CHAPTER II
EXPERIMENTAL

2.1 Instruments and Equipment

Melting points were determined on a Fisher-Johns melting point apparatus or Electrothermal digital melting point apparatus model IA9100 and are uncorrected. Column chromatography was carried out on silica gel (Merck Kieselgel 60, 70-230 mesh). Thin-layer chromatography (TLC) was performed on aluminum sheets precoated with silica gel (Merck Kieselgel 60 PF_{254}). The FT-IR spectra were recorded on a Fourier-Transformed Infrared Spectrophotometer model Impact 410: solid samples were incorporated to potassium bromide to form a pellet. The ^{1}H-NMR and ^{13}C-NMR spectra were obtained in deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) with tetramethylsilane (TMS) as an internal reference on a Bruker model ACF 200 Spectrometer which was operated at 200.13 MHz for ^{1}H and 50.32 MHz for ^{13}C nuclei. The chemical shifts were assigned by comparison with residue solvent protons ($CDCl_3$/DMSO-d_6 means that DMSO-d_6 is added dropwise to a suspension of the compound in $CDCl_3$ until a clear solution is obtained). Elemental analysis (EA) were carried out on a Perkin Elmer PE 2400 Series II : option CHN on. Mass spectra (70 eV) were acquired from a Fissons Instrument mass spectrometer Model VG TRIO 2000 in EI mode.

2.2 Chemicals

All solvents used in this research were purified prior to use by standard methodology except for those which were reagent grades. The reagents used for synthesizing the precursors, dicoumarols and 3-substituted-4-hydroxycoumarins were purchased from Fluka Chemical Company or otherwise stated and were used without further purification.

2.3 Synthesis

2.3.1 Synthesis of Dicoumarols

General Procedure :

Method A : (for aromatic aldehydes)[13]

4-Hydroxycoumarin (2 mol-equiv) was dissolved in ethanol and the aldehyde was added. The solution was refluxed approximately 24 hours or until the solid began to precipitate. The solution was kept at that temperature for another 0.5 hour. After that the reaction mixture was cooled down. The product was filtered, washed with cold ethanol and recrystallized.

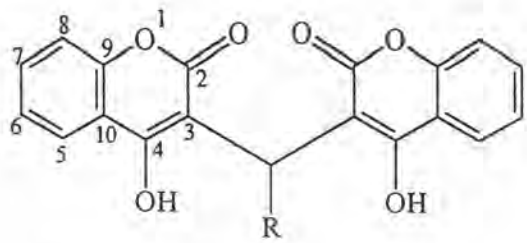
Method B : (for aliphatic aldehydes)[14]

The aldehyde was added to a mixture of 4-hydroxycoumarin (2 mol-equiv) and (0.05 mol-equiv) of ethylenediammonium diacetate in ethanol. The solution was stirred at room temperature for 4 days. The reaction mixture was allowed to cool. The product was filtered off, washed with cold ethanol and recrystallized.

Method C : (for formaldehyde)[9]

4-Hydroxycoumarin 1.00 g (6.17 mmol) was dissolved in 300 mL of boiling water, the solution was allowed to cool to 70°C and 10 mL of 40% aqueous formaldehyde was quickly added with stirring. The mixture was then chilled, the crude product was filtered off and washed well with water, dried and recrystallized with ethanol.

This research involves the SAR studies of fifty-five dicoumarols and analogues. All studied compounds are depicted in Fig 2.1. Compounds **2-5**, **7**, **8**, **10-16**, **18**, **19**, **21**, **22**, **24**, **25**, **27**, **28**, **45**, **46** and **48** were kindly supplied by S. Wattanasereekul.



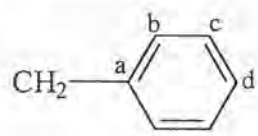
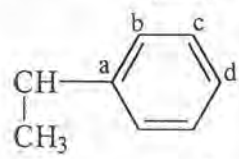
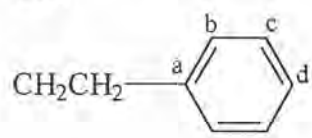
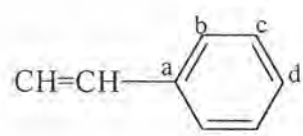
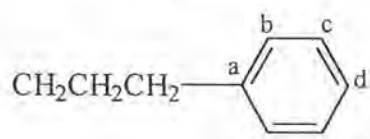
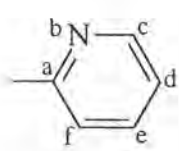
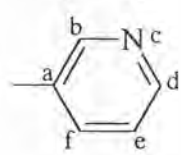
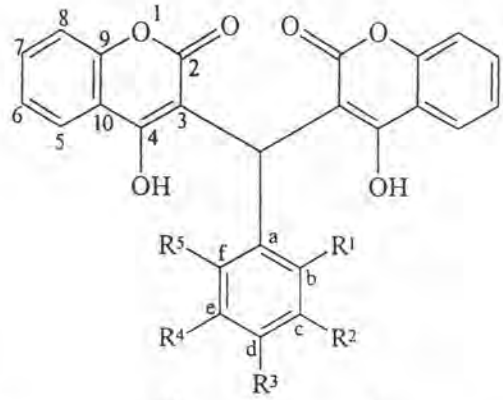
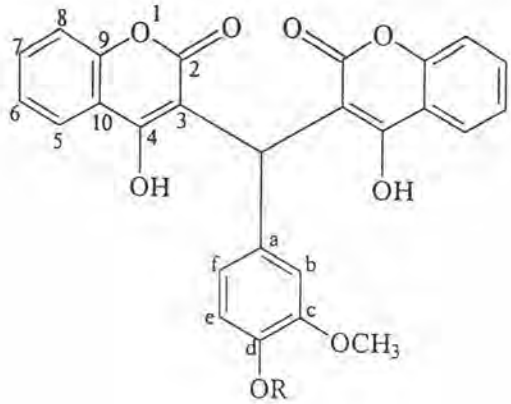
Compound	R
1	H
35	CH_3
36	$CH_2CH_2CH_3$
37	$CH(CH_3)_2$
38	$CH(CH_2CH_3)_2$
39	Cyclohexyl
40	
41	
42	
43	
44	
52	
53	

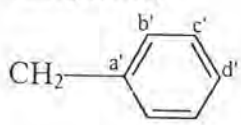
Fig 2.1 The structures of dicoumarols and analogues

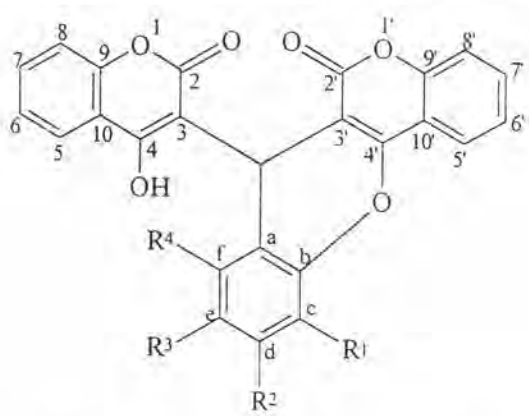


Compound	R^1	R^2	R^3	R^4	R^5
2	H	H	H	H	H
3	NO_2	H	H	H	H
4	H	NO_2	H	H	H
5	H	H	NO_2	H	H
6	H	F	H	H	H
7	H	H	F	H	H
8	Cl	H	H	H	H
9	H	Cl	H	H	H
10	H	H	Cl	H	H
11	Cl	H	Cl	H	H
12	Br	H	H	H	H
13	H	Br	H	H	H
14	H	H	Br	H	H
15	H	H	CH_3	H	H
16	H	H	$CH(CH_3)_2$	H	H
17	H	H	$C(CH_3)_3$	H	H
18	H	H	CF_3	H	H
19	OCH_3	H	H	H	H
20	H	OCH_3	H	H	H
21	H	H	OCH_3	H	H
22	H	H	OH	H	H
24	H	OH	OH	H	H
25	H	$-OCH_2O-$		H	H
26	H	OCH_3	OCH_3	OCH_3	H

Fig 2.1 (cont.)

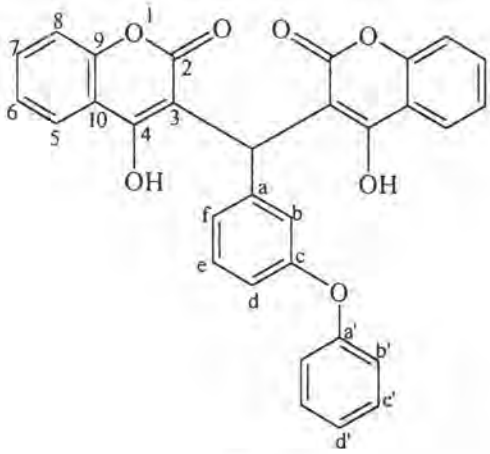


Compound	R
27	H
28	CH_3
29	CH_2CH_3
30	$(CH_2)_3CH_3$
31	$(CH_2)_5CH_3$
32	$(CH_2)_7CH_3$
33	$(CH_2)_{11}CH_3$
34	



Compound	R^1	R^2	R^3	R^4
45	H	H	H	H
46	OCH_3	H	H	H
47	H	H	H	Cl

Fig 2.1 (cont.)



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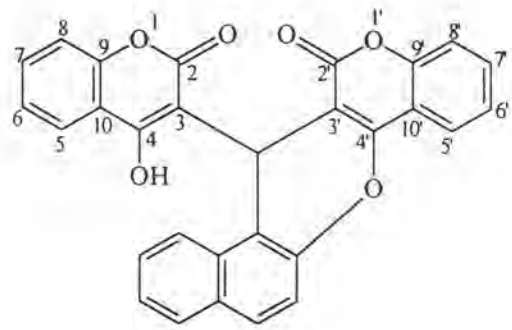

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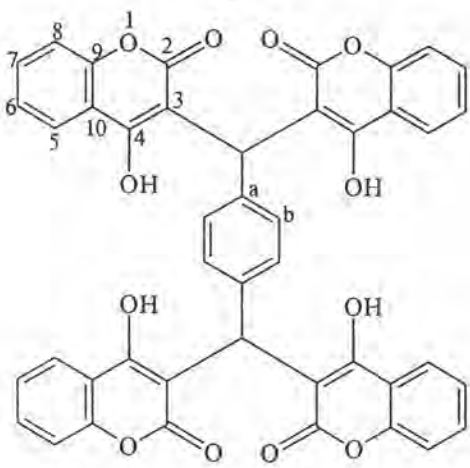

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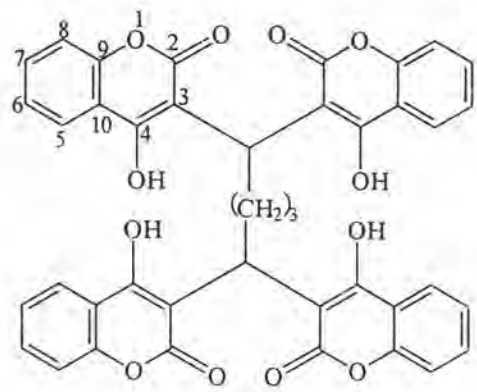

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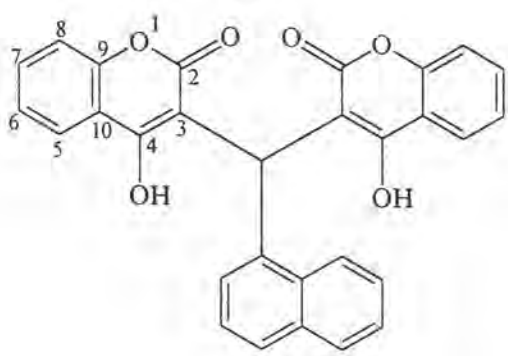

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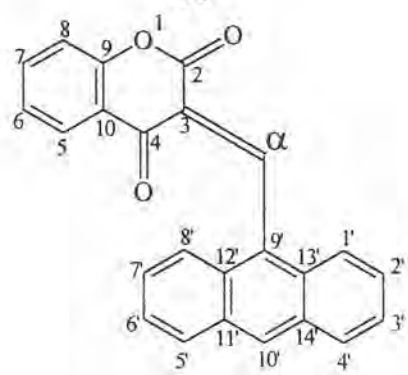

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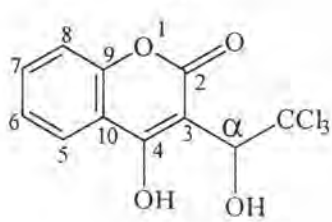

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Fig 2.1 (cont.)

