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Synthesis and anti-oxidant activity of coumarinyl chalcones

Raj Keshwar Prasad* and Kavita R. Loksh

Abstract

Background: The ability to inhibit oxidative stress has been established as the prime mechanism in treatment of several disease conditions. In view of this, two new series of coumarin–chalcone hybrid molecules (**5a–o** and **6a–o**) were synthesized using various aromatic aldehydes. The structures of the compounds were confirmed using IR, ¹H NMR and mass spectral analyses. The compounds were evaluated for their antioxidant potential against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radicals in scavenging assays.

Results: Compounds **5o** and **5k** exhibited significant antioxidant potential as compared to the standard drug (ascorbic acid).

Conclusions: It can be concluded that the coumarin–chalcone treatment have the potential to be optimized further to generate scaffolds capable to treat many pathological conditions.

Keywords: Coumarin, Chalcone, Antioxidant, Free radical, Vilsmeier–Haack, Claisen–Schmidt condensation

Background

Coumarins (2H-1-benzopyran-2-one) (**I**) contribute to more than 1300 secondary metabolites obtained from plants, bacteria, and fungi and therefore represent the largest class of phenolic substances found in plants [1]. The widespread availability of coumarins in nature has been instrumental for the wide spectrum biological activities exhibited by the natural coumarins. Several synthetic derivatives of coumarins have been explored for activities including antibacterial [2], antifungal [3], anticancer [4], anti-HIV [5], anti-inflammatory [6] etc. Al-Majedy et al. have reported a detailed review on the antioxidant action of synthetic coumarin derivatives [7].

Chalcones (**II**) are molecules containing a 1,3-diphenylprop-2-en-1-one obtained from the flavonoid class of natural products. Several chalcone compounds have been isolated from plant sources and plenty synthetic derivatives have been produced in laboratories by substituting on the benzene rings. Most of the biological actions

exhibited by chalcones as owed to their antioxidant potential [8].

Reports have been made where linking two distinct moieties together using various functional groups or spacers has resulted in synergizing the action of the resulting molecules [9–17]. In persuasion to the reports, we envisaged to fuse coumarin and chalcone nucleuses to form novel conjugates and evaluate the antioxidant potential of the conjugates.

Methods

General

Melting points were determined using open capillary tubes and the reported results are uncorrected. Infrared spectra (KBr) were obtained on Bruker FTIR spectrophotometer. ¹H NMR spectra were recorded on Bruker AVANCE-III 400 MHz spectrometer in the suitable solvent using TMS as the internal standard and the mass spectra were obtained on Applied Biosystems 3200 Q-Trap spectrometer. Purity of the compounds was checked by TLC.

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Chemistry

The synthesis of the coumarin–chalcone hybrid molecules was accomplished according to the reaction depicted in Scheme 1. The steps of the present scheme were adapted with modifications from the scheme reported by Tandel et al. [18] and Srikrishna et al. [19].

4-chloro-2-oxo-2H-chromene-3-carbaldehyde (2)

To a cooled solution of DMF (25 mL) and POCl₃ (5 mL) at 0–5 °C was added 4-hydroxycoumarin (**1**) (5 mmol). The reaction mixture was stirred for 2–3 h at room temperature. On completion of reaction, as monitored on TLC (chloroform–acetone 3:2), the mixture was poured into ice-cold water (30 mL). The separated solid was filtered, washed with two 15 mL portions of water and dried to obtain. The crude product was recrystallized from a methanol gave pure carbaldehyde.

4-Morpholino-2-oxo-2H-chromene-3-carbaldehyde (3)

A solution of morpholine (21.75 g, 20 mmol) in 10 mL of dichloromethane was gradually added under constant stirring to an ice-cooled mixture of **2** (2.09 g, 10 mmol) in 25 mL of dichloromethane. After stirring for 30 min at 0–5 °C, the mixture was washed with three 10 mL portions of water to remove any unreacted morpholine and its salt. The organic phase was dried over MgSO₄ and the solvent was evaporated under reduced pressure. The dry residual flakes were recrystallized from 1,4-dioxane to obtain pure **3** [20].

(E)-3-(3-(4-aminophenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one (4)

4-Morpholino-2-oxo-2H-chromene-3-carbaldehyde (**3**, 0.031 mmol) and 4-amino acetophenone (0.03 mmol) were dissolved in chloroform (30 mL). A catalytic amount of piperidine (0.02 mmol) was added and the reaction mixture was refluxed for 1.5 h. Chloroform was distilled out from the mixture and the residue was washed with methanol to obtain the pure chalcone (**4**) [21].

