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Synthesis, Characterization, and Anti-oxidant Evaluation of Novel Coumarin–Pyrazoline Derivatives

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Abstract. General Background: Oxidative stress is a major contributor to the pathogenesis of chronic diseases such as cancer and cardiovascular disorders. Specific Background: Coumarin and pyrazoline derivatives are known for their pharmacological activities, particularly their antioxidant potential. Knowledge Gap: However, limited studies have explored the synthesis and evaluation of coumarin–pyrazoline hybrids as potent free radical scavengers. Aims: This study aimed to synthesize and characterize novel coumarin–pyrazoline derivatives and evaluate their antioxidant activity using the DPPH assay. Results: Six new compounds were synthesized and structurally confirmed by ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. Compounds 2 and 5 exhibited superior antioxidant activity compared to ascorbic acid, with IC_{50} values of 18.3 µM and 19.7 µM, respectively, versus 23.9 µM for ascorbic acid. Novelty: The enhanced activity was attributed to specific functional groups: the methoxy group in compound 2 and the dimethylamino group in compound 5. Implications: These findings support the potential of coumarin–pyrazoline hybrids as promising antioxidant agents and lay the groundwork for future therapeutic development targeting oxidative stress-related conditions.

Highlights:

1. Coumarin–pyrazoline compounds 2 and 5 showed stronger antioxidant activity than ascorbic acid.
2. Structures were confirmed through ^{1}H NMR, ^{13}C NMR, and mass spectrometry.
3. Antioxidant efficiency is linked to specific functional groups like OCH_3 and $N(CH_3)_2$.

Keywords: Anti-oxidant activity, Coumarin, DPPH assay, Pyrazoline, IC50

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Introduction

Anti-oxidants play a critical role in protecting cellular organelles from damage caused by oxidative stress induced by free radicals. These radicals, such as hydroxyl radicals, superoxide anions, and hydrogen peroxide, are highly reactive species that may originate from metabolic processes or external sources, including environmental toxins and certain food components. If not neutralized, free radicals can cause significant damage to essential biomolecules such as DNA, proteins, and lipids, thereby contributing to the development of various chronic diseases, including cancer, hypertension, and coronary heart disease [1]. The human body is equipped with endogenous defense systems, including enzymes such as catalase, superoxide dismutase, and the glutathione peroxidase system, which collectively scavenge and neutralize these reactive species during metabolism [2]. Among naturally occurring compounds with potent anti-oxidant properties, coumarins have received considerable attention. Belonging to the lactone family with a benzopyrone core, coumarins can be isolated from natural sources or synthesized chemically [3]. Due to their broad conjugated π-electron systems, coumarins are effective in donating electrons or hydrogen atoms to neutralize free radicals [4]. In addition to their anti-oxidant potential, coumarins exhibit diverse pharmacological properties, including antibacterial [5], antitumor [6], anti-HIV [7], anti-inflammatory [8], and antidepressant [9]. Their wide range of bioactivities and synthetic versatility has made them attractive targets in medicinal chemistry [10]. Pyrazoline, on the other hand, is a five-membered heterocycle with two adjacent nitrogen atoms and three carbon atoms. [11]. Pyrazoline derivatives have demonstrated a wide spectrum of biological activities, including analgesic, antipyretic, anti-cancer, antihyperglycemic, antimicrobial, and anti-oxidant effects [12]. The molecular hybridization approach, which involves the combination of two or more pharmacophores into a single hybrid structure, has emerged as a powerful strategy in drug discovery [13]. By combining the coumarin and pyrazoline moieties into a single molecule, researchers aim to enhance pharmacological efficacy and develop novel agents for the treatment of multifactorial diseases [14].

Method

All chemicals used in this study were of high purity, ranging from 99% to 99.9%, and were purchased from BDH, Merck AG, and Thomas Baker. Melting points were

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determined using an electrothermal melting point apparatus (SMP30). The 1HNMR, and 13CNMR spectra were recorded on a Bruker spectrometer (Bruker, Switzerland), using DMSO-d_6 and $CDCl_3$ as solvents. Tetramethyl silane was employed as the internal standard. The electron ionization mass spectra (EI-MS) of the synthesized compounds were recorded at the Faculty of Chemistry, University of Tehran.

A. Synthesis of 3-acetylcoumarin

A solution of ethyl acetoacetate (0.06mole) and salicylic acid (0.06mole) was mixed, and 10 drops of dimethylamine were added as a catalyst with stirring for 30 minutes. As the reaction progressed, a yellow solid appeared. The precipitate was obtained by filtration and recrystallisation with methanol, yielding 51% [15].

B. Synthesis of Coumarin Chalcone

A mixture (0.06mole) of coumarin chalcone compounds and (15mL) of glacial acetic acid was added to a (100ml) round-bottom flask containing (0.12mole) of hydrazine hydrate (80%). The mix was refluxed for 2–8 hours. TLC (ethyl acetate-n-hexane, 4:8) was used to monitor the reaction under ultraviolet light. Add to ice-cold water, filter, and recrystallize with a suitable solvent [17], as in Figure 1.

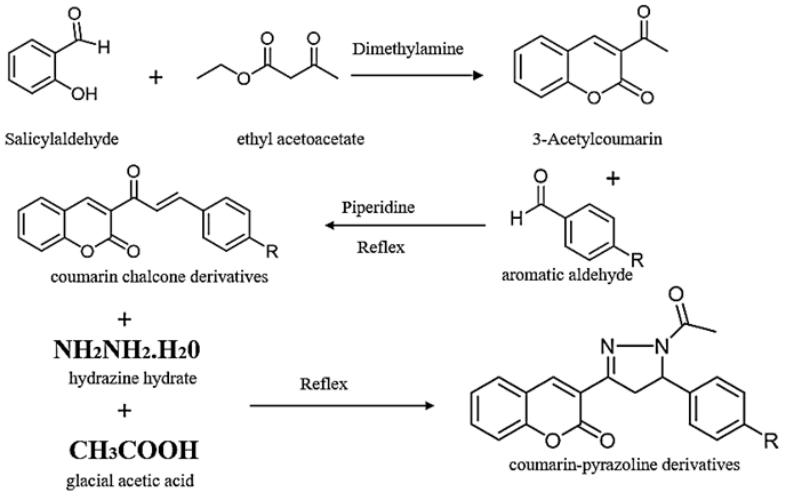

Figure 1. General synthesis of coumarin-pyrazoline derivatives (compounds 1-6)

Results and Discussion

A coumarin-pyrazoline compounds was successfully synthesized starting from 3-

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acetylcoumarin and various aromatic aldehydes to form coumarin–chalcone intermediates, which then underwent cyclisation with hydrazine hydrate (80%) in glacial acetic acid. The physical properties of the synthesized compounds are summarized in Table 1. The structures of the synthesized compounds 1-6 were confirmed through detailed spectroscopic analysis, including 1HNMR, 13CNMR, and mass spectrometry (MS).

