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Design and Synthesis of Novel Pegylated 4-Methylcoumarins

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Coumarins are well known for their antioxidant and anti-edema activities. Their antioxidant property gets enhanced with a methyl group at the C-4 position of the pyran ring. To increase the antioxidant potential and their hydrophilicity, a lipase (Novozyme 435) catalyzed copolymerization of 4-methylcoumarin diesters and polyethylene glycols (PEGs) has been carried out to give novel copolymers.

Keywords: 4-methylcoumarin; PEG; antioxidant; lipase

1 Introduction

Coumarins comprise a very large class of phenolic substances found in plants that are made of fused benzene and α-pyrone rings, and are well-known for a variety of biological activities, such as antioxidant, antithrombophletic, anti-inflammatory and anti-edema activity (1, 6). It is evident from the literature that the simple coumarins possess a diverse array of pharmacological and biochemical properties (2, 5), some of which may be of potential pharmaceutical interest. It is also equally evident that their specificity of action is rather limited (7), in the sense that individual members of the coumarin family sometimes exert multiple actions, often within similar ranges of concentration. This suggests that these compounds are not likely to lead to specific high potency pharmaceuticals with unique modes of action. Thus, the "pharmacological promiscuity" of individual coumarins may not necessarily limit their exploitation. In fact, it could be argued that several of the exhibited properties could be beneficial if combined. In view of their well-established low toxicity, ready availability, presence in the diet and occurrence in various herbal remedies, coumarins appear to be candidates for evaluation of their properties and applications further. Therefore, we have designed the combination of coumarin moieties with poly(ethylene glycols) (PEGs). These PEGs have unique properties, including solubility in wide range of solvents, lack of toxicity, absence of antigenicity and immunogenicity, non-interference with enzymatic activities thus making them ideal combination for further applications as drug discovery and drug delivery agents.

The use of enzymes in organic synthesis has several advantages, such as superior catalytic power and high selectivity under mild conditions with regard to temperature, pressure, and pH, to give high substrate conversion efficiency, high diastero-, regio-, and chemoselectivity. Many naturally occurring polymers are produced *in vivo* by enzymatic catalysis. These features allow the generation of functional compounds for pharmaceutical and agrochemical sectors employing non-toxic natural catalysts with "green appeal". Additional advantages include catalyst recyclability and use in bulk reaction media avoiding organic solvents (8–10). Therefore, we have performed the condensation polymerization of polyethylene glycols with 4-methylcoumarin diesters **6–7** catalyzed by *Candida antarctica lipase (CAL).*

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2 Experimental

2.1 Materials

Resorcinol, 2-methylresorcinol, diethyl 2-acetylglutarate, polyphosphoric acid (PPA), anhydrous potassium carbonate, acetonitrile and polyethylene glycols (PEG 600, 900, 1500) were purchased from Aldrich (Milwaukee, WI). Anhydrous potassium carbonate was fused over night at 200°C before use, where as polyethylene glycols were dried under vacuum at 60°C for 3 h prior to their use. All other chemicals and solvents were of analytical grade and were used without further purification. Novozyme 435 was obtained as a gift from Novozymes A/S.

2.2 Characterization

Gel permeation chromatography (GPC) was used to determine the molecular weight and molecular weight distribution (Mw/Mn) of polymers using THF as solvent and polystyrene as the standard. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DPX 500 spectrometer operating at 500 and 125 MHz, respectively. ^{1}H-^{1}H COSY NMR spectra were recorded on 250 MHz Bruker Instrument using TMS as internal standard. Fluorescence spectra were recorded on a FLUOROLOG (ISA Instruments) spectrofluorometer. Infrared spectra were recorded as KBr pellets on a Nicolet 750 series Fourier transform infrared (FT-IR) spectrometer. UV spectra were recorded on Perkin-Elmer Lambda-9-UV-Vis-Near IR spectrophotometer.

2.3 General Procedure for the Synthesis of 7-Hydroxy-4-methylcoumarin and 7-Hydroxy-4,8-dimethylcoumarin Derivatives 4 and 5

Polyphosphoric acid was added to a solution of resorcinol (**1**) or 2-methylresorcinol (**2**) and diethyl 2-acetylglutarate (**3**) in a three-neck round bottom flask with a water condenser with constant stirring. The resulting mixture was heated at 75–80°C for 30 min and then poured into ice-water to give a pale yellow colored solid. This was filtered, washed with cold water, dried at 60°C and recrystallized from dilute ethanol.

