Synthesis of Novel Fatty Substituted 4-methyl-2H-Chromen-2-one via Cross Metathesis: Potential Antioxidants and Chemotherapeutic Agents

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Abstract: A series of novel fatty substituted 4-methyl-2H-chromen-2-one (coumarins) were synthesized by employing cross metathesis, a key step in the synthesis. The antioxidant activities of the title compounds were compared with the commercial antioxidants, namely butylated hydroxy toluene (BHT) and α-tocopherol, glycosidic and other substituted 4-methyl-2H-chromen-2-ones. Among the different 4-methyl-2H-chromen-2-ones, the glycosidic substituted 4-methyl-2H-chromen-2-one was excellent, while those with aliphatic fatty acid chain and hydroxyl substituents were good. Among the substituted 4-methyl-2H-chromen-2-ones, glycosidic, hydroxyl and cyano containing 4-methyl-2H-chromen-2-ones exhibited good, while fatty substituted exhibited moderate anticancer activities against the four different cancer cell lines tested, namely DU145 (Prostate carcinoma cancer cell), HepG2 (Hepato cellular carcinoma cancer cell), SKOV3 (Ovarian cancer cell) and MDA-MB 231 (Human breast cancer cell). The study reveals that these substituted coumarins can be potential candidates in a number of food and pharmaceutical formulations.

Key words: 4-methyl-2H-chromen-2-one, cross metathesis, fatty acids, anticancer activity, antioxidant activity

1 Introduction

Coumarins (known as 1, 2-benzo pyrones or O-hydroxy-cinnamic acid 8-lactones) constitute a major class of phenolic derivatives found in plants comprising of benzene and α-pyrene rings¹⁻¹⁰. They are natural substances that are known to show anti-tumour activity in vivo, with the effect due to its metabolites (e.g., 7-hydroxy coumarin). Owing to their wide range of applications in many fields of everyday life such as pharmaceuticals, cosmetics, perfumery and nutrition¹¹⁻¹², their chemistry is being investigated and many natural and non-natural coumarins are being synthesized in recent years¹³⁻¹⁴.

The importance of free radicals, especially reactive oxygen species (ROS) in the pathogenicity of various diseases¹⁵,¹⁶ including hepatic and vascular diseases¹⁷ has attached greater attention of researchers to explore more and more anti-oxidants. In this context, it was observed that additives of 4-methyl-2H-chromen-2-one possessing dihydroxy, diacetoxy and hydroxy-amino groups in the benzene ring at ortho position exhibited very good antioxidant and radical scavenging properties comparably better than α-tocopherols¹⁸. Thio and oxo analogues of isopsoralen containing 4-methyl-2H-chromen-2-one with 7-oxo and 7-thio moieties derived from the 8-(allyloxy)-4-methyl-2H-chromen-2-one were found to show enhanced activity than those which have seen general clinical use¹⁹,²⁰. Matos and coworkers²¹ synthesized novel amino/nitro substituted 3-aryl-2H-chromen-2-one with nitro, methyl, methoxy, amino and bromo substituents at various positions on the 3-aryl-2H-chromen-2-one scaffold and some of the derivatives exhibited good antibacterial activity against Gram positive and Gram negative bacteria.

As various coumarin derivatives are known to exhibit photophysical and biological activities²², the interest in the synthesis²³,²⁴ of these important ring derivatives continues to increase. Also, the interesting biological activities of coumarins and their derivatives have made these coumarins interesting intermediates in organic synthesis. Several synthetic strategies have been employed for the synthesis of coumarins. They are synthesized via Pechman reaction²⁵, Pechman reaction²⁶ or by Knovengel condensation of salicylaldehyde with melonic acid²⁶,²⁷, melonicesters²⁸,²⁷.

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cyano acetic esters\(^{28}\) or meldrums acid\(^{32}\). Witting reaction in N, N-diethyl aniline was also employed for the synthesis of coumarins\(^{30,32}\).

