

# Synthesis and Biological Studies of 4-Methyl-7-Ethylcoumarin Derivatives Containing Azo Group

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## Abstract

New 4-methyl-7-ethylcoumarin derivatives bearing the azo group were synthesized through series of sequential reactions and tested for their biological activity. Starting from 4-methyl-7-ethylcoumarin prepared from a reaction of m-ethyl phenol and ethyl acetoacetate by pechmann condensation reaction, nitration of 4-methyl-7-ethyl coumarin using nitric acid was carried out in the presence of sulfuric acid to produce one isomer from 4-methyl-7-ethyl-8-nitrocoumarin under the cold condition at (2-5C°). Then reducing nitro group used iron metal in an acidic medium to form corresponding amino coumarin, which was converted to azo dyes by reacting its diazonium salt with different phenol derivatives. Mass, FT-IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, UV spectra, and TLC chromatography signposted the preparation compounds. The synthetic compounds' biological activities were evaluated against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) at 1×10<sup>-3</sup>M. It was found that compounds 4 and 6 have a broad spectrum against different types of bacteria, *Staphylococcus aureus*, and *Escherichia coli*, compared to the standard drug vancomycin. In contrast, all compounds showed moderate activity against fungi compared with nystatin. The newly synthesized compounds also showed powerful antioxidants compared with ascorbic acid as a standard, especially compound 7, which showed high effectiveness as an antioxidant compared to the same reference Ascorbic Acid.

**Keywords:** Amino coumarin, Anti-microbial activity, Azo dyes, coumarin, Diazonium salt.

## Introduction

Chemically, coumarins (2H-1-benzopyran-2-one) belong to the subgroup of lactones<sup>1, 2</sup>. Simple coumarins, furanocoumarins, pyrano coumarins (linear and angular types), dihydrofurano coumarins, phenyl coumarins, and bicoumarins are the six essential categories that can be used to classify natural coumarins<sup>3, 4</sup>. The first isolated parent coumarin was from Tonka bean in (*Dipteryx odorata*) 1820 by Vogel<sup>5, 6</sup>. Coumarins are extensively distributed in nature and are secondary

metabolites in various plant parts, including the roots, flowers, leaves, peels, seeds, and fruits<sup>7, 8</sup>. In contrast, most recovered coumarins exhibit biological activity, and coumarin derivatives are increasingly being synthesized because extracting them from plants is time-consuming and uneconomical (many operation steps are required to have the final product)<sup>9,10</sup>.

There are numerous ways to make coumarins, including the Perkin reaction, Vilsmeier-Haack-and-Suzuki cross-coupling process<sup>11, 12</sup>, Knoevenagel condensation, Pechmann condensation, Wittig reaction, Baylis-Hillman reaction, Knoevenagel condensation<sup>13, 14</sup>. This study used a series of sequential processes to create coumarin derivatives containing azo compounds (nitration reaction, reduction reaction, and diazonium salt formation reaction)<sup>15, 16</sup>. Azo dye compounds are utilized in various industries, including nonlinear optics, liquid crystal displays<sup>17,18</sup>, cosmetics, food coloring, polymers, optical switches, and acid-base indicators<sup>19-21</sup>. Additionally, heteroaryl-based azo dyes have been studied for their potential biological applications, such as antioxidant<sup>22-24</sup>, antimicrobial<sup>25-28</sup>, antitumor<sup>29</sup>, antidiabetics<sup>30</sup>, and antiviral activities<sup>31</sup>. A diazonium salt, created when a primary amine

acts as an electrophile<sup>32</sup>, was typically coupled with a nucleophilic coupling component, such as an amine or phenol, to create an azo dye<sup>33</sup>. Compared to simple aromatic compounds, these azo dye compounds create a wide range of colors over the visible spectrum because the heterocyclic azo dyes have a substantial bathochromic impact<sup>34</sup>. Different colors can be made by altering the functional groups added to the azo molecule<sup>35</sup>. Also, compared to other dye molecules, these dyes are far more stable and resistant to light deterioration over time<sup>36</sup>. In this work, we will be able to synthesized of new 7-ethyl-4-methylcoumarin derivatives containing azo group by using nitration reaction applied on 7-ethyl-4-methylcoumarin in an easy methods with a good yield after reducing it to corresponding amine including this in the synthesis of many azo chemical compounds *which* could be antioxidant, antifungal, and antibacterial.

## Materials and Methods

The starting chemical compounds Fluka or Aldrich were used to obtain all utilized chemicals. Melting points (MP) were measured with a Thomas capillary melting point device using Gallenkamp in open glass capillaries<sup>37</sup>. The SHIMADZU FTIR-8400 Fourier transform infrared spectrophotometer was used to capture KBr disc FTIR spectra in the Department of Chemistry, University of Baghdad. All major components were pure and readily available on the market<sup>38</sup>. <sup>1</sup>H-NMR and 500 MHz spectrometer recorded <sup>13</sup>C-NMR spectra<sup>39</sup> in the College of Education of Pure Sciences, the University of Basrah, and the University of Isfahan. Dimethyl sulfoxide solvent (DMSO- d<sub>6</sub>) was used to record Agilent Technologies model ultra-shield nuclear magnetic resonance (NMR) spectra, and the chemical shifts are given in  $\delta$  (ppm) using tetramethyl silane (TMS) as references<sup>40,41</sup>.

### Preparation of 7-ethyl-4-methylcoumarin<sup>42</sup>.

The title compound was prepared as literature. The physical properties and FT-IR of the compound [1] are listed in Table 1.

### Synthesis of 7-ethyl-4-methyl-8-nitrocoumarin **2**<sup>43</sup>

A mixture of compound [1] (1g, 0.0053 mol) and H<sub>2</sub>SO<sub>4</sub> (7.5 ml) was stirred for 15 min, then the nitration reagent (1:3) mixture from HNO<sub>3</sub>:H<sub>2</sub>SO<sub>4</sub> was added dropwise to the mixture at a temperature not exceeding 5°C; stirring for 4 hrs, then stirring at

room temperature for 4 hrs as well. The TLC monitored the reaction. After the reaction, it was poured over ice, left for several hrs, filtered by a Buechner funnel, and the filtrate was washed with cold distilled water. The physical properties and FT-IR of the compound [2] are listed in Table.1 and Table.2, respectively.

### Synthesis of 7-ethyl-4-methyl-8-aminocoumarin.<sup>44</sup>

A mixture of Iron 0.3 g, 2.3 ml of water, and 1.2 ml of glacial acetic acid was refluxed for 15 min. After that, compound [2] (1g, 0.004 mol.) solution in 15 ml of ethanol was added to the mixture and refluxed for 10 hrs. The mixture was filtered to remove the remaining iron, and then sodium bicarbonate was added to the filtrate to neutralize the mixture. The mixture was left to evaporate the ethanol and washed with cold distilled water. The physical properties and FT-IR of the compound [3] are listed in Table 1 Table 2 respectively.

