Reactions of 4-methylchromene-2,7,8-trione with phosphonium ylides. Synthesis and evaluation of fused 1,3-dioxolocoumarins as antioxidants and antiinflammatories

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4-Methylchromene-2,7,8-trione **1** reacts with stabilized **2a**–**c** and non-stabilized ylides **11a**–**c** bearing an α-methylene group to give 7,8-fused 1,3-dioxolocoumarins **4a**–**c** and **12a**–**c** along with betaine **6** and 7-hydroxycoumarin derivative **9**. The reaction of 4,5-fused 1,3-dioxolo-*o*-benzoquinone **13** with the ylide **14** leads to 4-ethoxycarbonylayapin **18** and benzofuranone **19**. Deethoxycarbonylation of **18** gives ayapin **20**. Compounds **4b**, **12a**–**c**, **18** and **20** were tested for their ability to interact with 1,1-diphenyl-2-picrylhydrazyl stable free radical (DPPH), to scavenge superoxide anion radicals, to compete with DMSO for hydroxyl radicals, and to inhibit proteolysis, β-glucuronidase and soybean lipoxygenase activity *in vitro*. These compounds were also tested for their effect on the ferrous ion-stimulated peroxidation of linoleic acid. They showed a potent inhibitory effect (55–57%) against inflammation induced by carrageenan in the rat paw edema model. On the contrary their reducing ability was found to be low and no inhibition on soybean lipoxygenase was recorded.

The coumarin molecule has been shown to possess unique antiedema and antiinflammatory activities, and these make it particularly effective in the treatment of all high-protein edemas.[1,2] Several natural or synthetic coumarins with various hydroxy and other substituents were found to inhibit lipid peroxidation and to scavenge hydroxyl radicals, superoxide radicals and hypochlorous acid.[3] The dihydroxylated coumarins[4] (vicinal OH groups) and flavonoids[4] (a closely related class) were all also active. Furthermore furocoumarins, pyranocoumarins, geiparvarin analogues[5] and some more newly synthesized coumarins condensed with an heteroaromatic ring were found to act as antiinflammatories,[6–8] antioxidants,[6–8] cytostatic agents[5] and to inhibit viral proteases[9] and viral replication.[9] Several heterocycles containing a dioxolane ring were also synthesized and reported as possible antiviral agents.[10] Ayapin is a representative structure of this type with reported antifungal,[11] trypanomicidal[12] and hemocoagulant[13] activities.

In connection to our previous work on the synthesis of coumarin derivatives,[14–20] we have recently studied the synthesis and biological activities of 4-substituted coumarins with an heterocyclic ring.[6–8,21] These derivatives were found to possess significant antiinflammatory and antioxidant activities, as well as inhibitory activity on soybean lipoxygenase. Very recently[22] we found that the reactions of phenanthrene-9,10-quinone with the phosphonium ylides 1-phenylethylidene(triphenyl)phosphorane and cyclopentylidene(triphenyl)phosphorane, bearing an α-H to the ylidic carbanion, afforded 1,3-dioxolane derivatives besides the expected intermediate mono-Wittig adduct. These results prompted us to design and synthesize novel coumarins with a 7,8- or 6,7-fused dioxolane ring in order to investigate the biological activity and the possible role of the dioxolane ring on the biological results.

Results and discussion

Synthesis of fused 1,3-dioxolocoumarins

The title reactions studied and the products obtained are depicted in Schemes 1 and 2. Treatment of *o*-quinone **1** with ylide **2a** (1 mol equiv.) in DCM at −10 °C for 4 h resulted in the formation of white crystals of betaine **6** in 28% yield. Separation of the filtrate by column chromatography gave dioxolane **4a** (7%) followed by 7,8-dihydroxy-4-methylcoumarin **8** (8%). No Wittig product was detected in the reaction mixture. This reaction seems to be analogous with reactions of quinone **1** with semistabilized ylides (allylidene- and benzylidenetriphenylphosphoranes), which gave dioxolanes, while reactions with stabilized ylides (alkoxycarbonyl- and acylmethylenetriphenylphosphoranes) gave Wittig products.[17]

Similar treatment (at −10 °C) of *o*-quinone **1** with ylide **2b** produced the corresponding dioxolane **4b** (12.5%) following separation of the reaction mixture by column chromatography, the phenol **9** (16%) eluted first. By repeating the same reaction at room temperature only compound **4b** (12%) was obtained followed by dihydroxycoumarin **8** (10%). A similar reaction of *o*-quinone **1** with ylide **2c** at room temperature gave dioxolane **4c** (5%) along with the coumarin **8** (16%). The same reaction at −10 °C for 6 h resulted in the formation of **4c** (2%), while coumarin **8** (42%) was also separated from the reaction mixture.

The analytical and spectral data of the new dioxolocoumarins **4a**–**c**, betaine **6** and phenol derivative **9** are consistent with the proposed structures. For betaine **6** the structure of 1,4-dioxaphosphorane **7** could be a possible alternative, but ^{31}P NMR (δ_P 36.8) ruled out this possibility. The ESI-MS also agree with the alkoxide structure **6** and not with a possible

