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RESEARCH ARTICLE

Synthesis and biological evaluation of fused oxepinocoumarins as free radicals scavengers

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Abstract

Some fused dihydrooxepino[f]-, [g]-, and [h]coumarins were obtained from the ring-closing metathesis of the corresponding *o*-allyl-allyloxycoumarins under the treatment with the first generation Grubbs' catalyst. These compounds were tested *in vitro* for their antioxidant activity, and they present significant scavenging activity. They were also showed to inhibit *in vitro* soybean lipoxygenase.

Keywords: Oxepinocoumarins, allyloxycoumarins, claisen rearrangement, ring-closing metathesis, antioxidant activity

Introduction

Coumarin derivatives are an interesting class of heterocyclic system, since the coumarin ring is an essential core moiety for a variety of natural and synthetic biologically active compounds[1–3]. In particular, coumarins fused with a ring containing an O-atom such as furocoumarins and pyranocoumarins are important as photochemotherapeutic[4–10] agents and exhibit antitumorial[11], antifungal[12], insecticidal[12], anticancer[12], anti-HIV[6,13], anti-inflammatory[3,14], and antioxidant[3,14] activities. The synthesis of those coumarins has been achieved mainly by formation of furan or pyran ring starting from hydroxycoumarins and using the tandem Claisen rearrangement-cyclization reaction[15,16] of the intermediate propargyloxy- or allyloxycoumarins[17–21]. The Ru-catalyzed ring-closing metathesis (RCM)[22–26] has been applied in the synthesis of furan and pyran ring during the last decade[25,27–30] and especially in the synthesis of fused furo- or pyranocoumarins[25,27,29]. With this method, oxepines[25,27,31–34], oxocines[25,27,34,35], or larger O-containing rings[25,36–38] have also been prepared. In the course of our interest on the synthesis[21,39] of coumarin derivatives and the study[3,14,40,41] of their biological activities and in continuation to our previous work on RCM[37,42], we wish to report here the synthesis of [6,5]-, [7,6]-, [7,8]- and [8,7]-fused oxepinocoumarins through the combination of Claisen rearrangement, allylation, and RCM starting from allyloxycoumarins.

There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body. Free radicals are molecules produced when human body breaks down foods, or by environmental exposures such as tobacco smoke and radiation and have been implicated in the pathology of more than 50 human diseases. Oxidative stress, occurring when antioxidant defenses are inadequate, can damage lipids, proteins, carbohydrates, and DNA. Several antioxidants are available for therapeutic use. They include molecules naturally

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present in the body as well as synthetic antioxidants[43]. Thus, we found interesting the biological screening of the resulted compounds as possible free-radicals scavengers and lipoxygenase (LOX) inhibitors. It is well known that free radicals play an important role in inflammatory process[44]. Consequently compounds with antioxidant properties could be expected to offer protection in rheumatoid arthritis and inflammation and to lead to potentially effective drugs. The reactions studied and the products received are depicted in Schemes 1 to 3.

Materials and methods

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Infrared (IR) spectra were obtained with a Perkin–Elmer 1310 spectrophotometer as KBr pellets or Nujol mulls. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AM 300 (300 and 75 MHz for ^{1}H and ^{13}C, respectively) using $CDCl_3$ as solvent and tetramethylsilane (TMS) as an internal standard. *J* values are reported in Hertz. Mass spectra were determined on a LCMS-2010 EV Instrument (Shimadzu) under electrospray ionization (ESI) conditions or on a VG-250 spectrometer at 70 eV under electron impact (EI) conditions. Microanalyses were performed on a Perkin–Elmer 2400-II Element analyzer. Silica gel (no. 60, Merck A.G.) was used for column chromatography. All the reagents used were commercially available. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and nordihydroguaiaretic acid (NDGA) were purchased from the Aldrich Chemical Co. (Milwaukee, WI). Soybean LOX, linoleic acid sodium salt, and 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH) were obtained from Sigma Chemical, Co. (St. Louis, MO).