General method of synthesis of 3-((E)-3-(4-((Z)-benzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one (5a–o)

In a round bottom flask compound **4** (0.0329 mol) was dissolved in methanol (20 mL). Separately, substituted benzaldehyde (0.0329 mol) was dissolved in methanol (20 mL) in a beaker. The solution of substituted benzaldehyde was added drop by drop in to the solution of **4** with continuous stirring. On completion of addition, the mixture was allowed to reflux for 4 h. On completion of reaction, the reaction mixture was poured in to an evaporating dish and

the excess of solvent was removed under reduced pressure. The solid obtained crystallized using methanol [22].

3-((E)-3-(4-((Z)-4-hydroxybenzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one, 5a

Color: Dark Yellow; IR (KBr, cm⁻¹): 3420 (–OH str), 1652 (–C=O str, chalcone), 1712 (–C=O str, coumarin), 1098 (–C–O–C str, morpholine), 1468 (–C=C str, aromatic); ¹H NMR (DMSO,δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine), 4.9 (OH); Mass: 481.6 (M+1).

3-((E)-3-(4-((Z)-2-nitrobenzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one, 5b

Color: Dark Yellow; IR (KBr, cm⁻¹): 1658 (–C=O str, chalcone), 1719 (–C=O str, coumarin), 1098 (–C–O–C str, morpholine), 1465 (–C=C str, aromatic); ¹H NMR (DMSO,δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.4 (H, imine), 8.2 (H, adj. NO₂); Mass: 510.3 (M+1).

3-((E)-3-(4-((Z)-3-nitrobenzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one, 5c

Color: Pale Yellow; IR (KBr, cm⁻¹): 1650 (–C=O str, chalcone), 1710 (–C=O str, coumarin), 1095 (–C–O–C str, morpholine), 1468 (–C=C str, aromatic); ¹H NMR (DMSO,δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine), 8.5 (H, adj. NO₂); Mass: 510.6 (M+1).

3-((E)-3-(4-((Z)-4-nitrobenzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one, 5d

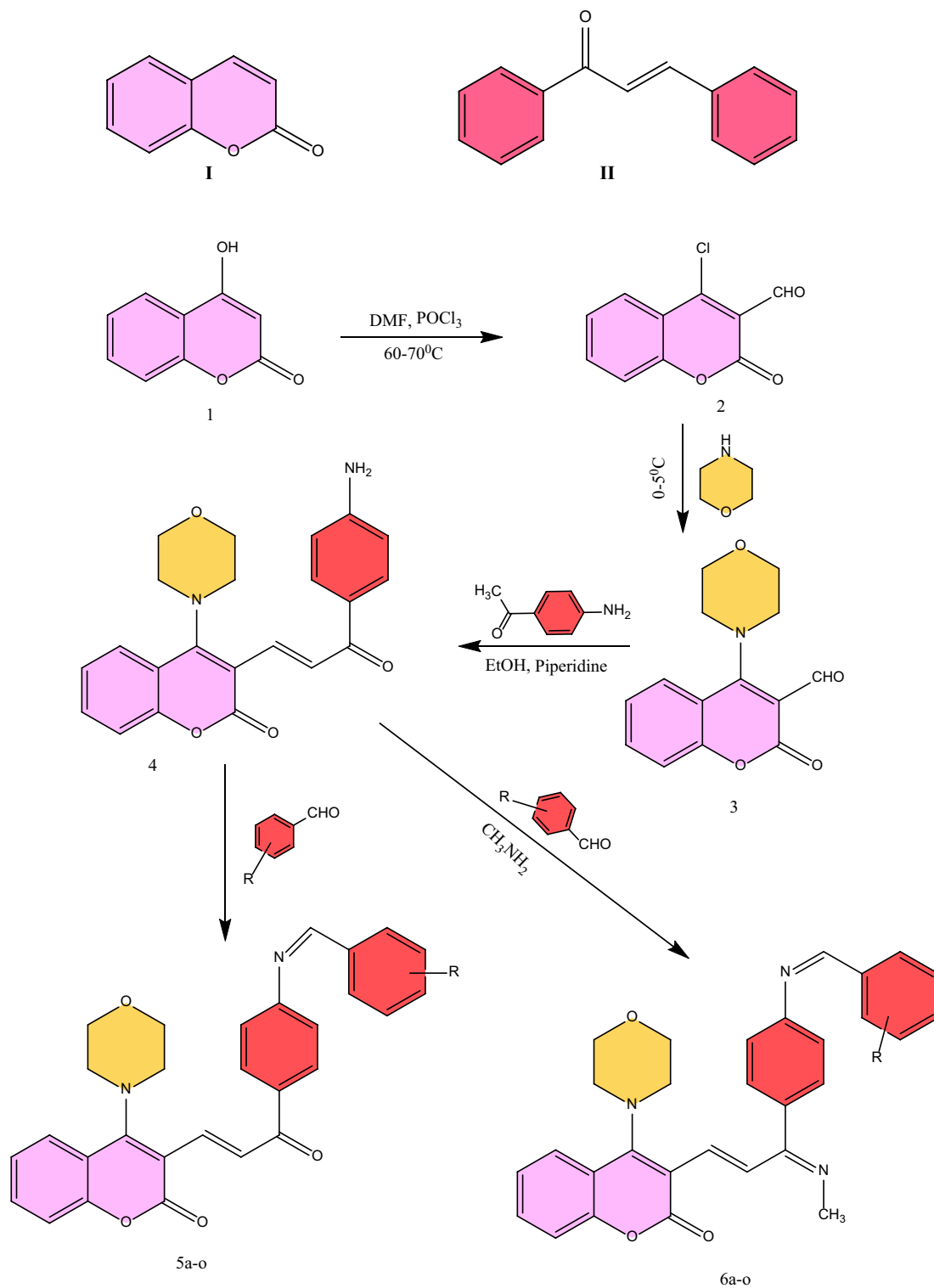
Color: Dark Yellow; IR (KBr, cm⁻¹): 1658 (–C=O str, chalcone), 1712 (–C=O str, coumarin), 1098 (–C–O–C str, morpholine), 1470 (–C=C str, aromatic); ¹H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.4 (H, imine), 8.2 (H, adj. NO₂); Mass: 510.5 (M+1).