A new strategic route for the synthesis of polycyclic coumarin derivatives by the combination of Claisen rearrangement, ring closing metathesis and Diels-Alder reaction was developed by Shiital and co-workers\(^{20}\). Metathesis reaction, across a double bond helps in the expansion of diversity and avoids complex reaction steps. Most of the coumarin derivatives prepared by cross metathesis involved allylic functionalities and employed Grubbs’ second generation catalyst\(^{25,33}\). Further, Voigtritter et al employed copper salts to enhance the rate of cross metathesis reactions. They observed an increase in reaction rates and yields when CuI was used along with Grubbs’ second generation catalyst. Use of Cu salts not only reduces the rate of reaction but also avoids the use of chlorinated solvents\(^{34-37}\). Further carbohydrate conjugated with proteins or lipids play a crucial role in various cellular processes. Oliver et al.\(^{38}\) employed highly selective cross metathesis reaction to develop a variety of glycolipid moieties which were expected to effect the immune system specially the CD-1 media and T-cell activation.

Fatty acids are also known to play a very important role in the synthesis of a number of oleochemicals with industrial and biological applications\(^{39}\). Also, there exists no reports on the synthesis of glycosidic and fatty acid derivatives of coumarins and hence with this background an attempt was made to synthesize hydroxyl, benzyl, glycosidic and fatty acid derivatives of 7-(allyloxy)-4-methyl-2H-chromen-2-one employing conventional method followed by cross metathesis in presence of CuI\(^{40}\).

2 Materials and Methods

2.1 Materials

Resorcinol, allyl bromide, potassium carbonate, ethyl acetoacetate, Cis-oleic acid methyl ester (99%), acrylonitrile, styrene, acrylic acid, allyl alcohol, erucic acid, sulfuric acid, tricyclohexylphosphine\(^{1,3}\) bis\(\{2,4,6\)-trimethylphenyl\} -4,5-dihydroimidazo[2,1-b]pyridine hexane, ethyl acetate, dry methanol, dry ethyl ether used in the synthesis were of analytical grade and were purchased from M/s Sigma Aldrich Chemical Pvt. Ltd., St. Louis, USA. Castor oil was purchased from M/s Ramcharan Industries Pvt. Ltd., Hyderabad.

2.2 General procedure for self metathesis of fatty acids

Fatty acid (0.83 mmol) and Grubbs’ second generation catalyst (0.03 mmol) were taken into a two neck round bottom flask (100 mL). One neck is fixed with septa and another neck with condenser. Two N\(_2\) balloons one with needle arranged to the septa and another to the condenser. 20 mL of dry toluene is added through the septa with the help of syringe followed by Grubbs’ second generation catalyst to the reaction mixture maintaining the temperature at 90°C and the reaction was carried out for 4 h. Completion of the reaction was monitored by TLC eluted with hexane: ethyl acetate (90:10 v/v). The reaction mixture was extracted with ethylacetate, washed with water. Solvent was evaporated using rotary evaporator and dried under vacuum to obtain the product in 92% yield.

(Z)-7,12-dihydroxystearic acid C\(_{18}\)H\(_{35}\)O\(_{3}\); FT-IR (neat, cm\(^{-1}\)) : 2989,1735, 1021,758; \(^{13}\)C NMR (δ/ppm, CDCl\(_3\)) : 5.50 – 5.40 (m, 1H), 3.73 – 3.65 (m, 1H), 2.63 – 2.50 (m, 1H), 2.43 – 2.30 (m, 2H), 2.33 – 2.21 (m, 1H), 2.11 – 1.92 (m, 2H), 1.80 – 1.69 (m, 1H), 1.73 – 1.56 (m, 3H), 1.45 (d, J = 4.9 Hz, 1H), 1.35 – 1.11 (m, 1H); \(^{13}\)C NMR (δ/ppm, CDCl\(_3\)) : 174.40, 128.23, 72.28, 37.36, 36.73, 33.80, 28.99, 25.64, 24.71; ESI-MS (m/z): 346.12 [M + H]\(^{+}\), HRMS: Calcd. for C\(_{18}\)H\(_{35}\)O\(_{3}\)[M + H]\(^{+}\): 346.09.

(Z)-octadec-9-ene C\(_{18}\)H\(_{32}\); FT-IR (neat, cm\(^{-1}\)) : 3151, 1731, 756; \(^{13}\)C NMR (δ/ppm, CDCl\(_3\)) : 5.36 (m, 1H), 2.32 (t, J = 5.5 Hz, 1H), 2.10 – 2.02 (m, 1H), 1.45 (m, 1H), 1.39 – 1.30 (m, 1H), 1.31 – 1.25 (m, 1H), 1.28 (s, 2H); \(^{13}\)C NMR (δ/ppm, CDCl\(_3\)) : 174.40, 130.83, 33.60, 29.47, 28.41, 27.30, 24.65; ESI-MS (m/z): 312.45[M\(^{+}\)]; HRMS: Calcd. for C\(_{18}\)H\(_{35}\)O\(_{3}\)[M\(^{+}\)]: 312.89.