Synthesis

General procedure for the Claisen rearrangement of allyloxycoumarins

A solution of 2.133 g (10.5 mmol) of 7-allyloxycoumarin[45] in ethyleneglycol (90 mL) was refluxed under stirring for 9 h. Cold water (100 mL) was added and it was refrigerated overnight. The precipitated solid was filtered and separated by column chromatography [silica gel No. 60, hexane/ethyl acetate (2:1)] to give unreacted starting material (214 mg, 10%), 6-allyl-7-hydroxy-2H-chromen-2-one (**4**) (363 mg, 17%), m.p. 140–141°C (DCM–CH_3OH) (lit[45]. 137–139°C) and 8-allyl-7-hydroxy-2H-chromene-2-one (**3a**) (1.45 g, 68%), m.p. 164–166°C (DCM–CH_3OH) (lit[46]. 165–166°C).

General procedure for the allylation of o-hydroxy-allylcoumarins

To a solution of compound **3a** (340 mg, 1.68 mmol) in dry acetone (30 mL) anhydrous K_2CO_3 (1.12 g, 8.1 mmol) was added, followed by allyl bromide (0.87 mL, 1.25 g, 10.3 mmol). The mixture was heated under reflux and stirring for 2 h and filtered while hot. The filtrate was concentrated and the residue was left for crystallization in the freezer to give 8-allyl-7-(allyloxy)-2H-chromene-2-one (**5a**) (325 mg, 80%), m.p. 82–84°C (DCM–hexane); IR(Nujol) 3060, 1710, 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.64 (d, J= 6.4 Hz, 2H), 4.65 (d, J= 5.1 Hz, 2H), 4.99 (d, J= 11.5 Hz, 1H), 5.09 (d, J= 19.1 Hz, 1H), 5.31 (d, J= 8.9 Hz, 1H), 5.44 (d, J= 19.1 Hz, 1H), 5.92–6.10 (m, 2H), 6.25 (d, J= 8.9 Hz, 1H), 6.83 (d, J= 8.9 Hz, 1H), 7.30 (d, J= 8.9 Hz, 1H), 7.62 (d, J= 8.9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 27.0, 69.3, 108.6, 110.0, 113.2, 115.5, 117.7, 120.9, 126.5, 132.5, 139.2, 143.7, 148.9, 159.3, 161.9; MS (EI) 242 ($M^{+\cdot}$, 20%), 214 (9), 201 (63), 187 (58), 173 (44), 145 (30), 117 (17), 115 (100). Anal. Calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.83. Found: C, 74.70; H, 6.14.

General procedure for the RCM reaction of o-allyl-allyloxycoumarins

The catalyst **6** (12.2 mg, 0.015 mmol) was added to a solution of derivative **5a** (105 mg, 0.43 mmol) in dry dichloromethane (DCM) (50 mL) after removing of the air by a pump and introducing Argon (repeating in three cycles). The solution was stirred (the air was removed at the beginning and Argon was passed in the solution for three times again) for 4 h, a new amount of the catalyst **6** (3.3 mg, 0.004 mmol, 4.3 mol% totally) was added and the stirring was continued for 20 h more (totally 24 h stirring). After the evaporation of the solvent, the residue was separated by column chromatography (silica gel No. 60, DCM) to give 8,11-dihydro-2H-oxepino[2,3-h]chromen-2-one (**7a**), (83 mg, 90%), m.p. 119–121°C (DCM–hexane); IR(Nujol) 3070, 1695, 1595 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.78 (d, J= 3.8 Hz, 2H), 4.65 (d, J= 3.8 Hz, 2H), 5.58 (dt, J_1= 11.5 Hz, J_2= 3.8 Hz,1H), 5.87–5.97 (m, 1H), 6.31 (d, J= 8.9 Hz, 1H), 6.99 (d, J= 8.9 Hz, 1H), 7.30 (d, J= 8.9 Hz, 1H), 7.67 (d, J= 8.9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 22.2, 70.8, 114.2, 115.2, 118.2, 123.4, 125.9, 126.6, 127.4, 143.7, 151.4, 160.8, 162.1; MS (EI) 214 ($M^{+\cdot}$, 87%), 186 (20), 185 (43), 170 (100), 160 (16), 158 (25), 157 (30), 142 (55). Anal. Calcd for $C_{13}H_{10}O_3$: C, 72.89; H, 4.71. Found: C, 72.97; H, 4.55.

8-Allyl-7-hydroxy-4-methyl-*2H*-chromen-2-one (3b) (80% yield), m.p. 197–199°C (EtOH) (lit[46]. 198–199°C)
8-Allyl-7-(allyloxy)-4-methyl-2*H*-chromene-2-one (**5b**) (90% yield), 92–93°C (acetone) (lit[27]. 94°C).

