Synthesis of coumarin derivatives containing pyrazole and indenone rings as potent antioxidant and antihyperglycemic agents

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Received 8 August 2013; accepted 31 May 2014

Abstract The present work describes the synthesis of 6-substituted-3-(1-(4-substituted)-4-((Z)-(5, 6-dimethoxy-1-oxo-1H-inden-2(3H)-ylidene)methyl)-1H-pyrazol-3-yl)-2H-chromen-2-one derivatives (5a-l) by Claisen–Schmidt condensation of 3-(6-substituted-2-oxo-2H-chromen-3-yl)-1-(4-substituted)-1H-pyrazole-4-carbaldehyde derivatives (4a-l) with 5,6-dimethoxy-2,3-dihydro-1H-inden-1-one at reflux temperature for 8 h. The synthesized compounds were screened for in vitro antioxidant activity. Compounds 5e and 5g showed promising DPPH radical scavenging activity with IC50 54.14–56.19 lg/mL, ferrous ion chelating ability with IC50 53.75–56.89 lg/mL and reductive capability with IC50 58.01–62.57 lg/mL. The selected compounds were also tested for in vivo antihyperglycemic activity against Streptozotocin–nicotinamide induced Adult Wistar rats. Compounds 5e and 5g showed significant decrease in glucose concentration (115 and 138 mg/dL) with the dose of 100 mg/kg.

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1. Introduction

Human bodies are protected from oxidative stress by natural enzymatic and non-enzymatic antioxidant defensive system, whose capacity is affected by age, diet, and health status of the individual (Chun et al., 2003). Therefore, only endogenous antioxidant defenses are not absolutely efficient. Dietary antioxidants are required to diminish the cumulative effects of oxidative damage due to excess ROS (reactive oxygen species) that remains in our system (Lim and Murtijaya, 2013).
2. Materials and methods

2.1. Chemistry

5,6-Dimethoxyindenone with 98% purity was purchased from Sigma Aldrich Company. Melting points were recorded on an electro thermal melting point apparatus and are uncorrected. 

$^1$H and $^{13}$C NMR spectra were recorded on a Bruker 400 MHz spectrometer and chemical shifts are shown in $\delta$ values (ppm) with tetramethylsilane (TMS) as internal standard. LC–MS was obtained using an Agilent LC/MS instrument (Electronic Ionization method). The FT-IR spectra were taken in KBr pellets (100 mg) using a Shimadzu FT-IR spectrophotometer. Column chromatography was performed using silica gel (230–400 mesh), silica gel GF254 plates from Merck were used for TLC and spots were identified either by UV or dipping the plates in potassium permanganate solution.

2.1.1. General procedure for the synthesis of 3-(6-substituted-2-oxo-2H-chromen-3-yl)-1-(4-substituted)-1H-pyrazole-4-carbaldehyde derivatives (4a–l)

To the cold solution of DMF (1.0 mL, 0.014 mol), POCl$_3$ (1.3 mL 0.014 mol) was added drop wise for half an hour by maintaining the temperature at 0–5 °C. To this solution, 3-[(E)-1-(2-phenylhydrazinylidene) ethyl]-2H-chromen-2-one (0.0035 mol) (3a–l) was added and the reaction mixture was stirred. After complete addition, the stirring was continued for 30 min, at 0–5 °C and then the temperature was raised to 80 °C for about 6 h. Completion of reaction was confirmed by TLC, reaction mixture was poured into crushed ice and neutralized with 10% NaOH solution. The solid precipitated out was filtered, dried and recrystallized from ethanol.

The formation of compounds 4a (m.p.; 183–186 °C) and 4b (m.p.; 185 to 187 °C) was confirmed by comparing its melting points with the literature value (Vijaya Laksmi et al., 2015).

2.1.2. 1-(4-Chlorophenyl)-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazole-4-carbaldehyde (4c)

Yield 70%; m.p. 189–192 °C; Mol. Formula: C$_{17}$H$_{14}$ClN$_2$O$_3$; IR (KBr, cm$^{-1}$): 1714 (C=O), 1675 (CHO), 1625 (C=N), 1590 (C=C); $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$: 7.30–8.10 (m, 9H), 9.43 (s, 1H), 10.22 (s, 1H). MS: m/z = 370 [M$^+$], 372 [M + 2].