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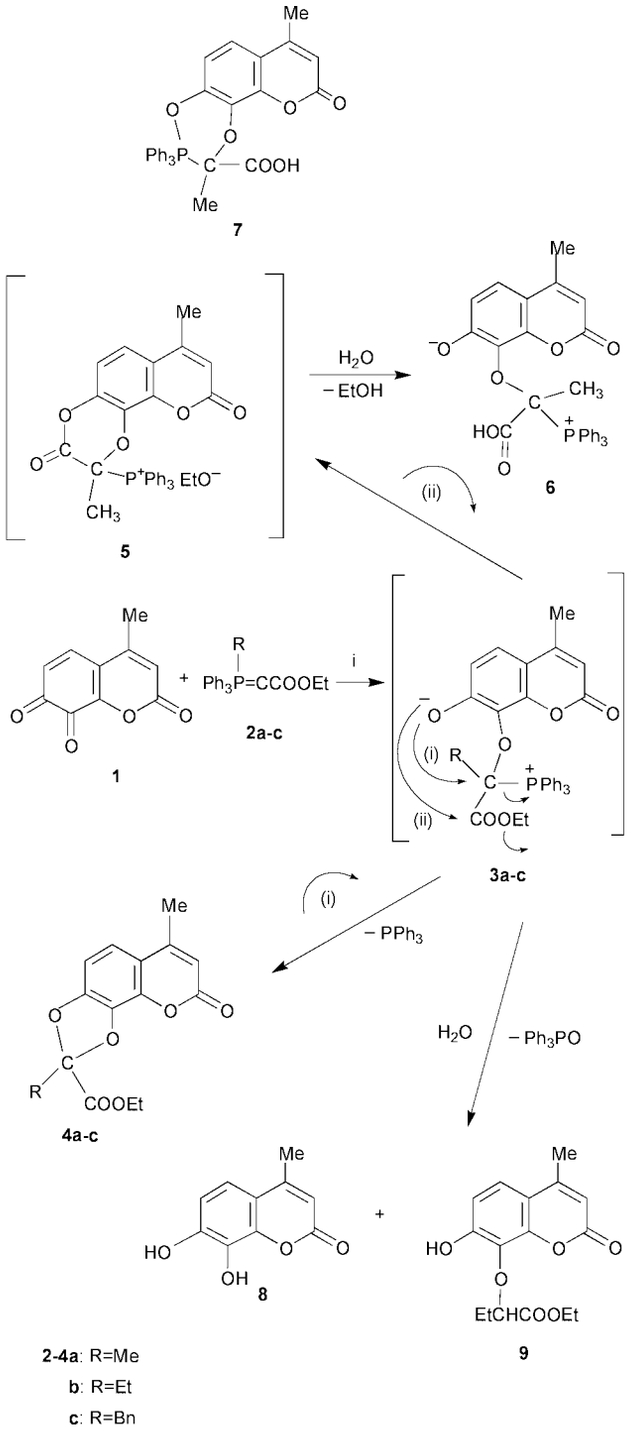
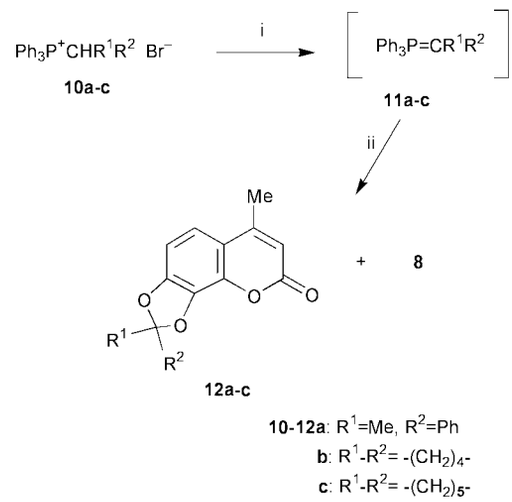


Scheme 1 *Reagents and conditions*: i, DCM, −10 °C, 4 h.



Scheme 2 *Reagents and conditions*: i, BuLi, argon atmosphere, anhydrous THF, rt or 0 °C or −40 °C, 5 min or 30 min; ii, **1** in THF, rt or 0 °C or −40 °C, 1 h, 4–37%.

structure like **5**. The coumarin ring and COOH group might be responsible for the stability of betaine **6** .

The formation of dioxolanes **4a**–**c**, betaine **6** and phenol **9** could be explained by a 1,4-addition of ylide carbanion (Scheme 1) to give the intermediate betaines **3a**–**c**. Elimination of triphenylphosphine resulted in dioxolanes **4a**–**c**. Phenol **9** was obtained by reductive hydrolysis of betaine **3b**. Intramolecular nucleophilic attack of the oxyanion, in betaine **3a**, to the carboxylic ester carbon followed by hydrolysis of the resulting 4-oxapyran-2-one **5** could give the new betainic acid **6**.

We studied next (Scheme 2) the reactions of *o*-quinone **1** with the non-stabilized ylides **11a**–**c**, prepared *in situ* from the corresponding phosphonium salts **10a**–**c** (1 equiv.) in THF by treatment with BuLi under an argon atmosphere. The reaction of **1** with ylide **11a** at room temperature for 1 h resulted in the formation of dioxolane **12a** (37%). An analogous reaction of quinone **1** with ylide **11b** at 0 °C for 1 h gave compound **12b** (4%). From the reaction of quinone **1** with ylide **11c** at −40 °C for 1 h dioxolane **12c** (20%) was obtained. From all the above reactions dihydroxycoumarin **8** was separated in 1%, 9%, 7% yields respectively. No Wittig products were detected in the reaction mixtures.

The formation of dioxolanes **12a**–**c** could be explained by the same mechanism as for dioxolanes **4a**–**c**. A possible explanation for the absence of any Wittig products, compared with the reactions of alkoxycarbonylmethylenetriphenylphosphorane,[17] is that ylides **2a**–**c** and **11a**–**c** have a stronger ylidic carbanion character. However, these bulky molecules can not attack the carbonyl carbon of the *o*-quinone **1** easily and thus they attack the carbonyl oxygen in a type of 1,4-addition leading to aromatization of the benzene ring.

Although the above prepared dioxolanes **4a**–**c** and **12a**–**c** were obtained in moderate to low yields (4–37%), the methods used for dioxolane preparation in the literature (fusion[23] of catechol with dichloro compounds or condensation[24] of catechol with ketones or ketone dimethyl acetals in the presence of acid catalyst) are not so efficient. For example, compound **12c** was isolated in 11% yield (86% of 7,8-dihydroxycoumarin **8** remained unreacted) by refluxing a toluene solution of **8** with cyclohexanone in the presence of a catalytic amount of toluene-*p*-sulfonic acid (*p*-TSA) for 12 h with a Dean–Stark trap. An analogous effort for the preparation of **12a** from the reaction of **8** with acetophenone failed even after 26 h of reflux. Compound **12a** was prepared in only 4% yield (91% of catechol **8** remained unreacted) by heating under reflux a benzene solution of **8** with acetophenone dimethyl acetal in the presence of a catalytic amount of *p*-TSA for 48 h using a Dean–Stark trap.

