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https://doi.org/10.31925/farmacia.2026.2.7 **ORIGINAL ARTICLE**

ANTIOXIDANT COUMARIN-CHALCONE HYBRIDS WITH LOW ACUTE TOXICITY

SUZANA JANIĆEVIĆ [1], VIDOSLAV DEKIĆ [1]*, NIKO RADULOVIĆ [2], BILJANA DEKIĆ [1], EMILIJA PECEV-MARINKOVIĆ [2], MILENKO RISTIĆ [1], MARIJA AKSIĆ [1]

[1]*University of Priština in Kosovska Mitrovica, Faculty of Sciences and Mathematics, Kosovska Mitrovica, Serbia*
[2]*University of Niš, Faculty of Sciences and Mathematics, Niš, Serbia*

**corresponding author: vidoslav.dekic@pr.ac.rs*

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Abstract

The synthesis of new products by combining different physiologically active structural cores is of great interest to medicinal chemists due to the potential for synergistic effects, which can result in hybrids with enhanced pharmaceutical activities. Eight coumarin-chalcone hybrids were synthesized by reacting 4-hydroxy-3-acetylcoumarin with various substituted aromatic aldehydes. The structures of the synthesized products were fully characterized using spectral methods. Detailed assignment of ^{1}H- and ^{13}C-NMR resonances was achieved through a combination of ^{1}H-^{1}H COSY, NOESY, HSQC and HMBC experiments. Antioxidant activities were evaluated using the DPPH free radical scavenging assay.

Rezumat

Sinteza de noi compuși prin combinarea unor nuclee structurale diferite, cu activitate fiziologică, prezintă un interes deosebit pentru cercetătorii din domeniul chimiei medicale, datorită potențialului de apariție a unor efecte sinergice, care pot conduce la obținerea unor hibrizi cu activități farmaceutice îmbunătățite. Au fost sintetizați opt hibrizi cumarină–chalconă prin reacția dintre 4-hidroxi-3-acetilcoumarina și diverși aldehizi aromatici substituiți. Structurile compușilor sintetizați au fost complet caracterizate prin metode spectrale. Atribuirea detaliată a semnalelor din spectrele RMN de ^{1}H și ^{13}C a fost realizată prin combinarea experimentelor ^{1}H-^{1}H COSY, NOESY, HSQC și HMBC. Activitatea antioxidantă a fost evaluată prin testul de captare a radicalului liber DPPH.

Keywords: coumarin-chalcone hybrids, spectral characterization, antioxidant activities, acute toxicity

Introduction

Chalcones (1,3-diphenyl-2-propen-1-ones) represent a significant group of biologically active natural products (1, 2). They are also referred to as "open-chain flavonoids" because their structure, featuring two aromatic rings connected *via* an α,β-unsaturated ketone unit, serves as a precursor for the biosynthesis of flavonoids and isoflavonoids (3). Many chalcones exhibit notable physiological activities, including antimicrobial and antifungal (4-7), anti-inflammatory (8-11), antioxidant (5, 12-14), antiparasitic (15-17), antimalarial (18-20) and anticancer properties (21-24). Due to their substantial biological activity, chalcones attract considerable attention from organic synthetic chemists. Chalcones possess readily replaceable hydrogens and often have one or more phenolic groups on their aromatic rings, making them valuable synthons and bioactive scaffolds for structural modifications. Furthermore, the α,β-unsaturated ketone unit enables the linkage of diverse structural cores, allowing for the creation of novel scaffolds that serve as templates for synthesizing compounds with enhanced physiological effects.

Coumarin and its derivatives represent an important group of organic compounds widely distributed in the plant world as secondary metabolites. Additionally, numerous synthetic derivatives have been obtained through various chemical transformations of the coumarin core. These compounds are well known for their diverse physiological activities, including antimicrobial (25-27), antioxidant (28-31), anti-inflammatory (32-34), anticoagulant (35, 36), anti-HIV (37, 38), anticancer (37), antiviral (39, 40) and antianxiety effects (41, 42). Coumarin derivatives with a hydroxy group at position 4 on the coumarin core are particularly noteworthy due to their physiological activities. Compounds such as warfarin, dicoumarol and bromadiolone, which feature this structure, are recognized as effective rodenticides due to their anticoagulant effects. In previous studies, we synthesized a series of asymmetric azines containing a 4-hydroxycoumarin moiety (43). These compounds exhibit significant antimicrobial and antioxidant properties and have shown minimal effects on the survival of brine shrimp.

The structural features of coumarins and chalcones make them excellent building blocks for hybridiza-

tion, resulting in products with notable pharmacological activities (44). Comprehensive spectral characterization is crucial for determining the structures of new products, with the complete assignment of ^{1}H- and ^{13}C-NMR spectra playing a key role. However, many reported coumarin-chalcone hybrids lack adequate spectral data. Due to partial or complete signal overlaps between protons from the coumarin moiety and other parts of the molecule, ^{1}H-NMR spectral data is often incomplete or presented in a limited form. A similar issue arises with ^{13}C-NMR spectroscopy.