2.1.3. 3-(6-Bromo-2-oxo-2H-chromen-3-yl)-1-(4-chlorophenyl)-1H-pyrazole-4-carbaldehyde (4d)

Yield 75%; m.p. 189–192 °C; Mol. Formula: C$_{19}$H$_{12}$BrClN$_2$O$_3$; IR (KBr, cm$^{-1}$): 1700 (C=O), 1671 (CHO), 1621 (C=N), 1592 (C=C); $^1$H NMR (DMSO-d$_6$, 400 MHz) $\delta$: 7.31–8.15 (m, 9H), 9.45 (s, 1H), 10.23 (s, 1H). MS: m/z = 430 [M$^+$], 432 [M + 2], 434 [M + 4].

2.1.4. 1-(4-Methylphenyl)-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazole-4-carbaldehyde (4e)

Yield 72%; m.p. 194–197 °C; Mol. Formula: C$_{20}$H$_{14}$N$_2$O$_3$; IR (KBr, cm$^{-1}$): 1710 (C=O), 1689 (CHO), 1625 (C=N), 1588 (C=C); $^1$HMIR (DMSO-d$_6$, 400 MHz) $\delta$: 3.20 (s, 3H, CH$_3$), 7.20–7.93 (m, 9H), 9.40 (s, 1H), 9.97 (s, 1H). MS: m/z = 330 [M$^+$].
2.1.5. 3-(6-Bromo-2-oxo-2H-chromen-3-yl)-1-(4-methylphenyl)-1H-pyrazole-4-carbaldehyde (4f)
Yield 68%; m.p. 199–202 °C; Mol. Formula: C_{20}H_{17}BrN_{2}O_5; IR (KBr, cm^{-1}): 1715 (C=O), 1691 (CHO), 1612 (C=CN), 1585 (C=C); ¹H NMR (DMSO-d_{6}, 400 MHz) δ: 3.25 (s, 3H, OCH_{3}), 7.22–8.10 (m, 8H), 9.43 (s, 1H), 10.10 (s, 1H); MS: m/z = 408 [M⁺]+, 410 [M + 2].

2.1.6. 1-(4-Methoxyphenyl)-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazole-4-carbaldehyde (4g)
Yield 77%; m.p. 177–180 °C; Mol. Formula: C_{20}H_{18}BrN_{2}O_5; IR (KBr, cm^{-1}): 1718 (C=O), 1685 (CHO), 1618 (C=CN), 1595 (C=C); ¹H NMR (DMSO-d_{6}, 400 MHz) δ: 3.35 (s, 3H, OCH_{3}), 7.13–8.06 (m, 9H), 9.42 (s, 1H), 9.94 (s, 1H); MS: m/z = 346.33 [M⁺].

2.1.7. 3-(6-Bromo-2-oxo-2H-chromen-3-yl)-1-(4-methoxyphenyl)-1H-pyrazole-4-carbaldehyde (4h)
Yield 78%; m.p. 181–183 °C; Mol. Formula: C_{20}H_{18}BrN_{2}O_5; IR (KBr, cm^{-1}): 1712 (C=O), 1687 (CHO), 1620 (C=CN), 1591 (C=C); ¹H NMR (DMSO-d_{6}, 400 MHz) δ: 6.90–8.0 (m, 9H), 9.43 (s, 1H), 10.01 (s, 1H); MS: m/z = 424 [M⁺]+, 426 [M + 2].

2.1.8. 1-(4-Nitrophenyl)-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazole-4-carbaldehyde (4i)
Yield 75%; m.p. 189–192 °C; Mol. Formula: C_{19}H_{16}BrN_{2}O_5; IR (KBr, cm^{-1}): 1721 (C=O), 1681 (CHO), 1620 (C=CN), 1599 (C=C); ¹H NMR (DMSO-d_{6}, 400 MHz) δ: 6.90–8.0 (m, 9H), 9.43 (s, 1H), 10.01 (s, 1H); MS: m/z = 361 [M⁺].

2.1.9. 3-(6-Bromo-2-oxo-2H-chromen-3-yl)-1-(4-nitrophenyl)-1H-pyrazole-4-carbaldehyde (4j)
Yield 76%; m.p. 187–189 °C; Mol. Formula: C_{19}H_{16}BrN_{2}O_5; IR (KBr, cm^{-1}): 1719 (C=O), 1684 (CHO), 1630 (C=CN), 1625, 1520 (N=O), 1585 (C=C); ¹H NMR (DMSO-d_{6}, 400 MHz) δ: 6.95–8.05 (m, 8H), 9.48 (s, 1H), 10.15 (s, 1H); MS: m/z = 439 [M⁺]+, 441 [M + 2].