Finally, we studied the reaction of 1,3-dioxolo[*d*]benzoquinone **13** with ylide **14**, which give us the opportunity[15] to have 6,7-fused dioxolocoumarins. Treatment of quinone **13** with ylide **14** (1 equiv.) in dry DCM for 12 h at room temperature gave after separation of the reaction mixture by column chromatography the new compounds **19** (15%) and **18** (30%). By repeating this reaction of **13** with **14** (2.5 equiv.), compounds **19** and **18** were received in 24% and 52% yields respectively. Analytical and spectral data for compounds **18** and **19** are consistent with the proposed structures and they are analogous to the data of the corresponding compounds prepared earlier by us from the reactions of 1,2-naphthoquinone or 4,6-bis(*tert*-butyl)-1,2-benzoquinone with the ylide **14**.[15] The synthesis of compounds **18** and **19** could be explained by assuming a Wittig monoolefination at first to form **15**, followed by a Michael addition of a second ylide molecule to **16** and subsequent nucleophilic attack of the OH group of the intermediate phenol

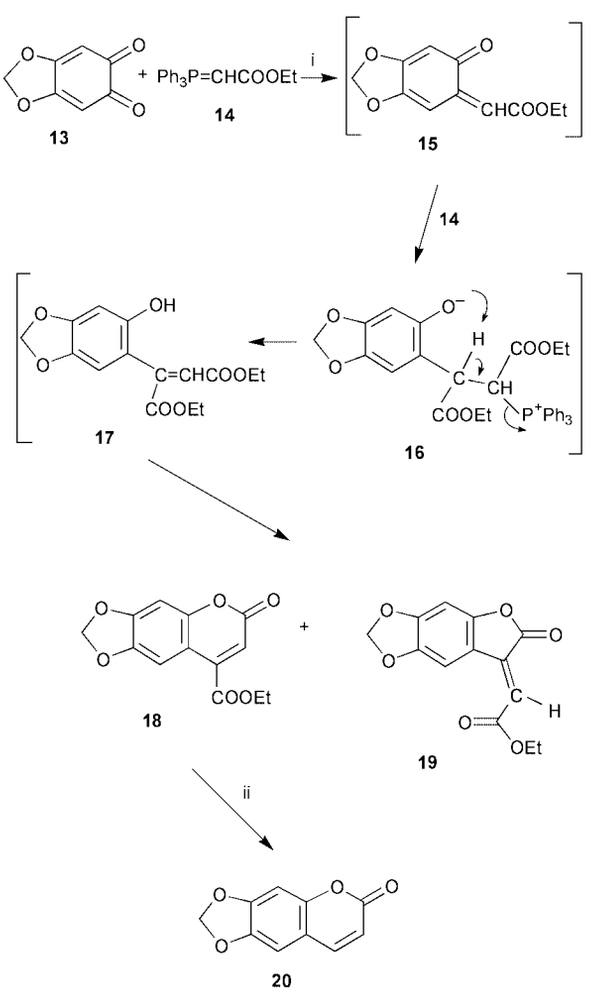
Table 1 Reduction ability (RA %), inhibition of Fe^{2+}-stimulated oxidation of linoleic acid (Fe^{2+} INH), competition with DMSO for hydroxyl radical ($HO^{\bullet}$ %), *in vivo* inhibition of carrageenan rat paw edema (CPE %). Lipophilicity values (clog *P* and R_M)[a]

Compound	RA %[b,c]	Fe^{2+} INH[b,d]	$HO^{\bullet}$ %[b,e]	CPE %[f,g]	clog *P*	R_M
4b	28 (0.1mM), 35 (0.5 mM)	No	40.1	55	2.480	0.094
12a	17.7 (0.1 mM), 32.5 (0.5 mM)	50.3	No	57.4	4.307	0.0836
12b	14 (0.1 mM), 13 (0.5 mM)	22.6	74.7	Nt	3.821	0.045
12c	3.1 (0.1 mM), 22.4 (0.5 mM)	47.9	No	Nt	4.380	0.131
18	17 (0.1 mM), 44 (0.5 mM)	44.3	No	Nt	1.620	−0.213
20	14 (0.1 mM)	Nt	41.6	Nt	1.160	Nt

[a] Nt: not tested; No: no action under the experimental conditions. [b] Data are the means of two or three independent experiments and the deviation in absorbance values were less than 10%. [c] Acetylsalicylic acid as a standard 80.5% (0.1 mM), nordihydroguaeretic acid 94.4% (0.1 mM). [d] Tocopherol acetate as a standard 83.4%. [e] DMSO as a standard 78.5%. [f] Each value represents the mean of two independent experiments with 6 animals in each group, statistical studies were done with student's T-test $p < 0.01$. [g] Indomethacin as a standard 47%.

17 to each of the carbonyl ester carbon atoms to give compounds **18** and **19**.

Coumarin derivative **18** is the 4-ethoxycarbonyl derivative of the natural product ayapin **20** (Scheme 3).[25–27] Heating of compound **18** with copper powder in quinoline at 190 °C for 10 h resulted in deethoxycarbonylation to give ayapin **20** in 69% yield.



Scheme 3 *Reagents and conditions*: i, dry DCM, rt, 12 h; ii, Cu, quinoline, 190 °C, 10 h.

Biological results and discussion

Lipophilicity, an important physicochemical parameter correlated with biological activity, was determined experimentally as R_M values, from reversed phase TLC (RPTLC). It is known that the lipophilic character of a compound can influence its antioxidant activity. Thus theoretical calculations of lipophilicity as clog *P* (Biobyte)[28] using the method of additivity[29] were also performed.

