CHAPTER III
RESULTS AND DISCUSSION

3.1 Synthesis

3.1.1 Synthesis of Dicoumarols and Their Analogues

A class of 3-substituted-4-hydroxycoumarin contained another group of 4-hydroxycoumarin linkage with CH-bridging at 3-position is called dicoumarols. These compounds were generally synthesized by condensation between two-mole equivalent of 4-hydroxycoumarins and one mole equivalent of interested aldehydes. In this research, three different procedures for preparation of these compounds as shown in Fig 3.1 were employed. The first one was the condensation of 4-hydroxy coumarin with aromatic aldehyde in hot ethanol (method I).[13] This method is also useful for aromatic aldehydes since the insoluble products were easily separated by filtration. The next procedure (method II) was similarly performed to that of method I, but using ethylene diammonium diacetate (EDDA) as a catalyst. This method was useful for aliphatic aldehydes because derived dicoumarols were generally soluble in hot ethanol and reactive to get more by products which may cause a lower yield.[14,30] However, stirring at room temperature could solve this problem. The last one was used for formaldehyde which was rapidly reacted with 4-hydroxycoumarin in hot water (method III).[9]

In this research, fifty-five compounds including forty-seven dicoumarols, four fused-rings, two tetramers and two unexpected compounds were synthesized. Twenty-four compounds were kindly supplied by S. Wattanasereekul.[21] Eleven new compounds (**17**, **23**, **29**, **30**, **31**, **32**, **33**, **38**, **44**, **50** and **54**) based upon no report of those compounds available in chemical literature can be synthesized (structures showed in Fig 3.2). The structures of all synthesized compounds were well characterized using various spectroscopic techniques including IR, ^{1}H-NMR, ^{13}C-NMR and MS which will be discussed in the forthcoming section. The comparative results of the synthetic compounds in this research are tabulated in Table 3.1.

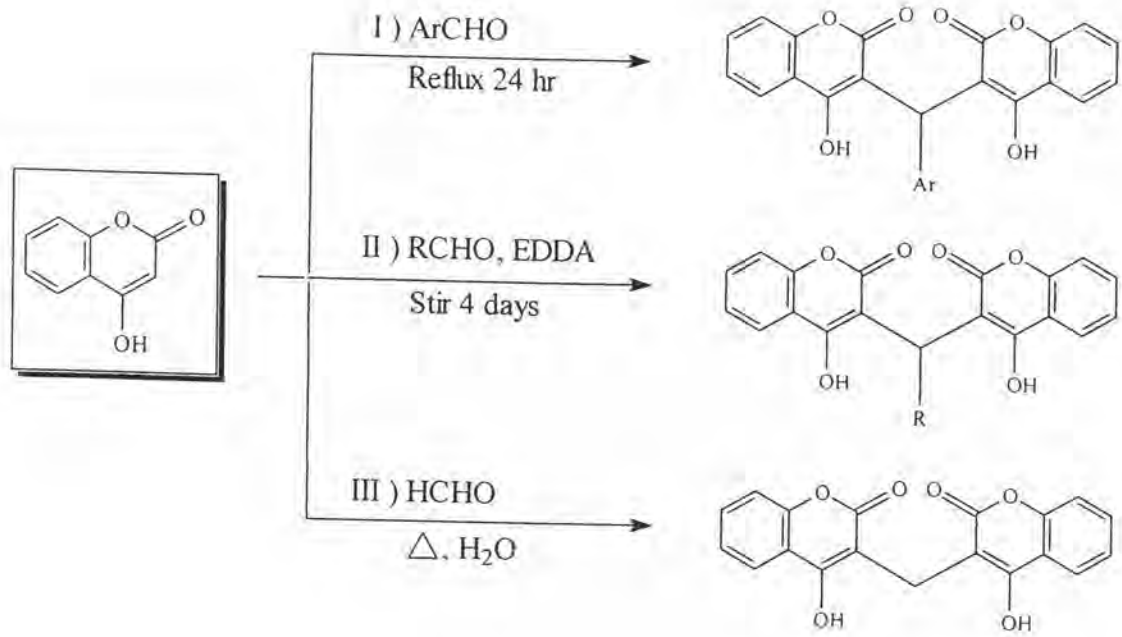
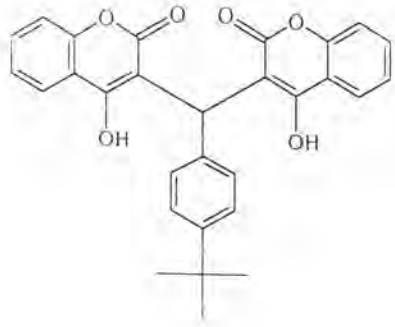


Fig 3.1 The general synthesis of dicoumarols



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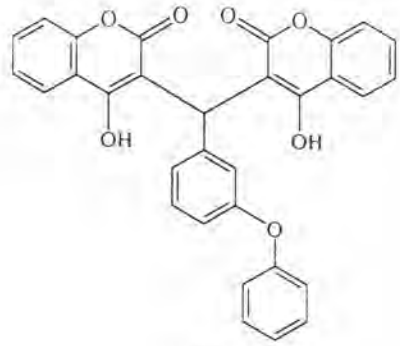

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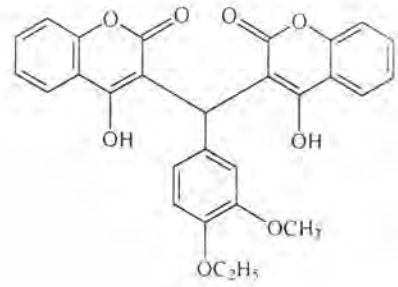

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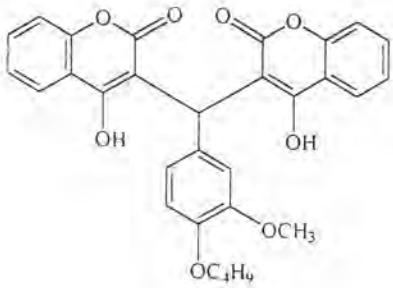

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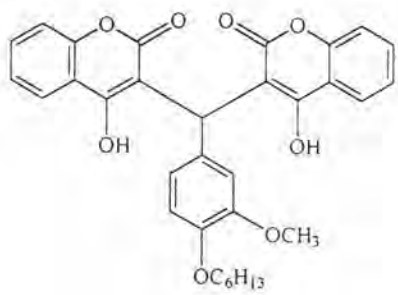

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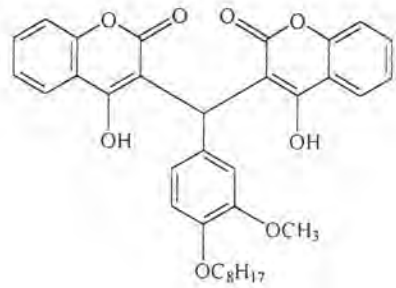

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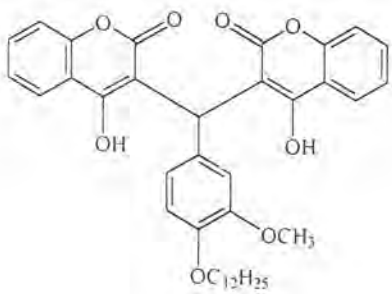

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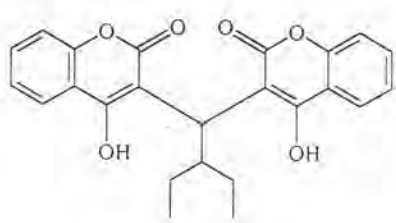

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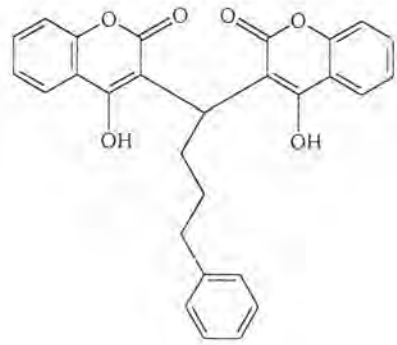

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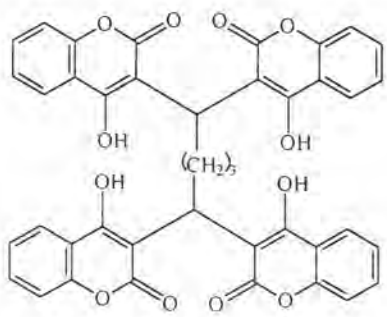

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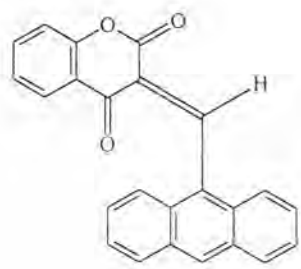

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Fig 3.2 The new synthetic dicoumarols and analogues

Table 3.1 The physical properties and % yield of synthesized compounds

Cpd	Physical Properties		% Yield	Method
	Appearance	m.p. (°C)		
1	white prism	288-290	92	III
6	white crystal	237-238	64	I
9	small white crystal	231-232	85	I
17	white crystal	252-254	60	I
20	white crystal	256-257	89	I
23	white crystal	182-183	84	I
26	small white crystal	237-239	79	I
29	yellow amorphous solid	137-139	85	I
30	yellow amorphous solid	169-170	86	I
31	light yellow solid	142-144	82	I
32	white amorphous solid	141-142	61	I
33	white amorphous solid	110-112	76	I
34	light yellow solid	207-209	94	I
35	white crystal	176-178	64	II
36	white crystal	116-118	47	II
37	white crystal	202-205	27	II
38	small white crystal	165-167	41	II
39	small white crystal	209-211	37	II
40	white crystal	184-185	42	II
41	white crystal	186-188	54	II
42	small white crystal	198-199	40	II
43	white needle	174-175	90	II
44	white crystal	162-164	40	II
47	small white crystal	306-307	49	I
49	white amorphous solid	299-301	56	I
50	small white crystal	229-230	18	I

Table 3.1 (cont.)

Cpd	Physical Properties		% Yield	Method
	Appearance	m.p. (°C)		
51	white amorphous solid	208-209	67	I
52	light yellow powder	239-240	64	I
53	white needle	268-269	44	I
54	small red crystal	236-237	84	I
55	white crystal	206-208	20	II

The condensation products between 4-hydroxycoumarins and aromatic aldehydes were generally found to achieve in high yield (76-94 %) except for Compounds **6**, **17**, **32** and **51** which were obtained in moderate yield (60-67 %). Melting points of most aromatic dicoumarols are over 200 °C, except for those of **23** and **29-33** (110-183 °C). For dicoumarols which were synthesized from aliphatic aldehydes, low to moderate yield of these were provided (27-64 %) except for **43** (90 %) which contained unsaturated moiety. The low yield of the desired products was obtained which possibly due to the fact that those compounds were highly soluble in a reaction mixture and further reacted to give some unidentified by product.[30] Melting points of aliphatic dicoumarols were below 210 °C and had a tendency to be lower when increasing the length of the alkyl chain (**39** > **40** > **43** >**44**).

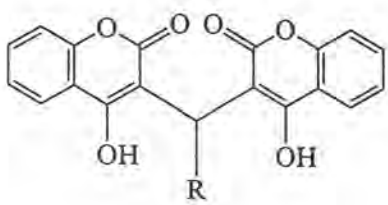
These synthetic compounds **45-48** may not actually be called dicoumarol because the hydroxy group in one part of 4-hydroxycoumarin was condensed with the hydroxy, halide or nitro group at the 2-position of aromatic aldehyde, to give the analogues product and perhaps can be called as fused compounds.[13] These compounds have high melting point above 240 °C. In this research Compound **47** was synthesized in moderate yield (51 %) and had high melting point (306-307 °C).

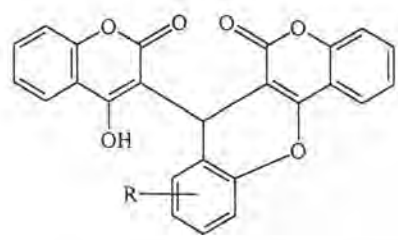
From the reports about anti HIV-1 enzyme inhibitor of dicoumarols and related compounds, it was indicated that tetramer analogues exhibited high activity against HIV-1 protease and integrase.[18,19] Thus, two tetrameric compounds were also synthesized to study in this research. Tetramers **49** and **50** gave moderate and low yield (56 and 18 %, respectively) and had melting point higher than 200°C.

Two dicoumarols **52** and **53** which contained *N*-heteroaromatic substituents can be synthesized using pyridine-2-carboxaldehyde and pyridine-3-carboxaldehyde in moderate yield (64 and 44 %, respectively). 2-Pyridyl derivative did not give similar fused ring compound as that reported by Litvan,[32] but provided the dicoumarol product.[35] Moreover, the condensation of 4-hydroxycoumarin with anthracene-9-carboxaldehyde and with chloral (or trichloroacetaldehyde) provided 1:1 condensed products. The unexpected compound **54** (84%) was observed by lossing 1 molecule of water to form the 2,4-dione which was stabilized by conjugation. However, the yield of **55** (20%) was similar to that reported when acetal of chloral ($Cl_3CCH(OH)_2$) was used.[35]

3.1.2 Spectroscopy of Dicoumarols and Analogues

Infrared Spectroscopy (IR)

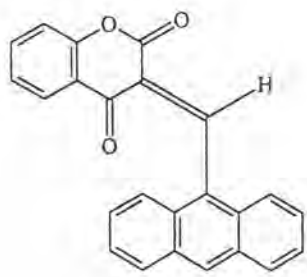




The IR absorption pattern for all dicoumarols and fused compounds displayed the characteristic of common functional groups containing in the structure. O-H Stretching vibrations were presented around 3670-2400 cm^{-1} (br, w). The C-H stretching vibration of aromatic at 3090-3020 cm^{-1} and that of aliphatic at 2991-2830 cm^{-1} were detected. C=O Stretching vibration of pyrane ring at 1678-1642 cm^{-1} and that of C=C ring stretching at 1524-1488 cm^{-1} were also found. Other absorption peaks of C-O stretching vibration at 1133-1047 cm^{-1} were also visualized. Moreover, Compounds **52** and **53** which contained pyridine ring, C=N stretching vibration at 1700 and 1737 cm^{-1} was observed, respectively.

The FT-IR characteristic absorption peaks of unexpected products **54** and **55** were also detected. Compound **54** did not show a broad band O-H stretching vibration and C-H aliphatic stretching vibration and showed strong C=O peak belonging to lactone and ketone apart from the above mentioned characteristic at

1748 and 1667 cm^{-1}, respectively. O-H Stretching vibration of Compound **55** was also detected at 3500-2600 cm^{-1} and other absorption patterns were similar to those of dicoumarols. The FT-IR absorption band assignments of dicoumarols and analogues are tabulated in Table 3.2.



