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Synthesis of hydroxycoumarins and hydroxybenzo[*f*]- or [*h*]coumarins as lipid peroxidation inhibitors

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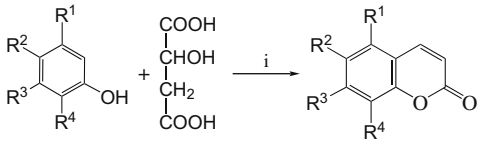
ABSTRACT

Substituted hydroxycoumarins and 7- or 8-hydroxybenzo[*f*]coumarins were prepared by the treatment of phenols and naphthalenediols, respectively, with malic acid and H_2SO_4 under microwave irradiation. 7- or 8-Hydroxybenzo[*f*]coumarins and 6-hydroxybenzo[*h*]coumarin were synthesized by the reaction of naphthalenediols with ethylpropiolate in the presence of $ZnCl_2$ in refluxing dioxane. The compounds were tested in vitro for their ability: (i) to interact with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical, (ii) to inhibit lipid peroxidation, (iii) to scavenge the superoxide anion, (iv) to inhibit the activity of soybean lipoxygenase and (v) to inhibit in vivo the carrageenin-induced rat paw edema. Most of them are potent superoxide anion scavengers and inhibit in vitro lipid peroxidation. The majority of the compounds did not show high lipoxygenase inhibitory activity. No differences were observed between biological responses of hydroxycoumarins and hydroxybenzocoumarins. Compound **3i** was found to present a promising antioxidant profile.

Coumarin derivatives exhibit a broad range of biological activities[1–7] including anticoagulation, antibiotic, antifungal, antipsoriasis, cytotoxic, anti-HIV, anti-inflammatory. Especially 7-hydroxycoumarin has antioxidant[3,4,7] properties and cytostatic,[5,6] antibacterial,[4] antiviral,[4] xanthine oxidase inhibitor,[4] antihyperglycemic,[8] casein kinase 2 inhibitor[9] activities. There have been reported many synthetic routes[3,4] to coumarins including the Pechmann, Perkin, Knoevenagel, Reformatsky and Wittig reactions. One of the most attractive methods is the Pechmann condensation,[10] which starts from phenols and β-ketoesters or malic acid or alkynoates[11,12] in the presence mainly of concentrated H_2SO_4 or Lewis acids. The main drawbacks of this method stem from the requirement for excess of acids, the high temperature and the long reaction time. For these reasons, there have been attempts[4] to replace acids with solid acid catalysts[13,14] (in high temperature) or to use microwave (MW)[15–18] irradiation of the reactants (phenols and β-ketoesters[15,18] or malic acid[17] or propiolic acid[15,16,19]) on solid support[15,16] or H_2SO_4.[17–19] From the above methods only malic acid,[17] propiolic acid[15,19] and propiolate[12] resulted to unsubstituted (in the pyrone ring) 7-hydroxycoumarins. In the course of our interest on the synthesis[20,21] of coumarin derivatives and the study[3,22,23] of their biological activities we wish to report here the synthesis of hydroxycoumarins or hydroxybenzo[*f*]coumarins from phenols or naphthalenediols and malic acid in the presence of small amount of H_2SO_4 under microwave irradiation. We wish also to refer to the synthesis of hydroxybenzo[*f*]- or [*h*]coumarins by the reaction of naphthalenediols with ethylpropiolate in the presence of $ZnCl_2$.



1a-h **2** **3a-h**

	R^1	R^2	R^3	R^4	Yield(%)
1,3a	H	H	OH	H	92
b	H	H	H	OH	52
c	H	OH	Me	H	89
d	Me	H	OH	H	85
e	H	H	OH	Me	72
f	$(CH=CH)_2$		OH	H	43
g	$(CH=CH)_2$		H	OH	3
h	H	OH	$(CH_2)_4$		3

Scheme 1. Reagent and condition: (i) concd H_2SO_4, MW, 80 W, 0.5–4 min.