Table 1. Physical properties of coumarin-coumarin pyrazoline compounds 1-6

Compound	Molecular Formula	Appearance	Yield %	Melting Point (^{0}C)
1	$C_{20}H_{16}N_2O_3$	Yellow powder	23.9	196-198
2	$C_{21}H_{18}N_2O_4$	Yellow powder	46.7	191-192
3	$C_{20}H_{15}N_2Br\ O_3$	Yellow crystal	39.2	181-182
4	$C_{22}H_{19}N_3O_4$	Yellow powder	38.7	229-231
5	$C_{23}H_{25}N_3O_3$	Yellow crystal	19.5	192-194
6	$C_{20}H_{15}N_2O_4$	Yellow crystal	27.4	245-247

A. 1HNMR Spectroscopy of coumarin-pyrazoline 1-6

The 1H-NMR spectra of the synthesized coumarin–pyrazoline derivatives exhibited a singlet signal corresponding to the N-acetyl group (–$COCH_3$), which was observed in the range of 2.30–2.44 ppm, integrating for three protons[18]. A doublet of doublets was observed in the region of 3.85–3.96 ppm, with coupling constants of approximately 19 Hz (geminal coupling) and 12 Hz (vicinal coupling), corresponding to a single proton (Ha) at the C-4″ position of the pyrazoline ring. Another doublet of doublets appeared in the region of 3.18–3.42 ppm, with coupling constants of approximately 19 Hz (geminal) and 4.8 Hz (vicinal), attributed to a single proton (Hb) also located at C-4″. A third doublet of doublets was observed in the region of 5.43–5.59 ppm, with coupling constants of approximately 12 Hz and 4.8 Hz, resulting from vicinal coupling with the Ha and Hb protons. This signal, integrating for one proton(Hc), corresponds to the proton at C-5″ of the pyrazoline ring[17][19]. shown in figures (2-7).

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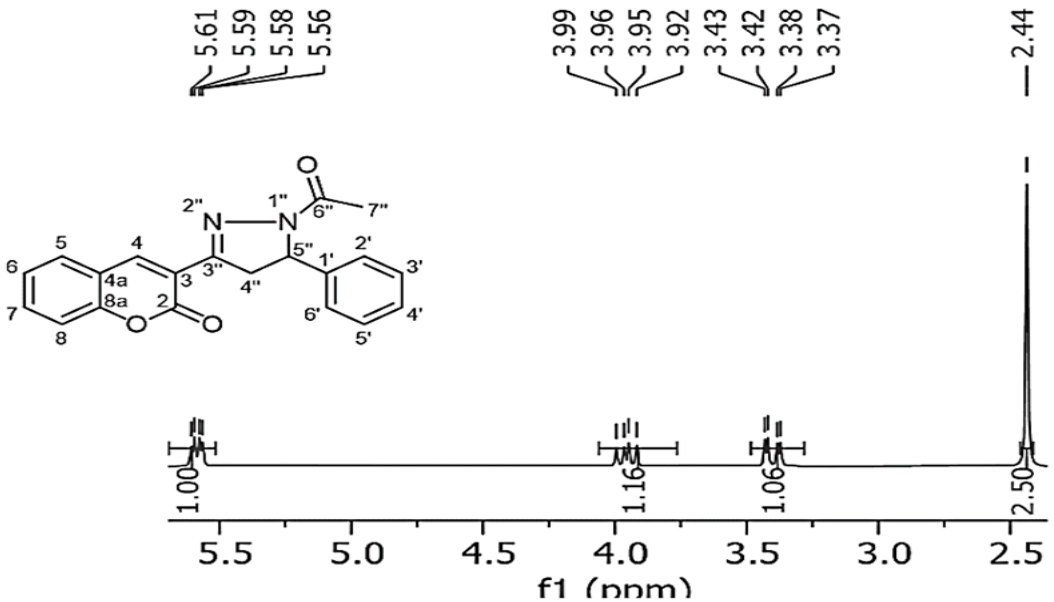

Figure 2. [1]HNMR of compound 1

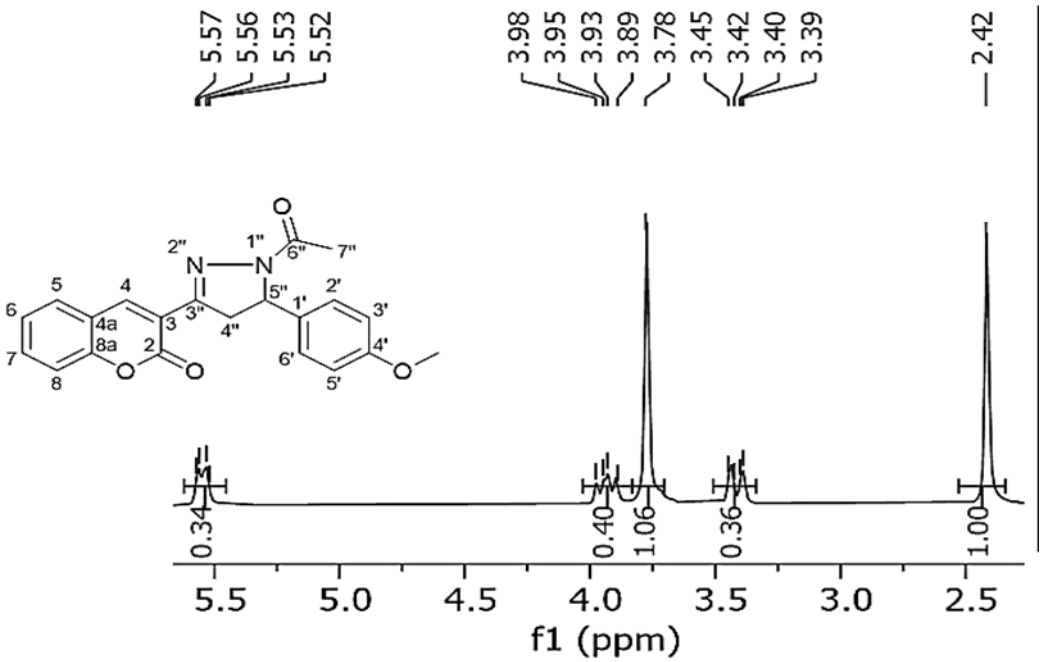

Figure 3. [1]HNMR of compound 2

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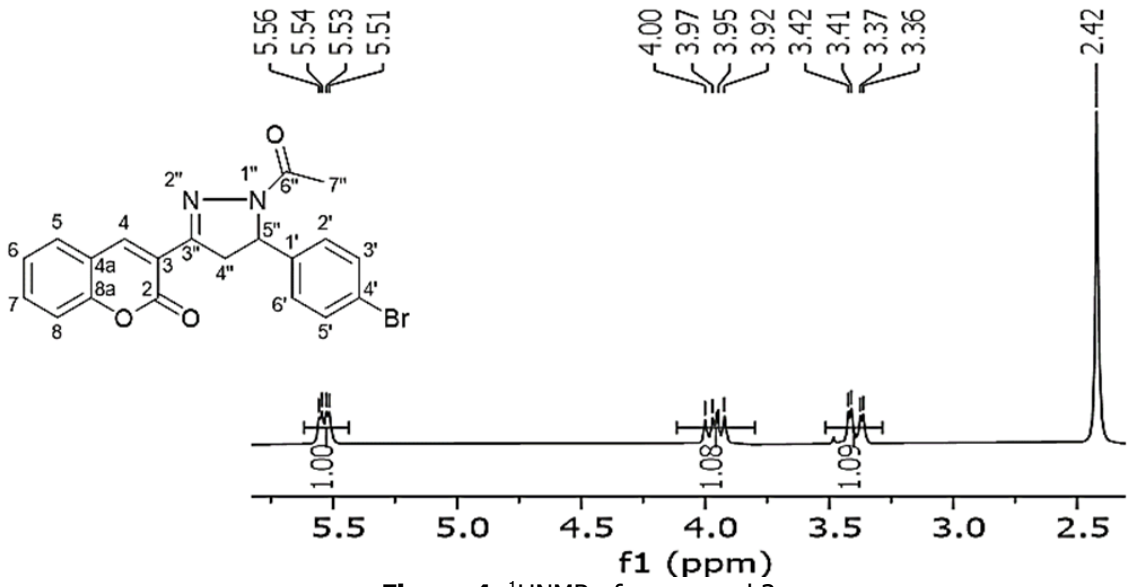