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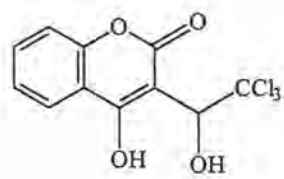

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Table 3.2 The FT-IR absorption band assignments of dicoumarols and analogues

Cpd	Wave number (cm^{-1})					
	O-H	Ar-H	C-H str.	C=O	benzo	C-O
1	3400-2500 (s)	3066 (w)	-	1652 (s)	1501 (m)	1110 (s)
6	3300-2500 (s)	3090 (w)	-	1657 (s)	1490 (m)	1095 (s)
9	3300-2500 (s)	3079 (w)	2933, 2859 (w)	1671 (s)	1499 (m)	1058 (s)
17	3300-2500 (s)	3080 (w)	2969 (w)	1669 (s)	1498 (m)	1100 (s)
20	3300-2500 (s)	3083 (w)	2962-2830 (w)	1671 (s)	1488 (m)	1102 (s)
23	3300-2600 (s)	3076, 3024 (w)	2903 (w)	1667 (s)	1495 (m)	1099 (s)
26	3300-2600 (s)	3072 (w)	2951-2834 (w)	1656 (s)	1510 (m)	1128 (s)
29	3670-3290 (s)	3076 (w)	2980-2867 (w)	1667 (s)	1521 (m)	1095 (s)
30	3640-2600 (s)	3076 (w)	2958-2881 (w)	1675 (s)	1517 (m)	1095 (s)
31	3650-2500 (s)	3079 (w)	2958-2863 (w)	1678 (s)	1517 (m)	1095 (s)
32	3250-2600 (s)	3072, 3013 (w)	2936, 2867 (w)	1667 (s)	1521 (m)	1099 (s)
33	3300-2500 (s)	3068 (w)	2922, 2852 (w)	1667 (s)	1524 (m)	1099 (s)
34	3660-2500 (s)	3068, 3028 (w)	2940, 2874 (w)	1671 (s)	1521 (m)	1095 (s)
35	3400-2600 (s)	3080 (w)	2991, 2878 (w)	1644 (s)	1491 (m)	1127 (s)
36	3300-2500 (s)	3083 (w)	2955, 2874 (w)	1656 (s)	1495 (m)	1124 (s)
37	3300-2500 (s)	3079 (w)	2984, 2870 (w)	1664 (s)	1499 (m)	1132 (s)
38	3300-2500 (s)	3079 (w)	2966, 2874 (w)	1667 (s)	1495 (m)	1080 (s)
39	3300-2500 (s)	3079 (w)	2929, 2852 (w)	1660 (s)	1495 (m)	1099 (s)
40	3400-2400 (s)	3090, 3040 (w)	2950, 2850 (w)	1650 (s)	1510 (m)	1100 (s)
41	3300-2400 (s)	3090, 3020 (w)	2950, 2850 (w)	1650 (s)	1500 (m)	1100 (s)
42	3300-2400 (s)	3080, 3050 (w)	2975-2850 (w)	1650 (s)	1500 (m)	1100 (s)
43	3320-2600 (s)	3083, 3028 (w)	2914 (w)	1675 (s)	1499 (m)	1102 (s)
44	3300-2500 (s)	3083, 3028 (w)	2977-2856 (w)	1667 (s)	1502 (m)	1102 (s)
47	3550-3200 (s)	3083 (w)	2984-2896 (w)	1645 (s)	1500 (m)	1117 (s)
49	3600-2500 (s)	3076, 3043 (w)	2988, 2896 (w)	1664 (s)	1499 (m)	1099 (s)
50	3300-2500 (s)	3079 (w)	2973-2863 (w)	1671 (s)	1491 (m)	1117 (s)

Table 3.2 (cont.)

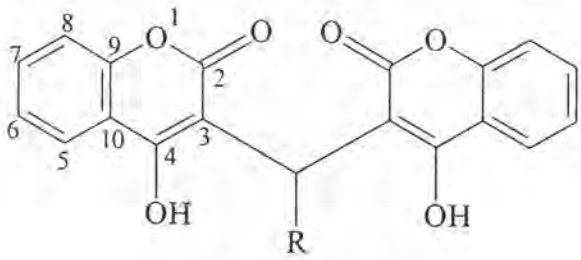
Cpd	Wave number (cm^{-1})					
	O-H	Ar-H	C-H str.	C=O	benzo	C-O
51	3600-2500 (s)	3079 (w)	-	1664 (s)	1495 (m)	1095 (s)
52[a]	3600-3300 (s)	3123-3068 (w)	2940 (w)	1642 (s)	1495 (m)	1113 (s)
53[b]	3650-3300 (s)	3145-3061 (w)	2984, 2922 (w)	1678 (s)	-	1106 (s)
54[c]	-	3050, 3024 (w)	-	-	1462 (s)	1133 (s)
55[d]	3500-2600 (s)	-	2947 (w)	1671 (s)	1502 (m)	1047 (s)

Other stretching vibration : [a]C=N 1700 cm^{-1} (s), [b]C=N 1737 cm^{-1} (s),
[c]C=O 1748 cm^{-1} (s), 1667 cm^{-1} (s),
[d]C-Cl 827 cm^{-1} (s).

- not assigned.

Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H-NMR



Most of dicoumarols are soluble in $CDCl_3$. DMSO-d_6 was used as a solvent for dicoumarols which are insoluble in $CDCl_3$. On the dicoumarol moiety, the 2H integration of H-5 was observed as doublet or broad singlet around 7.78-8.11 ppm (J = 7.16-8.24 Hz) and 2H integration of H-6 was also found as triplet at 7.54-7.62 ppm (J = 7.80-8.24 Hz). The overlapped 4H integration of H-7 and H-8 at 7.20-7.45 ppm was generally detected. The typical 1H integration of CH methylene bridge was exhibited in a wide range between 3.84-6.66 ppm depending upon the R substituent at the bridge carbon. The 2H integration of 4-OH as two broad singlet signals was also observed. This can possibly be explained that the substituent at the bridge carbon exhibited a double hindered rotation around the bonds connecting to this carbon as represented in Fig 3.3. When DMSO-d_6 was used as a solvent, the formation of intermolecular H-bonds between the hydroxy groups and solvent molecules will lower the barrier of intramolecular hydrogen bond that caused disappearance of the hydroxy pattern.[22]

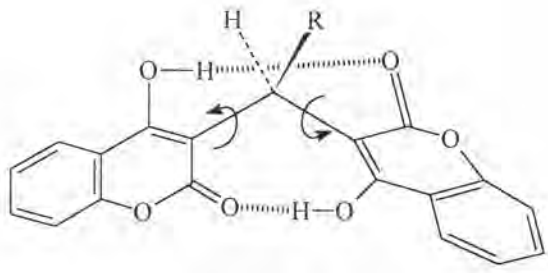
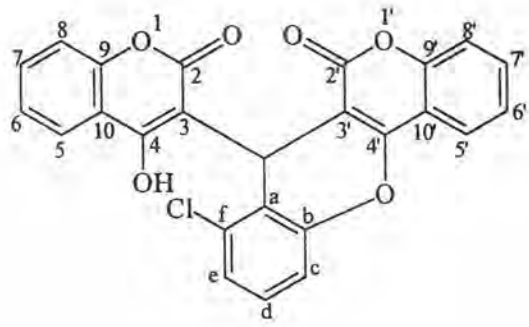


Fig 3.3 Dynamic structure of substituted dicoumarols



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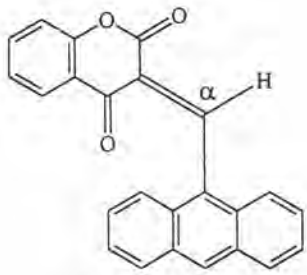
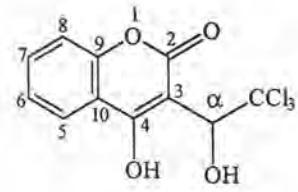
For the ^{1}H-NMR spectra of fused ring dicoumarols, the pattern of proton was non-equivalent between two 4-hydroxycoumarin residues and the overlapped spectral signals were difficult for clearly interpreting. Other proton signals of the substituent at the bridge carbon were also tentatively assigned. The ^{1}H-NMR spectral assignments of synthesized compounds are tabulated in Table 3.3.



54 (±)-isomer



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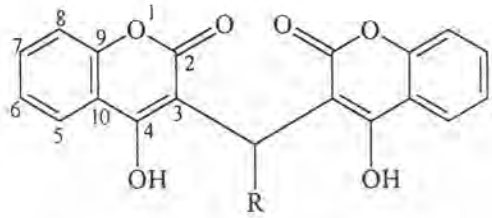
The ^{1}H-NMR spectra of two-unexpected compounds (**54** and **55**) were measured. Compound **54** did not exhibit the proton of CH-bridge like dicoumarols. It provided the complicated signals around 7.12-9.62 ppm that could not be assigned for the exact position of proton. However, it showed a pair of spectra due to its racemic mixture. Compound **55** exhibited the ^{1}H-NMR spectrum very close to those of dicoumarols which could be assigned for H-5, H-6, H-7-H-8 and α-H at 7.92, 7.60, 7.28-7.42 and 5.69 ppm, respectively.

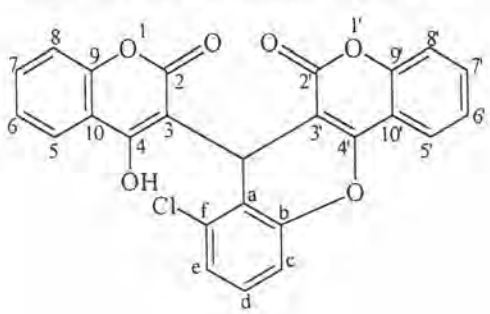
Table 3.3 The ^{1}H-NMR spectral assignments of dicoumarols and analogues

Cpd	chemical shift (ppm)					
	H-5	H-6	H-7, H-8	CH-bridge	4-OH	Others
1	7.99(d)	7.59(t)	7.33-7.40(m)	3.84(s)	11.3(s)	-
6	8.02(br)	7.62(dt)	7.33-7.41(m)	6.04(s)	11.30(s), 11.58(s)	6.88-7.01(m), 7.20-7.27(m) (Ar-H)
9	8.02(br)	7.62(dt)	7.37-7.41(m)	6.03(s)	11.29(s), 11.56(s)	7.02-7.24(m) (Ar-H)
17	8.02(br)	7.61(dt)	7.33-7.40(m)	6.05(s)	11.28(s), 11.50(s)	1.29(s) (CH_3), 7.12(d), 7.2(d) (Ar-H)
20	8.03(br)	7.58(t)	7.34-7.42(m)	6.07(s)	11.26(s), 11.57(s)	3.74(s) (OCH_3), 6.78-6.83(m), 7.25(s) (Ar-H)
23	8.01(br)	7.60(dt)	7.35-7.40(m)	6.07(s)	11.29(s), 11.63(s)	6.86-6.99(m), 7.21-7.31(m) (Ar-H)
26	8.03(br)	7.62(t)	7.35-7.42(m)	6.06(s)	11.28(s), 11.53(s)	3.70(s), 3.83(s) (OCH_3), 6.40(s) (Ar-H)
29	8.01(d)	7.60(t)	7.32-7.40(m)	6.05(s)	11.48(s)	1.43(t) (CH_3), 3.71(s) (OCH_3), 4.06(q) (OCH_2), 6.69-6.82(m) (Ar-H)
30	8.01(d)	7.60(dt)	7.32-7.40(m)	6.05(s)	11.49(s)	0.95(t) (CH_3), 1.46(m), 1.80(m) (CH_2), 3.70(s) (OCH_3), 3.98(t) (OCH_2), 6.69-6.82(m) (Ar-H)
31	8.02(d)	7.62(t)	7.35-7.42(m)	6.07(s)	11.30(s), 11.51(s)	0.89(t) (CH_3), 1.24-1.80(m) (CH_2), 3.77(s) (OCH_3), 3.99(t) (OCH_2), 6.71-6.84(m) (Ar-H)
32	8.02(br)	7.61(t)	7.33-7.41(m)	6.05(s)	11.29(s), 11.47(s)	0.86(t) (CH_3), 1.29-1.81(m) (CH_2), 3.70(s) (OCH_3), 3.97(t) (OCH_2), 6.69-6.82(m) (Ar-H)
33	8.00(br)	7.60(dt)	7.32-7.41(m)	6.04(s)	11.29(s), 11.49(s)	0.85(t) (CH_3), 1.24-1.80(m) (CH_2), 3.70(s) (OCH_3), 3.97(t) (OCH_2), 6.69-6.82(m) (Ar-H)
34	8.02(br)	7.62(t)	7.25-7.42(m)	6.05(s)	11.30(s), 11.51(s)	3.74(s) (OCH_3), 5.13(s) (OCH_2), 6.68-6.85(m), 7.25-7.42(m) (Ar-H)
35	7.99(d)	7.56(t)	7.29-7.37(m)	4.70(q)	11.23(s), 12.03(s)	1.84(d) (CH_3)
36	7.97(d)	7.55(t)	7.29-7.36(m)	4.48(t)	11.18(s), 12.01(s)	0.92(t) (CH_3), 1.30(m), 2.34(m) (CH_2)
37	7.99(d)	7.56(t)	7.29-7.37(m)	4.00(d)	11.13(s), 12.00(s)	0.93(d), 0.96(d) (CH_3), 3.31(m) (CH)
38	7.98(dd)	7.56(dt)	7.29-7.38(m)	4.30(d)	11.18(s), 12.05(s)	0.80(t) (CH_3), 1.17-1.53(m) (CH_2), 3.08(m) (CH)
39	8.00(d)	7.58(t)	7.21-7.40(m)	4.14(d)	11.13(s), 12.03(s)	0.89-2.95(m) (cyclohexyl ring)
40	7.92(d), 8.01(d)	7.55(t)	7.25-7.37(m)	4.83(t)	11.17(s), 12.26(s)	3.69(m) (CH_2), 7.08-7.20(m) (Ar-H)
41	7.86(d), 8.03(d)	7.58	7.27-7.45(m)	4.69(d)	11.00(s), 11.30(s)	1.24(d), 1.27(d) (CH_2), 4.47(m) (CH), 7.03-7.22(m) (Ar-H)
42	7.97(dd)	7.56(t)	7.29-7.37(m)	4.48(t)	11.15(s), 12.05(s)	2.64-2.79(m) (CH_2), 7.02-7.22(m) (Ar-H)
43	8.01(d)	7.59(t)	7.35-7.42(m)	5.47(dd)	11.27(s), 11.76(s)	6.52(dd), 6.76(dd) (CH=CH), 7.17-7.31(m) (Ar-H)
44	7.97(d)	7.56(t)	7.33-7.36(m)	4.49(t)	11.18(s), 12.01(s)	1.62(q) (CH_2), 2.41(m), 2.64(t) (CH_2), 7.10-7.21(m) (Ar-H)
47	7.99(d), 8.11(d)	7.57(t)	7.26-7.44(m)	5.39(s)	10.19(s)	7.14-7.23(m) (Ar-H)
49	7.87(d)	7.56(t)	7.25-7.35(m)	6.29(s)	-	6.99(s) (Ar-H)
50	7.80(d)	7.56(t)	7.24-7.31(m)	4.81(t)	-	1.17(br), 2.11(d) (CH_2),
51	7.28-8.14(m)			6.66(s)	11.14(s), 11.40(s)	7.28-8.14(m) (naphthyl ring)
52	7.80(d)	7.57(t)	7.22-7.34(m)	6.50(s)	-	7.88(d), 8.44(t), 8.62(d) (pyridyl ring)
53	7.80(d)	7.54(t)	7.21-7.31(m)	6.41(s)	-	7.92(t), 8.35(d), 8.64-8.71(m) (pyridyl ring)

^{13}C-NMR



Like the hydroxy pattern in ^{1}H-NMR, the ^{13}C-NMR spectra of dicoumarols showed an unequal signal between the same position of two sides of 4-hydroxy coumarin residue regarding to their substituent on the bridge carbon except for a parent compound (**1** R = H). The signal of carbon bridge was detected around 33.8-37.6 ppm. The two benzopyrane ring signals (18C) were observed above 100 ppm (103.2-169.3 ppm) as C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-9 and C-10 (2C each) at 164.4-166.5, 103.2-106.1, 166.4-169.3, 124.2-124.7, 124.6-125.6, 131.8-133.7, 116.3-117.3, 152.1-153.3 and 116.2-117.6 ppm, respectively. Other relevant carbons substituted at a bridge carbon were also detected. The ^{13}C-NMR spectral assignments of dicoumarols are presented in Table 3.4.