3,3′-(Methylene)*bis*-4-hydroxycoumarin (**1**)[10,22]

White prism (88 %), m.p. 288-290 °C (lit.[22] m.p. 288-289 °C), R_f 0.40 (ethyl acetate). IR (KBr) 3400-2500, 3066, 1652, 1597, 1567, 1501, 1451, 1346 and 1110 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.84 (2H, s), 7.33-7.40 (4H, m), 7.59 (2H, t, J = 7.08 Hz), 7.99 (2H, d, J = 8.31 Hz) and 11.30 (2H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 19.9 (1C, CH_2), 102.9 (2C,C-3), 116.4 (2C,C-10), 116.7 (2C, C-8), 124.0 (2C, C-5), 124.8 (2C, C-6), 132.6 (2C, C-7), 152.3 (2C,C-9), 164.4 (2C, C-2) and 168.7 (2C, C-4).

3,3′-(3-Fluorobenzylidene)*bis*-4-hydroxycoumarin (**6**)[23]

White crystal (64 %), m.p. 237-238 °C (lit[23] m.p. 240-241 °C), R_f 0.44 (ethyl acetate). IR (KBr) 3300-2500, 3090, 1657, 1577, 1490, 1300 and 1095 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.04 (1H, s), 6.88-7.01 (3H, m), 7.20-7.27 (1H, m), 7.33-7.40 (4H, m), 7.62 (2H, dt, J = 7.76, 1.64 Hz), 8.02 (2H, s, br), 11.30 (1H, s, br) and 11.58 (1H, s, br); ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.1 (1C, CH), 103.5, 105.2 (2x1C, C-3), 113.6 (1C, C-d), 114.0 (1C, C-b), 116.7 (4C, 2C-10, 2C-8), 122.1 (C-f), 124.4 (2C, C-5), 125.0 (2C, C-6), 130.1 (1C, C-e), 133.0 (2C, C-7), 138.1 (1C, C-a), 152.5 (2C, C-9), 164.7 (1C, C-2), 165.6 (1C, C-c), 166.0 (1C, C-2), 166.8 and 169.2 (2x1C, C-4).

3,3′-(3-Chlorobenzylidene)*bis*-4-hydroxycoumarin (**9**)[24]

Small white crystal (85 %), m.p. 231-232 °C (dichloromethane-ethanol), R_f 0.44 (ethyl acetate). IR (KBr) 3300-2500, 3079, 2933, 2859, 1671, 1623, 1568, 1499, 1356, 1312 and 1058 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.03 (1H, s), 7.07-7.24 (4H, m), 7.37-7.41 (4H, m), 7.62 (2H, dt, J = 7.77, 1.56 Hz), 8.02 (2H, s, br), 11.29 (1H, s, br) and 11.56 (1H, s, br); ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.0 (1C, CH), 103.4, 105.1 (2x1C, C-3), 116.3 (2C, C-10), 116.7 (2C, C-8), 124.4 (2C, C-5), 124.8 (1C, C-f), 125.0 (2C, C-6), 126.7 (1C, C-d), 127.1 (1C, C-b), 129.8 (1C, C-e), 133.0 (2C, C-7), 134.7 (1C, C-c), 137.6 (1C, C-a), 152.3, 152.5 (2x1C, C-9), 164.7, 166.0 (2x1C, C-2), 166.8 and 169.1 (2x1C, C-4).

3,3′-(4-*t*-Butylbenzylidene)*bis*-4-hydroxycoumarin (**17**)

White crystal (60 %), m.p. 252-254 °C (dichloromethane-ethanol), R_f 0.45 (ethyl acetate). IR (KBr) 3300-2600, 3080, 2969, 1669, 1620, 1571, 1498, 1320 and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.29 (9H, s), 6.05 (1H, s), 7.12 (2H, d, J = 8.36 Hz), 7.29 (2H, d, J = 8.35 Hz), 7.33-7.41 (4H, m), 7.61 (2H, dt, J = 7.64, 1.68 Hz), 8.02 (2H, s, br), 11.28 (1H, s, br) and 11.50 (1H, s, br); ^{13}C-NMR ($CDCl_3$) δ (ppm): 31.3 (3C, CH_3), 34.4 (1C, C-(CH_3)$_3$), 104.0, 105.8 (2x1C, C-3), 116.6 (4C, C-8, C-10), 124.4 (2C, C-5), 124.9 (2C, C-6), 125.6 (2C, C-c), 126.2 (2C, C-b), 132.1 (1C, C-a), 132.8 (2C, C-7), 149.7 (1C, C-d), 152.4 (2C, C-9), 164.5, 165.6 (2x1C, C-2), 166.9 and 169.3 (2x1C, C-4); MS m/z (% rel.int.): 468 (M^+, 80), 305 (30), 249 (60), 162 (100) and 120 (60); Elemental analysis found %C 74.18 and %H 5.42; calcd. for $C_{29}H_{24}O_6$ (MW. 468.50): %C 74.35 and %H 5.16.

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **17** are shown in Figs 1-4, respectively.

3,3′-(3-Methoxybenzylidene)*bis*-4-hydroxycoumarin (**20**)[25]

White crystal (89 %), m.p. 256-257 °C (dichloromethane-ethanol) (lit.[25] m.p. 249-250 °C), R_f 0.45 (ethyl acetate). IR (KBr) 3300-2500, 3083, 3002, 2962, 2936, 2830, 1671, 1612, 1572, 1488, 1455, 1440, 1352 and 1102 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.74 (3H, s), 6.07 (1H, s), 6.78-6.83 (3H, m), 7.25 (1H, s), 7.34-7.42 (4H, m), 7.58 (2H, t, J = 8.78 Hz), 8.03 (2H, s, br), 11.26 (1H, s, br) and 11.57 (1H, s, br); ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.1 (1C, CH), 55.2 (1C, OCH_3), 103.8, 105.7 (2x1C, C-3), 111.2 (1C, C-d), 113.4 (1C, C-b), 116.6 (4C, C-8, C-10), 118.9 (1C, C-f), 124.4 (2C, C-5), 124.8 (2C, C-6), 129.6 (1C, C-e), 132.8 (2C, C-7), 137.0 (1C, C-a), 152.5 (2C, C-9), 159.9 (1C, C-c), 164.6, 165.7 (2x1C, C-2), 166.8 and 169.3 (2x1C, C-4).

3,3′-(3-Phenoxybenzylidene)*bis*-4-hydroxycoumarin (**23**)

White crystal (84 %), m.p. 182-183 °C (dichloromethane-ethanol), R_f 0.47 (ethyl acetate). IR (KBr) 3300-2600, 3076, 3024, 2903, 1667, 1612, 1565, 1495, 1444, 1363 and 1099 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.07 (1H,s), 6.86-6.99 (6H, m), 7.21-7.31 (3H, m), 7.35-7.40 (4H, m), 7.60 (2H, dt, J = 8.82, 1.70 Hz), 8.01 (2H, s, br), 11.29 (1H, s, br) and 11.63 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 36.1 (1C, CH), 103.7, 105.5 (2x1C, C-3), 116.4 (2C, C-10), 116.6 (2C, C-8), 117.2 (1C, C-d), 117.6 (1C, C-b), 118.6 (2C, C-b′), 121.4 (1C, C-f), 123.1 (1C, C-d′), 124.4 (2C, C-6), 124.8 (2C, C-5), 129.7 (2C, C-c′), 129.9 (1C, C-e), 132.9 (2C, C-7), 137.5 (1C, C-a), 152.3, 152.5 (2x1C, C-9), 157.0 (1C, C-c), 157.4 (1C, C-a′), 164.7, 165.8 (2x1C, C-2), 166.9 and 169.2 (2x1C, C-4); MS *m/z* (% rel. int.): 504 (M^+, 5), 341 (30), 249 (80), 162 (100), 120 (90) and 92 (90); Elemental analysis found %C 73.41 and %H 3.86; calcd. for $C_{31}H_{20}O_7$ (MW. 504.50): %C 73.80 and %H 4.00.

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **23** are shown in Figs 5-8, respectively.

3,3′-(3,4,5-Trimethoxybenzylidene)*bis*-4-hydroxycoumarin (**26**)[26,27]

Small white crystal (79 %), m.p. 237-239 °C (dichloromethane-ethanol) (lit.[26] m.p. 246-248 °C), R_f 0.37 (ethyl acetate). IR (KBr) 3300-2600, 3072, 2951, 2933, 2834, 1656, 1568, 1510, 1451, 1348 and 1128 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.70 (6H, s), 3.83 (3H, s), 6.06 (1H, s), 6.40 (2H, s), 7.35-7.42 (4H, m), 7.62 (2H, t, J = 8.62 Hz), 8.03 (2H, s, br), 11.28 (1H, s, br) and 11.53 (1H, s, br); ^{13}C-NMR ($CDCl_3$) δ (ppm): 32.6 (1C, CH), 56.3 (2C, 3-OCH_3), 60.9 (1C, 4-OCH_3), 104.3 (3C, C-3, 2C-b), 105.6 (1C, C-3), 116.7 (4C, C-8, C-10), 124.3 (2C, C-5), 125.0 (2C, C-6), 130.9 (1C, C-a), 132.9 (2C, C-7), 137.2 (1C, C-d), 152.4 (2C, C-9), 153.4 (2C, C-c), 164.7, 165.7 (2x1C, C-2), 166.7 and 169.1 (2x1C, C-4).