2.1.10. 1-(4-Fluorophenyl)-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazole-4-carbaldehyde (4k)
Yield 72%; m.p. 161–164 °C; Mol. Formula: C_{19}H_{15}BrN_{2}O_5; IR (KBr, cm^{-1}): 1709 (C=O), 1680 (CHO), 1611 (C=CN), 1603 (C=C); ¹H NMR (DMSO-d_{6}, 400 MHz) δ: 7.13–8.06 (m, 9H), 9.45 (s, 1H), 9.98 (s, 1H); MS: m/z = 334 [M⁺].

2.1.11. 3-(6-Bromo-2-oxo-2H-chromen-3-yl)-1-(4-fluorophenyl)-1H-pyrazole-4-carbaldehyde (4l)
Yield 68%; m.p. 166–168 °C; Mol. Formula: C_{19}H_{15}BrN_{2}O_5; IR (KBr, cm^{-1}): 1705 (C=O), 1682 (CHO), 1618 (C=CN), 1589 (C=C); ¹H NMR (DMSO-d_{6}, 400 MHz) δ: 7.10–8.0 (m, 8H), 9.42 (s, 1H), 10.12 (s, 1H); MS: m/z = 412 [M⁺]+, 414 [M + 2].

2.1.12. Synthesis of 3-(4-((Z)-(5,6-dimethoxy-1-oxo-1H-inden-2(3H)-ylidene) methyl)-1-(4-substituted-phenyl-1H-pyrazole-4-carbaldehyde (4a–l)
The mixture of 5,6-dimethoxy-2,3-dihydro-1H-inden-1-one (0.5 g 0.002 mol) and 3-(4-substituted-2-oxo-2H-chromen-3-yl)-1-(4-substituted)-1H-pyrazole-4-carbaldehyde (4a–l) (0.002 mol) was stirred in sodium methoxide solution under reflux condition for 8 h. and completion of the reaction was confirmed by TLC. The reaction mixture was poured into cold water and neutralized with dilute HCl. The solid separated was filtered, dried and recrystallized with ethanol to furnish the title compounds.
3.24 (3H, s, OCH3), 3.37 (3H, sOCH3), 4.71 (2H, s, CH2), 7.35–8.85 (10H, m, Ar–H), 8.86 (1H, s, CH methine), 9.74 (1H, s, C–C–H), 11CNMR (CDCl3) δ: 45.0 (CH2), 57.9 (2-OCH3), 102.6, 104.7, 109.7, 112.0 (2C), 114.7, 116.1, 120.5, 122.0, 123.7, 125.0, 127.9, 130.0, 132.5 (2C), 134.1, 136.7, 139.5, 142.0, 144.2, 150.4, 153.1, 156.4, 162.2, 165.1, 169.0 (coumarin C=O), 175.5 (indenone C=O). MS: m/z 602 [M]+, 604 [M + 2], 606 [M + 4].

2.1.17. 3-(4-((Z)-(5,6-Dimethoxy-1-oxo-1H-inden-2(3H)-ylidene) methyl)-1-p-tolyl-pyrazol-3-yl)-2H-chromen-2-one (5e)

Yield 72%; m.p. 242–245 °C, Mol. Formula: C31H23N2O5; IR (KBr, cm−1): 3060 (Ar–H), 1703 (coumarin C=O), 1667 (indenone C=O), 1615 (C=C–N), 1605 (C=C–C), 1H NMR (DMSO-d6, 400 MHz) δ: 2.52 (3H, s, CH3)2.22 (3H, s, OCH3), 3.35 (3H, s, OCH3), 4.72 (2H, s, CH2), 7.36–8.86 (11H, m, Ar–H), 8.88 (1H, s, CH methine), 9.73 (1H, s, C–C–H). 13CNMR (CDCl3) δ: 25.6 (CH3), 43.0 (CH2), 57.1 (2-OCH3), 103.2, 105.2, 108.6, 112.0 (2C), 114.7, 116.1, 119.5, 122.0, 125.3, 125.9, 127.9, 130.0, 132.2 (2C), 134.7, 136.5, 138.2, 142.3, 144.7, 151.1, 154.1, 157.1, 161.4, 165.1, 168.0 (coumarin C=O), 174.2 (indenone C=O). MS: m/z 504 [M]+.