4-Methyl-8,11-dihydro-2*H*-oxepino[2,3-h] chromen-2-one (**7b**) (90% yield– 0.9 mol% of **6** was added at once), m.p. 109–111°C (DCM); IR(Nujol) 3060, 1690, 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.41 (s, 3H), 3.78 (s, 2H), 4.65 (s, 2H), 5.56 (d, J_1= 11.4 Hz, 1H), 5.87–5.98 (m, 1H), 6.19 (s, 1H), 7.01 (d, J= 7.6 Hz, 1H), 7.43 (d, J= 7.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 18.8, 22.3, 70.8, 113.0, 116.3, 117.8, 123.2, 123.4, 126.0, 127.3, 150.8, 152.6, 160.8, 161.9; MS (EI) 228 ($M^{+\cdot}$, 94%), 213 (92), 200 (29), 199 (74), 186 (97), 185 (78), 184 (14), 156 (10), 128 (100). Anal. Calcd for $C_{14}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 73.61; H, 5.12.

6-Allyl-7-(allyloxy)-2*H*-chromene-2-one (**8**) (91% yield), m.p. 94–95°C (acetone); IR(Nujol) 3060, 1700,

1600 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.42 (d, J= 5.9 Hz, 2H), 4.61 (d, J= 4.9 Hz, 2H), 5.09 (d, J= 12.8 Hz, 1H), 5.10 (d, J= 11.8 Hz, 1H), 5.34 (d, J= 11.8 Hz, 1H), 5.46 (d, J= 18.7 Hz, 1H), 5.85–6.14 (m, 2H), 6.24 (d, J= 8.9 Hz, 1H), 6.77 (s, 1H), 7.22 (s, 1H), 7.64 (d, J= 8.9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 33.7, 69.1, 99.7, 112.0, 112.9, 116.3, 117.9, 126.3, 128.0, 132.1, 135.8, 143.4, 154.5, 159.3, 161.3; MS (EI) 242 (M^{+}·, 64%), 214 (8), 201 (61), 173 (39), 145 (30), 117 (44), 115 (100), 91 (49). Anal. Calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.83. Found: C, 74.52; H, 5.59.

6,9-Dihydro-2*H*-oxepino[3,2-g]chromen-2-one (9) (97% yield– 3.9 mol% of **6** was added in four portions during 15 h), m.p. 118–120°C (DCM–hexane); IR (KBr) 3080, 2927, 1732, 1622, 1561 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.53 (d, J= 3.0, 2H), 4.67 (dd, J_1 = 3.0, J_2 = 2.0, 2H), 5.52 (dt, J_1 = 11.8 Hz, J_2 = 2.0 Hz,1H), 5.86–5.97 (m, 1H), 6.34 (d, J= 9.9 Hz, 1H), 7.04 (s, 1H), 7.21 (s, 1H), 7.43 (d, J= 9.9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 31.1, 71.6, 110.2, 115.0, 115.1, 125.8, 127.2, 127.7, 132.9, 143.0, 155.0, 160.9, 161.8; MS (ESI) 215 $[M+H]^+$, 237 $[M+Na]^+$. Anal. Calcd for $C_{13}H_{10}O_3$: C, 72.89; H, 4.71. Found: C, 73.01; H, 4.97.

5-Allyl-6-hydroxy-4-methyl-2*H*-chromen-2-one (**12**) (78% yield, after the reflux of compound **11** in ethyleneglycol for 16 h), m.p. 176–178°C (EtOH) (lit[47]. 176–177°C).

5-Allyl-6-(allyloxy)-4-methyl-2*H*-chromene-2-one (**13**) (83% yield, after 4 h of refluxing), m.p. 62–64°C (acetone); IR (KBr) 3097, 2978, 2934, 1695, 1598, 1563 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.67 (s, 3H), 3.87 (m, 2H), 4.57 (dd, J_1 = 4.9 Hz, J_2 = 2.0 Hz, 2H), 4.75 (dq, J_1 = 18.7 Hz, J_2 = 2.0 Hz, 1H), 5.08 (dq, J_1 = 10.8 Hz, J_2 = 2.0 Hz, 1H), 5.28 (dt, J_1 = 11.8 Hz, J_2 = 3.0 Hz, 1H), 5.41 (dt, J_1 = 18.7 Hz, J_2 = 2.0 Hz, 1H), 5.96–6.13 (m, 2H), 6.25 (s, 1H), 7.12 (d, J= 8.9 Hz, 1H), 7.23 (d, J= 8.9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 24.2, 30.6, 70.2, 115.5, 116.3, 116.4, 117.3, 117.7, 120.0, 126.1, 133.0, 136.8, 149.1, 153.4, 153.6, 160.3; MS (EI) 256 (M^{+}·, 73%), 215 (27), 201 (21), 200 (42), 187 (16), 171 (18), 115 (48), 91 (40), 41 (100). Anal. Calcd for $C_{16}H_{16}O_3$: C, 74.97; H, 6.30. Found: C, 75.12; H, 6.27.

