Lipase catalysed substituted coumarins with antioxidant activity

Mazaahir Kidwai*, Vaishali & Roona Poddar
Green Chemistry Research Laboratory, Department of Chemistry, University of Delhi, Delhi 110 007, India
E-mail: kidwai.chemistry@gmail.com

Received 19 November 2009; accepted (revised) 11 March 2011

Lipase has been employed as a green catalyst to esterify 7- and 6-hydroxy-4-methyl-coumarin with different fatty and acetic acid. The esterified coumarins have been evaluated for antioxidant activities using DPPH and ABTS assays, among them 3a and 5a are known as active antioxidants.

Keywords: ABTS, acyl coumarins, antioxidant, DPPH, esterfication, lipase

Oxidation reaction is an important process for sustaining life. However, some oxidation reaction forms free radicals or singlet oxygen which could act as precursor of chain reactions causing damage to the cells. The unbalance between formation and detoxification of free radicals and singlet oxygen result in progression to oxidative stress and wide spectrum of pathogenicity of various diseases such as cancer, cardiovascular disorders, aging, immunosuppression and diabetes that are considered to be consequences of oxidative stress. In relation to this, minor dietary constituent especially plant based foods, vitamin C, vitamin E, β-carotene, lycopene are of active consideration.

Polyphenolic compounds have drawn considerable interest to counter ill effects of oxygen radicals acting as antioxidant. Coumarin moiety is found as an important structural component in bioactive natural compounds and in synthetic approaches for promising compound in the field of medicinal chemistry. Now a days, flavones and functionalized coumarins derivatives are highly cited for their radical scavenging effects.

Among different type of coumarins 7-acetoxo-4-methyl coumarin and 6-acetoxo-4-methyl coumarin are useful as they are found to possess radical scavenging property. To synthesize esterified derivatives of coumarins, a green approach has been employed by using lipase as catalyst which has been recently found to have extensive potential application in industrial progress as well as in synthetic organic chemistry.

Herein, we explore more of chemo-enzymatic esterification reactions of bioactive coumarin moieties using lipase and evaluate their antioxidant properties. Acyl derivatives of 7-hydroxy-4-methyl-chromen-2-one 3a as well as of 6-hydroxy-4-methyl-chromen-2-one 5a are found to possess the antioxidant activity.

Result and Discussion

7-Hydroxy-4-methyl-chromen-2-one 1 is synthesized as per well known Pechmann reaction, using resorcinol and ethyl acetoacetate in the presence of conc. H₂SO₄ with greater yield that constitutes foremost part of our plan.

In the realm of green chemistry, esterified coumarins were synthesized using 1 and different acids 2a-g via green catalyst lipase which is known to play a pivotal role in esterification as well as in reverse reaction. The amount of catalyst was standardized and it was found that 10 mol % of lipase was most favourable for catalytic activity, as no enhancement in rate of reaction was observed with increase in amount of lipase. Further, to optimize reaction conditions, a number of solvents were investigated and alcoholic solvents were found to restrict the progress of reaction being acylated. Compound 1 was found to be less soluble in for diisopropyl ether, benzene and toluene, whereas 1,4-dioxane provided the required optimum conditions and also found suitable for enzymes as they were easily recovered.

For esterification, a number of acids mainly fatty acids ranging carbon chain length from C-2 to C-20 were investigated and more preferably for acetic acid which is well known for its antioxidant properties and the results are summarized in Table I.
Encouraged from these positive results, we further, synthesized 6-hydroxy-4-methyl-chromene-2-one 4 using hydroquinone and ethyl acetoacetate. However, yield was found to be near about 40% and from its recrystallized product, we proceeded to synthesize its ester derivative using different fatty acids and acetic acid and employing lipase under same reaction condition as done earlier (Schemes I and II). The results are summarized in Table II.