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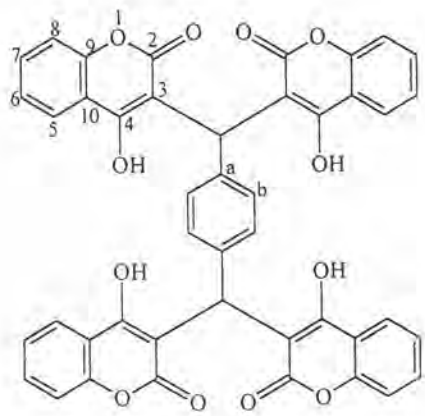
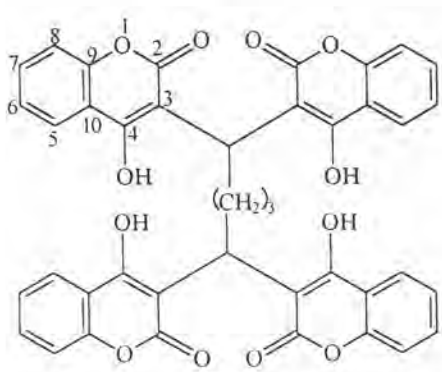


49



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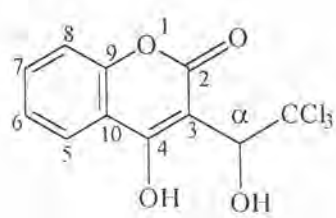


55

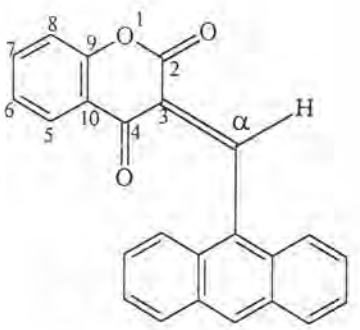
Table 3.4 The ^{13}C-NMR spectral assignments of dicoumarols and analogues

Cpd	Chemical shift (ppm)										
	CH-bridge	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	Others
1	19.9	164.4	102.9	168.7	124.0	124.8	132.6	116.7	152.3	116.4	-
6	36.1	164.7 166.0	103.5 105.2	166.8 169.2	124.4	125.0	133.0	116.7	152.5	166.6	113.6, 114.0, 122.1, 130.1, 138.1, 165.6 (C-Ar)
9	36.6	164.7 166.0	103.4 105.1	166.8 169.1	124.4	125.0	133.0	116.7	152.3 152.5	116.3	124.8, 126.7, 127.1, 129.8, 134.7, 137.6 (C-Ar)
17	35.8	164.5 165.6	104.0 105.8	166.9 169.3	124.4	124.9	132.8	116.6	152.4	116.6	31.3 (CH_3), 34.4 (C-(CH_3)$_3$), 125.6, 126.2, 132.1, 149.7 (C-Ar)
20	36.1	164.6 165.7	103.8 105.7	166.8 169.3	124.4	124.8	132.8	116.6	152.5	116.6	55.2 (OCH_3), 111.2, 113.4, 118.9, 129.6, 137.0, 159.9 (C-Ar)
23	36.1	164.7 165.8	103.7 105.5	166.9 169.2	124.8	124.4	132.9	116.6	152.3 152.5	116.4	118.6, 123.1, 129.7, 157.4 (C-Ar'), 117.2, 117.6, 121.4, 129.9, 137.5, 157.0 (C-Ar)
26	32.6	164.7 165.7	104.3 105.6	166.7 169.1	124.3	125.0	132.9	116.7	152.4	166.7	56.3, 60.9 (OCH_3), 104.3, 130.9, 137.2, 153.4 (C-Ar)
29	35.7	164.8 165.5	104.2 105.3	166.9 169.3	124.3	124.9	132.8	116.6	152.4	166.6	14.8 (CH_3), 56.2 (OCH_3), 64.3 (OCH_2), 110.8, 112.7, 119.0, 127.5, 147.4, 149.4 (C-Ar)
30	35.7	164.7 165.5	104.2 105.8	166.8 169.3	124.3	124.9	132.8	116.6	152.4	166.6	13.9 (CH_3), 19.2, 31.3 (CH_2), 56.4 (OCH_3), 68.7 (OCH_2), 111.1, 112.8, 119.0, 127.4, 147.7, 149.5 (C-Ar)
31	35.7	164.9 165.5	104.2 105.8	167.5 169.3	124.3	124.9	132.8	116.6	152.4	116.6	14.0 (CH_3), 22.6, 25.7, 30.2, 31.6 (CH_2), 56.4 (OCH_3), 69.0 (OCH_2), 111.1, 112.9, 119.0, 127.4, 147.7, 149.5 (C-Ar)
32	35.5	164.6 165.6	104.4 105.8	166.8 169.0	124.3	124.9	132.8	116.6	152.4	116.6	14.1 (CH_3), 20.0, 22.6, 26.0, 29.2, 29.4, 31.8 (CH_2), 56.4 (OCH_3), 69.0 (OCH_2), 111.1, 112.9, 119.0, 127.4, 147.7, 149.5 (C-Ar)
33	35.7	164.5 165.5	104.2 105.8	166.9 169.3	124.3	124.9	132.8	116.6	152.4	116.6	15.7 (CH_3), 20.0, 22.7, 26.0, 29.2, 29.4, 29.6, 31.9 (CH_2), 56.3 (OCH_3), 69.0 (OCH_2), 111.1, 112.9, 119.0, 127.4, 147.7, 149.5 (C-Ar)
34	35.8	164.7 165.6	104.1 105.8	166.8 169.2	124.3	124.9	132.9	116.6	152.4	116.6	56.3 (OCH_3), 71.1 (OCH_2), 128.1, 128.5, 137.2 (C-Ar'), 111.1, 114.0, 119.0, 127.3, 147.3, 149.8 (C-Ar)
35	26.1	164.2	106.3	166.8	123.5	124.0	131.7	115.7	151.6	116.8	14.9 (CH_3)
36	32.6	164.3 164.7	105.8 106.0	167.5 169.2	124.0 124.2	124.7	132.4 132.5	116.5	152.0 152.3	117.2	13.8 (CH_3), 21.8, 30.5 (CH_2)

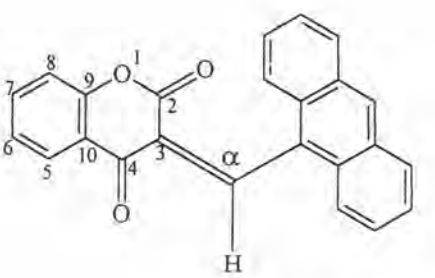
Table 3.4 (Cont.)

Cpd	Chemical shift (ppm)										
	CH-bridge	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	Others
37	41.4	163.9 165.2	105.5 106.0	167.5 169.4	124.1 124.3	124.7 124.8	132.5	116.5	152.1 152.3	116.3 117.0	21.7, 21.9 (CH_3), 25.7 (CH)
38	35.7	164.2 165.1	105.1 105.8	167.7 169.4	124.1 124.3	124.7 124.8	132.4 132.5	116.5	152.1 152.3	116.4 117.0	9.3, 9.4 (CH_3), 21.9, 22.2 (CH_2), 35.9 (CH)
39	39.7	163.9 165.3	104.8 105.5	167.5 169.4	124.1 124.2	124.7 124.8	132.4	116.5	152.1 152.3	117.0	25.8, 25.9, 26.2, 31.8, 32.3, 34.5 (Cyclohexyl ring)
40	34.9	164.5 165.0	105.5	167.8 169.1	124.0 124.3	124.8	132.5 132.6	116.5	152.0 152.3	116.2 117.1	34.4 (CH_2), 126.6, 128.5, 128.6, 139.0 (C-Ar)
41	37.1 37.3	163.9- 165.8	104.9- 106.2	167.6- 169.5	123.8- 124.9	123.8- 124.9	132.2- 132.7	115.9- 117.1	151.8- 152.4	115.9 117.1	21.6, 21.8 (CH_3), 40.3, 40.4 (CH), 126.6, 126.7, 128.5, 128.6, 144.2, 144.5 (C-Ar)
42	35.0	164.3 164.8	105.7 105.8	167.5 169.1	124.0 124.2	124.7 124.8	132.5 132.6	116.5	152.1 152.3	117.1	30.3, 32.6 (CH_2), 126.0, 128.4, 140.8 (C-Ar)
43	34.6	164.3	105.1 106.3	167.0 168.9	124.3	124.8	132.7	116.6	152.3	116.6	125.0, 132.3 (CH=CH), 126.4, 127.7, 128.6, 136.7 (C-Ar)
44	35.5	164.3 164.9	105.6 105.8	167.5 169.2	124.0 124.2	124.7 124.8	132.5 132.6	116.5	152.1 152.3	117.1	28.0, 30.5, 32.9 (CH_2), 125.9, 128.3, 141.7 (C-Ar)
47	28.9	158.3 161.3	100.4 106.3	152.3 165.6	123.3 123.9	124.5 125.1	132.0 132.9	115.8 116.2	152.1 153.1	114.2 116.6	117.0, 119.8, 126.2, 129.1, 133.6, 162.1 (C-Ar)
49	35.6	164.8	104.2	164.8	123.8	123.8	132.0	116.0	152.1	117.6	126.6, 136.7 (C-Ar)
50	31.4	164.0	105.1	164.9	123.5	123.8	131.8	115.9	151.8	117.3	26.0, 28.9 (CH_2)
51	35.2	164.6 165.0	105.3 107.3	166.9 168.9	124.5	124.9	133.2	116.6	152.2	116.6	122.8, 125.0, 125.5, 126.3, 126.5, 129.4, 129.7, 130.8, 131.2, 134.6 (naphthyl ring)
52	36.6	163.8	100.4	168.5	123.2	124.3	131.8	115.8	152.8	119.3	125.7, 141.8, 146.2, 157.5 (C-Ar)
53	34.6	164.0	101.6	168.0	123.1	124.1	131.5	115.7	152.7	119.5	126.6, 139.1, 140.3, 142.8, 144.7 (C-Ar)

However, the ^{13}C-NMR spectra of fused compounds were found to be unlike those observed for other dicoumarols. The signals of a part of 4-hydroxycoumarin which fused with aromatic ring were occurred at lower chemical shifts, except for C-3′ signal. The two tetramers **49** and **50** which DMSO-d_6 was used as solvent showed normal signals of C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-9 and C-10 at 164.4-166.5, 103.2-106.1, 166.4-169.3, 124.2-124.7, 124.6-125.6, 131.8-133.7, 116.3-117.3, 152.1-153.3 and 116.2-117.6 ppm, respectively. The ^{13}C-NMR of Compound **55** showed a pattern of signals similar to that of tetramers due to DMSO-d_6 solvent as C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-9, C-10, C-Cl and α-C at 162.5, 103.3, 165.9, 123.9, 124.0, 132.9, 116.3, 153.2, 115.9, 80.3 and 98.9 ppm, respectively.



(E)-isomer



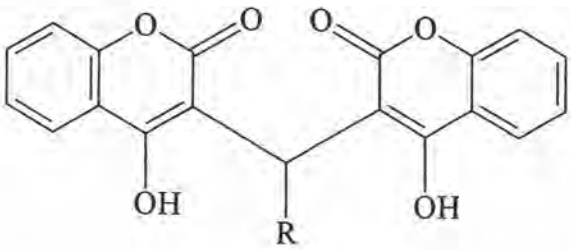
(Z)-isomer

The product derived from the condensation of 4-hydroxycoumarin with anthracene-9-carboxaldehyde did not show the expect pattern like those of dicoumarols. The ^{13}C-NMR spectrum of **54** exhibited a pair of signals belonging to both (E) and (Z) isomers. Signals of ketone form for C-4 were visualized at 177.8 and 179.9 ppm, while the signals of CH-double bond were attributed at 127.8 and 128.1 ppm. The ^{13}C-NMR spectral assignments of Compound **54** are tabulated in Table 3.5.

Table 3.5 The ^{13}C-NMR spectral assignments for Compound **54**

Position	Chemical shift (ppm)
C-2	157.7, 162.1
C-3	119.7, 120.7
C-4	177.8, 179.9
C-5	124.9, 125.0
C-6	124.9, 125.0
C-7	130.7, 130.9
C-8	117.6, 117.9
C-9	158.9, 159.1
C-10	128.2, 128.3
C-α	127.8, 128.1
C-1′, C-8′	127.2
C-2′, C-7′	125.0, 125.1
C-3′, C-6′	125.6
C-4′, C-5′	129.3, 129.4
C-9′	154.7, 155.7
C-10′	136.5, 137.2
C-11′, C-14′	128.8, 129.1
C-12′, C-13′	130.9, 131.0

Mass Spectrometry (MS)



The mass spectrum was scanned at 70 eV to confirm the structures of new compounds. MS displayed the molecular ion (M^+) with small relative intensity or sometimes it could not be observed as found in Compounds **32, 33** and **50**. This was possibly due to the easy fragmentation of alkyl chain moiety. Other relevant ions were observed at M^+-162, 162, 121 and 120. The data are tabulated in Table 3.6 and the possible fragmentation pattern of dicoumarols is presented in Schemes 3.1 and 3.2.

Table 3.6 Mass spectral assignments of Compounds **17, 23, 29-33, 38, 44, 50** and **54**

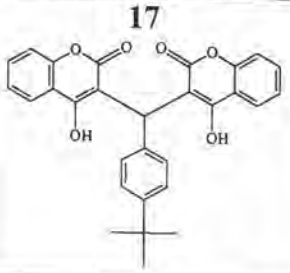
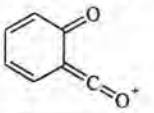
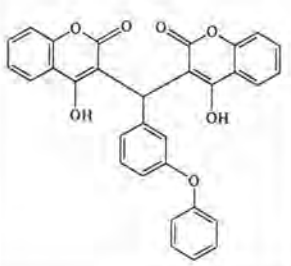
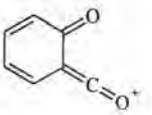
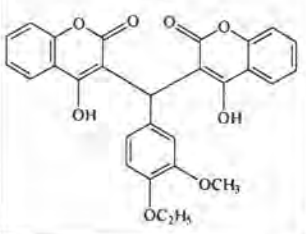
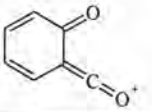
Cpd	peak (m/z)	Assignments
17 	468 305 162 120	M^+ M^+-162-H 4-hydroxycoumarin moiety 
23 	504 342 162 120	M^+ M^+-162 4-hydroxycoumarin moiety 
29 	486 324 162 120	M^+ M^+-162 4-hydroxycoumarin moiety 

Table 3.6 (cont.)

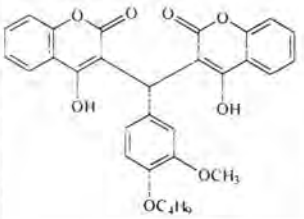
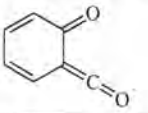
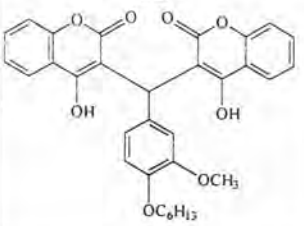
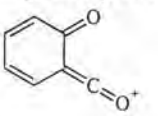
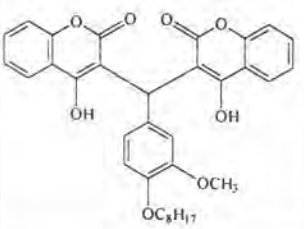
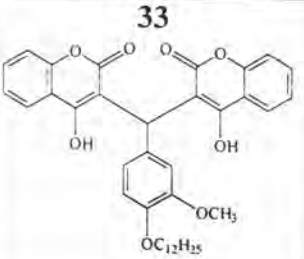
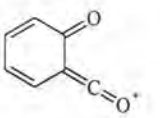
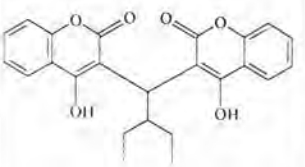
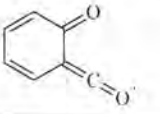
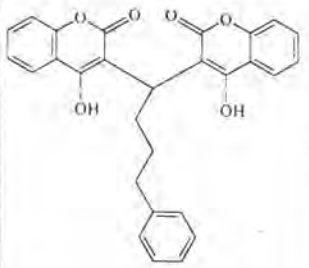
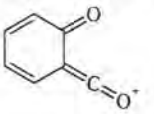
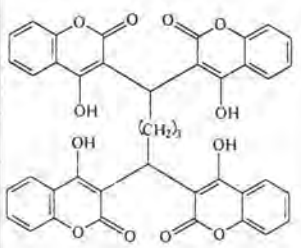
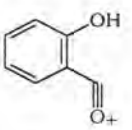
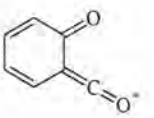
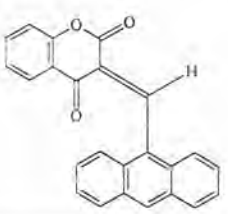
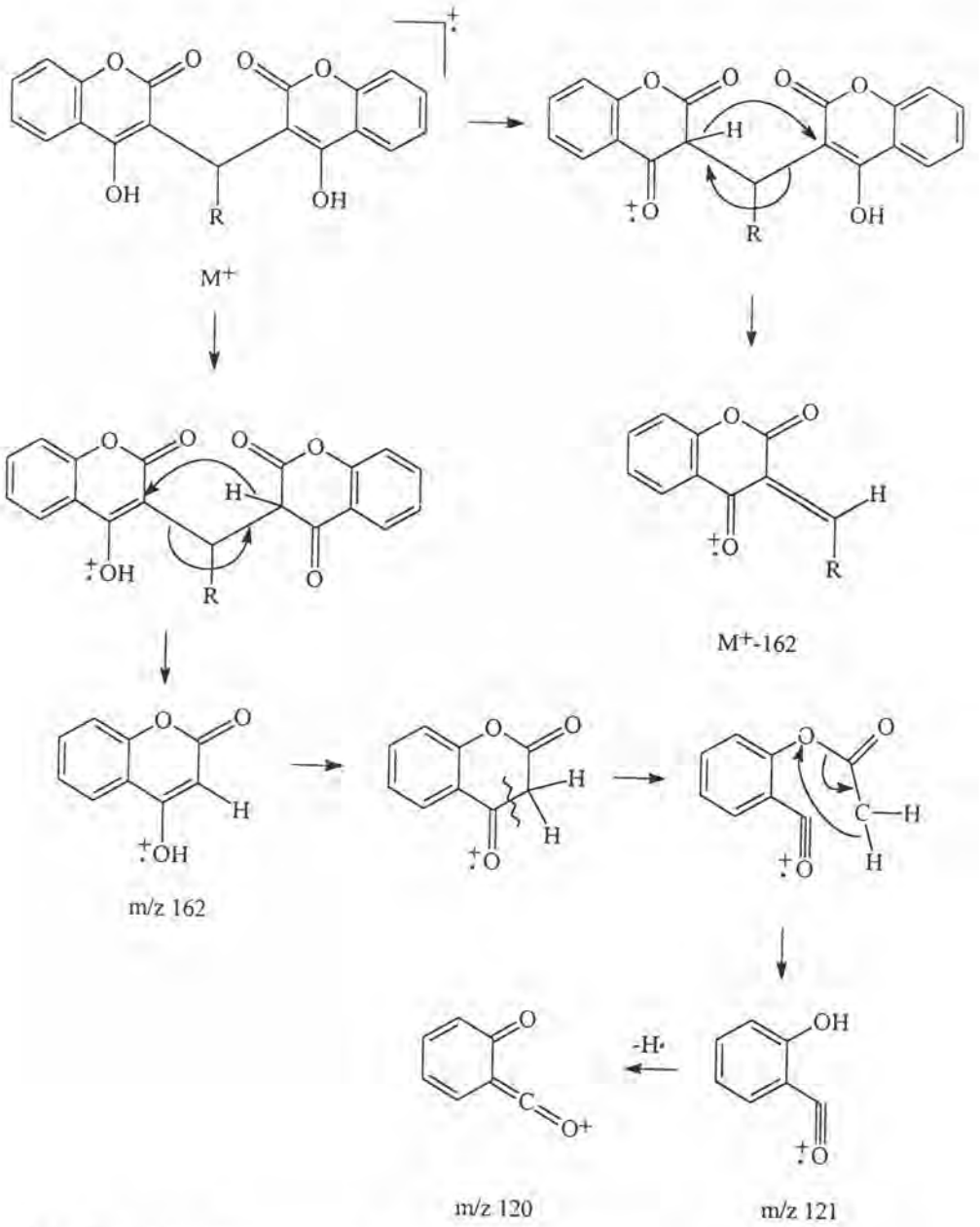
Cpd	peak (m/z)	Assignments
30 	514 352 162 120	M^+ M^+-162 4-hydroxycoumarin moiety 
31 	542 380 162 120	M^+ M^+-162 4-hydroxycoumarin moiety 
32 	365 161	M^+-162-C_2H_5 4-hydroxycoumarin moiety
33 	464 162 120	M^+-162 4-hydroxycoumarin moiety 
38 	406 335 244 162 120	M^+ M^+-C_5H_{11} M^+-162 4-hydroxycoumarin moiety 