Compound 4 was obtained by using resorcinol (10.0 g, 90.0 mmol), diethyl 2-acetyl- glutarate (21.0 g, 91.0 mmol) and PPA (158.0 g, 1580.0 mmol), m.pt. 88–90°C.

^{1}H-NMR (($_H$ CD_3OD, 500 MHz) 1.25 (t, 3H, $J = 7.0$ Hz -OCH_2CH_3), 2.44 (s, 3H, -CH_3) 2.61 (t, 2H, $J = 7.5$ Hz, *H-2′*), 2.98 (t, 2H, $J = 7.5$ Hz, *H-3′*), 4.16 (q, 2H, $J = 7.0$ Hz, -OCH_2CH_3), 6.83 (d, 1H, $J = 8.6$ Hz, *H-6*), 6.95 (s, 1H, *H-8*), 7.49 (d, 1H, $J = 8.6$ Hz, *H-5*).

^{13}C-NMR (δ_c CD_3OD, 125 MHz) 13.4, 14.1, 22.7, 32.4, 60.4 (OCH_2), 102.0 (*C*H), 113.5 (*C*H), 113.9 (q), 119.7 (q), 126.0 (*C*H), 148.9 (q), 153.6 (q), 161.5 (q), 162.7 (q), 173.2 (>C═O).

IR (KBr) νmax 3261, 2928, 2854, 1720, 1709, 1620, 1602, 1384, 1283, 1247, 1164, 1100, 869 cm^{-1}.

UV λmax (MeOH): 364, 276, 232 nm.

Compound 5 was prepared by using 2-methylresorcinol (10.0 g, 80.0 mmol), diethyl 2-acetylglutarate (18.5 g, 80.6 mmol) and PPA (140.0 g, 1400.0 mmol), m.pt. 143–145°C.

^{1}H-NMR (δ_H, CD_3OD, 500 MHz) 1.27 (t, 3H, $J = 7.0$ Hz -OCH_2CH_3), 2.32 (s, 3H, Ar-CH_3), 2.43 (s, 3H, -CH_3), 2.64 (t, 2H, $J = 6.7$ Hz, *H-2′*), 2.99 (t, 2H, $J = 6.7$ Hz, *H-3′*), 4.16 (q, 2H, $J = 7.0$ Hz, -OCH_2CH_3), 6.79 (d, 1H, $J = 8.5$ Hz, *H-6*), 7.33 (d, 1H, $J = 8.5$ Hz, *H-5*).

^{13}C-NMR (δ_c CD_3OD, 125 MHz) 8.34 (ArCH_3), 14.5, 15.3, 23.5, 33.2, 61.0 (OCH_2), 111.8 (q), 112.2 (*C*H), 114.3 (q), 120.9 (q), 122. 9 (*C*H), 148.5 (q), 152.1(q), 156.9 (q), 162.6 (q), 173.7(>C═O).

IR (KBr) νmax 3240, 2987, 2854, 1724, 1707, 1675, 1605, 1576, 1460, 1378, 1298, 1192, 1103, 871 cm^{-1}.

UV λmax (MeOH): 379, 326, 257 nm.

2.4 General Procedure for the Synthesis of 4-Methylcoumarin Diesters 6 and 7

Hydroxycoumarin ***(4 or 5)*** was dissolved in anhydrous acetonitrile in a three-neck round bottom flask under the inert atmosphere of N_2 and fused potassium carbonate was added, followed by the dropwise addition of a solution of α-bromo ethyl acetate in acetonitrile with constant stirring. The resulting mixture was refluxed for 9 h, cooled and filtered to remove the salts. The filtrate was concentrated to get the desired product as an amorphous solid which was further purified by column chromatography using chloroform as solvent, the product was obtained in methanol/chloroform (1:9) mixture as a pale yellow solid.

Compound 6 was prepared by using compound **4** (10.0 g, 36.0 mmol), α-bromoethyl acetate (6.0 g, 35.9 mmol) and potassium carbonate (5.0 g, 36.2 mmol), m.pt. 66–68°C

^{1}H-NMR (δ_H $CDCl_3$, 500 MHz) 1.25 (t, 3H, $J = 7.0$ Hz, -OCH_2CH_3), 1.32 (t, 3H, $J = 7.0$ Hz, -OCH_2CH_3), 2.44 (s, 3H, -CH_3), 2.61 (t, 2H, $J = 7.5$ Hz, *H-2′*), 2.98 (t, 2H, $J = 7.5$ Hz, *H-3′*), 4.16 (q, 2H, $J = 7.0$ Hz, -OCH_2CH_3), 4.29 (q, 2H, $J = 7.0$ Hz, -OCH_2CH_3), 4.68 (s, 2H, *H-2″*), 6.77 (d, 1H, $J = 8.6$ Hz, *H-6*), 6.92 (s, 1H, *H-8*), 7.56 (d, 1H, $J = 8.6$ Hz, *H-5*).