3-((E)-3-(4-((Z)-2-hydroxybenzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one, 5e

Color: Dark Yellow; IR (KBr, cm⁻¹): 3422 (–OH str), 1648 (–C=O str, chalcone), 1710 (–C=O str, coumarin), 1098 (–C–O–C str, morpholine), 1468 (–C=C str, aromatic); ¹H NMR (DMSO,δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine), 4.9 (OH); Mass: 481.3 (M+1).

3-((E)-3-(4-((Z)-3,4-dimethoxybenzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one, 5f

Color: Pale Yellow; IR (KBr, cm⁻¹): 1652 (–C=O str, chalcone), 1712 (–C=O str, coumarin), 1098 (–C–O–C



R (a-o) = 4-OH, 2-NO₂, 3-NO₂, 4-NO₂, 2-OH, 2,4-dimethoxy, 2-Cl, 3-Cl, H, 4-OCH₃, 3-methoxy-4-OH, 3-Br, 4-Br, 4-Cl, 4-N(CH₃)₂

Scheme 1 Reaction scheme for the synthesis of coumarin-chalcone conjugates

str, morpoline), 1468 (–C=C str, aromatic); ^1H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.3 (H, imine), 3.8 (CH₃, methoxy); Mass: 525.4 (M + 1).

3-((E)-3-(4-((Z)-2-chlorobenzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one, 5g

Color: Dark Yellow; IR (KBr, cm⁻¹): 1650 (–C=O str, chalcone), 1715 (–C=O str, coumarin), 1097 (–C–O–C str, morpoline), 1463 (–C=C str, aromatic), 765 (C–Cl); ^1H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine); Mass: 500.1 (M + 1).

3-((E)-3-(4-((Z)-3-chlorobenzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one, 5h

Color: Dark Yellow; IR (KBr, cm⁻¹): 1650 (–C=O str, chalcone), 1715 (–C=O str, coumarin), 1097 (–C–O–C str, morpoline), 1463 (–C=C str, aromatic), 755 (C–Cl); ^1H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine); Mass: 500.1 (M + 1).

3-((E)-3-(4-((Z)-benzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one, 5i

Color: Dark Yellow; IR (KBr, cm⁻¹): 1652 (–C=O str, chalcone), 1712 (–C=O str, coumarin), 1098 (–C–O–C str, morpoline), 1468 (–C=C str, aromatic); ^1H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine); Mass: 467.3 (M + 1).

3-((E)-3-(4-((Z)-4-methoxybenzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one, 5j

Color: Yellow; IR (KBr, cm⁻¹): 1655 (–C=O str, chalcone), 1718 (–C=O str, coumarin), 1095 (–C–O–C str, morpoline), 1466 (–C=C str, aromatic); ^1H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine), 3.8 (H, methoxy); Mass: 498.0 (M + 1).

3-((E)-3-(4-((Z)-4-hydroxy-3-methoxybenzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one, 5k

Color: Dark Yellow; IR (KBr, cm⁻¹): 3600 (–OH str), 1650 (–C=O str, chalcone), 1717 (–C=O str, coumarin), 1097 (–C–O–C str, morpoline), 1468 (–C=C str, aromatic); ^1H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine), 5.1 (OH), 3.7 (H, methoxy); Mass: 513.8 (M + 1).

3-((E)-3-(4-((Z)-3-bromobenzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one, 5l

Color: Dark Yellow; IR (KBr, cm⁻¹): 1650 (–C=O str, chalcone), 1710 (–C=O str, coumarin), 1094 (–C–O–C str, morpoline), 1459 (–C=C str, aromatic), 610 (C–Br); ^1H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine); Mass: 543.3 (M + 1).