2.1.18. 6-Bromo-3-(4-((Z)-(5,6-dimethoxy-1-oxo-1H-inden-2(3H)-ylidene) methyl)-1-p-tolyl-pyrazol-3-yl)-2H-chromen-2-one (5f)

Yield 68%; m.p. 251–253 °C, Mol. Formula: C31H22BrN2O5; IR (KBr, cm−1): 3068 (Ar–H), 1710 (coumarin C=O), 1669 (indenone C=O), 1617 (C=C–N), 1600 (C=C–C), 1H NMR (DMSO-d6, 400 MHz) δ: 2.50 (3H, s, CH3)3.23 (3H, s, OCH3), 4.72 (2H, s, CH2), 7.35–8.85 (10H, m, Ar–H), 8.87 (1H, s, CH methine), 9.77 (1H, s, C–C–H). 13CNMR (CDCl3) δ: 24.3 (CH3), 44.0 (CH2), 57.6 (2-OCH3), 102.2, 105.2, 107.6, 112.0 (2C), 113.7, 116.1, 119.5, 121.0, 123.5, 125.0, 127.0, 130.0, 132.2 (2C), 134.1, 136.5, 138.2, 142.3, 144.7, 151.4, 157.1, 161.4, 165.1, 168.9 (coumarin C=O), 175.3 (indenone C=O). MS: m/z 582 [M]+, 584 [M + 2].

2.1.19. 3-(4-((Z)-(5,6-Dimethoxy-1-oxo-1H-inden-2(3H)-ylidene) methyl)-1-(4-methoxy phenyl)-1H-pyrazol-3-yl)-2H-chromen-2-one (5g)

Yield 74%; m.p. 228–231 °C, Mol. Formula: C31H22BrN2O4; IR (KBr, cm−1): 2989 (Ar–H), 1712 (coumarin C=O), 1687 (indenone C=O), 1624 (C=N), 1603 (C=C), 1H NMR (DMSO-d6, 400 MHz) δ: 2.78 (3H, s, OCH3)3.30 (3H, s, OCH3), 3.40 (3H, s, OCH3), 4.34 (2H, s, CH2), 7.40–8.90(11H, m, Ar–H), 8.90 (1H, s, CH methine), 9.82 (1H, s, C–C–H). 13CNMR (CDCl3) δ: 25.3 (OCH3), 45.0 (CH2), 58.6 (2-OCH3), 103.2, 105.2, 107.8, 112.0 (2C), 114.7, 116.1, 119.0, 121.0, 122.9, 125.7, 127.0, 129.7, 132.2 (2C), 134.1, 136.3, 139.2, 142.3, 146.7, 152.1, 155.1, 157.1, 161.4, 166.1, 168.9 (coumarin C=O), 174.3 (indenone C=O). MS: m/z 520 [M]+.

2.1.20. 6-Bromo-3-(4-((Z)-(5,6-dimethoxy-1-oxo-1H-inden-2(3H)-ylidene)methyl)-1-(4-methoxy phenyl)-1H-pyrazol-3-yl)-2H-chromen-2-one (5h)

Yield 76%; m.p. 234–237 °C, Mol. Formula: C31H22BrN2O4; IR (KBr, cm−1): 2995 (Ar–H), 1709 (coumarin C=O), 1681 (indenone C=O), 1621 (C=N), 1600 (C=C), 687 (C=Br); 1H NMR (DMSO-d6, 400 MHz) δ: 2.76 (3H, s, CH3)3.29 (3H, s, OCH3), 3.42 (3H, s, OCH3), 4.76 (2H, s, CH2), 7.42–8.91 (10H, m, Ar–H), 8.92 (1H, s, CH methine), 9.84 (1H, s, C–C–H). 13CNMR(CDCl3) δ: 25.5 (OCH3), 46.0 (CH2), 57.2 (2-OCH3), 102.3, 105.2, 106.8, 111.0 (2C), 114.7, 116.1, 118.0, 121.0, 123.5, 125.0, 127.0, 129.3, 132.0 (2C), 134.2, 136.9, 139.2, 142.5, 145.6, 151.1, 155.2, 158.4, 161.4, 165.4, 169.0 (coumarin C=O), 175.3 (indenone C=O). MS: m/z 598 [M]+, 600 [M + 2].