^{13}C-NMR (δ_c, $CDCl_3$, 125 MHz) 14.4 (2XCH_3), 15.4, 23.5, 32.5, 60.9 (OCH_2), 61.8 (OCH_2), 65.7 (*C-2″*), 101.8 (*C*H), 112.9 (*C*H), 115.3 (q), 122.4 (q), 126.0 (*C*H), 148.0 (q), 153.9 (q), 160.2 (q), 162.4 (q), 168.4 (q), 173.2 (>C═O).

IR (KBr) νmax 2982, 2931, 1739, 1721, 1708, 1620, 1602, 1378, 1286, 1247, 1165, 1100, 1026, 869, 765 cm^{-1}.

UV λmax (MeOH): 361, 325, 309, 229 nm.

Compound 7 was by using compound **5** (10.0 g, 34.4 mmol), α-bromoethyl acetate (5.7 g, 34.4 mmol) and potassium carbonate (4.7 g, 34.4 mmol), m.pt.105–106°C.

^{1}H-NMR (δ_H $CDCl_3$, 500 MHz) 1.25 (t, 3H, $J = 7.0$ Hz, -OCH_2CH_3), 1.32 (t, 3H, $J = 7.0$ Hz -OCH_2CH_3), 2.31 (s, 3H, Ar-CH_3), 2.44 (s, 3H, -CH_3), 2.62 (t, 2H,

$J = 7.5$ Hz, *H-2′*), 2.99 (t, 2H, $J = 7.5$ Hz, *H-3′*), 4.14 (q, 2H, $J = 7.0$ Hz, -OCH_2CH$_3$), 4.30 (q, 2H, $J = 7.0$ Hz, -OCH_2CH$_3$), 4.74 (s, 2H, *H-2″*), 6.73 (d, 1H, $J = 8.8$ Hz, *H-6*), 7.43 (d, 1H, $J = 8.8$ Hz, *H-5*).

^{13}C-NMR (δ_c, $CDCl_3$, 125 MHz) 8.70 (Ar*C*H$_3$), 14.5 (2X*C*H$_3$), 15.3, 23.5, 33.0, 60.8 (O*C*H$_2$), 61.8 (O*C*H$_2$), 66.3 (*C2″*), 108.0 (*C*H), 115.2 (q), 115.5 (q), 122.3 (q), 122.8 (*C*H), 147.6 (q), 151.9 (q), 158.1(q), 162.0 (q), 168.8 (q), 173.3 (>C═O).

IR (KBr) νmax 2980, 2945, 1743, 1720, 1707, 1620, 1604, 1450, 1377, 1296, 1206, 1133, 1100, 860 cm^{-1}

UV λmax (MeOH): 369, 319, 254, 244 nm.

2.5 General Procedure for Copolymerization of 4-Methylcoumarin Diesters 6 and 7 with Polyethylenegycols

The coumarin diester **(6** or **7)** and polyethylene glycol (PEG 600, 900 or 1500) were placed in a round-bottom flask and enzyme (10 wt% with respect to monomers), was added to this mixture, and the flask was then placed in a constant temperature oil bath maintained at 90°C under vacuum. The reaction was allowed to proceed for 48 h, after which it was quenched by adding water and filtering off the enzyme and any unreacted monomer **6** or **7** under vacuum. The filtrate was dialyzed using membrane (MWCO 6000–8000). After the completion of dialysis, the product polymers **8–13** were obtained as semisolids after freeze drying.

Copolymer 8 was prepared by using compound **6** (1.0 g, 2.7 mmol), PEG-600 (1.65 g, 2.7 mmol) and lipase (Novozyme-435, 0.265 g).

