European Journal of Medicinal Chemistry 46 (2011) 5894–5901

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological evaluation of (2,5-dihydro-1*H*-pyrrol-1-yl)-2*H*-chromen-2-ones as free radical scavengers☆

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ARTICLE INFO

Article history:
Received 22 February 2011
Received in revised form
26 September 2011
Accepted 29 September 2011
Available online 6 October 2011

Keywords:
Ring Closing Metathesis
Grubbs' catalysts
Allylation
(2,5-Dihydro-1*H*-pyrrol-1-yl)coumarins
(1*H*-Pyrrol-1-yl)coumarins
Free radicals scavengers

ABSTRACT

The allylation of aminocoumarins in the presence of excess of anhydrous K_2CO_3 and allyl bromide to diallylaminocoumarins is described. The Ring Closing Metathesis reaction of the later with the Grubbs' 1rst generation catalyst under reflux or MW irradiation has resulted mainly to (2,5-dihydro-1*H*-pyrrol-1-yl)coumarins and (1*H*-pyrrol-1-yl)coumarins. The new 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 LO and (v) to scavenge hydroxyl radicals. Most of them were found to be potent lipid peroxidation inhibitors in vitro. The majority of the compounds showed significant hydroxyl radical scavenging activity. Compounds **11a** and **12c** presenting higher LO inhibitory activity as well as compound **17** were found to present a promising antioxidant and LO inhibitory profile.

1. Introduction

Reactive oxygen species (ROS) play an essential role in the control of cell functions. Many components of the vascular system, such as leukocytes, monocytes and endothelial cells are able to release ROS upon the appropriate stimulation. In vivo molecular oxygen is easily converted to reactive free radicals, which contain the superoxide anion ($O_2^{-\bullet}$), hydroxyl radical (HO•) and they are highly reactive substances that react with lipids, proteins and DNA, provoking irreversible changes of their biomolecular structure [1]. Tissues with high oxygen consumption rate are more easily susceptible to oxidative damage, due to the presence of excitatory amino acids, elevated iron stores, cell membranes rich in polyunsaturated fatty acids and low levels of the natural antioxidant glutathione in neurons [2]. Furthermore, blood brain barrier reduces the permeability and the protective efficacy of most antioxidants [3]. Therefore it is evident that the treatment of various diseases could benefit from the use of drugs that present free radical scavenging activity. This has already been proven for a number of commercially available Non Steroidal Anti-Inflammatory Drugs (NSAIDs) which simultaneously possess radical scavenging activity [4].

Coumarin derivatives are an interesting class of heterocyclic systems, since coumarin ring consist an essential core moiety for a variety of natural and synthetic biologically active compounds [5–8]. The biological activities include anticoagulation, antibiotic, antifungal, antipsoriasis, antitumor, anti-HIV, anti-inflammatory, etc. Functionalized pyrrolines are an important class of bioactive compounds with diverse biological activities [9–12]. A variety of synthetic strategies has been developed for the synthesis of those compounds [10–20]. Two of them apply the Ring Closing Metathesis (RCM) [10,13,18], a powerful synthetic method [21] for the construction of different ring systems. Moreover, since the combination of two pharmacophores on the same scaffold is a well-established approach to the synthesis of more potent drugs [22–27], we decided to incorporate the Δ^3-pyrroline ring moiety in the coumarin derivatives and to examine the influence of this modification on their activity as free radical scavengers.

2. Chemistry

The reactions studied and the title new compounds received are depicted in Schemes 1–3. 6-Aminocoumarin (**1a**) [prepared [28]

☆ Preliminary communication presented at 1st Hellenic Symposium Organic Synthesis, November 4–6, 2004, Athens, Greece, Book of Abstracts, p. 84.

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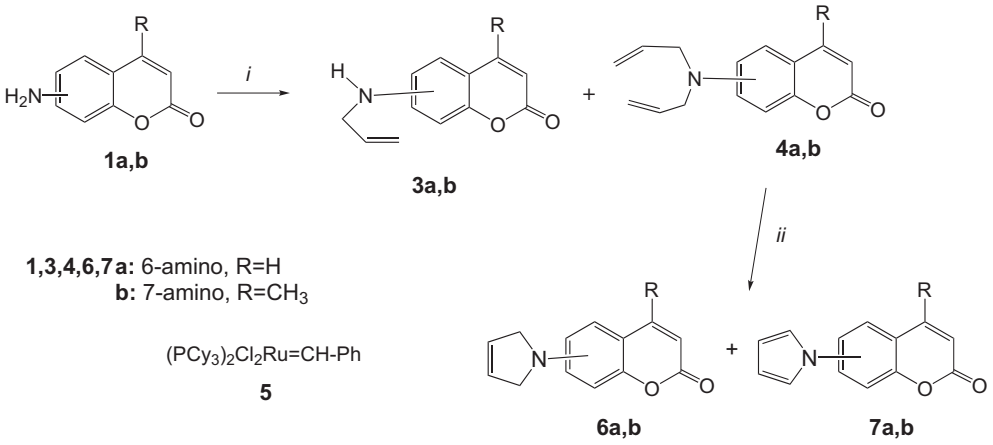
doi:10.1016/j.ejmech.2011.09.053