Materials and Methods

Chemistry

All reagents and solvents were obtained from commercial sources (Aldrich, USA; Merck, Germany; Fluka, Germany) and used as supplied. Melting points were determined on a Kofler hot-plate apparatus and were uncorrected. The purity of the synthesized compounds was checked by thin layer chromatography (TLC) on precoated aluminium silica gel plates (Kieselgel 60 F254, 0.2 mm, Merck, Germany). Purification of the synthesized compounds was performed by column chromatography (silica gel 60, particle size 0.040 - 0.063 mm, Fluka). High-resolution mass spectrometry (HRMS) measurements were carried out on a JEOL MStation JMS-700 mass spectrometer operating at 70 eV ionization energy, with the ion-trap current set to 300 μA and the ion-source temperature maintained at 230°C. Perfluorokerosene (Sigma-Aldrich, USA) served as the internal reference for mass calibration in the HRMS experiments. Exact masses were obtained by averaging results from 5 - 10 scans; values were derived from the mass centroids of the molecular ion ($M^{+\bullet}$) together with the remaining observed peaks. For each proposed elemental composition, the corresponding mass error is reported in millimass units (mmu), as computed by the software integrated into the instrument's data system. The IR spectra were recorded on a Thermo Nicolet model 6700 FT-IR spectrometer (Waltham, USA). ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100.6 MHz) spectra were recorded on Bruker Avance III spectrometer (Fällanden, Switzerland) at 25°C in $CDCl_3$ and DMSO-d_6 as solvents, using TMS as the internal standard. Chemical shifts are reported in ppm (δ) relative to TMS.

Synthesis of 3-acetyl-4-hydroxy-2H-chromen-2-one

To a solution of 4-hydroxy-2*H*-chromen-2-one (1) (3 g, 18.6 mmol) in glacial acetic acid, phosphorus oxychloride (5.6 mL) was added. The reaction mixture was refluxed in an oil bath for 30 minutes. After cooling in an ice bath, the precipitate that formed was collected by filtration. Recrystallization from ethanol yielded white crystals of 3-acetyl-4-hydroxy-2*H*-chromen-2-one (2) with a yield of 2.9 g (90%). The melting point was determined to be 135°C.

General procedure for the synthesis of coumarin-chalcone hybrids

A mixture of 3-acetyl-4-hydroxy-2*H*-chromen-2-one (2) (0.82 g, 4 mmol) and an appropriate aromatic aldehyde (3a-h) (4 mmol) was dissolved in 10 mL of chloroform. A catalytic amount of piperidine (1.5 mmol) was added, and the reaction mixture was refluxed for 1.5 hours, with monitoring by TLC (hexane/ethyl acetate, 2:1, v/v). After the reaction was complete, the chloroform was evaporated, and the solid residue was washed with methanol. The resulting products were purified by column chromatography on silica gel (hexane/ethyl acetate, 1.5:1, v/v). A series of chalcones (4a-h) was obtained with high yields ranging from 82% to 89%.

Antioxidant activity

The antioxidant activity of the synthesized compounds was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Stock solutions of tested products and ascorbic acid (0.1 mg/mL) were diluted to obtain solutions in the concentration range of 10 - 1000 μg/mL. Subsequently, 2 mL of a methanolic solution of DPPH (0.004%) and 1 mL of the solutions containing varying concentrations of the tested compounds or ascorbic acid (used as a positive control) were mixed in a test tube and incubated for 30 minutes. The percentage inhibition of the DPPH radical was determined spectrophotometrically by measuring the absorbance at 517 nm against a blank solution (methanol). The results were calculated using the following equation:

$$\text{inhibition (\%)} = [(Ac\text{-}As)/Ac] \cdot 100.$$

Ac is the absorbance of the control (1 mL of methanol and 2 mL of DPPH), while As is the absorbance of the tested compounds (1 mL of sample solution and 2 mL of DPPH). The measurements were performed in triplicate, and the results were given as IC_{50} values (mmol/L).

Acute toxicity

Acute toxicity was assessed in the *Artemia salina* model. In 1 L of artificial seawater, approximately 10 grams (one spoon) of lyophilized *A. salina* cysts was added. The suspension was maintained at 25°C, aerated and constantly illuminated so that most cysts were transformed after 48 hours. Shrimp were not fed during the test period. The tested compounds were dissolved in DMSO and then diluted with artificial seawater to prepare solutions at final concentrations of 1, 0.5 and 0.05 mg/mL, ensuring the DMSO concentration remained below 1% (v/v). Subsequently, 20 hatched nauplii were transferred to Petri dishes containing the solutions of the tested compounds (4a-h). The Petri dishes were kept at room temperature under constant light and without aeration. Dead shrimp were counted after 24 and 48 hours. As a

positive control, different concentrations of sodium dodecyl sulphate were used. The results were analysed to determine the LC_{50} values (mg/mL), which indicate the concentration of the tested compounds required to cause mortality in 50% of the shrimp population.

Results and Discussion

Synthesis

Eight coumarin-chalcone hybrids were synthesized by reacting 4-hydroxy-3-acetylcoumarin with various substituted aromatic aldehydes (Figure 1).