### Table I — Synthesis of acyl derivative of 7-hydroxy-4-methyl-chromen-2-one 1 using lipase

<table>
<thead>
<tr>
<th>Compd</th>
<th>Acid</th>
<th>Time (hr)</th>
<th>Yield(b) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>Acetic acid</td>
<td>4.5</td>
<td>86</td>
</tr>
<tr>
<td>3b</td>
<td>Caprylic acid</td>
<td>4</td>
<td>84</td>
</tr>
<tr>
<td>3c</td>
<td>Capric acid</td>
<td>4</td>
<td>88</td>
</tr>
<tr>
<td>3d</td>
<td>Lauric acid</td>
<td>3.5</td>
<td>94</td>
</tr>
<tr>
<td>3e</td>
<td>Myristic acid</td>
<td>3.5</td>
<td>95</td>
</tr>
<tr>
<td>3f</td>
<td>Palmitic acid</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>3g</td>
<td>Oleic acid</td>
<td>2</td>
<td>96</td>
</tr>
</tbody>
</table>

\(a\)Reaction condition: Equimolar amount of 7-hydroxy-4-methyl-chromen-2-one 1 and acids 2a-g were dissolved in 10 mL 1,4-dioxane and stirred with lipase (10 mol %), at RT. 
\(b\)Isolated and unoptimised yields

### Scheme I

R: a = CH\(_3\), b = CH\(_3\)(CH\(_2\))\(_6\), c = CH\(_3\)(CH\(_2\))\(_8\), d = CH\(_3\)(CH\(_2\))\(_10\), e = CH\(_3\)(CH\(_2\))\(_{12}\), f = CH\(_3\)(CH\(_2\))\(_{14}\), g = CH\(_3\)CH\(_2\)CH=CH(CH\(_2\))\(_7\)

### Table II — Synthesis of acyl derivative of 6-hydroxy-4-methyl-chromen-2-one 4 using lipase

<table>
<thead>
<tr>
<th>Compd</th>
<th>Acid</th>
<th>Time (hr)</th>
<th>Yield(b) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>Acetic acid</td>
<td>4</td>
<td>88</td>
</tr>
<tr>
<td>5b</td>
<td>Caprylic acid</td>
<td>4.5</td>
<td>89</td>
</tr>
<tr>
<td>5c</td>
<td>Capric acid</td>
<td>4</td>
<td>82</td>
</tr>
<tr>
<td>5d</td>
<td>Lauric acid</td>
<td>3</td>
<td>93</td>
</tr>
<tr>
<td>5e</td>
<td>Myristic acid</td>
<td>3.5</td>
<td>97</td>
</tr>
<tr>
<td>5f</td>
<td>Palmitic acid</td>
<td>2.5</td>
<td>98</td>
</tr>
<tr>
<td>5g</td>
<td>Oleic acid</td>
<td>2</td>
<td>94</td>
</tr>
</tbody>
</table>

\(a\)Reaction condition: Equimolar amount of 6-hydroxy-4-methyl-chromen-2-one 4 and acids 2a-g were dissolved in 10 mL 1,4-dioxane and stirred with lipase (10 mol %), at RT. 
\(b\)Isolated and unoptimised yields

### Antioxidant activity

Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods. The main characteristic of an antioxidant is its ability to trap free radicals which is generally measured by using free radicals such as DPPH, ABTS, TBARS, MDA, etc or abstraction of proton of these free radicals from antioxidants. Herein, we report two methods for evaluating antioxidant activity DPPH and ABTS both of which are free radicals. The general mechanism which can be outlined for the antioxidant activity is thought be as\(^{13}\):

\[
\text{ Radical + RXH} \rightarrow \text{ RadicalH} + \text{ RX}^+ \\
\text{ Radical + RXH} \rightarrow \text{ Radical}^+ + \text{ RXH}^+ \\
\text{ Radical + RXH} \rightarrow \text{ RadicalH} + \text{ RX}^+ 
\]

Ascorbic acid and gallic acid were used as reference standard. The scavenging activity of the samples corresponded to the intensity of quenching of radicals DPPH and ABTS. The percent inhibition was calculated from the following\(^{14}\) in both the cases.