(Z)-octadec-9-ene C\(_{18}\)H\(_{35}\)O\(_{3}\); FT-IR (neat, cm\(^{-1}\)) : 3241, 2284, 945; \(^{1}H\) NMR (δ/ppm, CDCl\(_3\)) : 5.39 (m, 1H), 2.12 – 2.00 (m, 2H), 1.40 – 1.30 (m, 2H), 1.34 – 1.21 (m, 10H), 0.94 – 0.85 (m, 3H); \(^{13}\)C NMR (δ/ppm, CDCl\(_3\)) : 130.83, 31.68, 29.51, 28.92, 28.41, 27.30, 22.48, 14.03; ESI-MS (m/z): 252.27[M\(^{+}\)]; HRMS: Calcd. for C\(_{18}\)H\(_{35}\)O\(_{3}\)[M\(^{+}\)]: 252.94.

(Z)-hexacos-13-ene C\(_{28}\)H\(_{53}\); FT-IR (neat, cm\(^{-1}\)) : 3451, 1733, 956; \(^{1}H\) NMR (δ/ppm, CDCl\(_3\)) : 5.39 (m, 1H), 2.32 (t, J = 8.1 Hz, 2H), 2.10 – 2.02 (m, 2H), 1.50 – 1.35 (m, 2H), 1.39 – 1.32 (m, 1H), 1.36 – 1.26 (m, 10H), 1.29 – 1.20 (m, 5H); \(^{13}\)C NMR (δ/ppm, CDCl\(_3\)) : 174.40, 130.83, 33.60, 29.62, 29.57, 28.41, 27.30, 24.65; ESI-MS (m/z): 424.07[M + H\(^{+}\)]; HRMS: Calcd. for C\(_{28}\)H\(_{53}\)O\(_{3}\)[M + H\(^{+}\)]: 424.09.

2.3 Synthesis of 7-Hydroxy-4-methyl-2H-chromen-2-one (5)

To an ice-cold solution of resorcinol (5.5 g, 0.05 mol) in dioxane, conc. H\(_{2}\)SO\(_{4}\) (2 mL) was added drop wise under 25°C. After the addition of concentrated sulphuric acid, ethyl acetoacetate (7 mL) was added and the mixture was heated to 60°C for 4 h. The mixture was then poured into cold water and the precipitate was filtered and dried under reduced pressure. The resulting mixture was recrystallized from methanol to give white needle like crystals, 7-hydroxy-4-methyl-coumarin with 91% yield. C\(_{10}\)H\(_{8}\)O\(_{3}\); FT-IR (neat, cm\(^{-1}\)) : 3021, 2931, 1731, 1438, 1215, 910, 759; \(^{1}H\)
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NMR (δ/ppm, CDCl₃): 7.52 (d, J = 7.4 Hz, 1H), 7.00 (s, 1H), 6.91 (m, J = 7.5, 2.0 Hz, 1H), 6.80 (d, J = 2.0 Hz, 1H), 6.24 (q, J = 1.0 Hz, 1H), 2.39 (d, J = 1.0 Hz, 3H); ¹³C NMR (δ/ppm, CDCl₃): 170.39, 157.92, 154.92, 153.87, 125.40, 114.79, 114.00, 112.03, 101.29, 20.03; ESI-MS (m/z): 177.04 [M + H]^+, HRMS: Calcd. for C₉H₇O₃[M + H]^+, 177.19.

