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Synthesis and Biological Studies of Schiff Bases Derived from 4-methyl 7-Ethylcoumarin

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Abstract

New 7-ethyl-4-methyl coumarin derivatives bearing an azo methene group (C=N) were synthesized by a four-step process. The reaction between 7-ethylphenol and ethyl acetoacetate in the presence of sulfuric acid afforded 7-ethyl-4-methylcoumarin **1** in 90% yield. Nitration conditions (nitric acid and sulfuric acid) were then applied to product **1** to produce 6-nitro-7-ethyl-4-methycoumarin **2** and 8-nitro-4-methyl-7-ethylcoumarin **3**. The temperature, the amount of starting materials, and the time of reaction were essential factors in obtaining a high yield of isomer **2** compared to the second isomer **3**. The nitro groups at compounds **2** and **3** were then reduced with iron metal in an acidic medium to form the corresponding amino compounds **4** and **5**, which were converted to Schiff base derivatives **6**-**17** *via* reaction with different substituted aromatic aldehydes. The synthesized compounds were characterized by FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopy. Evaluating the biological activities of the synthetic compounds was carried out against two bacteria: gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*) at 1×10^{-3} M; compound **15** showed a broad spectrum against these two types of bacteria compared to Vancomycin and Amikacin and against fungi compared to nystatin as a standard drug, but compound **7** showed stronger activities against fungi than the other synthesized compounds. In contrast, the rest of the compounds showed moderate activity against fungi compared with the same standard drag; in the antioxidant study, some new compounds showed powerful antioxidants compared with ascorbic acid as a stander reference by calculating the IC_{50}. Compound **8** showed very good results as an antioxidant agent compared with the same standard.

Keywords: Aminocoumarin, Antioxidant, Coumarin, Schiff base, Nitrocomarin.

تخليق و دراسات بيولوجية لقواعد شف مشتقة من 4-مثيل-7-اثيل كومارين

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الخلاصة

حضرت مشتقات جديدة من 7-إثيل-4-مثيل كومارين التي تحمل مجموعة الآزو ميثين (C=N) بعملية من أربع خطوات. التفاعل بين 7-إثيل فينول وإثيل أسيتواسيتات في وجود حامض الكبريتيك أعطى 7-إثيل-4-مثيل كومارين **1** بمنتوج 90%. تم بعد ذلك تطبيق شروط النترتة (حمض النيتريك وحمض الكبريتيك) على المنتج **1** لإنتاج 6-نيترو-7-إثيل-4-مثيل كومارين **2** و8- نيترو-7-إثيل -4-مثيل كومارين **3**. درجة

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الحرارة، كمية المواد الأولية، وزمن التفاعل كانوا عوامل أساسية في الحصول على ناتج عالي للأيزومر **2** مقارنة بالأيزومر الثاني **3**. تم بعد ذلك اختزال مجموعات النيترو في المركبين **2** و **3** بمعدن الحديد في وسط حامضي لتكوين المركبات الأمينية المقابلة **4** و **5** والتي تم تحويلها إلى مشتقات قاعدة شيف **6** – **17** عن طريق التفاعل مع ألديهايدات عطرية مختلفة. شخصت المركبات المحضرة بواسطة مطيافية FT–IR، ^{1}HNMR، ^{13}CNMR. تم تقييم النشاط البيولوجي للمركبات المخلقة ضد نوعين من البكتيريا: البكتيريا إيجابية الجرام (*Staphylococcus aureus*) والبكتيريا سالبة الجرام (*Escherichia coli*) عند 1×10^{-3} M ؛ أظهر المركب **15** طيفاً واسعاً ضد هذين النوعين من البكتيريا مقارنة Vancomycin و Amikacin كعقار قياسي وكذلك ضد الفطريات مقارنة Nystatin كدواء قياسي، لكن المركب **7** أظهر نشاطًا أقوى ضد الفطريات من المركبات المخلقة الأخرى. في المقابل أظهرت بقية المركبات نشاطاً متوسطاً ضد الفطريات مقارنة بنفس المرجع ؛ في دراسة مضادات الأكسدة، أظهرت بعض المركبات الجديدة مضادات أكسدة قوية مقارنة بحامض الأسكوربيك كمرجع قياسي من خلال حساب IC_{50}. أظهر المركب **8** نتائج جيدة جداً كعامل مضاد للأكسدة مقارنة بنفس المرجع.

الكلمات المفتاحية: فواعد سف، كومارين، نايتروكومارين، امينوكومارين، مضادات الاكسدة

1. Introduction

Coumarins are oxygen-containing fused heterocycles that are common in plants [1]. Many of them have been widely recognized as important building blocks in the design of synthetic drug candidates because of their dynamic pharmacological activities, such as being anti-inflammatory [2], anti-cancer [3], anti-HIV [4], and anti-malarial [5]. Coumarin and its derivatives are essential compounds with antibacterial, anti-carcinogenic, and analgesic activity [6]. The first isolated parent coumarin was from tonka bean (Dipteryx odorata) in 1820 by Vogel [7]. Coumarins are widely distributed and can be found as secondary metabolites in various plants [8,9]. Coumarins could be synthesized by the Perkin reaction, Knoevenagel condensation, Pechmann condensation, Baylis-Hillman reaction, Claisen rearrangement, Wittig reaction, and Vilsmeier-Haack and Suzuki cross-coupling reactions [10,11]. Because most extracted coumarins have biological activity, coumarin derivatives are increasingly being synthesized, as extraction from plants is unprofitable and time-consuming [12]. This research synthesized coumarin derivatives bearing an azo methane group (R_2-C=N-) through sequence reactions (nitration reaction, reduction reaction, and Schiff base's formation reaction). Schiff bases are substances with an azomethine group; they are synthesized when a primary amine and a carbonyl compound condense [13]. Their biological characteristics result from the imine group's presence in them. Schiff base derivatives show significant biological activities like antimalarial [14], antibacterial [15], antifungal [16], antitumor [17], and antioxidant [18] activities. Because the ring structure moves the free electrons around, aromatic Schiff bases have a greater chance of being used in biological applications [19]. They are essential molecules in the fields of medicine and pharmaceuticals. Furthermore, it has been proposed that the azomethine linkage is responsible for their biological activities [20]. In this work, we will be able to obtain two different nitro isomers in an easy way and with a good yield by controlling the reaction conditions without using the previous separation process such as column chromatography and then including those in the synthesis of many chemical compounds, which could be antibacterial, antifungal, and antioxidant.