4-Methyl-8,11-dihydro-3*H*-oxepino[3,2-f]chromen-3-one (**14**) (98% yield– 2.2 mol% of **6** was added in four portions during 15 h), m.p. 126–128°C (DCM–hexane); IR (KBr) 3079, 2939, 2843, 1732, 1593, 1568 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.63 (s, 3H), 3.90 (d, J= 5.9 Hz, 2H), 4.64 (t, J= 2.0 Hz, 2H), 5.46 (dt, J_1 = 10.8 Hz, J_2 = 2.0 Hz, 1H), 5.88–5.98 (m, 1H), 6.26 (s, 1H), 7.19 (d, J= 8.9 Hz, 1H), 7.28 (d, J= 8.9 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 25.0, 26.1, 72.0, 116.4, 117.5, 120.8, 124.6, 124.9, 128.4, 136.2, 150.9, 152.7, 155.5, 159.1; MS (ESI) 229 $[M+H]^+$, 251 $[M+Na]^+$. Anal. Calcd for $C_{14}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 73.85; H, 5.51.

7-Allyl-8-hydroxy--2*H*-chromen-2-one (**17**) (89% yield, after the reflux of compound **16** in ethyleneglycol for 20 h), m.p. 151–153°C (DCM–hexane) (lit[48]. 154°C).

7-Allyl-8-(allyloxy)-2*H*-chromene-2-one (**18**) (82% yield), m.p. 51–53°C (acetone); IR (Nujol) 3040, 1715, 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.50 (d, J= 6.9 Hz, 2H), 4.70 (d, J= 5.9 Hz, 2H), 5.07 (d, J= 17.7 Hz, 1H), 5.09 (d, J= 8.9 Hz, 1H), 5.25 (d, J= 10.8 Hz, 1H), 5.40 (d, J= 15.8 Hz, 1H), 5.88–6.0 (m, 1H), 6.05–6.18 (m, 1H), 6.36 (d, J= 9.8 Hz, 1H), 7.08 (d, J= 7.9 Hz, 1H), 7.15 (d, J= 7.9 Hz, 1H), 7.67 (d, J= 9.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 34.3, 74.6, 115.6, 116.5, 118.2, 118.4, 122.3, 125.4, 128.4, 133.5, 135.9, 137.5, 143.7, 147.1, 160.1; MS (ESI) 265 $[M+Na]^+$. Anal. Calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.83. Found: C, 74.41; H, 5.96.

7,10-Dihydro-2*H*-oxepino[3,2-h]chromen-2-one (19) (83% yield– 6.9 mol% of **6** was added in three portions over 12 h), m.p. 109–111°C (ethyl acetate); IR (Nujol) 3050, 1710, 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.58 (d, J= 2.6, 2H), 4.71 (d, J= 2.6, 2H), 5.53 (dt, J_1 = 12.0 Hz, J_2 = 2.6 Hz,1H), 5.80–5.91 (m, 1H), 6.39 (d, J= 9.5 Hz, 1H), 7.02 (d, J= 7.7 Hz, 1H), 7.15 (d, J= 7.7 Hz, 1H), 7.69 (d, J= 9.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 31.7, 70.8, 114.7, 117.0, 121.5, 123.7, 125.2, 126.6, 128.6, 142.5, 144.5, 155.3, 160.5; MS (ESI) 237 $[M+Na]^+$. Anal. Calcd for $C_{13}H_{10}O_3$: C, 72.89; H, 4.71. Found: C, 73.08; H, 4.60.

Biological assay

In vitro *experiments*

In the *in vitro* assays, each experiment was performed at least in triplicate and the standard deviation of absorbance was <10% of the mean.