2.4 Synthesis of 7-allyloxy-4-methyl-2H-chromen-2-one (6)

To a solution of 7-hydroxy-4-methyl-2H-chromen-2-one (656 mg, 3.73 mmol) in acetone (35 mL), allyl bromide (520 mg, 4.29 mmol) and solid K₂CO₃ (1290 mg, 9.33 mmol) were added and the mixture was stirred at 60°C for 6 h. The solvent was evaporated and the residue was extracted into ethyl acetate (35mL). The mixture was passed through anhydrous Na₂SO₄, concentrated and dried under reduced pressure which furnished compound 7-allyloxy-4-methyl-2H-chromen-2-one as off-white solid prismatic needles in 81% yield. C₁₅H₁₄O₃; FT-IR (neat, cm⁻¹): 3415, 1736, 1598, 1390, 956; ¹³C NMR (δ/ppm, CDCl₃): 7.60 (s, 1H), 6.97 (s, 1H), 6.24 (d, J = 0.9 Hz, 1H), 6.05 (t, J = 16.5 Hz, 1H), 5.63-5.54 (m, 1H), 5.39-5.29 (m, 1H), 4.69 (s, 2H), 2.39 (s, 3H); ¹³C NMR (δ/ppm, CDCl₃): 170.39, 159.94, 155.06, 153.87, 134.52, 125.35, 117.35, 115.16, 114.20, 112.04, 101.22, 70.02, 20.03; ESI-MS (m/z): 217.07 [M + H]^+, HRMS: Calcd. for C₁₀H₈O₃[M + H]^+, 217.06.

2.5 General Procedure for the Synthesis of compound 9a-9f

A mixture of 7-allyloxy-4-methyl-2H-chromen-2-one (0.50 mmol), compounds 7a-7f (1.50 mmol), Grubbs' second generation catalyst (8.5 mg, 10.0 μmol), and Cu (2.9 mg, 15.0 μmol) under N₂ atmosphere distilled ethyl ether (5.0 mL) were added, mixture was stirred at 40°C for 5 h in a reflux condenser. After cooling to room temperature, the reaction mixture was concentrated. The solution was heated at 40°C for 5 h followed by cooling to room temperature, then the reaction mixture was passed through anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by column chromatography and eluted with hexane: ethyl acetate (60:40 v/v) to obtain the pure product with 60-78% isolated yields.

(E)-7-(4-hydroxybuto-2-enyloxy)-4-methyl-2H-chromen-2-one (9a)

C₁₀H₈O₄Na·Na[+M + Na]^+, 269.32.

C₂₉H₃₄O₁₂; Yield 75.2%; FT-IR (neat, cm⁻¹): 3301, 2928, 2857, 1738, 1488, 1241, 753; ¹³C NMR (δ/ppm, CDCl₃): 7.59 (d, J = 7.5 Hz, 1H), 7.30 (m, J = 5.7, 4.7, 1.7 Hz, 2H), 7.25 – 7.16 (3H), 7.01 (m, J = 7.5, 2.0 Hz, 1H), 6.92 (d, J = 2.0 Hz, 1H), 6.62 (m, J = 15.2, 0.9 Hz, 1H), 6.36 (m, J = 15.0, 6.2 Hz, 2H), 6.24 (q, J = 1.0 Hz, 1H), 4.71 (m, J = 6.1, 1.0 Hz, 2H), 2.39 (d, J = 1.1 Hz, 2H); ¹³C NMR (δ/ppm, CDCl₃): 170.39, 159.94, 155.06, 153.87, 147.78, 125.35, 118.31, 115.16, 114.20, 112.04, 101.22, 68.72, 20.03; ESI-MS (m/z): 264.24 [M + Na]^+, HRMS: Calcd. for C₁₉H₁₆O₃Na[M + Na]^+, 264.37.

(E)-7-(cinnamoyloxy)-4-methyl-2H-chromen-2-one (9c)

C₁₀H₈O₄Na·Na[+M + Na]^+, 269.32.

C₂₉H₃₄O₁₂; Yield 76.7%; FT-IR (neat, cm⁻¹): 3051, 2968, 2852, 1738, 1488, 1241, 753; ¹³C NMR (δ/ppm, CDCl₃): 7.59 (d, J = 7.5 Hz, 1H), 7.30 (m, J = 5.7, 4.7, 1.7 Hz, 2H), 7.25 – 7.16 (3H), 7.01 (m, J = 7.5, 2.0 Hz, 1H), 6.92 (d, J = 2.0 Hz, 1H), 6.62 (m, J = 15.2, 0.9 Hz, 1H), 6.36 (m, J = 15.0, 6.2 Hz, 2H), 6.24 (q, J = 1.0 Hz, 1H), 4.71 (m, J = 6.1, 1.0 Hz, 2H), 2.39 (d, J = 1.1 Hz, 2H); ¹³C NMR (δ/ppm, CDCl₃): 170.39, 159.94, 155.06, 153.87, 147.78, 125.35, 118.31, 115.16, 114.20, 112.04, 101.22, 68.72, 20.03; ESI-MS (m/z): 315.32 [M + Na]^+, 315.47.