### Synthesis of 7-ethyl-4-methyl-8-substituted diazenylcoumarin derivatives<sup>45</sup>.

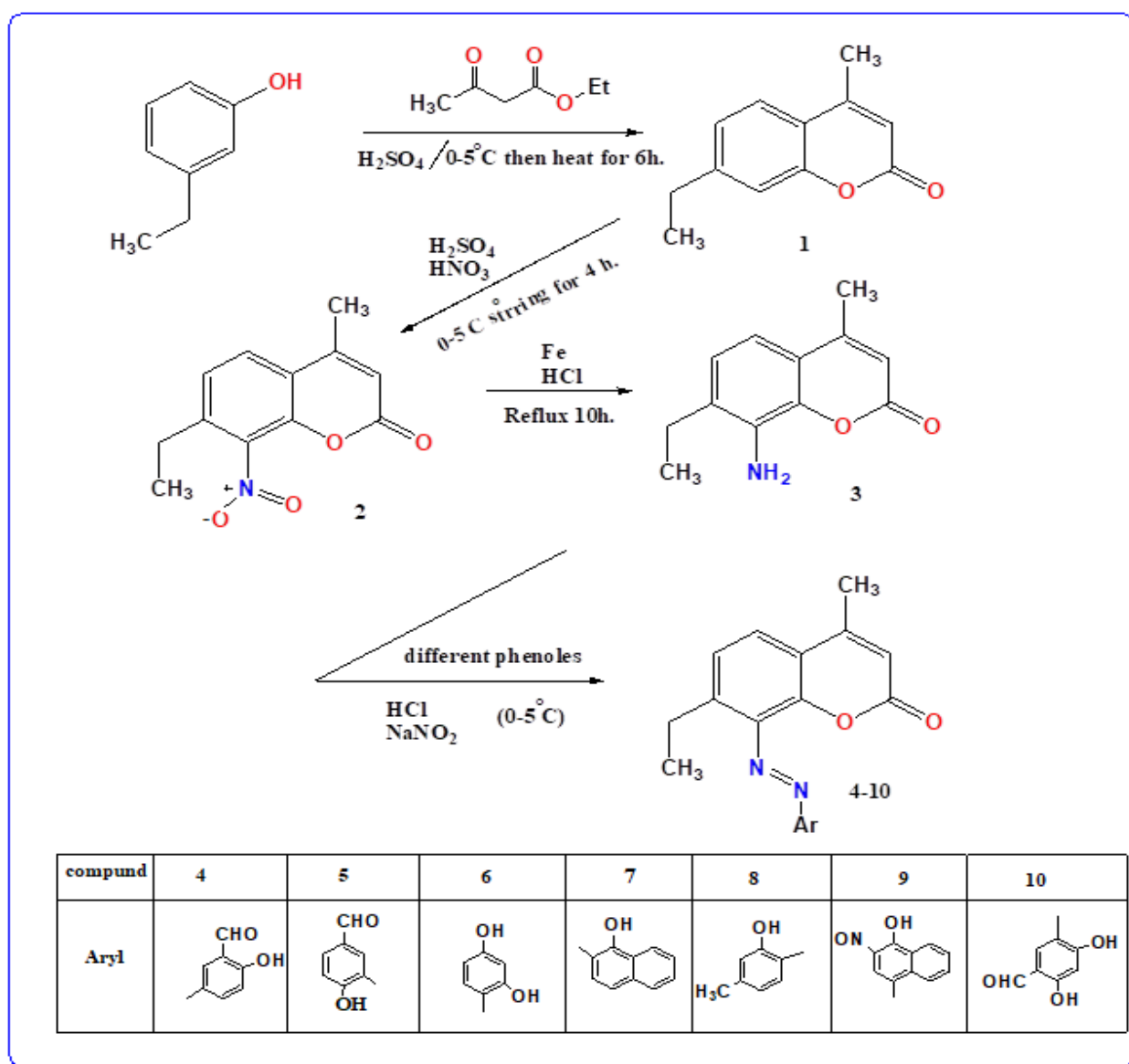
7-ethyl-4-methyl-8-aminocoumarin [3] (0.6 g, 0.002 mol) was dissolved in conc. HCl (5 ml) and water (2 ml) and stirred for 15 min. The solution was cooled to 0-5°C and was diazotized below 4°C in aqueous sodium nitrite (10 ml) with continuous stirring; the temperature was kept to 0-5°C for 30 min to form diazonium salt. Then the cooled

solution was added slowly by stirring a mixture of phenol derivatives in water (10 ml) at 0-4°C. The pH of the mixture must be kept at nine by neutralizing it with 10% NaOH aqueous solution;

## Results and Discussion

The research synthesized a new series of coumarin derivatives bearing the azo group through several sequential reactions, as shown in Scheme 1.

after stirring for 1hr, the mixture was filtered and washed with cold distilled water. The physical properties and FT-IR of compounds [4-9] are listed in Table 1 and 2, respectively.



**Scheme 1. Synthesis of 4-methyl-7-ethyl coumarin derivatives 4-10**

7-ethyl-4-methylcoumarin was prepared by reacting ethyl acetoacetate with 3-ethylphenol<sup>46</sup>. The FT-IR spectrum diagnosed the compound in Table 2, showing disappearance of the  $\nu(\text{OH})$  band and the appearance of a strong absorption band for a new  $\nu(\text{C}=\text{O})$  at  $1731\text{ cm}^{-1}$  for the lactone ring. In the second step, the nitration of 7-ethyl-4-

methylcoumarin is by using nitric acid in the presence of  $\text{H}_2\text{SO}_4$  conc. To produce 7-ethyl-4-methyl-8-nitrocoumarin, the temperature, reaction time, and amount of reactants were crucial for a pure compound with a high percentage yield. The FTIR spectrum Table 2 of this compound showed

two absorption bands  $\nu(\text{NO}_2)$  at  $1523 \text{ cm}^{-1}$  and  $1357 \text{ cm}^{-1}$  for asym. and sym. Respectively, Fig. 1.

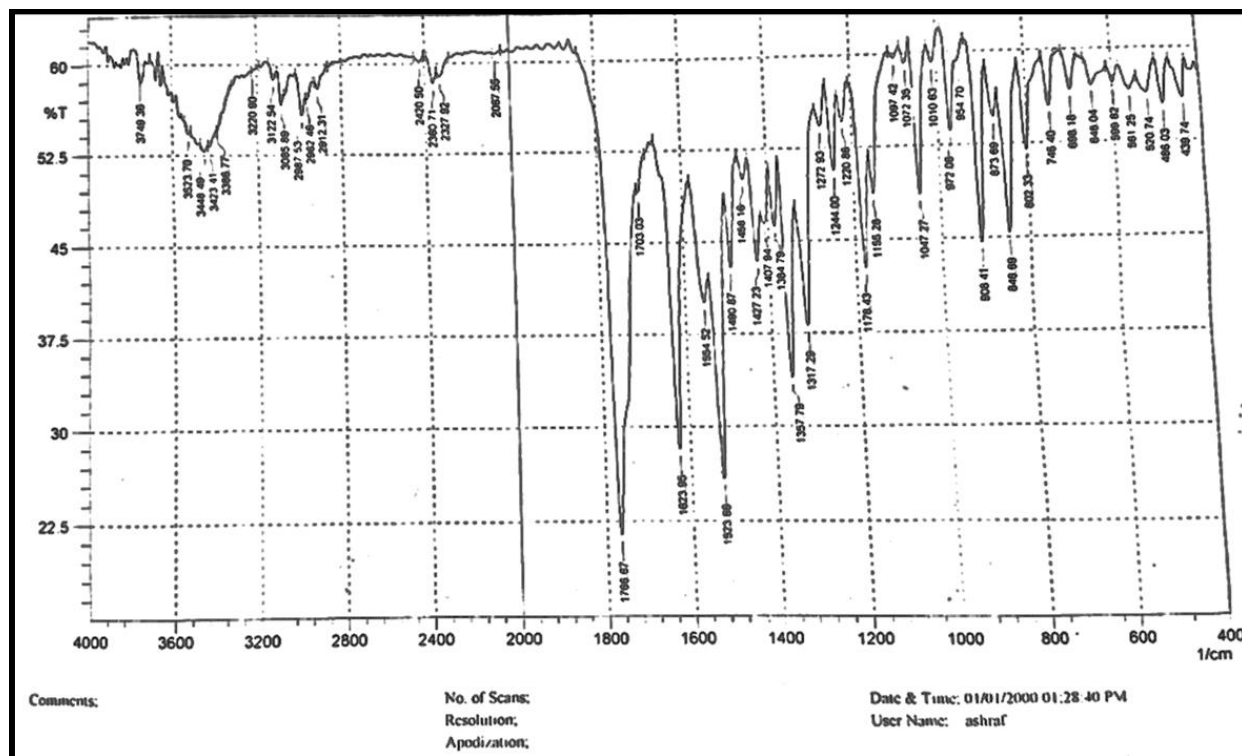


Figure 1. The FTIR spectrum of compound 2

$^1\text{H-NMR}$  spectrum Table, 3 of compound 2, showed a triplet signal at 1.2 ppm due to the methyl group ( $\text{CH}_3\text{-CH}_2$ ), a quartet signal at 3.3 ppm due to a methylene group ( $\text{CH}_3\text{-CH}_2$ ), a singlet signal at 2.4

ppm owing to ( $\text{CH}_3$ ) and multiplet signals at 8.2-6.5ppm due to aromatic rings (Ar-H) protons Fig. 2.  $^{13}\text{C-NMR}$  spectrum Table 3 showed a signal at 159.32 ppm for ( $\text{C=O}$ ) group Fig. 3.