3-((E)-3-(4-((Z)-4-bromobenzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one, 5m

Color: Dark Yellow; IR (KBr, cm⁻¹): 1650 (–C=O str, chalcone), 1710 (–C=O str, coumarin), 1094 (–C–O–C str, morpoline), 1459 (–C=C str, aromatic), 610 (C–Br); ^1H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine); Mass: 543.3 (M + 1).

3-((E)-3-(4-((Z)-4-chlorobenzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one, 5n

Color: Dark Yellow; IR (KBr, cm⁻¹): 1652 (–C=O str, chalcone), 1710 (–C=O str, coumarin), 1097 (–C–O–C str, morpoline), 1463 (–C=C str, aromatic), 763 (C–Cl); ^1H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine); Mass: 500.1 (M + 1).

3-((E)-3-(4-((Z)-4-(dimethylamino)benzylideneamino)phenyl)-3-oxoprop-1-enyl)-4-morpholino-2H-chromen-2-one, 5o

Color: Pale Yellow; IR (KBr, cm⁻¹): 1652 (–C=O str, chalcone), 1712 (–C=O str, coumarin), 1096 (–C–O–C str, morpoline), 1468 (–C=C str, aromatic); ^1H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine), 2.9 (H, N–CH₃); Mass: 508.6 (M + 1).

General method of synthesis of 3-((1E,3E)-3-(4-((Z)-benzylideneamino)phenyl)-3-(methylimino)prop-1-enyl)-4-morpholino-2H-chromen-2-one (6a–o)

A mixture of **5(a–o)** (5 mmol), methylamine (5 mmol) and acetic acid (10 mL) was stirred at room temperature for 10 min. Then, the mixture was poured into ice-cold water (30 mL). The separated solid was filtered, washed with two 5 mL portions of aqueous acetic acid (1:1) and dried. The crude product was recrystallized from suitable 1,4-dioxane to obtain pure compounds **6a–o** [19, 22].

3-((1E,3E)-3-(4-((Z)-4-hydroxybenzylideneamino)phenyl)-3-(methylimino)prop-1-en-1-yl)-4-morpholino-2H-chromen-2-one, 6a

Color: Dark Yellow; IR (KBr, cm⁻¹): 3420 (–OH str), 1652 (–C=O str, chalcone), 1712 (–C=O str,

coumarin), 1098 (–C–O–C str, morpholine), 1468 (–C=C str, aromatic); ¹H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine), 4.9 (OH), 3.5 (N-CH₃); Mass: 493.5 (M + 1).

3-((1E,3E)-3-(methylimino)-3-(4-(((Z)-2-nitrobenzylidene)amino)phenyl)prop-1-en-1-yl)-4-morpholino-2H-chromen-2-one, 6b

Color: Dark Yellow; IR (KBr, cm⁻¹): 1658 (–C=O str, chalcone), 1719 (–C=O str, coumarin), 1098 (–C–O–C str, morpholine), 1465 (–C=C str, aromatic); ¹H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.4 (H, imine), 8.2 (H, adj. NO₂), 3.4 (N-CH₃); Mass: 525.7 (M + 1).

3-((1E,3E)-3-(methylimino)-3-(4-(((Z)-3-nitrobenzylidene)amino)phenyl)prop-1-en-1-yl)-4-morpholino-2H-chromen-2-one, 6c

Color: Pale Yellow; IR (KBr, cm⁻¹): 1650 (–C=O str, chalcone), 1710 (–C=O str, coumarin), 1095 (–C–O–C str, morpholine), 1468 (–C=C str, aromatic); ¹H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine), 8.5 (H, adj. NO₂), 3.5 (N-CH₃); Mass: 525.6 (M + 1).

3-((1E,3E)-3-(methylimino)-3-(4-(((Z)-4-nitrobenzylidene)amino)phenyl)prop-1-en-1-yl)-4-morpholino-2H-chromen-2-one, 6d

Color: Dark Yellow; IR (KBr, cm⁻¹): 1658 (–C=O str, chalcone), 1712 (–C=O str, coumarin), 1098 (–C–O–C str, morpholine), 1470 (–C=C str, aromatic); ¹H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.4 (H, imine), 8.2 (H, adj. NO₂), 3.5 (N-CH₃); Mass: 525.5 (M + 1).

3-((1E,3E)-3-(4-(((Z)-2-hydroxybenzylidene)amino)phenyl)-3-(methylimino)prop-1-en-1-yl)-4-morpholino-2H-chromen-2-one, 6e

Color: Dark Yellow; IR (KBr, cm⁻¹): 3422 (–OH str), 1648 (–C=O str, chalcone), 1710 (–C=O str, coumarin), 1098 (–C–O–C str, morpholine), 1468 (–C=C str, aromatic); ¹H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine), 4.9 (OH), 3.5 (N-CH₃); Mass: 494.4 (M + 1).