^{1}H-NMR (δ_H $CDCl_3$, 500 MHz) 1.26 (t, 3H, -OCH$_2$C*H*$_3$ end group), 2.43 (s, 3H, -C*H*$_3$), 2.63 (t, 2H, $J = 7.5$ Hz, *H-2′*), 2.95 (t, 2H, J = 7.5 Hz, *H-3′*), 3.55–3.74 (bs, methylene protons of PEG main chain), 4.21 (q, 2H, $J = 7.0$ Hz, -OCH_2PEG), 4.38 (q, 2H, $J = 7.0$ Hz, -OCH_2PEG), 4.72 (s, 2H, *H-2″*), 6.77 (d, 1H, $J = 8.6$ Hz, *H-6*), 6.91 (s, 1H, *H-8*), 7.55 (d, 1H, $J = 8.6$ Hz, *H-5*).

^{13}C-NMR (δ_c, $CDCl_3$, 125 MHz) 14.5 (OCH$_2$*C*H$_3$ end group), 15.3, 23.5, 33.0, 61.4, 61.9 (-O*C*H$_2$CH$_3$ end group), 64.8, 65.6, 69.4, 70.8–70.9 (methylene carbons of PEGmain), 72.7, 101.9 (*C*H), 112.8 (*C*H), 115.3 (q), 122.4 (q), 126.3 (*C*H), 147.6 (q), 154.0 (q), 160.2 (q), 162.2 (q), 168.3 (q), 173.3 (>C═O).

IR (KBr) νmax 2979, 2939, 2871, 1730, 1709, 1610, 1452, 1351, 1288, 1105, 952, 849 cm^{-1}.

Copolymer 9 was prepared by using compound **6** (1.0 g, 2.7 mmol), PEG-900 (2.47 g, 2.7 mmol) and lipase (Novozyme-435, 0.347 g).

^{1}H-NMR (δ_H $CDCl_3$, 500 MHz) 1.23 (t, 3H, -OCH$_2$C*H*$_3$ end group), 2.41 (s, 3H, -C*H*$_3$) 2.62 (t, 2H, $J = 7.5$ Hz, *H-2′*), 2.93 (t, 2H, $J = 7.5$ Hz, *H-3′*), 3.55–3.71 (bs, methylene protons of PEG main Chain), 4.19 (q, 2H, $J = 7.0$ Hz, -OCH_2 PEG), 4.35 (q, 2H, $J = 7.0$ Hz, -OCH_2 PEG), 4.70 (s, 2H, *H-2″*), 6.74 (d, 1H, $J = 8.6$ Hz, *H-6*), 6.89 (s, 1H, *H-8*), 7.53 (d, 1H, $J = 8.6$ Hz, *H-5*).

^{13}C-NMR (δ_c, $CDCl_3$, 125 MHz) 14.5 (OCH$_2$*C*H$_3$ end group), 15.3, 23.4, 33.0, 61.4, 61.9 (-O*C*H$_2$CH$_3$ end group), 64.8, 65.6, 69.4, 70.6–70.9 (methylene carbons of PEGmain), 71.8, 101.9 (*C*H), 112.8 (*C*H), 115.2 (q), 122.3 (q), 126.3 (*CH*), 147.6 (q), 154.1 (q), 160.2 (q), 162.2 (q), 168.3 (q), 173.2 (>C═O).

IR (KBr) νmax 2972, 2930, 2873, 1739, 1708, 1608, 1460, 1352, 1286, 1295, 1108, 956, 852 cm^{-1}.

Copolymer 10 was prepared by using compound **6** (1.0 g, 2.7 mmol), PEG-1500 (4.13 g, 2.7 mmol) and lipase (Novozyme-435, 0.513 g).

^{1}H-NMR (δ_H $CDCl_3$, 500 MHz) 1.27 (t, 3H,-OCH$_2$C*H*$_3$ end group), 2.41 (s, 3H, -C*H*$_3$), 2.63 (t, 2H, $J = 7.5$ Hz, *H-2′*), 2.95 (t, 2H, $J = 7.5$ Hz, *H-3′*), 3.55–3.76 (bs, methylene protons of PEG main chain), 4.20 (q, 2H, $J = 7.0$ Hz, -OCH_2PEG), 4.36 (q, 2H, $J = 7.0$ Hz, -OCH_2*P*EG), 4.70 (s, 2H, *H-2″*), 6.75 (d, 1H, $J = 8.6$ Hz, *H-6*), 6.90 (s, 1H, *H-8*), 7.54 (d, 1H, $J = 8.6$ Hz, *H-5*).