Figure 4. [1]HNMR of compound 3

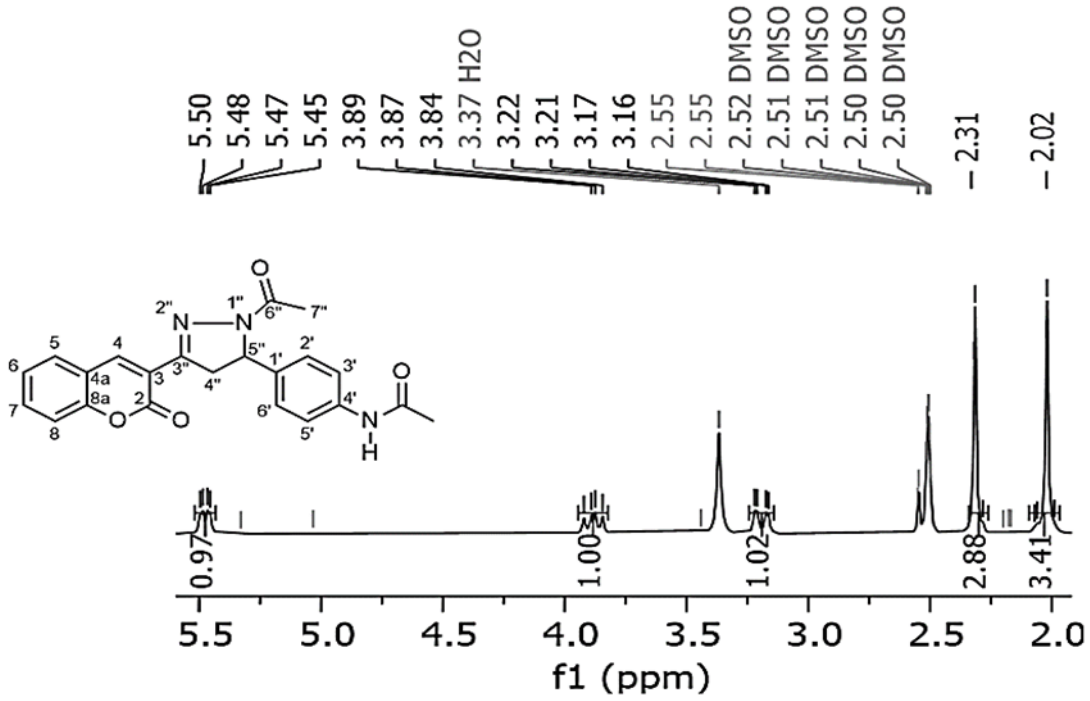

Figure 5. [1]HNMR of compound 4

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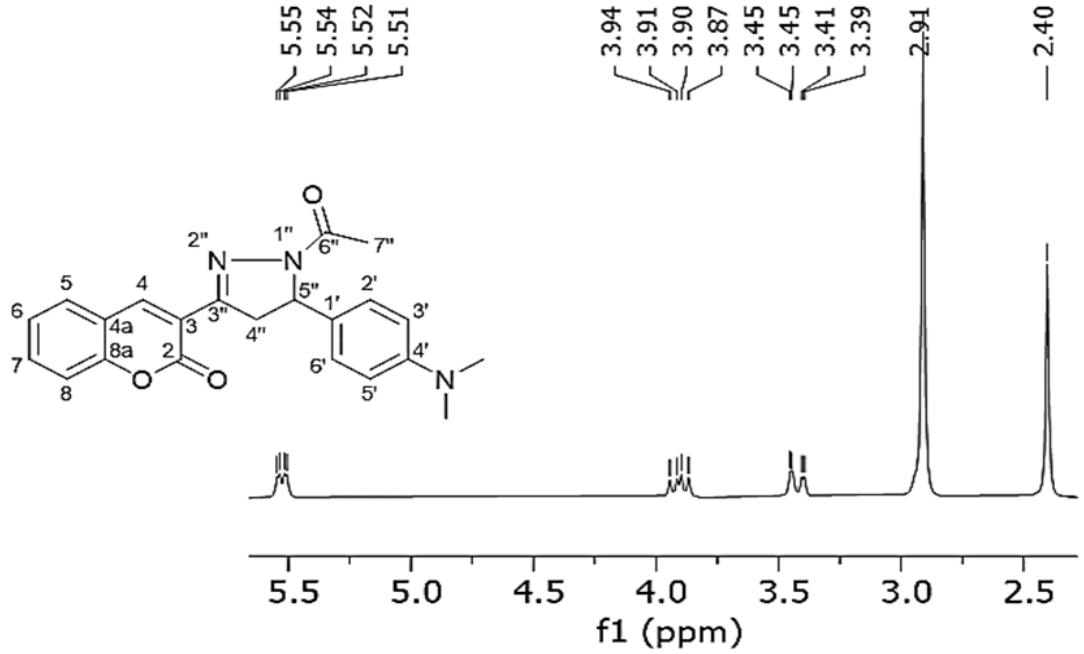

Figure 6. [1]HNMR of compound 5

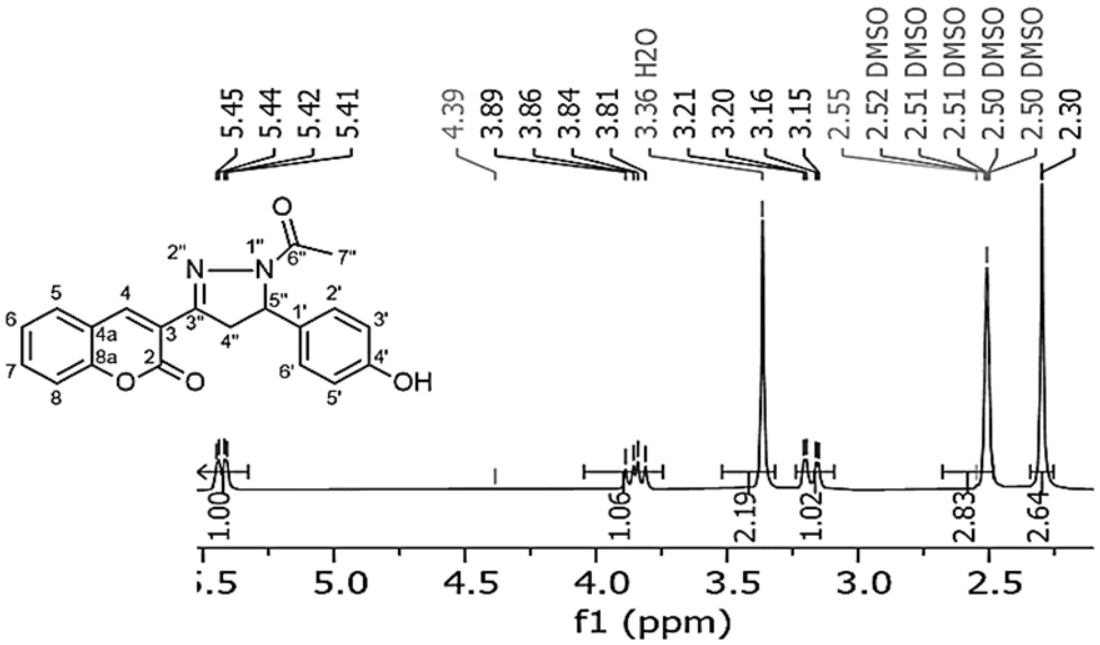

Figure 7. [1]HNMR of compound 6

B. The [13]C NMR Spectroscopy of coumarin pyrazoline 1-6

[13]CNMR spectra of the synthesized coumarin–pyrazoline derivatives show signals at approximately 44 ppm and 59 ppm, which are attributed to the formation of the pyrazoline ring, corresponding to C-4″ and C-5″, respectively[20].