3-((1E,3E)-3-(4-(((Z)-3,4-dimethoxybenzylidene)amino)phenyl)-3-(methylimino)prop-1-en-1-yl)-4-morpholino-2H-chromen-2-one, 6f

Color: Pale Yellow; IR (KBr, cm⁻¹): 1652 (–C=O str, chalcone), 1712 (–C=O str, coumarin), 1098 (–C–O–C str, morpholine), 1468 (–C=C str, aromatic); ¹H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂),

6.7–7.8 (H, Ar), 8.3 (H, imine), 3.8 (CH₃, methoxy), 3.4 (N-CH₃); Mass: 538.8 (M + 1).

3-((1E,3E)-3-(4-(((Z)-2-chlorobenzylidene)amino)phenyl)-3-(methylimino)prop-1-en-1-yl)-4-morpholino-2H-chromen-2-one, 6g

Color: Dark Yellow; IR (KBr, cm⁻¹): 1650 (–C=O str, chalcone), 1715 (–C=O str, coumarin), 1097 (–C–O–C str, morpholine), 1463 (–C=C str, aromatic), 765 (C–Cl); ¹H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine), 3.4 (N-CH₃); Mass: 513.1 (M + 1).

3-((1E,3Z)-3-(4-(((Z)-3-chlorobenzylidene)amino)phenyl)-3-(methylimino)prop-1-en-1-yl)-4-morpholino-2H-chromen-2-one, 6h

Color: Dark Yellow; IR (KBr, cm⁻¹): 1650 (–C=O str, chalcone), 1715 (–C=O str, coumarin), 1097 (–C–O–C str, morpholine), 1463 (–C=C str, aromatic), 755 (C–Cl); ¹H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine), 3.5 (N-CH₃); Mass: 513.4 (M + 1).

3-((1E,3Z)-3-(4-(((Z)-benzylidene)amino)phenyl)-3-(methylimino)prop-1-en-1-yl)-4-morpholino-2H-chromen-2-one, 6i

Color: Dark Yellow; IR (KBr, cm⁻¹): 1652 (–C=O str, chalcone), 1712 (–C=O str, coumarin), 1098 (–C–O–C str, morpholine), 1468 (–C=C str, aromatic); ¹H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine), 3.4 (N-CH₃); Mass: 479.1 (M + 1).

3-((1E,3Z)-3-(4-(((Z)-4-methoxybenzylidene)amino)phenyl)-3-(methylimino)prop-1-en-1-yl)-4-morpholino-2H-chromen-2-one, 6j

Color: Yellow; IR (KBr, cm⁻¹): 1655 (–C=O str, chalcone), 1718 (–C=O str, coumarin), 1095 (–C–O–C str, morpholine), 1466 (–C=C str, aromatic); ¹H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine), 3.8 (H, methoxy), 3.4 (N-CH₃); Mass: 508.3 (M + 1).

3-((E)-3-(4-(((Z)-4-hydroxy-3-methoxybenzylidene)amino)phenyl)-3-oxoprop-1-en-1-yl)-4-morpholino-2H-chromen-2-one, 6k

Color: Dark Yellow; IR (KBr, cm⁻¹): 3600 (–OH str), 1650 (–C=O str, chalcone), 1717 (–C=O str, coumarin), 1097 (–C–O–C str, morpholine), 1468 (–C=C str, aromatic); ¹H NMR (DMSO, δ): 3.1–3.5 (–N(CH₂)₂), 3.7–3.9 (–O(CH₂)₂), 6.7–7.8 (H, Ar), 8.2 (H, imine), 5.1 (OH), 3.7 (H, methoxy), 3.4 (N-CH₃); Mass: 511.4 (M + 1).

3-((1E,3Z)-3-(4-(((Z)-3-bromobenzylidene)amino)phenyl)-3-(methylimino)prop-1-en-1-yl)-4-morpholino-2H-chromen-2-one, 6l

Color: Dark Yellow; IR (KBr, cm^{-1}): 1650 ($-\text{C}=\text{O}$ str, chalcone), 1710 ($-\text{C}=\text{O}$ str, coumarin), 1094 ($-\text{C}-\text{O}-\text{C}$ str, morpholine), 1459 ($-\text{C}=\text{C}$ str, aromatic), 610 (C-Br); ^1H NMR (DMSO, δ): 3.1–3.5 ($-\text{N}(\text{CH}_2)_2$), 3.7–3.9 ($-\text{O}(\text{CH}_2)_2$), 6.7–7.8 (H, Ar), 8.2 (H, imine), 3.4 (N- CH_3); Mass: 557.3 (M+1).