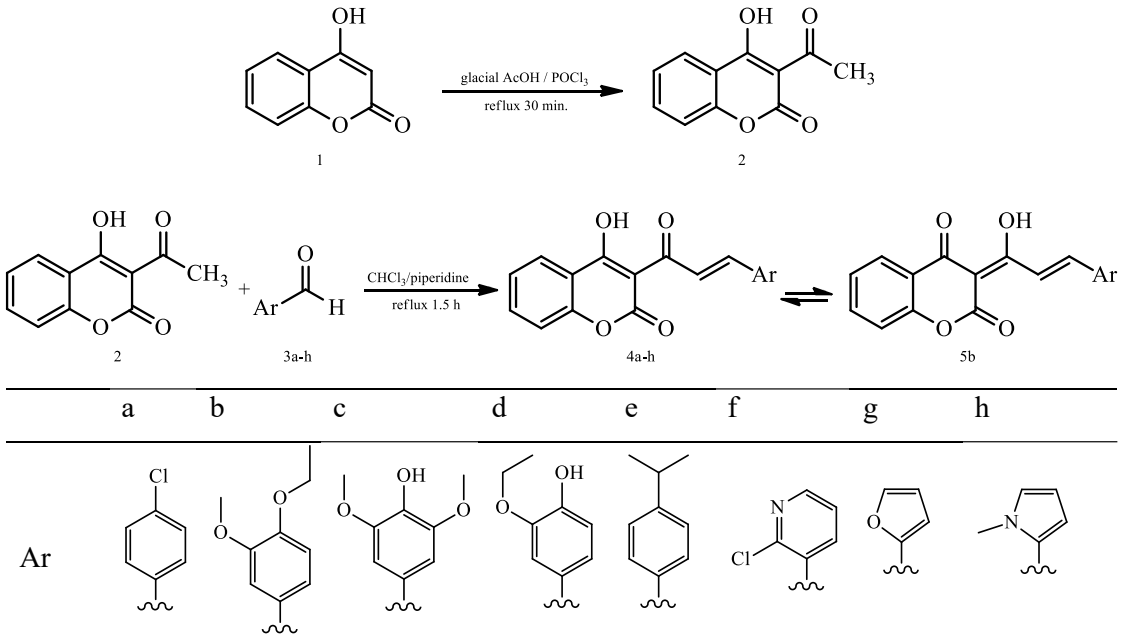

Figure 1.
Synthesis of coumarin-chalcone hybrids (4a-h)

The synthesis involved two reaction steps. First, 3-acetyl-4-hydroxycoumarin (2) was prepared by acetylation of 4-hydroxycoumarin (1) according to a previously published method (43). Subsequently, through Claisen-Schmidt condensation between 3-acetyl-4-hydroxycoumarin (2) and the corresponding aromatic aldehydes (3a-h), the final products (4a-h) include: 3-(3-(4-chlorophenyl)acryloyl)-4-hydroxy-2*H*-chromen-2-one (4a), 3-(3-(4-ethoxy-3-methoxyphenyl)acryloyl)-4-hydroxy-2*H*-chromen-2-one (4b), 4-hydroxy-3-(3-(4-hydroxy-3,5-dimethoxyphenyl)-acryloyl)-2*H*-chromen-2-one (4c), 3-(3-(3-ethoxy-4-hydroxyphenyl)acryloyl)-4-hydroxy-2*H*-chromen-2-one (4d), 4-hydroxy-3-(3-(4-isopropylphenyl)acryloyl)-2*H*-chromen-2-one (4e), 3-(3-(2-chloropyridin-3-yl)-acryloyl)-4-hydroxy-2*H*-chromen-2-one (4f), 3-(3-(furan-2-yl)acryloyl)-4-hydroxy-2*H*-chromen-2-one (4g), and 4-hydroxy-3-(3-(1-methyl-1*H*-pyrrol-2-yl)-acryloyl)-2*H*-chromen-2-one (4h). X-ray diffraction analysis of compound 4b reveals prototropic tautomerism, where a hydrogen atom migrates from the hydroxyl group on the coumarin core to the adjacent keto group (45). This migration causes the structure of the synthesized coumarin-chalcone hybrids to appear as the corresponding dione 5b in the crystal state.

One of the obtained compounds is new (4f), while four known compounds (4a (46, 47), 4b (48), 4c (49) and 4h (50)) had previously been published with scarce or incomplete NMR spectral characterization. Additionally, three compounds (4d (49), 4e (47), and 4g (51)) were not accompanied by any NMR spectral data. A complete spectral analysis was performed for all compounds, ensuring that all synthesized products were fully characterized spectrally for the first time.

The structures of the obtained products were fully characterized using HRMS-EI, IR, and 1D and 2D NMR spectroscopy, and the spectral data completely confirmed the proposed structures. A detailed structural elucidation is presented for the new coumarin–chalcone hybrid 4f, highlighting the key correlations that enabled the complete assignment of the ^{1}H- and ^{13}C-NMR signals. High-resolution electron impact mass spectrometry (HR-EIMS) of 4f suggests a molecular formula of $C_{17}H_{10}ClNO_4$, with the $[M]^+$ ion observed at *m/z* - 327.0305, Δ = + 0.7 mmu. The IR spectrum of 4f shows an absorption band at 3087 cm^{-1} corresponding to Ar-H vibrations, and carbonyl vibrations at 1717 and 1610 cm^{-1} attributed to the lactone carbonyl of the coumarin core and the α,β-unsaturated ketone, respectively. Based on the NOESY spectrum, the proton resonances can be divided into two spin systems (Figure 2).