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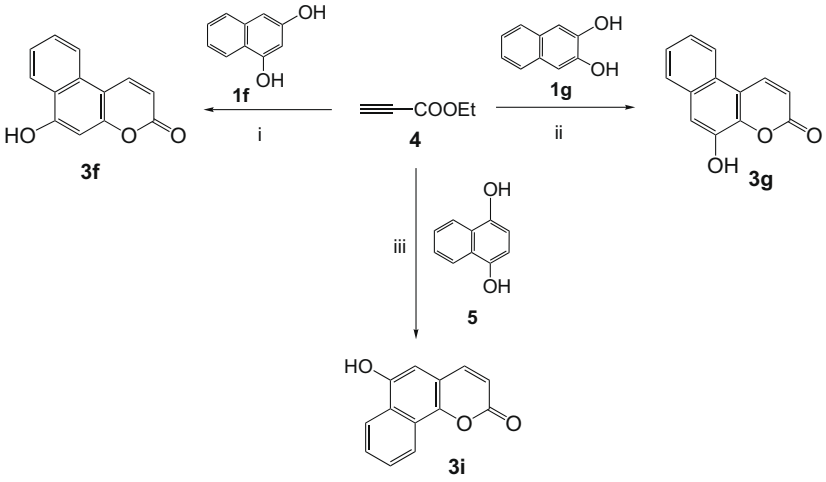

Scheme 2. Reagents and conditions: (i) $ZnCl_2$, dioxane, reflux, 4 d; (ii) $ZnCl_2$, dioxane, reflux, 7 d; (iii) $ZnCl_2$, dioxane, reflux, 9 d.

The reactions studied and the prepared compounds are depicted in Schemes 1 and 2. The synthesis of hydroxycoumarins was achieved by modifying slightly (~3 equiv and not 1 equiv of H_2SO_4) the earlier[17] reported synthesis of 7-hydroxycoumarin (**3a**). A mixture of equimolar amounts of phenol **1** and malic acid (**2**) with a small amount of concentrated H_2SO_4 (~3 equiv) was exposed to MW irradiation (80 W) for a few minutes.[24] The addition of this mixture after cooling into crushed ice and scratching resulted to the precipitation as solids of the hydroxycoumarin derivatives[24] **3** in good yields in most of the cases (Scheme 1). Compound **3a** was prepared in 92% yield [much better from the former[17] preparation (57%) or the preparation[15] from propiolic acid and Dowex (69%)], while compound **3e** was received in 72% yield (37% yield from propiolic[19] acid, H_2SO_4, heating at 120 °C). Naphthalene-2,3-diol (**1g**) gave too low yield, as expected[10] for β-naphthol and pyrocatechol.

The 5-hydroxy-3*H*-benzo[*f*]chromen-3-one (**3g**)[24] was obtained in 31% yield by refluxing **1g**, ethylpropiolate (**4**) and $ZnCl_2$ in dioxane[12] (Scheme 2). Analogous reactions of naphthalene-1,3-diol (**1f**) or naphthalene-1,4-diol (**5**) with **4** in the presence of $ZnCl_2$ in dioxane gave 6-hydroxy-3*H*-benzo[*f*]chromen-3-one (**3f**)[24] (83%) or 6-hydroxy-*2H*-benzo[*h*]chromen-2-one (**3i**)[24] (26%).

Oxidation is one of the most important processes, which produces free radicals in food, chemicals and in living systems. Antioxidants are defined as substances that even at low concentration significantly delay or prevent oxidation of easy oxidizable substrates. There is an increased interest of using antioxidants for medical purposes in the recent years. Several methods are used for the estimation of efficiency of synthetic/natural antioxidants, like the 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH)/linoleic acid assay,[29] 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.[30] DPPH assay is one of the best-known, frequently employed, and accurate methods. DPPH is a stable free radical because of its spare electron delocalization over the whole molecule. The delocalization causes a deep violet color with λ_{max} around 520 nm. Compounds with antioxidant properties could be expected to offer protection in rheumatoid arthritis and inflammation and to lead to potentially effective drugs. Reduction of DPPH stable free radical by the examined compounds was studied by the use of the latter at 0.05 mM and 0.1 mM after 20 and 60 min (Table 1), [the percentage of DPPH reduction DPPH % = $(A_{standard} - A_{sample}/A_{standard}) \times 100$]. The results indicate their radical scavenging ability in an iron-free system and they are time and concentration independent with the exception of compounds **3c**, **3d**, **3f**, **3g** and **3i**. We tested the new derivatives with regard to their antioxidant ability and in comparison to a well known antioxidant agent, for example, nordihydroguaiaretic acid (NDGA). The presented % values are low compared to NDGA with the exception of compounds **3d**, **3f** and **3h** (Table 1). Compound **3a** did not present any activity.[7] No significant differences are observed among hydroxycoumarins and hydroxybenzo[*f*]- or [*h*]coumarins with the exception of **3i**. Free coumarin as well as 7-CH_3-coumarin presented very low reducing abilities. Phenolic compounds present antioxidant activity. The presence of the phenolic hydroxyl group (6-, 7- or 8-) seems to support the antioxidant activity. The results are independent of the position of hydroxyl group. Lipophilicity also influences reducing ability (calculated log*P* values).[31] It seems that the benzo derivative **3i** is more potent than the cyclohexyl **3h**.