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Additionally, a signal at 153 ppm indicates the presence of a C=N group [21]. Also, two signals appear at 168.8 and 22.2 ppm; these signals are attributed to the ketone carbonyl (C=O) and the methyl group at the pyrazoline ring[22][23]. Shown in figures (8–13).

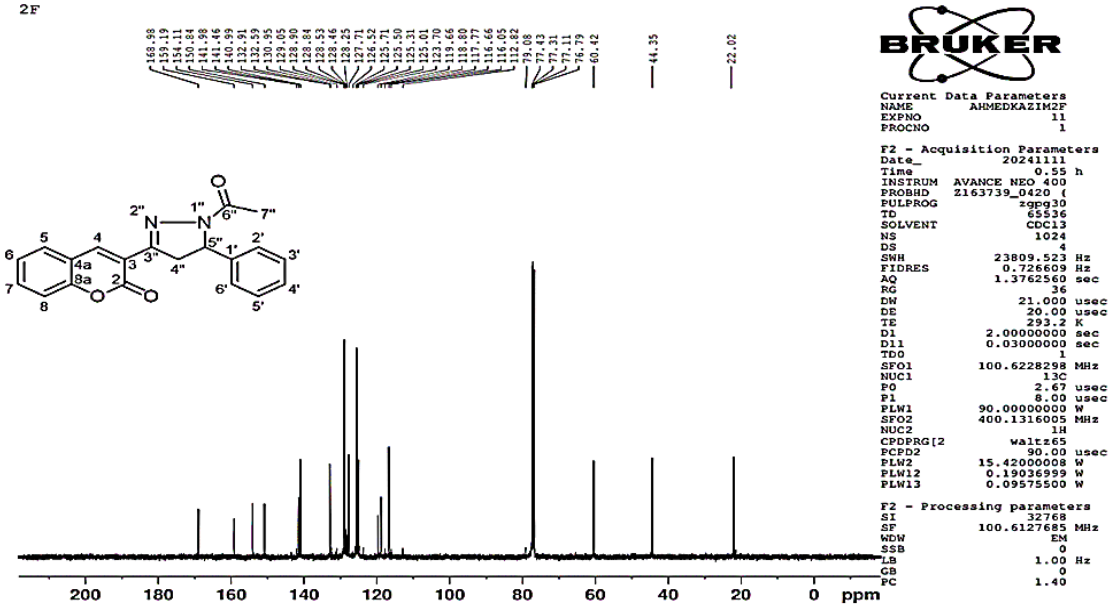

Figure 8. ^{13}CNMR of compound 1

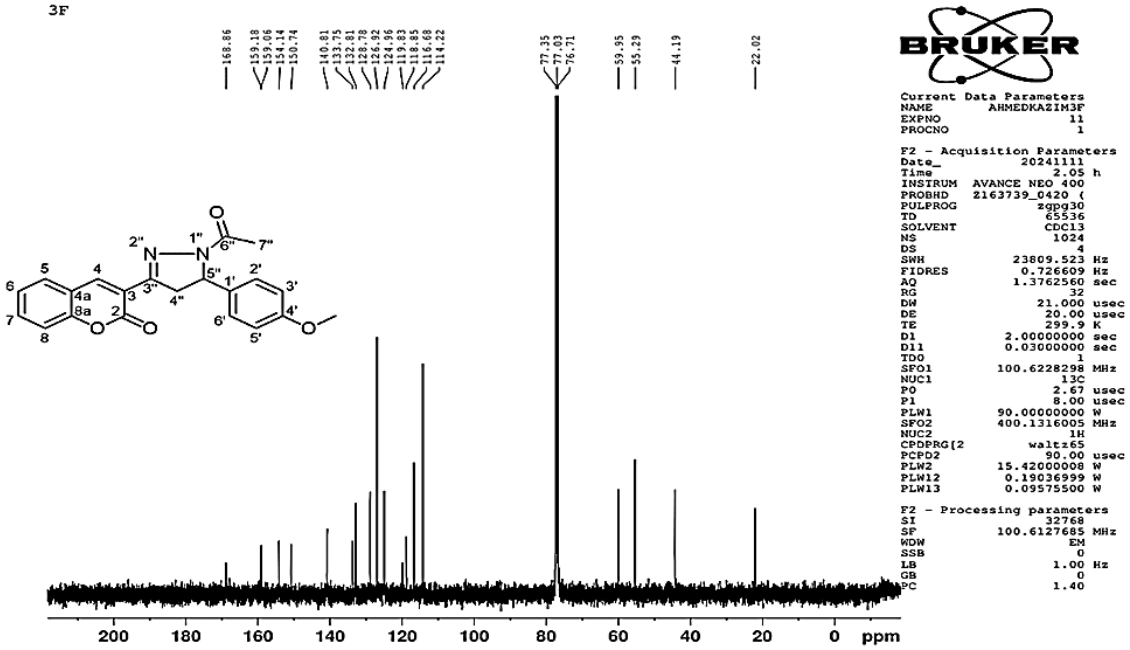

Figure 9. ^{13}CNMR of compound 2

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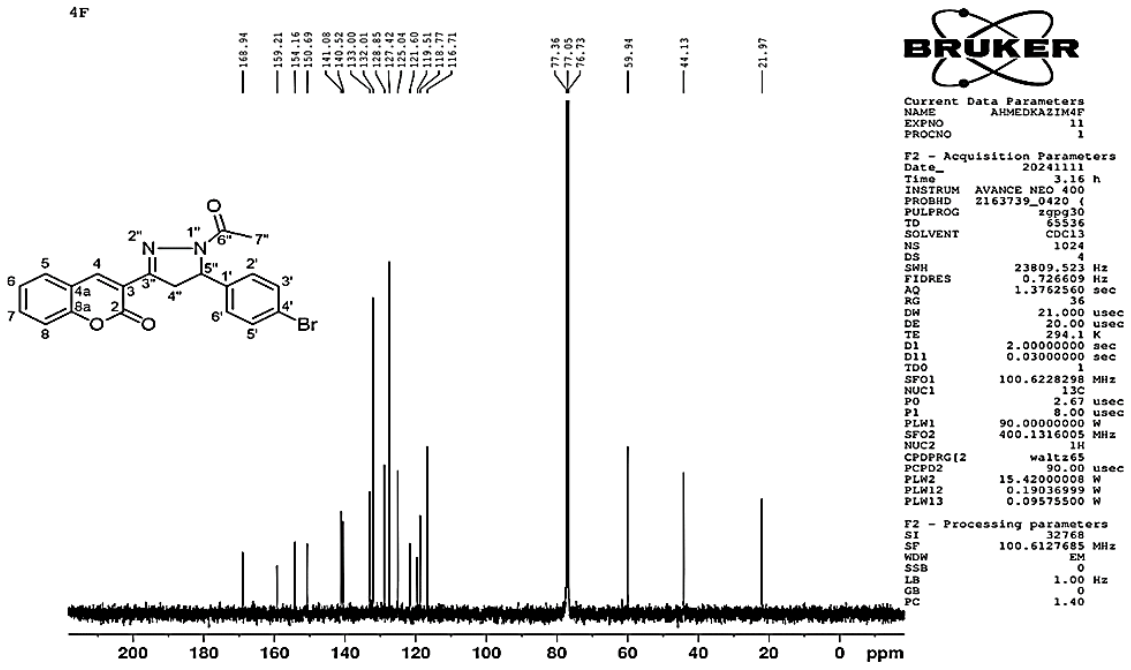