Table 1. Interaction % with DPPH at 0.1 mM; competition % of compounds with DMSO for hydroxyl radical (HO %); inhibition of lipid peroxidation at 0.1 mM (LP %); *in vitro* inhibition of soybean LOX IC_{50} μM.

No.	DPPH % (20/60 min 0.1 mM)	HO· % (0.1 mM)	LP % at 0.1 mM	LOX inhibitor (IC_{50} μM)	Clog *P*[a]
7a	19/24	94	nd	310	2.56
7b	3/12	61	nd	180	3.06
9	13/13	nd	65	nd	2.56
14	no	nd	87	nd	3.06
19	10/10	nd	92	nd	2.56
NDGA	81/83				
CA				600	
Trolox		88	73		

[a]Biobyte Corp., C-QSAR Database 201 West 4th Str., Suite 204, Claremont CA, California 91711, USA.
NDGA, nordihydroguaiaretic acid; CA, caffeic acid; nd, not detectable under the reported experimental conditions; each experiment was performed at least in triplicate and the standard deviation of absorbance was <10% of the mean.

Determination of the reducing activity of the stable radical DPPH[41]

An equal volume of the compounds dissolved in dimethylsulfoxide (DMSO) was added to a solution of DPPH (0.1 mM) in absolute ethanol. Ethanol was used as the control solution. The concentration of the solutions of the compounds was 0.1 mM. After 20 and 60 min at room temperature, the absorbance was recorded at 517 nm (Table 1). NDGA was used as a standard.

Competition of the tested compounds with DMSO for hydroxyl radicals[49]

The hydroxyl radicals generated by the Fe^{3+}/ascorbic acid system, were detected according to Nash, by the determination of formaldehyde produced from the oxidation of DMSO. The reaction mixture contained EDTA (0.1 mM), Fe^{3+} (167 mM), DMSO (33 mM) in phosphate buffer (50 mM, pH 7.4), the tested compounds (concentration 0.1 mM), and ascorbic acid (10 mM). After 30 min of incubation (37°C), the reaction was stopped with CCl_3COOH (17% w/v) (Table 1). Trolox was used as a standard.

Inhibition of linoleic acid lipid peroxidation[41]

Production of conjugated diene hydroperoxide by oxidation of linoleic acid sodium salt in an aqueous solution was monitored at 234 nm. AAPH was used as a free-radical initiator. Ten microliters of the 16 mM linoleic acid sodium salt solution was added to the UV cuvette containing 0.93 mL of 0.05 M phosphate buffer, pH 7.4 prethermostated at 37°C. The oxidation reaction was initiated at 37°C under air by the addition of 50 μL of 40 mM AAPH solution. Oxidation was carried out in the presence of aliquots (10 μL) of oxepincoumarins. In the assay without antioxidant, lipid oxidation was measured in the presence of the same level of DMSO. The rate of oxidation at 37°C was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxides.

Soybean LOX inhibition study in vitro

In vitro study was evaluated as reported previously[41]. The tested compounds dissolved in ethanol were incubated at room temperature with sodium linoleate (0.1 mM) and 0.2 mL of enzyme solution ($1/9 \times 10^{-4}$ w/v in saline). The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with the appropriate standard inhibitor caffeic acid (IC_{50} 600 mM) (Table 1).

Results and discussion

Synthesis

The treatment of 8-allyl-7-hydroxycoumarin (**3a**) [prepared[45] by the Claisen rearrangement under reflux in ethyleneglycol of 7-allyloxycoumarin (**2a**), received from umbelliferone (**1a**)] with allyl bromide and K_2CO_3 in dry acetone resulted in the 8-allyl-7-(allyloxy) coumarin (**5a**) in 80% yield (Scheme 1). The RCM of **5a** with the first generation Grubbs' catalyst **6** (4.3 mol%, added in two portions during 4 h) in dichloromethane solution under stirring at room temperature furnished the dihydrooxepin derivative **7a** in 90% yield.