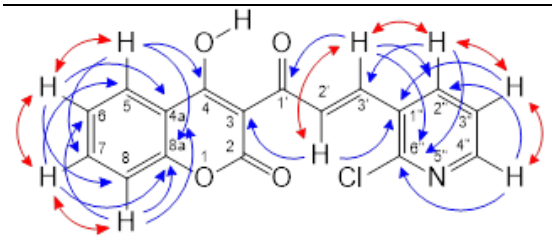

Figure 2.
Crucial NOESY (H↷H) and HMBC (H⌒C) correlations observed in the NOESY and HMBC spectrum of compound 4f

The first spin system contains the coumarin-core protons (H-5–H-8), consistent with their number, intensity and multiplicities. The second spin system comprises the α,β-unsaturated ketone linker and the 2-chloropyridin-3-yl fragment (two olefinic and three pyridyl protons). The olefinic protons are assigned primarily from their line-shapes and coupling constant as two doublets at 8.34 (H-2′) and 8.10 ppm (H-3′) with a large ^{3}J = 16.0 Hz, consistent with an *E* (*trans*) configuration of the C=C bond. Their assignment was enabled by a NOESY cross-peak between the signal at 8.10 ppm (H-3′) and the resonance at 8.35 ppm (*dd*, H-2″), indicating spatial proximity at the enone–pyridyl junction. This key NOESY contact not only supports the assignment of H-3′, but also facilitates the assignment of the pyridyl protons, which is further corroborated by sequential NOESY correlations between H-2″ (8.35 ppm) and H-3″ (*dd*, 7.60 ppm), and between H-3″ (7.60 ppm) and H-4″ (*dd*, 8.53 ppm). Based on the combined HSQC and HMBC analyses, the above assignment is supported by key long-range correlations that establish the connectivity between the coumarin core, the enone linker, and the 2-chloropyridinyl ring. The key HMBC correlation of H-2′ (8.34 ppm) with C-3 of the coumarin core (100.6 ppm) indicates that the enone moiety is attached at C-3 of the chromen-2-one scaffold, while the correlation of H-2′ with the enone carbonyl carbon C-1′ (191.7 ppm) further supports the assigned enone framework. In addition, the linkage between the enone and the heteroaryl fragment is evidenced by HMBC correlations involving the pyridyl unit: H-2′ (8.34 ppm) shows a long-range correlation to the pyridyl *ipso*-carbon C-1″ (129.5 ppm), and H-3′ (8.10 ppm) correlates with C-2″ (138.0 ppm), supporting the connection of the α,β-unsaturated ketone unit to the 2-chloropyridin-3-yl ring. Finally, HMBC correlations between H-3″ (7.60 ppm) and C-1″ (129.5 ppm), and between H-2″ (8.35 ppm) and C-6″ (148.2 ppm), support the internal assignment of the pyridyl fragment around the attachment point, completing the overall spectral assignment.

3-(3-(4-chlorophenyl)acryloyl)-4-hydroxy-2*H*-chromen-2-one (4a) (46, 47): Light yellow crystals. M.p. 252 - 254°C (Lit. (47): m.p. 254 - 256°C). Yield: 83%. HR-EI-MS: 326.0365 ([*M*]$^{+}$, $C_{18}H_{11}ClO_4^{+}$; calc. 326.0346). IR (KBr, ν, cm^{-1}): 3087 (Ar-H), 1717 (C=O, lactone carbonyl of coumarin), 1610 (C=O, α,β-unsaturated ketone), 1526, 1495, 1435, 1063, 748. ^{1}H-NMR (400 MHz, $CDCl_3$): 18.85 (*brs*, OH); 8.45 (*d*, *J* = 16.0 Hz, H-C(2')); 8.13 (*dd*, *J* = 8.0, 1.6 Hz, H-C(5)); 8.02 (*d*, *J* = 16.0 Hz H-C(3')); 7.73 (*ddd*, *J* = 8.4, 7.6, 1.6 Hz, H-C(7)); 7.68 (*d*, *J* = 8,4 Hz, 2H, H-C(2"/6")); 7.44 (*d*, *J* = 8,4 Hz, 2H, H-C(3"/5")); 7.37 (*td*, *J* = 8.0, 7.6, 2.0 Hz, H-C(6)); 7.34 (*dd*, *J* = 8.4, 2.0 Hz, H-C(8)) ppm. ^{13}C-NMR (100.6 MHz, $CDCl_3$): 192.5 (C(1')); 181.5 (C(4)); 160.2 (C(2)); 154.8 (C(8a)); 145.7 (C(3')); 137.4 (C(4")); 136.2 (C(7)); 133.2 (C(1")); 130.5 (2C, C(2"/6")); 129.4 (2C, C(3"/5")); 125.8 (C(5)); 124.5 (C(6)); 123.3 (C(2')); 117.1 (C(8)); 116.2 (C(4a)); 100.8 (C(3)) ppm.