3,3′-(4-Ethoxy-3-methoxybenzylidene)*bis*-4-hydroxycoumarin (**29**)

Yellow amorphous solid (85 %), m.p. 137-139 °C (dichloromethane-ethanol), R_f 0.42 (ethyl acetate). IR (KBr) 3670-3290, 3076, 2980, 2933, 2903, 2867, 1667, 1620, 1565, 1521, 1451, 1352, 1143 and 1095 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.43 (3H, t, J = 6.95 Hz), 3.71 (3H, s), 4.06 (2H, q, J = 6.97 Hz), 6.05 (1H, s), 6.69-6.82 (3H, m), 7.32-7.40 (4H, m), 7.60 (2H, t, J = 8.01 Hz), 8.01 (2H, d, J = 6.64 Hz) and 11.48 (2H, s, br); ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.8 (1C, CH_3), 35.7 (1C, CH), 56.2 (1C, OCH_3), 64.3 (1C, OCH_2), 104.2, 105.3 (2x1C, C-3), 110.8 (1C, C-b), 112.7 (1C, C-f), 116.6 (4C, 2C-8, 2C-10), 119.0 (1C, C-e), 124.3 (2C, C-5), 124.9 (2C, C-6), 127.5 (1C, C-a), 132.8 (2C, C-7), 147.4 (1C, C-d), 149.4 (1C, C-c), 152.4 (2C, C-9), 164.8, 165.5 (2x1C, C-2), 166.9 and 169.3 (2x1C, C-4); MS m/z (% rel. int.): 486 (M^+, 1), 324 (10), 295 (10), 162 (60), 120 (100) and 92 (80).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **29** are shown in Figs 9-12, respectively.

3,3′-(4-*n*-Butoxy-3-methoxybenzylidene)*bis*-4-hydroxycoumarin (**30**)

Yellow amorphous solid (86 %), m.p. 169-170 °C (dichloromethane-ethanol), R_f 0.45 (ethyl acetate). IR (KBr) 3640-3370, 3300-2600, 3076, 2958, 2936, 2881, 1675, 1605, 1565, 1517, 1455, 1352, 1143 and 1095 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.95 (3H, t, J = 7.22 Hz), 1.46 (2H, m), 1.80 (2H, m), 3.70 (3H, s), 3.98 (2H, t, J = 6.72 Hz), 6.05 (1H, s), 6.69-6.82 (3H, m), 7.32-7.40 (4H, m), 7.60 (2H, dt, J = 7.26, 1.66 Hz), 8.01 (2H, d, J = 6.08 Hz) and 11.49 (2H, s, br); ^{13}C-NMR ($CDCl_3$) δ (ppm): 13.9 (1C, CH_3), 19.2, 31.3 (2C, CH_2), 35.7 (1C, CH), 56.4 (1C, OCH_3), 68.7 (1C, OCH_2), 104.2, 105.8 (2x1C, C-3), 111.1 (1C, C-b), 112.8 (1C, C-f), 116.6 (4C, 2C-8, 2C-10), 119.0 (1C, C-e), 124.3 (2C, C-5), 124.9 (2C, C-6), 127.4 (1C, C-a), 132.8 (2C, C-7), 147.7 (1C, C-d), 149.5 (1C, C-c), 152.4 (2C, C-9), 164.7, 165.5 (2x1C, C-2), 166.8 and 169.3 (2x1C, C-4); MS m/z (% rel. int.): 514 (M^+, 1), 352 (10), 295 (10), 162 (50), 120 (70) and 92 (100); Elemental analysis found %C 69.61 and %H 5.20; calcd. for $C_{30}H_{26}O_8$ (MW. 514.53): %C 70.03 and %H 5.09.

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **30** are shown in Fig 13-16, respectively.

3,3′-(4-*n*-Hexyloxy-3-methoxybenzylidene)*bis*-4-hydroxycoumarin (**31**)

Light yellow solid (82 %), m.p. 142-144 °C (dichloromethane-ethanol), R_f 0.48 (ethyl acetate). IR (KBr) 3650-3320, 3300-2500, 3079, 2958, 2933, 2863, 1678, 1612, 1605, 1572, 1517, 1455, 1352, 1146 and 1095 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.89 (3H, t, J = 6.66 Hz), 1.24-1.49 (5H, m, br), 1.68 (1H, s), 1.80 (2H, m), 3.77 (3H, s), 3.99 (2H, t, J = 6.80 Hz), 6.07 (1H, s), 6.71-6.84 (3H, m), 7.35-7.42 (4H, m), 7.62 (2H, t, J = 7.70 Hz), 8.02 (2H, d, J = 5.50 Hz), 11.30 (1H, s, br) and 11.51 (1H, s, br); ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.0 (1C, CH_3), 22.6, 25.7, 30.2, 31.6 (4C, CH_2), 35.7 (1C, CH), 56.4 (1C, OCH_3), 69.0 (1C, OCH_2), 104.2, 105.8 (2x1C, C-3), 111.1 (1C, C-b), 112.9 (1C, C-f), 116.6 (4C, 2C-8, 2C-10), 119.0 (1C, C-e), 124.3 (2C, C-5), 124.9 (2C, C-6), 127.4 (1C, C-a), 132.8 (2C, C-7), 147.7 (1C, C-d), 149.5 (1C, C-c), 152.4 (2C, C-9), 164.9, 165.5 (2x1C, C-2), 167.5 and 169.3 (2x1C, C-4); MS m/z (% rel. int.): 380 (20), 295 (20), 162 (80) and 120 (100).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **31** are shown in Figs 17-20, respectively.

3,3′-(3-Methoxy-4-*n*-octyloxybenzylidene)*bis*-4-hydroxycoumarin (**32**)

White amorphous solid (61 %), m.p. 141-142 °C (dichloromethane-ethanol), R_f 0.50 (ethyl acetate). IR (KBr) 3250-2600, 3072, 3013, 2936, 2867, 1667, 1620, 1572, 1521, 1451, 1352, 1143 and 1099 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.86 (3H, t, J = 6.72 Hz), 1.27 (9H, s), 1.59 (1H, s), 1.81 (2H, m), 3.70 (3H, s), 3.97 (2H, t, J = 6.80 Hz), 6.05 (1H, s), 6.69-6.82 (3H, m), 7.33-7.41 (4H, m), 7.61 (2H, t, J = 7.81 Hz), 8.02 (2H, s, br), 11.29 (1H, s, br) and 11.47 (1H, s, br); ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.1 (1C, CH_3), 20.0, 22.6, 26.0, 29.2, 29.4, 31.8 (6C, CH_2), 35.5 (1C, CH), 56.4 (1C, OCH_3), 69.0 (1C, OCH_2), 104.4, 105.8 (2x1C, C-3), 111.1 (1C, C-b), 112.9 (1C, C-f), 116.6 (4C, 2C-8, 2C-10), 119.0 (1C, C-e), 124.3 (2C, C-5), 124.9 (2C, C-6), 127.4 (1C, C-a), 132.8 (2C, C-7), 147.7 (1C, C-d), 149.5 (1C, C-c), 152.4 (2C, C-9), 164.6, 165.6 (2x1C, C-2), 166.8 and 169.0 (2x1C, C-4). MS m/z (% rel. int.): 365 (1), 178 (100), 161 (45) and 105 (35); Elemental analysis found %C 71.42 and %H 5.82; calcd. for $C_{34}H_{34}O_8$ (MW. 570.63): %C 71.56 and %H 6.01.

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **32** are shown in Figs 21-24, respectively.

3,3′-(4-*n*-Dodecyloxy-3-methoxybenzylidene)*bis*-4-hydroxycoumarin (**33**)

White amorphous solid (77 %), m.p. 110-112 °C (dichloromethane-ethanol), R_f 0.50 (ethyl acetate). IR (KBr) 3300-2500, 3068, 2922, 2852, 1667, 1623, 1524, 1455, 1348, 1146 and 1099 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.85 (3H, t, J = 6.36 Hz), 1.24 (17H, s, br), 1.80 (3H, m), 3.70 (3H, s), 3.97 (2H, t, J = 6.82 Hz), 6.04 (1H, s), 6.69-6.82 (3H, m), 7.32-7.41 (4H, m), 7.60 (2H, dt, J = 7.81, 1.56 Hz), 8.00 (2H, s, br), 11.29 (1H, s, br) and 11.49 (1H, s, br); ^{13}C-NMR ($CDCl_3$) δ (ppm): 15.7 (1C, CH_3), 20.0, 22.7, 26.0, 29.2, 29.4, 29.6, 31.9 (10C, CH_2), 35.7 (1C, CH), 56.3 (1C, OCH_3), 69.0 (1C, OCH_2), 104.2, 105.8 (2x1C, C-3), 111.1 (1C, C-b), 112.9 (1C, C-f), 116.6 (4C, 2C-8, 2C-10), 119.0 (1C, C-e), 124.3 (2C, C-5), 124.9 (2C, C-6), 127.4 (1C, C-a), 132.8 (2C, C-7), 147.7 (1C, C-d), 149.5 (1C, C-c), 152.4 (2C, C-9), 164.5, 165.5 (2x1C, C-2), 166.9 and 169.3 (2x1C, C-4); MS m/z (% rel. int.): 464 (10), 295 (10), 178 (10), 162 (80) and 120 (100); Elemental analysis found %C 72.62 and %H 6.90; calcd. for $C_{38}H_{42}O_8$ (MW. 626.74): %C 72.82 and %H 6.75.

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **33** are shown in Figs 25-28, respectively.

3,3′-(4-Benzyloxy-3-methoxybenzylidene)*bis*-4-hydroxycoumarin (**34**)[28]

Light-yellow solid (94 %), m.p. 207-209 °C (dichloromethane-ethanol), R_f 0.45 (ethyl acetate). IR (KBr) 3660-3280, 3200-2500, 3068, 3028, 2940, 2874, 1671, 1623, 1605, 1565, 1521, 1458, 1345, 1143 and 1095 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.74 (3H, s), 5.13 (2H, s), 6.05 (1H, s), 6.68-6.85 (3H, m), 7.25-7.42 (9H, m), 7.62 (2H, t, J = 7.72 Hz), 8.02 (2H, s, br), 11.30 (1H, s, br) and 11.51 (1H, s, br); ^{13}C-NMR ($CDCl_3$) δ (ppm): 35.8 (1C, CH), 56.3 (1C, OCH_3), 71.1 (1C, OCH_2), 104.1, 105.8 (2x1C, C-3), 111.1 (1C, C-b), 114.0 (1C, C-f), 116.6 (4C, 2C-8, 2C-10), 119.0 (1C, C-e), 124.3 (2C, C-5), 124.9 (2C, C-6), 127.3 (1C, C-a), 128.1 (1C, C-d′), 128.5 (4C, 2C-b′, 2C-c′), 132.9 (2C, C-7), 137.2 (1C, C-a′), 147.3 (1C, C-d), 149.8 (1C, C-c), 152.4 (2C, C-9), 164.7, 165.6 (2x1C, C-2), 166.8 and 169.2 (2x1C, C-4).