The Claisen rearrangement of 7-allyloxy-4-methylcoumarin (**2b**)[50] in refluxing ethyleneglycol for 9 h, in analogous way to **2a**, gave 8-allyl-7-hydroxy-4-methylcoumarin (**3b**) (80% yield). Allylation of **3b** provided 8-allyl-7-(allyloxy)-4-methylcoumarin (**5b**)[27] (90%). From the RCM of **5b** with the catalyst **6** (0.9 mol%, added at once) in dichloromethane solution under stirring at room temperature, the dihydrooxepin derivative **7b** was obtained in 90% yield. The same reaction was performed in refluxing dichloromethane (10 mol% of catalyst), and the product **7b** was received in 33% yield along with a pyrano[7,8]coumarin derivative (24%)[27].

The 6-allyl-7-hydroxycoumarin (**4**) (isolated[45] also from the mixture of the Claisen rearrangement reaction of **2a**) allylated with allyl bromide and gave the 6-allyl-7-(allyloxy) coumarin (**8**) (91%). The RCM reaction of derivative **8** with the catalyst **6** (3.9 mol%) (added in four portions during 15 h) in dichloromethane solution at room temperature resulted in the dihydrooxepin compound **9** in 97% yield.

The allylation of 5-allyl-6-hydroxy-4-methylcoumarin (**12**)[47] (prepared in 78% yield by the heating under reflux of allyloxy derivative **11** in ethyleneglycol) provided the 5-allyl-6-allyloxy-4-methylcoumarin (**13**) (83%). The RCM reaction of 5-allyl-6-(allyloxy)coumarin derivative **13** with the catalyst **6** (2.2 mol%, added in four portions during 15 h) led to the dihydrooxepin derivative **14** in 98% yield (Scheme 2).

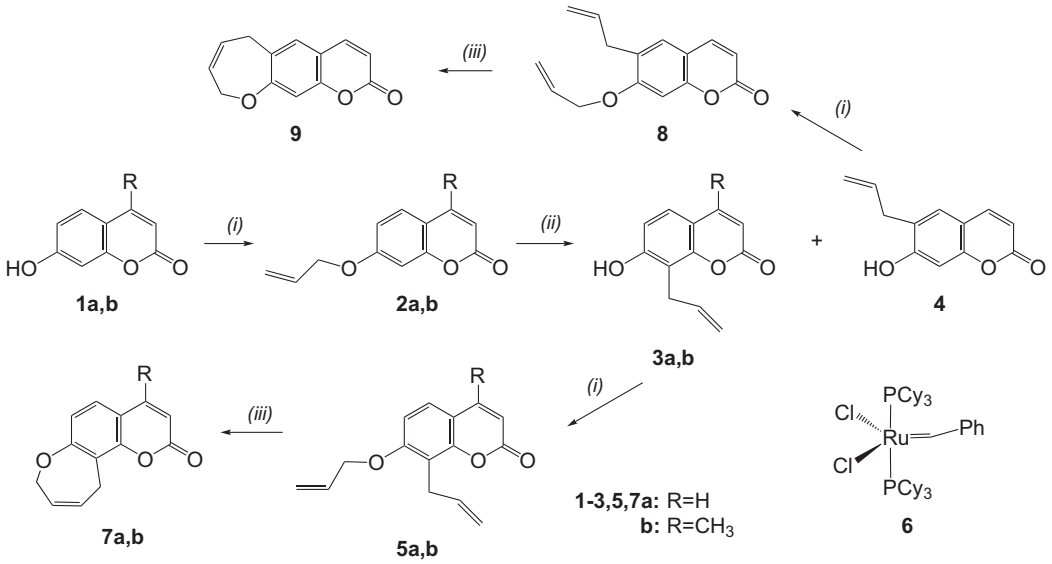
The Claisen rearrangement of 8-allyloxycoumarin (**16**)[48] in refluxing ethyleneglycol resulted in 7-allyl-8-hydroxycoumarin (**17**) (89%)[48]. The later allylated with allyl bromide and gave 7-allyl-8-(allyloxy)coumarin (**18**) (82%), which by RCM reaction with the catalyst **6** (6.9 mol%, added in three portions over 12 h) furnished the dihydrooxepin derivative **19** in 83% yield (Scheme 3).

In all the above cases, the RCM reaction product received as a sole product in excellent yield. The loading of the catalyst usually is more than one portion.