Please cite this article in press as: Kenchappa, R. et al., Synthesis of coumarin derivatives containing pyrazole and indenone rings as potent antioxidant and anti-inflammatory agents. Arabian Journal of Chemistry (2014), http://dx.doi.org/10.1016/j.arabjc.2014.05.029
Synthesis of coumarin derivatives containing pyrazole and indenone rings

2.2. Pharmacology

2.2.1. Antioxidant activity

2.2.1.1. Free radical scavenging activity by DPPH method. Free radical scavenging capacities of synthesized compounds were determined according to the reported procedure (Braca et al., 2001). The newly synthesized compounds at different concentrations (25–100 μg/mL) were added to each test tube and volume was made up to 4 mL using methanol. To this, 3 mL of 0.004% DPPH in methanol was added and the mixtures were incubated at room temperature under dark condition for 30 min. The absorbance was recorded at 517 nm using a UV–Visible spectrophotometer (Shimadzu UV-1800, Japan). Butylated hydroxytoluene (BHT), dissolved in distilled water was used as a reference. Control sample was prepared using the same volume without any compound and BHT, 95% methanol served as blank. Test was performed in triplicate and the results were averaged. Radical scavenging activity was calculated using the formula:

\[
\% \text{ of radical scavenging activity} = \left( \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right) \times 100
\]

where \( A_{\text{control}} \) is the absorbance of the control sample (DPPH solution without test sample) and \( A_{\text{test}} \) is the absorbance of the test sample (DPPH solution + test compound).

2.2.2. Chelating effect on ferrous ions

The chelating effect was determined according to the literature method (Nevcihan et al., 2010). The test solution (2 mL) of different concentrations (25–100 μg/mL) in methanol was added to a solution of 2 mM FeCl\(_2\) (0.05 mL) and the reaction was initiated by adding 5 mM ferrozine (0.2 mL) and the total volume was adjusted to 5 mL with methanol. Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was measured spectrophotometrically at 562 nm. EDTA was used as a standard. The inhibition percentage of ferrozine-Fe\(^{2+}\) complex formations was calculated using the formula:

\[
\% \text{ of metal chelating activity} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

where \( A_{\text{control}} \) is the absorbance of control (control contains FeCl\(_2\) ferrozine complex) and \( A_{\text{sample}} \) is the absorbance of test compounds. Test was performed in triplicate, and the results were averaged.

2.2.3. Total reductive capability

The reductive capability of the compounds was determined according to the literature method (Oyaizu, 1986). The solution of test compound (1 mL) at different concentrations (25–100 μg/mL) in methanol was mixed with phosphate buffer (2.5 mL, 0.2 mol/L pH 6.5) and potassium ferricyanide (2.5 mL, 1%) and the mixture was incubated at 50 °C for 20 min. At the end of the incubation, trichloroacetic acid (2.5 mL, 10%) was added to the mixture, and centrifuged at 3000 rpm for 10 min. The upper layer solution was collected and mixed with distilled water (2.5 mL) and 0.1% ferric chloride (0.5 mL, 0.1%) solution. The absorbance was measured at 700 nm against a blank. Test was performed in triplicate, and the results were averaged.

2.2.4. In-vivo anti-hyperglycemic activity

2.2.4.1. Animal housing and maintenance. Adult Wistar rats weighing between 200 and 300 g were used in this study. Animals were taken care as per the Regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The ‘Form B’ for carrying out animal experimentation was reviewed and approved by the Institutional Animal Ethics Committee (IAEC). Animals were maintained in a controlled environment with 22–25 °C temperature, 50–60% humidity, a light/dark cycle of 12 h each and 15–20 air changes per hour. Animals were fed, \textit{ad libitum}, with certified Irradiated Laboratory Rodent Diet (Nutri lab brand, Tetragon Chemie Pvt. Ltd, Bangalore) except during the fasting & study period.

2.2.4.2. Oral glucose tolerance test in normal rats. Overnight fasted normal rats were divided into twenty groups of six rats each. They were orally administered with vehicle, six synthesized compounds \(5a, 5c\) and \(5e-5h\) (10, 50 and 100 mg/kg) and metformin (60 mg/kg), respectively. Glucose (2 g/kg) was fed 30 min after the administration of compounds (Shirwaikar and Rajendran, 2006). Blood was withdrawn through the tail vein at 0, 30, 60 and 120 min of glucose administration to observe changes in the blood glucose level.

