	(C=N)	(C=C)	(C - H _{Aro.})	(O=C - N _{of cytosine})	(C - Cl)	Compound
IR ν (cm ⁻¹)	1610	1470	3075	1634	775	D ₁
The Bond	(C=N)	(C=C)	(C - H _{Aro.})	(O=C - N _{of cytosine})	(C - OH)	Compound
IR ν (cm ⁻¹)	1611	1460	3072	1640	3290	D ₂
The Bond	(C=N)	(C=C)	(C - H _{Aro.})	(O=C - N _{of cytosine})	[C - N (CH ₃) ₂]	Compound
IR ν (cm ⁻¹)	1590	1473	3057	1639	855	D ₃
The Bond	(C=N)	(C=C)	(C - H _{Aro.})	(O=C - N _{of cytosine})	- C - O - C - furan	Compound
IR ν (cm ⁻¹)	1560	1464	3087	1633	1247	D ₄

Table 6.

The Bond	(C - N)	(C=C)	(C = O) Cytosine	(C - N) Quartet ring	C - C	(C=O)	(C=C) benzene ring	C - Cl	- CH ₃	Compound	from
¹³ C ppm	65	122	195	72	41	79	130 - 145	85	-	B ₁	Fig. 6
The Bond	(C - N)	(C=C)	(C = O) Cytosine	(C - N) Quartet ring	C - C	(C=O)	(C=C) benzene ring	C - N	- CH ₃	Compound	From
¹³ C ppm	66	146	195	71	42	186	126 - 143	75	36	B ₃	-
The Bond	(C - N)	(C=C)	(C = O) Cytosine	(C - N) Azule ring	C - O	(C=O)	(C=C) benzene ring	C - OH	- CH ₃	Compound	From
¹³ C ppm	42	121	195	55	75	205	115 - 139	90	-	C ₂	Fig. 8
The Bond	(C - N)	(C=C)	(C = O) Cytosine	(C - N) Seven ring	C - C	(C=O)	C - O	(C=C) furan ring	C - O furan	Compound	From
¹³ C ppm	51	118	197	58	36	189	65	132	81	D ₄	Fig. 10

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Table 7.

Position	a (1H,d)	b (1H,d)	c (1H, s) cytosine	d (1H,d) quartet ring	e (1H , d)	f (1H ,dd) benzene	h (1H , dd)	g (1H ,s)	Compound	From
Signals ppm	4	4.3	3.8	3.7	3.2	7.1 – 7.5	7.6 – 8.0	-	B ₁	Fig. 7
Position	a (1H,d)	b (1H,d)	c (1H,s) cytosine	d (1H,d) quartet ring	e (1H , d)	f (1H ,dd) benzene	h (1H , dd)	g (1H ,s)	Compound	From
Signals ppm	4.2	4.4	4.1	3.9	3.1	6.5 – 6.9	7.1 – 7.5	-	B ₃	-
Position	a (1H,d)	b (1H,d)	c (1H,s) cytosine	d (2H,s) azole ring	e (1H , s)	f (1H ,dd) benzene	h (1H , dd)	g (1H ,s)	Compound	From
Signals ppm	4.7	4.5	4.1	2.8	3.2	7.1 – 7.6	7.9 – 8.0	10.1	C ₂	Fig. 9
Position	a (1H,d)	b (1H,d)	c (1H,s) cytosine	d (2H,s) seven ring	e (2H , t)	f (1H , s) furfural	h (1H , d)	g (1H , t)	Compound	From
Signals ppm	4.1	4.4	3.8	2.8	2.6	2,9	6.5	6.1	D ₄	Fig. 11
Position	I (1H, d)	-	-	-	-	-	-	-	Compound	From
Signals ppm	6.7	-	-	-	-	-	-	-	D ₄	Fig. 11