\[
\% \text{ Inhibition} = \left( \frac{\text{Absorbance of control} - \text{Absorbance sample}}{\text{Absorbance control}} \right) \times 100
\]

### DPPH radical scavenging activity

The ability of antioxidant to scavenge free radicals is measured by the procedure described by Yen and Heish\(^{15}\) where free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) is supposed to abstract the proton from the sample and convert it into 1,1-diphenyl-2-picrylhydrazine\(^{14}\). This reaction could be visualized by change in colour from deep violet to light yellow and is monitored spectrophotometrically at characteristic wavelength of 517 nm. The
absorbance was found to be decreased upon reduction by antioxidant (Table III).

### ABTS radical cation activity

The 2,2′-azinobis(-ethyl-benzthioazoline-6-sulphonate) (ABTS) involves the reaction between ABTS and potassium persulphate giving blue/green long lived ABTS radical (ABTS\(^{+}\)), stable over wide range of pH. Decolourisation of solution is attained on addition of antioxidant and measured spectrophotometrically at 734 nm\(^{13}\) (Table III).

### Experimental Section

\(^1\)H and \(^13\)C NMR spectra were recorded on Bruker Top Spin 300 MHz and 75.6 MHz spectrometer with chemical shifts values (\(\delta\)) in ppm downfield from TMS using CDCl\(_3\) and DMSO-\(d_6\) as solvent. IR spectra of sample recorded on a model Perkin-Elmer FTIR-1710 spectrometer using KBr. Elemental analysis was carried out using Hereous CHN Rapid Analyzer. EI mass spectra were recorded on TOF MS mass spectrometer. Melting points were taken on Thomas-Hoover melting point apparatus and are uncorrected. The homogeneity of compounds was checked on silica gel coated aluminium plates (Merck TLC: mass particle size 10-12 µm; particle distribution 5-20 µm; layer thickness 250 µM plate height 30 µm). 2,2′-azinobis(3-ethylbenzthiazoline sulfonic acid) (98% pure) and 1,1-diphenyl-2-picryl hydrazine (90% pure) were purchased from Sigma Aldrich. Acetic acid (98.5% pure), lauric acid (98.5% pure), capric acid (95.5% pure), caprylic acid (95.5% pure), myristic acid (>98.5% pure), palmitic acid (>95.5% pure), oleic acid (>98.5% pure) were purchased from Loba Chemie and solvent was HPLC quality and was supplied by Spectrochem.

### Lipase

A lipase from *Candida antarctica* (Lipase B, a non specific lipase) immobilised on macroporous acrylic resin designated “Novozyme 435”, was gifted from Novozymes, Denmark.

### General synthesis for 7-hydroxy-4-methyl-chromen-2-one and 6-hyroxy-4-methyl-chromen-2-one

In a 100 mL round bottom flask, 5.5 g (50 mmol) of resorcinol/hydroquinone and equimolar amount of ethyl acetoacetate (6.5 g) were taken and added to 10-12 mL of conc. H\(_2\)SO\(_4\) acid placed on crushed ice. The reaction was set up on stirrer at 10°C using ice bath for 5-6 hr and left overnight. Workup was done using ice cold water by pouring the solution into it with constant stirring. The product obtained was filtered, dried in vacuo and recrystallized from ethanol.

### Esterification of coumarins

The precursor 7/6-hydroxy-4-methyl-chromen-2-one (5 mmol) and equimolar amount of acids 2a-g were taken in small conical flask to which 10 mL of conc. H\(_2\)SO\(_4\) acid placed on crushed ice. The reaction was set up on stirrer at 10°C using ice bath for 3-4 hr. The product formation was checked by TLC from time to time. Work up was done using ice cold water by pouring the solution into it with constant stirring. The product obtained was filtered, washed with saturated NaHCO\(_3\) solution dried in vacuo and recrystallized from ethanol.