^{13}C-NMR (δ_c, $CDCl_3$, 125 MHz) 14.5 (OCH$_2$*C*H$_3$ end group), 15.3, 23.4, 33.0, 61.4, 61.9 (-O*C*H$_2$CH$_3$ end group), 64.8, 65.6, 69.4, 70.6–70.9 (methylene carbons of PEG main), 71.7, 101.9 (*CH*), 112.8 (*C*H), 115.3 (q), 122.3 (q), 126.3 (*C*H), 147.6 (q), 154.1 (q), 160.2 (q), 162.2 (q), 168.3 (q), 173.2 (>C═O).

IR (KBr) νmax 2977, 2933, 2883, 1734, 1710, 1611, 1466, 1345, 1281, 1243, 1290, 1113, 956, 839 cm^{-1}.

Copolymer 11 was prepared by using compound **7** (1.0 g, 2.6 mmol), PEG-600 (1.50 g, 2.6 mmol) and lipase (Novozyme-435, 0.250 g).

^{1}H-NMR (δ_H $CDCl_3$, 500 MHz) 1.25 (t, 3H, -OCH$_2$C*H*$_3$ end group), 2.39 (s, 3H, Ar-C*H*$_3$), 2.43 (s, 3H,-C*H*$_3$), 2.67 (t, 2H, $J = 7.5$ Hz, *H-2′*), 2.99 (t, 2H, J = 7.5 Hz, *H-3′*), 3.52–3.74 (bs, methylene protons of PEG main chain), 4.24 (q, 2H, $J = 7.0$ Hz, -OCH_2PEG), 4.39 (q, 2H, $J = 7.0$ Hz, -OCH_2PEG), 4.78 (s, 2H, *H-2″*), 6.79 (d, 1H, $J = 8.6$ Hz, *H-6*), 7.44 (d, 1H, $J = 8.6$ Hz, *H-5*).

^{13}C-NMR (δ_c, $CDCl_3$, 125 MHz); 8.7 (Ar-*C*H$_3$), 14.5 (OCH$_2$*C*H$_3$ end group), 15.3, 23.4, 33.0, 61.3, 61.9 (-O*C*H$_2$CH$_3$ end group), 64.6, 66.4, 69.4, 70.7–70.9(methylene carbons of PEG main), 71.9, 108.5 (*C*H), 115.0 (q), 115.4

(q), 122.6 (q), 123.2 (*CH*), 147.8 (q), 155.0 (q), 158.7 (q), 162.5 (q), 168.7 (q), 173.2 (>C═O).

IR (KBr) νmax 2981, 2943, 2878, 1733, 1707, 1613, 1457, 1349, 1286, 1109, 959, 853 cm^{-1}.

Copolymer 12 was prepared by using compound **7** (1.0 g, 2.6 mmol), PEG-900 (2.38 g, 2.6 mmol) and lipase (Novozyme-435, 0.338 g).

^{1}H-NMR (δ_H $CDCl_3$, 500 MHz) 1.27 (t, 3H,-OCH_2CH_3 end group), 2.39 (s, 3H, Ar-CH_3), 2.43 (s, 3H,-CH_3) 2.67 (t, 2H, *J* = 7.5 Hz, *H-2′*), 2.99 (t, 2H, *J* = 7.5 Hz, *H-3′*), 3.52–3.74 (bs, methylene protons of PEG main chain), 4.24 (q, 2H, *J* = 7.0 Hz, -OCH_2PEG), 4.39 (q, 2H, *J* = 7.0 Hz, -OCH_2PEG), 4.78 (s, 2H, *H-2″*), 6.79 (d, 1H, *J* = 8.6 Hz,), 7.44 (d, 1H, *J* = 8.6 Hz, *H-5*).

^{13}C-NMR (δ_c, $CDCl_3$, 125 MHz) 8.7 (ArCH_3), 14.5 (OCH_2CH_3 end group), 15.3, 23.4, 33.0, 61.4, 61.9 (-OCH_2CH_3 end group), 64.7, 66.3, 69.4, 70.6–70.9 (methylene carbons of PEG main), 71.9, 108.3 (*C*H), 115.0 (q), 115.3 (q), 122.4 (q), 123.0 (*C*H), 147.5 (q), 154.3 (q), 158.7 (q), 162.4 (q), 168.8 (q), 173.4 (>C═O).

IR (KBr) νmax 2979, 2938, 2889, 1736, 1709, 1605, 1462, 1359, 1289, 1109, 959, 856 cm^{-1}.

Copolymer 13 was prepared by using compound **7** (1.0 g, 2.6 mmol), PEG-1500 (3.97 g, 2.6 mmol) and lipase (Novozyme-435, 0.497 g).