3,3′-(Ethylidene)*bis*-4-hydroxycoumarin (**35**)[13,22]

White crystal (64 %), m.p. 176-178 °C (dichloromethane-ethanol) (lit.[13] m.p. 176-178 °C), R_f 0.39 (ethyl acetate). IR (KBr) 3400-2600, 3080, 2991, 2878, 1644, 1619, 1565, 1491, 1452, 1349 and 1127 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.84 (3H, d, J = 7.42 Hz), 4.70 (1H, q, J = 7.40 Hz), 7.29-7.37 (4H, m), 7.56 (2H, t, J = 7.92 Hz) 7.99 (2H, d, J = 7.63 Hz), 11.23 (1H, s) and 12.03 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 14.9 (1C, CH_3), 26.1 (1C, CH), 106.3 (2C, C-3), 115.7 (2C, C-8), 116.8 (2C, C-10), 123.5 (2C, C-5), 124.0 (2C, C-6), 131.7 (2C, C-7), 151.6 (2C, C-9), 164.2 (2C, C-2) and 166.8 (2C, C-4).

3,3′-(*n*-Butylidene)*bis*-4-hydroxycoumarin (**36**)[13,29]

White crystal (47 %), m.p. 116-118 °C (dichloromethane-ethanol) (lit.[29] m.p. 121-123 °C), R_f 0.46 (ethyl acetate). IR (KBr) 3300-2500, 3083, 2955, 2874, 1656, 1616, 1605, 1568, 1495, 1451, 1323 and 1124 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.92 (3H, t, J = 7.15 Hz), 1.30 (2H, m), 2.34 (2H, m), 4.48 (1H, t, J = 7.86 Hz), 7.29-7.36 (4H, m), 7.55 (2H, t, J = 7.36 Hz), 7.97 (2H, d, J = 8.38 Hz), 11.18 (1H, s) and 12.01 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 13.8 (1C, CH_3), 21.8, 30.5 (2x1C, CH_2), 32.6 (1C, CH), 105.8, 106.0 (2x1C, C-3), 116.5 (2C, C-8), 117.2 (2C, C-10), 124.0, 124.2 (2x1C, C-5), 124.7 (2C, C-6), 132.4, 132.5 (2x1C, C-7), 152.0, 152.3 (2x1C, C-9), 164.3, 164.7 (2x1C, C-2) and 167.5, 169.2 (2x1C, C-4).

3,3′-(*Iso*butylidene)*bis*-4-hydroxycoumarin (**37**)[13,22]

White crystal (27 %), m.p. 202-205 °C (dichloromethane-ethanol) (lit.[22] m.p. 209-210 °C), R_f 0.49 (ethyl acetate). IR (KBr) 3300-2500, 3079, 2984, 2955, 2933, 2870, 1664, 1601, 1557, 1499, 1455 and 1132 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.93 (3H, d, J = 6.30 Hz), 0.96 (3H, d, J = 6.40 Hz), 3.31 (1H, m), 4.00 (1H, d, J = 11.22 Hz), 7.29-7.37 (4H, m), 7.56 (2H, t, J = 7.90 Hz), 7.99 (2H, d, J = 8.40 Hz), 11.13 (1H, s, br) and 12.00 (1H, s, br); ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.7, 21.9 (2x1C, CH_3), 25.7 (1C, CH-<u>C</u>H-(CH_3)$_2$), 41.4 (1C, <u>C</u>H-CH-(CH_3)$_2$), 105.5, 106.0 (2x1C, C-3), 116.3 (1C, C-10), 116.5 (2C, C-8), 117.0 (1C, C-10), 124.1, 124.3 (2x1C, C-5), 124.7, 124.8 (2x1C, C-6), 132.5 (2C, C-7), 152.1, 152.3 (2x1C, C-9), 163.9, 165.2 (2x1C, C-2) and 167.5, 169.4 (2x1C, C-4).

3,3'-(2-Ethyl-1-butylidene)*bis*-4-hydroxycoumarin (**38**)

Small white crystal (41 %), m.p. 165-167 °C (dichloromethane-ethanol), R_f 0.50 (ethyl acetate). IR (KBr) 3300-2500, 3079, 2966, 2874, 1667, 1601, 1568, 1495, 1455, 1348 and 1080 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.80 (6H, t, J = 7.38 Hz), 1.17-1.53 (4H, m), 3.08 (1H, m), 4.30 (1H, d, J = 11.72 Hz), 7.29-7.38 (4H, m), 7.56 (2H, dt, J = 7.50, 2.56 Hz), 7.98 (2H, dd, J = 8.36, 1.86 Hz), 11.18 (1H, s) and 12.05 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 9.3, 9.4 (2x1C, CH_3), 21.9, 22.2 (2x1C, CH_2), 35.7, 35.9 (2x1C, CH), 105.1, 105.8 (2x1C, C-3), 116.4 (1C, C-10), 116.5 (2C, C-8), 117.0 (1C, C-10), 124.1, 124.2 (2x1C, C-5), 124.7, 124.8 (2x1C, C-6), 132.4, 132.5 (2x1C, C-7), 152.1, 152.3 (2x1C, C-9), 164.2, 165.1 (2x1C, C-2), 167.7 and 169.4 (2x1C, C-4); MS *m/z* (% rel. int.): 406 (M^+, 25), 335 (55), 244 (33), 241 (70), 215 (90), 162 (40), 121 (100), 120 (65) and 92 (50); Elemental analysis found %C 70.88 and %H 5.66; calcd. for $C_{24}H_{22}O_6$ (MW. 406.43): %C 70.93 and %H 5.46.

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **38** are shown in Figs 29-32, respectively.

3,3'-(Cyclohexylmethylidene)*bis*-4-hydroxycoumarin (**39**)[30]

Small white crystal (37 %), m.p. 209-211 °C (dichloromethane-ethanol) (lit[30] m.p. 201°C), R_f 0.52 (ethyl acetate). IR (KBr) 3300-2500, 3079, 2929, 2852, 1660, 1612, 1565, 1495, 1447, 1323 and 1099 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.89 (2H, m), 1.28 (2H, m), 1.69 (6H, m), 2.95 (1H, m), 4.14 (1H, d, J = 11.2 Hz), 7.21-7.40 (4H, m), 7.58 (2H, t, J = 9.08 Hz), 8.00 (2H, d, J = 7.82 Hz), 11.13 (1H, s) and 12.03 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 25.8, 25.9, 26.2, 31.8, 32.3, 34.5 (6C, cyclohexyl moeity), 39.7 (1C, CH-cyclohexyl), 104.8, 105.5 (2x1C, C-3), 116.5 (2C, C-8), 117.0 (2C, C-10), 124.1, 124.2 (2x1C, C-5), 124.7, 124.8 (2x1C, C-6), 132.4 (2C, C-7), 152.1, 152.3 (2x1C, C-9), 163.9,165.3 (2x1C, C-2), 167.5 and 169.4 (2x1C, C-4).

3,3′-(2-Phenylethylidene)bis-4-hydroxycoumarin **(40)**[13,22]

White crystal (40 %), m.p. 184-185 °C (dichloromethane-ethanol) (lit.[22] m.p. 190-192 °C), R_f 0.47 (hexane/ethyl acetate [1:9]). IR (KBr) 3400-2400, 3090, 3040, 2950, 2850, 1650, 1600, 1550, 1510, 1300 and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 3.69 (2H, m), 4.83 (1H, t, J = 8.20 Hz), 7.08-7.20 (5H, m), 7.25-7.37 (4H, m), 7.55 (2H, t, J = 6.94 Hz), 7.92 (1H, d, J = 8.06 Hz), 8.01 (1H, d, J = 8.36 Hz), 11.17 (1H, s) and 12.26 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 34.4 (1C, CH_2), 34.9 (1C, CH), 105.5 (2C, C-3), 116.2 (1C, C-10), 116.5 (2C, C-8), 117.1 (1C, C-10), 124.0, 124.3 (2x1C, C-5), 124.8 (2C, C-6), 126.6 (1C, C-d), 128.5 (2C, C-b), 128.6 (2C, C-c), 132.5, 132.6 (2x1C, C-7), 139.0 (1C, C-a), 152.0, 152.3 (2x1C, C-9), 164.5, 165.0 (2x1C, C-2), 167.8 and 169.1 (2x1C, C-4).

(±)-3.3′-(2-phenylpropylidene)*bis*-4-hydroxycoumarin **(41)**[31]

White crystal (54 %), m.p. 186-188 °C (dichloromethane-ethanol), R_f 0.44 (ethyl acetate). IR (KBr) 3300-2400, 3090, 3020, 2950, 2850, 1650, 1600, 1550, 1500, 1300 and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.24 (3H, d, J = 7.02 Hz), 1.27 (3H, d, J = 6.92 Hz), 4.47 (2H, m), 4.63 (1H, d, J = 4.98 Hz), 4.69 (1H, d, J = 5.06 Hz), 7.03-7.22 (10H, m), 7.27-7.45 (8H, m), 7.58 (4H, m), 7.86 (2H, m), 8.03 (2H, d, J = 7.92 Hz), 11.0 (1H, s), 11.3 (1H, s), 12.11 (1H, s) and 12.37 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 21.6, 21.8 (2x1C, CH_3), 37.1, 37.3, 40.3, 40.4 (4x1C, CH), 104.9, 105.2, 105.6, 106.2 (4x1C, C-3), 115.9, 116.3, 116.5, 116.8, 117.1 (8C, C-8, C-10), 123.8, 124.1, 124.3, 124.6, 124.8, 124.9 (8C, C-5, C-6), 126.6, 126.7 (2x1C, C-d), 128.5, 128.6 (2x2C, C-b, C-c), 132.2, 132.3, 132.6, 132.7 (4x1C, C-7), 144.2, 144.5 (2x1C, C-a), 151.8, 152.1, 152.4 (C-9), 163.9, 164.3, 164.5, 165.8 (4x1C, C-2), 167.6, 167.8, 169.1 and 169.5 (4x1C, C-4); MS *m/z* (% rel. int.): 440 (M^+, 23), 335 (100), 278 (20), 241 (80), 162 (20) and 121 (53).

3,3′-(3-Phenylpropylidene)*bis*-4-hydroxycoumarin **(42)**[13]

Small white crystal (40 %), m.p. 198-199 °C (dichloromethane-ethanol) (lit.[13] m.p. 197-198 °C), R_f 0.45 (hexane/ethyl acetate [1:9]). IR (KBr) 3300-2400, 3080, 3050, 2975, 2920, 2850, 1650, 1600, 1560, 1500, 1450, 1350 and 1100 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.64-2.79 (4H, m), 4.48 (1H, t, J = 7.34 Hz), 7.02-7.22 (5H, m), 7.29-7.37 (4H, m), 7.56 (2H, t, J = 7.74 Hz), 7.97 (2H, dd, J = 8.12, 1.64 Hz), 11.15 (1H, s) and 12.05 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 30.3, 32.6 (2x1C, CH_2), 35.0 (1C, CH), 105.7, 105.8 (2x1C, C-3), 116.5 (2C, C-8), 117.1 (2C, C-10), 124.0, 124.2 (2x1C, C-5), 124.7, 124.8 (2x1C, C-6), 126.0 (1C, C-d), 128.4 (4C, C-b, C-c), 132.5, 132.6 (2x1C, C-7), 140.8 (1C, C-a), 152.1, 152.3 (2x1C, C-9), 164.3, 164.8 (2x1C, C-2), 167.5 and 169.1 (2x1C, C-4).