### DPPH antioxidant activity

DPPH scavenging activity was studied using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH). Various concentration of test solution in 0.1 mL was added to 0.9 mL of 0.15 mM solution of DPPH in methanol. Methanol only (0.1 mL) was used as experimental control. After 30 min of incubation at RT, scavenge in the number of free radical was measured, reading the absorbance at 517 nm\(^{16}\).

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**Table III — DPPH and ABTS radical scavenging activity (RSA%) of the coumarins 3a-g and 5a-g**

<table>
<thead>
<tr>
<th>Compd</th>
<th>DPPH (1 mg/mL)</th>
<th>(0.5 mg/mL)</th>
<th>ABTS (1 mg/mL)</th>
<th>(0.5 mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>11.7</td>
<td>6.83</td>
<td>82.4</td>
<td>72.3</td>
</tr>
<tr>
<td>3b</td>
<td>13.7</td>
<td>6.02</td>
<td>51.3</td>
<td>49.2</td>
</tr>
<tr>
<td>3c</td>
<td>19.0</td>
<td>8.3</td>
<td>57.2</td>
<td>55.3</td>
</tr>
<tr>
<td>3d</td>
<td>13.3</td>
<td>6.31</td>
<td>55.3</td>
<td>47.2</td>
</tr>
<tr>
<td>3e</td>
<td>16.8</td>
<td>11.2</td>
<td>54.5</td>
<td>51.1</td>
</tr>
<tr>
<td>3f</td>
<td>22.5</td>
<td>16.0</td>
<td>50.1</td>
<td>49.3</td>
</tr>
<tr>
<td>3g</td>
<td>9.6</td>
<td>7.8</td>
<td>58.7</td>
<td>56.2</td>
</tr>
<tr>
<td>5a</td>
<td>10.3</td>
<td>9.1</td>
<td>80.2</td>
<td>77.7</td>
</tr>
<tr>
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<td>9.0</td>
<td>82.3</td>
<td>70.4</td>
</tr>
<tr>
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<td>21.2</td>
<td>7.2</td>
<td>82.3</td>
<td>80.2</td>
</tr>
<tr>
<td>5d</td>
<td>7.8</td>
<td>5.7</td>
<td>79.3</td>
<td>73.6</td>
</tr>
<tr>
<td>5e</td>
<td>7.9</td>
<td>6.0</td>
<td>83.8</td>
<td>64.8</td>
</tr>
<tr>
<td>5f</td>
<td>23.6</td>
<td>10.1</td>
<td>67.8</td>
<td>66.6</td>
</tr>
<tr>
<td>5g</td>
<td>10.2</td>
<td>7.2</td>
<td>66.3</td>
<td>54.6</td>
</tr>
</tbody>
</table>

* Standard used for ABTS is Gallic acid and for DPPH is Ascorbic acid
ABTS antioxidant activity

ABTS$^+$ was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at RT during 12-16 hr before use. The ABTS$^{+*}$ solution was diluted with 5 mM phosphate buffered saline (pH 7.4) to an absorbance of \(0.70 \pm 0.02\) at 734 nm and equilibrated at 30°C. For the photometric assay, 3 mL of the ABTS$^+$ solution and 0.03 mL of samples were mixed for 45 s and measured immediately after 5 min at 734 nm.

7-Hydroxy-4-methyl-chromen-2-one, 1: White solid: m.p. 179-81°C (uncorrected), TLC (hexane/ethyl acetate 8:2 v/v), IR (KBr pellet): 3115.53 (OH), 1671.42 (C=O), 1349.07 (C-O), 1259.94 cm\(^{-1}\) (C-O); \(^1\)H NMR (DMSO-d\(_6\), 300 MHz): \(\delta 2.58\) (d, 1H, Ar), 7.03 (d, 1H, Ar). Found: C, 74.44; O, 17.38%. Anal. Calcd for C\(_{10}\)H\(_8\)O\(_2\): C, 74.58; O, 17.38%.