3-((1E,3Z)-3-(4-(((Z)-4-bromobenzylidene)amino)phenyl)-3-(methylimino)prop-1-en-1-yl)-4-morpholino-2H-chromen-2-one, 6m

Color: Dark Yellow; IR (KBr, cm^{-1}): 1650 ($-\text{C}=\text{O}$ str, chalcone), 1710 ($-\text{C}=\text{O}$ str, coumarin), 1094 ($-\text{C}-\text{O}-\text{C}$ str, morpholine), 1459 ($-\text{C}=\text{C}$ str, aromatic), 610 (C-Br); ^1H NMR (DMSO, δ): 3.1–3.5 ($-\text{N}(\text{CH}_2)_2$), 3.7–3.9 ($-\text{O}(\text{CH}_2)_2$), 6.7–7.8 (H, Ar), 8.2 (H, imine), 3.5 (N- CH_3); Mass: 557.6 (M+1).

3-((1E,3Z)-3-(4-(((Z)-4-chlorobenzylidene)amino)phenyl)-3-(methylimino)prop-1-en-1-yl)-4-morpholino-2H-chromen-2-one, 6n

Color: Dark Yellow; IR (KBr, cm^{-1}): 1652 ($-\text{C}=\text{O}$ str, chalcone), 1710 ($-\text{C}=\text{O}$ str, coumarin), 1097 ($-\text{C}-\text{O}-\text{C}$ str, morpholine), 1463 ($-\text{C}=\text{C}$ str, aromatic), 763 (C-Cl); ^1H NMR (DMSO, δ): 3.1–3.5 ($-\text{N}(\text{CH}_2)_2$), 3.7–3.9 ($-\text{O}(\text{CH}_2)_2$), 6.7–7.8 (H, Ar), 8.2 (H, imine), 3.5 (N- CH_3); Mass: 523.2 (M+1).

3-((1E,3Z)-3-(4-(((Z)-4-(dimethylamino)benzylidene)amino)phenyl)-3-(methylimino)prop-1-en-1-yl)-4-morpholino-2H-chromen-2-one, 6o

Color: Pale Yellow; IR (KBr, cm^{-1}): 1652 ($-\text{C}=\text{O}$ str, chalcone), 1712 ($-\text{C}=\text{O}$ str, coumarin), 1096 ($-\text{C}-\text{O}-\text{C}$ str, morpholine), 1468 ($-\text{C}=\text{C}$ str, aromatic); ^1H NMR (DMSO, δ): 3.1–3.5 ($-\text{N}(\text{CH}_2)_2$), 3.7–3.9 ($-\text{O}(\text{CH}_2)_2$), 6.7–7.8 (H, Ar), 8.2 (H, imine), 2.9 (H, N- CH_3), 3.5 (N- CH_3); Mass: 521.6 (M+1).

In-vitro antioxidant activity

The in vitro antioxidant activity of the synthesized compounds **5a–o** and **6a–o** was determined by two different methods using ascorbic acid as the standard.

DPPH method

The free radical scavenging activity of the synthesized molecules was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH [23]. The test samples (10–100 μL) were prepared in DMSO and were mixed with 1.0 mL of DPPH solution and filled up with methanol to a final volume of 4 mL.

Absorbance of the resulting solution was measured at 517 nm in a visible spectrophotometer. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\%inhibition = \frac{(A_o - A_t)}{A_o} \times 100$$

where A_o is the absorbance of the control (blank, without sample) and A_t is the absorbance in the presence of the test samples. All tests were performed in triplicate and the results were expressed as mean values \pm standard deviations.

Hydroxyl radical scavenging method

The test samples (10–100 μL) were prepared in DMSO and 1 mL of iron EDTA solution, 0.5 mL of EDTA solution, 1 mL of DMSO and 0.5 mL of ascorbic acid were added to it. The mixture was incubated in a boiling water bath at 80 to 90 $^\circ\text{C}$ for 15 min. After incubation, 1 mL of ice cold TCA and 3 mL of Nash reagent were added and the reaction mixture was incubated at room temperature for 15 min. The absorbance was read at 412 nm. The % hydroxyl radical scavenging activity is calculated by the following formula

$$\%HRSA = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

where, HRSA is the Hydroxyl Radical Scavenging Activity, Abs control is the absorbance of control and Abs sample is the absorbance of the test solution.

Results

Chemistry

Table 1 presents the physical data and chemical structures of all the synthesized compounds.

Antioxidant action

The antioxidant activity displayed by the synthesized compounds against DPPH and hydroxyl radicals is presented in Tables 2 and 3.