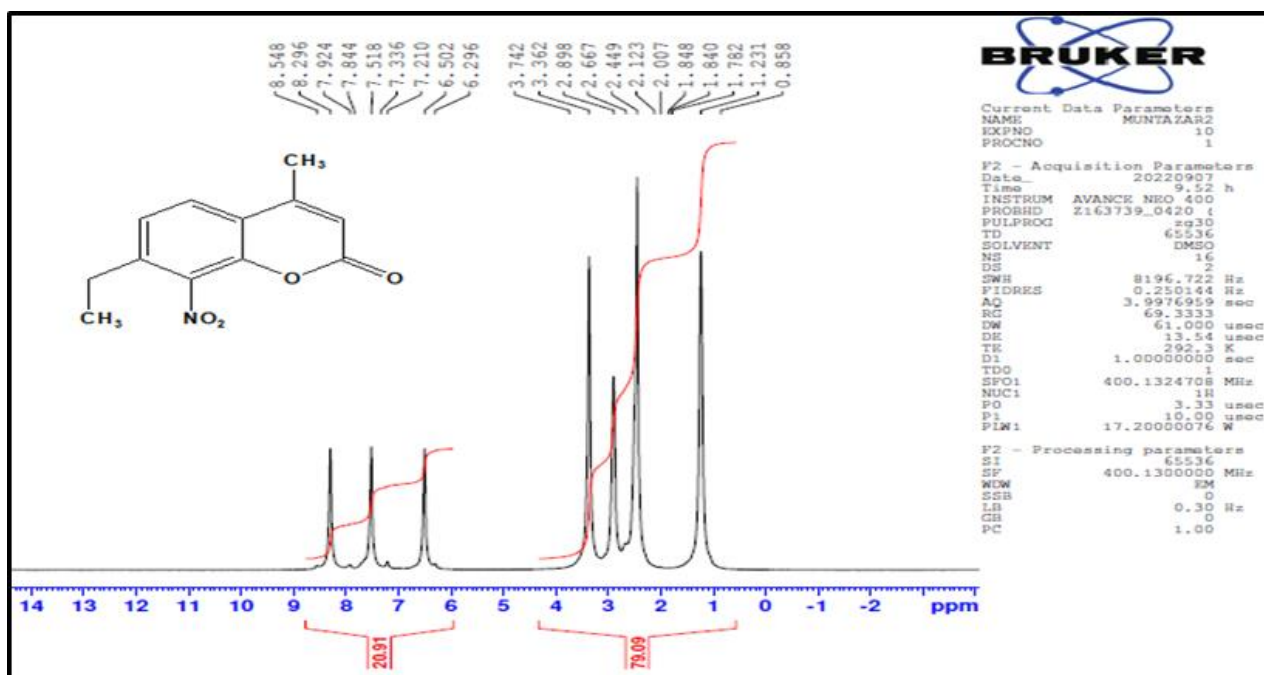


Figure 2. The  $^1\text{H-NMR}$  spectrum of compound 2

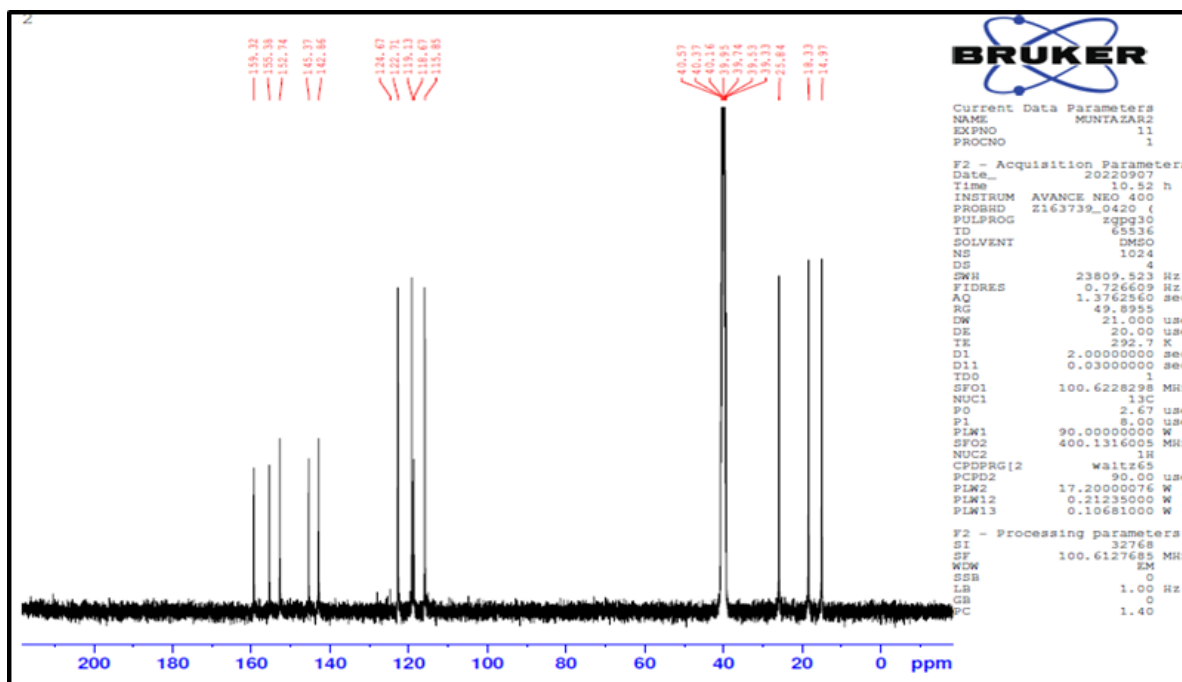


Figure 3. The  $^{13}\text{C}$ -NMR spectrum of the compound 2

Compound 2 was subjected to a reducing reaction by iron metal and hydrochloric acid to form 7-ethyl-4-methyl-8-aminocoumarin 3. The FTIR spectrum of compound 3 showed asymmetric and symmetric stretching bands of  $\nu(\text{NH}_2)$  absorption bands at  $3444\text{ cm}^{-1}$  asym. Moreover,  $3355\text{ cm}^{-1}$  sym. and stretching band of  $\nu(\text{C}=\text{O})$  at ( $1687\text{ cm}^{-1}$ ), the reason for the lower frequency for  $\nu(\text{C}=\text{O})$  of the lactone

ring because the effect of activated amino as releasing group which made the low order bond of the carbonyl group in comparing with nitro as an electron-withdrawing group in compound 2 which makes  $\nu(\text{C}=\text{O})$  at high frequency ( $1766\text{ cm}^{-1}$ )<sup>47</sup>. The  $^1\text{H}$ -NMR spectrum for the same compound showed a signal at 5.04 ppm for the  $\text{NH}_2$  group Fig. 4.