3-(3-(4-ethoxy-3-methoxyphenyl)acryloyl)-4-hydroxy-2*H*-chromen-2-one (4b) (48): Orange crystals. M.p. 170 - 172°C (Lit. (48): m.p. 169 - 171°C). Yield: 85%. HR-EI-MS: 366.1109 ([*M*]$^{+}$, $C_{21}H_{18}O_6^{+}$; calc. 366.1103). IR (KBr, ν, cm^{-1}): 3072 (Ar-H), 1707 (C=O, lactone carbonyl of coumarin), 1619 (C=O, α,β-unsaturated ketone), 1532, 1486, 1445, 1071, 753. ^{1}H-NMR (400 MHz, DMSO-d_6): 12.20 (*brs*, OH); 8.16 (*d*, *J* = 15.6 Hz, H-C(2')); 8.04 (*dd*, *J* = 8.0, 1.6 Hz, H-C(5)); 7.81 (*dt*, *J* = 8.4, 7.6, 1.6 Hz, H-C(7)); 7.42 (*m*, 2H, H-C(6), H-C(8)); 7.40 (*dd*, *J* = 7.6, 1.6 Hz, H-C(6")); 7.33 (*dd*, *J* = 1.6 Hz, H-C(2")); 7.17 (*d*, *J*=15.6 Hz, H-C(3')); 7.08 (*d*, *J*=7.6 Hz, H-C(5")); 4.10 (*q*, *J*=7.2 Hz, 2H, $OC\underline{H}_2CH_3$); 3.83 (*s*, 3H, $OC\underline{H}_3$); 1.36 (*t*, 3H, *J* = 7.2 Hz, $OCH_2C\underline{H}_3$) ppm. ^{13}C-NMR (100.6 MHz, DMSO-d_6): 191.8 (C(1')); 182.3 (C(4)); 161.3 (C(2)); 154.6 (C(8a)); 150.8 (C(4")); 149.5 (C(3")); 136.7 (C(7)); 127.4 (C(1")); 125.9 (C(5)); 124.9 (C(6)); 124.4 (C(6")); 123.2 (C-(3')); 120.3 (C(2')); 117.3 (C(8)); 117.1 (C(4a)); 113.2 (C(5")); 112.1 (C(2")); 100.8 (C(3)); 64.4 ($O\underline{C}H_2CH_3$); 56.0 ($O\underline{C}H_3$); 15.1 ($OCH_2\underline{C}H_3$) ppm.

4-hydroxy-3-(3-(4-hydroxy-3,5-dimethoxyphenyl)acryloyl)-2*H*-chromen-2-one (4c) (49): Dark yellow powder. M.p. > 300°C (Lit. (49): m.p. > 350°C). Yield: 82%. HR-EI-MS: 368.0890 ([*M*]$^{+}$, $C_{20}H_{16}O_7^{+}$; calc. 368.0896). IR (KBr, ν, cm^{-1}): 3349 (OH), 3110 (Ar-H), 2966 (CH_3), 2939(CH_3), 1704 (C=O, lactone carbonyl of coumarin), 1608 (C=O, α,β-unsaturated ketone), 1486, 1420, 1030, 980, 767, 631. ^{1}H-NMR (400 MHz, $CDCl_3$): 19.08 (*brs*, OH); 8.35 (*d*, *J* = 15.2 Hz, H-C(2')); 8.13 (*dd*, *J* = 8.0, 0.8 Hz, H-C(5)); 8.05 (*d*, *J* = 15.2 Hz, H-C(3')); 7.70 (*dt*, *J* = 8.4, 8.0, 1.2 Hz, H-C(7)); 7.36 (*dt*, *J* = 8.4, 8.0, 0.8 Hz, H-C(6),); 7.32 (*dd*, *J* = 8.0, 1.2 Hz, H-C(8)); 7.00 (*s*,2H, H-C(2"/6")); 5.98 (*s*, OH); 4.00 (s, $OC\underline{H}_3$) ppm. ^{13}C-NMR (100.6 MHz, $CDCl_3$): 191.9 (C(1')); 181.3 (C(4)); 160.7 (C(2)); 154.7 (C(8a)); 148.2 (C-(3')); 147.3 (2C, C(3"/5")); 138.6 (C (4")); 136.2 (C(7)); 126.4 (C(1")); 125.8

(C(5)); 124.4 (C(6)); 120.1 (C(2')); 117.0 (C(8)); 116.6 (C(4a)); 106.6 (2C, C(2"/6")); 100.4 (C(3)); 56.5 (2C, O<u>C</u>H$_3$) ppm.

3-(3-(3-ethoxy-4-hydroxyphenyl)acryloyl)-4-hydroxy-2*H*-chromen-2-one (4d) (49): Orange powder. M.p. 122 - 125°C (Lit. (49): m.p. 123 - 125°C). Yield: 83%. HR-EI-MS: 352.0939 ([*M*]$^+$, $C_{20}H_{16}O_6^+$; calc. 352.0946). IR (KBr, ν, cm^{-1}): 3396 (OH), 3111 (Ar-H), 2917 (CH$_3$), 1728 (C=O, lactone carbonyl of coumarin), 1613 (C=O, α,β-unsaturated ketone), 1489, 1420, 1033, 980, 752, 669. ^{1}H-NMR (400 MHz, DMSO-d_6): 10.06 (*brs*, OH); 8.14 (*d*, *J* = 15.6 Hz, H-C(2')); 8.06 (*d*, *J* = 15.6 Hz, H-C(3')); 8.03 (*dd*, *J* = 8.0, 1.6 Hz, H-C(5)); 7.82 (*dt*, *J* = 8.0, 7.2, 1.6 Hz, H-C(7)); 7.40 - 7.46 (*m*, 2H, H-C(6/8)); 7.28 - 7.35 (*m*, 2H, H-C(2"/6")); 6.93 (*d*, H-C(5")); 4.10 (q, 2H, OC<u>H$_2$</u>CH$_3$); 1.38 (t, 3H, OCH$_2$C<u>H$_3$</u>) ppm. ^{13}C-NMR (100.6 MHz, DMSO-d_6): 191.1 (C(1')); 181.8 (C(4)); 160.1 (C(2)); 154.6 (C(8a)); 151.0 (C(1")); 148.9 (C-(3')); 147.7 (C (4")); 137.0 (C(7)); 126.3 (C(3")); 125.8 (C(5)); 125.1 (C(6)); 124.7 (C(6")); 118.5 (C(2')); 117.3 (C(8)); 116.7 (C(5")); 116.0 (C(4a)); 114.5 (C(2")); 100.7 (C(3)); 64.4 (O<u>C</u>H$_2$CH$_3$); 15.1 (OCH$_2$<u>C</u>H$_3$) ppm.