(E)-7-(acetoxyethylmethyl)-6-(5-(4-methyl-2-oxo-2H-chromen-7-yl)oxy)pent-3-enyltetrahydro-2H-pyran-3,4,5-triylic triacetate (9f)

C₂₉H₃₄O₁₂; Yield 67.3%; FT-IR (neat, cm⁻¹): 3121, 2738, 2867, 1736, 1508, 1236, 723; ¹³C NMR (δ/ppm, CDCl₃): 7.59
A mixture of 7-allyloxy-4-methyl-2H-chromen-2-one (0.50 mmol), compounds 8a-8d (1.0 mmol), Grubbs’ second generation catalyst (8.5 mg, 10.0 μmol), and Cul (2.9 mg, 15.0 μmol) under an N₂ atmosphere distilled ethyl ether (5.0 mL) were added, mixture was stirred at 40°C for 5 h in a reflux condenser. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The crude product was purified by column chromatography and eluted with hexane: ethyl acetate (90:10 v/v) to obtain the pure product with 60-78% isolated yields.

(E)-4-methyl-7-(undec-2-enyloxy)-2H-chromen-2-one (10a)

C₂₂H₂₈O₅; Yield 75.1%; FT-IR (neat, cm⁻¹): 3155, 2857, 1725, 1237, 726; ¹H NMR (δ/ppm, CDCl₃): 6.98 – 6.89 (m, 1H), 6.24 (q, J = 1.0 Hz, 1H), 5.77 – 5.63 (m, 1H), 4.71 (m, J = 5.1, 1.0 Hz, 1H), 2.39 (d, J = 1.1 Hz, 1H), 2.11 – 2.03 (m, 1H), 1.40 – 1.28 (m, 1H), 1.31 – 1.21 (m, 4H), 0.92 – 0.86 (m, 1H); ¹³C NMR (δ/ppm, CDCl₃): 170.38, 159.94, 155.06, 153.88, 132.17, 129.84, 127.15, 115.16, 114.19, 112.03, 101.22, 69.37, 28.92, 28.41, 22.48, 20.03, 14.03; ESI-MS (m/z): 597.30[M + Na]⁺, HRMS: Calcd. for C₂₁H₂₈O₅Na[M + Na]⁺, 597.47.

(E)-7-(5-hydroxyundec-2-enyloxy)-4-methyl-2H-chromen-2-one (10d)

C₂₅H₃₉O₅Na; Yield 75.7%; FT-IR (neat, cm⁻¹): 3251, 2928, 2857, 1725, 1508, 1217, 715; ¹H NMR (δ/ppm, CDCl₃): 7.59 (m, J = 7.7, 1.1 Hz, 1H), 6.94 (s, 1H), 6.96 – 6.90 (m, 1H), 6.24 (q, J = 1.0 Hz, 1H), 5.79 – 5.62 (m, 2H), 4.74 – 4.68 (m, 2H), 2.39 (d, J = 1.0 Hz, 2H), 2.32 (t, J = 8.1 Hz, 2H), 2.07 (m, 2H), 1.50 – 1.23 (m, 5H), 1.28 (s, 3H); ¹³C NMR (δ/ppm, CDCl₃): 174.40, 170.38, 159.94, 155.06, 153.87, 132.17, 129.84, 125.35, 115.15, 114.20, 112.04, 101.22, 69.37, 33.60, 31.60, 29.49, 28.41, 24.65, 20.03; ESI-MS (m/z): 395.49[M + Na]⁺, 395.67.

2.7 Biological activity

2.7.1 Antioxidant activity

The antioxidant activity of the synthesized compounds were measured by three in vitro methods such as 2, 2-diphenyl-1-picylhydrazyl (DPPH) radical activity, superoxide (SO) free radical scavenging activity, and inhibition of lipid peroxidation. The EC₅₀ values represent the concentration of the drug at which 50% of the radicals were scavenged which means the lower EC₅₀ value indicates highest antioxidant activity. For comparison purpose, the EC₅₀ values of commercial antioxidants, namely BHT and α-tocopherol were also determined. The tested results are shown in Tables 1 and 2.