Table 1
Reduction % of 1,1-diphenyl-2-picrylhydrazyl (DPPH %). Final concentrations of the tested compounds 0.05 mM and 0.1 mM

Compound	DPPH % 20 min 0.05 mM	DPPH % 60 min 0.05 mM	DPPH % 20 min 0.1 mM	DPPH % 60 min 0.1 mM	Clog*P*[31]
3a	No	No	2	2	1.61
3b	31	30	33	32	1.61
3c	22	20	51	51	2.11
3d	43	43	31	54	2.11
3e	14	15	26	27	2.11
3f	28	31	55	51	2.79
3g	22	20	46	54	2.79
3h	35	38	44	40	1.94
3i	65	72	77	89	2.79
NDGA	68	72	81	83	
Coumarin	No	No	5	21	
7-CH_3-coumarin	No	No	2	2	

NDGA, nordihydroguaiaretic acid; No, no results under the reported experimental conditions; each experiment was performed at least in triplicate and the standard deviation of absorbance was less than 10% of the mean.

Azo compounds generating free radicals through spontaneous thermal decomposition are useful for free radical production studies in vitro. The water soluble azo compound AAPH has been extensively used as a clean and controllable source of alkylperoxyl free radicals. In our studies AAPH was used as a free radical initiator to follow oxidative changes of linoleic acid to conjugated diene hydroperoxide.[29] In our experiments compounds **3a**, **3g**, **3f** and **3i** showed excellent inhibition on lipid peroxidation at 100 μM (Table 2) compared to trolox, used as a standard (73%), whereas compounds **3b, 3c** and **3d** were found to present low inhibition. Compound **3e** was inactive. No differences were

Table 2
Inhibition of lipid peroxidation at 100 μM (LP %); superoxide radical scavenging activity ($O_2^{-\cdot}$ %) at 100 μM; in vitro inhibition of soybean lipoxygenase (LO) % at 100 μM; inhibition % of induced carrageenin rat paw edema (CPE %) at 0.01 mmol/kg body weight

No	LP % at 100 μM	$O_2^{-\cdot}$ % at 100 μM	LO % at 100 μM	CPE %[a]
3a	83	65	IC_{50} = 43 μM	No
3b	28	56	37%	18*
3c	24	54	3%	
3d	30	61	27%	
3e	No	60	25%	
3f	57	No	38%	15*
3g	100	79	No	18*
3h	42	68	12%	
3i	100	61	IC_{50} = 100 μM	39*
Coumarin	nt	nt	15%	30*
7-CH_3-coumarin	nt	nt	89%	55*
CA		71	IC_{50} = 600 μM	
Trolox	73			
IMA				47*

CA, caffeic acid; IMA, indomethacin; No, no result under the experimental conditions; each experiment was performed at least in triplicate and the standard deviation of absorbance was less than 10% of the mean.
[a] Statistical studies were done with student's *t*-test.
* $p < 0.01$.

observed between the hydroxycoumarins and the hydroxybenzocoumarins.

It is consistent that rates of reactive oxygen species (ROS) production are increased in most diseases.[32] Cytotoxicity of $O_2^{-\cdot}$ and H_2O_2 in living organisms is mainly due to their transformation into **.**OH, reactive radical metal complexes and $^1O_2$. During the inflammatory process, phagocytes generate the superoxide anion radical at the inflamed site. Enzymatic superoxide anion radicals were generated by a hypoxanthine and xanthine oxidase (XOD) reaction system.[33] At pH 7.4 superoxide anion reduces the tetrazolium blue into formasan blue (λ_{max} = 560 nm). The production of superoxide was estimated by the nitroblue tetrazolium method. The majority of the compounds present high scavenging activity at 100 μM (54–79%) compared to caffeic acid used as a standard (71%) (Table 2), with the exception of compound **3f** which does not show any activity.