Table 3.6 (cont.)

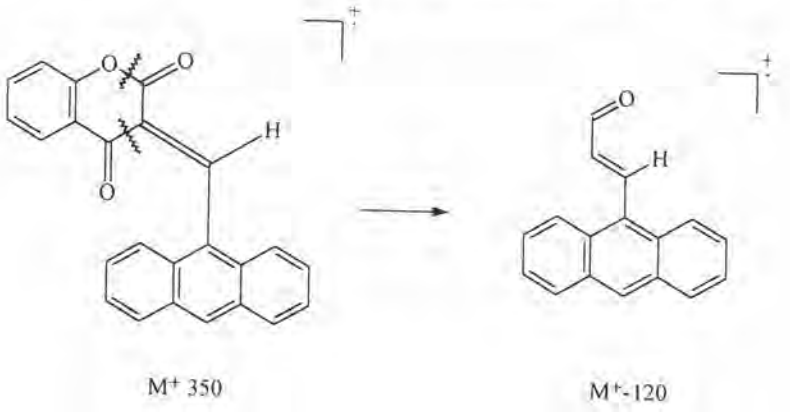
Cpd	peak (m/z)	Assignments
44 	454 292 201 162 120	M^+ M^+-162 M^+-162-C_7H_7 4-hydroxycoumarin moiety 
50 	162 121 120	4-hydroxycoumarin moiety  
54 	350 230 200	M^+ M^+-120 M^+-120-CHO

Most of dicoumarols show a weak molecular ion [M^+] and a medium peak formed by loss of 4-hydroxycoumarin [M^+-162]. This fragmentation pattern gives a strong m/z peak at 162 of 4-hydroxycoumarin moiety. This signal could be firmly be a characteristic peak owing to the lost of 42 mass units of C_2H_2O from the pyrone ring to provide probably as ketene (m/z 120).

The fragmentation pattern of Compound **54** is unlike the above processes. Molecular ion [M^+ 350, 100%] is observed as a base peak. Another peak is [M-120] $^+$ which formed by cleavage of the ketene mass unit.



Scheme 3.1 The proposed mass fragmentation pattern of dicoumarols



Scheme 3.2 The possible mass fragmentation pattern of Compound **54**

3.1.3 Synthesis of 3-Alkyl-4-hydroxycoumarins

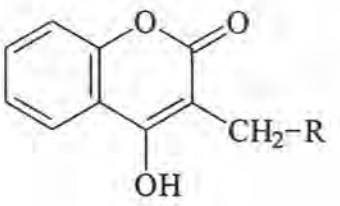
Another group of 3-substituted-4-hydroxycoumarin conducted in this research is the 4-hydroxycoumarins whose alkyl groups are substituted at 3-position. This class of compounds has been reported to possess the high anticoagulant activity.[49,50] Although, 3-alkyl-4-hydroxycoumarins can be synthesized by various methods and gave variably low-high yield, the direct alkylation of 4-hydroxycoumarin with alkyl bromide to form the desired product was reported to obtain in low yield.[40] By products were obtained because of their ambident anions as the O-alkylated, C,O-dialkylated, α,α-disubstituted-*o*-hydroxy acetophenones etc.[51] In order to solve these problems, dicoumarols are reduced by sodium cyanoborohydride to cleave a 4-hydroxycoumarin moiety resulted in 3-alkyl-4-hydroxycoumarin in high yield.[14] In this research, fifteen 3-alkyl-4-hydroxy coumarins synthesized from dicoumarols using this methodology were obtained in high yield (70-93%) except for **R1** and **R14** (35 and 20%, respectively). Each compound was examined by IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. **R12** is found to be a new compound after searching from chemical literature. All 3-alkyl-4-hydroxycoumarins are white solid and their melting points are in the range of 130-230 °C. Reduction time of an alkyl group substituted at 3-position **R1-R5** (48-168 hours) was generally found to be longer than aromatic substituent on C-α **R6**, **R11-R15** (10-46 hours). The phenyl ring substituted on an alkyl chain (**R7-R9**) needed a reduction time in the range of 14-190 hours. However, the reaction time was very fast when a double bond conjugated to an aromatic ring **R10**. The data are compilated in Table 3.7.

Table 3.7 The physical properties and % yield of synthesized 3-alkyl-4-hydroxy coumarins

Cpd	Physical properties		% Yield	Reaction time (hours)
	Appearance	m.p. (°C)		
R1	White solid	229-230	35	168
R2	White solid	153-154	78	48
R3	White solid	155-156	89	70
R4	White solid	137-139	79	96
R5	White solid	184-186	85	75
R6	White solid	204-205	92	46
R7	White solid	208-209	74	14
R8	White solid	158-159	70	190
R9	White solid	156-157	93	82
R10	White solid	193-194	87	5
R11	White solid	183-184	76	28
R12	White solid	207-208	80	32
R13	White solid	179-183	78	10
R14	White solid	200-201	20	30
R15	White solid	211-212 (dec)	72	13

3.1.4 Spectroscopy of 3-Alkyl-4-hydroxycoumarins

Infrared Spectroscopy (IR)



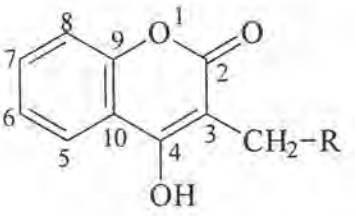
The FT-IR spectra of 3-alkyl-4-hydroxycoumarins generally reveal the absorption band of O-H stretching vibration at 3500-2700 cm^{-1}(s). C-H Aromatic stretching vibration at 3098-3029 cm^{-1} (w) and C-H aliphatic stretching vibration at 2999-2807 cm^{-1} were presented. The C=O stretching vibration of pyran ring was observed at 1690-1656 cm^{-1}, while C=C aromatic ring stretching vibration at 1524-1488 cm^{-1} and the C-O stretching vibration at 1139-1047 cm^{-1} were also detected. The FT-IR absorption band assignments of 3-alkyl-4-hydroxycoumarins are tabulated in Table 3.8.

Table 3.8 The FT-IR absorption band assignments of 3-alkyl-4-hydroxycoumarins

Cpd	Wave number (cm^{-1})					
	O-H	Ar-H	C-H str.	C=O	benzo	C-O
R1	3365-2936 (s)	-	-	1671 (s)	1502 (m)	1091 (s)
R2	3428-3000 (s)	-	2977-2870 (w)	1678 (s)	1499 (m)	1100 (s)
R3	3400-3000 (s)	-	2955-2807 (w)	1675 (s)	1499 (m)	1095 (s)
R4	3480-3000 (s)	-	2955-2870 (w)	1690 (s)	1506 (m)	1117 (s)
R5	3500-3000 (s)	-	2951-2856 (w)	1678 (s)	1499 (m)	1084 (s)
R6	-	3098-3032 (w)	2966-2933 (w)	1660 (s)	1495 (m)	1084 (s)
R7	-	3087-3032 (w)	2936-2867 (w)	1660 (s)	1495 (m)	1088 (s)
R8	3440-2800 (s)	3087-3028 (w)	2966-2903 (w)	1667 (s)	1502 (m)	1047 (s)
R9	3380-2800 (s)	3072-3024 (w)	2936-2859 (w)	1671 (s)	1495 (m)	1099 (s)
R10	3430-2800 (s)	3079-3028 (w)	2973-2903 (w)	1660 (s)	1502 (m)	1117 (s)
R11	3420-2800 (s)	3046-3024 (w)	2969-2929 (w)	1667 (s)	1499 (m)	1117 (s)
R12	3450-2700 (s)	3065-3032 (w)	2925-2859 (w)	1660 (s)	1517 (m)	1113 (s)
R13	3350-2800 (s)	3076 (w)	2999-2837 (w)	1656 (s)	1510 (m)	1113 (s)
R14	-	3032 (w)	2966-2845 (w)	1675 (s)	1524 (m)	1139 (s)
R15	3400-2800 (s)	3035 (w)	2903 (w)	1671 (s)	1488 (m)	1110 (s)

Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H-NMR

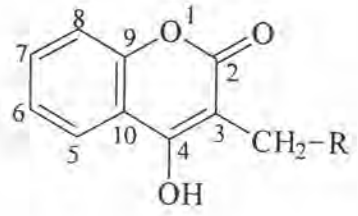


All 3-alkyl-4-hydroxycoumarins were dissolved in DMSO-d_6 to measure the NMR spectra. The methylene signal was detected around 2.45-3.82 ppm. H-5 and H-6 were observed at 7.62-7.97 ppm and at 7.43-7.60 ppm, respectively. The overlapped signals of H-7 and H-8 were also detected around 7.03-7.39 ppm. The O-H signal was not observed which was similar to those of dicoumarol when DMSO-d_6 was used as a solvent. Other protons of a substituent group were also presented. The ^{1}H-NMR spectral assignments of 3-alkyl-4-hydroxy coumarins are tabulated in Table 3.9.

Table 3.9 The ^{1}H-NMR spectral assignments of 3-alkyl-4-hydroxycoumarins

Cpd	R-	Chemical shift (ppm)				
		H-5	H-6	H-7, H-8	CH_2	Others
R1	H	7.88(d)	7.57(t)	7.28-7.36(m)	-	1.98(s) (CH_3)
R2	CH_3	7.81(d)	7.51(t)	7.24-7.32(m)	2.61(q)	1.19(t) (CH_3)
R3	$CH_3CH_2CH_2$	7.92(d)	7.53(t)	7.27-7.32(m)	1.20-1.42(m)	0.86(t) (CH_3), 1.20-1.42(m) (CH_2)
R4	$(CH_3)_2CH$	7.82(d)	7.52(t)	7.24-7.32(m)	2.45(d)	0.98(d) (CH_3), 2.02(m) (CH)
R5	Cyclohexyl	7.84(d)	7.51(t)	7.26-7.32(m)	2.48(d)	0.98-1.28(m), 1.66-1.77(m) (Cyclohexyl moeity)
R6	C_6H_5	7.93(d)	7.46(t)	7.03-7.26(m)	3.78(s)	7.03-7.26(m) (Ar-H)
R7	$C_6H_5CH_2$	7.62(d)	7.50(t)	7.17-7.32(m)	2.88-2.91(m)	2.83-2.91(m) (CH_2), 7.17-7.32(m) (Ar-H)
R8	$C_6H_5(CH_3)CH$	7.90(d)	7.56(t)	7.08-7.35(m)	3.11(m)	1.17(d) (CH_2), 2.79(m) (CH), 7.08-7.35(m) (Ar-H)
R9	$C_6H_5CH_2CH_2$	7.84(d)	7.51(t)	7.18-7.37(m)	2.72(t)	1.95(m) (CH_2), 2.57(t) (CH_2), 7.18-7.37(m) (Ar-H)
R10	$C_6H_5CH{=}CH$	7.87(d)	7.43(t)	7.08-7.27(m)	3.49(d)	6.26(dt), 6.45(d) (CH=CH), 7.08-7.27(m) (Ar-H)
R11	4-$(CH_3)C_6H_4$	7.97(d)	7.60(t)	7.31-7.39(m)	3.82(s)	2.25(s) (CH_3), 7.04(d), 7.12(d) (Ar-H)
R12	4-$(OH)C_6H_4$	7.95(d)	7.60(t)	7.31-7.38(m)	3.75(s)	6.63(d), 7.03(d) (Ar-H), 9.13(br) (OH)
R13	4-$(OCH_3)C_6H_4$	7.96(d)	7.49(t)	7.19-7.26(m)	3.74(s)	3.66(s) (OCH_3), 6.74(d), 7.17(d) (Ar-H)
R14	3,4-$(OCH_3)_2C_6H_3$	7.96(d)	7.60(t)	7.31-7.38(m)	3.80(s)	3.67(s), 3.69(s) (OCH_3), 6.69-6.89(m) (Ar-H)
R15	3,4-$(OCH_2O)C_6H_3$	7.96(d)	7.59(t)	7.30-7.36(m)	3.77(s)	5.91(s) (CH_2), 6.68-6.80(m) (Ar-H)

^{13}C-NMR

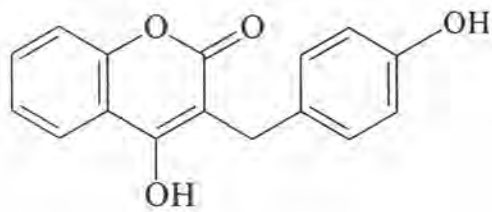


The ^{13}C-NMR spectra of 3-alkyl-4-hydroxycoumarins also provided common characteristic patterns. To illustrate this, the signals belonging to CH_2 at 3-position were detected at 17.0-37.7 ppm. The typical chemical shifts of nine carbon atoms on benzopyran ring were observed above 100 ppm (100.2-166.1 ppm) designated as C-2, C-3, C-4, C-5, C-6, C-7, C-8, C-9 and C-10 at 159.3-163.8, 100.2-106.4, 162.6-166.2, 122.3-123.3, 122.5-124.0, 130.1-131.9, 115.0-116.5, 151.2-152.7 and 115.6-119.8 ppm, respectively. Other carbons of a substituent were manifestly detected. The ^{13}C-NMR spectral assignments of 3-alkyl-4-hydroxycoumarins are presented in Table 3.10.