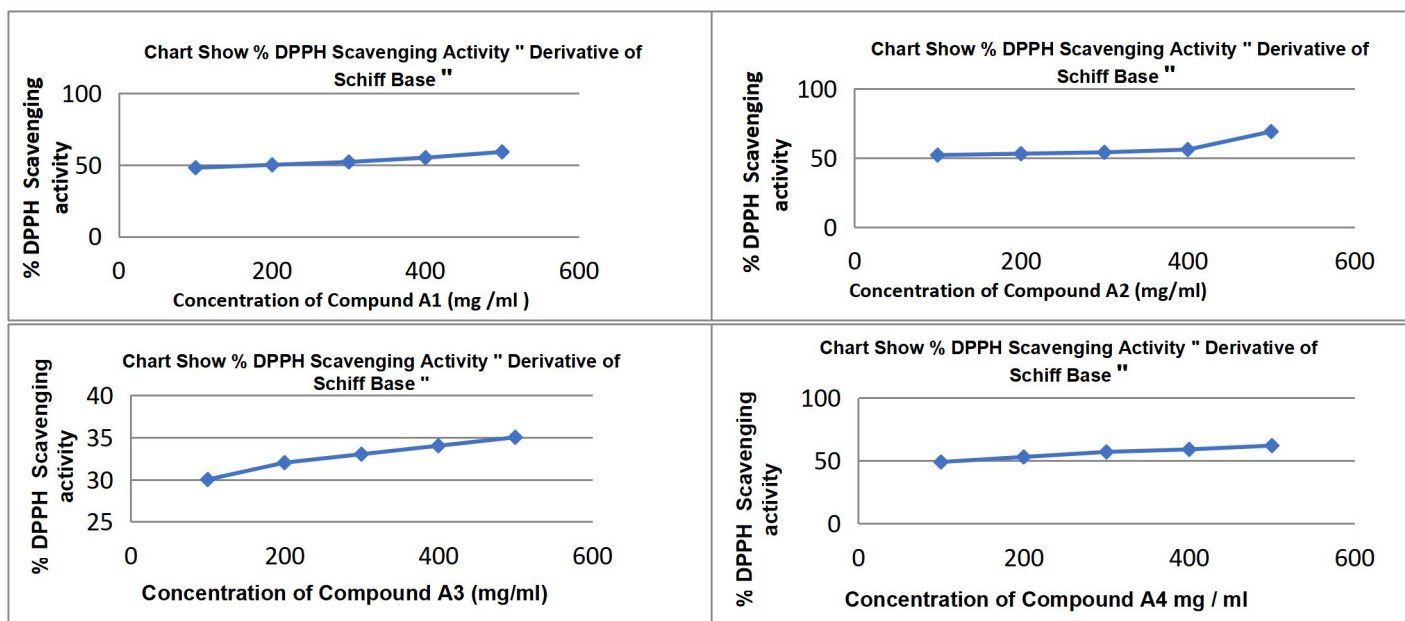


Fig. 1. The (% DPPH) Radical Scavenging Activity for Compound (A₁ – A₄).

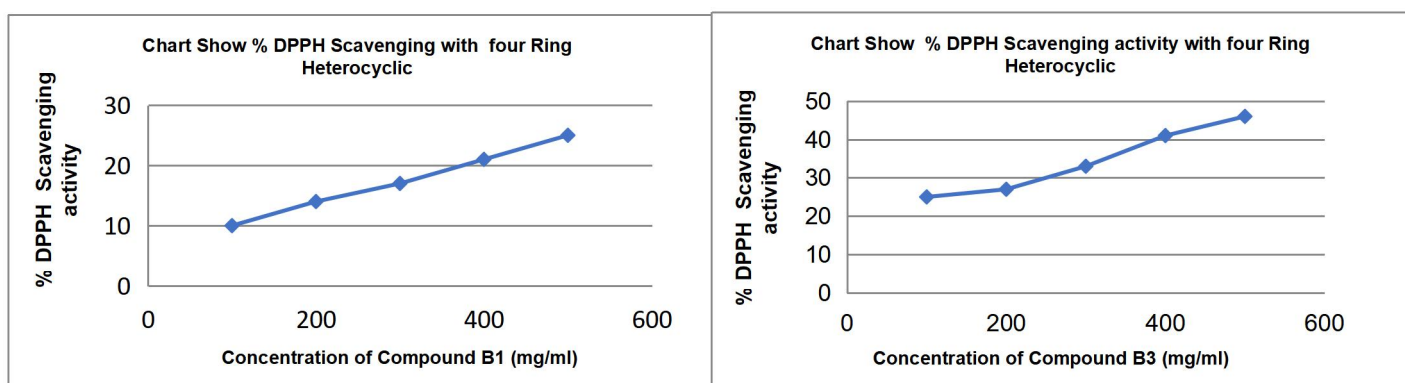


Fig. 2. The (% DPPH) Radical Scavenging Activity for Compound (B₁, B₃).