Compounds were further evaluated for inhibition of soybean lipoxygenase LO by the UV absorbance based enzyme assay.[22] Lipoxygenases oxidize certain fatty acids at specific positions to hydroperoxides that are the precursors of leukotrienes, which contain a conjugated triene structure. It is known that soybean lipoxygenase, which converts linoleic to 13-hydroperoxylinoleic acid, is inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs)[34,35] in a qualitatively similar way to that of the rat mast cell lipoxygenase and may be used in a reliable screen[35] for such activity. For compounds **3a** and **3i** the IC_{50} values were determined. Compound **3a** is the most potent derivative. The most of the LO inhibitors are antioxidants or free radical scavengers,[36] since lipoxygenation occurs via a carbon-centered radical. Some studies suggest a relationship between LO inhibition and the ability of the inhibitors to reduce Fe^{3+} at the active site to the catalytically inactive Fe^{2+}. LOs contain a "non-heme" iron per molecule in the enzyme active site as high-spin Fe^{2+} in the native state and the high-spin Fe^{3+} in the activated state. Several LO inhibitors are excellent ligands for Fe^{3+}. Many flavonoids and other phenolics such as hydroxycoumarin derivatives inhibit soybean lipoxygenase. 7-Hydroxycoumarin also inhibits the activity of LO.[3] This inhibition is related to their ability to be reduced from the iron species in the active site to the catalytically inactive ferrous form.[36] Thus the presence of a free hydroxyl group could explain the inhibition results of the examined compounds. Compounds **3b** and **3f** present equipotent inhibition. The majority of the derivatives did not show high inhibitory activity. No differences were observed between the hydroxycoumarins and the hydroxybenzocoumarins.

Compounds **3a,b,f,g** and **3i** were tested for their anti-inflammatory activity in vivo (dose ip 0.01 mmol/kg body weight). The in vivo anti-inflammatory effects of the tested coumarins were assessed by using the functional model of carrageenin-induced rat paw edema (CPE)[22] and are presented in Table 2, as percent inhibition of induced rat paw edema. After 3.5 h, compounds **3b, 3f** and **3g** induced very low protection (15–18%) against carrageenin-induced paw edema while the reference drug indomethacin (IMA) induced 47% protection at an equivalent dose. Compound **3a** did not inhibit carrageenin-induced rat paw edema whereas compound **3i** (a hydroxyl benzocoumarin) presents the higher inhibition among the tested compounds (39%). The side of ring's condensation seems to influence the biological response [*f*] < [*h*] (compounds **3f**, **3g** < **3i**).

In conclusion, the broad spectrum of the observed antioxidant activity of the majority of the examined coumarins allows us to propose them as templates in the design of compounds useful in treating human diseases that involves reactive oxygen species (ROS)**.** Their synthesis is almost simple with moderate to high yields. Most of them are potent superoxide anion scavengers and inhibit in vitro lipid peroxidation. Antioxidant power might be important in the inhibition of lipid peroxidation. Compound **3i** presenting higher LO inhibitory activity among the tested hydroxybenzocoumarins, was found to present a promising antioxidant profile and 39% inhibition on carrageenin-induced rat paw edema.