Table 3.10 The ^{13}C-NMR spectral assignments of 3-alkyl-4-hydroxycoumarins

Cpd	Chemical shift (ppm)											
		CH_2	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	Others
R1	H	-	159.7	100.2	163.1	122.9	123.8	131.4	116.0	151.6	116.3	9.7 (CH_3)
R2	CH_3	17.0	159.3	106.4	162.7	123.1	123.8	131.5	116.0	151.7	116.3	12.8 (CH_3)
R3	$CH_3CH_2CH_2$	30.2	161.1	104.3	163.1	123.3	123.4	131.1	115.9	151.9	117.2	13.9 (CH_3), 22.1, 23.4 (CH_2)
R4	$(CH_3)_2CH$	32.1	160.1	104.2	163.0	123.1	123.7	131.5	116.0	151.8	116.2	22.1 (CH_2), 27.2 (CH)
R5	Cyclohexyl	36.3	160.0	103.8	163.0	123.1	123.7	131.4	116.0	151.8	116.2	25.8, 26.1, 30.7, 32.4, 36.6 (Cyclohexyl moeity)
R6	C_6H_5	29.5	163.8	100.7	166.2	122.7	124.0	130.5	115.7	152.7	119.8	125.1, 127.8, 128.2, 142.0 (C-Ar)
R7	$C_6H_5CH_2$	32.8	159.3	103.8	162.6	122.3	122.5	130.1	115.0	151.2	115.6	24.8 (CH_2), 124.7, 127.1, 127.4, 140.8 (C-Ar)
R8	$C_6H_5(CH_3)CH$	37.7	160.3	103.7	162.8	123.2	123.8	131.6	116.1	151.8	116.1	20.8 (CH_3), 32.0 (CH), 125.9, 126.8, 128.1, 146.8 (C-Ar)
R9	$C_6H_5CH_2CH_2$	35.4	159.6	105.1	164.1	123.0	123.9	131.6	116.5	152.2	115.7	23.2, 29.8 (CH_2), 126.0, 128.5, 141.8 (C-Ar)
R10	$C_6H_5CH=CH$	27.1	160.8	103.4	164.0	123.2	123.6	131.3	116.4	152.5	116.6	126.7, 126.9 (CH=CH), 126.0,128.4, 130.5, 137.4 (C-Ar)
R11	4-$(CH_3)C_6H_4$	28.6	160.3	104.4	162.8	123.3	123.9	131.8	116.2	151.9	116.2	20.6 (CH_3), 128.0, 128.7, 134.5, 136.7 (C-Ar)
R12	4-$(OH)C_6H_4$	28.2	160.1	104.9	162.6	123.3	123.8	131.7	116.1	151.9	116.3	114.9, 129.0, 129.8, 155.5 (C-Ar)
R13	4-$(OCH_3)C_6H_4$	28.5	163.6	102.3	164.4	123.0	123.9	130.8	115.8	152.5	118.9	54.9 (OCH_3), 113.3, 129.1, 133.4, 157.2 (C-Ar)
R14	3,4-$(OCH_3)_2C_6H_3$	28.6	160.2	104.6	162.9	123.3	123.9	131.8	116.2	151.9	116.2	55.4, 55.5 (OCH_3), 111.8, 112.4, 119.8, 132.2, 147.1, 148.5 (C-Ar)
R15	3,4-$(OCH_2O)C_6H_3$	28.7	160.7	104.3	163.0	123.3	123.9	131.9	116.2	151.9	116.3	100.6 (OCH_2O), 108.0, 108.6, 120.8, 133.6, 145.3, 147.0 (C-Ar)

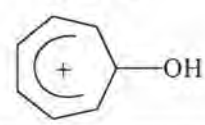
Mass Spectroscopy (MS)



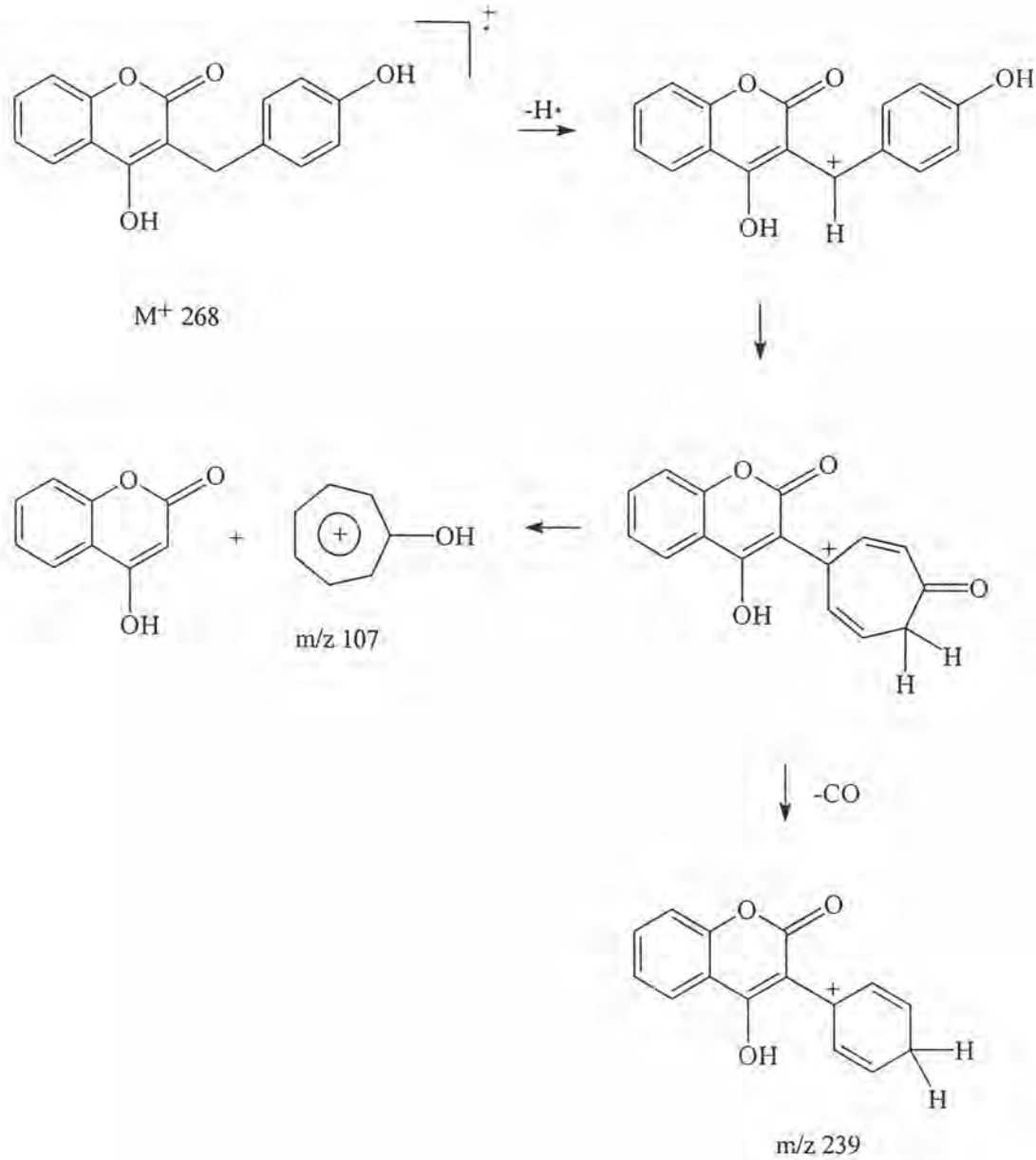
R12

MS was utilized to confirm the structure of a new compound. The MS spectrum of **R12** showed a molecular ion at m/z 268 (100 %). Other relevant ions were observed at m/z 239 (40), 121 (60) and 107 (50). The MS spectral assignments for this new 3-alkyl-4-hydroxycoumarins are tabulated in Table 3.11. The fragmentation pattern of **R12** is proposed as shown in Scheme 3.3.

Table 3.11 Mass spectral assignments for **R12**

Cpd	peak (m/z)	Assignments
R12	268	M^+
	239	M^+-29 (CHO)
	121	M^+-118
	107	

The fragmentation pattern of Compound **R12** is unlike that of dicoumarols. To illustrate this, the molecular ion peak [M^+ 268] was also found to be a base peak. The phenolic ring was rearranged and gave m/z 107 of $[C_7H_7O]^+$ or loss of CO group to generate m/z 239.



Scheme 3.3 The proposed mass fragmentation pattern of Compound **R12**

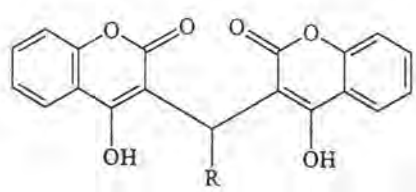
3.2 Biological Activity

As previously mentioned, 3-substituted-4-hydroxycoumarins are generally well-recognized to possess anticoagulant activity.[9,24] In this research, three bioassays, namely, Brine Shrimp Cytotoxic Lethality Test (BSCLT), Antibacterial activity and Antiviral activity were preliminary examined.

3.2.1 Brine Shrimp Cytotoxic Lethality Test against *Artemia salina* Leach.

To our best knowledge, the brine shrimp lethality test of 3-substituted-4-hydroxycoumarins has never been reported in literatures before. Generally, lethality test bioassays depend on the ability to measure the amount of survived brine shrimp from test samples. Low lethality efficiency is observed when the LC_{50} value is high and *vice versa*.

Dicoumarol



Fifty five synthesized dicoumarols and analogues were subjected to brine shrimp lethality test. The results are summarized as shown in Table 3.12. Dicoumarol **1** (R = H) was found to display low activity (LC_{50} = 173.4 μg/mL). The introduction of substituent markedly increased the activity, except for Compounds **48** and **52** (LC_{50} = 207.4 and 404.4 μg/mL, respectively). Among tested dicoumarols, four compounds, namely, **31**, **43**, **50** and **55** exhibited high activity with LC_{50} 4.882, 0.045, 0.094 and 4.309 μg/mL, respectively.

Table 3.12 The LC_{50} value at 24h of tested dicoumarols and analogues

Cpd	R	LC_{50} (μg/mL)	Bioactivity
1	H	173.4	Medium
2	C_6H_5	13.18	Medium
3	2-$(NO_2)C_6H_4$	23.03	Medium
4	3-$(NO_2)C_6H_4$	25.21	Medium
5	4-$(NO_2)C_6H_4$	31.62	Medium
6	3-FC_6H_4	29.56	Medium
7	4-FC_6H_4	35.98	Medium
8	2-ClC_6H_4	31.62	Medium
9	3-ClC_6H_4	28.69	Medium
10	4-ClC_6H_4	22.09	Medium
11	2,4-$(Cl)_2C_6H_3$	31.62	Medium
12	2-BrC_6H_4	19.38	Medium
13	3-BrC_6H_4	17.77	Medium
14	4-BrC_6H_4	27.87	Medium
15	4-$(CH_3)C_6H_4$	31.62	Medium
16	4-(*i*-Pr)C_6H_4	31.62	Medium
17	4-(*t*-Bu)C_6H_4	25.48	Medium
18	4-$(CF_3)C_6H_4$	25.21	Medium
19	2-$(OCH_3)C_6H_4$	19.38	Medium
20	3-$(OCH_3)C_6H_4$	11.82	Medium
21	4-$(OCH_3)C_6H_4$	27.62	Medium
22	4-$(OH)C_6H_4$	31.62	Medium
23	3-$(OC_6H_5)C_6H_4$	18.37	Medium
24	3,4-$(OH)_2C_6H_3$	31.62	Medium
25	3,4-methylenedioxybenzyl	118.2	Low
26	3,4,5-$(OCH_3)_3C_6H_2$	27.62	Medium
27	3-(OCH_3)-4-$(OH)C_6H_3$	31.62	Medium
28	3,4-$(OCH_3)_2C_6H_3$	30.63	Medium
29	3-(OCH_3)-4-$(OC_2H_5)C_6H_3$	22.09	Medium

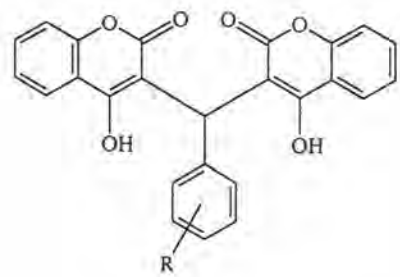
Table 3.12 (cont.)

Cpd	R	LC_{50} (μg/mL)	Bioactivity
30	3-(OCH_3)-4-(OC_4H_9)C_6H_3	11.82	Medium
31	3-(OCH_3)-4-(OC_6H_{13})C_6H_3	4.882	High
32	3-(OCH_3)-4-(OC_8H_{17})C_6H_3	22.04	Medium
33	3-(OCH_3)-4-($OC_{12}H_{25}$)C_6H_3	31.68	Medium
34	3(OCH_3)-4($OCH_2C_6H_5$)C_6H_3	25.47	Medium
35	CH_3	27.52	Medium
36	$CH_3CH_2CH_2$	17.76	Medium
37	$(CH_3)_2CH$	36.22	Medium
38	$(CH_3CH_2)_2CH$	26.43	Medium
39	Cyclohexyl	15.66	Medium
40	$C_6H_5CH_2$	17.00	Medium
41	(±)-$C_6H_5(CH_3)CH$	16.25	Medium
42	$C_6H_5CH_2CH_2$	38.27	Medium
43	(E)-C_6H_5CH=CH	0.045	High
44	$C_6H_5CH_2CH_2CH_2$	49.98	Medium
45	C_6H_4(fuse)	21.23	Medium
46	3-(OCH_3)C_6H_3 (fuse)	40.54	Medium
47	6-ClC_6H_3 (fuse)	12.79	Medium
48	naphthyl (fuse)	207.4	Low
49	C_6H_4 (Tetramer)	13.06	Medium
50	$CH_2CH_2CH_2$ (Tetramer)	0.094	High
51	1-Naphthyl	19.94	Medium
52	2-Pyridinyl	404.4	Low
53	3-Pyridinyl	23.61	Medium
54	(±)-9-Anthracenyl	31.59	Medium
55	Cl_3C	4.309	High

Note : LC_{50} < 10 μg/mL = High activity, LC_{50} < 100 μg/mL = Medium activity
LC_{50} < 1000 μg/mL = Low activity, LC_{50} > 1000 μg/mL = Inactive

Generally, the position and type of substituents were major factors to influence the biological activity. To make the SAR study more comprehendable, the comparison of various substituents of fifty five compounds could be summarized as follows:

1) Substituent on a benzylidene ring



Using Compound **2** (R = H) as a reference, the introduction of various substituents at 4-position lucidly reduced activity. The activity was observed in the series of:

$$H > Cl > CF_3 > t\text{-Bu} > Br > OMe > OH = i\text{-Pr} = Me = NO_2 > F$$

Comparing with the compounds having the same substituents but different in the position, it was found that the substituents bearing on the 3-position of the benzylidene ring had little effect on activity observed. For instance, 3-OMe showed highest activity in this series and the activity of 3-Br was higher than that of 3-Cl. The comparative activity is exhibited as follows:

$$OMe > H > Br > OPh > NO_2 > Cl > F$$

The effects of the position of the same functional group could also be visualized in Fig 3.4. The substituents such as OMe and Br had similar tendency of activity from high to low when they were substituted at *meta* > *ortho* > *para*, while the activity observed by the influence of the NO_2 group could be arranged as *ortho* > *meta* > *para*. However, Cl was found to be differed from the above, i.e., the activity was observed as *para* > *meta* > *ortho*. The addition of two Cl atoms at *ortho* and *para* positions revealed less activity than that at *para* position only.