The biological activities of the compounds were screened by *in vitro* and *in vivo* assays. Inhibitory activities were measured against isolated enzymes (trypsin, β-glucuronidase and soybean lipoxygenase). Proteases are intimately involved in the initiating events, cellular recruitment and degenerative aspects of inflammation.[30]

Antiinflammatory agents have been reported to exhibit antiproteolytic ability.[31] No inhibition was observed on proteolysis, on β-glucuronidase activity and on soybean lipoxygenase (LOX) under our experimental conditions.

The reducing abilities of the examined dioxolane compounds were determined by their interaction with the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). Antioxidants can react with DPPH and produce 1,1-diphenyl-2-picrylhydrazine.[32] Due to its odd electron DPPH gives a strong absorption band at 517 nm. As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes and the resulting decolorization is stoichiometric with respect to the number of electrons taken up. The change of absorbance produced in this reaction is assessed to evaluate the antioxidant potential of test samples and this assay is useful as a primary screening system. All compounds, compared with acetylsalicylic acid (80.5%) or nordihydroguaeretic acid (94.4%) used as standard drugs, were found to interact slightly (3.12–28%, Table 1) with DPPH in a concentration dependent manner (the interaction increases with the increase of the concentration of the tested compounds). Compounds **4b** and **12a** were found to be more active, and they have been screened *in vivo* (0.01 mmol kg^{-1}) for their antiinflammatory activity using the functional model of carrageenan-induced rat paw edema. The results are given as percentage of weight increase at the right hind paw in comparison to the uninjected left hind paw. Both induced a 55–57.4% protection against carrageenan-induced paw edema while the reference drug indomethacin, induced 47% protection at equivalent concentration. It seems that the magnitude of the ring of the alicyclic substituents affects efficacy (**12b** > **12c**). For steric reasons, it may be possible that the enlargement of the ring (**12c**) leads to lower biological response.

Under the reported experimental conditions none of the examined compounds scavenged the superoxide anion. Because $HO^{\bullet}$ is one of the most potent oxidizing agents and under certain conditions it might be implicated in lipid peroxidation, and because hydrogen peroxide as a source of $HO^{\bullet}$ has been implicated in inflammation,[33] we attempted to investigate the ability of the synthesized compounds to compete with DMSO for hydroxyl radicals. The competition of the compounds **4b**, **12b** and **20** for hydroxyl radicals was high (40–74.7%), whereas compounds **12a**, **12c** and **18** did not show any effect. It is remarkable that for compounds **4b** and **20** the results are almost equal. In compound **12b** the presence of a spiro 5-membered ring significantly affects the competition in comparison to **4b**, **12a**, **18** and **20**. More experiments are in progress in order to

delineate the role of structural requirements and their correlation with the $HO^{\bullet}$ competition.

We also determined the inhibition of Fe^{2+}–stimulated oxidation of linoleic acid by compounds **4b**, **12a**–**c** and **18** in order to find out if the tested compounds act as antioxidants in a nonbiological system. All compounds (except **4b**) inhibited this type of lipid peroxidation (22–50.7%). Compound **12a** showed the highest inhibition. No attempt was made to find the concentration of compound which produces maximal inhibition.

Our attempt to correlate our biological results with some physicochemical parameters was unsuccessful. However our preliminary results seem to correlate with the literature values for potency of the 7,8 disubstituted coumarinic derivatives. Certain similarities in the biological screening exist between the ayapin structure and **4b**. It could be possible that no specificity exists in the presence of a 6–7 or a 7–8 fused ring. Furthermore lipophilicity in our case does not seem to affect predominantly the biological activity. On the contrary sterimol parameters (expressing steric requirements) are more important.

Experimental

Mps were measured on a Kofler hot-stage apparatus and are uncorrected. IR spectra were obtained using a Perkin-Elmer 1310 spectrophotometer as Nujol mulls except otherwise stated. NMR spectra were recorded on a Bruker AM 300 (300 MHz and 75 MHz for ^{1}H and ^{13}C respectively) or on a Bruker AMX-400 (162 MHz for ^{31}P NMR) spectrometers using $CDCl_3$ as solvent and TMS as an internal standard. *J* values are reported in Hz. Mass spectra were determined on a VG-250 spectrometer at 70 eV under Electron Impact (EI) conditions, or on a Perkin-Elmer API 100 Sciex Single quadrupole under Electronspray (ESI) conditions. High resolution mass spectra (HRMS) were recorded on an Ionspec mass spectrometer under matrix-assisted laser desorption/ionization Fourier transform mass spectrometry (MALDI-FTMS) conditions with 2,5-dihydroxybenzoic acid (DHB) as the matrix. Microanalyses were performed on a Perkin-Elmer 2400-II Element analyzer. THF was refluxed over sodium and benzophenone and distilled when the mixture turned blue. Silica gel N^0 60, Merck A. G. has been used for column chromatographies. *o*-Quinones **1**[34] and **13**[35] were prepared according to the literature method.

Synthesis

Reaction of quinone 1 with ylide 2a. Synthesis of ethyl 2,6-dimethyl-8-oxo-8*H*-[1,3]dioxolo[4,5-*h*]chromene-2-carboxylate 4a and 8-[1-carboxy-1-(triphenylphosphonio)ethoxy]-4-methyl-2-oxo-2*H*-chromen-7-olate 6. A mixture of quinone **1** (0.19 g, 1 mmol) and ylide **2a** (0.362 g, 1 mmol) in DCM (10 cm^3) was stirred at −10 °C for 4 h. After the consumption of quinone the mixture was concentrated and treated with DCM–hexane to give pure **6** as white crystals (0.147 g, 28%), mp 126–128 °C (methanol–ethyl acetate) (Found: C, 70.9; H, 4.8. $C_{31}H_{25}O_6P$ requires C, 71.0; H 4.8%); ν_{max}/cm^{-1} 3040, 2710, 2660, 2590, 1710, 1690, 1630, 1580; ν_{max}/cm^{-1} (KBr) 3056, 2988, 2920, 2850, 2750, 2670, 2560, 1724, 1710, 1642, 1618, 1590; δ_H 2.32 (d, 3H, *J* = 19.1), 2.35 (s, 3H), 5.99 (s, 1H), 6.94 (d, 1H, *J* = 8.9), 7.27 (d, 1H, *J* = 8.9), 7.57–7.68 (m, 6H), 7.70–7.76 (m, 3H), 8.01–8.09 (m, 6H), 14.68 (br s, 1H); δ_C 19.0, 21.3 (d, *J* = 2.4), 91.9 (d, *J* = 47.2), 110.0, 112.4, 116.3, 118.9 (d, *J* = 83.1), 122.1, 129.5 (d, *J* = 12.8), 130.4 (d, *J* = 4.9), 134.3 (d, *J* = 2.9), 135.6 (d, *J* = 9.4), 149.3, 153.7, 158.4 (d, *J* = 2.4), 160.5, 170.9 (d, *J* = 7.3); δ_P 36.8; ESIMS: *m*/*z* 525 $(M + H)^+$, 547 $(M + Na)^+$; MALDIHRMS (DHB): *m*/*z* 507.1355 $[MH^+ - H_2O]^+$. $C_{31}H_{24}O_5P$ requires *m*/*z* 507.1356.