3,3′-((E)-3-Phenylprop-2-enylidene)*bis*-4-hydroxycoumarin **(43)**[25,32]

White needle crystal (90 %), m.p. 174-175 °C (dichloromethane-ethanol) (lit.[32] m.p. 220-224 °C), R_f 0.39 (hexane/ethyl acetate [1:9]). IR (KBr) 3320-2600, 3083, 3028, 2914, 1675, 1601, 1572, 1499, 1455, 1352, and 1102 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.47 (1H, dd, J = 4.45, 1.96 Hz), 6.52 (1H, dd, J = 16.07, 1.87 Hz), 6.76 (1H, dd, J = 16.06, 4.46 Hz), 7.17-7.31 (5H, m), 7.35-7.42 (4H, m), 7.59 (2H, t, J = 7.81 Hz), 8.01 (2H, d, J = 7.34 Hz), 11.27 (1H, s) and 11.76 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 34.6 (1C, CH), 105.1, 106.3 (2x1C, C-3), 116.6 (4C, C-8, C-10), 124.3 (2C, C-5), 124.8 (2C, C-6), 125.0 (1C, CH=CHAr), 126.4 (2C, C-c), 127.7 (1C, C-d), 128.6 (2C, C-b), 132.3 (1C, CH=CHAr), 132.7 (2C, C-7), 136.7 (1C, C-a), 152.3 (2C, C-9), 164.3 (2C, C-2), 167.0 and 168.9 (2x1C, C-4).

3,3′-(4-Phenyl-1-butylidene)*bis*-4-hydroxycoumarin (**44**)

White crystal (37 %), m.p. 162-164 °C (dichloromethane-ethanol), R_f 0.56 (ethyl acetate). IR (KBr) 3300-2500, 3083, 3028, 2977, 2955, 2856, 1667, 1616, 1601, 1568, 1502, 1458, 1352, 1133 and 1102 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.62 (2H, q, J = 7.76 Hz), 2.41 (2H, m), 2.64 (2H, t, J = 7.66 Hz), 4.49 (1H, t, J = 8.11 Hz), 7.10-7.21 (5H, m), 7.33-7.36 (4H, m), 7.56 (2H, t, J = 7.95 Hz), 7.97 (2H, d, J = 8.42 Hz), 11.18 (1H, s) and 12.01 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 28.0, 30.5, 32.9 (3C, CH_2), 35.5 (1C, CH), 105.6, 105.8 (2x1C, C-3), 116.4 (1C, C-10), 116.5 (2C, C-8), 117.1 (1C, C-10), 124.0, 124.2 (2x1C, C-5), 124.7, 124.9 (2x1C, C-6), 125.9 (1C, C-d), 128.3 (4C, C-b, C-c), 132.5, 132.6 (2x1C, C-7), 141.7 (1C, C-a), 152.1, 152.3 (2x1C, C-9), 164.3, 164.9 (2x1C, C-2), 167.5 and 169.2 (2x1C, C-4); MS *m/z* (% rel. int.): 454 (M^+, 1), 292 (40), 162 (75) and 120 (100); Elemental analysis found %C 73.93 and %H 4.87; calcd. for $C_{28}H_{22}O_6$ (MW. 454.48): %C 74.00 and %H 4.88.

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **44** are shown in Figs 33-36, respectively.

3-[2-Chloro-6-oxo(1)benzopyrano(4,3-*b*)-(1)benzopyran-7-yl]4-hydroxy coumarin (**47**)[33]

Small white crystal (49 %), m.p. 306-307 °C (dichloromethane-ethanol) (lit.[33] m.p. 318-320 °C), R_f 0.58 (ethyl acetate). IR (KBr) 3550-3200, 3083, 2984, 2944, 2896, 1711, 1645, 1609, 1565, 1500, 1451, 1392, 1117 and 1055 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 5.39 (1H, s), 7.14-7.23 (3H, m), 7.26-7.44 (4H, m), 7.57 (2H, t, J = 6.95 Hz), 8.02 (1H, d, J = 8.36 Hz), 8.08 (1H, d, J = 8.06 Hz) and 10.19 (1H, s, br); ^{13}C-NMR ($CDCl_3$) δ (ppm): 28.9 (1C, CH), 100.4 (1C, C-3), 106.3 (1C, C-3′), 114.2 (1C, C-10′), 115.0 (1C, C-8′), 116.2 (1C, C-8), 116.6 (1C, C-10), 117.0 (1C, C-c), 119.8 (1C, C-a), 123.3 (1C, C-5′), 123.9 (1C, C-5), 124.5 (1C, C-6′), 125.1 (1C, C-6), 126.2 (1C, C-e), 129.1 (1C, C-d), 132.0 (1C, C-7′), 132.9 (1C, C-7), 133.6 (1C, C-f), 152.1 (1C, C-9′), 152.3 (1C, C-4′), 153.1 (1C, C-9), 158.3 (1C, C-2′), 161.3 (1C, C-2), 162.1 (1C, C-b) and 165.6 (1C, C-4).

3,3',3'',3'''-(1,4-Phenylmethylidene)*tetrakis*-4-hydroxycoumarin (**49**)[19,34]

White amorphous solid (74 %), m.p. 299-301 °C (ethanol), R_f 0.60 (chloroform/ethanol [1:1]). IR (KBr) 3600-3350, 3300-2500, 3076, 3043, 2988, 2896, 1664, 1620, 1601, 1565, 1499, 1458, 1352 and 1099 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.29 (2H, s), 6.99 (4H, s), 7.25-7.35 (8H, m), 7.56 (4H, t, J = 7.30 Hz) and 7.87 (4H, d, J = 7.62 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 35.6 (2C, CH), 104.2 (4C, C-3), 116.0 (4C, C-8), 117.6 (4C, C-10), 123.8 (8C, C-5, C-6), 126.6 (4C, C-b), 132.0 (4C, C-7), 136.7 (2C, C-a), 152.1 (4C, C-9) and 164.8 (8C, C-2, C-4).

3,3',3'',3'''-(1,5-Pentylidene)*tetrakis*-4-hydroxycoumarin (**50**)

Small white crystal (18 %), m.p. 229-230 °C (ethyl acetate-ethanol), R_f 0.67 (chloroform/ethanol [1:1]). IR (KBr) 3300-2500, 3079, 2973, 2933, 2863, 1671, 1609, 1568, 1491, 1455, 1352 and 1117 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.17 (2H, br), 2.11 (4H, d, br, J = 6.61 Hz), 4.81 (2H, t, br, J = 8.15 Hz), 7.24-7.31 (8H, m), 7.56 (4H, t, J = 7.52 Hz) and 7.80 (4H, d, J = 7.23 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 26.0 (1C, CH_2), 28.9 (2C, CH_2), 31.4 (2C, CH), 105.1 (4C, C-3), 115.9 (4C, C-8), 117.3 (4C, C-10), 123.5 (4C, C-5), 123.8 (4C, C-6), 131.8 (4C, C-7), 151.8 (4C, C-9), 164.0 (4C, C-2) and 164.9 (4C, C-4); MS m/z (% rel. int.): 162 (70), 121 (30) and 120 (100); Elemental analysis found %C 67.37 and %H 3.97; calcd. for $C_{41}H_{28}O_{12}.H_2O$(MW. 730.67): %C 67.40 and %H 4.14.

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **50** are shown in Figs 37-40, respectively.

3,3'-(1-Naphthalenylmethylidene)*bis*-4-hydroxycoumarin (**51**)[35]

White amorphous solid (67 %), m.p. 208-209 °C (lit.[35] m.p. 214-215 °C), R_f 0.44 (ethyl acetate). IR (KBr) 3600-3300, 3300-2500, 3079, 1664, 1612, 1572, 1495, 1308 and 1095 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 6.66 (1H, s), 7.28-8.14 (15H, m), 11.13 (1H, br, s) and 11.40 (1H, br, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 35.2 (1C, CH), 105.3, 107.3 (2x1C, C-3), 116.6 (4C, C-8, C-10), 122.8 (1C, naphthyl moiety), 124.5 (2C, C-5), 124.9 (2C, C-6), 125.0, 125.5, 126.3, 126.5, 129.4, 129.7, 130.8, 131.2 (8C, naphthyl moiety), 133.2 (2C, C-7), 134.6 (1C, naphthyl moiety), 152.2 (2C, C-9), 164.6, 165.0 (2x1C, C-2), 166.9 and 168.9 (2x1C, C-4).

3,3'-(2-Pyridinylmethylidene)*bis*-4-hydroxycoumarin (**52**)[33,36]

Light-yellow powder (64 %), m.p. 239-240 °C (ethyl acetate-ethanol) (lit.[36] m.p. 276 °C), R_f 0.64 (chloroform/ethanol [1:1]). IR (KBr) 3600-3300, 3123, 3098, 3068, 2940, 2874, 1700, 1642, 1616, 1543, 1495, 1458, 1183, 1113 and 1040 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.50 (1H, s), 7.22-7.34 (4H, m), 7.57 (2H, t, J = 7.09 Hz), 7.80 (2H, d, J = 7.63 Hz), 7.88 (2H, d, J = 7.67 Hz), 8.44 (1H, t, J = 7.94 Hz) and 8.62 (1H, d, J = 5.55 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 36.6 (1C, CH), 100.4 (2C, C-3), 115.8 (2C, C-8), 119.3 (2C, C-10), 123.2 (2C, C-5), 124.3 (2C, C-10), 125.7 (2C, C-d, C-f), 131.8 (2C, C-7), 141.8 (1C, C-e), 146.2 (1C, C-a), 152.8 (2C, C-9), 157.5 (1C, C-c), 163.8 (2C, C-2) and 168.5 (2C, C-4).

3,3'-(3-Pyridinylmethylidene)*bis*-4-hydroxycoumarin (**53**)[33,36]

White needle crystal (44 %), m.p. 268-269 °C (ethyl acetate-ethanol) (lit.[33] m.p. 278-281°C), R_f 0.55 (chloroform/ethanol [1:1]). IR (KBr) 3650-3300, 3145, 3109, 3061, 2984, 2922, 1737, 1678, 1612, 1561, 1458, 1187, 1106 and 1047 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 6.41 (1H, s), 7.21-7.31 (4H, m), 7.54 (2H, t, J = 7.67 Hz), 7.80 (2H, d, J = 7.62 Hz), 7.92 (1H, t, J = 6.88 Hz), 8.35 (1H, d, J = 8.13 Hz), 8.64-8.71 (2H, m); ^{13}C-NMR (DMSO-d_6) δ (ppm): 34.6 (1C, CH), 101.6 (2C, C-3), 115.7 (2C, C-8), 119.5 (2C, C-10), 123.1 (2C, C-5), 124.1 (2C, C-6), 126.6 (1C, C-e), 131.5 (2C, C-7), 139.1 (1C, C-f), 140.3 (1C, C-a), 142.8 (1C, C-d), 144.7 (1C, C-b), 152.7 (2C, C-9), 164.0 (2C, C-2) and 168.0 (2C, C-4).