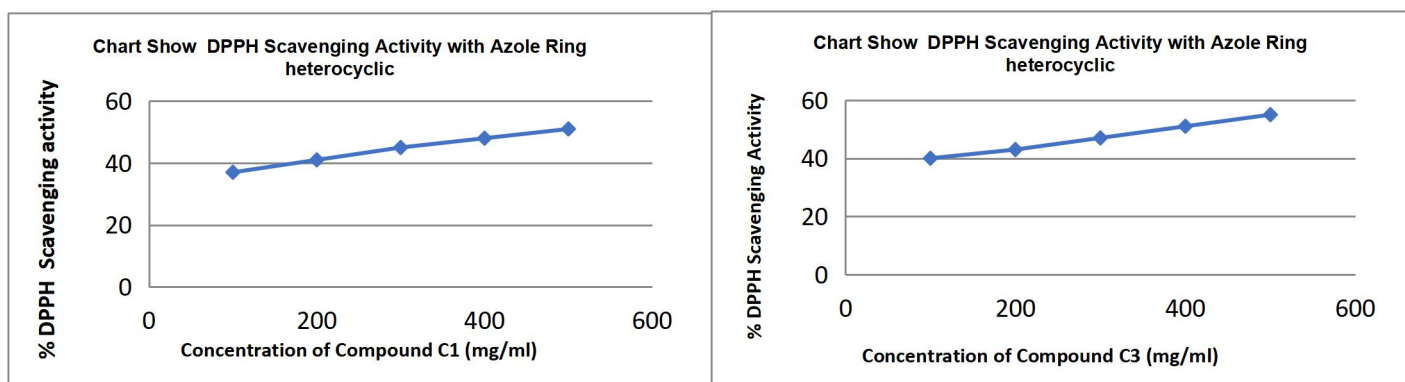


Fig. 3. The (% DPPH) Radical Scavenging Activity for Compound (C₁, C₃).

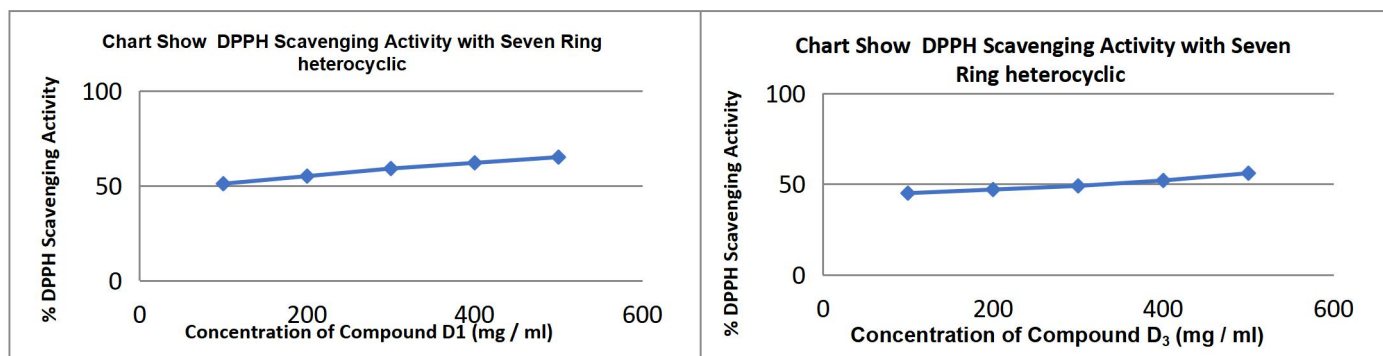


Fig. 4. The (% DPPH) Radical Scavenging Activity for Compound (D1, D3).

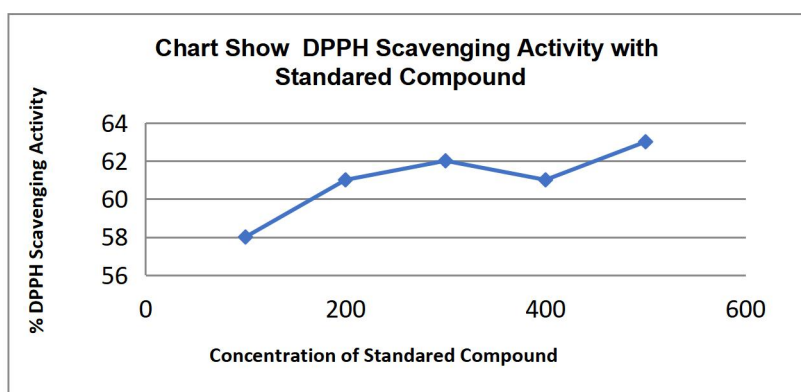


Fig. 5. The (% DPPH) Radical Scavenging Activity with Standard Compound.