The filtrate was separated by column chromatography [hexane–ethyl acetate (5 : 2)] to give after elution of triphenylphosphine (8 mg, 3%) compound **4a** (20 mg, 7%) mp 117–119 °C (DCM–hexane) (Found: C, 62.0; H, 5.1. $C_{15}H_{14}O_6$ requires C, 62.05; H 4.85%); ν_{max}/cm^{-1} 3050, 1740, 1720, 1635; δ_H 1.30 (t, 3H, *J* = 7.6), 1.97 (s, 3H), 2.39 (s, 3H), 4.28 (q, 2H, *J* = 7.6), 6.15 (s, 1H), 6.83 (d, 1H, *J* = 8.3), 7.15 (d, 1H, *J* = 8.3); δ_C 13.9, 19.1, 21.9, 62.7, 78.4, 105.1, 112.7, 113.1, 118.6, 134.9, 150.6, 152.6, 154.2, 160.3, 178.5; ESIMS: *m*/*z* 291 $(M + H)^+$, 313 $(M + Na)^+$. From the next band compound **8** (16 mg, 8%) was isolated, mp 236–238 °C (from ethanol) (lit.[17] mp 236–238 °C).

Reactions of quinone 1 with ylide 2b. Synthesis of ethyl 2-ethyl-6-methyl-8-oxo-8*H*-[1,3]dioxolo[4,5-*h*]chromene-2-carboxylate 4b and ethyl 2-(7-hydroxy-4-methyl-2-oxo-2*H*-chromen-8-yloxy)butanoate 9. A. A mixture of quinone **1** (0.38 g, 2 mmol) and ylide **2b** (0.752 g, 2 mmol) in DCM (10 cm^3) was stirred at room temperature for 30 min (consumption of quinone) and the mixture was then concentrated. Column chromatography [hexane–ethyl acetate (4 : 1 to 1 : 1)] gave after the elution of triphenylphosphine (52 mg, 10%) compound **4b** (71 mg, 12%), mp 60–61 °C (hexane–ether) (Found: C, 63.3; H, 5.4. $C_{16}H_{16}O_6$ requires C, 63.15; H, 5.3%); ν_{max}/cm^{-1} 3050, 1740, 1715, 1630; δ_H 1.08 (t, 3H, *J* = 7.4), 1.31 (t, 3H, *J* = 7.1), 2.30 (q, 2H, *J* = 7.4), 2.39 (s, 3H), 4.28 (q, 2H, *J* = 7.1), 6.15 (s, 1H), 6.84 (d, 1H, *J* = 8.4), 7.14 (d, 1H, *J* = 8.4); δ_C 6.2, 14.0, 19.1, 28.3, 62.6, 77.9, 105.0, 112.6, 112.8, 118.7, 133.9, 146.4, 152.8, 156.6, 162.0, 177.2; EIMS: *m*/*z* 304 (48%, M^+), 258 (19), 231 (94), 230 (58), 203 (93), 201 (46), 91 (98), 57 (100). Coumarin **8**[17] (39 mg, 10%) was eluted next.

B. A mixture of quinone **1** (95 mg, 0.5 mmol) and ylide **2b** (0.188 g, 0.5 mmol) in DCM (4 cm^3) was stirred at −10 °C for 4 h. After evaporation of the solvent the residue was separated by column chromatography [hexane–ethyl acetate (6 : 1)] to give after the elution of triphenylphosphine (16 mg, 12%) compound **9** (25 mg, 16%), mp 102–104 °C (DCM–hexane) (Found: C, 63.0; H, 6.1. $C_{16}H_{18}O_6$ requires C, 62.75; H, 5.9%); ν_{max}/cm^{-1} 3230, 3050, 1710, 1690, 1590; δ_H 1.24 (t, 3H, *J* = 7.2), 1.28 (t, 3H, *J* = 7.1), 2.05–2.2 (m, 1H), 2.3–2.5 (m, 1H), 2.38 (s, 3H), 4.19 (dq, 1H, J_1 = 7.1, J_2 = 10.7), 4.29 (dq, 1H, J_1 = 7.1, J_2 = 10.7), 4.76 (dd, 1H, J_1 = 4.4, J_2 = 6.3), 6.11 (s, 1H), 6.92 (d, 1H, *J* = 8.8), 7.27 (d, 1H, *J* = 8.8), 8.75 (s, 1H); δ_C 9.1, 14.0, 18.9, 26.7, 62.3, 82.3, 111.4, 113.2, 113.8, 120.8, 132.8, 147.5, 153.1, 153.3, 160.2, 175.2; ESIMS: *m*/*z* 307 $(M + H)^+$, 329 $(M + Na)^+$. Compound **4b** (19 mg, 12.5%) was eluted next.