Figure 10. ^{13}CNMR of compound 3

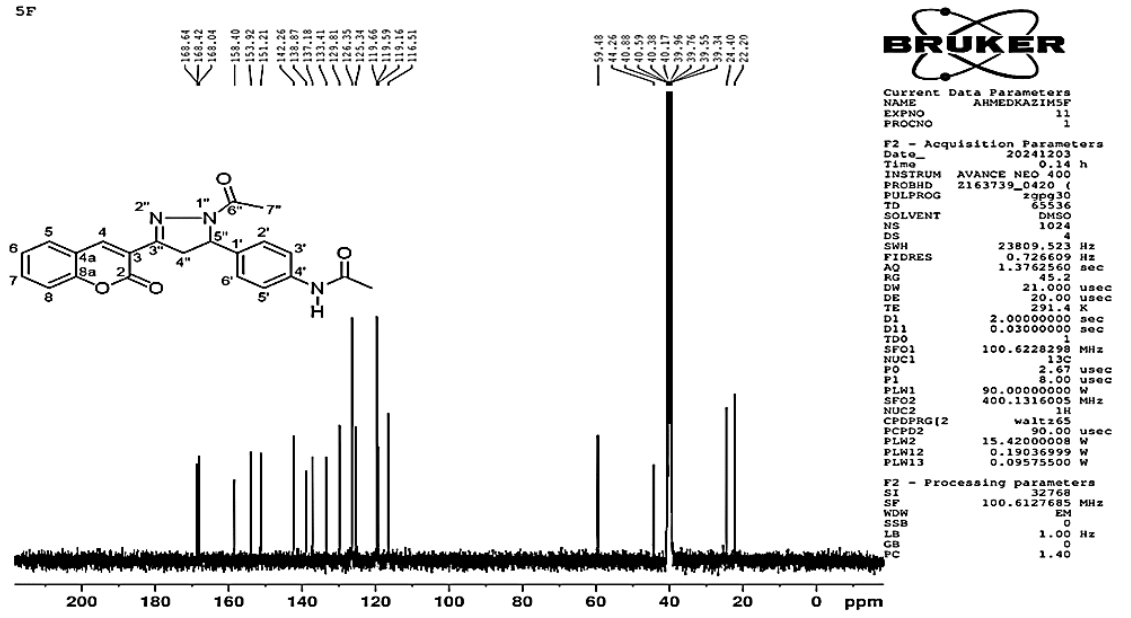

Figure 11. ^{13}CNMR of compound 4

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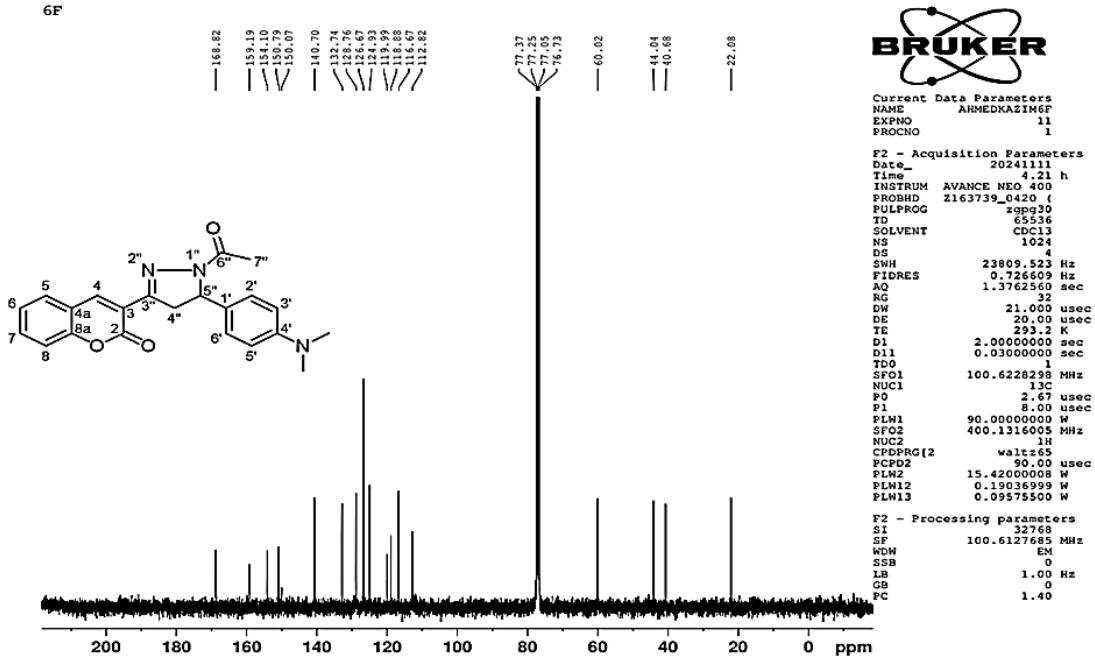