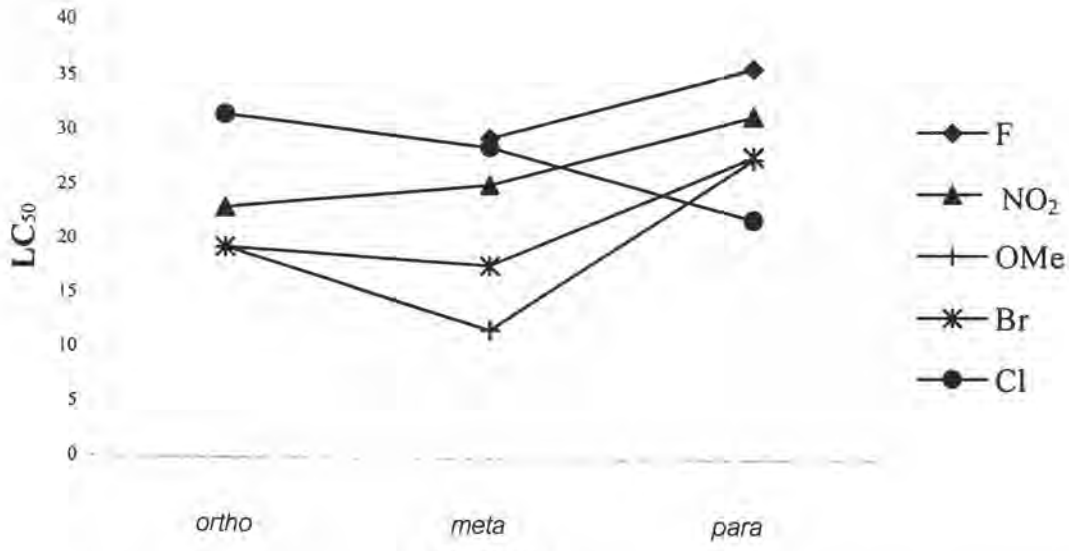
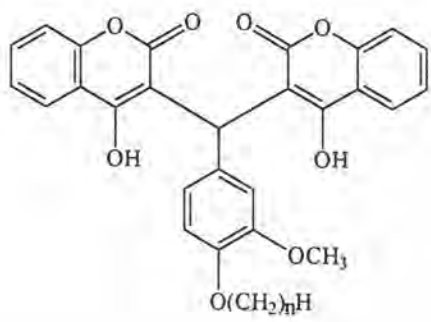


Fig 3.4 Comparison of LC_{50} value of substituents on a benzylidene ring of dicoumarols

Considering of hydroxy and methoxy groups, the activity was found to be variable in the range of medium activity around 11-31 μg/mL. In the case of Compound **25** which contained 3,4-OCH_2O-bridge substituted group, the activity was dropped to low potency.

The variation of the numbers of carbon atoms in a benzylidene ring of an alkoxy group at 4-position was also investigated. It was found that in a series of Compounds **27-33** which carbon atoms increased from 0, 1, 2, 4, 6, 8 and 12, Compound **31** displayed the highest activity. The optimization of the number of carbon atoms in chain can be deduced that six carbon atoms exhibited the highest activity. The normal curve was plotted as a bell-shape. The results are shown in Fig 3.5.



27 n = 0
28 n = 1
29 n = 2
30 n = 4
31 n = 6
32 n = 8
33 n = 12

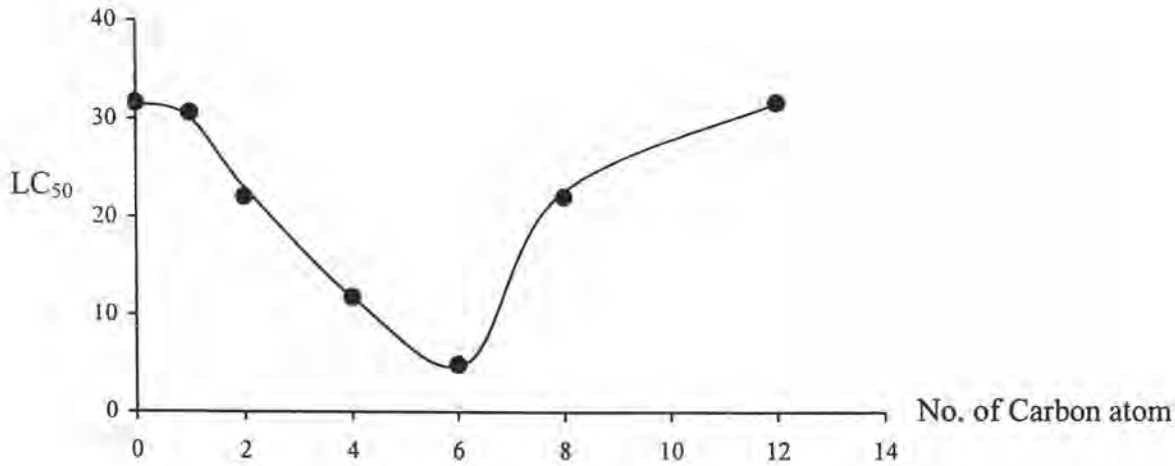
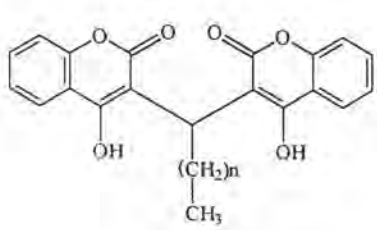


Fig 3.5 Effect of carbon atom on LC_{50} value of dicoumarols **27-33**

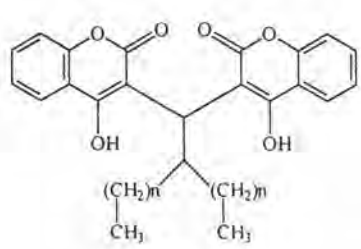
2) Effect on alkyl substituents

The examination on the effects of alkyl substituents was performed employing a series of 10 compounds: Compounds **35-44**. Compared with Compound **1** (R = H), Compounds **35** and **36** which were of a longer chain of carbon atoms revealed higher activity. The similar result was observed when the examination of branch chain substituent was carried out. Increasing the branch chain of carbon atoms in Compounds **37** to **38** provided the increasing of brine shrimp lethality. The cyclohexyl ring substituent on the benzylidene ring in Compound **39** was also promoted the activity.



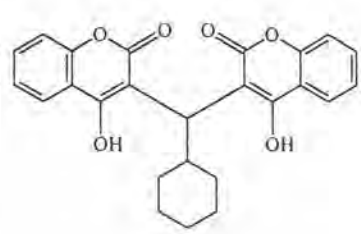
35 n = 0

36 n = 1



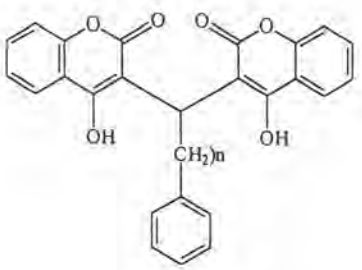
37 n = 0

38 n = 1

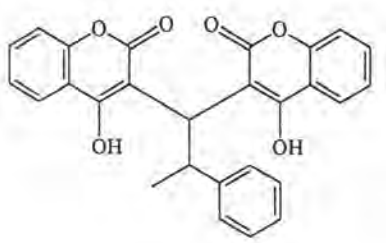


39

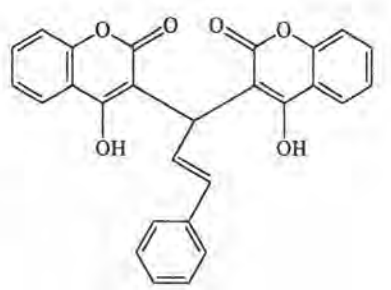
The comparison of the activity of Compounds **2**, **40-44** which contained the aromatic ring at the end of chain length in the benzylidene portion was another point of examination. When the alkyl chain was extended, the activity of these compounds was found to be diminished. The activity was found to render as: **2** > **40** > **42** > **44**. The branch chain moiety in Compound **41** did not show the difference in the magnitude of activity from that containing the straight chain (Compound **40**). However, Compound **43** which constitutes of the chain with a double bond conjugated system exhibited the highest activity in this series.



2 n = 0
40 n = 1
42 n = 2
44 n = 3



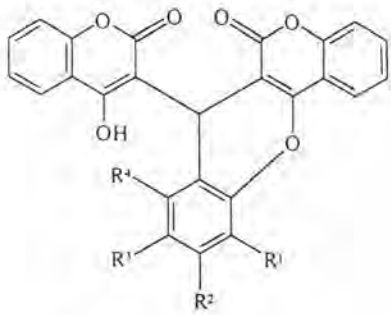
41

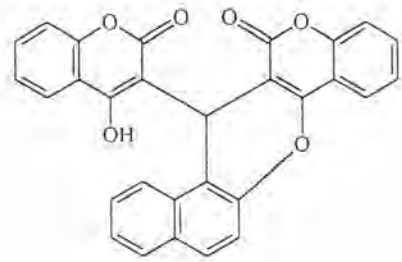


43

3) Fused ring compounds

The fused ring compounds (Compounds **45-48**) were prepared. Comparison of Compound **45** with Compound **2**, it was found that the constrained conformations proved little deleterious to lethality potency. The addition of a methoxy group at 3-position in Compound **46** caused the loss of potency, since 6-Cl substituted to aromatic ring (Compound **47**) showed better potency than the parent Compound **2**. The addition of the large group such as naphthyl moiety (Compounds **48**) rendered the activity down more than 20 folds.





45 $R^1 = R^2 = R^3 = R^4 = H$

46 $R^1 = OCH_3$, $R^2 = R^3 = R^4 = H$

47 $R^1 = R^2 = R^3 = H$, $R^4 = Cl$

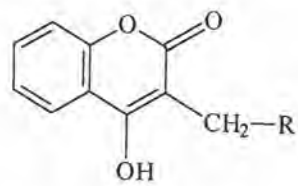
48

4) Miscellaneous compounds

Tetrameric Compound **49** has been reported recently as the development of inhibitors for HIV-1 integrase.[4,18] Two tetrameric Compounds **49** and **50** were thus synthesized to observe the cytotoxicity activity. The tetramer which contained a phenyl ring showed low activity than **2**, while that contained an alkyl chain exhibited very high activity. Replacement of a phenyl ring in Compound **2** with 1-naphthyl ring in Compound **51** exhibited less potency. The change of a phenyl ring with a pyridine ring decreased the activity, with 2-pyridinyl (Compound **52**) showed extremely low activity, and with 3-pyridinyl in Compound **53** showed less active than the phenyl ring.

The two unexpected products, Compounds **54** and **55** were also synthesized and tested. Compound **54** exhibited medium cytotoxicity activity while Compound **55** showed high activity.

3-Alkyl-4-hydroxycoumarins



3-Alkyl-4-hydroxycoumarins have also been recalled to exhibit anticoagulant activity. Some were found more potent than their dicoumarol analogues. The LC_{50} value for the lethality of brine shrimp from fifteen 3-alkyl-4-hydroxycoumarins were determined. The results are summarized in Table 3.13. Most 3-alkyl-4-hydroxy coumarins showed high activity, except for **R4**, **R6**, **R11** and **R15** which were exhibited medium activity.

Table 3.13 The LC_{50} value at 24h of tested 3-alkyl-4-hydroxycoumarins

Cpd	R	LC_{50} (μg/mL)	Bioactivity
R1	H	0.145	High
R2	CH_3	0.045	High
R3	$CH_3CH_2CH_2$	1.381	High
R4	$(CH_3)_2CH$	11.27	Medium
R5	Cyclohexyl	0.045	High
R6	C_6H_5	12.84	Medium
R7	$C_6H_5CH_2$	0.077	High
R8	$C_6H_5(CH_3)CH$	0.051	High
R9	$C_6H_5CH_2CH_2$	0.067	High
R10	$C_6H_5CH{=}CH$	0.089	High
R11	$4\text{-}(CH_3)C_6H_4$	15.91	Medium
R12	$4\text{-}(OH)C_6H_4$	0.145	High
R13	$4\text{-}(OCH_3)C_6H_4$	2.026	High
R14	$3,4\text{-}(OCH_3)_2C_6H_3$	0.525	High
R15	3,4-methylenedioxybenzyl	42.53	Medium

To gain more information for discussion, 3-alkyl-4-hydroxycoumarins were classified according to their substituents into three types as follows:

1) Alkyl chain length

The length of an alkyl chain substituted at 3-position of the main skeleton was explored. It was found that Compound **R2** gave the highest activity than **R1** and **R3**. This may imply that the only two carbons chain length had the great potent. When the alkyl group bearing a branch chain as those observed in Compounds **R4** and **R5** was examined, it was observed that the branched chain containing compound (Compound **R4**) dropped the activity more than 9 folds. Whereas the inflexible structure in Compound **R5** gave the high activity comparably equal to Compound **R2**.

2) Phenyl substituent on an alkyl chain

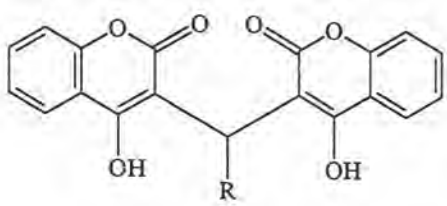
The effect of chain elongation next to the phenyl ring was seen from the comparative study of Compounds **R6**, **R7** and **R9**. It was found that the activity was increased when increasing carbon atom from 1 to 3. The branch chain moiety in Compound **R8** gave the highest activity while the introduction of a conjugated system on C-2 and C-3 of the carbon skeleton in Compound **R10** decreased the activity.

3) Substituent group on a phenyl ring

Further studies on the effect of a substituent group on a phenyl ring were observed compared with the parent compound **R6**. The activity was increased when the benzene moiety contained electron donating group such as hydroxy and methoxy groups (Compounds **R12-R14**). The weak electron donating group such as a methyl group bearing at 4-position of a benzene ring such as in Compound **R11** displayed slightly decreased in activity from the parent compound **R6**, while the OCH_2O bridge containing compound (**R15**) rendered the activity more than 3.5 fold.

4) Comparison with dicoumarols

The structure and activity comparison between the corresponding dicoumarols and 3-alkyl-4-hydroxycoumarins which derived from those dicoumarols was the next target to be explored. It was observed that all 3-alkyl-4-hydroxycoumarins exhibited higher activity than those dicoumarols, except for Compound **43** which contained a cinnamyl moiety showed more or less equal observed activity. The comparative data is tabulated in Table 3.14.



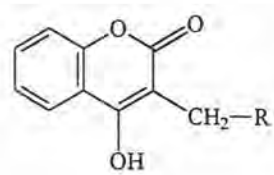


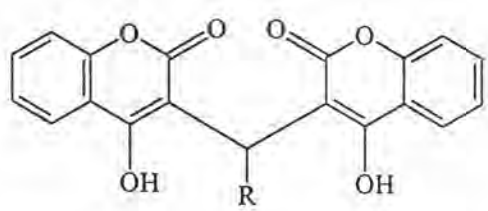
Table 3.14 The comparative results of the LC_{50} values of dicoumarols and 3-alkyl-4-hydroxycoumarins

R	Dicoumarol		3-Alkyl-4-hydroxycoumarin	
	Cpd	LC_{50} (μg/mL)	Cpd	LC_{50} (μg/mL)
H	**1**	173.4	**R1**	0.145
CH_3	**35**	27.52	**R2**	0.045
$CH_3CH_2CH_2$	**36**	17.76	**R3**	1.381
$(CH_3)_2CH$	**37**	36.22	**R4**	11.27
Cyclohexyl	**39**	15.66	**R5**	0.045
C_6H_5	**2**	13.18	**R6**	12.84
$C_6H_5CH_2$	**40**	17.00	**R7**	0.077
$C_6H_5(CH_3)CH$	**41**	16.25	**R8**	0.051
$C_6H_5CH_2CH_2$	**42**	38.27	**R9**	0.067
$C_6H_5CH=CH$	**43**	0.045	**R10**	0.089
$4\text{-}(CH_3)C_6H_4$	**15**	31.62	**R11**	15.91
$4\text{-}(OH)C_6H_4$	**24**	31.62	**R12**	0.145
$4\text{-}(OCH_3)C_6H_4$	**21**	27.62	**R13**	2.026
$3,4\text{-}(OCH_3)_2C_6H_3$	**28**	30.63	**R14**	0.525
3,4-methylenedioxybenzyl	**25**	118.2	**R15**	42.53

3.2.2 Antibacterial Results

There were a few reports concerning with the SAR study of 4-hydroxy coumarin derivatives and their antibacterial properties. For instance, Gu et al., in 1989, reported that twenty-six hydroxycoumarins have been tested against *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Streptococcus hemolyticus*, *Diplococcus pneumoniae*, *Neisseria gonorrhoeae*, *Escherichia coli*, *Salmonella typhosa*, *Shigella dysenteriae* and *Shigella paradysenteriae*.[52] 3-*N*-Cabamoyl-4-hydroxycoumarins were found to be useful as bactericidal and fungicidal properties.[53] In addition, in 1951, Ukita et al. found that 3-butyl and 3-decyl-4-hydroxycoumarins were tested against nine bacteria.[54] In this research, fifty five dicoumarols and their analogues and fifteen 3-alkyl-4-hydroxycoumarins were investigated against seven bacteria which have been known to the general public to cause food poisoning. Those bacteria can be distinguished into three groups: 1) gram-positive bacteria: *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococus aureus*; 2) gram-negative bacteria: *Escherichia coli*, *Escherichia coli* O157:H7 and *Salmonella derby*; and 3) Flat sour spoilage. The method applied for this assay is using paper disc method. Diameter of clear zone was measured after 24 hours of incubation. High inhibition was occurred when a diameter of clear zone was more than 10 mm. Weak inhibition was established in case of 7-10 mm diameter of clear zone. While a diameter of inhibition zone was less than 7 mm the test compounds were inactive.