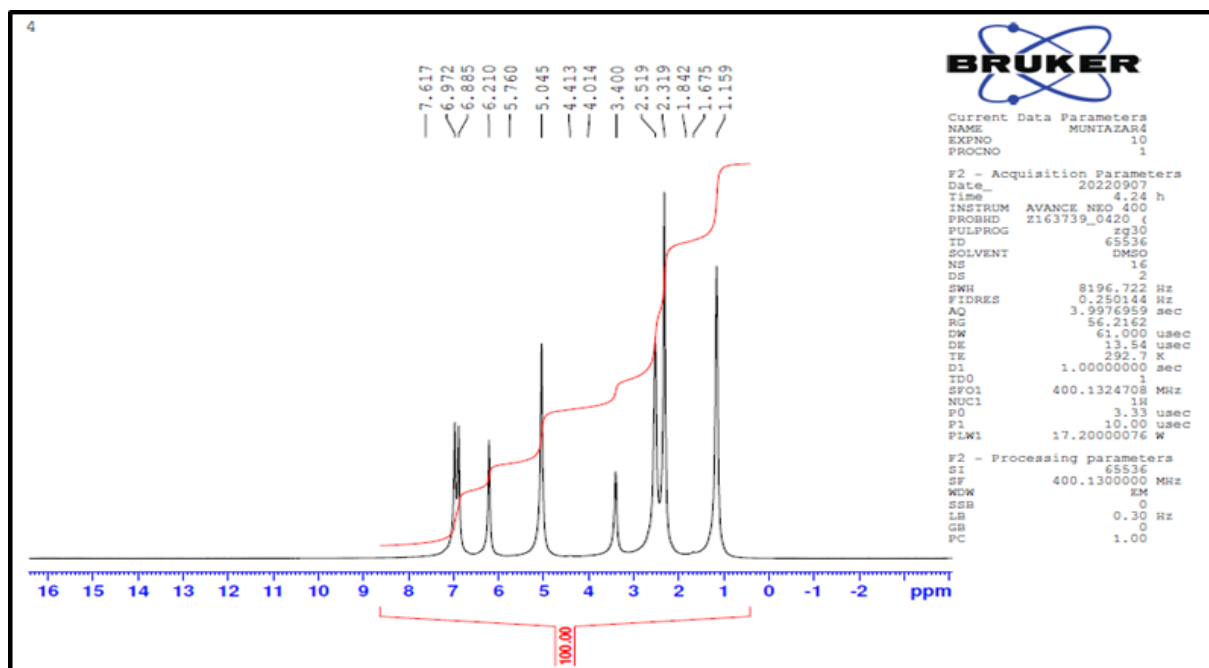


Figure 4. The  $^1\text{H}$ -NMR spectrum of compound 3

The mass spectrum for the same compound showed the value of  $[M+H]^+$  ion absorption signal, 204.1,

which was consistent with the theoretical value (204.09) for  $C_{12}H_{14}NO_2^+$  Fig. 5.

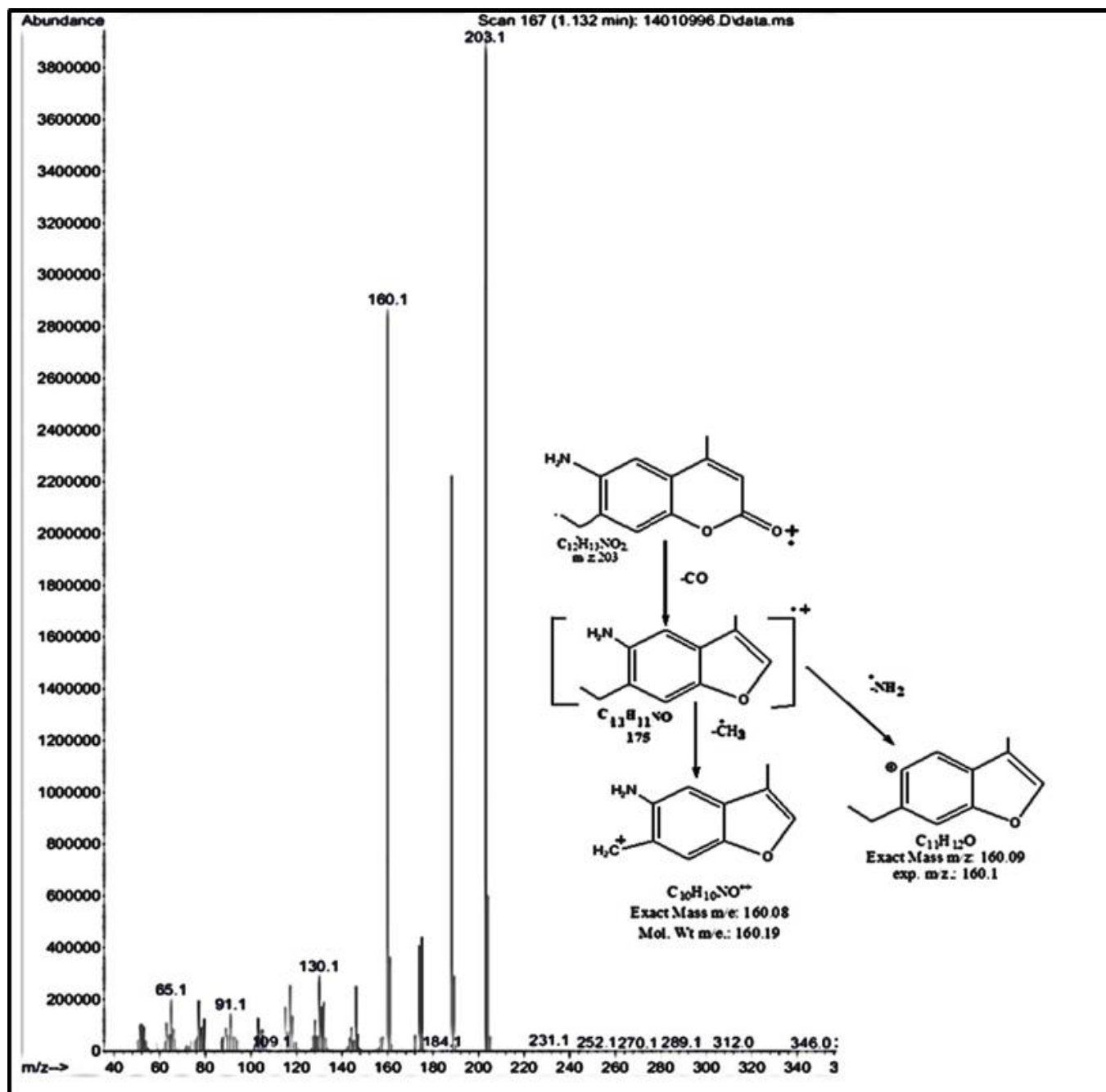
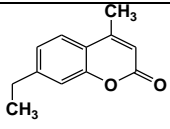
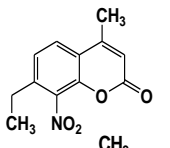
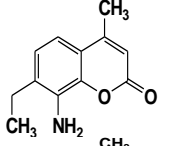
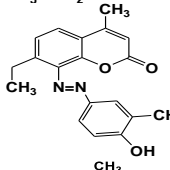
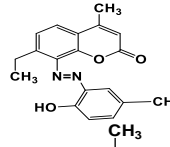
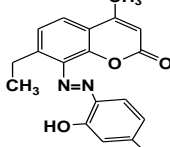
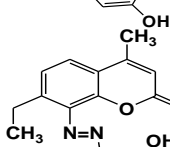
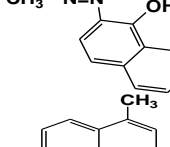
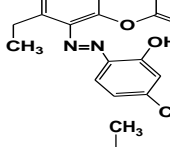
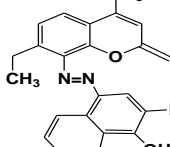


Figure 5. Mass spectrum and its fragmentation for 7-ethyl-4-methyl-8-aminocoumarin

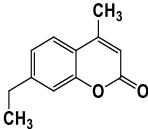
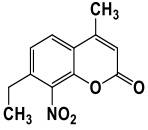
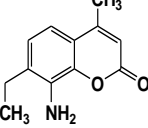
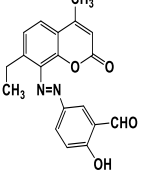
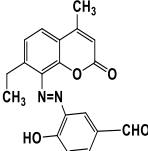
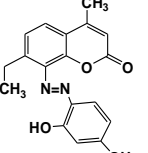
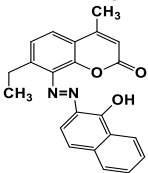
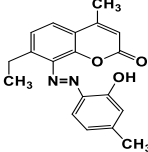
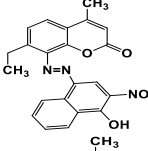
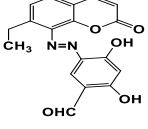
Finally, 7-ethyl-4-methyl-8-aminocoumarin 3 was converted to diazonium salt and then coupled with different phenol derivatives to form different azocoumarin derivatives. The chemical structure of the compounds 4-9 was verified by FT-IR spectra,

Table 1, which showed a disappearing band of  $\nu$ the ( $NH_2$ ) group and appearing absorption bands at  $3442-3440\text{ cm}^{-1}$  for  $\nu(OH)$ , at  $1485-1517\text{ cm}^{-1}$  for azo groups  $\nu(N=N)$ .