2. Materials and methods

2.1. Materials

Chemical materials were purchased from Fluka or Aldrich as starting chemical compounds. Melting points (MP) were marked using Gallenkamp in open glass capillaries

using an uncorrected Thomas capillary melting point apparatus. Fourier transform infrared (FTIR) spectra were recorded on a SHIMADZU FTIR-8400 spectrophotometer as KBr discs. The total primary components and reagents were pure and commercially available. A 500 MHz spectrometer recorded ^{1}H NMR and ^{13}C NMR spectra. Dimethyl sulfoxide solvent (DMSO-d_6) was utilized to record Agilent Technologies model ultra-shield nuclear magnetic resonance (NMR) spectra, and the chemical shifts are given in δ (ppm) downfield using tetramethyl silane (TMS) as references.

2.2. Methods

*2.2.1. Synthesis of 7-ethyl-4-methylcoumarin (**1**)*

This compound (**1**) was prepared according to the method reported in the literature [21] with some modification using a mixture of 7-ethylphenol (0.1 mol) with ethyl acetoacetate (0.1 mol) and concentrated sulphuric acid (45 mL) under cold condition for 2 hours. and heated at 60-80 ºC for 6 hours, then the resulting solution was poured into the ice with stirring, after that the precipitate was filtered and wash with cold water producing: 90%; m.p 72-74 ºC, R_f = 0.8; gray precipitate, recrystallization from EtOH FTIR (KBr,cm^{-1}): 1706(C=O).

*2.2.1. Synthesis of 7-ethyl-4-methyl-6-nitrocoumarin (**2**) [22]*

A mixture of compound **1** (1 g, 0.0053 mol) and H_2SO_4 (7.5 mL) was stirred for 15 minutes. The nitration reagent [HNO_3 (0.4 mL) and H_2SO_4 (1.25 mL)] was then added dropwise to this mixture at a temperature not exceeding 3 °C and stirred for 4 hours. The reaction mixture was stirred for another 4 hours at room temperature. The reaction was monitored by TLC (eluent with *n*-hexane:ethyl acetate, 7:3). After completion of the reaction, the mixture was poured into ice and left for several hours. It was then filtered by a Buechner funnel, and the precipitate was washed with cold distilled water. The physical properties and FT-IR of compound **2** are listed in Table 2. Yield: 79%; m.p 140-141ºC, R_f = 0.8; off-white color. Recrystallization from EtOH; FT-IR (KBr, cm^{-1}): 1766 (C=O); 1525 asym. and 1352 sym. (NO_2).

*2.2.3. Synthesis of 7-ethyl-4-methyl-8-nitrocoumarin (**3**) [23]*

7-Ethyl-4-methylcoumarin 3 was prepared in the same way as compound **2**, but the nitration reagent mixture (0.8 mL HNO_3 and 2.5 mL H_2SO_4) was added drop by drop at a temperature of no more than 8 °C. After completion of the reaction, the mixture was poured into ice, filtered, and the precipitate was washed with water. The product that did not dissolve in hot ethanol was recrystallized from acetic acid. Yield: 50%; m.p. 273-275 ºC, R_f = 0.78; yellow color; FT-IR (KBr, cm^{-1}): 1766 (C=O); 1523 asym. and 1357 sym. (NO_2).

*2.2.4. Synthesis of 7-ethyl-4-methyl-6-aminocoumarin (**4**) and 7-ethyl-4-methyl-8-aminocoumarin (**5**) [24]*

A mixture of iron (0.3 g), water (2.3 mL), and glacial acetic acid (1.2 mL) was refluxed for 15 minutes. After that, a solution of compound **2** (0.004 mol) in ethanol (15 ml) was added and refluxed for 10 hours. The mixture was filtered to get rid of iron before adding sodium bicarbonate to the filtrate to neutralize the mixture (the desired pH is 6 -7). The mixture was left to evaporate the ethanol and washed with cold distilled water. The physical properties and FT-IR of compounds **4** and **5** are listed in Table 1. Compound **4**: Yield: 83%; m.p. 273-275ºC, R_f = 0.8; yellow color, recrystallization from EtOH; FT-IR (KBr, cm^{-1}): 1733 (C=O); 3444 asym. and 3355 sym. (NH_2).

Compound **5**: Yield: 60%; m.p 325-328ºC, R_f = 0.2, green color, recrystallization from EtOH; FT-IR (KBr, cm^{-1}): 1687 (C=O); 3444 asym. and 3355 sym. ν(NH_2).