Reactions of quinone 1 with ylide 2c. Synthesis of ethyl 2-benzyl-6-methyl-8-oxo-8*H*-[1,3]dioxolo[4,5-*h*]chromene-2-carboxylate 4c. A. A mixture of quinone **1** (0.19 g, 1 mmol) and ylide **2c** (0.424 g, 1 mmol) in DCM (10 cm^3) was stirred at room temperature for 24 h. The reaction mixture was concentrated and the residue was separated by column chromatography [hexane–ethyl acetate (75 : 25 to 0 : 100)] to give compound **4c** (17 mg, 5%), mp 150–151 °C (DCM–hexane); ν_{max}/cm^{-1} (KBr) 3060, 2923, 2850, 1732, 1715, 1640; δ_H 0.86 (t, 3H, *J* = 7.6), 2.34 (s, 3H), 3.55 (s, 2H), 4.24 (q, 2H, *J* = 7.6), 6.12 (s, 1H), 6.76 (d, 1H, *J* = 8.9), 7.06 (d, 1H, *J* = 8.9), 7.15–7.4 (m, 5H); δ_C 14.0, 19.0, 29.7, 62.8, 81.4, 105.0, 111.5, 112.8, 118.6, 121.0, 128.5, 130.0, 130.8, 130.9, 143.7, 150.4, 156.8, 166.0, 177.4; EIMS: *m*/*z* 366 (24%, M^+), 365 (22), 293 (100), 275 (32), 231 (10), 203 (36), 91 (98). MALDIHRMS (DHB): *m*/*z* 367.1190 $[M + H]^+$. $C_{21}H_{19}O_6$ requires *m*/*z* 367.1176. Coumarin **8**[17] (30 mg, 16%) was then eluted.

B. Quinone **1** (0.19 g, 1 mmol) was added at once to a solution of ylide **2c** (0.424 g, 1 mmol) in dry DCM (10 cm^3) at −10 °C and the reaction mixture was stirred at this temperature for 6 h (consumption of quinone). The solvent was then evaporated and the residue was separated by column chromatography [hexane–ethyl acetate (80 : 20 to 0 : 100)] to give triphenylphosphine (16 mg, 6%), compound **4c** (8 mg, 2%) and coumarin **8**[17] (80 mg, 42%).

Reaction of quinone 1 with ylide 11a. Synthesis of 2,6-dimethyl-2-phenyl-8*H*-[1,3]dioxolo[4,5-*h*]chromene-8-one 12a. A 1.6 M solution of BuLi in hexane (0.204 g, 3.2 mmol, 2 cm^3)

was added at once at room temperature under an argon atmosphere to a mixture of salt **10a** (1.341 g, 3 mmol) in THF (40 cm^3) and the mixture was stirred for 5 min. This red solution was added at once to a solution of quinone **1** (0.57 g, 3 mmol) in THF (25 cm^3) under an argon atmosphere. The mixture was stirred at room temperature for 1 h. After evaporation of the solvent the residue was treated with water (25 cm^3), extracted with DCM (4 × 25 cm^3), dried and the solvent was removed *in vacuo*. The residue was chromatographed on a column [hexane–ethyl acetate (10 : 1)] to give after the elution of triphenylphosphine (0.142 g, 18%) compound **12a** (0.326 g, 37%), mp 129–131 °C (ether–hexane) (Found: C, 73.8; H, 4.6. $C_{18}H_{14}O_4$ requires C, 73.45; H, 4.8%); ν_{max}/cm^{-1} 3040, 1725, 1625, 1580, 1170; δ_H 2.07 (s, 3H), 2.36 (s, 3H), 6.12 (s, 1H), 6.80 (d, 1H, J = 8.3), 7.09 (d, 1H, J = 8.3), 7.33–7.45 (m, 3H), 7.63 (d, 2H, J = 6.3); δ_C 19.0, 27.2, 80.0, 105.1, 112.3, 115.9, 118.1, 120.1, 124.8, 128.4, 129.2, 131.1, 133.7, 150.7, 152.8, 159.9; EIMS: *m*/*z* 294 (32%, M^+), 279 (8), 193 (11), 192 (100), 164 (15), 105 (13), 103 (61), 77 (14). Compound **8**[17] (6 mg, 1%) was then eluted.

Reactions of quinone 1 with ylide 11b. Synthesis of 6′-methylspiro[cyclopentane-1,2′-8′*H*-[1,3]dioxolo[4,5-*h*]chromene-8′-one] 12b. A. A 1.6 M solution of BuLi (0.204 g, 3.2 mmol, 2 cm^3) was added at 0 °C under an argon atmosphere to a mixture of phosphonium salt **10b** (1.233 g, 3 mmol) in THF (40 cm^3) and stirred for 30 min. A cold (0 °C) solution of **1** (0.57 g, 3 mmol) in THF (60 cm^3) was then added to the former red solution and the mixture was stirred for 1 h at 0 °C. After concentration, the residue was treated with water (25 cm^3), extracted with DCM (4 × 25 cm^3), dried (Na_2SO_4) and the solvent was evaporated. The residue was chromatographed on a column [hexane–ethyl acetate (7 : 1)] to give, after the elution of triphenylphosphine (35 mg, 4%), compound **12b** (33 mg, 4%), mp 131–133 °C (ether–hexane) (Found: C, 69.9; H, 5.6. $C_{15}H_{14}O_4$ requires C, 69.75; H, 5.6%); ν_{max}/cm^{-1} 3060, 1730, 1630, 1585, 1260, 1105; δ_H 1.80–1.99 (m, 4H), 2.06–2.29 (m, 4H), 2.38 (s, 3H), 6.11 (s, 1H), 6.73 (d, 1H, J = 8.3), 7.08 (d, 1H, J = 8.3); δ_C 19.1, 23.2, 37.1, 89.8, 104.8, 112.2, 117.7, 126.9, 130.6, 149.4, 152.8, 156.4, 158.8; EIMS: *m*/*z* 258 (71%, M^+), 257 (100), 230 (72), 216 (43), 203 (46), 202 (30), 192 (73). Coumarin **8**[17] (50 mg, 9%) was eluted next.

B. The above reaction was repeated at room temperature to give compound **12b** (8 mg, 1%) and coumarin **8**[17] (7 mg, 1%).