(±)-3-(9-Anthracenylmethylidene)-chroman-2,4-dione (**54**)

Red crystal (84 %), m.p. 236-237 °C (dichloromethane-ethanol), R_f 0.72 (ethyl acetate). IR (KBr) 3050, 3024, 1748, 1667, 1612, 1580, 1462, 1363 and 1133 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.19-8.08 (15H, m), 8.21 (1H, dd, J = 6.44, 1.67 Hz), 8.56 (2H, d, J = 3.41 Hz), 9.59 (1H, s) and 9.68 (1H, s); ^{13}C-NMR ($CDCl_3$) δ (ppm): 117.6, 117.9 (1C, C-8), 119.7, 120.7 (1C, C-3), 124.9 (2C, C-5, C-6), 125.0 (4C, C-5, C-6, C-2′, C-7′), 125.1 (2C, C-2′, C-7′), 125.6 (2C, C-3′, C-6′), 127.2 (2C, C-1′, C-8′), 127.8, 128.1 (1C, C-α), 128.2, 128.3 (1C, C-10), 128.8, 129.1 (2C, C-11′, C-14′), 129.3, 129.4 (2C, C-4′, C-5′), 130.7 (1C, C-7), 130.9 (3C, C-7, C-12′, C-13′), 131.0 (2C, C-12′, C-13′), 136.5, 137.2 (1C, C-10′), 154.7, 155.7 (1C, C-9′), 157.7 (1C, C-2), 158.9, 159.1 (1C, C-9), 162.1 (1C, C-2), 177.8 and 179.9 (1C, C-4); MS m/z (% rel. int.): 350 (M^+, 100), 230 (65), 229(85), 202(50) and 200 (65).

The FT-IR, ^{1}H-NMR, ^{13}C-NMR, expanded ^{13}C-NMR, DEPT 135 and mass spectra of **54** are shown in Figs 41-46, respectively.

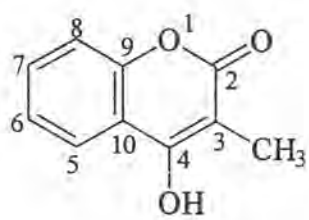
3-(1-Hydroxy-2-trichloroethylidene)-4-hydroxycoumarin (**55**)[26,36]

White crystal (20 %), m.p. 206-208 °C (dichloromethane-ethanol) (lit.[36] m.p. 208 °C), R_f 0.77 (chloroform/ethanol [1:1]). IR (KBr) 3500-2600, 2947, 1671, 1623, 1572, 1502, 1407, 1348, 1308, 1047, 1000 and 827 cm^{-1}; ^{1}H-NMR ($CDCl_3$/DMSO-d_6) δ (ppm): 5.69 (1H, s), 7.28-7.42 (2H, m), 7.60 (1H, t, J = 7.57 Hz) and 7.92 (1H, d, J = 8.21 Hz); ^{13}C-NMR ($CDCl_3$/DMSO-d_6) δ (ppm): 80.3 (1C, C-Cl), 98.9 (1C, α C-OH), 103.3 (1C, C-3), 115.9 (1C, C-10), 116.3 (1C, C-8), 123.9 (1C, C-5), 124.0 (1C, C-6), 132.9 (1C, C-7), 153.2 (1C, C-9), 162.5 (1C, C-2) and 165.9 (1C, C-4); MS m/z (% rel. int.): 291(2), 220 (20), 178 (45), 161 (20), 121 (30) and 57 (100).

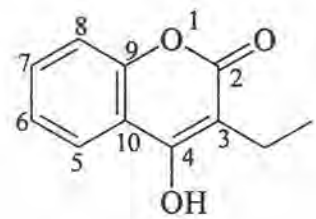
2.3.2 Synthesis of 3-Alkyl-4-hydroxycoumarins

General Procedure[14] **:**

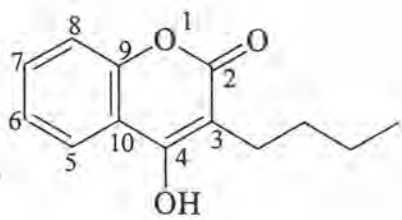
Sodium cyanoborohydride (2 mol-equiv) was added to the suspension of the dicoumarols in methanol. The reaction mixture was refluxed up to the complete disappearance of dicoumarols (TLC control (hexane/ethyl acetate [1:9]). The solvent was then removed on steam-bath, and saturated ammonium chloride solution was added to the residue. The mixture was extracted four times with ethyl acetate and the organic phase was washed twice with saturated sodium hydrogen carbonate. After washing with brine and drying over anhydrous sodium sulfate, the solvent was removed under vacuum. The residue was purified by column chromatography.



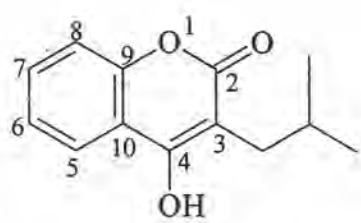
R1



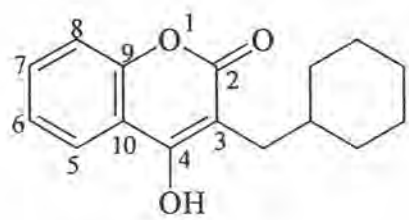
R2



R3

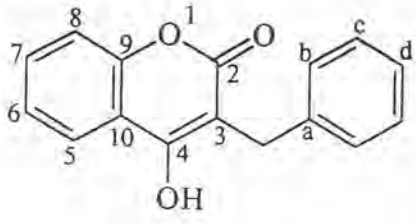


R4

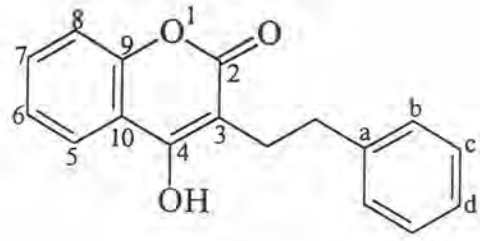


R5

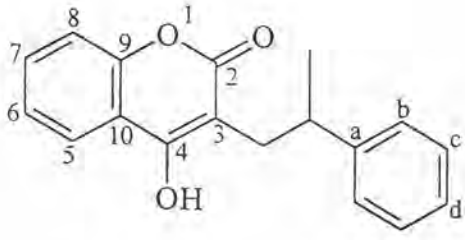
Fig 2.2 The structures of 3-alkyl-4-hydroxycoumarins



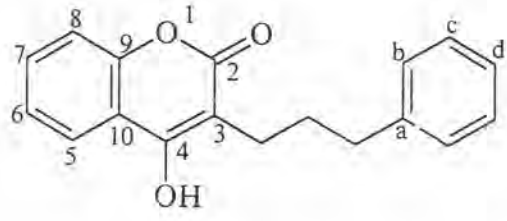
R6



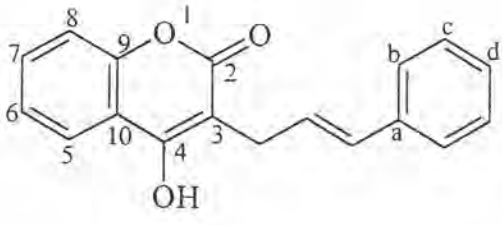
R7



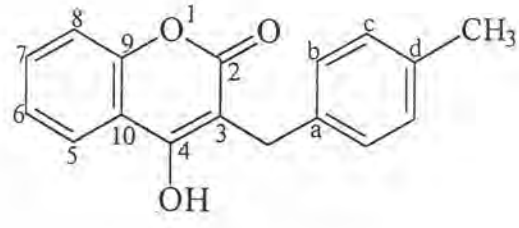
R8



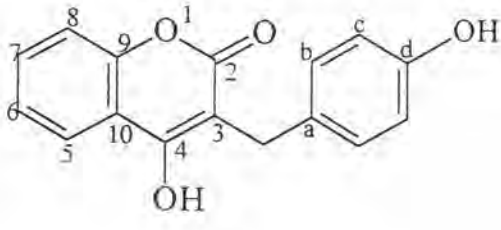
R9



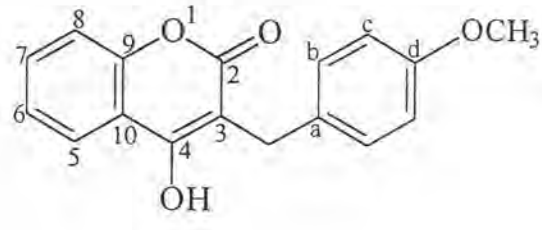
R10



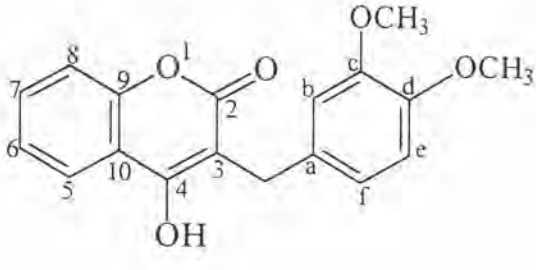
R11



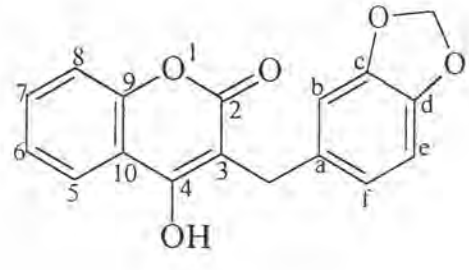
R12



R13



R14



R15

Fig 2.2 (cont.)

3-Methyl-4-hydroxycoumarin (**R1**)[10,37]

White solid (35 %), m.p. 229-230 °C (hexane/ethyl acetate [1:1]) (lit.[37] m.p. 228-230 °C), R_f 0.50 (ethyl acetate). IR (KBr) 3365-2936, 1671, 1616, 1572, 1502, 1458, 1378, 1242 and 1091 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.98 (3H, s), 7.28-7.36 (2H, m), 7.57 (1H, t, J = 7.77 Hz) and 7.88 (1H, d, J = 7.03 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 9.7 (1C, CH_3), 100.2 (1C, C-3), 116.0 (1C, C-8), 116.3 (1C, C-10), 122.9 (1C, C-5), 123.8 (1C, C-6), 131.4 (1C, C-7), 151.6 (1C, C-9), 159.7 (1C, C-2) and 163.1 (1C, C-4).