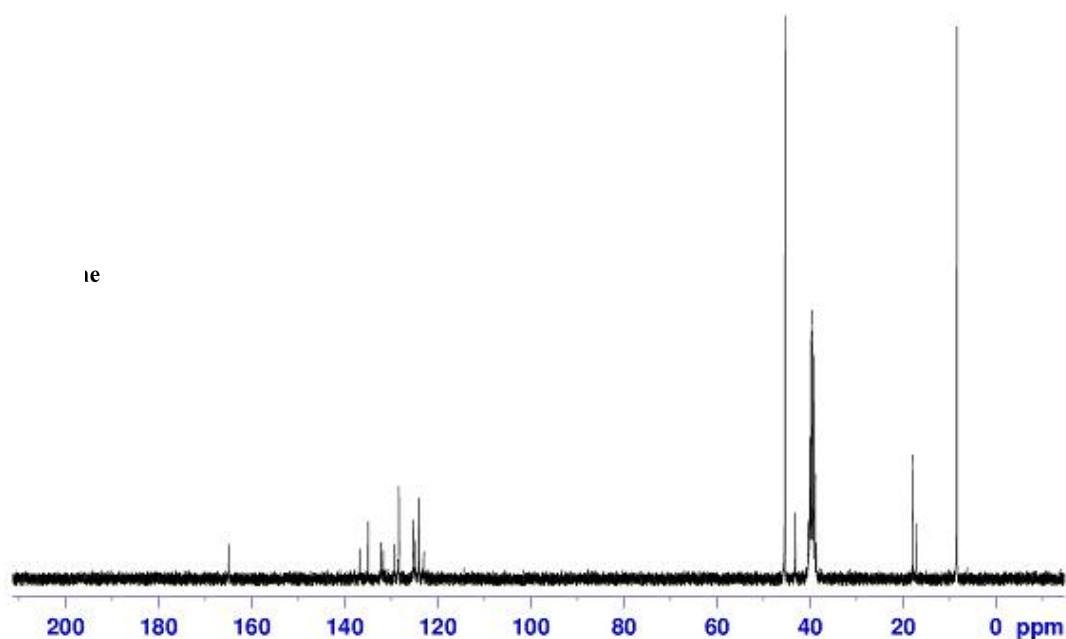


Fig. 6. The ^{13}C NMR for compound B1.