Discussion

Chemistry

Two series of newer coumarin-chalcone conjugates **5a–o** and **6a–o** were synthesized utilizing Scheme 1. Carbaldehyde **2** resulted by the reaction of 4-hydroxy coumarin

Table 1 Physical characterization data and chemical structure of 5a–o & 6a–o

Compound	Structure	Melting point (°C)	Yield (%)
5a		139-142	69
5b		161-163	61
5c		154-157	63
5d		159-162	64
5e		136-139	72
5f		171-174	76
5g		127-130	67
5h		129-131	64

Table 1 (continued)

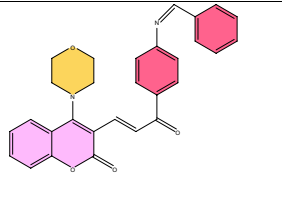
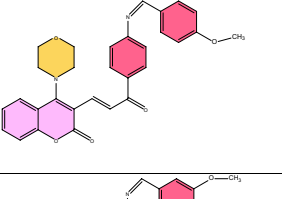
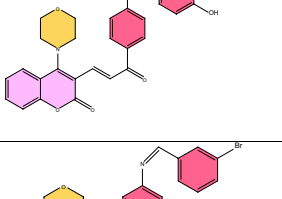
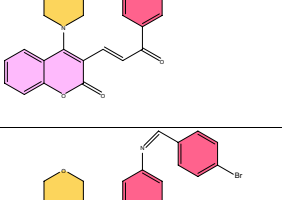
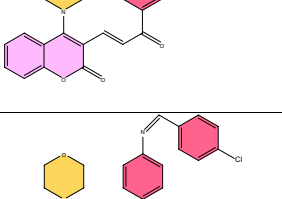
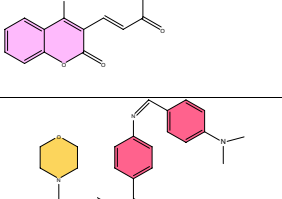
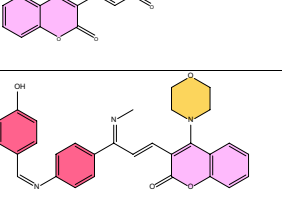
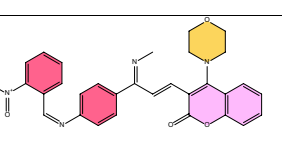

5i		119-122	63
5j		139-141	70
5k		151-154	71
5l		129-133	64
5m		127-130	61
5n		128-131	60
5o		145-147	66
6a		169-172	59
6b		172-175	67

Table 1 (continued)

6c		165-167	54
6d		181-183	61
6e		143-145	60
6f		191-194	56
6g		140-143	72
6h		146-149	70
6i		138-141	64
6j		151-153	62
6k		167-170	60
6l		153-156	66

Table 1 (continued)

6m		147-149	61
6n		143-146	63
6o		161-163	65

Table 2 IC₅₀ values of 5a–o

Compound	IC ₅₀	
	DPPH	HRSA
5a	17.1 ± 0.19	16.9 ± 0.01
5b	37.4 ± 0.08	33.8 ± 0.10
5b	35.7 ± 0.21	33.1 ± 0.35
5d	35.2 ± 0.11	33.0 ± 0.56
5e	17.9 ± 0.03	16.4 ± 0.17
5f	19.6 ± 0.07	18.1 ± 0.73
5g	27.2 ± 0.61	26.7 ± 0.21
5h	27.1 ± 0.26	25.9 ± 0.11
5i	26.7 ± 0.29	24.9 ± 0.14
5j	21.3 ± 0.47	19.8 ± 0.25
5k	16.6 ± 0.10	15.5 ± 0.66
5l	29.1 ± 0.53	28.6 ± 0.05
5m	29.5 ± 0.67	27.7 ± 0.26
5n	27.1 ± 0.30	26.0 ± 0.13
5o	15.3 ± 0.26	14.4 ± 0.29
Ascorbic acid	13.8 ± 0.11	15.7 ± 0.65

Table 3 IC₅₀ values of 6a–o

Compound	IC ₅₀	
	DPPH	HRSA
6a	23.9 ± 0.15	19.1 ± 0.36
6b	46.5 ± 0.04	44.8 ± 0.34
6c	44.9 ± 0.17	42.1 ± 0.76
6d	46.1 ± 0.56	43.7 ± 0.18
6e	24.3 ± 0.11	19.3 ± 0.21
6f	18.1 ± 0.54	16.0 ± 0.08
6g	34.1 ± 0.01	32.9 ± 0.40
6h	32.7 ± 0.35	32.0 ± 0.08
6i	28.2 ± 0.13	26.5 ± 0.16
6j	19.9 ± 0.26	16.3 ± 0.02
6k	17.5 ± 0.08	15.2 ± 0.97
6l	39.6 ± 0.05	37.2 ± 0.29
6m	39.2 ± 0.26	36.5 ± 0.76
6n	32.9 ± 0.32	32.5 ± 0.01
6o	16.0 ± 0.03	14.9 ± 0.12
Ascorbic acid	13.8 ± 0.11	15.7 ± 0.65