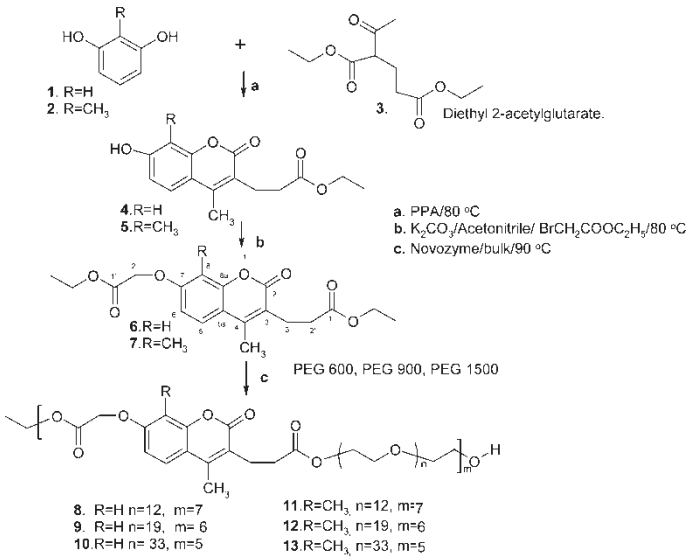
^{1}H-NMR (δ_H $CDCl_3$, 500 MHz) 1.26 (t, 3H, -OCH_2CH_3 end group), 2.37 (s, 3H, Ar-CH_3), 2.42 (s, 3H, -CH_3), 2.66 (t, 2H, *J* = 7.5 Hz, *H-2′*), 2.97 (t, 2H, *J* = 7.5 Hz, *H-3′*), 3.53–3.74 (bs, methylene protons of PEG main chain), 4.23 (q, 2H, *J* = 7.0 Hz, -OCH_2PEG), 4.37 (q, 2H, *J* = 7.0 Hz, -OCH_2PEG), 4.78 (s, 2H, *H-2″*), 6.79 (d, 1H, *J* = 8.6 Hz, *H-6*), 7.44 (d, 1H, *J* = 8.6 Hz, *H-5*).

^{13}C-NMR (δ_c, $CDCl_3$, 125 MHz) 8.70 (ArCH_3), 14.5 (OCH_2CH_3 end group), 15.3, 23.5, 32.9, 61.6, 61.9 (-OCH_2CH_3 end group), 64.7, 66.1, 69.4, 70.7–70.9 (methylene carbons of PEG main), 71.9, 108.0 (*C*H), 115.1 (q), 115.4 (q), 122.2 (q), 122.9 (*C*H), 147.4 (q), 154.1 (q), 158.1 (q), 162.5 (q), 168.7 (q), 173.1 (>C═O).

IR (KBr) νmax 2974, 2936, 2884, 1734, 1708, 1605, 1466, 1339, 1281, 1243, 1114, 959, 837 cm^{-1}.

3 Results and Discussion

Considering the proven antioxidant properties of 4-methylcoumarins, (11, 12) we designed this project to combine the properties of PEGs with these coumarins to make them water soluble so that their applications may be explored in various sectors. 4-Methylcoumarins have been synthesized (Scheme 1) starting from resorcinol (**1** or 2-methylresorcinol, **2**) and diethyl 2-acetylglutarate (**3**). Diethyl 2-acetylglutarate (**3**) has been chosen because it could provide the ester group



Sch. 1. Synthesis of pegylated 4-methylcoumarins.