**Table 1. Physical properties of prepared compounds 1-9**

No. Comp.	Structure	Molecular formula	Yield %	M.P	Color	R <sub>f</sub> (ethylacetate: Hexane 3:7)	Solvent for Recrystallization
1		C <sub>12</sub> H <sub>12</sub> O <sub>2</sub>	90	72-74	Gray	0.8	EtOH
2		C <sub>12</sub> H <sub>11</sub> NO <sub>4</sub>	79	160-161	off-white	0.8	EtOH
3		C <sub>12</sub> H <sub>13</sub> NO <sub>2</sub>	60	325-328	Green	0.205	MeOH
4		C <sub>19</sub> H <sub>16</sub> N <sub>2</sub> O <sub>4</sub>	66	182-186	brown	0.694	EtOH
5		C <sub>19</sub> H <sub>16</sub> N <sub>2</sub> O <sub>4</sub>	60	d.c. >360	Dark brown	0.325	EtOH
6		C <sub>18</sub> H <sub>16</sub> N <sub>2</sub> O <sub>4</sub>	85	d.c. >360	Dark Green	0.48	EtOH
7		C <sub>22</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub>	65	d.c. >360	Gray	0.75	EtOH
8		C <sub>19</sub> H <sub>18</sub> N <sub>2</sub> O <sub>3</sub>	75	224-226	Greenish Yellow	0.906	EtOH
9		C <sub>22</sub> H <sub>17</sub> N <sub>3</sub> O <sub>4</sub>	65	d.c. >230	dark green	0.72	EtOH
10		C <sub>19</sub> H <sub>16</sub> N <sub>2</sub> O <sub>5</sub>	75	d.c. >250	dark yellow	0.769	EtOH

**Table 2. FT-IR spectral data  $\text{cm}^{-1}$  of prepared compounds 1-9**

No.	Structure	$\nu$ (OH)	$\nu$ (C-H) Arom.	$\nu$ (C-H) Aliph.	$\nu$ (C=O)	$\nu$ (C=C)	$\nu$ (N=N)	Other bonds
1		–	3058	2962 asym. And 2931 Sym.	1731	1620 And 1456	–	1191 (C-O-C)
2		–	3085	2987 asym. And 2912 Sym.	1766	1623 And 1554	–	$\text{NO}_2$ 1523 asym. and 1357 sym.
3		–	–	2979 asym. And 2920 Sym.	1687	1612 And 1556	–	$\text{NH}_2$ 3444 asym. And 3355 sym.
4		3442	3066	2958 asym. And 2929 Sym	1724	1610 And 1456	1456	(C=O) Aldehyde 1650, (C-H) Aldehyde 2869 (C=O) Aldehyde 1680 (C-H) Aldehyde 2852 (C-O) 1153 (C-O) 1151
5		3429	–	2968 asym. And 2925 Sym	1731	1612 And 1413	1560	(C=O) Aldehyde 1680 (C-H) Aldehyde 2852 (C-O) 1153 (C-O) 1151
6		3444	–	2972 asym. And 2921 Sym	1683	1608 And 1485	1555	(C=O) Aldehyde 1680 (C-H) Aldehyde 2852 (C-O) 1153 (C-O) 1151
7		3433	3064	2970 asym. And 2929 Sym	1728	1577 And 1444	1512	C-O-C 1228 –
8		3438	3060	2968 asym. And 2925 Sym	1739	1612 And 1487	1579	(C-O) 1155
9		3473	3064	2970 asym. And 2933 Sym	1726	1610 And 1450	1517	(C-O) 1153 (C-N) 1404
10		3438	3058	2968 asym. And 2933 Sym	1710	1575 And 1429	1517	(C=O) Aldehyde 1680 (C-H) Aldehyde 2881



The  $^1\text{H}$ -NMR spectrum for compound 4 showed a singlet signal at 10.39 ppm for the proton of the aldehydic group and a singlet signal at 11.61 ppm for one proton of hydroxyl group, Table.3  $^1\text{H}$ -NMR spectrum Table, .2 of compound 6, showed a singlet

signal at 10.62 ppm and 12.2 ppm for two hydroxyl groups Fig. 6.  $^{13}\text{C}$ -NMR spectrum for the same compound showed a signal at 163.71 ppm for carbon carbonyl group Fig. 7.