2.7.2 Anticancer activity

The synthesized coumarin derivatives 9a-9f, 10a-10d were further evaluated for in vitro anticancer activity against different cell lines using MTT assay against a panel of tumour cell lines namely DU145, HepG2, SKOV3 and MDA-MB 231.
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ing 1,3-dihydroxy benzene with ethyl acetoacetate with 95% yield, in step 3 7-hydroxy-4-methyl-2H-chromen-2-one with allyl bromide to yield 7-(allyloxy)-4-methyl-2H-chromen-2-one in 91% yield. While, step 4, a key step in the synthesis is the cross metathesis of 7-(allyloxy)-4-methyl-2H-chromen-2-one with different allylic substitutions such as cyano, hydroxyl, glycosidic and self metathesized fatty acid molecules to yield different coumarin derivatives 9a-9f and 10a-10d in 60-78% yields (Scheme 2).

With an intention to study the effect of aliphatic fatty acids and aliphatic hydroxyl fatty acids, the double bond in the molecule was coupled to 7-(allyloxy)-4-methyl-2H-chromen-2-one via cross-metathesis. All the molecules were synthesized employing a simple procedure avoiding multi-step reactions in good yields by selecting cross metathesis (Step 4). All the synthesized coumarins were thoroughly characterized using FT-IR, $^1$H-NMR, $^{13}$C-NMR and mass spectral studies. Coumarin derivatives are known for their good anti-oxidant, anti-cancer and anti-inflammatory behavior. Hence the coumarins synthesized in the present study were evaluated for antioxidant and anticancer properties.

### Table 1 Antioxidant activity of coumarin derivatives 9a-9f.

| Compounds | EC$_{50}$ (µg/mL) | DPPH FRSA | Superoxide FRSA | Inhibition of lipid peroxidation |
|-----------|------------------|-----------|-----------------|-------------------------------|
| 9a        | 237.1 ± 0.32     | 120.0 ± 0.22 | 255.3 ± 0.34   |                               |
| 9b        | 17.2 ± 0.41      | 17.9 ± 0.18  | 18.2 ± 0.18     |                               |
| 9c        | 107.8 ± 0.29     | 100.1 ± 0.36 | 112.2 ± 0.26    |                               |
| 9d        | 359.5 ± 0.54     | 348.3 ± 0.52 | 325.3 ± 0.35    |                               |
| 9e        | 48.7 ± 0.36      | 32.6 ± 0.36  | 43.1 ± 0.26     |                               |
| 9f        | 8.7 ± 0.26       | 6.6 ± 0.16   | 9.2 ± 0.21      |                               |
| BHT       | 28.1 ± 0.21      | 14.1 ± 0.22  | 40.5 ± 0.22     |                               |
| $\alpha$-Tocopherol | 10.5 ± 0.32 | 7.1 ± 0.24 | 20.2 ± 0.12 |                               |

### Table 2 Antioxidant activity of coumarin derivatives 10a-10d.

| Compounds | EC$_{50}$ (µg/mL) | DPPH FRSA | Superoxide FRSA | Inhibition of lipid peroxidation |
|-----------|------------------|-----------|-----------------|-------------------------------|
| 10a       | 139.2 ± 0.44     | 104.2 ± 0.44 | 98.8 ± 0.38   |                               |
| 10b       | 15.0 ± 0.42      | 15.2 ± 0.44  | 15.9 ± 0.52     |                               |
| 10c       | 15.8 ± 0.18      | 15.1 ± 0.12  | 14.9 ± 0.26     |                               |
| 10d       | 12.9 ± 0.62      | 12.2 ± 0.32  | 12.5 ± 0.48     |                               |
| BHT       | 28.1 ± 0.21      | 14.1 ± 0.22  | 25.2 ± 0.27     |                               |
| $\alpha$-Tocopherol | 11.5 ± 0.12 | 7.1 ± 0.24 | 20.1 ± 0.22 |                               |

Scheme 1 Self-metathesis of fatty acids.
3.1 Antioxidant activity

DPPH radical scavenging method is a simple and widely employed method because of the commercial availability of DPPH radical. The antioxidant behavior of the coumarin derivatives were compared with the commercial antioxidants, BHT and α-tocopherol, as positive controls. It was found that all the compounds exhibited radical scavenging activity in all the three assays performed. Among the different coumarins studied, 9a-9f and 10a-10d, 9f, glycosidic derivative of 7-(allyloxy)-4-methyl-2H-chromen-2-one showed the higher antioxidant activity and was better than BHT. The higher antioxidant activity of 9f can be attributed to the fact that the glycosidic molecule coupled to the 4-methyl-2H-chromen-2-one scaffold active than the native phenolic hydroxyl moiety which could be explained similar to the activities shown by novel coumarin-benzimidazoline derivatives reported by Arora et al.51.