4-hydroxy-3-(3-(4-isopropylphenyl)acryloyl)-2*H*-chromen-2-one (4e) (47): Yellow powder. M.p. 288 - 290°C (Lit. (47): m.p. 288 - 290°C). Yield: 88%. HR-EI-MS: 334.1199 ([*M*]$^+$, $C_{21}H_{18}O_4^+$; calc. 334.1205). IR (KBr, ν, cm^{-1}): 2958 (CH$_3$), 1730 (C=O, lactone carbonyl of coumarin), 1608 (C=O, α,β-unsaturated ketone), 1548, 1418, 992, 751. ^{1}H-NMR (400 MHz, CDCl$_3$): 19.01 (*brs*, OH); 8.44 (*d*, *J* = 15.6 Hz, H-C(2')); 8.12 (*dd*, *J* = 8.0, 1.6 Hz, H-C(5)); 8.08 (*d*, *J* = 15.6 Hz, H-C(3')); 7.66 - 7.72 (*m*,3H, H-C(7,2"/6")); 7.29 - 7.34 (*m*, 4H, H-C(6,8,3"/5")); 2.96 (*m*, *J* = 6.8 Hz, H-C<u>H</u>(CH$_3$)$_2$); 1.30 (*d*, *J* = 6.8 Hz, H-CH(C<u>H$_3$</u>)$_2$) ppm. ^{13}C-NMR (100.6 MHz, CDCl$_3$): 192.5 (C(1')); 181.7 (C(4)); 160.3 (C(2)); 154.7 (C(8a)); 153.2 (C (4")); 147,7 (C-(3')); 136,0 (C(7)); 132.4 (C(1")); 129.7 (C(2"/6"));127.2 (C(3"5")); 125.8 (C(5)); 124.4 (C(6)); 121.6 (C(2')); 117.0 (C(8)); 116,5 (C(4a)); 100.7 (C(3)); 34.3(<u>C</u>H(CH$_3$)$_2$); 23.7 (CH(<u>C</u>H$_3$)$_2$) ppm.

3-(3-(2-chloropyridin-3-yl)acryloyl)-4-hydroxy-2*H*-chromen-2-one (4f): Yellow powder. M.p. 188 - 190°C. Yield: 89%. HR-EI-MS: 327.0305 ([*M*]$^+$, $C_{17}H_{10}ClNO_4^+$; calc. 327.0298). IR (KBr, ν, cm^{-1}): 3087 (Ar-H), 1717 (C=O, lactone carbonyl of coumarin), 1610 (C=O, α,β-unsaturated ketone), 1495, 1435, 1063, 978, 757, 666. ^{1}H-NMR (400 MHz, DMSO-d_6): 17.63 (*brs*, OH); 8.53 (*dd*, *J* = 4.8, 2.0 Hz, H-C(4")); 8.35 (*dd*, *J* = 7.2, 2.0 Hz, H-C(2")); 8.34 (*d*, *J* = 16.0 Hz, H-C(2')); 8.10 (*d*, *J* = 16.0 Hz, H-C(3')); 8.08 (*dd*, *J* = 7.6, 1.6 Hz, H-C(5)); 7.86 (*dt*, *J* = 8.4, 8.0, 1.6 Hz, H-C(7)); 7.45 (*dt*, *J* = 8.0, 7.6, 2.0 Hz, H-C(6)); 7.38 (*dd*, *J* = 8.4, 2.0 Hz, H-C(8)); 7.60 (*dd*, *J* = 7.2, 4.8 Hz, H-C(3")) ppm. ^{13}C-NMR (100.6 MHz, DMSO-d_6): 191.7 (C(1')); 180.3 (C(4)); 161.9 (C(2)); 154.6 (C(8a)); 151.0 (C(1")); 148.2 (C(6")); 139.1 (C-(3')); 138.0 (C(2")); 137.4 (C(7)); 129.5 (C(4")); 128.2 (C(2')); 125.9 (C(5)); 125.4 (C(6)); 124.6 (C(3")); 117.4 (C(8)); 115.1 (C(4a)); 100.6 (C(3)) ppm.