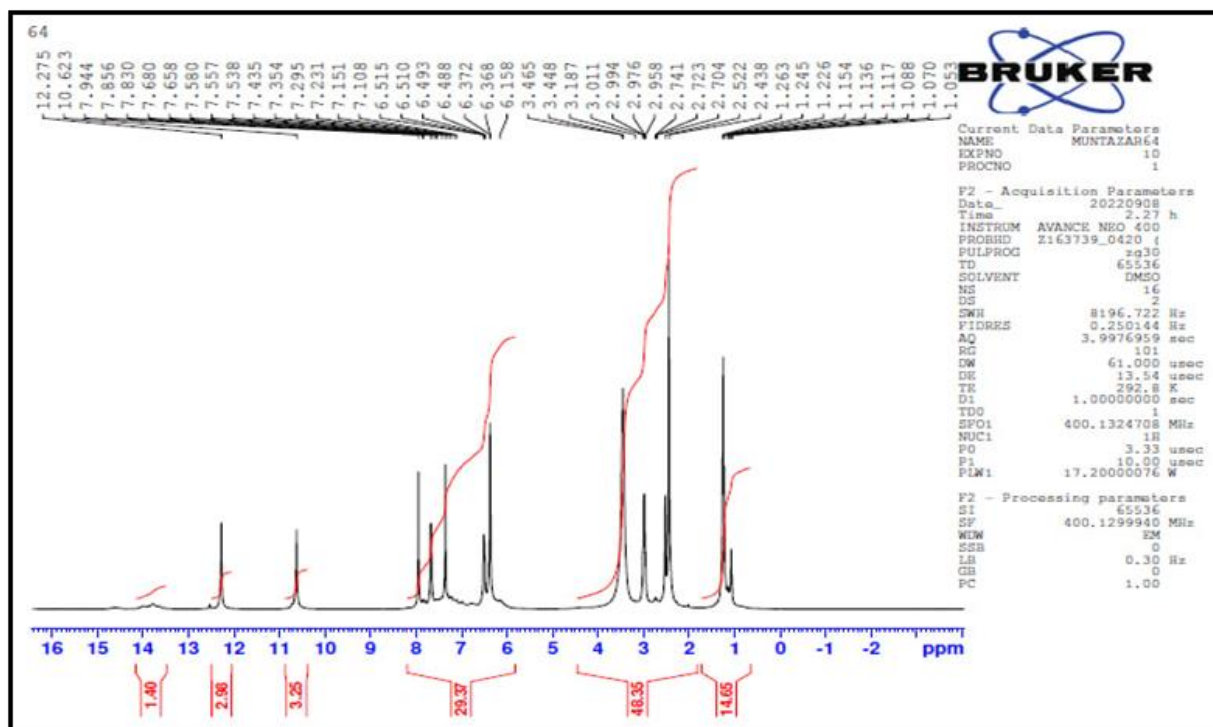


Figure 6. The  $^1\text{H}$ -NMR spectrum of compound 6

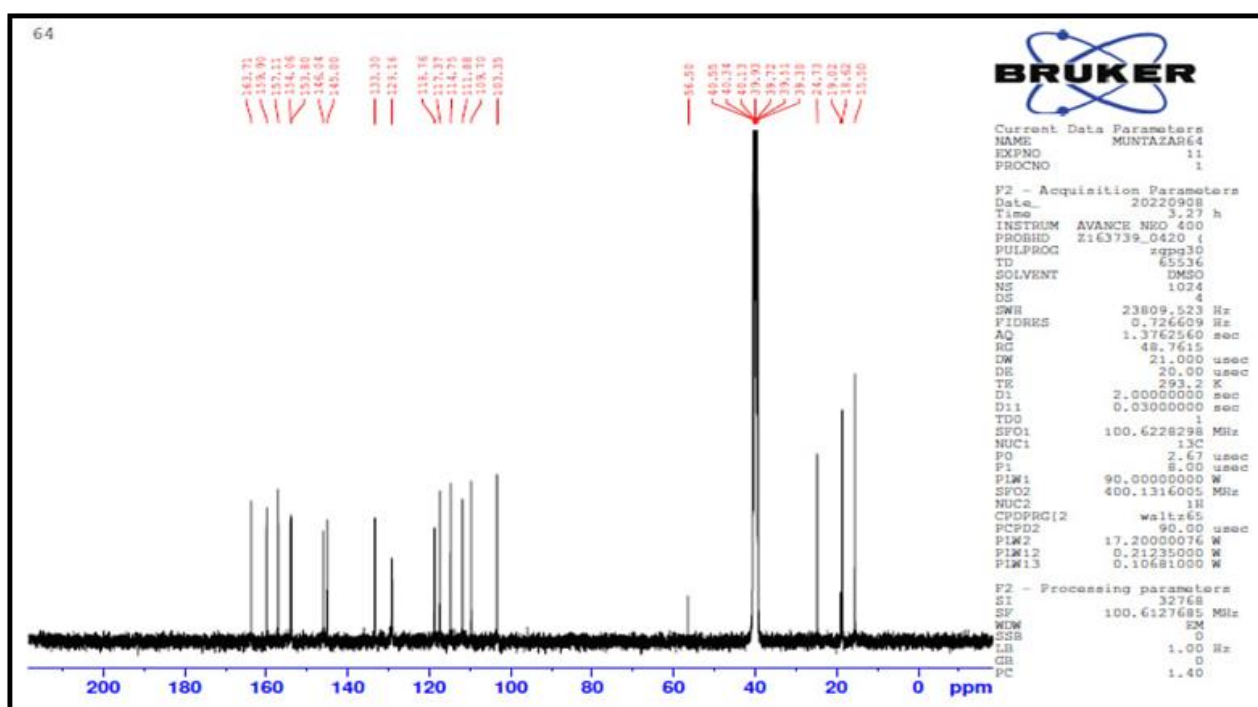
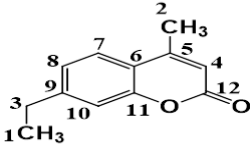
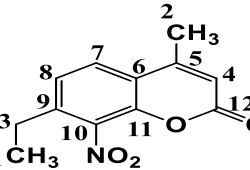
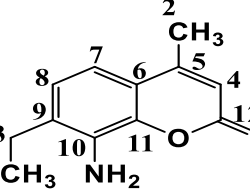
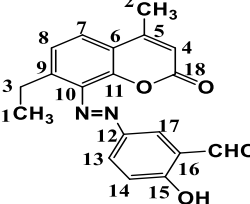
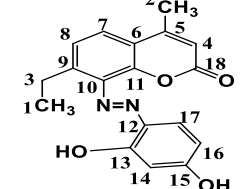
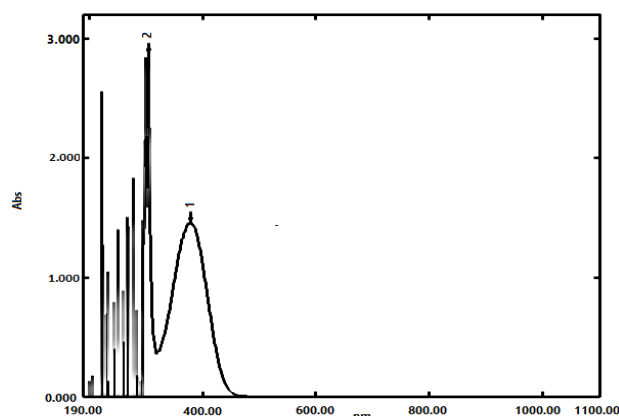


Figure 6. The  $^{13}\text{C}$ -NMR spectrum of compound 6

**Table 3. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra data of compounds 2-4 and 9**

No.	Structure	<sup>1</sup> H-NMR spectral data ( $\delta$ ppm)	<sup>13</sup> C-NMR spectral data ( $\delta$ ppm)
1		1.3(t,3H,CH <sub>3</sub> ), 2.55(s,3H,CH <sub>3</sub> ), 2.75(q,2H,CH <sub>2</sub> ), 6.35(s,1H, H lactone ring), 6.3,7.3 and 7.8(s,s,s,3H,Ar-H)	15(C1),18(C2), 28(C3),113(C4), 115(C5),117(C6), 124(C7),125(C8),148(C9), 153(C10),154(C11),160(C12)
2		1.23(t, 3H, <u>CH<sub>3</sub></u> -CH <sub>2</sub> ); 2.97(q, 2H, <u>CH<sub>2</sub></u> -CH <sub>3</sub> ); 2.47(s, 3H, CH <sub>3</sub> ); 6.53- 8.34(m, 3H, Ar-H).	14.57(C1), 18.33(C2), 25.84(C3), 115.85- 155.38(C4,C5,C6,C7,C8, C9,C10,C11), 159.32(C12).
3		1.15 (t, 3H, <u>CH<sub>3</sub></u> -CH <sub>2</sub> ); 2.51(q, 2H, <u>CH<sub>2</sub></u> -CH <sub>3</sub> ); 2.31(s, 3H, CH <sub>3</sub> ); 5.04(s, 2H, NH <sub>2</sub> ); 6.21- 7.61(m, 3H, Ar-H).	13.08(C1), 18.53(C2), 23.98(C3); 108.07- 153.26(C4,C5,C6,C7,C8, C9,C10,C11), 160.9(C12).
4		1.28(t, 3H, <u>CH<sub>3</sub></u> -CH <sub>2</sub> ); 3.18(q, 2H, <u>CH<sub>2</sub></u> -CH <sub>3</sub> ); 2.48(s, 3H, CH <sub>3</sub> ); 6.45- 8.23(m, 6H, Ar-H). 10.39(s, 1H, CHO), 11.61(s,1H,OH) 1H, H).	15.50(C1),18.62(C2), 24.73(C3), 113.35- 157.9(C4,C5,C6,C7,C8,C9,C10, C11,C12,C13,C14,C15,C16,C17), 159.85(C18).
6		1.11 (t, 3H, <u>CH<sub>3</sub></u> -CH <sub>2</sub> ); 2.95(q, 2H, <u>CH<sub>2</sub></u> -CH <sub>3</sub> ); 2.52(s, 3H, CH <sub>3</sub> ); 6.15- 7.94(m, 6H, Ar-H); 10.62(s, 1H, 2OH).	15.5(C1), 18.62(C2), 24.73(C3), 103.25- 159.9(C4,C5,C6,C7,C8,C9,C10, C11,C12,C13,C14,C15,C16,C17), 163.71(C18).

The UV-vis spectrum of 6-amino coumarin **3** in DMSO within the 200-1100 nm range shows two peaks; the first was at 305nm, which belongs to the coumarin ring's mild energy ( $\pi$ - $\pi^*$ ) transition Fig. 8. Due to the amino group, the second peak for the ( $n$ - $\pi^*$ ) transition appeared at 378 nm<sup>48, 49</sup>. The electronic spectra of the azo compounds **4-10** showed absorption maxima at 205-293 nm due to the ( $\pi$ - $\pi^*$ ) another absorption maxima at 368-704 nm due to  $n$ - $\pi^*$ , which was shifted to a higher wavelength compared to compound **3** Fig. 9-11.