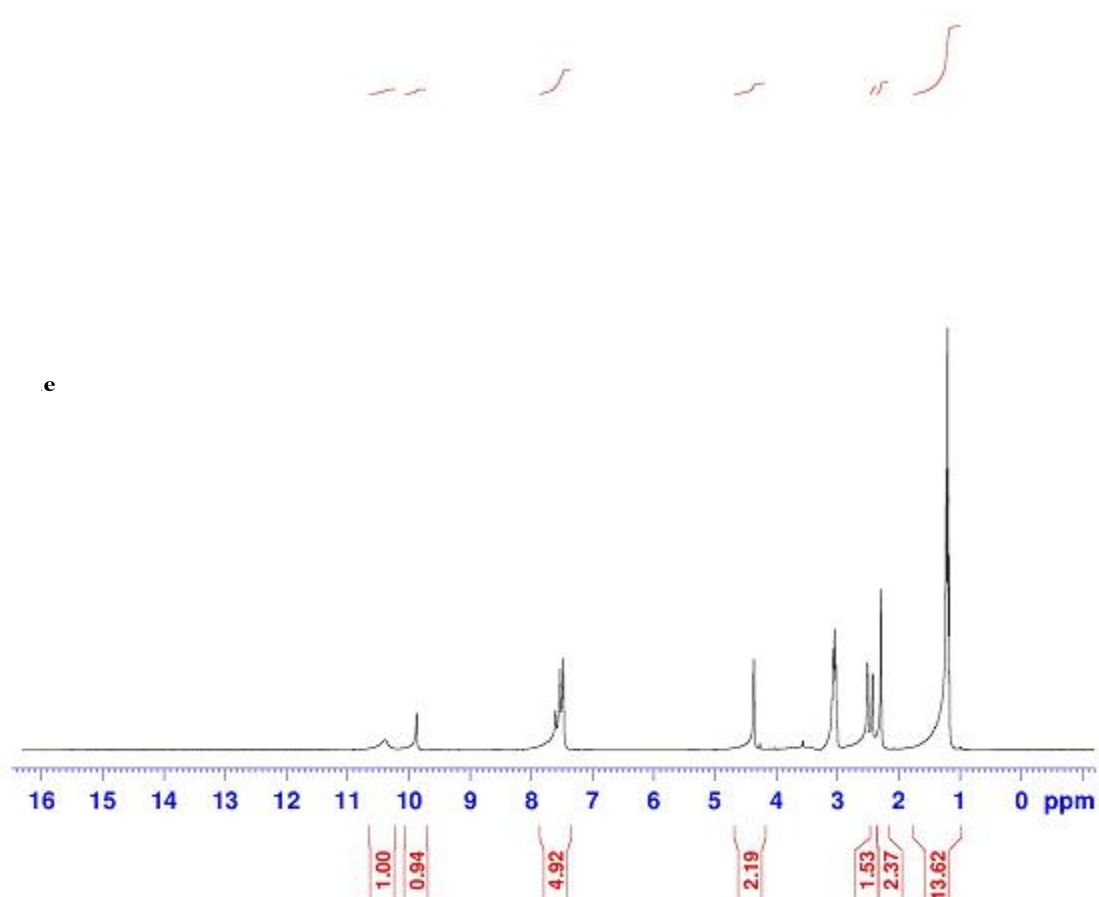


Fig. 7. The ¹H NMR for compound B1.

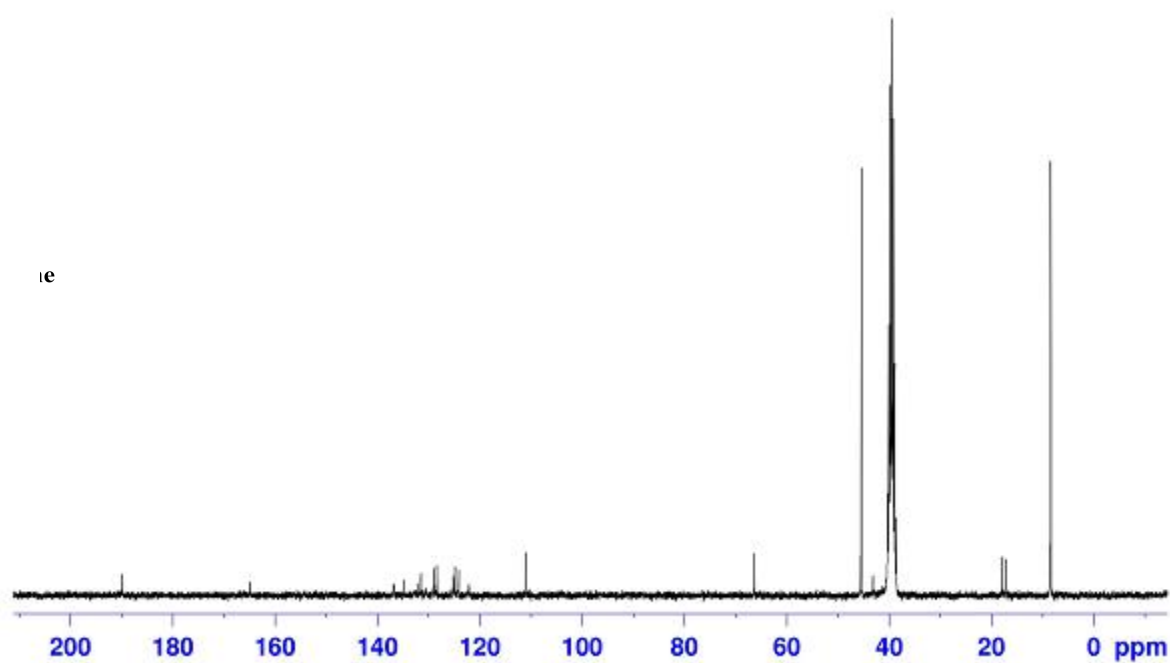


Fig. 8. The ¹³C NMR for compound C2.