Acetic acid-4-methyl-2-oxo-2H-chromen-7-yl-ester, 3a: White solid: m.p. 48-50°C (uncorrected), TLC (hexane/ethyl acetate 8:2 v/v), IR (KBr pellet): 3501.44 (CH\(_3\)), 1671.91 (CO), 1607.41 (C=O), 1488.06 (C=C), 1349.07 (C-O), 1259.94 cm\(^{-1}\) (C-O); \(^1\)H NMR (DMSO-d\(_6\), 300 MHz): \(\delta 1.95\) (s, 3H, CH\(_3\)), 2.52 (s, 3H, CH\(_3\)), 6.23 (s, 1H, C=H), 6.67 (s, 1H, Ar), 7.30 (dd, 1H, Ar), 7.62 (d, 1H, Ar); MS: \(m/z\) 176.0179 (M\(^+\)). Anal. Calcd for C\(_{12}\)H\(_{10}\)O: C, 68.18; H, 4.58; O, 27.16%. Found: C, 68.28; H, 4.53; O, 27.16%.

Tetradecanoic acid-4-methyl-2-oxo-2H-chromen-7-yl-ester, 3e: White solid: m.p. 50°C (uncorrected), TLC (hexane/ethyl acetate 8:2 v/v); IR (KBr pellet): 3125.77 (CH\(_3\)), 1701.62 (COOR), 1609.32 (C=O), 1508.06 (C=C), 1410.45 (CH\(_3\)), 1135.22 cm\(^{-1}\) (C-O); \(^1\)H NMR (DMSO-d\(_6\), 300 MHz): \(\delta 0.94\) (t, 3H, CH\(_3\)), 1.26 (m, 12H, CH\(_2\)) chain), 2.35 (s, 3H, CH\(_3\)), 2.51 (t, 2H, CH\(_2\)CO), 6.16 (s, 1H, C=H), 6.68 (s, 1H, Ar), 6.87 (d, 1H, Ar), 7.49 (dd, 1H, Ar); MS: \(m/z\) 358.8246 (M\(^+\)). Anal. Calcd for C\(_{26}\)H\(_{24}\)O\(_2\): C, 73.71; H, 8.44; O 17.07. Found: C, 73.24; H, 8.53; O, 17.38%.

Hexadecanoic acid-4-methyl-2-oxo-2H-chromen-7-yl-ester, 3f: White solid: m.p. 63°C (uncorrected), TLC (hexane/ethyl acetate 8:2 v/v); IR (KBr pellet): 2917.95 (CH\(_3\) chain), 1713.01 (C=O), 1612.57 (COOR), 1395.04 (C-O), 1208.06 cm\(^{-1}\) (C-O); \(^1\)H NMR (DMSO-d\(_6\), 300 MHz): \(\delta 0.84\) (t, 3H, CH\(_3\)), 1.22 (m, 14H, CH\(_2\) chain), 2.25 (t, 2H, CH\(_2\)CO), 2.57 (s, 3H, CH\(_3\)), 6.05 (s, 1H, C=H), 6.74 (s, 1H, Ar), 6.80 (d, 1H, Ar), 7.44 (dd, 1H, Ar); MS: \(m/z\) 374.8903 (M\(^+\)). Anal. Calcd for C\(_{26}\)H\(_{24}\)O\(_2\): C, 74.58; H, 8.87; O, 16.56. Found: C, 74.24; H, 8.53; O, 16.38%.