*2.2.5. Synthesis of Schiff base derivatives **6**-**17** [25]*

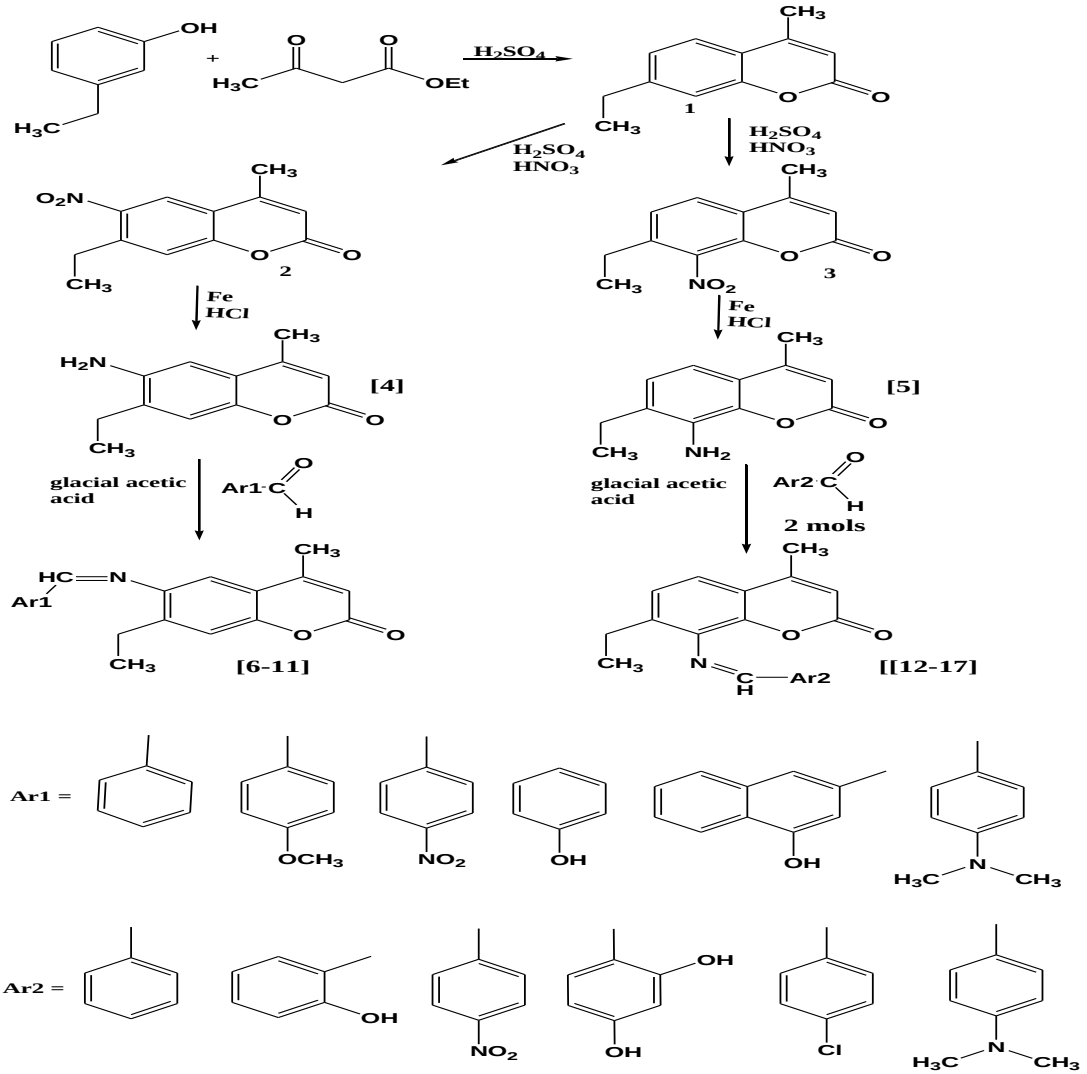
A solution of substituted aldehydes (0.01 mol) and 6- or 8-nitrocoumarin (0.01 mol) in absolute methanol (20 mL) and glacial acetic acid (2-3 drops) was refluxed for 4 hours. The mixture was then cooled to room temperature, and the solid crude material was washed with cold water, and recrystallized from ethanol to afford compounds **6-17**.

*2.2.5.1. Synthesis of 6-(benzylidene-amino)-7-ethyl-4-methyl-chromen-2-one (**6**)*
Yield: 50%; m.p. 126-128 ºC, R_f = 0.66, brown color, recrystallization from EtOH; FT-IR (KBr, cm^{-1}): 1735 (CO); 1627 (C=N).
*2.2.5.2. Synthesis of 7-ethyl-6-[(4-methoxy-benzylidene)-amino]-4-methyl-chromen-2-one (**7**)*
Yield: 65%; m.p. 324-326 ºC, R_f = 0.4, light nutty color, recrystallization from EtOH; FT-IR (KBr, cm^{-1}): 1731 (CO); 1627 (C=N).
*2.2.5.3. Synthesis of 7-ethyl-6-[(4-nitro-benzylidene)-amino]-4-methyl-chromen-2-one (**8**)*
Yield: 70%; m.p. 220-222 ºC, R_f = 0.56, green color, recrystallization from EtOH; FT-IR (KBr, cm^{-1}): 1720 (CO); 1631 (C=N).
*2.2.5.4. Synthesis of 7-ethyl-6-[(4-hydroxy-benzylidene)-amino]-4-methyl-chromen-2-one (**9**)*
Yield: 75%; m.p. 164-166 ºC, R_f = 0.74, yellow color, recrystallization from EtOH; FT-IR (KBr, cm^{-1}): 1685 (CO); 1600 (C=N).
*2.2.5.5. Synthesis of 7-ethyl-6-[(4-hydroxy-naphthalen-2-ylmethylene)-amino]-4-methyl-chromen-2-one (**10**)*
Yield: 65%; m.p. 228-230 ºC, R_f = 0.72, brown color, recrystallization from EtOH; FT-IR (KBr, cm^{-1}): 1691 (CO); 1643 (C=N).
*2.2.5.6. Synthesis of 6-[(4-dimethylamino-benzylidene)-amino]-7-ethyl-4-methyl-chromen-2-one (**11**)*
Yield: 70%; m.p. 232-234 ºC, R_f = 0.79, yellow color, recrystallization from EtOH; FT-IR (KBr, cm^{-1}): 1735 (CO); 1600 (C=N).
*2.2.5.7. Synthesis of 8-(benzylidene-amino)-7-ethyl-4-methyl-chromen-2-one (**12**)*
Yield: 60%; m.p. 120-122 ºC, R_f = 0.68, peggy color, recrystallization from EtOH; FT-IR (KBr, cm^{-1}): 1715 (CO); 1629 (C=N).
*2.2.5.8. Synthesis of 7-ethyl-4-methyl-8-[(4-nitro-benzylidene)-amino]-chromen-2-one (**13**)*
Yield: 67%; m.p. 235-237 ºC, R_f = 0.72, orange color, recrystallization from EtOH; FT-IR (KBr, cm^{-1}): 1724 (CO); 1629 C=N; 1521 asym. and 1384 sym. $\nu(NO_2)$.
*2.2.5.9. Synthesis of 7-ethyl-8-[(2-hydroxy-benzylidene)-amino]-4-methyl-chromen-2-one (**14**)*
Yield: 73%; m.p 158-160 ºC, R_f = 0.78, white color, recrystallization from EtOH; FT-IR (KBr, cm^{-1}): 1733 (CO); 1616 (C=N); 3414 (OH).
*2.2.5.10. Synthesis of 8-[(2,4-dihydroxy-benzylidene)-amino]-7-ethyl-4-methyl-chromen-2-one (**15**)*
Yield: 68%; m.p. 226-228 ºC, R_f = 0.78, off white color, recrystallization from EtOH; FT-IR(KBr, cm^{-1}): 1710 (CO); 1622 (C=N); 3436 and 3406 (OH).
*2.2.5.11. Synthesis of 8-[(4-chloro-benzylidene)-amino]-7-ethyl-4-methyl-chromen-2-one (**16**)*
Yield: 55%; m.p. 225-227 ºC, R_f = 0.63, peggy color, recrystallization from EtOH; FT-IR (KBr, cm^{-1}): 1695 (CO); 1629 (C=N).
*2.2.5.12. Synthesis of 8-[(2,4-dihydroxy-benzylidene)-amino]-7-ethyl-4-methyl-chromen-2-one (**17**)*
Yield: 65%; m.p. 250-252ºC, R_f =0.7, green color; recrystallization from EtOH; FT-IR (KBr, cm^{-1}): 1735 (CO); 1600 (C=N).