3-Ethyl-4-hydroxycoumarin (**R2**)[10,38]

White solid (78 %), m.p. 153-154 °C (hexane/ethyl acetate [1:4]) (lit.[38] m.p. 153 °C), R_f 0.58 (hexane/ethyl acetate [1:9]). IR (KBr) 3428-3000, 2977, 2936, 2870, 1678, 1616, 1572, 1499, 1447, 1227, 1209 and 1100 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.19 (3H, t, J = 7.51 Hz), 2.61 (2H, m), 7.24-7.32 (2H, m), 7.51 (1H, t, J = 6.84 Hz) and 7.81 (1H, d, J = 7.88 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 12.8 (1C, CH_3), 17.0 (1C, CH_2), 106.4 (1C, C-3), 116.0 (1C, C-8), 116.3 (1C, C-10), 123.1(1C, C-5), 123.8 (1C, C-6), 131.5 (1C, C-7), 151.7 (1C, C-9), 159.3 (1C, C-2) and 162.7 (1C, C-4).

3-*n*-Butyl-4-hydroxycoumarin (**R3**)[10,39]

White solid (89 %), m.p. 155-156 °C (hexane/ethyl acetate [1:4]) (lit.[39] m.p. 157 °C), R_f 0.67 (hexane/ethyl acetate [1:9]). IR (KBr) 3400-3000, 2955, 2925, 2867, 1675, 1616, 1568, 1499, 1462, 1205, 1180, 1095 and 1047 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.86 (3H, t, J = 6.67 Hz), 1.20-1.42 (6H, m), 7.27-7.32 (2H, m), 7.53 (1H, t, J = 7.98 Hz) and 7.92 (1H, d, J = 7.24 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 13.9 (1C, CH_3), 22.1, 23.4, 30.2 (3C, CH_2), 104.3 (1C, C-3), 115.9 (1C, C-8), 117.2 (1C, C-10), 123.3 (1C, C-5), 123.4 (1C, C-6), 131.1 (1C, C-7), 151.9 (1C, C-9), 161.1 (1C, C-2) and 163.1 (1C, C-4).

3-*Iso*butyl-4-hydroxycoumarin (**R4**)[40]

White solid (79 %), m.p. 137-139 °C (hexane/ethyl acetate [1:4]), R_f 0.71 (hexane/ethyl acetate [1:9]). IR (KBr) 3480-3000, 2955, 2870, 1690, 1671, 1609, 1568, 1506, 1462, 1389, 1209, 1180, 1117 and 1040 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.98 (6H, d, J = 6.54 Hz), 2.02 (1H, m), 2.45 (2H, d, J = 7.35 Hz), 7.24-7.33 (2H, m), 7.52 (1H, t, J = 7.12 Hz) and 7.82 (1H, d, J = 7.51 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 22.1 (2C, CH_3), 27.2 (1C, CH), 32.1 (1C, CH_2), 104.2 (1C,C-3), 116.0 (1C, C-8), 116.2 (1C, C-10), 123.1 (1C, C-5), 123.7 (1C, C-6), 131.5 (1C, C-7), 151.8 (1C, C-9), 160.1 (1C, C-2) and 163.0 (1C, C-4); MS *m/z* (% rel. int.): 218 (M^+, 40), 203 (33), 175 (100), 162 (55), 120 (50) and 107 (45).

3-(Cyclohexylmethyl)-4-hydroxycoumarin (**R5**)[14]

White solid (83 %), m.p. 184-186 °C (hexane/ethyl acetate [1:4]) (lit.[14] m.p. 182-184 °C), R_f 0.69 (hexane/ethylacetate [1:9]). IR (KBr) 3500-3000, 2951, 2922, 2856, 1678, 1616, 1565, 1499, 1458, 1286, 1213, 1084 and 1036 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 0.98-1.28 (4H, m), 1.66-1.77 (7H, m), 2.48 (2H, d, J = 6.98 Hz), 7.26-7.32 (2H, m), 7.51 (1H, t, J = 6.76 Hz) and 7.84 (1H, d, J = 7.98); ^{13}C-NMR (DMSO-d_6) δ (ppm): 25.8 (2C, C-c), 26.1 (1C, C-d), 30.7 (1C, C-a), 32.4 (2C, C-b), 36.6 (1C, CH_2), 103.8 (1C, C-3), 116.0 (1C, C-8), 116.2 (1C, C-10), 123.1 (1C, C-5), 123.7 (1C, C-6), 131.4 (1C, C-7), 151.8 (1C, C-9), 160.0 (1C, C-2) and 163.0 (1C, C-4).

3-Benzyl-4-hydroxycoumarin (**R6**)[10,41]

White solid (92 %), m.p. 204-205 °C (hexane/ethyl acetate [1:4]) (lit.[41] m.p. 202-204 °C), R_f 0.64 (hexane/ethyl acetate [1:9]). IR (KBr) 3098, 3068, 3032, 2966, 2933, 1660, 1612, 1495, 1455, 1187, 1133 and 1084 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.78 (2H, s), 7.03-7.26 (7H, m), 7.46 (1H, t, J = 7.54 Hz) and 7.93 (1H, d, J = 8.26 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 29.5 (1C, CH_2), 100.7 (1C, C-3), 115.7 (1C, C-8), 119.8 (1C, C-10), 122.7 (1C, C-5), 124.0 (1C, C-6), 125.1 (1C, C-d), 127.8 (2C, C-b), 128.2 (2C, C-c), 130.5 (1C, C-7), 142.0 (1C, C-a), 152.7 (1C, C-9), 163.8 (1C, C-2) and 166.2 (1C, C-4).

3-(2-Phenylethyl)-4-hydroxycoumarin (**R7**)[42]

White solid (74 %), m.p. 208-209 °C (hexane/ethyl acetate [1:4]) (lit.[42] m.p. 195-198 °C), R_f 0.65 (hexane/ethyl acetate [1:9]). IR (KBr) 3087, 3032, 2936, 2867, 1660, 1612, 1568, 1495, 1198 and 1150 cm^{-1}; ^{1}H-NMR ($CDCl_3$/DMSO-d_6) δ (ppm): 2.83-2.91 (4H, m), 7.17-7.32 (7H, m), 7.50 (1H, d, J = 7.08 Hz) and 7.62 (1H, t, J = 7.90 Hz); ^{13}C-NMR ($CDCl_3$/DMSO-d_6) δ (ppm): 24.8, 32.8 (2C, CH_2), 103.8 (1C, C-3), 115.0 (1C, C-8), 115.6 (1C, C-10), 122.3 (1C, C-5), 122.5 (1C, C-6), 124.7 (1C, C-d), 127.1 (2C, C-b), 127.4 (2C, C-c), 130.1 (1C, C-7), 140.8 (1C, C-a), 151.2 (1C, C-9), 159.3 (1C, C-2) and 162.6 (1C, C-4).

(±)-3-(2-Phenylpropyl)-4-hydroxycoumarin (**R8**)[43]

White solid (70 %), m.p. 158-159 °C (hexane/ethyl acetate [1:4]) (lit.[43] 159-161 °C), R_f 0.71 (ethyl acetate). IR (KBr) 3440-2800, 3087, 3028, 2966, 2929, 2903, 1667, 1620, 1568, 1502, 1458, 1216, 1169 and 1047 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 1.17 (3H, d, J = 6.89 Hz), 2.79 (1H, m), 3.11 (2H, m), 7.08-7.35 (7H, m), 7.56 (1H, t, J = 7.75 Hz) and 7.90 (1H, d, J = 7.08 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 20.8 (1C, CH_3), 32.0 (1C, CH), 37.7 (1C, CH_2), 103.7 (1C, C-3), 116.1 (2C, C-8, C-10), 123.2 (1C, C-5), 123.8 (1C, C-6), 125.9 (1C, C-d), 126.8 (2C, C-b), 128.1 (2C, C-c), 131.6 (1C, C-7), 146.8 (1C, C-a), 151.8 (1C, C-9), 160.3 (1C, C-2) and 162.8 (1C, C-4).

3-(3-Phenylpropyl)-4-hydroxycoumarin (**R9**)[14,31]

White solid (93 %), m.p. 156-157 °C (hexane/ethyl acetate [1:4]) (lit.[14] m.p. 137 °C), R_f 0.65 (hexane/ethyl acetate [1:9]). IR (KBr) 3380-2800, 3072, 3024, 2936, 2859, 1671, 1616, 1565, 1495, 1455, 1213, 1183 and 1099 cm^{-1}; ^{1}H-NMR ($CDCl_3$/DMSO-d_6) δ (ppm): 1.95 (2H, m), 2.57 (2H, t, J = 7.31 Hz), 2.72 (2H, t, J = 7.31 Hz), 7.18-7.37 (7H, m), 7.51 (1H, t, J = 7.43 Hz) and 7.84 (1H, d, J = 8.47 Hz); ^{13}C-NMR ($CDCl_3$/DMSO-d_6) δ (ppm): 23.2, 29.3, 35.4 (3C, CH_2), 105.1 (1C, C-3), 115.7 (1C, C-10), 116.5 (1C, C-8), 123.0 (1C, C-5), 123.9 (1C, C-6), 126.0 (1C, C-d), 128.5 (4C, C-b, C-c), 131.6 (1C, C-7), 141.8 (1C, C-a), 152.2 (1C, C-9),159.6 (1C, C-2) and 164.1 (1C, C-4).

3-((E)-3-Phenylprop-2-enylidene)-4-hydroxycoumarin (**R10**)[32]

White solid (87 %), m.p. 193-194 °C (hexane/ethyl acetate [1:4]) (lit.[32] m.p. 164-168 °C), R_f 0.60 (ethyl acetate). IR (KBr) 3430-2800, 3079, 3054, 3028, 2973, 2903, 1660, 1627, 1572, 1502, 1458, 1400 1176, 1117 and 1088 cm^{-1}; ^{1}H-NMR ($CDCl_3$/DMSO-d_6) δ (ppm): 3.49 (2H, d, J = 5.94 Hz), 6.26 (1H, dt, J = 15.8, 5.97 Hz), 6.45 (1H, d, J = 15.9 Hz), 7.08-7.27 (7H, m), 7.43 (1H, t, J = 7.74 Hz) and 7.87 (1H, d, J = 7.86 Hz); ^{13}C-NMR ($CDCl_3$/DMSO-d_6) δ (ppm): 27.1 (1C, CH_2), 103.4 (1C, C-3), 116.4 (1C, C-8), 116.6 (1C, C-10), 123.2 (1C, C-5), 123.6 (1C, C-6), 126.0 (2C, C-b), 126.7, 126.9 (2C, HC=CH), 128.4 (1C, C-d), 130.5 (2C, C-c), 131.3 (1C, C-7), 137.4 (1C, C-a), 152.5 (1C, C-9), 160.8 (1C, C-2) and 164.0 (1C, C-4).