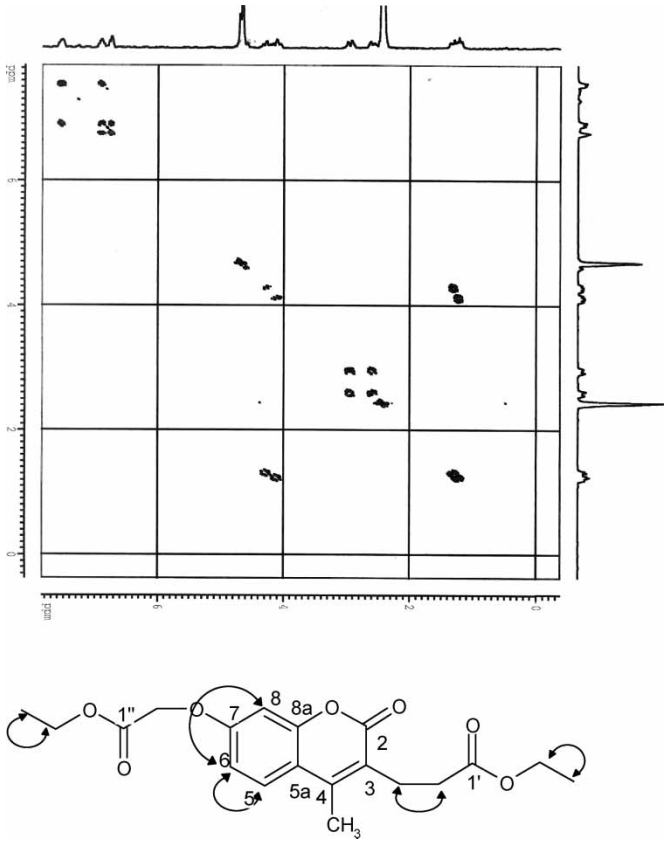


Fig. 1. ^{1}H-^{1}H correlation of compound 6.

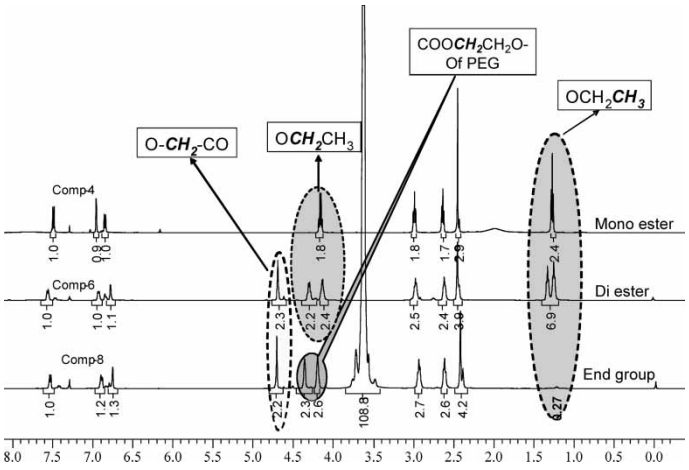


Fig. 2. Characterization of copolymer 9 by ^{1}H-NMR.

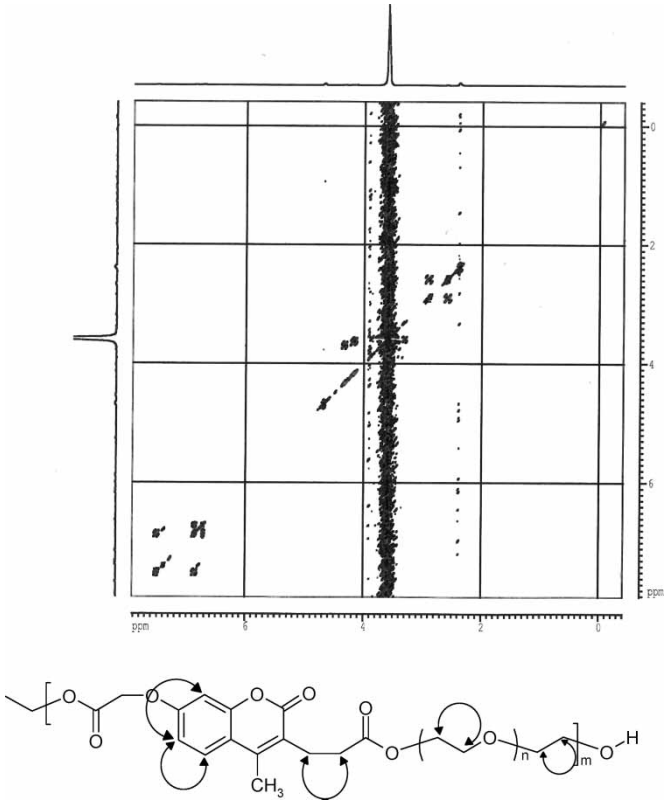


Fig. 3. ^{1}H- ^{1}H correlation of copolymer 9.

at the C-3 position on pyran ring with a two carbon spacer from the coumarin nucleus and therefore, facilitate the copolymerization reaction with enzyme. The structures of 3-ethoxycarbonylethyl-7-hydroxy-4-methylcoumarin (**4**) and 3-ethoxycarbonylethyl-7-hydroxy-4, 8-dimethylcoumarin (**5**) has been determined from their ^{13}C-NMR spectra which showed a lactone carbonyl at (162.7 and an ester carbonyl at (173.2. In compound **4,** they appeared at (162.6 and 173.7. In the case of compound **5,** IR spectrum also confirmed this observation and showed peaks at 1709 and 1720 cm^{-1} for the presence of lactone carbonyl and ester carbonyl in the molecule **4,** respectively. The UV and ^{1}H-NMR spectrum of these compounds were also found to be in accordance with the desired products **4** and **5**.