Figure 12. ^{13}CNMR of compound 5

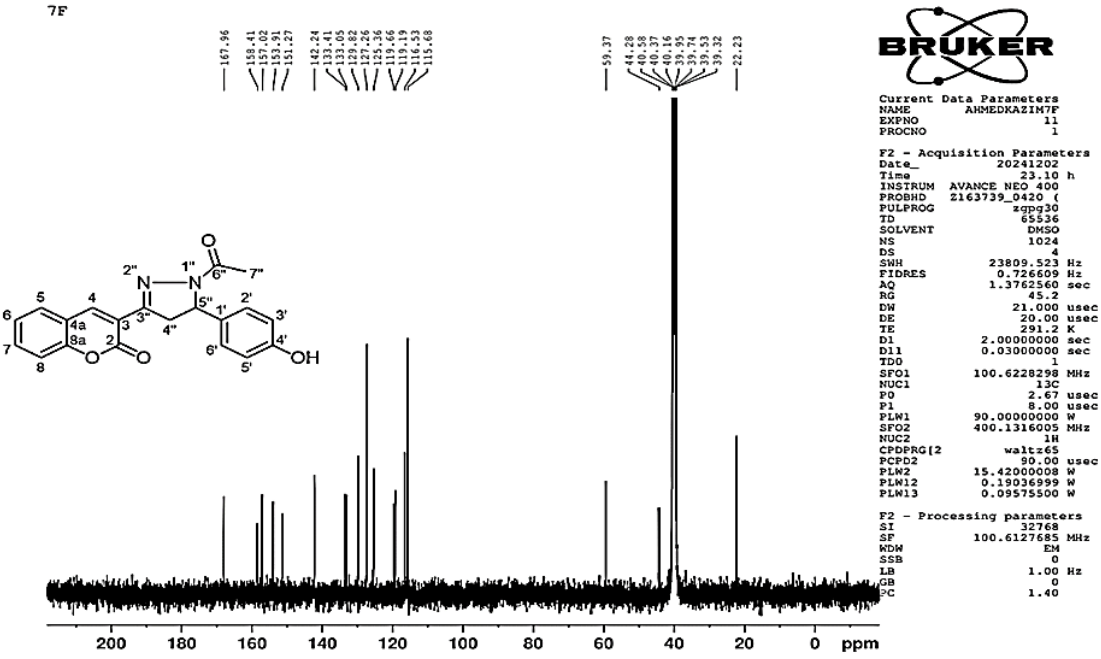

Figure 13. ^{13}CNMR of compound 6

C. Mass Spectrometry of coumarin pyrazoline 1-6

The mass spectra of the synthesized compounds are presented in Figures 27–34. The experimental m/z values listed in Table 2 show excellent agreement with the calculated values, confirming the successful synthesis of the target compounds [24]. Shown in figures. (14–19)

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Table 2. Mass spectra of coumarin pyrazoline 1-6

Compound	1	2	3	4	5	6
Molecular weight	332	362	411	389	375	348
Molecular ions M.+	332.1	362.1	411.1	389.1	375.4	348.3