1 with Vilsmeier-Haack reagent leading to formylation of the electron rich ring [24]. Compound **2** undergoes nucleophilic aromatic substitution with morpholine to yield the compound **3** which on condensation with amino acetophenone under the conditions of Claisen-Schmidt reaction yielded the chalcone conjugates **4**. The coumarin-chalcone conjugates were further condensed at reflux conditions with aromatic aldehydes and methyl amine to obtain compounds **5a–o** and **6a–o**. The optimization of the reaction for completion and purity was performed throughout using TLC. The structures of the

synthesized molecules were characterized by ¹H NMR, IR and mass spectral studies.

The ¹H NMR spectra of compound **5a–o** exhibited peaks in the region of 3.1–3.9 corresponding to -N(CH₂)₂ and -O(CH₂)₂; 6.7–7.8 due to aromatic protons, the peaks due to α, β-unsaturation of chalcones; 8.2 owing to the imine protons. Additionally, the signals due to the hydroxyl and methoxy protons were also found in the corresponding compounds. In the ¹H NMR spectra of compound **6a–o** additional peak at 3.3–3.5 was obtained due the methyl protons of methylamine. The mass

spectra of all the compounds displayed $M + 1$ peaks corresponding to their molecular formula.

Antioxidant action

As known, free radical scavenging is one of most perceived mechanisms for any antioxidant to inhibit oxidation of lipids. The standard assay protocols to evaluate the free radical scavenging activity include the DPPH and the hydroxyl radical scavenging activity assays. The antioxidant potential of the compounds **5a–o** and **6a–o** was assessed using these two methods using ascorbic acid as the standard.

DPPH radicals are stable free radicals whose radical character is neutralized in the presence of molecules that may donate H atoms. The reduction of the DPPH radical can be spectrophotometrically determined by the decreased absorbance at 517 nm caused due to antioxidants.

Hydroxyl radical scavenging assay is used to find the antioxidant activity of test compounds against free hydroxyl radicals like hydrogen peroxide (known cause damage to the cells). The model used is ascorbic acid-iron-EDTA model of hydroxyl radical generating system. This is a totally aqueous system wherein ascorbic acid, iron and EDTA combine with each other to generate hydroxyl radicals.

As it can be seen from results tabulated in Table 3, compounds **5a**, **5e**, **5f**, **5k** and **5o** had DPPH and hydroxyl radical scavenging activity comparable to the standard drug ascorbic acid while the remaining compounds exhibited very high IC_{50} values. It was also observed that the IC_{50} values of the compounds were lower for hydroxyl radical scavenging (14.4 to 33.8 $\mu\text{g/mL}$) as compared to DPPH radical scavenging (15.3 to 37.4 $\mu\text{g/mL}$). Compounds **5o** and **5k** were found to be exhibiting better inhibition of the hydroxyl radical with IC_{50} values 14.4 and 15.5 respectively as compared to ascorbic acid (15.7).

The results highlighted the importance of the carbonyl group of the chalcone molecule in the antioxidant action as the IC_{50} values of compounds **6a–o** was comparatively poor to that of **5a–o**. The absence of the carbonyl carbon could be attributed to the decreased antioxidant action of **6a–o**. A similar decrease in antioxidant potential was reported by Lahsasni et al. [25] where they condensed the carbonyl carbon and the double bond into pyridine ring to obtain compounds which were less potential antioxidants when compared to the corresponding α , β -unsaturated carbonyl compounds. The excellent antioxidant capacity of **5a**, **5e**, **5f**, **5k** and **5o** reaffirmed the concept that organic molecules containing electron donating groups have better capacity to neutralize free radicals and oppose oxidation [26].

Conclusions

Two series of coumarin-chalcone hybrid molecules were synthesized in good yields using Vilsmeier-Haack, nucleophilic aromatic substitution and Claisen-Schmidt condensation reaction and characterized by spectral studies. The synthesized compounds exhibited good antioxidant potential against DPPH and hydroxyl radicals in the scavenging assays with compounds **5o** and **5k** being the most significant against the tested radicals.

Abbreviations

FTIR: Fourier-Transform Infrared Spectroscopy; DMF: Dimethylformamide; TLC: Thin layer chromatography; NMR: Nuclear Magnetic Resonance; HRSA: Hydroxyl Radical Scavenging Activity; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; DMSO: Dimethyl sulfoxide; EDTA: Ethylene Diamine Tetra Acetic Acid.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43094-021-00340-1>.

Additional file 1. Scheme 1.

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Authors' contributions

KRP performed all the synthetic and biological activity work. KRL was involved in interpreting the results and preparing the manuscript. All authors read and approved the manuscript.

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Declarations

Ethics approval and consent to participate

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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