2.2.4.3. Hypoglycemic activity assay. Test substance and reference standard (metformin) suspension were prepared with vehicle containing 0.5% (w/v) of carboxymethyl cellulose and 0.1% Tween 80. Streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5) and nicotinamide in normal saline solution (0.9% NaCl solution). Diabetes was induced in overnight fasted rats by a single intraperitoneal (i.p.) injection of 60 mg/kg of streptozotocin and 120 mg/kg of nicotinamide in Wistar rats (of both sexes) (Shirwaikar and Rajendran, 2006). Hyperglycemia was confirmed by the elevated non fasting glucose levels in blood, determined 48 h after diabetes induction using a Glucometer (Accu-check Advantage, Roche diagnostics Mannheim, Germany) and strips. Animals with blood glucose concentrations more than 250 mg/dL were used for the study. Each study group involved 6 diabetic rats. Cage cards indicating the study number, animal number & treatment group details were affixed to the corresponding cages. Standard drug and test compounds were given orally at 10, 50 and 100 mg/kg. The change in the body weight of rats after induction diabetes was noted. The blood sample was collected from caudal vein of the tested rats. Reduction in blood glucose produced by the compound was recorded on 1st, 2nd, 3rd, 4th, 7th and 14th day. At the end of experiment, animals were euthanized by CO\(_2\) asphyxiation and organ weights were recorded.

Statistical analysis was performed by One-way ANOVA followed by Dunnett’s multiple comparison tests. \( P < 0.05 \) was considered as a significant change.
3. Result and discussion

3.1. Chemistry

Reaction sequences employed for the synthesis of title compounds is shown in Scheme 1. The key intermediates, 6-substituted-3-[(1E)-1-[(4-substituted) hydrazinylidene] ethyl]-2H-chromen-2-one (3a–l) were synthesized by stirring the reaction mixture of 6-substituted 3-acetyl coumarin (1a–b) with p-substituted phenyl hydrazine hydrochloride (2a–f) in dry ethanol using sodium acetate at room temperature (Goudarshivannavar et al., 2009). Vilsmeier formylation of these compounds (3a–l) at reflux temperature for 6 h gave the expected formyl pyrazole derivatives (4a–l) in good yield. Further, Claisen–Schmidt condensation of 3-(6-substituted-2-oxo-2H-chromen-3-yl)-1-(4-substituted)-1H-pyrazole-4-carbaldehyde derivatives (4a–l) with 5,6-dimethoxy-2,3-dihydro-1H-inden-1-one at room temperature for 8 h led to the formation of corresponding 6-substituted-3-(1-(4-substituted)-4-((Z)-(5,6-dimethoxy-1-oxo-1H-inden-2(3H)-ylidene)methyl)-1H-pyrazol-3-yl)-2H-chromen-2-one derivatives (5a–l).

All pyrazole coumarin indenone analogs provided satisfactory spectral data. In IR spectra, bands in the region 1671–1691 cm\(^{-1}\)/C\(_{0}\) were attributed to the –CHO group of (4a–l) series and bands in the region 1625, 1520 cm\(^{-1}\)/C\(_{0}\) were attributed to symmetric and asymmetric frequency of the NO\(_2\) group of the compound 4j. Bands at 1703–1713 cm\(^{-1}\)/C\(_{0}\) obtained from the lactone ring of coumarin C\(_{0}\), 1667–1687 cm\(^{-1}\)/C\(_{0}\) stretching frequencies correspond to the C\(_{0}\) groups of indenone. In \(^1\)H NMR spectra, the singlet between \(\delta\) 9.94–10.23 ppm corresponds to the presence of the –CHO group of the compounds 4a–l. The absence of aldehyde proton signal at \(\delta\) 9.94–10.21 ppm and the presence of a signal in the range \(\delta\) 9.68–9.91 ppm (C\(_{0}\)–C\(_{1}\)) support the formation of compounds 5a–l and the singlet in the range of 8.50–9.2 ppm due to the methine proton. In \(^13\)C-NMR, signal at \(\delta\) 171.5–176.3 assigned to carbonyl carbon in indenone, signal at \(\delta\) 168.2–169.2 confirmed lactone carbonyl of \(5a–l\) and the signal between 43-49 ppm due to CH\(_{2}\) carbon in the indenone ring. Signal that appeared between 56.2–58.6 corresponds to the OCH\(_{3}\) carbon in indenone ring. Other signals are in good agreement with the target compounds.

3.2. Pharmacology

3.2.1. Free radical scavenging activity by DPPH method

The newly synthesized compounds (5a–l) were screened for their radical scavenging activity by the DPPH method. The activity results of the newly synthesized compounds are represented in Fig. 1.