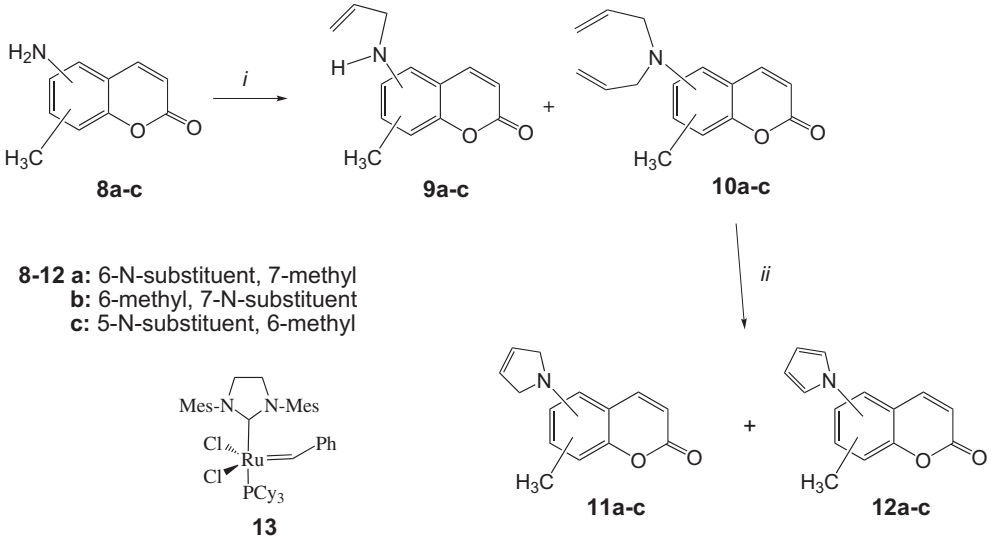


Scheme 1. Reagents and conditions: (i) Allyl bromide (**2**), K_2CO_3, acetone, reflux, 5 days. (ii) Grubbs' catalyst, first generation (**5**), DCM, reflux, 3 days (r.t., 5 days for **4b**) or MW, 100 °C, 1 h.

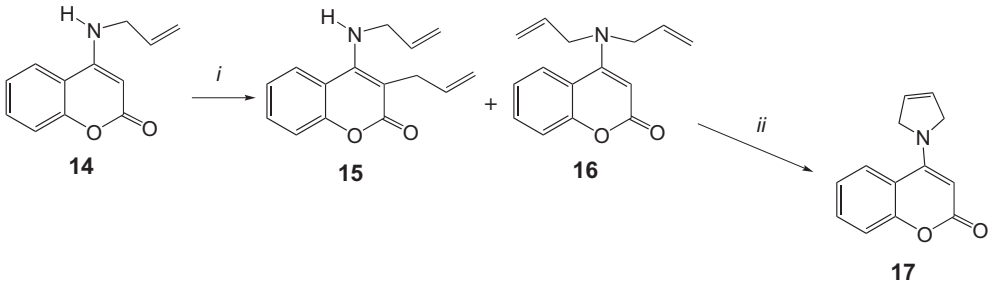
from coumarin quantitavely by nitration with HNO_3 and H_2SO_4 through 6-nitrocoumarin followed by reduction (in 78% yield) of the later with Et_3N/HCO_2H in the presence of 10% Pd-C] allylated by allyl bromide (**2**) (2 equiv.) in the presence of K_2CO_3 (4 equiv.) in refluxing dry acetone for 5 days to give 6-allylaminocoumarin (**3a**) (18%) and 6-diallylaminocoumarin (**4a**) (64%) (Scheme 1). Analogous allylation of the commercially available 7-amino-4-methylcoumarin (**1b**) resulted to 7-allylamino-4-methylcoumarin (**3b**) (41%) and 7-diallylamino-4-methylcoumarin (**4b**) (56%).