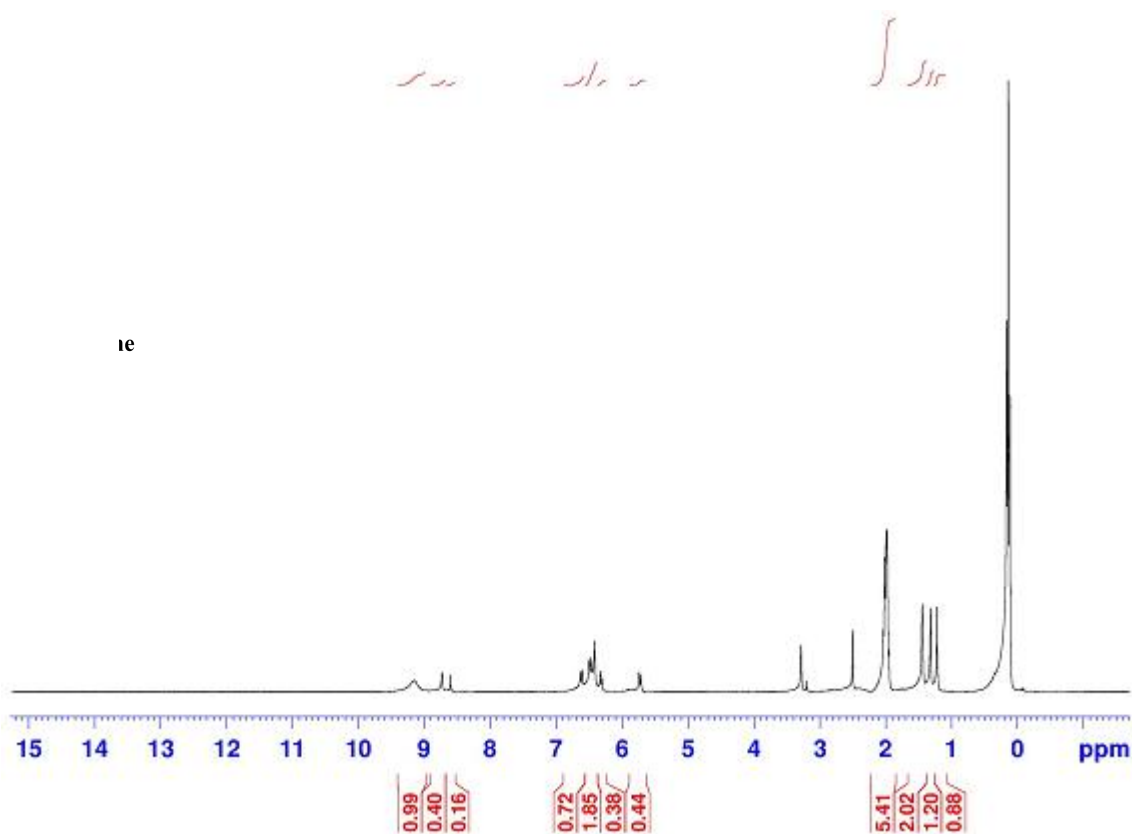


Fig. 9. The ^1H NMR for compound C2

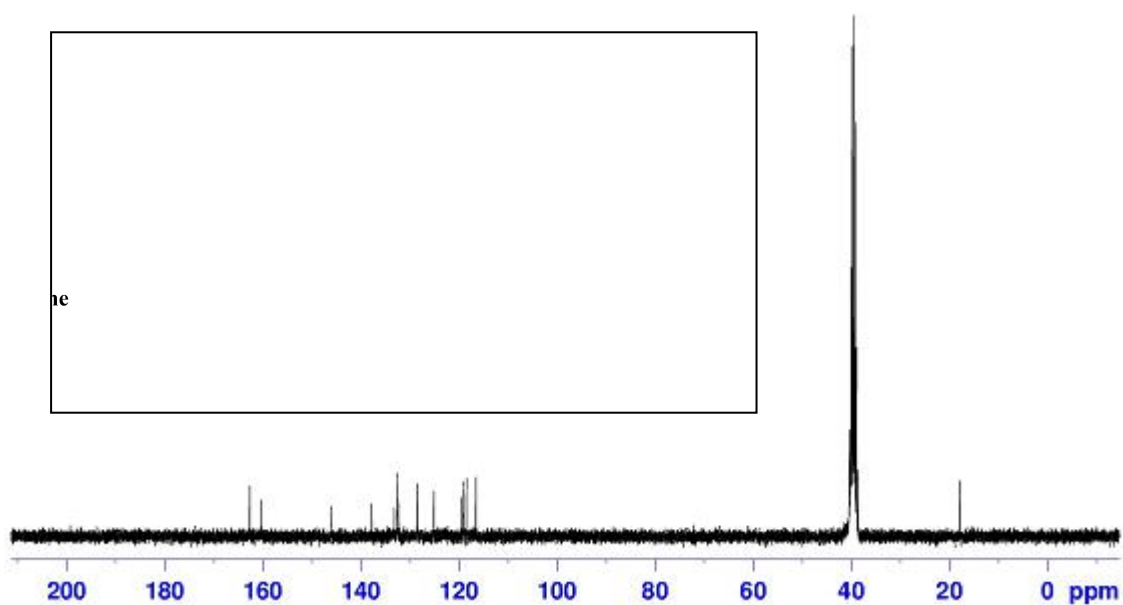