Dicoumarol



Most of dicoumarols tested provided selectively inhibition against four bacteria including *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococus aureus* and Flat sour spoilage. The parent Compound **1** showed weak activity while Compound **2** exhibited high activity against these bacteria. The data of antibacterial activity result are tabulated in Table 3.15.

Table 3.15 Antibacterial properties of dicoumarols and analogues

Cpd	R	Activity (diameter (mm))			
		B	L	S	F
1	H	9	10	×	×
2	C_6H_5	12	15	×	11
3	2-$(NO_2)C_6H_4$	8	16	×	×
4	3-$(NO_2)C_6H_4$	0	10	×	×
5	4-$(NO_2)C_6H_4$	0	0	×	×
6	3-FC_6H_4	11	14	×	×
7	4-FC_6H_4	10	12	×	11
8	2-ClC_6H_4	15	15	×	13
9	3-ClC_6H_4	8	0	10	8
10	4-ClC_6H_4	10	8	×	8
11	2,4-$(Cl)_2C_6H_3$	10	13	×	10
12	2-BrC_6H_4	12	10	×	8
13	3-BrC_6H_4	10	8	×	8
14	4-BrC_6H_4	10	8	×	9
15	4-$(CH_3)C_6H_4$	11	10	×	×
16	4-(*i*-Pr)C_6H_4	10	8	×	×
17	4-(*t*-Bu)C_6H_4	10	8	×	×
18	4-$(CF_3)C_6H_4$	10	10	×	9
19	2-$(OCH_3)C_6H_4$	10	10	×	8
20	3-$(OCH_3)C_6H_4$	8	10	8	×
21	4-$(OCH_3)C_6H_4$	9	7	×	7
22	4-$(OH)C_6H_4$	7	15	×	0
23	3-$(OC_6H_5)C_6H_4$	0	0	0	0
24	3,4-$(OH)_2C_6H_3$	14	0	×	0
25	3,4-methylenedioxybenzyl	7	0	×	0
26	3,4,5-$(OCH_3)_3C_6H_2$	8	10	0	×
27	3-(OCH_3)-4-$(OH)C_6H_3$	10	13	×	12
28	3,4-$(OCH_3)_2C_6H_3$	10	0	×	0

Table 3.15 (cont.)

Cpd	R	Activity (diameter (mm))			
		B	L	S	F
29	3-(OCH_3)-4-(OC_2H_5)C_6H_3	0	0	8	0
30	3-(OCH_3)-4-(OC_4H_9)C_6H_3	0	0	0	0
31	3-(OCH_3)-4-(OC_6H_{13})C_6H_3	0	0	0	0
32	3-(OCH_3)-4-(OC_8H_{17})C_6H_3	0	0	0	0
33	3-(OCH_3)-4-($OC_{12}H_{25}$)C_6H_3	0	0	0	0
34	3(OCH_3)-4($OCH_2C_6H_5$)C_6H_3	0	0	0	0
35	CH_3	8	12	12	×
36	$CH_3CH_2CH_2$	8	8	8	×
37	$(CH_3)_2CH$	0	0	0	×
38	$(CH_3CH_2)_2CH$	10	10	8	×
39	Cyclohexyl	0	0	0	×
40	$C_6H_5CH_2$	12	11	×	×
41	(±)-$C_6H_5(CH_3)CH$	8	0	×	×
42	$C_6H_5CH_2CH_2$	8	9	×	×
43	(*E*)-C_6H_5CH=CH	8	15	13	8
44	$C_6H_5CH_2CH_2CH_2$	8	0	10	0
45	C_6H_4(fuse)	15	15	×	12
46	3-(OCH_3)C_6H_3 (fuse)	10	7	×	0
47	6-ClC_6H_3 (fuse)	13	10	20	15
48	naphthyl (fuse)	12	14	×	8
49	C_6H_4 (Tetramer)	12	0	10	0
50	$CH_2CH_2CH_2$ (Tetramer)	8	10	8	×
51	1-Naphthyl	0	0	0	0
52	2-Pyridinyl	8	10	8	×
53	3-Pyridinyl	8	10	8	×
54	(±)-9-Anthracenyl	0	8	0	0
55	Cl_3C	12	20	0	0

Table 3.15 (cont.)

Cpd	Activity (diameter (mm))			
	B	L	S	F
DMSO	0	0	0	0
CH_2Cl_2	0	0	0	0
Streptomycin 10 μg/mL	15	0	17	14
Kanamycin 10 μg/mL	15	20	15	22

Note : B = *Bacillus cereus*

L = *Listeria monocytogenes*

S = *Staphylococcus aureus*

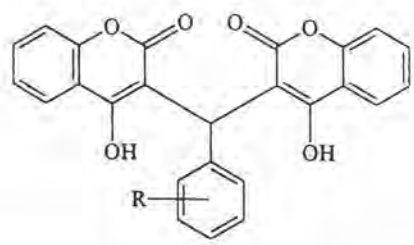
F = Flat sour spoilage

0 = Inactive

× = not tested

Although all antibacterial activity data against *Staphylococcus aureus* and Flat sour spoilage were not available, the complete bioassay results for *Bacillus cereus* and *Listeria monocytogenes* inhibition were gathered enough to discuss. Based on substituents on dicoumarols, the relationship between antibacterial activity and dicoumarol derivatives were classified into various types as follows:

1) Substituent on a benzylidene ring



Considering Compound **2** as a reference compound of these groups, this compound showed high activity against those bacteria. The effect of substituents and their position on antibacterial activity have further studied. In the case of nitro group, the substituent at 2-position showed high activity and decreased when the position of the substituent was altered to 3 and 4-position, respectively. The series of halogen

groups (**6-14**) has also studied, 2-Cl was expressed the highest inhibition activity in this series. The activity observed when the Cl group involved could be arranged as 2-position > 2,4-position > 4-position > 3-position. In the case of fluoro, bromo and methoxy groups, the similar activity as 2-position > 3-position > 4-position was observed, respectively. The activity of these series was represented in Fig 3.6 and 3.7.

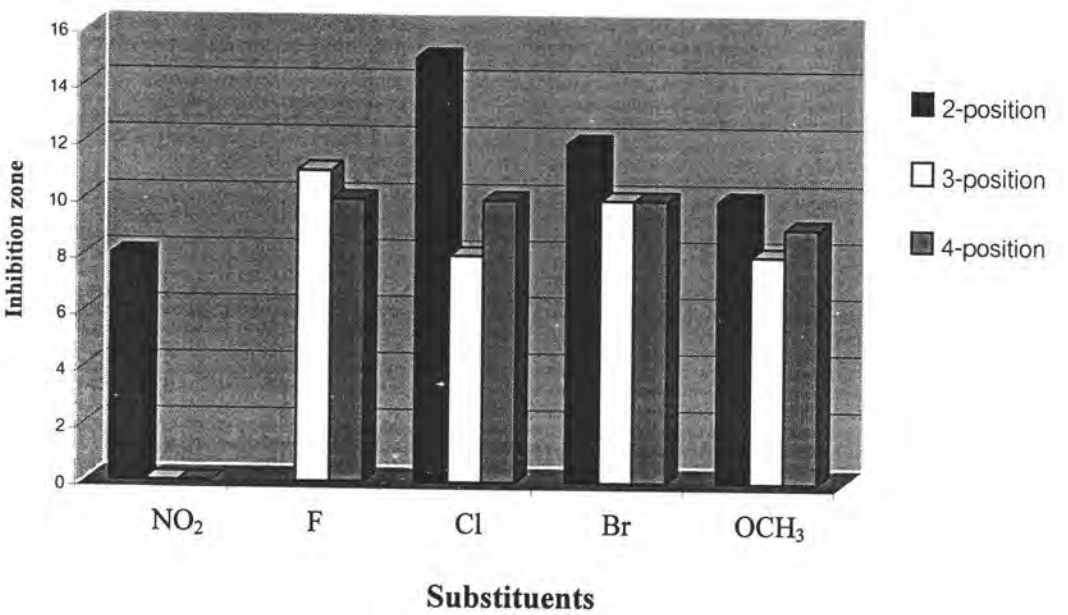


Fig 3.6 Effect of substituent on a benzylidene ring against *B. cereus*

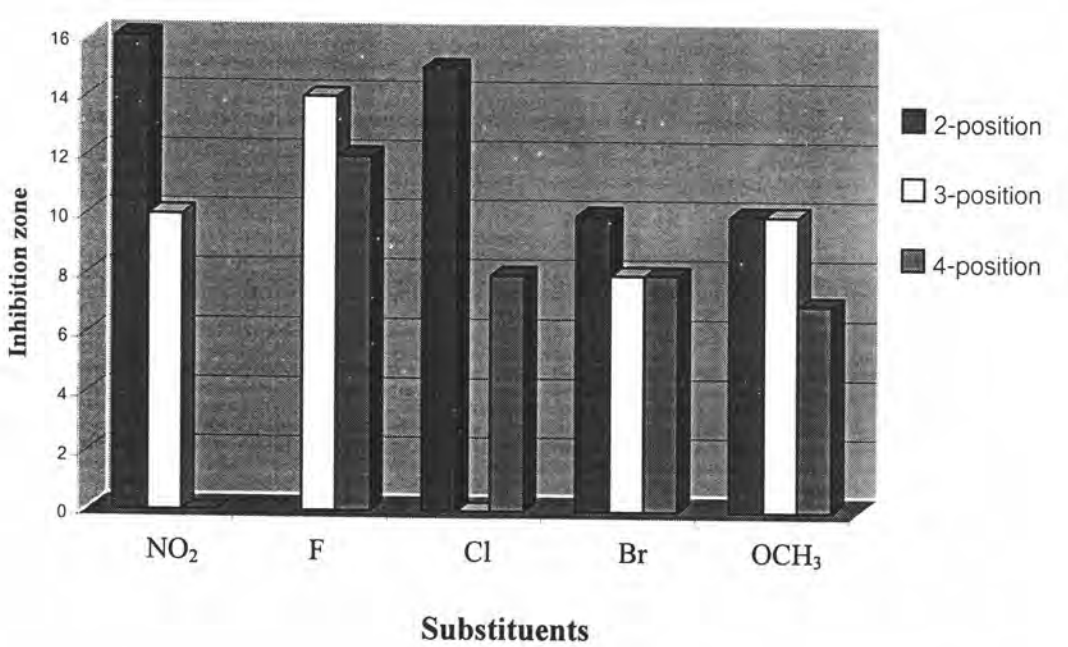


Fig 3.7 Effect of substituent on a benzylidene ring against *L. monocytogenes*

The substituents at 4-position were compared. The compound with a hydroxy group (**22**) showed the highest activity against *Listeria monocytogenes* and that containing a methyl group (**15**) displayed the highest activity for *Bacerus cereus*, while that with nitro group (**5**) provided the weakest inhibition against both bacteria. The activity could be arranged as follows:

Bacerus cereus : $CH_3 > F = Cl = Br = CF_3 = i\text{-}Pr = t\text{-}Bu > OMe > OH > NO_2$

Listeria monocytogenes : $OH > F > CH_3 = CF_3 > Cl = Br = i\text{-}Pr = t\text{-}Bu > OMe > NO_2$

When more functional groups substituted on a benzylidene ring such as the 3-methoxy-4-hydroxy group in Compound **27**, the highest activity against *Listeria monocytogenes* was observed. Moreover, the similar tendency could be noticed from the compound bearing 3,4-dihydroxy groups (**24**) which displayed the highest activity for *Bacerus cereus*, while 3-methoxy-4-alkoxy group (**29-34**) was inactive with both bacteria. The activity of these derivatives may be represented as follows:

Bacerus cereus :

$3,4\text{-}(OH)_2 > 3\text{-}OCH_3\text{-}4\text{-}OH > 3,4,5\text{-}(OCH_3)_3 > 3,4\text{-}OCH_2O > 3\text{-}OCH_3\text{-}4\text{-}OR$

Listeria monocytogenes :

$3\text{-}OCH_3\text{-}4\text{-}OH > 3,4,5\text{-}(OCH_3)_3 > 3,4\text{-}OCH_2O = 3,4\text{-}(OH)_2 > 3\text{-}OCH_3\text{-}4\text{-}OR$

2) Effect on alkyl substituents

Using Compound **1**, which has an unsubstituted on CH-bridge as a reference, the comparison of alkyl substituents (**35-39**) could be possibly made. The results showed that Compounds **35** and **38** exhibited high inhibition against *Bacerus cereus* and *Listeria monocytogenes*, respectively. Compounds **37** and **39** were inactive against both bacteria, the activity of these compounds may be arranged as follows:

Bacerus cereus :

$CH(CH_2CH_3)_2 > H > CH_3 = CH_2CH_2CH_3 > CH(CH_3)_2 = Cyclohexyl$

Listeria monocytogenes :

$CH_3 > H = CH(CH_2CH_3)_2 > CH_2CH_2CH_3 > CH(CH_3)_2 = Cyclohexyl$

Considering dicoumarols **40-44** compared with Compound **2**, the results showed that when the alkyl chain was longer, the less inhibition was observed. Except for Compound **43**, which contained double bond conjugated with phenyl ring, exhibited high potency against *Listeria monocytogenes*, but weak inhibition with *Bacerus cereus*.

3) Fused ring compounds

For analogues of dicoumarols, the tendency of the activity for fused ring compounds (**45-48**) revealed that the compound with no substituent showed the highest inhibition whereas 3-OCH_3 substituent exhibited the weakest activity in this series. The activity of these derivatives may be represented as follows:

Bacerus cereus :

$$C_6H_4 > \text{Naphthyl} > 6\text{-}ClC_6H_4 > 3\text{-}OCH_3C_6H_4$$

Listeria monocytogenes :

$$C_6H_4 > 6\text{-}ClC_6H_4 > \text{Naphthyl} > 3\text{-}OCH_3C_6H_4$$

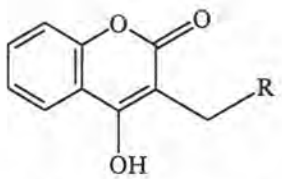
4) Miscellaneous compounds

The two tetramers synthesized (**49** and **50**) did not show high potency against those bacteria, except for phenyl tetramer (**49**) which exhibited high activity with *Bacerus cereus*. The effect of other aromatic groups instead of benzene ring in Compound **2** could be seen. The pyridine rings in Compounds **51** and **52** exhibited less activity than Compound **2** while a large substituent such as naphthyl ring in Compound **53** was inactive with all bacteria.

Compounds **54** and **55** were also tested against these bacteria. The results showed that Compound **54** displayed weak potency against *Listeria monocytoganes* and was not active with *Bacerus cereus*, while Compound **55** exhibited high activity against both bacteria.

Moreover, the comparison of dicoumarols with antibiotic drugs included Streptomycin and Kanamycin showed that all synthesized dicoumarols and analogues did not exhibit higher activity than these two drugs, except for Streptomycin which was inactive with *Listeria monocytoganes*.

3-Alkyl-4-hydroxycoumarins



Like dicoumarols, 3-alkyl-4-hydroxycoumarins were also tested against most bacteria except for Flat sour spoilage. Interestingly, the results showed selectively inhibition with three gram-positive bacteria: *Listeria monocytogenes*, *Bacerus cereus* and *Stapphylococus aureus*. The data were accumulated as shown in Table 3.16.