The Ring Closing Metathesis (RCM) of compound **4a** in the presence of the Grubbs' catalyst first generation **5** (11 mol %, added in 7 portions every 9–10 h) in refluxing dichloromethane (DCM) for 3 days led to the dihydropyrrolocoumarin derivative **6a** in 66% yield and the pyrrolocoumarin derivative **7a** (27%). When the same reaction with the catalyst **5** (6 mol %) is performed at r.t. for 3 days the yields for compounds **6a** and **7a** are 12% and 2% respectively, while 78% of the starting material **4a** recovered. By application of MW irradiation with the same catalyst **5** (3.5 mol %, added in 2 portions after 30 min) in DCM at 100 °C for 1 h derivative **6a** is isolated in 81% yield accompanied from **7a** (6%) and unreacted compound **4a** (9%). The analogous RCM reaction of coumarin derivative **4b** performed with the catalyst **5** (17 mol %) at r.t. for 5 days to give the dihydropyrrolocoumarin **6b** (70%) and the pyrrolocoumarin **7b** (6%), while 9% of the starting material remained unchanged. For the elucidation of compounds **6a** and **7a**, in the ^{1}H-NMR spectra there are two singlets at 4.13 (4H) and 5.98 (2H) for 3-pyrroline **6a** and two triplets at 6.38 (2H, $J = 2.1$ Hz) and 7.06 (2H, $J = 2.1$ Hz) for pyrrole **7a**. The formation of pyrrole derivative **7a** could be explained by the oxidation of dihydropyrrolocoumarin **6a** under the reaction conditions, although the reactions were performed under Argon atmosphere.

The allylation of 6-amino-7-methylcoumarin (**8a**) [28] resulted to 6-allylamino-7-methylcoumarin (**9a**) (32%) and 6-diallylamino-7-methylcoumarin (**10a**) (52%) (Scheme 2). The products **9a** and **10a** received in 54% and 25% yield respectively, when the reaction repeated with equimolar amount of anhydrous K_2CO_3, The RCM reaction of the later with the first generation's Grubbs catalyst [11,12] **5** (8 moles %) in DCM solution under argon atmosphere at room temperature for 4 days gave the 6-(2,5-dihydro-1*H*-pyrrol-1-yl)-7-methyl-2*H*-chromen-2-one (**11a**) in 49% yield and 7-methyl-6-(1*H*-pyrrol-1-yl)-2*H*-chromen-2-one (**12a**) (45%). The same reaction under reflux for 2 days with the catalyst **5** (7% mol %) led to



Scheme 2. Reagents and conditions: (i) Allyl bromide (**2**), K_2CO_3, acetone, reflux, 5 days. (ii) Grubbs' catalyst, first generation (**5**), DCM, r.t., 3 days (reflux, 2 days for **10a** or MW, 100 °C, 1 h for **10a,c**).



Scheme 3. Reagents and conditions: (i) Allyl bromide (**2**), K_2CO_3, acetone, reflux, 12 days. (ii) Grubbs' catalyst, first generation (**5**), DCM, r.t., 3 days.

compounds **11a** (83%) and **12a** (7%), while under MW irradiation (catalyst **5** 3.5 mol % at 100 °C for 1 h) resulted to derivatives **11a** (75%) and **12a** (9%) (12% of the starting material recovered).

The 6-methyl-7-nitrocoumarin (starting material for the synthesis of aminocoumarin **8b**) is received by the nitration of 6-methylcoumarin with a mixture of HNO_3 and H_2SO_4 in 22% yield after the separation by column chromatography from the main nitration product 6-methyl-5-nitrocoumarin [28] (49%). This nitrocoumarin by reduction resulted to aminocoumarin **8b** (56%), which by allylation gave 7-allylamino-6-methylcoumarin (**9b**) (48%) and 7-diallylamino-6-methylcoumarin (**10b**) (51%) (Scheme 2). The RCM reaction of the later at r.t. for 4 days gave the 7-(2,5-dihydro-1*H*-pyrrol-1-yl)-6-methyl-2*H*-chromen-2-one (**11b**) in 64% yield and 6-methyl-7-(1*H*-pyrrol-1-yl)-2*H*-chromen-2-one (**12b**) (21%) (14% of the starting material **10b** recovered) after the separation by PTLC.

The 5-allylamino-6-methylcoumarin (**9c**) (80%) and 5-diallylamino-6-methylcoumarin (**10c**) (7%) (Scheme 2) are synthesized by the allylation of aminocoumarin **8c** [29] with equimolar amounts of anhydrous K_2CO_3 and allyl bromide (**2**). The RCM reaction of diallylcoumarin **10c** at r.t. for 4 days in the presence of the catalyst **5** (12 mol %) resulted to 5-(2,5-dihydro-1*H*-pyrrol-1-yl)-6-methyl-2*H*-chromen-2-one (**11c**) (56%), 6-methyl-5-(1*H*-pyrrol-1-yl)-2*H*-chromen-2-one (**12c**) (21%) and unreacted starting material **10c** (19%) after the separation of the reaction mixture by PTLC. The same reaction for 15 days gave **11c** (37%), **12c** (44%) and **10c** (18%). This reaction was performed under MW irradiation (catalyst **5** 3.5 mol % at 100 °C for 1 h) and led to pyrrolino derivative **11c** (65%), pyrrolo derivative **12c** (10%) and unreacted compound **10c** (19%). When we use the second generation Grubbs' catalyst **13** (12 mol %) under stirring at r.t. for 2 days we received again the compounds **11c** (46%), **12c** (20%) and the deallylation products **9c** (20%) and **8c** (13%). The tertiary amine's deallylation has been referred earlier in the literature by using the catalyst **5** as a method of choice [30].