3. Results and discussion

3.1. Chemistry

The synthesis of the coumarin derivatives **6-17** is shown in Scheme 1. The product 7-ethyl-4-methylcoumarin **1** was prepared in excellent yield (90%) *via* the reaction of ethyl acetoacetate with 3-ethylphenol. The FT-IR spectrum revealed the disappearance of OH stretching absorption and the appearance of new absorptions at 1731 cm^{-1} and 2962 cm^{-1} belong to the C=O group and an C-H bond, respectively. In the second step, the nitration of 7-ethyl-4-methylcoumarin **1** using nitric acid in the presence of H_2SO_4 produced 7-ethyl-4-methyl-6-nitrocoumarin **2** and 7-ethyl-4-methyl-8-nitrocoumarin **3**. When the reaction was carried out at 3 ºC for 4 hours, and then at 25 ºC for 4 hours using 0.4 mL of the nitration mixture, the product **2** was obtained with a 79% yield. In contrast, with the use of a double amount of the nitration mixture (0.8 mL) and a temperature of not more than 8 ºC, product **3** was isolated as a major product with a 50% yield. The FT-IR spectral data of compounds **2** and **3** showed two absorption bands at 1523 cm^{-1} and 1357 cm^{-1} attributed to the asymmetric and symmetric absorptions of the NO_2 group. The ^1H NMR analysis data of compound **2** showed a triplet signal at 1.19 ppm due to the CH_3 protons of the ethyl group, a quartet signal at 2.89 ppm due to the CH_2 protons of the ethyl group [26], and a singlet signal at 2.4 ppm owing to CH_3 at the ring. Multiple signals from 8.49 to 6.29 ppm are for aromatic protons (Figure 1). The ^{13}C NMR spectrum showed a signal at 159 ppm for the C=O group. All the ^1H NMR and ^{13}C NMR data are shown in Table 3.



Scheme 1: Synthesis of coumarin derivatives **1-17**

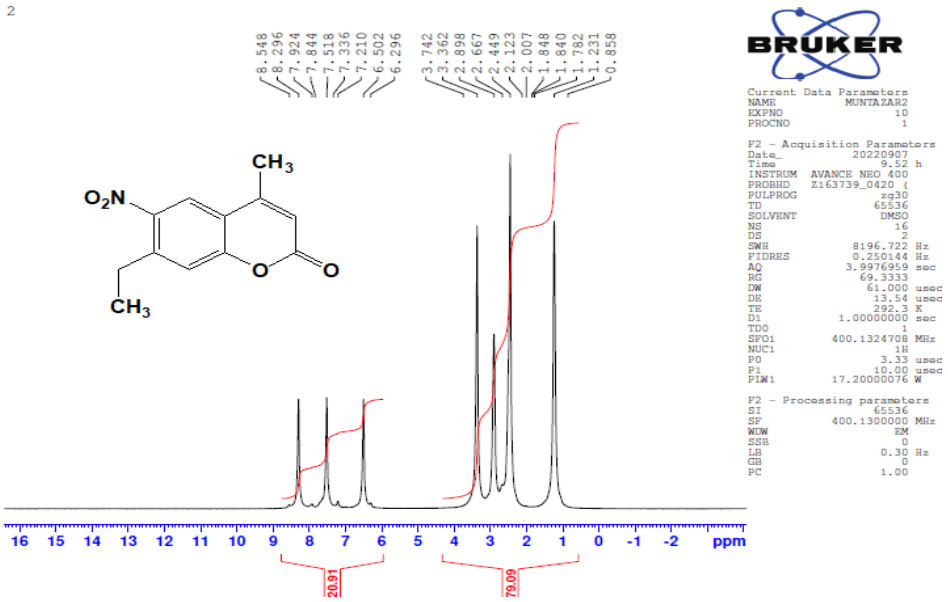


Figure 1: ^{1}H NMR spectrum of compound **2**

The ^{1}H NMR spectrum of compound **3** showed a triplet signal at 1.1ppm belonging to the CH_3 protons of the ethyl group and a quartet signal at 2.90 ppm for the CH_2 protons of the ethyl group. Two doublet signals at 7.5 and 8.54 ppm are due to aromatic ring protons and a singlet signal at 6.3 is due to one proton of the lactone ring (Figure 2). The ^{13}C NMR spectrum showed a signal at 159.3 ppm for the C=O group.