3.2 Anti cancer activity

Among the various substituents coupled to 7-(allyloxy)-4-methyl-2H-chromen-2-one scaffold, the benzyl, 9e; glycosidyl 9f and hydroxyl aliphatic chain containing coumarin, 10d exhibited very good anticancer activities against all tested cell lines. The glycosidic derivatives of 7-(allyloxy)-4-methyl-2H-chromen-2-one exhibited good anticancer activity since the glycolipid is previously known for a number of biological activities52–54. Coumarin derivatives with alkyl and benzyl substituents also known to exhibited good anticancer activity as observed in case of compounds 9e and 10d of the present study.55. The fatty substituted coumarin derivatives exhibited moderate activity against the tested cell lines (Table 3).
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4 Conclusion

A simple step three procedure was employed for the synthesis of a panel of 10 novel coumarin derivatives. Different molecules such as allyloxy, benzyl, acrylonitrile, acrylic acid and fatty acid analogues were coupled to 4-methyl-2H-chromen-2-one using cross metathesis in step 3 employing Grubb’s second generation catalyst in the presence of CuI salt. This is a key step in the synthesis which avoids multi step reactions to obtain the title compounds.

The title compounds exhibited extraordinary to good antioxidant activity against BHT and α-tocopherols which are commercially used antioxidants in a number of pharmaceutical formulations. Coumarin derivatives with glycosidic moiety exhibited extraordinary antioxidant activity, while aliphatic fatty acid and alcohol containing derivatives exhibited good antioxidant activities as compared to BHT and α-tocopherol.

The title compounds when evaluated for anticancer activities against four cancer cell lines, namely DU145 (Prostate carcinoma cancer cell), HepG2 (Hepato cellular carcinoma cancer cell), SKOV3 (Ovarian cancer cell) and MDA-MB 231 (Human breast cancer cell) indicated that the glycosidic, hydroxyl and benzyl substituted 4-methyl-2H-chromen-2-ones exhibited good anticancer activities, while other substituted coumarins showed moderate activities, making these candidates potential for use in a number of food and pharmaceutical formulations.

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Supporting Information

This material is available free of charge via the Internet at http://dx.doi.org/jos.65.10.5650/jos.ess.15221.

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Table 3  Anticancer activity of coumarin derivatives 9a-9f, 10a-10d.

| Compounds | IC_{50} values in (μM) |
|-----------|------------------------|
|           | DU145^{a} | HepG2^{b} | SKOV3^{c} | MDA-MB 231^{d} |
| 9a        | 25.3 ± 0.32 | 13.6 ± 0.26 | 18.2 ± 0.22 | 17.7 ± 0.18 |
| 9b        | 46.5 ± 0.55 | 44.4 ± 0.38 | 39.5 ± 0.25 | 31.66 ± 0.26 |
| 9c        | 56.5 ± 0.55 | 54.5 ± 0.38 | 59.5 ± 0.25 | 51.26 ± 0.25 |
| 9d        | 32.8 ± 0.22 | 44.5 ± 0.55 | 39.5 ± 0.25 | 36.6 ± 0.26 |
| 9e        | 12.9 ± 0.24 | 13.2 ± 0.18 | 12.5 ± 0.16 | 10.6 ± 0.15 |
| 9f        | 14.9 ± 0.24 | 13.8 ± 0.18 | 12.8 ± 0.16 | 12.6 ± 0.25 |
| 10a       | 82.9 ± 0.21 | 89.2 ± 0.38 | 85.1 ± 0.22 | 80.5 ± 0.25 |
| 10b       | 78.2 ± 0.22 | 85.2 ± 0.22 | 77.7 ± 0.22 | 79.8 ± 0.25 |
| 10c       | 75.8 ± 0.22 | 82.1 ± 0.22 | 77.1 ± 0.22 | 80.1 ± 0.25 |
| 10d       | 19.0 ± 0.32 | 17.3 ± 0.26 | 22.6 ± 0.22 | 18.3 ± 0.24 |
| Doxorubicin (control) | 0.6 ± 0.11 | 0.8 ± 0.09 | 0.8 ± 0.12 | 0.7 ± 0.08 |

DU145^{a} (Prostate carcinoma cancer cell), HepG2^{b} (Hepato cellular carcinoma cancer cell), SKOV3^{c} (Ovarian cancer cell) and MDA-MB 231^{d} (Human breast cancer cell)
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