Octadecanoic acid-4-methyl-2-oxo-2H-chromen-7-yl-ester, 3g: White solid: m.p. 59-61°C (uncorrected), TLC (hexane/ethyl acetate 8:2 v/v); IR (KBr pellet): 2925.84 (CH\(_3\) chain), 1670.33 (C=O), 1607.06 (COOR), 1277.40 (C-O), 1394.67 cm\(^{-1}\) (CH\(_3\)); \(^1\)H NMR (DMSO-d\(_6\), 300 MHz): \(\delta 0.96\) (t, 3H, CH\(_3\)), 1.29 (m, 18H CH\(_2\) chain), 2.49 (t, 2H, CH\(_2\)CO), 4.68 (d, 1H, CH=CH), 6.01 (s, 1H, C=H), 6.74 (s, 1H, Ar), 6.85 (d, 1H, Ar), 7.44 (dd, 1H, Ar); MS: \(m/z\) 440.1832 (M\(^+\)).
6-Hydroxy-4-methyl-2-oxo-2H-chromen-2-one, 4: White solid: m.p. 185-88°C (uncorrected), TLC (hexane/ethyl acetate 8:2 v/v); IR (KBr pellet): 3267.32 (CH₃), 1682.72 (COOR), 1667.32 (C=O), 1408.06 (C=C), 1366.94 (C=C), 1445.74 cm⁻¹ (C=O); ¹H NMR: (DMSO-d₆, 300 MHz): δ 1.77 (t, 3H, CH₃), 2.39 (t, 2H, CH₂CO), 6.23 (s, 1H, C=H), 6.87 (d, 1H, Ar), 7.03 (dd, 1H, Ar), 7.27 (d, 1H, Ar); MS: m/z 218 1248 (M⁺). Anal. Calcd for C₁₂H₁₀O₄: C, 66.05; H, 4.62; O, 29.33. Found C, 66.38; H, 4.25; O, 28.88%.

Acetic acid-4-methyl-2oxo-2H-chromen-6-yester, 5a: White solid: m.p. 47-49°C (uncorrected), TLC (hexane/ethyl acetate 8:2 v/v); IR (KBr pellet): 2935.06 (8 CH₂ chain), 1732.72 (COOR), 1577.23 (C=O), 1508.06 (C=C), 1354.40 (CH₃), 1228.6 cm⁻¹ (C=O); ¹H NMR: (DMSO-d₆, 300 MHz): δ 1.22 (t, 3H, CH₃), 1.58 (t, 8H, CH₂), 2.45 (s, 2H, CH₂CO), 6.13 (s, 1H, C=H), 6.71 (s, 1H, Ar), 7.28 (d, 1H, Ar), 7.32 (dd, 1H, Ar); MS: m/z 302.9644 (M⁺). Anal. Calcd for C₁₂H₁₂O₃: C, 71.50; H, 7.33; O, 21.17. Found C, 71.07; H, 6.93; O, 21.36%.

Decanoic acid-4-methyl-2oxo-2H-chromen-6-yester, 5c: White solid: m.p. 63-65°C (uncorrected), TLC (hexane/ethyl acetate 8:2 v/v); IR (KBr pellet): 2918.43 (12 CH₂ chain), 1725.06 (COOR), 1578.49 (C=O), 1442.06 (C=C), 1395.74 (CH₃), 1266.21 cm⁻¹ (C=O); ¹H NMR: (DMSO-d₆, 300 MHz): δ 0.95 (t, 3H, CH₃), 1.79 (s, 10H, CH₂), 2.33 (t, 2H, CH₂CO), 6.23 (s, 1H, C=H), 6.78 (s, 1H, Ar), 6.44 (d, 1H, Ar), 7.49 (dd, 1H, Ar); MS: m/z 330.5572 (M⁺). Anal. Calcd for C₁₄H₁₄O₃: C, 72.30; H, 7.93; O, 19.17. Found C, 72.12; H, 7.48; O, 19.72%.

Dodecanoic acid-4-methyl-2oxo-2H-chromen-6-yester, 5d: White solid: m.p. 50-52°C (uncorrected), TLC (hexane/ethyl acetate 8:2 v/v); IR (KBr pellet): 2918.43 (12 CH₂ chain), 1700.08 (COOR), 1578.64 (C=O), 1439.05 (CH₃), 1449.06 (C=C), 1305.43 cm⁻¹ (C=O); ¹H NMR: (DMSO-d₆, 300 MHz): δ 0.86 (t, 3H, CH₃), 1.35 (t, 11H, CH₂), 2.37 (t, 2H,CH₂CO), 4.55 (s, 3H, CH₃) 6.02 (s, 1H, C=H), 6.43 (s, 1H, Ar), 6.78 (d, 1H, Ar), 7.54 (dd, 1H, Ar); MS: m/z 357.9324 (M⁺). Anal. Calcd for C₁₆H₁₆O₅: C, 73.71; H, 8.44; O, 17.07. Found: C, 73.22; H, 8.43; O, 17.45%.