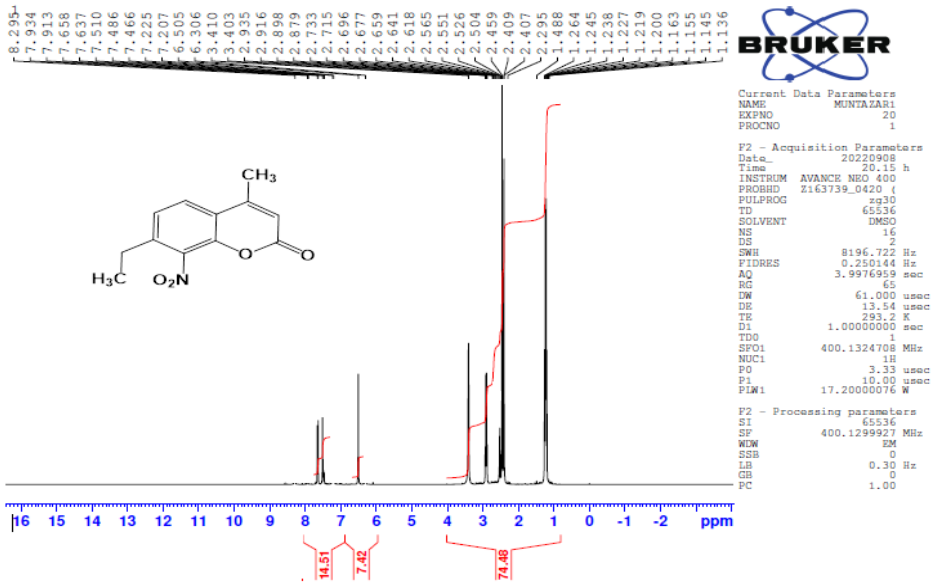


Figure 2: ^{1}H NMR spectrum of compound **3**

The reduction of the nitro group at compounds **2** and **3** was carried out using iron metal and hydrochloric acid to form 7-ethyl-4-methyl-6-aminocoumarin **4** and 7-ethyl-4-methyl-8-aminocoumarin **5**, respectively. The FT-IR spectra of compounds **4** and **5** showed asymmetric and symmetric stretching bands of the NH_2 group at 3444 and 3355 cm^{-1}, respectively, and the disappearing of the asymmetric and symmetric stretching bands of the NO_2 group. The ^1H NMR spectra of compounds **4** and **5** showed singlet signals at 5.0 and 5.1 ppm due to two protons of NH_2, respectively (Figure 3). The ^{13}C NMR spectrum showed a signal at 160 ppm for the carbonyl group (Figure 4).

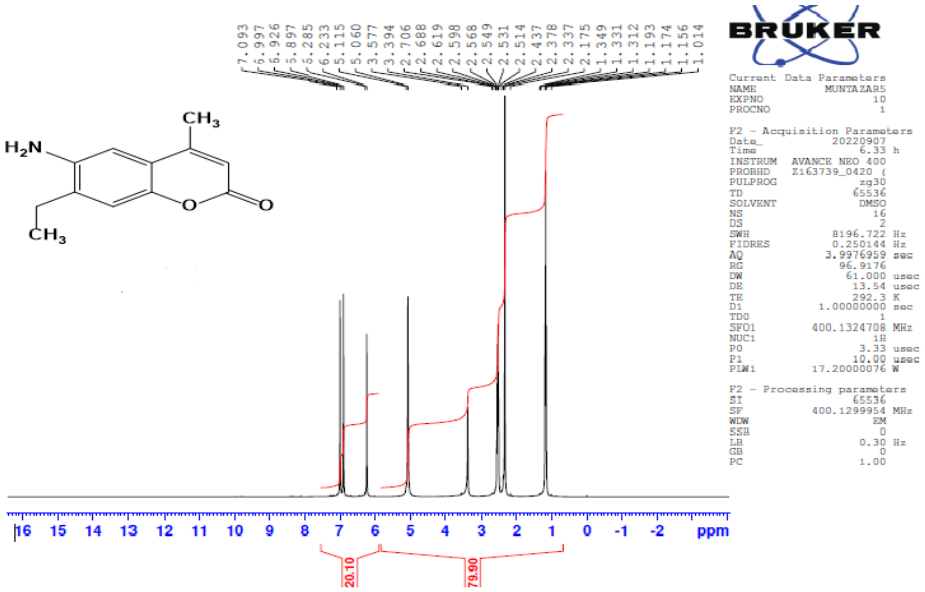


Figure 3: ^1H NMR spectrum of compound **4**

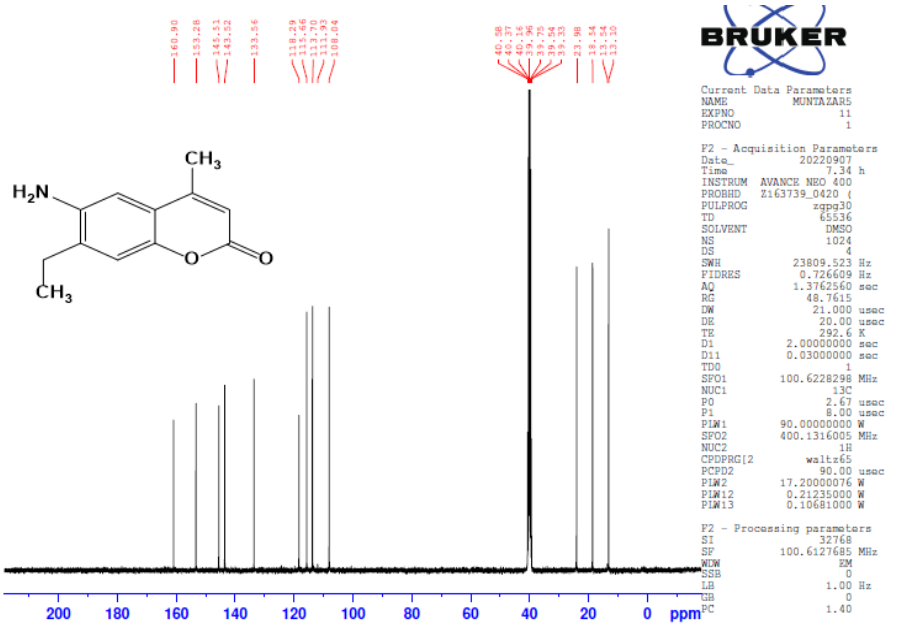


Figure 4: ^{13}C NMR spectrum of compound **4**

The mass spectrum for the same compound showed that the value of the $M+H^+$ ion absorption signal was m/z 204, consistent with the theoretical value (204.09) for $C_{12}H_{14}NO_2^+$ (Figure 5).