3-(3-(furan-2-yl)acryloyl)-4-hydroxy-2*H*-chromen-2-one (4g) (51): Dark yellow powder. M.p. 203 - 205°C (Lit. (51): m.p. 204 - 205°C). Yield: 86%. HR-EI-MS: 282.0526 ([*M*]$^+$, $C_{16}H_{10}O_5^+$; calc. 282.0528). IR (KBr, ν, cm^{-1}): 3105 (Ar-H), 2921 (CH$_3$), 1716 (C=O, lactone carbonyl of coumarin), 1601 (C=O, α,β-unsaturated ketone), 1487, 1029, 952, 753. ^{1}H-NMR (400 MHz, DMSO-d_6): 18.91 (*brs*, OH); 8.12 (*d*, *J* = 15.6 Hz, H-C(2')); 8.05 (*dd*, *J* = 8.0, 1.6 Hz, H-C(5)); 8.00 (*d*, *J* = 1.6 Hz, H-C(5")); 7.88 (*d*, *J* = 15.6 Hz, H-C(3')); 7.81 (*dt*, *J* = 8.8, 7.6, 1.6 Hz, H-C(7)); 7.41 - 7.47 (*m*,2H, H-C(6,8)); 7.22 (*d*, *J* = 3.2 Hz, H-C(3")); 6.77 (*dd*, *J* = 3.2, 1.6 Hz, H-C(4")) ppm. ^{13}C-NMR (100.6 MHz, DMSO-d_6): 191.1 (C(1')); 181.5 (C(4)); 160.0 (C(2)); 154.7 (C(8a)); 151.6 (C(2")); 148.6 (C(5")); 137.1 (C(7)); 133.1 (C-(3')); 125.9 (C(5)); 125.2 (C(6)); 120.9 (C(3")); 119.1 (C(2')); 117.3 (C(8)); 116.6 (C(4a)); 114.3 (C(4")); 101.1 (C(3)) ppm.

4-hydroxy-3-(3-(1-methyl-1*H*-pyrrol-2-yl)acryloyl)-2*H*-chromen-2-one (4h) (50): Red powder. M.p. 205 - 206°C (Lit. (50): m.p. 204 - 206°C). Yield: 86%. HR-EI-MS: 295.0841 ([*M*]$^+$, $C_{17}H_{13}NO_4^+$; calc. 295.0845). IR (KBr, ν, cm^{-1}): 3185 (Ar-H), 1704 (C=O, lactone carbonyl of coumarin), 1587 (C=O, α,β-unsaturated ketone), 1385, 1049, 994, 897, 761. ^{1}H-NMR (400 MHz, DMSO-d_6): 15.82 (*brs*, OH); 8.07 (*d*, *J* = 15.2 Hz, H-C(2')); 8.03 (*dd*, *J* = 8.0, 1.6 Hz, H-C(5)); 7.99 (*d*, *J* = 15.2 Hz, H-C(3')); 7.80 (*dt*, *J* = 8.8, 8.0, 1.6 Hz, H-C(7)); 7.40 - 7.46 (*m*,2H, H-C(6,8)); 7.32 (*d*, *J* = 2.4 Hz, H-C(3")); 7.02 (*d*, *J* = 4.0 Hz, H-C(5")); 6.31 (*dd*, *J* = 4.0, 2.4 Hz, H-C(4")); 3.83 (*s*, C<u>H$_3$</u>) ppm. ^{13}C-NMR (100.6 MHz, DMSO-d_6): 189.6 (C(1')); 181.9 (C(4)); 160.3 (C(2)); 154.5 (C(8a)); 136.6 (C(7)); 136.1 (C-(3')); 132.9 (C(3")); 130.8 (C(2")); 125.8 (C(5)); 125.0 (C(6)); 117.4 (2C C(5") and (C(8)); 117.2 (C(4a)); 114.4 (C(2')); 111,7 (C(4")); 100.0 (C(3)); 34.8 (<u>C</u>H$_3$) ppm.

Antioxidant activity

The antioxidant activity of the synthesized compounds was determined spectrophotometrically by monitoring their reaction with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Neutralization of the DPPH radical causes a colour change from purple to yellow, which can be observed by measuring the decrease in absorbance at 517 nm. The free radical-scavenging activities of the synthesized compounds, as well as ascorbic acid, are presented in Table II.

Table II

Antioxidant activity of compounds 4a - 4h

Compound	DPPH scavenging activity IC_{50} (mmol/L)
4a	0.289
4b	1.234

Compound	DPPH scavenging activity IC_{50} (mmol/L)
4c	0.187
4d	0.184
4e	0.259
4f	0.233
4g	0.261
4h	2.842
Ascorbic acid	0.106

The synthesized coumarin–chalcone hybrids displayed a broad range of DPPH radical-scavenging activities, with IC_{50} values between 0.184 and 2.842 mmol/L, compared with 0.106 mmol/L for ascorbic acid. Within the present series, compounds 4c and 4d were the most active, while 4a, 4e, 4f and 4g also showed substantial activity; in contrast, 4b and especially 4h were clearly less effective. This pattern suggests that the antioxidant response is governed not only by the common 4-hydroxycoumarin unit, but also by the nature of the substituent attached through the enone bridge. In particular, the comparatively strong activity of 4c and 4d is consistent with the presence of an additional phenolic OH group on the aryl ring, which can directly participate in hydrogen-atom transfer and improve stabilization of the resulting radical. By contrast, methoxy- and ethoxy-substituted derivatives without a free phenolic OH on the aryl fragment, such as 4b, showed lower activity, while the N-methylpyrrolyl derivative 4h was the least active. This trend is broadly consistent with previous observations that antioxidant activity in coumarin- and chalcone-based systems is strongly influenced by the presence and position of free phenolic hydroxyl groups, whereas simple alkoxy substitution does not necessarily enhance radical scavenging to the same extent (29, 31, 52).