Table 1. Molecular weight and solubility of the copolymers

S. no.	Compound no.	Molecular weight (Dalton)	Solubility
1	Copolymer 08	7.0×10^3	Water
2	Copolymer 09	7.6×10^3	Water
3	Copolymer 10	9.2×10^3	Water
4	Copolymer 11	7.1×10^3	Water
5	Copolymer 12	7.8×10^3	Water
6	Copolymer 13	9.4×10^3	Water

The coumarins **4** and **5** were further treated with α-bromoethyl acetate in the presence of anhydrous potassium carbonate to get the 3-ethoxycarbonylethyl-7-ethoxycarbonylmethoxy-4-methylcoumarin (**6**) and 3-ethoxycarbonylethyl-7-ethoxycarbonylmethoxy-4, 8-dimethylcoumarin (**7**). The formation of the coumarin diesters has been confirmed by the presence of singlets at δ 4.68 and 4.74 due to the H-2″ protons and the presence of additional ethoxy moiety by observance of peaks at (4.29 and 4.30 for the methylene protons (-OCH_2CH$_3$) for both the compounds **6** and **7**. These observations were further confirmed by the presence of a peak at (65.7 (C-2′) and 61.8 (additional -OCH$_2$) in case of compound **6**, and at (66.3 (C-2′) and 61.8 (additional -OCH$_2$) for compound **7**. Formation of these diesters has also been confirmed from their ^{1}H-^{1}H correlation spectra as shown in Figure 1.

The diesters **6** and **7** were subjected to enzymatic copolymerization with polyethylene glycols (PEGs 600, 900, 1500) using *Candida antarctica* lipase (Novozyme −435) in bulk at 90°C under vacuum for 48 h. Formation of the pegylated

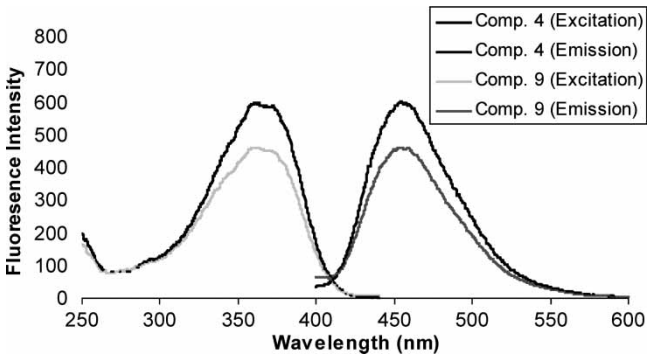


Fig. 4. Comparison of excitation and emission spectra of compound **4** and **9**.

4-methylcoumarins **8–13** has been shown by the loss of the ester methyl peak at (1.23–1.27 and appearance of new peaks at (4.19–4.24 and 4.35–4.39 due to the formation of a new ester with PEGs (Figure 2). The pegylated polymers have also been characterized from ^{1}H-^{1}H-NMR correlation spectrum, an example of which is shown in Figure 3. Formation of the pegylated coumarins **8-13** were also confirmed by all other spectroscopic techniques such as IR, UV, ^{1}H-NMR and ^{13}C-NMR, which showed all the relevant peaks in accordance with the structures of the products. The degree of polymerization was evaluated by comparison of integral ratio of peaks at δ 1.23–1.27 and δ 4.19–4.24, which suggested the molecular weight of these polymer should be in the range of 10,000–13,000 Daltons, whereas the GPC studies with these polymers suggested that they are in the range of 7,000–9,400 Daltons (Table 1).

Fluorescence studies (Figure 4) were conducted with both monomers **6** and **7** and pegylated polymers **8–13**, which showed excitation absorption maxima at 371 nm and emission maxima at 458.5 nm with same intensities, thus suggesting fluorescence with high quantum yield, which is indicative of the efficiency of fluorescence process.

4 Conclusions

Six novel pegylated 4-methylcoumarins **8–13** have been designed and successfully synthesized via enzymatic co-polymerization using Novozyme 435. They were fully characterized by detailed spectroscopic studies. All the novel polymers obtained were found to be water soluble and their antioxidant properties are under evaluation.

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