**Figure 8. The spectrum of UV-Vis of compound 3**

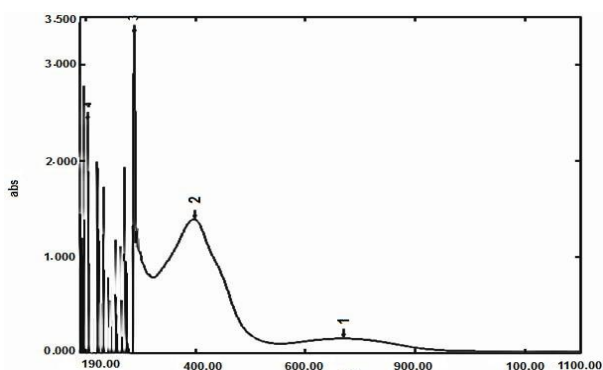


Figure 9. The spectrum of UV-Vis of compound 6

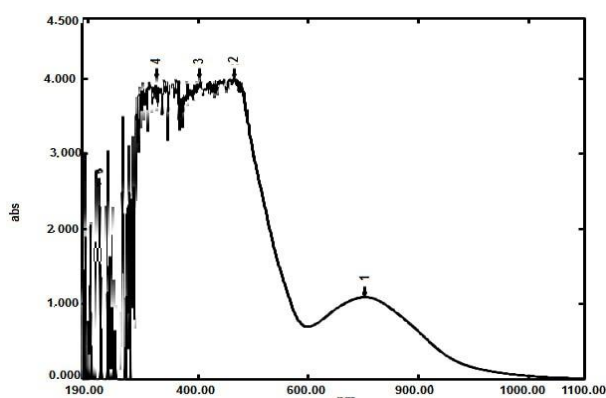


Figure 10. The spectrum of UV-Vis of compound 7

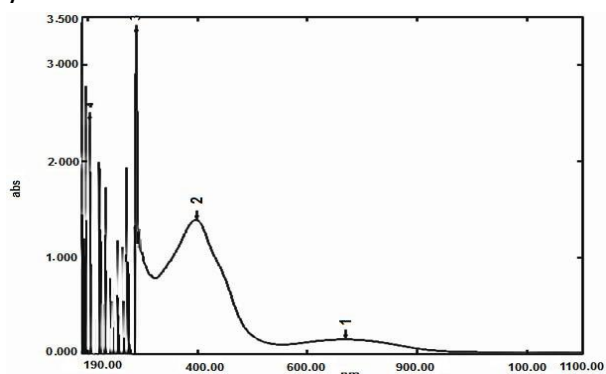


Figure 11. The spectrum of UV-Vis of compound 9

**Antimicrobial activity:**

Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*), and one type of fungal species (*Candida*) were used to determine the biological antimicrobial activity of all the prepared compounds by agar well diffusion method (well diameter was 5mm) using DMSO as a solvent. Vancomycin, Meropenem, Amikacin, and Nystatin were standard drugs at 37 °C for 24h.

**Antibacterial activity:**

All the prepared compounds showed good antibacterial activity compared to the standard antibiotic (Vancomycin), which has an inhibition area of 13mm against Gram-positive bacteria (*Staphylococcus aureus*). Compounds 1, 2, 3, 7, 8, and 9 showed moderate activities with an inhibitory zone of 5-7 mm compared to the used antibiotic, while compound 4 showed good activity with an inhibition zone of 10 mm, Table.4.

The biological activity of all the prepared compounds in Table 4 against Gram-negative bacteria (*Escherichia coli*) was also tested using two antibiotics, meropenem, and amikacin, which have an inhibition area of 25 and 21 mm, respectively. Compounds 1, 2, 3, 7, 8, and 9 possessed moderate inhibitory activity compared to the antibiotics that were used with an inhibition area of 6-8 mm, while compounds 4 and 6 showed high inhibitory activity with an inhibition area of 10 and 15 mm, respectively.

**Antifungal activity:**

All the compounds that were prepared showed a higher antifungal biological activity close to the standard antibiotic used (Nystatin), as the effectiveness of these compounds was tested against one type of fungi (*Candida albicans*). The compounds 1, 2, 3, 4, 6, and 7 showed good activity of (14, 15, 18, and 19) mm Table.4.

**Table 4. Bacterial and fungal inhibition zone in (mm) for prepared compounds (1-4) and (6-9)**

Com. No.	<i>Staphylococcus aureus</i> (mm)	<i>Escherichia coli</i> (mm)	<i>Candida</i> (mm)
1	10	12	10
2	10	14	11
3	11	14	10
4	15	16	13
6	15	21	13
7	11	13	10
8	11	12	10
9	12	13	10

Vancomycin	13	---	---
Meropenem	---	25	---
Amikacin	---	21	---
Nystatin	---	---	23

**Antioxidant activity:** <sup>50-52</sup>

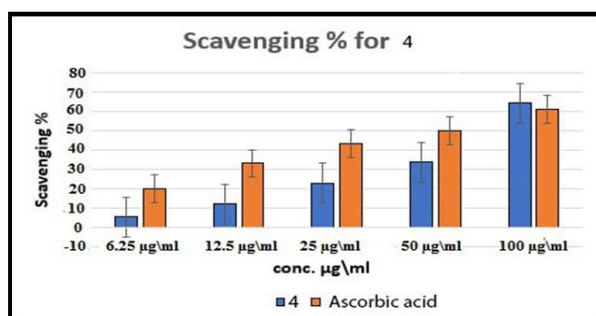
Antioxidant activity can stop oxidative stress by binding with free radicals and neutralizing their harmful effects through several chemical mechanisms created by natural activity. Oxidative degradation of organic materials, including biological molecules such as lipids, proteins, foods, and cosmetics, like any other radical chain reaction, the antioxidant comprises three steps: initiation, propagation, and termination of DPPH (2,2-Diphenyl-1-picrylhydrazyl) scavenging activity.

The antioxidant activity of some synthesized compounds was measured in comparison with vitamin C (Ascorbic acid). Compounds 5, 6, and 8

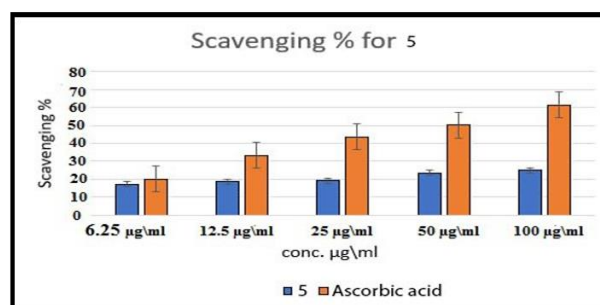
showed weak antioxidant activity, while compounds 9 showed intermediate antioxidant activity, while compounds 4, 7, and 10 showed anti-oxidant solid activity. All compounds were predestined by DPPH (2, 2-diphenyl-1- picrylhydrazyl) assay method at various concentrations (6.5, 12.5, 25, 50, 100 µg/mL). Depending on the reaction characterized by a change in its deep violet color (DPPH) or decolorization, the result is stoichiometric concerning several captured electrons. Compound 7 exhibited the best results among all compounds. Accordingly, the values of inhibitory concentrations (IC<sub>50</sub>) were recorded and tabulated in Table 5 Fig. 12-18.