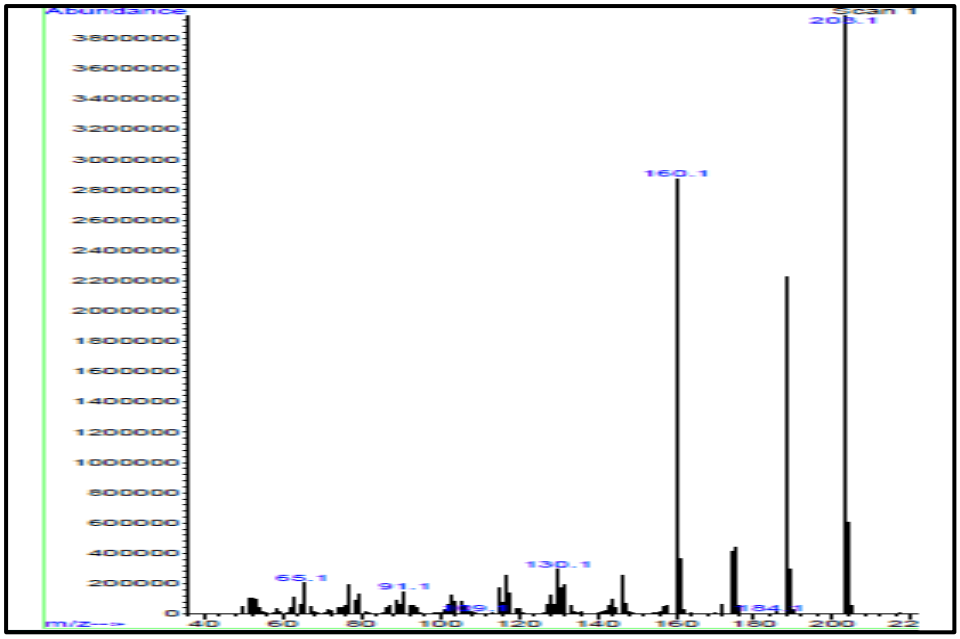
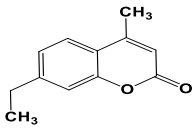
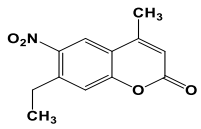
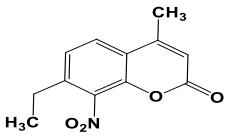
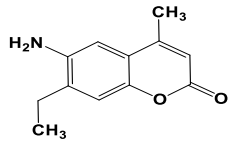
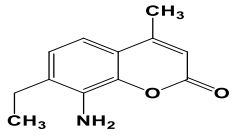
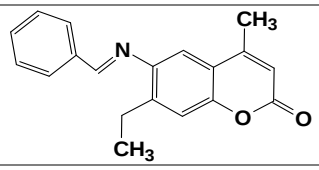
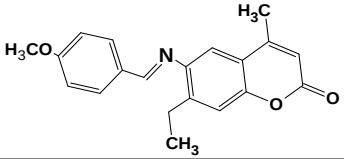
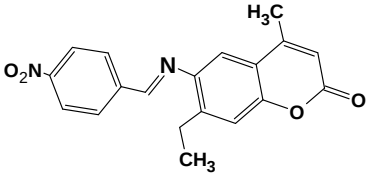
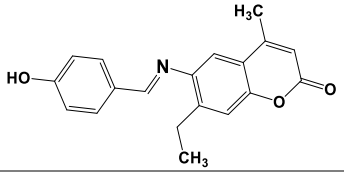
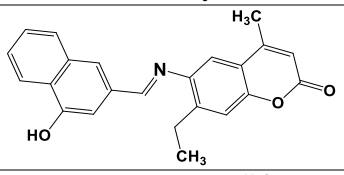
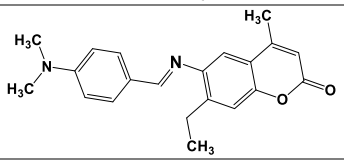
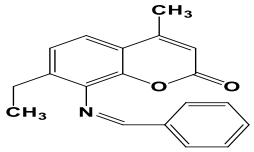
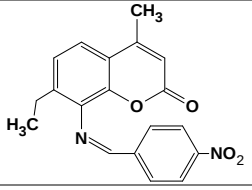
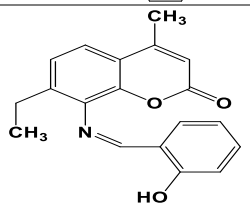


Figure 5: Mass spectrum of compound **4**

Finally, the products **4** and **5** were reacted with different substituted aromatic aldehydes in the presence of glacial acetic acid as a catalyst to form Schiff base derivatives **6-17** [27]. The chemical structure of these compounds (**6-17**) was verified by FT-IR spectroscopy (Table 1), which showed stretching absorption bands between 1643 and 1600 cm^{-1} for the imine bonds (CH=N) and the disappearance of the vibration of the NH_2 group. The ^1H NMR spectra of the synthesized compounds showed multiple signals from 8.4 to 6.1 ppm for the aromatic and imine protons. The ^1H NMR of compound **11** showed a new singlet signal at 3.0 ppm for the six protons of the two methyl groups. While the ^1H NMR of compound **14** showed a new singlet signal at 8.99 ppm for one proton of the phenolic hydroxyl group. Also, compound **17** showed a singlet signal at 3.44 ppm for the six protons of two methyl groups, as shown in Table 2. All synthesized compounds showed signals at 161.3-159.7 ppm for the carbon of carbonyl in the ^{13}C NMR spectra.

Table 1: FT-IR spectral data (cm^{-1}) of the prepared compounds (**1-17**)

No.	Structure	ν(C-H) Arom.	ν(C-H) Aliph.	ν(C=O)	ν(C=N) imine	ν(C=C)	Other bonds
1		3058	2964 asym. and 2939 Sym.	1706	-	1620 and 1456	ν(C-OC) 1191
2		3056	2987 asym. and 2921 Sym.	1766	-	1623 and 1458	ν(NO_2) 1525 asym. and 1352 sym.
3		3085	2987 asym. and 2921 Sym.	1766	-	1623 and 1458	NO_2 1523 asym. and 1357 sym.

4		3053	2970 asym. and 2935 Sym	1733	-	1596 and 1411	ν(NH_2) 3444 asym. and 3355 sym.
5		3063	2979 asym. and 2920 Sym.	1687	-	1612 and 1431	ν(NH_2) 3444 asym. and 3355 sym.
6		3056	2968 asym. and 2935 Sym	1735	1627	1575 and 1446	-
7		3068	2964 asym. and 2935 Sym	1731	1627	1604 and 1456	ν (C-O) 1230 δ*p*-position 827
8		3058	2972 asym. and 2935 Sym	1720	1631	1596 and 1456	ν (C-Cl) 1051 δ*p*-position 829
9		3058	2970 asym. and 2898 Sym	1685	1600	1581 and 1444	δ*p*-position 829 ν(OH) 344
10		3052	2964 asym. and 2929 Sym	1691	1643	1558 and 1463	ν(OH) 342
11		3053	2968 asym. and 2916 Sym	1735	1600	1590 and 1433	δ*p*-position 817
12		3056	2999 asym. and 2935 Sym	1715	1629	1575 and 1413	-
13		3058	2975 asym. and 2935 Sym	1724	1629	1598 and 1411	ν(NO_2) 1521 asym. and 1384 sym.
14		3053	2970 asym. and 2935 Sym	1733	1616	1571 and 1450	ν(OH) 341