We found that, most of the compounds showed considerable free radical scavenging activity. Compounds 5e–h have shown the strongest free radical scavenging activity among the tested compounds with IC\(_{50}\) ranging between 54.14 and

![Scheme 1](image-url)
59.76 µg/mL. The compound with no substitution on phenyl ring 5a with IC_{50} 64.75 µg/mL and the compounds 5c and 5k substituted with halogens showed good scavenging effect with IC_{50} 70.14 and 88.29 µg/mL. The other tested compounds displayed moderate to good scavenging activity. The IC_{50} value of the reference standard BHT was recorded as 46.95 µg/mL.

3.2.2. Chelating effects on ferrous ions

The iron chelating studies measure the ability of antioxidants to compete with ferrozine in chelating ferrous ion (Elmastaş et al., 2006). It was reported that the compounds containing two or more functional groups like OH, SH, COOH, C=O, NR_{2} and oxygen in a favorable structure–function configuration can show the activity of metal chelation (Yuan et al., 2005). The ferrous ion-chelating activity of the newly synthesized compounds is represented in Fig. 2. From the activity data it was observed that, the compounds substituted with electron donating groups on the indenone ring and phenyl ring 5e–5h exhibited excellent activity with IC_{50} value 53.75–62.30 µg/mL. Compounds 5a and 5k displayed satisfactory activity with IC_{50} 65.95 and 7.76 µg/mL. Compound 5c showed moderate activity with IC_{50} 93.94 µg/mL. The IC_{50} value of the reference standard EDTA was recorded as 44.11 µg/mL.

3.2.3. Total reductive capability

Fe(III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. In the reducing power assay, the presence of antioxidant in the synthesized compounds would result in the reduction of Fe^{3+} to Fe^{2+} complex by donating an electron. The amount of Fe^{2+} complexes was then monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reducing ability (Nabavi et al., 2008). The results are represented in Fig. 3. It was found that the reducing power of all the synthesized compounds increased with the increase in their concentrations. The best reducing power was presented by the compound 5g containing two methoxy groups on indenone and a methoxy group on phenyl ring with IC_{50} 58.01 µg/mL. Compounds substituted with electron donating groups 5e, 5f and 5h exhibited good activity with IC_{50} ranging between 50

![Figure 1](link)  
**Figure 1**  DPPH free radical scavenging assay (%) of test compounds (5a–l).

![Figure 2](link)  
**Figure 2**  Ferrous ion chelating assay (%) of test compounds (5a–l).
62.57 and 69.17 μg/mL. The other tested compounds showed less activity compared to standard. The IC50 value of the reference standard Butylated hydroxyl anisole BHA was recorded as 50.00 μg/mL.

IC50 values of DPPH radical scavenging ferrous ion chelating activity and total reductive capability of test compounds are given in Table 1.

3.2.4. Oral glucose tolerance test in normal rats

Oral glucose tolerance tests of synthesized compounds demonstrated the highest blood glucose level at 30 min following glucose administration, with decreasing blood glucose levels thereafter (Figs. 4–6). Compared with the groups, i.e. normal control, standard and with the tested compounds, compounds 5g and 5e showed a significant reduction in the blood glucose level. Additionally at 120 min following glucose administration, the 100 mg/kg 5g and 5e groups had significantly (P ≤ 0.005) a greater drop in blood glucose than the 10 and 50 mg/kg groups. The other tested groups exhibited moderate to good decrease in the blood glucose level.

3.2.5. In-vivo anti-hyperglycemic activity

The antidiabetic potential of the newly synthesized compounds was evaluated in STZ and nicotinamide induced T2DM in Wistar rats (Watcho et al. 2012). Blood glucose levels of compounds 5a, 5c and 5e–5h in diabetes in STZ/nicotinamide model were checked for 14 days.

Compounds 5g and 5e having an electron donating group on the phenyl ring showed prominent decrease in glucose concentration (115 and 138 mg/dL) with the dose of 100 mg/kg. It was interesting to note that the compound 5h substituted with electron withdrawing (Br) as well as electron donating (OCH3) groups showed moderate to good activity. No significant decrease in the plasma glucose level was observed in compound 5f. Compounds 5a and 5c showed less activity by increase in the plasma glucose level (278 mg/dL and 387 mg/dL).