Reaction of quinone 1 with ylide 11c. Synthesis of 6′-methylspiro[cyclohexane-1,2′-8′*H*-[1,3]dioxolo[4,5-*h*]chromene-8′-one] 12c. A 1.6 M solution of BuLi (0.204 g, 3.2 mmol, 2 cm^3) was added at −78 °C under an argon atmosphere to a mixture of salt **10c** (1.275 g, 3 mmol) in THF (60 cm^3) and stirred for 30 min at −40 °C. A previously cooled (−78 °C) solution of **1** (0.57 g, 3 mmol) in THF (60 cm^3) was then added at once to the former red ylide's solution and the resulting mixture was stirred for a further 1 h at −40 °C. Concentration of this mixture followed by treatment of the residue with water (25 cm^3), extraction with DCM (4 × 25 cm^3), drying (Na_2SO_4), evaporation of the solvent and column chromatography [hexane–ethyl acetate (10 : 1)] gave after the elution of triphenylphosphine (0.13 g, 17%) compound **12c** (0.16 g, 20%), mp 142–144 °C (ether–hexane) (Found: C, 70.5; H, 6.1. $C_{16}H_{16}O_4$ requires C, 70.6; H, 5.9%); ν_{max}/cm^{-1} 3060, 1730, 1635, 1585, 1275, 1100; δ_H 1.44–1.6 (m, 2H), 1.67–1.86 (m, 4H), 1.88–2.07 (m, 4H), 2.37 (s, 3H), 6.10 (s, 1H), 6.73 (d, 1H, J = 8.2), 7.06 (d, 1H, J = 8.2); δ_C 19.0, 23.0, 24.4, 35.2, 83.5, 105.1, 112.1, 117.6, 122.1, 133.4, 150.9, 152.9, 156.7, 159.3; EIMS: *m*/*z* 272 (37%, M^+), 271 (100), 244 (59), 230 (97), 216 (92), 192 (90). Coumarin **8**[17] (40 mg, 7%) followed compound **12c**.

Reaction of dihydroxycoumarin 8 with cyclohexanone. Synthesis of compound 12c. A mixture of dihydroxycoumarin **8** (50 mg, 0.26 mmol), cyclohexanone (31 mg, 0.32 mmol) and *p*-TSA (3 mg) in dry toluene (15 cm^3) was refluxed by using a Dean–Stark trap for 12 h. After cooling, the mixture was concentrated and separated by PTLC [hexane–ethyl acetate (1 : 2)] to give from the faster moving band compound **12c** (8 mg, 11%) and from the next band unreacted compound **8**[17] (43 mg, 86%).

Attempted reaction for compound 8 with acetophenone. A mixture of compound **8** (50 mg, 0.26 mmol), acetophenone (35 mg, 0.29 mmol) and *p*-TSA (4 mg) in dry toluene (25 cm^3) was heated under reflux by using a Dean–Stark trap. TLC check showed no reaction even after 26 h reflux.

Reaction of compound 8 with acetophenone dimethyl acetal. Synthesis of compound 12a. A mixture of acetophenone dimethyl acetal [61 mg (0.37 mmol) prepared according to the literature[24] by refluxing a dry methanol solution of acetophenone and trimethylorthoformate in the presence of a catalytic amount of *p*-TSA for 1 h] and compound **8** (82 mg, 0.43 mmol) in dry benzene (20 cm^3) was refluxed for 1 h and part of the solvent (2 cm^3) was distilled through a short Vigreux column. Heating was discontinued, the temperature was lowered to 40 °C and *p*-TSA (3 mg) was added. The reflux was started again by using a Dean–Stark trap and was continued for 48 h. After cooling the mixture was concentrated and separated by PTLC [hexane–ethyl acetate (1 : 2)] to give from the second band (after the elution of acetophenone) compound **12a** (5 mg, 4%) and from the next band unreacted catechol **8**[17] (75 mg, 91%).

Reaction of quinone 13 with ylide 14. Synthesis of ethyl 6-oxo-6*H*-[1,3]dioxolo[4,5-*g*]chromene-8-carboxylate 18 and ethyl 2-[6-oxofuro[2,3-*f*][1,3]benzodioxol-7(6*H*)-ylidene]acetate 19. A. A solution of quinone **13** (0.247 g, 1.625 mmol) and ylide **14** (0.564 g, 1.625 mmol) in dry DCM (10 cm^3) was stirred at room temperature for 12 h. The solvent was evaporated and the residue was separated by column chromatography [hexane–ethyl acetate (20 : 1 to 10 : 1)] to give from the faster moving band red crystals of compound **19** (62 mg, 15%), mp 220–222 °C (DCM–hexane) (Found: C, 59.5; H, 3.7. $C_{13}H_{10}O_6$ requires C, 59.55; H, 3.85%); ν_{max}/cm^{-1} 3050, 1785, 1698, 1595, 1280, 1182; δ_H 1.37 (t, 3H, J = 7.2), 4.31 (q, 2H, J = 7.2), 6.05 (s, 2H), 6.66 (s, 1H), 6.72 (s, 1H), 8.15 (s, 1H); δ_C 14.2, 61.3, 93.9, 102.2, 107.9, 114.0, 121.1, 121.8, 135.1, 149.8, 151.9, 161.7, 168.0; EIMS: *m*/*z* 262 (100%, M^+), 234 (80), 217 (37), 206 (68), 205 (17), 189 (71), 162 (87), 161 (77).

The second fraction was yellow crystals of compound **18** (0.128 g, 30%), mp 129–131 °C (DCM–hexane) (Found: C, 59.9; H, 3.7. $C_{13}H_{10}O_6$ requires C, 59.55; H, 3.85%); ν_{max}/cm^{-1} 3040, 1730, 1715, 1620, 1565, 1245, 1215; δ_H 1.43 (t, 3H, J = 7.0), 4.44 (q, 2H, J = 7.0), 6.09 (s, 2H), 6.82 (s, 1H), 6.85 (s, 1H), 7.71 (s, 1H); δ_C 14.1, 62.5, 98.4, 102.5, 104.3, 107.7, 109.8, 115.9, 119.2, 145.3, 151.5, 160.6, 164.2; EIMS: *m*/*z* 262 (94%, M^+), 234 (95), 217 (64), 206 (96), 205 (100), 189 (90), 162 (95), 161 (90).