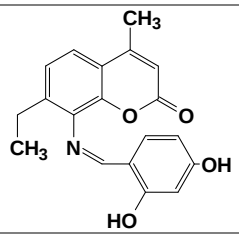
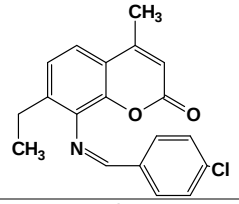
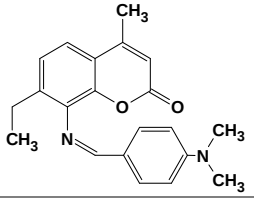
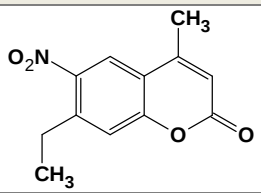
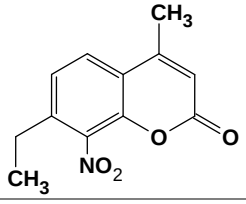
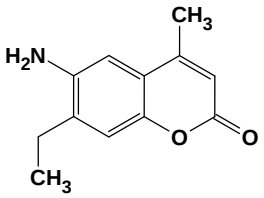
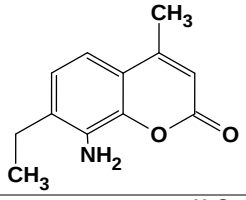
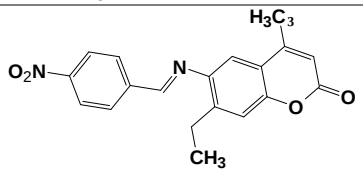
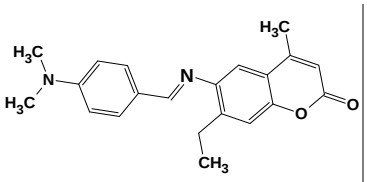
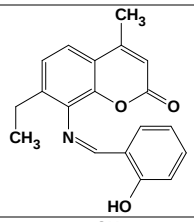
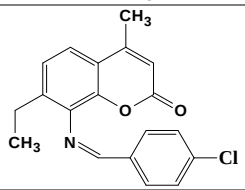
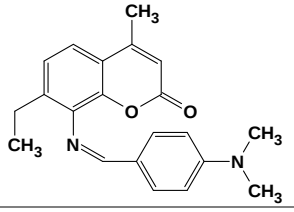
15		3068	2970 asym. and 2931 Sym	1710	1622	1581 and 1446	δ*p*-position 850 ν(OH) 3436 &340
16		3074	2970 asym. and 2933 Sym	1695	1629	1606 and 1433	δ*p*-position 840
17		3053	2970 asym. and 2881 Sym	1735	1600	1573 and 1433	δ*p*-position 817

Table 2: ^{1}H NMR and ^{13}C NMR spectral data (δ ppm) of compounds (**1**, **2**, **3**, **4**, **8**, **11**, **14**, **16**, and **17**)

No.	Structure	^{1}H-NMR spectral data (δ ppm)	^{13} C-NMR spectral data (δ ppm)
2		1.2 (t, J=7.1,3H, $\underline{CH_3}$-CH_2), 2.89 (q, J=7.1,2H, CH_3-$\underline{CH_2}$), 2.4 (s, 3H, CH_3), 6.29 (s, 1H, H-lactone ring), 7.5 (s, 1H, Ar-H), 8.4 (s, 1H, Ar-H)	14.9, 18.3, 25.84, 115.8, 118.6, 119.1, 122.7, 142.8, 145.3, 152.7, 155.4 159.32
3		1.2 (t, J=7.12,3H, $\underline{CH_3}$-CH_2), 2.9 (q, J=7.12,2H, CH_3-$\underline{CH_2}$), 2.5 (s, 3H, CH_3), 7.5 (d, J=8, 1H, Ar-H), 8.24 (d, J=8, 1H, Ar-H), 6.34 (s, 1H,H-lactone ring)	14.9, 18.3, 25.8, 115.8, 118.7, 119.6, 122.7, 148.7, 145.4, 152.8, 155.4, 195.4
4		1.15 (t, J=7.0,3H, $\underline{CH_3}$-CH_2), 2.51 (q, J=7.0,2H, CH_3-$\underline{CH_2}$), 2.31 (s, 3H, CH_3), 5.04 (s, 2H, NH_2), 6.21 (s, 1H, H-lactone ring), 6.9 (s, 1H, Ar-H), 7.61 (s, 1H, Ar-H)	13.0, 18.5, 23.9, 108.0, 111.9, 115.6, 118.2 133.5, 143.5, 145.5, 153.0, 160.9
5		1.15 (t, J=7.12,3H, $\underline{CH_3}$-CH_2), 2.6 (q, J=7.12,2H, CH_3-$\underline{CH_2}$), 2.3(s, 3H, CH_3), 5.4 (s, 2H, NH_2), 6.3 (s,1H,H-lactone ring), 7.2(d, J=7.8,1H, Ar-H), 7.6 (d, J=7.8, 1H, Ar-H)	13.1, 18.5, 24.0, 108.0, 113.7, 115.6, 118.3, 133.5, 143.5, 145.5, 153.3, 160.9
8		1.4 (t, J=7.3,3H, $\underline{CH_3}$-CH_2), 2.4 (q, J=7.3,2H, CH_3-$\underline{CH_2}$), 2.5 (s, 3H, CH_3), 6.35 (s, 1H, CH lactone), 7.36-8.86 (m, 7H, Ar-$\underline{H\ and}$-N=CH-)	15.1, 18.6, 24.8, 114.2, 114.3, 116.8, 118.7, 124.6, 130.1, 141.9, 144.0, 146.1, 149.3, 152.7, 153.9, 159.7, 160.3