3-((4-Methylphenyl)methyl)-4-hydroxycoumarin (**R11**)[14,44]

White solid (76 %), m.p. 183-184 °C (hexane/ethyl acetate [1:4]) (lit.[44] m.p. 183.5-184 °C), R_f 0.62 (hexane/ethyl acetate [1:9]). IR (KBr) 3420-2800, 3046, 3024, 2969, 2929, 1667, 1638, 1499, 1451, 1400, 1183, 1117 and 1073 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 2.22 (3H, s), 3.82 (2H, s), 7.04 (2H, d, J = 8.11 Hz), 7.12 (2H, d, J = 8.15 Hz), 7.31-7.39 (2H, m), 7.60 (1H, t, J = 7.66 Hz) and 7.97 (1H, d, J = 8.46 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 20.6 (1C, CH_3), 28.6 (1C, CH_2), 104.4 (1C, C-3), 116.2 (2C, C-8, C-10), 123.3 (1C, C-5), 123.9 (1C, C-6), 128.0 (1C, C-b), 128.7 (1C, C-c), 131.8 (1C, C-7), 134.8 (1C, C-d), 136.7 (1C, C-a), 151.9 (1C, C-9), 160.3 (1C, C-2) and 162.8 (1C, C-4).

3-((4-Hydroxyphenyl)methyl)-4-hydroxycoumarin (**R12**)

White solid (80 %), m.p. 207-208 °C (hexane/ethyl acetate [1:1]), R_f 0.59 (ethyl acetate). IR (KBr) 3450-2700, 3065, 3032, 2925, 2859, 1660, 1609, 1572, 1517, 1455, 1392, 1238, 1113 and 1066 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.75 (2H, s), 6.63 (2H, d, J = 8.39 Hz), 7.03 (2H, d, J = 8.39 Hz), 7.31-7.38 (2H, m), 7.60 (1H, t, J = 6.46 Hz), 7.95 (1H, dd, J = 7.82, 1.70 Hz) and 9.13 (1H, s, br); ^{13}C-NMR (DMSO-d_6) δ (ppm): 28.2 (1C, CH_2), 104.9 (1C,C-3), 114.9 (2C, C-c), 116.1 (1C, C-8), 116.3 (1C, C-10), 123.3 (1C, C-5), 123.8 (1C, C-6), 129.0 (2C, C-b), 129.8 (1C, C-a), 131.7 (1C, C-7), 151.9 (1C, C-9), 155.5 (1C, C-d), 160.1 (1C, C-2) and 162.6 (1C, C-4); MS *m/z* (% rel. int.): 268 (M^+, 100), 239 (40), 147 (50), 121 (60) and 107 (50); Elemental analysis found %C 71.26 and %H 4.77; calcd. for $C_{16}H_{12}O_4$ (MW 268.26): %C 71.64 and %H 4.51.

The FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra of **R12** are shown in Figs 47-50, respectively.

3-((4-Methoxyphenyl)methyl)-4-hydroxycoumarin (**R13**)[14]

White solid (78 %), m.p. 179-183 °C (hexane/ethyl acetate [3:7]) (lit.[14] m.p. 178-183 °C), R_f 0.58 (hexane/ethyl acetate [1:9]). IR (KBr) 3350-2800, 3076, 2999, 2955, 2936, 2911, 2837, 1656, 1627, 1572, 1510, 1451, 1392, 1249, 1172, 1113, 1084 and 1044 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.66 (3H, s), 3.74 (2H, s), 6.74 (2H, d, J = 8.54 Hz), 7.17 (2H, d, J = 8.59 Hz), 7.19-7.26 (2H, m), 7.49 (1H, t, J = 7.85 Hz) and 7.96 (1H, d, J = 7.74 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 28.5 (1C, CH_2), 54.9 (1C, OCH_3), 102.3 (1C, C-3), 113.3 (2C, C-c), 115.8 (1C, C-8), 118.9 (1C, C-10), 123.0 (1C, C-5), 123.9 (1C, C-6), 129.1 (2C, C-b), 130.8 (1C, C-7), 133.4 (1C, C-a), 152.5 (1C, C-9), 157.2 (1C, C-d), 163.6 (1C, C-2) and 164.4 (1C, C-4).

3-((3,4-Dimethoxyphenyl)methyl)-4-hydroxycoumarin (**R14**)[45]

White solid (20 %), m.p. 200-201 °C (hexane/ethyl acetate [1:1]), R_f 0.52 (ethyl acetate). IR (KBr) 3032, 2966, 2845, 1675, 1649, 1627, 1524, 1451, 1389, 1139 and 1029 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.67 (3H, s), 3.69 (3H, s), 3.80 (2H, s), 6.69-6.89 (3H, m), 7.31-7.38 (2H, m), 7.60 (1H, t, J = 7.53 Hz) and 7.96 (1H, d, J = 7.69 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 28.6 (1C, CH_2), 55.4, 55.5 (2x1C, OCH_3), 104.6 (1C, C-3), 111.8 (1C, C-b), 112.4 (1C, C-e), 116.2 (2C, C-8, C-10), 119.8 (1C, C-f), 123.3 (1C, C-5), 123.9 (1C, C-6), 131.8 (1C, C-7), 132.2 (1C, C-a), 147.1 (1C, C-d), 148.5 (1C, C-c), 151.9 (1C, C-9), 160.2 (1C, C-2) and 162.9 (1C, C-4).

3-(3,4-Methylenedioxybenzylidene)-4-hydroxycoumarin (**R15**)[14]

White solid (72 %), m.p. 211-212 °C (dec) (hexane/ethyl acetate [3:7]) (lit.[14] m.p. 211-214 °C), R_f 0.58 (hexane/ethyl acetate [1:9]). IR (KBr) 3400-2800, 3035, 2903, 1671, 1634, 1488, 1447, 1242, 1172, 1110 and 1040 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ (ppm): 3.77 (2H, s), 5.91 (2H, s), 6.68-6.80 (3H, m), 7.30-7.36 (2H, m), 7.59 (1H, t, J = 6.78 Hz) and 7.96 (1H, d, J = 7.34 Hz); ^{13}C-NMR (DMSO-d_6) δ (ppm): 28.7 (1C, CH_2), 100.6 (1C, OCH_2O), 104.3 (1C,C-3), 108.0 (1C, C-b), 108.6 (1C, C-e), 116.2 (1C, C-8), 116.3 (1C, C-10), 120.8 (1C, C-f), 123.3 (1C, C-5), 123.9 (1C, C-6), 131.9 (1C, C-7), 133.6 (1C, C-a), 145.3 (1C, C-d), 147.0 (1C, C-c), 151.9 (1C, C-9), 160.7 (1C, C-2) and 163.0 (1C, C-4).

2.4 Bioassay Procedures

Three important bioassays including brine shrimp cytotoxic lethality test, antibacterial bioassay and antiviral activity were selected to carry out in this research. The following bioassay experiments were performed.

2.4.1 Brine Shrimp Cytotoxic Lethality Test[46]

Brine shrimp lethality test is a procedure for general toxicity screening. This procedure is rapid, convenient, reliable, inexpensive and sensitive. It requires little material and is able to identify a broad spectrum of activities. Therefore, it is essential as a preliminary testing in the study of bioactive compounds. The aim of this method is to provide a front-line screen that can be backed up by more specific and more expensive bioassays. There were several techniques for this bioassay. A method of choice in this research, a microwell method has been developed for this experiment and was described in 2.4.1a-2.4.1d.

2.4.1a) Sample Preparation

Samples were prepared by dissolving 4 mg of tested compound in 80 μL of dimethyl sulfoxide (DMSO) or another appropriate solvent. Sea water was then added to the solution to make 4000 μL and allowed to shake-well to afford solution A (1000 ppm). Serial dilution of this stock solution was made to obtain solution B (100 ppm) and Solution C (10 ppm), respectively. Control solution was prepared using only solvent and sea water.

2.4.1b) Hatching the Shrimp

Brine shrimp eggs (*Artemia salina* Linn.) were hatched in an open shallow rectangular plastic box (13x18x4 cm) filled with artificial sea water (38 g of NaCl dissolved in 1 L of deionized or distilled water). The box was divided into two unequal compartments linked with 2 mm ϕ holes. The eggs were sprinkled into the larger compartment which was darkened with aluminum foil while the smaller was illuminated with the 20-watt lamp, and the box was kept at 22-29 ^{o}C. After 24 hours, nauplii were collected by disposable pipette from the smaller compartment.

2.4.1c) Bioassay

Five shrimps were transferred to each well of 24-well microplates by the disposable pipette, and tried to keep 100 μL of sea water. Six replications were made for each concentration. The covered plates were kept in the same condition as hatching. After 24 hours, numbers of dead nauplii in each well were counted under binocular microscope.

2.4.1d) LC_{50} Determinations

LC_{50} values were calculated by probit analysis program. In cases where data were insufficient for this program, LC_{50} values were estimated using logic transformation.

2.4.2 Antibacterial Bioassay*

This bioassay was performed by paper disc method,[47] unless otherwise stated. The compounds were tested with seven bacteria: *Escherichia coli*, *Bacillus cereus*, *Staphylococus aureus*, *Salmonella derby*, *Escherichia coli* O157:H7, *Listeria monocytogenes* and Flat sour spoilage. Stock solution was prepared by dissolving 10 mg of test sample in 1000 μL of proper solvent. 30 μL of stock solution were transferred by disposable pipette onto a disc. After 24 hours, diameter of clear zone was measured.

2.4.3 Anti HSV-1 and HSV-2 Assays**

This bioassay was carried out using the modified colorimetric method[48] by stained cell membrane of virus with drying agent and determined the optical density. The experiments were performed with HSV-1 (KOS strain) and HSV-2 (Baylor 185 strain) virus, and the cultures used in this assay was Vero cell line (African green monkey kidney cell line) which grown in Eagle's minimum essential medium (MEM) contianed 10% heat inactivated fetal bovine serum (FBS) and antibiotics. Virus (30 PFU) was mixed with various concentrations of test compounds (two-fold dilution) and DMSO as solvent in media. Experimental mixtures were plated in microplates and

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* This assay was performed by Ms. Siriporn Satanasaovapak at Institute of Food Research and Product Development, Kasetsart University.

** This assay was conducted by Associate Professor Wimolmas Lipipun, Ph.D. at Faculty of Pharmaceutical Science, Chulalongkorn University.

allowed to incubate in CO_2 incubator at 37 °C for 3 days. Following incubation, 50% cooled trichloroacetic acid (TCA) was gently added on top of the growth medium and chilled in refrigerator at 4 °C for 30 minutes. The cultures were washed 4 times with water, air dried and stained for 30 minutes with 0.4 % sulforhodamine B in 1 % acetic acid. After this period, sulforhodamine B was removed and cultures were quickly rinsed 4 times with 1 % acetic acid, then dried and solubilized with 10 mM Tris-base (pH 10). Absorbance of sample cultures was measured at 510 nm with ELISA microplate reader and calculated by comparing with sample-free control, and positive control (acyclovir). Cytotoxicity of sample compounds with host cell can be tested by this assay without viral infection.