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24. (a) Selected data: *General procedure. 7-Hydroxy-2H-chromen-2-one* (**3a**). A homogeneous mixture of resorcinol (**1a**) (10 mmol) and malic acid (**2**) (10 mmol) was wet by concentrated H_2SO_4 (1.5 ml, 2.76 g, 28 mmol) and irradiated in a household microwave oven at 80 W for 15 s. The progress of the reaction was monitored by tlc. The irradiation was repeated with pulses for 15 s and monitoring by tlc. The total irradiation was for 3 min and 30 s. After cooling the mixture was treated by ice (10 g) and the precipitated solid was compound **3a**, mp 226–228 °C (228 °C).[17]; (b) *8-Hydroxy-2H-chromene-2-one* (**3b**) (irradiation at 80 W for 30 s), mp 151–153 °C (153–154 °C).[25]; (c) *6-Hydroxy-7-methyl-2H-chromen-2-one* (**3c**) (irradiation at 80 W for 4 min), mp 207–209 °C (210 °C).[26]; (d) *7-Hydroxy-5-methyl-2H-chromene-2-one* (**3d**) (irradiation at 80 W for 3 min and 15 s), mp 246–248 °C (247–248 °C).[27]; (e) *7-Hydroxy-8-methyl-2H-chromen-2-one* (**3e**) (irradiation at 240 W for 4 min), mp 253–255 °C (253–256 °C).[28]; (f) Compound **3f** (irradiation at 80 W for 1 min and 30 s), mp 229–230 °C (dec.) (ethyl acetate), IR (KBr): 3100, 1715, 1595 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 6.30 (d, 1H, J = 10.0 Hz), 6.91 (s, 1H), 7.52 (t, 1H, J = 8.2 Hz), 7.68 (t, 1H, J = 8.2 Hz), 8.21 (d, 1H, J = 8.2 Hz), 8.30 (d, 1H, J = 8.2 Hz), 8.48 (d, 1H, J = 10.0 Hz), 10.95 (s, 1H, exchanged by D_2O); ^{13}C NMR ($CDCl_3$ + DMSO-d_6) δ 97.9, 105.6, 109.3, 120.3, 121.9, 122.3, 123.9, 124.5, 127.7, 139.0, 146.2, 157.6, 160.6; MS (ESI): 235 $[M+Na]^+$; Anal. Calcd for $C_{13}H_8O_3$: C, 73.85; H, 3.80. Found: C, 73.46; H, 3.91.*General procedure for the synthesis of* ***3f*** *from ethyl propiolate*. Ethyl propiolate (**4**) (442 mg, 0.46 ml, 4.5 mmol) and $ZnCl_2$ (405 mg, 3 mmol) was added to a solution of naphthalene-1,3-diol (**1f**) (722 mg, 4.5 mmol) in dry dioxan (15 ml) and the mixture was refluxed under stirring for 4 days. After cooling the mixture was poured in 5% HCl (15 ml), condensed to the half of the volume and left overnight in the refrigerator. The precipitated solid was recrystallised from ethyl acetate to give compound **3f** (83% yield).; (g) Compound **3g** (irradiation at 240 W for 30 s), mp 230–232 °C (ethyl acetate), IR (KBr): 3377, 1709, 1636 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 6.56 (d, 1H, J = 10.0 Hz), 7.45 (s, 1H), 7.46–7.52 (m, 2H), 7.73 (d, 1H, J = 9.1 Hz), 8.18 (d, 1H, J = 9.1 Hz), 8.59 (d, 1H, J = 10.0 Hz), 10.04 (s, 1H, exchanged by D_2O); ^{13}C NMR ($CDCl_3$ + DMSO-d_6) δ 113.6, 114.3, 120.4, 122.6, 124.1, 125.2, 126.0, 129.9, 136.4, 136.9, 138.9, 143.4, 159.2; MS (ESI): 235 $[M+Na]^+$; Anal. Calcd for $C_{13}H_8O_3$: C, 73.85; H, 3.80. Found: C, 73.64; H, 3.90.; (h) *6-Hydroxy-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one* (**3h**) (irradiation at 80 W for 1 min), mp 182–184 °C (dec.) (ethyl acetate), IR (Nujol): 3300, 1715, 1650, 1595 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.76–1.90 (m, 4H), 2.38–2.52 (m, 4H), 6.37 (d, 1H, J = 10.0 Hz), 7.23 (s, 1H), 7.78 (d, 1H, J = 10.0 Hz), 8.39 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.5, 21.8, 22.8, 23.6, 108.4, 115.5, 126.5, 131.4, 132.6, 138.3, 143.6, 146.6, 159.0; MS (ESI): 217 $[M+H]^+$; Anal. Calcd for $C_{13}H_{12}O_3$: C, 72.20; H, 5.60. Found: C, 72.44; H, 5.87.; (i) Compound **3i**: mp 250–252 °C (ethyl acetate), IR (Nujol): 3150, 1725, 1655, 1590 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 6.47 (d, 1H, J = 9.1 Hz), 6.87 (s, 1H), 7.58–7.68 (m, 2H), 7.79 (d, 1H, J = 9.1 Hz), 8.28 (d, 1H, J = 8.2 Hz), 8.43 (d, 1H, J = 8.2 Hz), 9.83 (s, 1H, exchanged by D_2O); ^{13}C NMR ($CDCl_3$ + DMSO-d_6) δ 103.0, 111.6, 115.1, 121.0, 122.1, 123.1, 124.2, 126.4, 126.6, 126.8, 143.8, 159.4, 160.5; MS (ESI): 235 $[M+Na]^+$; Anal. Calcd for $C_{13}H_8O_3$: C, 73.85; H, 3.80. Found: C, 73.98; H, 3.63.
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