B. By repeating the same reaction of quinone **13** (0.100 g, 0.66 mmol) and ylide **14** (0.573 g, 1.65 mmol) in dry DCM (5 cm^3) compounds **19** (42 mg, 24%) and **18** (88 mg, 52%) were isolated from the column chromatography.

Reaction of compound 18 with copper. Synthesis of ayapin 20. A mixture of coumarin derivative **18** (22 mg, 0.084 mmol), copper powder (36 mg, 0.57 mmol) and quinoline (1.5 cm^3) was heated at 190 °C for 10 h. After cooling the reaction mixture was diluted with ethyl acetate (100 cm^3), the residual copper powder was filtered and the filtrate was treated with 5% HCl (20 cm^3). The organic layer was separated out and the aqueous layer was extracted with ethyl acetate (4 × 50 cm^3). The combined organic layers were washed with water (100 cm^3), dried (anhydrous Na_2SO_4) and separated by column chrom-

atography [DCM–ethyl acetate (100 : 0 to 98 : 2)] to give from the faster moving band unreacted compound **18** (2 mg, 10%) and next ayapin **20** (11 mg, 69%), mp 221–223 °C (from ethanol) (lit.[27] 223–224 °C).

Biological experimental

Physicochemical studies. Reversed phase TLC (RPTLC) was performed on silica gel plates impregnated with 55% (v/v) liquid paraffin in light petroleum ether.[36] Mobile phase methanol–water mixture (70/30, v/v) contained 2% aqueous ammonia (27%). The plates were developed in closed chromatography tanks saturated with the mobile phase at 24 °C. Spots were detected under UV light or by iodine vapours. R_M values were determined from the corresponding R_f values (from ten individual measurements) using the equation $R_M = \log [(1/R_f) - 1]$ Table 1.

Inhibition of the carrageenan-induced edema.[6,7,8] Edema was induced in the right hind paw of Fisher 344 rats (150–180 g, 2–3 months old) by the intradermal injection of 0.1 cm^3 2% carrageenan in water. Both sexes were used. Pregnant females were excluded. The tested compounds, 0.1 mmol kg^{-1} body weight, were suspended in water and with a few drops of Tween 80 were given intraperitoneally simultaneously. The rats were killed 3.5 h after carrageenan injection. The difference between the weight of the injected and uninjected paws was calculated for each animal. The change in paw weight was compared with that in control animals (treated with water) and expressed as a percent inhibition of the edema CPE % values Table 1. Indomethacin in 0.01 mmol kg^{-1} (47%) was administered as a standard drug for comparison reasons. Values CPE % are the mean from two different experiments with a standard error of the mean less than 10% ($p < 0.01$ compared with control values).

Effects of the synthesized compounds on the ferrous ion-stimulated peroxidation of linoleic acid.[37] Linoleic acid sodium salt 90 mg was dissolved and diluted to 50 cm^3 with 0.2 M phosphate buffer pH 7.4. Oxygen (100%) was bubbled through this solution. 2 cm^3 aliquots of the linoleic acid sodium solution were incubated for 2 h at 37 °C with 2.5 mM Fe^{2+} ($FeSO_4$) and 1 mM of the tested compounds. The amount of peroxidation which occurred during this time was measured with the TBA test. The amount of TBA-reactive material in each sample was determined by reading the absorbance of the aqueous layer at 535 nm. The inhibition of Fe^{2+}-stimulated oxidation of linoleic acid caused by each compound is the mean value for three to five experiments.

Competition of the tested compounds with DMSO for hydroxyl radicals.[38] The hydroxyl radicals generated by the Fe^{3+}–ascorbic acid system, were detected according to Nash,[38] by the determination of formaldehyde produced from the oxidation of DMSO. The reaction mixture contained EDTA (0.1 mM), Fe^{3+} (167 μM), DMSO (33 mM) in phosphate buffer (50 mM, pH 7.4), the tested compounds and ascorbic acid (10 mM). After 30 min of incubation (37 °C) the reaction was stopped with CCl_3COOH (17% w/v).

Interaction of the tested compounds with 1,1-diphenyl-2-picrylhydrazyl (DPPH) stable free radical.[6,7,8] To a solution of DPPH (0.1 mM) in absolute ethanol an equal volume of the compounds dissolved in ethanol was added. As a control solution ethanol was used. After 20 min at room temperature the absorbance was recorded at 517 nm.

Scavenging activity on superoxide anion radical.[8] The superoxide anion was generated by a xanthine–xanthine oxidase system and measured by the nitroblue tetrazolium (NBT) method. After incubating for 10 min at room temperature the absorbance was recorded at 560 nm.

Inhibition on β-glucuronidase.[8] Compounds in acetate buffer (0.1 M, pH 7.4) were tested against β-glucuronidase (0.1 cm^3 of 1 U cm^{-3}) with 2.5 mM *p*-nitrophenyl-β-D-glucopyranosiduronic acid. After incubation at 37 °C for 30 min, 2 cm^3 of 0.5 M NaOH solution was added to the mixture and the absorbance of the mixture was measured at 410 nm.

Inhibition on proteolysis.[8] Tosyl arginine methyl ester (TAME) was used as substrate for trypsin. The reaction mixture consisted of 1.5 cm^3 buffer (0.1 M Tris-HCl, pH 7.8 in 50% methanol v/v) and 1.4 cm^3 TAME (0.01 M in 50% v/v methanol). Compounds dissolved in 50% methanol were added (0.1 mM). The reaction was started by addition of 0.1 cm^3 trypsin (1 mg cm^{-3} 0.001 M HCl). The increase in the absorbance at 256 nm was determined in the next 4 min.

Soybean lipoxygenase inhibition.[8] The conversion of sodium linoleate to 13-hydroxyperoxylinoleic acid at 234 nm, was recorded and compared with an appropriate standard.

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