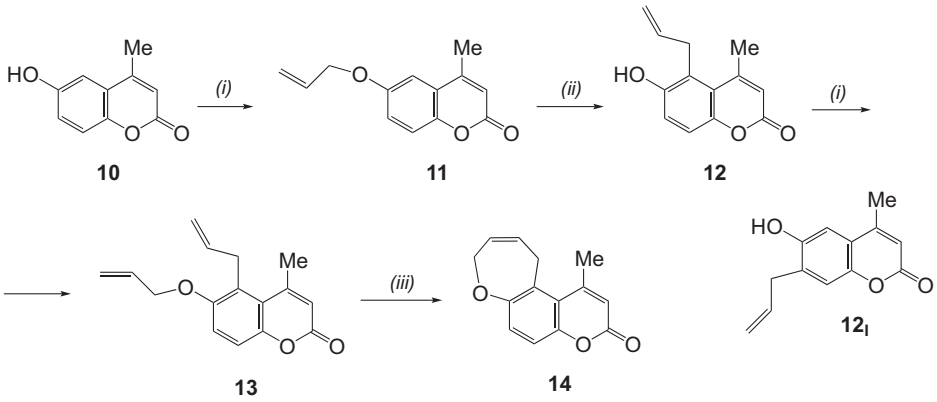
Biological studies

Herein the antioxidant activity was evaluated in several *in vitro* tests. In view of the differences among the test systems available, the results of a single assay can give only a suggestion on the protective potential of tested compounds. Thus, we have used three different types of assays to measure *in vitro* antioxidant activity of fused oxepino coumarins: (a) interaction with the stable free-radical DPPH, (b) competition with DMSO for hydroxyl radicals, and (c) interaction with the water soluble azo compound AAPH. All the assays require a spectrophotometric measurement and a certain reaction time to obtain reproducible results[51].

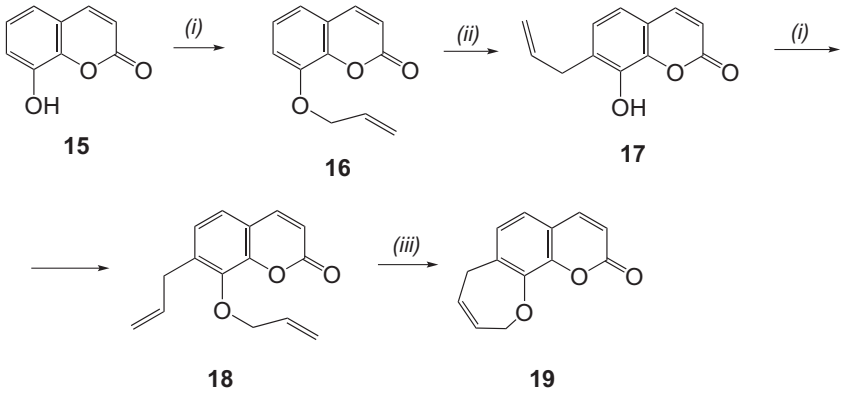
The DPPH test is very useful in the micromolar range demanding minutes to hours for both lipophilic and



Scheme 1. Reagents and conditions: (i) allyl bromide, K_2CO_3, acetone (dry), reflux, 2 h; (ii) ethyleneglycol, reflux, 9 h; and (iii) **6**, DCM (dry), room temperature, 24 h.



Scheme 2. Reagents and conditions: (i) allyl bromide, K_2CO_3, acetone (dry), reflux, 2 h; (ii) ethyleneglycol, reflux, 9 h; and (iii) **6**, DCM (dry), room temperature, 24 h.



Scheme 3. Reagents and conditions: (i) allyl bromide, K_2CO_3, acetone (dry), reflux, 2 h; (ii) ethyleneglycol, reflux, 9 h; and (iii) **6**, DCM (dry), room temperature, 24 h.

hydrophilic samples and indicates their reducing ability in an iron-free system. The interaction of the examined compounds with the stable free-radical DPPH was studied by the use of the stable 1,1-diphenyl-2-picrylhydrazyl radical DPPH[41] at 0.1 mM after 20 and 60 min (Table 1). The results showed that this interaction was very low, if any, compared with the reference compound NDGA. Small changes were observed with the time and only for compounds **7a** and **7b,** the rest remain unchanged (Table 1). Compound **14** does not present any interaction under the reported conditions.

The low interaction values compared with NDGA should be mainly attributed to the absence of easily oxidized functionalities like the ones present in NDGA (two catechol subunits). Lipophilicity is not well correlated with the results. There is no evidence for any structural characteristic of the tested compounds that is correlated with the antioxidant activity. The presence of the coumarin nucleus is implicated by itself in the reducing procedure.