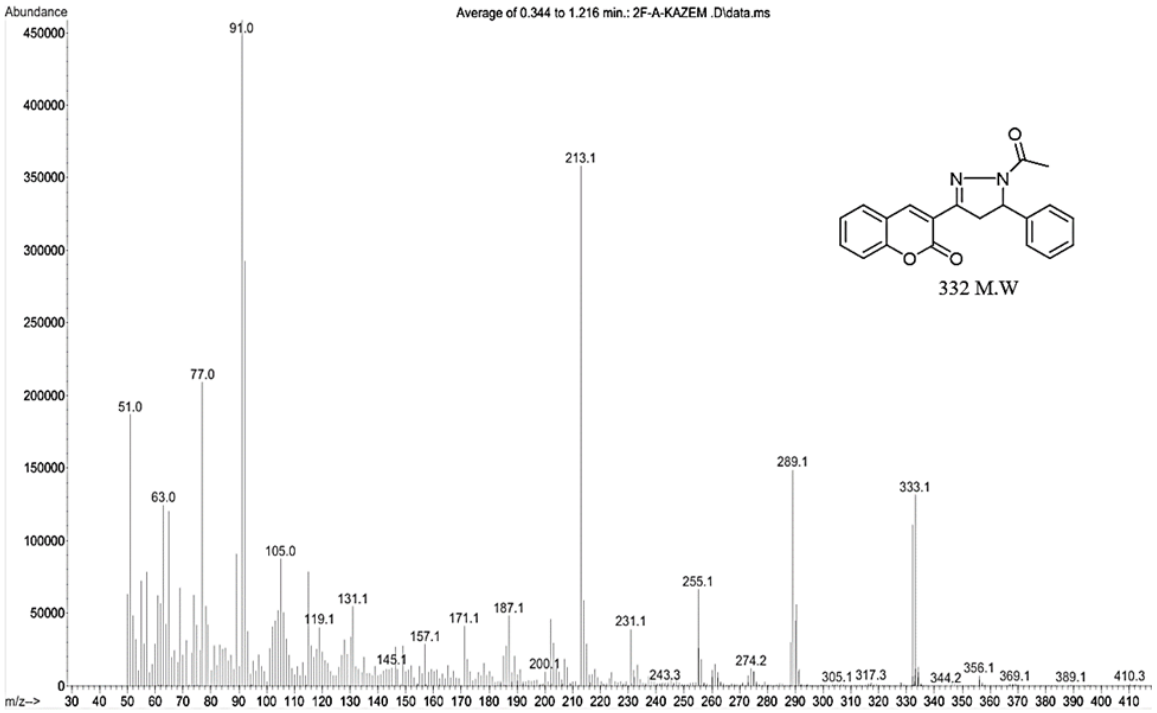

Figure 14. The mass spectra compound 1

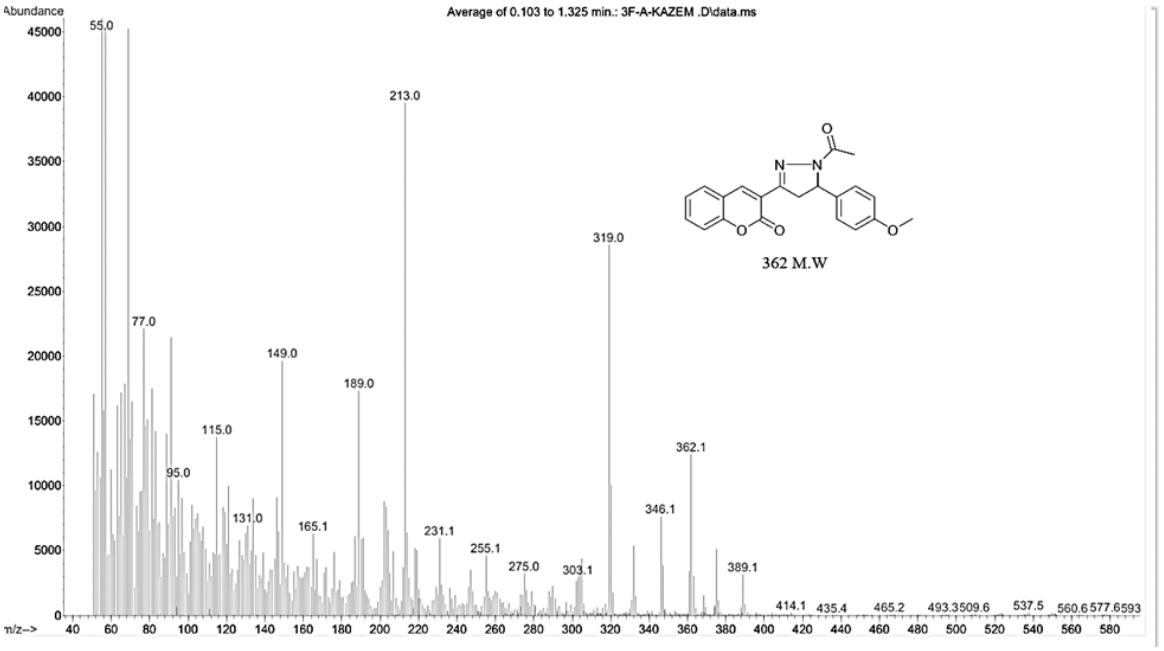

Figure 15. The mass spectra compound 2

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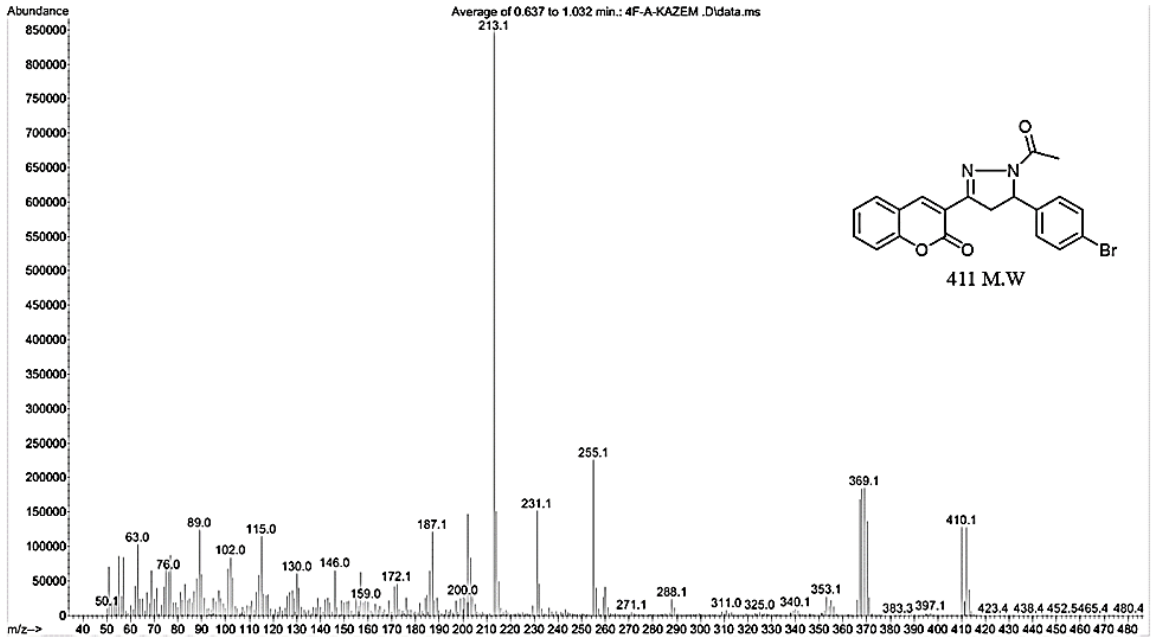

Figure 16. The mass spectra compound 3

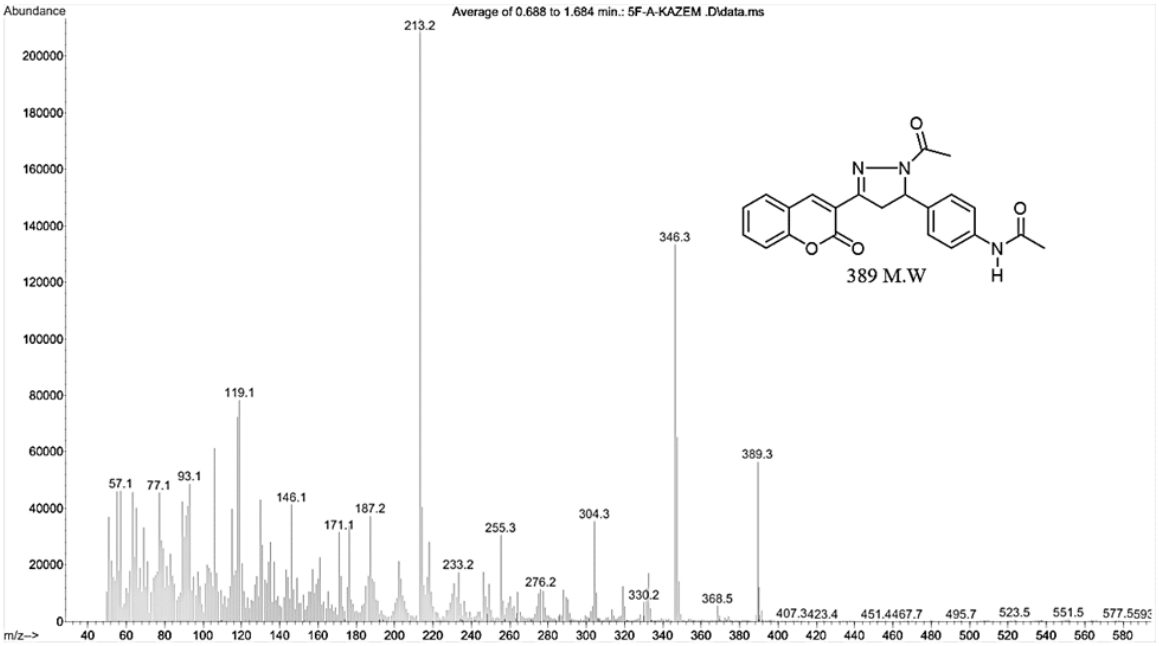

Figure 17. The mass spectra compound 4

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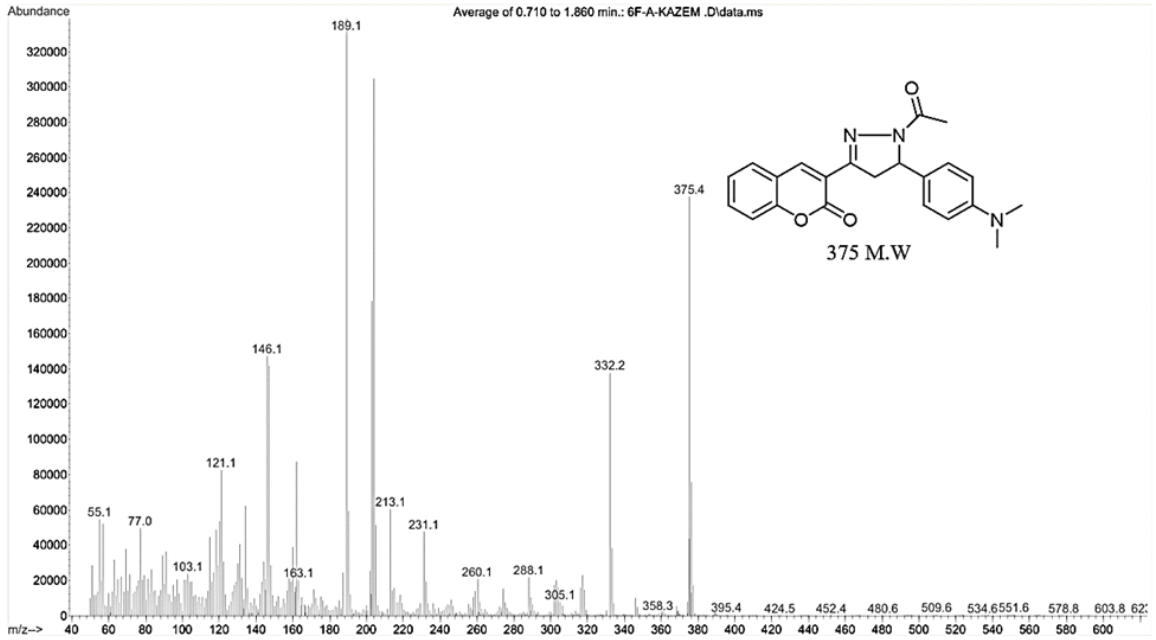