Fig. 10. The ^{13}C NMR for compound D4.

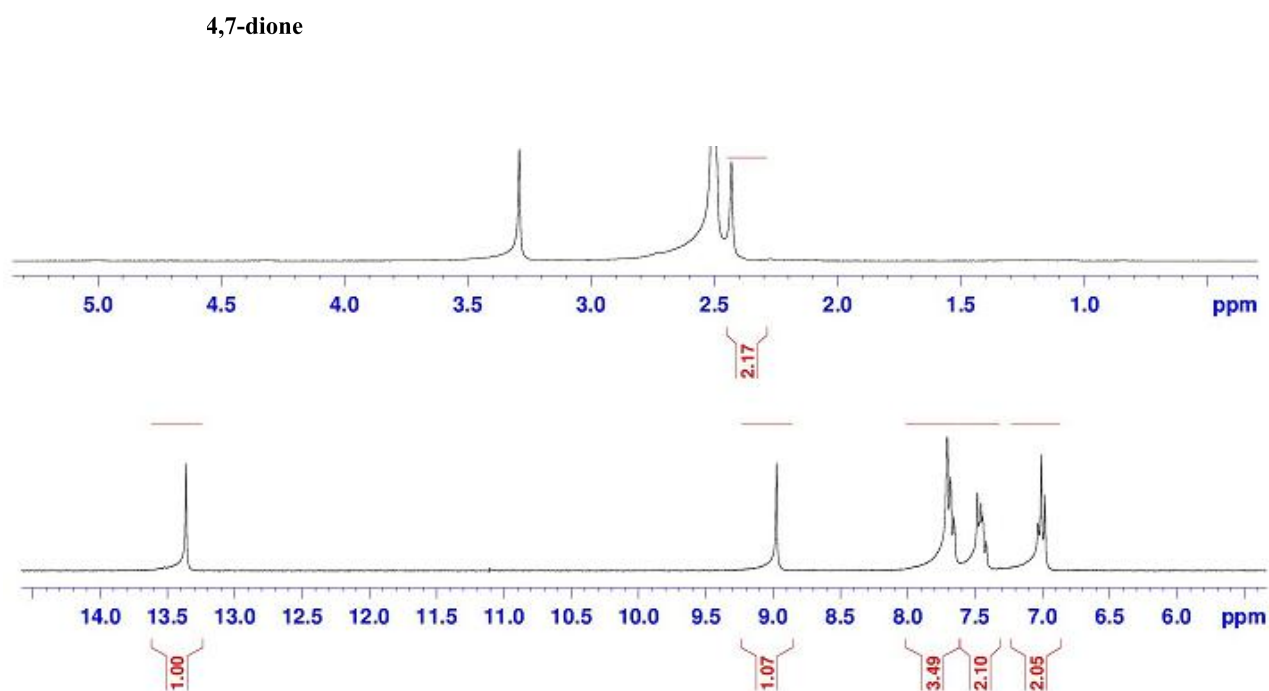


Fig. 11. The ¹H NMR for compound D4.