A plausible additional factor is the intramolecular hydrogen bond between the 4-hydroxy group of the coumarin core and the carbonyl oxygen of the enone system. If this interaction is strengthened, the ability of the molecule to donate hydrogen to DPPH may decrease. In that sense, the weaker activity of 4b may reflect stronger internal stabilization of the ground state, whereas in 4c and 4d the extra phenolic OH on the aryl ring may provide a more accessible site for radical quenching. The good activity of 4a and 4f further indicates that electron-withdrawing substituents can still be compatible with efficient scavenging, possibly by modulating electron distribution across the conjugated coumarin–chalcone framework. Overall, the present data support a substituent-dependent structure–activity relationship, in which both the electronic effects of the aryl fragment and the availability of a free phenolic hydrogen influence antioxidant performance. This interpretation also agrees with broader recent reviews describing coumarin–chalcone hybrids as biologically versatile scaffolds whose activity is highly sensitive to substitution pattern (53, 54).

Because antioxidant capacity was evaluated here only by the DPPH assay, the results should be interpreted as evidence of radical-scavenging potential under one test system, rather than as a complete description of antioxidant behaviour. DPPH mainly reflects the ability to quench a stable nitrogen-centred radical, while other mechanisms, such as reducing power or scavenging of other radical species, may lead to a different ranking of compounds. Recent data have assessed antioxidant properties using multiple complementary assays such as DPPH, CUPRAC, FRAP, ABTS, or Galvinoxyl, which highlights the value of a broader experimental panel when sufficient material is available. Therefore, further studies using additional methods would be desirable to define the antioxidant profile of these compounds more comprehensively (29, 55, 56).

Acute toxicity

The acute toxicity test was evaluated using the *Artemia salina* assay. Table III presents mortality rates (%) and LC_{50} values for the tested compounds.

Table III

Acute toxicity of compounds 4a - 4h in the *Artemia salina* model

Concentration (mg/mL)	Mortality (after 24 and 48 h)	Mortality rate (%) for the tested compounds							
		4a	4b	4c	4d	4e	4f	4g	4h
1.00	24	100.0	28.6	19.1	38.5	52.0	15.0	35.0	28.6
	48	100.0	40.0	33.3	42.9	58.3	55.6	60.9	52.0
0.50	24	37.5	26.1	15.0	33.4	40.0	10.0	15.0	23.8
	48	53.7	38.8	29.4	41.2	56.3	49.4	53.8	46.7
0.05	24	8.0	10.0	14.3	22.7	12.0	0.0	4.2	0.0
	48	47.8	29.4	23.5	37.5	47.3	35.3	47.1	44.0
LC_{50} (mg/mL)	24	0.53	> 1.00	> 1.00	> 1.00	0.88	> 1.00	> 1.00	> 1.00
	48	0.21	> 1.00	> 1.00	> 1.00	0.17	0.67	0.24	0.80

After 24 hours, the highest toxicity was observed with compound 4a, which exhibited an LC_{50} of 0.53 mg/mL, followed by 4e with an LC_{50} of 0.88 mg/mL. All other compounds showed low toxicity, with LC_{50} values exceeding 1 mg/mL. The acute toxicity after 48 hours revealed more significant effects. The lowest LC_{50} values were observed for compounds 4e (0.17 mg/mL), 4a (0.21 mg/mL) and 4g (0.24 mg/mL), while 4f and 4h displayed slightly higher LC_{50} values of 0.67 mg/mL and 0.80 mg/mL, re-

spectively. Compounds 4b, 4c and 4d exhibited LC_{50} values above 1 mg/mL. Based on these results, it is concluded that the synthesized products generally do not possess significant toxicity. According to the acute toxicity rating scale by FWS (57), compounds 4a, 4e, 4f, 4g and 4h can be classified as practically nontoxic, while 4b, 4c and 4d are categorized as relatively harmless. Despite their low toxicity, the mortality rate of shrimp is higher compared to previously studied compounds (43), where the coumarin core and the aryl substituent were connected by an azine bridge. This is likely due to the electrophilic nature of the α,β-unsaturated carbonyl system, which can form irreversible bonds with biological macromolecules, leading to potential toxic effects (58).

Conclusions

Eight coumarin-chalcone hybrids were synthesized and evaluated for their antioxidant activity and acute toxicity. All compounds were fully spectrally characterized, with the detailed assignment of ^{1}H- and ^{13}C-NMR spectra achieved using a combination of ^{1}H-^{1}H COSY, NOESY, HSQC and HMBC experiments. Antioxidant activity was assessed using the DPPH assay. The most active compounds, 4c and 4d, exhibited notable DPPH radical-scavenging activity, although they remained less active than the reference antioxidant ascorbic acid. The lower activity of 4b and 4h is likely due to the influence of strong electron-donating groups in the aryl moiety, which increases the electron density of the carbonyl oxygen and strengthens the hydrogen bond, thereby reducing the possibility of hydrogen abstraction. The compounds showed minimal toxicological effects on *Artemia salina* and were classified as practically nontoxic (4a, 4e, 4f, 4g and 4h) or relatively harmless (4b, 4c and 4d). These findings indicate that coumarin–chalcone hybrids can provide derivatives with promising radical-scavenging activity and generally low acute toxicity, warranting further optimization and broader biological evaluation.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Ethics approval and consent to participate

Not applicable.

AI use disclosure

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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