Figure 18. The mass spectra compound 5

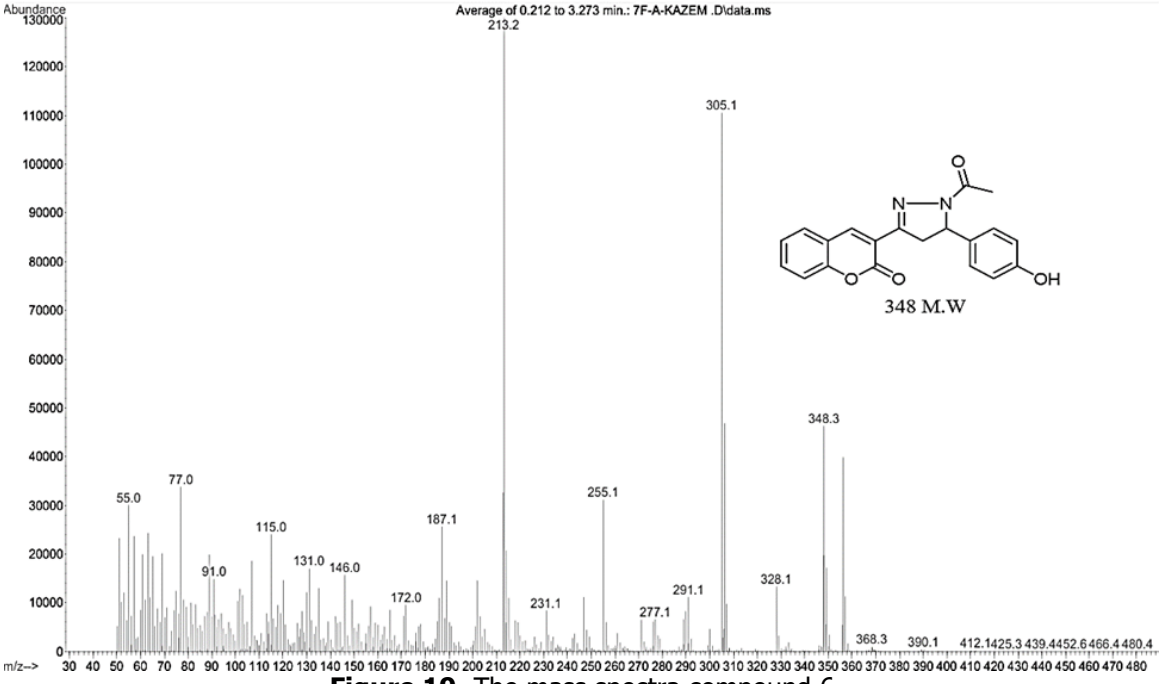

Figure 19. The mass spectra compound 6

D. Anti-oxidant evaluation of coumarin-pyrazoline 1-6

Studying the in vitro anti-oxidant activity of the coumarin-pyrazoline compounds 1-6, by using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. A methanolic solution of DPPH was prepared at a concentration of 16 µm/mL. Each coumarin-pyrazoline compounds were dissolved in methanol to prepare a series of concentrations: 1, 10, 25, 50, and 100 µM. For each test, 1 mL

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of the DPPH solution was mixed with 1 mL of the compound solution, and the mixtures were incubated in the dark at room temperature for 30 minutes to ensure a complete reaction. The anti-oxidant causes the DPPH purple coloring to change to yellow due to a decrease in absorptivity by giving up an electron. The degree of DPPH absorption decreases at 517 nm, and this indicates how well the tested substances can scavenge free radicals (109). Ascorbic acid was used as a standard anti-oxidant. It was tested under the same conditions and at the same concentrations. The equation (1.1) calculates the radical scavenging activity reported as a percentage.

Scavenging activity (%) = (Ac-As/Ac) *100 equation (1.1)

Ac: is the absorbance of the DPPH radical without the studied compounds. As is the absorbance of the DPPH after 30 minutes in the presence of coumarin pyrazoline 1-6 [25].

Table 3. The IC50 of coumarin-pyrazoline (compounds 1-6), against DPPH radical

Compounds	**1**	**2**	**3**	**4**	**5**	**6**	**Ascorbic Acid**
IC 50 (µM)	52.9	18.3	29.5	36.4	19.7	26.1	23.8

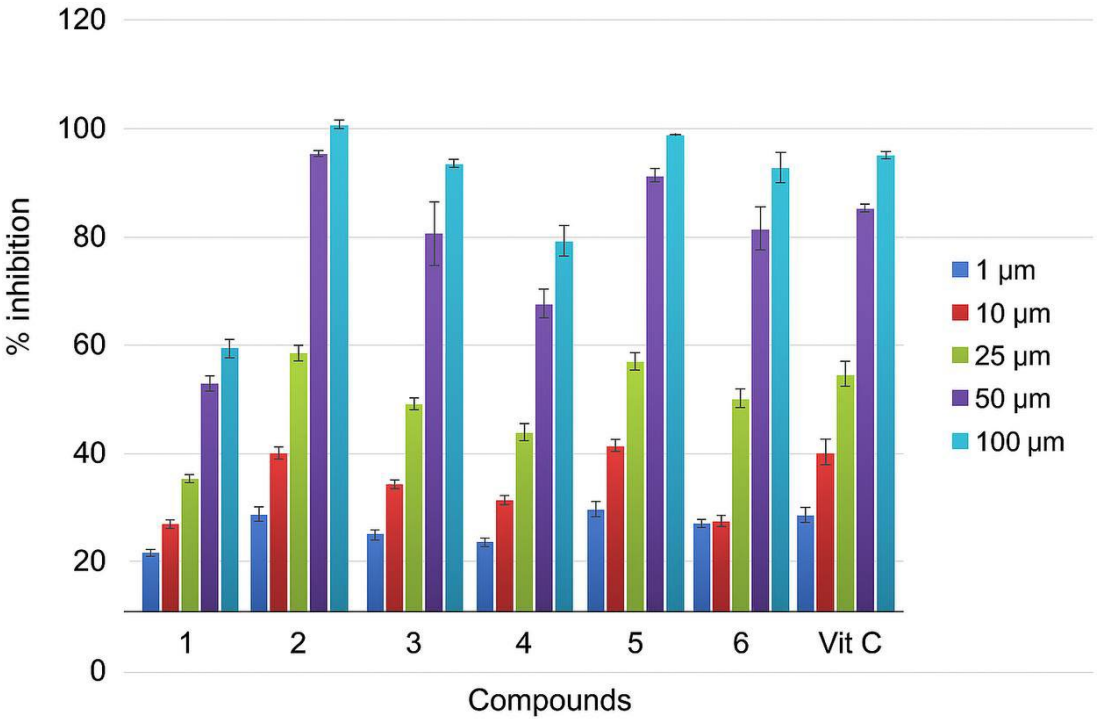

Figure 20. Percentage of DPPH radical inhibition by coumarin–pyrazoline (compounds 1-6) at various concentrations.

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Conclusions

This study presents the synthesis of coumarin–pyrazoline derivatives (compounds 1–6) and evaluates their antioxidant activity using the DPPH assay, with ascorbic acid as a reference. The structures were confirmed by 1HNMR,13CNMR, and MS techniques. Coumarin–pyrazoline compounds exhibited significant anti-oxidant activity. Compounds 2 and 5 showed more potent anti-oxidant activities than ascorbic acid (IC_{50} values of 23.9μM) with IC_{50} values of 18.3μM and 19.7μM, respectively; this enhanced activity can be attributed to the presence of the (OCH3) group in compound 2 and the (N(CH3)2) group in compound 5.

Acknowledgement

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