**Table 5. Antioxidant activity of some prepared compounds 4- 10**

Comp. NO.	Scavenging %					R2	Linear eq.	Ic50
	6.25 µg/ml	12.5 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml			
4	26.09	31.71	40.43	47.11	100.04	R <sup>2</sup> = 0.959	y = 0.7606x + 19.601	39.6
5	22.43	27.88	30.27	33.27	46.06	R <sup>2</sup> = 0.962	y = 0.2268x + 23.193	118.2
6	20	33.26	43.57	50.2	61.42	R <sup>2</sup> = 0.83	y = 0.3793x + 26.993	60.65
7	40.92	53.45	63.43	76.83	93.17	R <sup>2</sup> = 0.918	y = 0.5103x + 45.788	8.25
8	39.1	41.02	48.51	49.1	58.61	R <sup>2</sup> = 0.914	y = 0.194x + 39.75	52.84
9	32.23	35.81	37.08	57.34	76.34	R <sup>2</sup> = 0.975	y = 0.4858x + 28.936	53.65
10	32.92	41.74	63.92	70.07	85.11	R <sup>2</sup> = 0.832	y = 0.5087x + 39.04	21.55
Ascorbic acid	42.92	52.74	65.92	83.07	97.11	R <sup>2</sup> = 0.906	y = 0.55x + 47.04	5.38



**Figure 12. DPPH scavenging activity of compound 4 compared with vitamin C**



**Figure 13. DPPH scavenging activity of compound 5 compared with vitamin C**

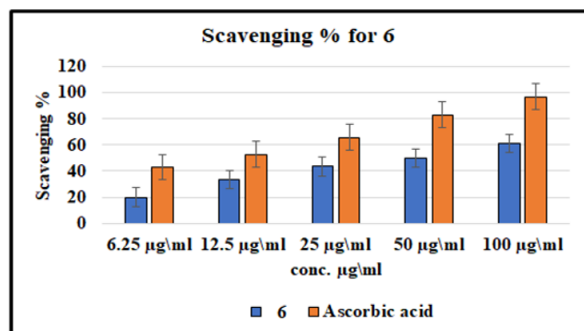


Figure 14. DPPH scavenging activity of compound 6 compared with vitamin C

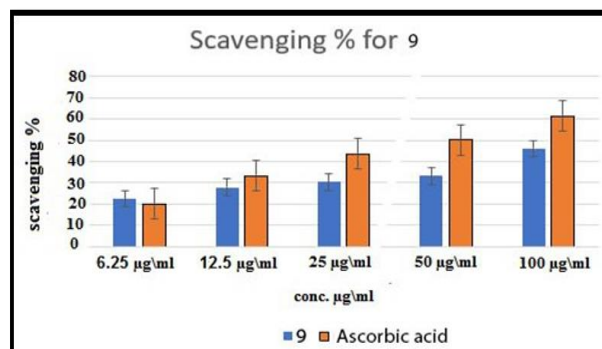


Figure 17. DPPH scavenging activity of compound 9 compared with vitamin C

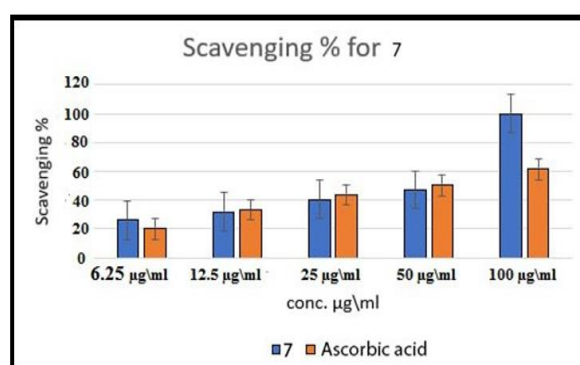


Figure 15. DPPH scavenging activity of compound 7 compared with vitamin C

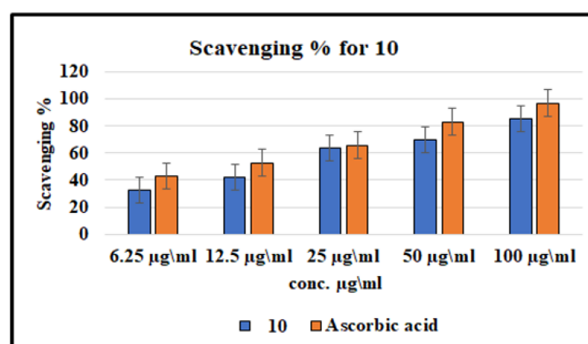


Figure 18. DPPH scavenging activity of compound 10 compared with vitamin C

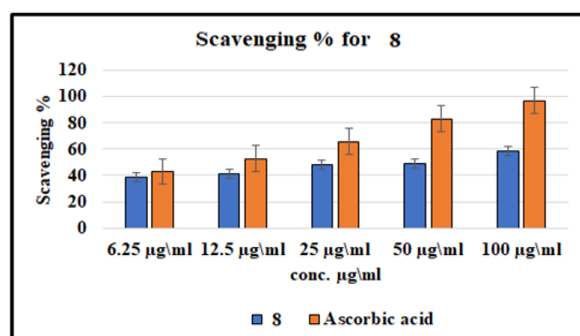


Figure 16. DPPH scavenging activity of compound 8 compared with vitamin C

## Conclusion

Synthesis of new 7-ethyl-4-methylcoumarin derivatives containing azo group, carried out by nitration of 7-ethyl-4-methylcoumarin, given one isomer of corresponding 8-nitro coumarin compound group. The reaction temperature, amount of coumarin, reaction time, and big substituent (ethyl group) were critical to give a high yield from 7-ethyl-4-methyl-8-nitrocoumarin compared with nitration of 7, 4-dimethyl coumarin, which gave two isomers as mention in<sup>21</sup>. The synthesized

compounds 4 and 6 showed a broad spectrum against different types of bacteria, *Staphylococcus aureus* and *Escherichia coli*, compared to the standard drug vancomycin, while all compounds showed moderate activity against fungi compared with nystatin. Some newly synthesized compounds showed good antioxidant activity, and compound 6 showed good antibacterial and antifungal activity<sup>23</sup>. It may be attributed to the presence of the releasing groups in the phenyl azo moiety.

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## Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for

re-publication, which is attached to the manuscript.

- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

## Authors' Contribution Statement

This work was carried out in collaboration between all authors; M.H. contributed to the revision, proofreading, and acquisition of data, and L.S.

contributed to the Conception, design, analysis, interpretation, and drafting of the MS. All the Authors read and approved the final manuscript.

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## تخليق ودراسات بيولوجية لمشتقات 4-مثيل-7-اثيل كومارين التي تحتوي على مجموعة الازو

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### الخلاصة

خلقت مشتقات الجديدة من 4-methyl-7-ethylcoumarin التي تحمل مجموعة azo من خلال سلسلة من التفاعلات المتسلسلة واختبار نشاطها البيولوجي. بدءاً من 7-اثيل-4-مثيل كومارين الذي حُضِر من تفاعل ميتا اثيل فينول مع اثيل اسيتو اسيتيت بواسطة تفاعل يكمان بعدها تم نيترة 7-اثيل-4-مثيل كومارين باستخدام حامض النيتريك بوجود  $H_2SO_4$  المركز لإنتاج ايزومر واحد 7-اثيل-4-مثيل-8-نايتروكومارين تحت التبريد في درجة حرار  $C^{\circ}$  (2-5)، ثم اختزال مجموعة النيترو باستخدام معدن الحديد في وسط حامضي لتكوين امينو كومارين المقابل الذي حُوّل الى أصباغ azo عن طريق تفاعل ملح الديازونيوم الخاص به مع مركبات الفينول المختلفة. شُخص المركبات المخلفة بواسطة اطياف الماس و FT-IR و  $^1H$ NMR و  $^{13}C$ -NMR، والأشعة فوق البنفسجية وتقنية كروماتوغرافيا الطبقة الرقيقة. قُيِّمت الفعالية البيولوجية للمركبات المخلفة تجاه نوعين من البكتريا كرام الموجبة والكرام السالبة بتركيز  $1 \times 10^{-3}$  M أظهرت ان المركبات المخلفة الجديدة 4 و 6 تمتلك طيف عريض ضد البكتريا الموجبة والسالبة مقارنة مع الدواء المستخدم كعقار vancomycin بينما كل المركبات أظهرت فعالية وسط تجاه الفطريات مقارنة مع العقار القياسي nystatin. كما أظهرت تلك المركبات المحضرة كمضادات أكسدة قوية جداً مقارنة بحمض الأسكوربيك كمرجع قياسي. خاصة مركب رقم 7 أظهر كفاءة قوية كمضاد للأكسدة لنفس المرجع.

**الكلمات المفتاحية:** أمينو كومارين، النشاط المضاد للميكروبات، أصباغ الأزو، الكومارين، ملح الديازونيوم.