It has been claimed that hydroxyl radical scavengers could serve as protectors, thus decreasing prostaglandin synthesis. During the inflammatory process, phagocytes generate the superoxide anion radical at the inflamed site and this is connected to other oxidizing species such as ·OH that are among the most reactive oxygen species and are considered to be responsible for some of the tissue damage occurring during inflammation[52]. The competition of compounds with DMSO for ·OH radicals[49], generated by the Fe^{3+}/ascorbic acid system, expressed as the inhibition of formaldehyde production was used for the evaluation of their hydroxyl radical scavenging activity. From the tested derivatives, only compounds **7a** and **7b** highly compete with DMSO (33 mM) at 0.1 mM in comparison with trolox (Table 1). Lower lipophilicity is well correlated with the results [clog *P* **7a** (2.56) < clog *P* **7b** (3.06); Table 1]. Antioxidants of hydrophilic or lipophilic character are both needed to act as radical scavengers in the aqueous phase or as chain-breaking antioxidants in biological membranes.

AAPH-induced linoleic acid oxidation has been developed as a quick and a reliable method for measuring the antioxidant activity and provides a measure of how efficiently antioxidants protect against lipid oxidation *in vitro*. Oxidation of exogenous linoleic acid by a thermal free radical producer (AAPH) is followed by UV spectrophotometry in a highly diluted sample[41]. Compounds **9**, **14**, and **19** effectively inhibit AAPH-induced lipid peroxidation, showing higher activity than the reference compound trolox (**14** and **19**, Table 1). Higher lipophilicity value (**14** > **9**) is correlated with higher lipid peroxidation inhibition (87% > 65%; Table 1). It also seems that angular analogues (**14** and **19**) are more potent than the linear **9** (Table 1). The standard inhibitor trolox obviously exerts its inhibitory effect on lipid peroxidation mainly through the ability of its 6-hydroxy-5,7,8-trimethylchromane moiety to break the radical chain. Although no phenol moieties are present in oxepinocoumarins **9**, **14**, and **19**, they could break the radical chain through the initial abstraction of hydrogen from the methyl group, e.g., compound **14,** or through other mechanisms. The new radicals thus created could be efficiently stabilized through resonance.

LOXs play a role in membrane lipid peroxidation by forming hydroperoxides in the lipid bilayer[53]. Inhibitors of LOX have attracted attention initially as potential agents for the treatment of inflammatory diseases, e.g. cancer and atheromatosis[54,55]. Our compounds were further evaluated for inhibition of soybean LOX by the UV absorbance-based enzyme assay[41]. Compound **7b** (IC_{50} 180 μM) is the most active within the set compared with the reference compound caffeic acid, whereas compounds **9**, **14,** and **19** do not present any inhibition. The majority of the LOX inhibitors act as: (a) antioxidants or free radical scavengers[56], (b) inhibitors to reduce Fe^{3+} at the active site to the catalytically inactive Fe^{2+} (LOXs contain a "non-heme" iron per molecule in the enzyme active site, and (c) excellent ligands for Fe^{3+}. On comparing our results, it seems that there is no correlation between their antioxidant ability and their LOX inhibitory activity (compounds **9**, **14,** and **19**). This is in accordance with the finding of Curini et al.[57] who have studied the antioxidant and LOX inhibitory activity of five natural prenyloxycarboxylic acids and showed that the most efficient LOX inhibitor (boropinic acid) is not the most active DPPH radical scavenger. Lipophilicity is referred[58] as an important physicochemical property for LOX inhibitors, and the above tested derivatives **7a** and **7b** seem to follow this concept (clog *P* **7b** > clog P **7a**, IC_{50} **7b** > IC_{50} **7a**).

Conclusion

In this study, fused dihydrooxepinocoumarins are prepared in excellent yields as a sole product of the RCM reactions. The loading of the catalyst is more than one portion. The newly synthesized compounds present interesting biological activities. Our study indicates that LOX or lipid peroxidation inhibitory activity is not always accompanied by DPPH radical scavenging activity. Thus, although compounds such as **9**, **14**, and **19** inhibit lipid peroxidation potently they present low, if any, DPPH and hydroxyl radical scavenging activity. However, a better correlation exists between LOX and hydroxyl radical scavenging activity for compounds **7a** and **7b**.

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Declaration of interest

The authors report no conflict of interest.

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