11		1.15 (t, J=7.12,3H, $\underline{CH_3}$-CH_2), 2.4(q, J=7.3,2H, CH_3-$\underline{CH_2}$), 2.5 (s, 3H, CH_3), 3.03(s, 6H, $2CH_3$), 6.3(s, 1H, CH lactone), 6.81- 8.44(m, 7H, Ar-H and, -N=CH-),	24.1, 18.7, 24.9, 40.6, 111.9, 113.6, 113.9, 116.4, 118.6, 124.3, 130.8, 143.3, 151.0, 152.9, 153.9, 160.5, 160.6.
14		1.19 (t, J=7.2,3H, $\underline{CH_3}$-CH_2), 2.79 (q, J=7. 2, 2H, CH_3-$\underline{CH_2}$), 2.49 (s, 3H, CH_3), 6.3 (s, 1H, CH lactone) 7.0-8.99 (m, 7H, Ar-$\underline{H}$ and, -N=CH-), 13 (s, 1H, OH)	14.8, 18.7, 25.1, 114.4, 114.9, 116.9, 117.0, 117.1, 118.9, 119.0, 119.7, 119.8, 133.0, 134.0, 143.1, 143.8, 153.8, 160.7, 164.5
16		1.19 (t, J=7.0,3H, $\underline{CH_3}$-CH_2), 2.83 (q, J=7.0,3H, CH_3-$\underline{CH_2}$), 2.4 (s, 3H, CH_3), 6.3 (s, 1H, CH lactone), 7.0-8.49 (m, 7H, Ar-H and -N=CH-)	14.90, 18.7, 24.9, 113.9, 114.0, 116.1, 116.4, 118.6, 127.6, 131.2, 143.2, 147.6, 151.3, 154.0, 160.4, 160.9, 161.3
17		1.18 (t, J=7.1,3H, $\underline{CH_3}$-CH_2), 3.02 (q, J=7.1,2H, CH_3-$\underline{CH_2}$), 2.67 (s, 3H, CH_3), 3.44 (s, 6H, $2CH_3$), 6.11 (s, 1H, CH lactone), 6.8-7.94 (m, 7H, Ar-H and-N=CH-),	24.1, 18.7, 24.9, 40.6,11.9, 113.6, 113.9, 116.4, 118.6, 124.3, 130.8, 143.3, 151.0, 152.9, 153.9, 160.5, 160.6

3.2. Biological activity [28-31]

3.2.1. 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. In the agar well diffusion method (well diameter was 5 mm) using DMSO as a solvent, Vancomycin, Meropenem, Amikacin, and Nystatin were used as standard drugs at 37 °C for 24 hours.

3.2.2. Antibacterial activity

The synthesized compounds generally show high antibacterial activity when compared to the gold standard antibiotic (Vancomycin), which has an inhibition area of 13 mm against gram-positive bacteria (*Staphylococcus aureus*). The compounds **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **13**, **14**, and **17** showed good activity close to that of the used antibiotic with an inhibition zone ranging from 10 to 12 mm, while compound **15** showed higher activity than the used antibiotic with an inhibition zone of 23 mm, as shown in Table 3. The biological activity of all the prepared compounds (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. The compounds **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **13**, **14**, and **17** showed moderate activities compared to the used antibiotics with an inhibition zone of 12 and 13 mm, while compound **15** showed good activity compared to the used antibiotics with an inhibition zone of 21 mm, as shown in Table 3.

3.2.3. Antifungal activity

All the compounds that were prepared showed higher antifungal biological activity than the standard antibiotic used (Nystatin), as their effectiveness was tested against one type of fungus (*Candida albicans*). The compounds (**1**, **2**, **3**, **4**, **5**, **6**, **8**, **13**, **14**, **15**, and **17**) showed moderate activity with an inhibition zone ranging from 10 to 14 mm, while compound **7**

showed good activity compared to the used antibiotic with an inhibition zone of (17 mm), as shown in Table 3.

Table 3: Inhibition zones in mm for the prepared compounds **1-17**

Compound No.	*Staphylococcus Aureus* (mm)	*Escherichia Coli* (mm)	Candida (mm)
1	11	12	10
2	11	13	11
3	10	13	10
4	10	12	10
5	10	13	10
6	11	12	12
7	11	12	17
8	11	13	11
13	11	12	10
14	12	13	10
15	23	21	14
17	11	12	12
Vancomycin,	13	-	-
Meropenem	-	25	-
Amikacin	-	21	-
Nystatin	-	-	23

3.2.3. Antioxidant activity [32]

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, foods, proteins, and cosmetics, is composed of three steps: initiation, propagation, and termination of DPPH scavenging activity. The antioxidant properties of a few produced compounds were compared to that of vitamin C (ascorbic acid). Compounds **2**, **4**, and **13** showed moderate antioxidant activity, while compound **8** showed weak antioxidant activity. All compounds were predestined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method at various concentrations (6.5, 12.5, 25, 50, and 100 µg/mL). Depending on whether the reaction is characterized by a change in its deep violet color (DPPH) or decolourization, the result is stoichiometric concerning several captured electrons. Accordingly, inhibitory concentration (IC_{50}) values were recorded and tabulated in Table 4.

Table 4: The antioxidant activity of some prepared compounds **2**, **4**, **8**, and **13**

Compound No.	Scavenging (%)					R2	Linear Equation	IC_{50}
	6.25 µg\mL	12.5 µg\mL	25 µg\mL	50 µg\mL	100 µg\mL			
2	30.95	32.23	36.06	41.18	56.52	$R^2 = 0.995$	y = 0.2714x + 28.872	77.85
4	34.53	35.55	35.55	46.8	65.98	$R^2 = 0.974$	y = 0.3481x + 30.192	56.9
8	17.11	18.47	19.1	23.42	24.8	$R^2 = 0.885$	y = 0.0824x + 17.387	17.5
13	5.5	12.12	23	33.78	64.41	$R^2 = 0.990$	y = 0.6042x + 4.3488	45.65
Ascorbic acid	42.92	52.74	65.92	83.07	97.11	$R^2 = 0.906$	y = 0.55x + 47.04	5.38

4. Conclusion

In summary, we have successfully synthesized new 7-ethyl-4-methylcoumarin derivatives bearing an azo methene group (-C=N-) which were synthesized through a series of sequential reactions through nitro-coumarin derivatives which were synthesized by very easy method then reduction nitro-coumarins were by Fe in the presence of glacial acetic acid to the corresponding amino coumarins, which were converted to Schiff bases by reacting them with aromatic aldehydes then they were tested of their biological activity. Compound 15 showed a broad spectrum against different types of bacteria, *Staphylococcus aureus,* and *Escherichia coli,* compared to vancomycin, meropenem and amikacin as standard drugs while nystatin was used a standard fungi drug. Compound 7 showed a stronger activity against fungi than the other synthesized compounds. In the antioxidant study, some new compounds showed powerful antioxidants compared with ascorbic acid as a stander by calculation of the IC50. Compound 8 showed very good as an antioxidant agent compared with the same stander.

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