Table 1  Half maximum inhibition concentration (IC50) values for DPPH free radical scavenging, ferrous ion chelating and total reductive capability activity of test compounds.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>DPPH (IC50 μg/mL)</th>
<th>Fe^{2+} ion Chelating activity (IC50 μg/mL)</th>
<th>Total reductive capability (IC50 μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>64.75 ± 0.11</td>
<td>65.95 ± 0.11</td>
<td>73.09 ± 0.11</td>
</tr>
<tr>
<td>5b</td>
<td>107.7 ± 0.32</td>
<td>105.01 ± 0.31</td>
<td>108.17 ± 0.31</td>
</tr>
<tr>
<td>5c</td>
<td>70.14 ± 0.10</td>
<td>93.94 ± 0.29</td>
<td>84.21 ± 0.29</td>
</tr>
<tr>
<td>5d</td>
<td>185.24 ± 0.15</td>
<td>189.29 ± 0.21</td>
<td>217.20 ± 0.21</td>
</tr>
<tr>
<td>5e</td>
<td>56.19 ± 0.18</td>
<td>53.75 ± 0.15</td>
<td>62.57 ± 0.15</td>
</tr>
<tr>
<td>5f</td>
<td>59.76 ± 0.21</td>
<td>59.45 ± 0.20</td>
<td>69.17 ± 0.20</td>
</tr>
<tr>
<td>5g</td>
<td>54.14 ± 0.13</td>
<td>56.89 ± 0.20</td>
<td>58.01 ± 0.20</td>
</tr>
<tr>
<td>5h</td>
<td>57.73 ± 0.13</td>
<td>62.30 ± 0.17</td>
<td>65.52 ± 0.17</td>
</tr>
<tr>
<td>5i</td>
<td>92.15 ± 0.17</td>
<td>117.36 ± 0.13</td>
<td>157.96 ± 0.13</td>
</tr>
<tr>
<td>5j</td>
<td>101.41 ± 0.13</td>
<td>153.87 ± 0.13</td>
<td>289.02 ± 0.13</td>
</tr>
<tr>
<td>5k</td>
<td>88.29 ± 0.21</td>
<td>70.76 ± 0.13</td>
<td>92.59 ± 0.13</td>
</tr>
<tr>
<td>5l</td>
<td>141.56 ± 0.13</td>
<td>131.02 ± 0.13</td>
<td>133.90 ± 0.13</td>
</tr>
<tr>
<td>Std a,b,c</td>
<td>46.95 ± 0.17</td>
<td>44.11 ± 0.15</td>
<td>50.00 ± 0.15</td>
</tr>
</tbody>
</table>

Each value is expressed as mean ± SD of three replicates.

a Std-BHT is used as standard for DPPH radical scavenging activity.
b Std-EDTA is used as a standard for Fe^{2+} ion chelating activity.
c Std-BHA is used as a standard for reductive capability.
with the dose of 100 mg/kg. The lowering of plasma glucose in diabetic rats after treatment with compounds 5a, 5c and 5e-h at concentrations 10, 50 and 100 mg/kg is represented in Fig. 7–9, respectively.

Treatment with the test substance for 14 days showed an increase in body weight (Table 2) compared to diabetic control; no significant increase in liver weight at 100 mg/kg; and no change in kidney weight were observed with treatment.

4. Conclusion

A new series of coumarin derivatives containing pyrazole and indenone moiety have been synthesized and the synthesized compounds were well characterized by IR, 1H NMR, 13C NMR and LC–MS spectroscopic methods. Our adopted method for the synthesis of target compounds was effective in terms of time and yield. The reaction conditions are simple, and they
Figure 7  Significant lowering of plasma glucose in diabetic rats treated with compounds at the concentration of 10 mg/kg of body weight.

Figure 8  Significant lowering of plasma glucose in diabetic rats treated with compounds at the concentration of 50 mg/kg of body weight.

Figure 9  Significant lowering of plasma glucose in diabetic rats treated with compounds at the concentration of 100 mg/kg of body weight.
are not sensitive to oxygen and water, which make it easy to operate at room temperature. The presence of pyrazole ring endows these species with important pharmacological and therapeutic interest. From the structure-activity relationship studies it revealed that, the presence of electron withdrawing group/halogen substituted compounds showed good antioxidant property. In case of antihiperglycemic activity, compounds 5g and 5e showed prominent antidiabetic activity. Further research needs to be done to fully understand how the synthesized compounds are working in the pancreas and by what mechanism it is able to lower glucose levels in Streptozotocin–nicotinamide induced diabetic mice.

Acknowledgements
The authors are thankful to the Chairman, Department of Industrial Chemistry, Kuvempu University, Shankaraghatta for providing the laboratory facilities. One of the authors (Kenchappa R.) is thankful to the UGC for the award of Rajiv Gandhi National Fellowship (RGNF).

References