Table 3.16 Antibacterial properties of 3-alkyl-4-hydroxycoumarins

Cpd	R	Activity (diameter (mm))		
		B	*L*	*S*
-	4-Hydroxycoumarin	0	0	0
R1	H	0	0	0
R2	CH_3	0	0	8
R3	$CH_3CH_2CH_2$	10	10	10
R4	$(CH_3)_2CH$	10	10	10
R5	Cyclohexyl	17	15	15
R6	C_6H_5	0	10	15
R7	$C_6H_5CH_2$	20	10	15
R8	$C_6H_5(CH_3)CH$	20	15	20
R9	$C_6H_5CH_2CH_2$	20	15	18
R10	$C_6H_5CH{=}CH$	12	12	20
R11	$4\text{-}(CH_3)C_6H_4$	12	10	15
R12	$4\text{-}(OH)C_6H_4$	0	0	8
R13	$4\text{-}(OCH_3)C_6H_4$	8	0	10
R14	$3,4\text{-}(OCH_3)_2C_6H_3$	0	0	8
R15	3,4-methylenedioxybenzyl	10	0	15
DMSO		0	0	0
Streptomycin 10 meq		15	0	17
Kanamycin 10 meq		15	20	15

Using 4-hydroxycoumarin as a reference compound, which was not active with all bacteria, the data showed that all 3-alkyl-4-hydroxycoumarins displayed a better activity except for **R1**. All these compounds could be classified into three categories according to their structures as follows:

1) Effect of number of carbon atom

The variation of the number of substituent carbon atom could be noticable from Compounds **R1-R5**. It was found that cyclohexyl substituent (**R5**) gave the highest activity, following by Compounds **R4**, **R3**, **R2** and **R1**, respectively. These results reflected that the more carbon atom, the more inhibition. The activity exhibited as follows:

For *Bacerus cereus* and *Listeria monocytogenes* :

$$\text{Cyclohexyl} > (CH_3)_2CH = CH_3CH_2CH_2 > CH_3 = H$$

For *Staphylococus aureus* :

$$\text{Cyclohexyl} > (CH_3)_2CH = CH_3CH_2CH_2 > CH_3 > H$$

2) Effect of phenyl ring substituted on alkyl chain

The effect of phenyl substituted on alkyl chain (**R6-R10**) was compared. The activity seems to be higher when increasing of the chain length. The branch chain (**R8**) gave the highest inhibition. The activity could be summarized as follows :

For *Bacerus cereus* :

$$C_6H_5(CH_3)CH = C_6H_5CH_2CH_2 > C_6H_5CH_2 > C_6H_5CH{=}CH > C_6H_5$$

For *Listeria monocytogenes* :

$$C_6H_5(CH_3)CH = C_6H_5CH_2CH_2 > C_6H_5CH{=}CH > C_6H_5CH_2 = C_6H_5$$

For *Staphylococus aureus* :

$$C_6H_5(CH_3)CH = C_6H_5CH{=}CH > C_6H_5CH_2CH_2 > C_6H_5CH_2 = C_6H_5$$

3) Effect of substituent group on 3-benzyl compounds

Further studies on Compounds **R11-R15** were set up in order to prove the effect of substituents on a benzylidene ring. The results demonstrated that the methyl group substituted at 4-position showed the highest activity against those three bacteria. The results could be arranged as follows:

For *Bacerus cereus* :

$$4\text{-}CH_3 > 3,4\text{-methylenedioxy} > 4\text{-}OCH_3 > 4\text{-OH} = 3,4\text{-}(OCH_3)_2$$

For *Listeria monocytogenes* :

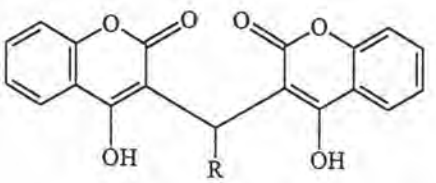
$$4\text{-}CH_3 > 4\text{-}OH = 3,4\text{-methylenedioxy} = 4\text{-}OCH_3 = 3,4\text{-}(OCH_3)_2$$

For *Stapphylococus aureus* :

$$4\text{-}CH_3 = 3,4\text{-methylenedioxy} > 4\text{-}OCH_3 > 4\text{-}OH = 3,4\text{-}(OCH_3)_2$$

4) Comparison with dicoumarols

The antibacterial activity of dicoumarols and 3-alkyl-4-hydroxycoumarins were compared. The results showed that most of 3-alkyl-4-hydroxycoumarins exhibited higher activity than dicoumarols. The comparative antibacterial activity results were tabulated in Table 3.17.



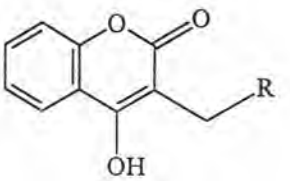


Table 3.17 The comparative antibacterial activity between dicoumarols and 3-alkyl-4-hydroxycoumarins

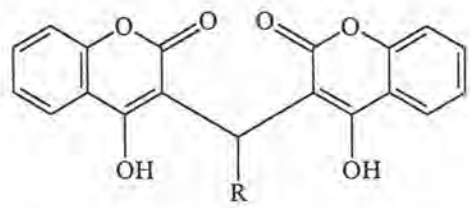
R	Dicoumarol		3-Alkyl-4-hydroxycoumarin	
	B	L	B	L
H	9	10	0	0
CH_3	8	12	0	0
$CH_3CH_2CH_2$	8	8	10	10
$(CH_3)_2CH$	0	0	10	10
Cyclohexyl	0	0	17	15
C_6H_5	12	15	0	10
$C_6H_5CH_2$	12	10	20	10
$C_6H_5(CH_3)CH$	8	0	20	15
$C_6H_5CH_2CH_2$	8	9	20	15
$C_6H_5CH{=}CH$	8	15	12	12
$4\text{-}(CH_3)C_6H_4$	11	10	12	10
$4\text{-}(OH)C_6H_4$	7	15	0	0
$4\text{-}(OCH_3)C_6H_4$	9	7	8	0
$3,4\text{-}(OCH_3)_2C_6H_3$	10	0	0	0
3,4-methylenedioxybenzyl	7	0	10	0

3.2.2 Antiviral Activity

Besides the well-recognized dicoumarols as the compounds useful for inhibition of HIV-1 integrase enzyme, the inhibition towards the similar DNA containing virus such as herpes group viruses has been also recently disclosed. There are four separate viruses of the herpes group which infect and cause disease in humans included: (1) Herpes Simplex Virus 1 and 2 (HSV-1 and HSV-2) are viruses that cause infection of herpes labialis and genital herpes, respectively; (2) Cytomegalovirus (CMV) is subclinical infections; (3) Varicellazoster virus (VZ) is associated with chicken-pox (varicella) and shingles (zoster) in humans and (4) Epstein-Barr virus (EB) is quite common and causes glandular fever: it is also believed to cause the genetic damage that leads to Burkitt's lymphoma. Examples of drugs used to treat herpes infections include: IUDR (5′-iodo-2′-deoxyuridine); Ara-C (1-[beta-D-arabinofuranosyl]-cytosine) and Acyclovir (9-[(2-hydroxyethoxy)methyl] guanine).[55] The antiviral agent against this virus by 3-substituted-4-hydroxy coumarins has never been reported. In this research, dicoumarols and 3-alkyl-4-hydroxycoumarins are tested against HSV-1 and HSV-2 using the modified colorimetric method.[48]

100% inhibition was occured as ++++ , more than 70% inhibition as +++ , 50 % inhibition as ++ , more than 30% inhibition as + and inactive was established as – .

Dicoumarol



Dicoumarols and analogues were tested for cytotoxicity with host cell and against some HSV-1 and HSV-2; however, the results obtained were not completed. Most dicoumarols inhibited HSV-1, while some derivatives were found to be active with HSV-2. A parent dicoumarol **1** (R = H) did not inhibit both virus, while Compound **2** (R = Ph) displayed only weak inhibition (+) against HSV-1, but not HSV-2. The highest activity (100% inhibition) against HSV-1 was observed in Compounds **3**, **18** and **46**. These data were tabulated in Table 3.18.

Table 3.18 Antiviral activity of dicoumarols and analogues

Cpd	R	Cytotoxicity (μg/ml)	HSV-1	HSV-2
1	H	>20	-	-
2	C_6H_5	>20	+	-
3	2-$(NO_2)C_6H_4$	>50	++++	-
4	3-$(NO_2)C_6H_4$	>50	+++	++
5	4-$(NO_2)C_6H_4$	>20	++	++
6	3-FC_6H_4	>20	+++	-
7	4-FC_6H_4	>20	+++	-
8	2-ClC_6H_4	>20	+	+
9	3-ClC_6H_4	×	×	×
10	4-ClC_6H_4	>20	+	-
11	2,4-$(Cl)_2C_6H_3$	>20	++	-
12	2-BrC_6H_4	>50	+++	+
13	3-BrC_6H_4	>20	+	-
14	4-BrC_6H_4	>20	++	-
15	4-$(CH_3)C_6H_4$	>50	++	-
16	4-(*i*-Pr)C_6H_4	>20	+	-
17	4-(*t*-Bu)C_6H_4	>20	++	-
18	4-$(CF_3)C_6H_4$	>20	++++	+
19	2-$(OCH_3)C_6H_4$	>50	+++	-
20	3-$(OCH_3)C_6H_4$	×	×	×
21	4-$(OCH_3)C_6H_4$	>50	+++	-
22	4-$(OH)C_6H_4$	>20	++	-
23	3-$(OC_6H_5)C_6H_4$	×	×	×
24	3,4-$(OH)_2C_6H_3$	>20	+	+
25	3,4-methylenedioxybenzyl	>50	-	-
26	3,4,5-$(OCH_3)_3C_6H_2$	>50	-	-
27	3-(OCH_3)-4-$(OH)C_6H_3$	>10	-	-
28	3,4-$(OCH_3)_2C_6H_3$	>50	-	-
29	3-(OCH_3)-4-$(OC_2H_5)C_6H_3$	×	×	×

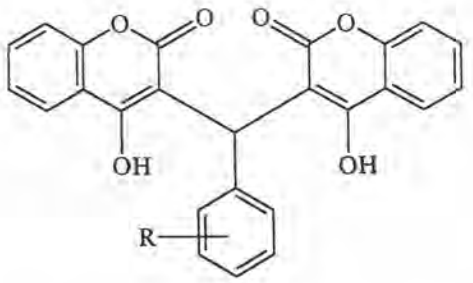
Table 3.18 (cont.)

Cpd	R	Cytotoxicity (μg/ml)	HSV-1	HSV-2
30	3-(OCH_3)-4-(OC_4H_9)C_6H_3	×	×	×
31	3-(OCH_3)-4-(OC_6H_{13})C_6H_3	×	×	×
32	3-(OCH_3)-4-(OC_8H_{17})C_6H_3	×	×	×
33	3-(OCH_3)-4-($OC_{12}H_{25}$)C_6H_3	×	×	×
34	3(OCH_3)-4($OCH_2C_6H_5$)C_6H_3	×	×	×
35	CH_3	>50	-	-
36	$CH_3CH_2CH_2$	>50	-	-
37	$(CH_3)_2CH$	>50	-	-
38	$(CH_3CH_2)_2CH$	>50	+++	++
39	Cyclohexyl	>20	+	-
40	$C_6H_5CH_2$	>10	-	-
41	(±)-$C_6H_5(CH_3)CH$	>10	-	-
42	$C_6H_5CH_2CH_2$	>20	++	-
43	(*E*)-C_6H_5CH=CH	×	×	×
44	$C_6H_5CH_2CH_2CH_2$	×	×	×
45	C_6H_4(fuse)	>20	-	-
46	3-(OCH_3)C_6H_3 (fuse)	>50	++++	++
47	6-ClC_6H_3 (fuse)	×	×	×
48	naphthyl (fuse)	>50	+	+
49	C_6H_4 (Tetramer)	×	×	×
50	$CH_2CH_2CH_2$ (Tetramer)	×	×	×
51	1-Naphthyl	×	×	×
52	2-Pyridinyl	×	×	×
53	3-Pyridinyl	>50	+++	++
54	(±)-9-Anthracenyl	×	×	×
55	Cl_3C	×	×	×

Note : × = not tested

For simplicity to comprehend, the relationship between antiviral activity and dicoumarols could be classified into many types as follows:

1) Substituent on a benzylidene ring



Using Compound **2** as a reference compound, the effects of substituents on a benzylidene ring were observed. For nitro group, it was interesting that the activity could be arranged similar to the brine shrimp lethality test and antibacterial activity as *ortho* > *meta* > *para*. In the case of halo compounds, fluoro substituent showed similar activity for both 3- and 4-positions against this virus. The chloro group at 2-position displayed weak inhibition with HSV-1 and HSV-2, while the chloro group at 4-position exhibited weak inhibition for HSV-1. A compound with two atoms of Cl substituted on 2- and 4-positions revealed the higher activity against this virus. The tendency of activity for bromo substituent from high to low could be rendered as *ortho* > *para* > *meta*.

Hydroxy and methoxy groups were also studied. Both 2- and 4-positon of methoxy substituents showed high activity (+++) with HSV-1, while hydroxy group at 4-position showed medium activity (++). Adding more hydroxy and methoxy group into a benzylidene ring would decrease the activity.

Comparing with the compounds having different substituents at 4-position, the activity observed could be ordered in the series of:

$$CF_3 > F = OCH_3 > NO_2 = Br = CH_3 = OH = t\text{-}Bu > Cl = i\text{-}Pr$$

2) Effect of alkyl substituents

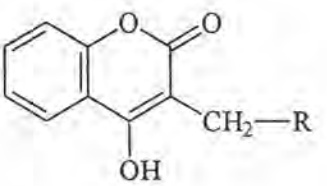
Compared with Compound **1**, Compounds **35-37** which contain small alkyl groups were inactive against these virus, while Compound **38** bearing a large branch alkyl chain revealed more activity. The cyclohexyl ring showed weak inhibition against HSV-1 and was inactive with HSV-2. Comparison of Compounds **40-42** with

Compound **2**, the results revealed that Compound **42** which contained two-carbon atoms exhibited medium activity whereas other compounds did not show the effect on both virus.

3) Miscellaneous compounds

The fused ring compounds were also tested. The results showed that Compound **46** which contained a methoxy group at 3-position displayed the highest inhibition against both HSV-1 and HSV-2. The naphthyl fused ring (**48**) exhibited weak activity, while phenyl fused ring **(45)** was inactive according to this assay. However, the activity of these compounds increased when a phenyl ring substituted in Compound **2** was replaced with a pyridine ring in Compound **53**.

3-Alkyl-4-hydroxycoumarins



Antiviral activity of 3-alkyl-4-hydroxycoumarins was found to be differed from that of dicoumarols. While dicoumarols inhibited the HSV-1 more than HSV-2, most 3-alkyl-4-hydroxycoumarins showed higher potency against HSV-2 than HSV-1. Compounds **R10** and **R15** expressed the highest activity in this series. The results are summarized in Table 3.19.

To aids the understanding this SAR study simply, 3-alkyl-4-hydroxy coumarins could be classified according to the substituents as follows:

1) Phenyl substituent on alkyl chain

The comparison of Compounds **R6-R10** was investigated. The activity of 3-benzyl substituent was medium potency. The introduction of a conjugated system on C-2 and C-3 of the carbon skeleton in Compound **R10** showed highest activity in this series. The order of activity of these compounds may present as follows:

$$C_6H_5CH{=}CH > C_6H_5(CH_3)CH > C_6H_5 > C_6H_5CH_2CH_2 > C_6H_5CH_2$$

Table 3.19 Antiviral activity of 3-alkyl-4-hydroxycoumarins

Cpd	R	Cytotoxicity (μg/ml)	HSV-1	HSV-2
R1	H	×	×	×
R2	CH_3	>20	-	+
R3	$CH_3CH_2CH_2$	>20	-	+
R4	$(CH_3)_2CH$	×	×	×
R5	Cyclohexyl	×	×	×
R6	C_6H_5	>50	++	++
R7	$C_6H_5CH_2$	>20	+	-
R8	$C_6H_5(CH_3)CH$	>50	++	+++
R9	$C_6H_5CH_2CH_2$	>50	+	+++
R10	$C_6H_5CH=CH$	>50	+++	+++
R11	4-$(CH_3)C_6H_4$	>20	+	+
R12	4-$(OH)C_6H_4$	>20	+	+
R13	4-$(OCH_3)C_6H_4$	>20	-	+
R14	3,4-$(OCH_3)_2C_6H_3$	>50	+++	++
R15	3,4-methylenedioxybenzyl	>50	+++	+++

2) Effect of substituent on 3-benzyl compounds

The effect of substituents on a benzylidene ring in Compounds **R11-R15** was investigated. The results demonstrated that 3,4-methylenedioxy group exhibited the highest activity against both HSV-1 and HSV-2 in this series, while a methoxy group substituted on 4-position was less potency. The order of activity from high to low of substituent could be arranged as:

3,4-methylenedioxy > 3,4-$(OCH_3)_2$ > 4-CH_3 = 4-OH > 4-OCH_3