Tetradecanoic acid-4-methyl-2oxo-2H-chromen-6-yel- ester, 5e: White solid: m.p. 58°C (uncorrected), TLC (hexane/ethyl acetate 8:2 v/v); IR (KBr pellet): 2918.57 (CH₂ chain), 1578.39 (C=O), 1690.29 (COOR), 1353.25 (CH₃), 1303.51 cm⁻¹ (C=O); ¹H NMR: (DMSO-d₆, 300 MHz): δ 0.86 (t, 3H, CH₃), 1.15 (m, 14H, CH₂ chain), 2.17 (s, 2H, CH₂CO), 6.21 (s, 1H, C=H), 6.73 (s, 1H, Ar), 6.86 (d, 1H, Ar), 7.22 (dd, 1H, Ar); MS: m/z 386.2430 (M⁺). Anal. Calcd for C₂₀H₂₆O₅: C, 74.58; H, 8.87; O, 16.56. Found C, 74.12; H, 8.46; O, 16.26%.

Hexadecanoic acid-4-methyl-2oxo-2H-chromen-6-yel- ester, 5f: White solid: m.p. 62-63°C (uncorrected), TLC (hexane/ethyl acetate 8:2 v/v); IR (KBr pellet): 2917.62 (CH₂ chain), 1700.08 (COOR), 1683.57 (C=O), 1371.16 (CH₃), 1295.36 cm⁻¹ (C=O); ¹H NMR: (DMSO-d₆, 300 MHz): δ 0.98 (t, 3H, CH₃), 1.44 (m, 14 CH₂ chain), 2.55 (s, 3H, CH₂CO), 3.88 (s, 3H, CH₃), 6.02 (d, 1H, C=H), 6.63 (s, 1H, Ar), 7.78 (d, 1H, Ar), 7.35(dd, 1H, Ar); MS: m/z 414.1269 (M⁺). Anal. Calcd for C₂₂H₂₈O₅: C, 75.32; H, 9.24; O, 15.44. Found C, 75.93; H, 9.45; O, 15.21%.

Octade-8-enoic acid-4-methyl-2oxo-2H-chromen-6-yel- ester, 5g: White solid: m.p. 58-60°C (uncorrected), TLC (hexane/ethyl acetate 8:2 v/v); IR (KBr pellet): 2918.43 (CH₂ chain), 1725.06 (COOR), 1690.70 (C=O), 1352.28 (CH₃), 1303.43 cm⁻¹ (C=O); ¹H NMR: (DMSO-d₆, 300 MHz): δ 0.88 (t, 3H, CH), 1.47 (m, 16 CH₂ chain), 2.33 (s, 2H, CH₂CO), 5.88 (d, 1H, CH=CH), 6.02 (s, 1H, C=H), 6.63 (s, 1H, Ar), 6.83 (d, 1H, Ar), 7.52 (dd, 1H, Ar); MS: m/z 440.1832 (M⁺). Anal. Calcd for C₂₄H₂₆O₅: C, 76.33; H, 9.15; O, 14.52. Found C, 76.23; H, 9.28; O, 14.31%.

Conclusion

Thus, we can say that we have designed an easy and eco-friendly method for biocatalytic transformations of coumarins and we have evaluated their antioxidant properties by screening them through ABTS and DPPH assays.

Acknowledgement

The authors (Vaishali and R Poddar) are thankful to UGC and CSIR, New Delhi for their junior and senior research fellowships respectively.
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