The allylation reaction of 4-allylaminocoumarin (**14**) [29] in the presence of K_2CO_3 with excess of allyl bromide (**2**) under reflux in acetone for 12 days resulted to 3-allyl-4-allylaminocoumarin (**15**) (31%) and 4-diallylaminocoumarin (**16**) (54%), while 14% of the starting material was recovered. Compound **15** is formed most probably after Claisen rearrangement of 4-allylaminocoumarin (**14**) followed by allylation of the intermediate 4-aminocoumarin derivative. The RCM reaction of coumarin **16** with the catalyst **5** (9 mol %) at r.t. for 3 days led to 4-(2,5-dihydro-1*H*-pyrrol-1-yl)-2*H*-chromen-2-one (**17**) (75%), while 23% of the starting compound was remained unchanged.

From the above reactions we can firstly conclude that the allylation with excess of anhydrous K_2CO_3 (4 equivalents) and allyl bromide (2 equivalents) leads mainly to diallylaminocoumarins, while with equimolar amounts of K_2CO_3 and allyl bromide the monoallylaminocoumarins are prepared. The 1rst generation Grubbs' catalyst is efficient in the RCM reactions for the preparation of dihydropyrrolocoumarins and pyrrolocoumarins in moderate to very good yields at r.t. or under reflux. By the application of MW irradiation the amounts of pyrrolocoumarins are diminished. The pyrrolocoumarins (oxidation products) are favored, by prolonged reaction time at r.t.

3. Biology

Taking the multifactorial character of oxidative stress into account, we decided to evaluate the in vitro antioxidant activity of the synthesized molecules using four different antioxidant assays. Coumarins are referred to affect the formation and scavenging of ROS, exhibiting tissues protective antioxidant properties, which may include numerous different molecular mechanisms and are probably related to their structural analogy with flavonoids and benzophenones [31]. Thus, they can bind Fe (III) and through this reaction to inhibit hydroxyl radical and hydrogen peroxide formation produced by Fenton's reactions.

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 [32], 1,1-dipheny l-1-picrylhydrazyl (DPPH) assay [33]. DPPH assay is one of the best-known, frequently employed and accurate methods. Reduction of DPPH stable free radical (50 μM) by the examined compounds at 50 and 100 μM [34], was studied after 20 and 60 min (Table 1). The results indicate their radical scavenging ability in an iron-free system. No result was observed at 50 μM. The results for the 100 μM are time independent with the exception of compounds **6a**, **6b** and **17**. We tested the new derivatives 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 compound **6a** (Table 1). No significant differences are observed among (2,5-dihydro-1*H*-pyrrol-1-yl)-2*H*-chromen-2-ones. Free coumarin as well as 7-methylcoumarin presented very low reducing abilities. The results are independent of the position of methyl- and/or of the Δ^3-pyrroline ring.

Hydroxyl radicals are among the most reactive oxygen species and are considered to be responsible for some of the tissue damage occurring in inflammation. It has been claimed that hydroxyl radical scavengers could serve as protectors. The competition of compounds with dimethyl sulfoxide (DMSO) for $^{\bullet}OH$ radicals generated by the Fe^{3+}/ascorbic acid system, expressed as the inhibition of formaldehyde production [35], was used for the evaluation of their hydroxyl radical scavenging activity. All the tested derivatives highly compete with DMSO (33 mM) at 100 μM in comparison to trolox (Table 1). Perusal of the % results shows that derivatives **6a**, **6b**, **11a**, **12b**, **12c** and **17** are more potent in comparison to trolox used as a standard. Compound **11b** presents lower activity (41%). The hydroxyl radical scavenging activity is similar for **6a** and **17**, regardless the position of Δ^3-pyrroline ring (4 or 7). No result is given by compound **11c,** whereas the activity for **7b** is very low. Lipophilicity, as theoretically

Table 1
Interaction % with 1,1-diphenyl-2-picrylhydrazyl (DPPH) at 0.1 mM; Competition % with DMSO for hydroxyl radical (HO· %) at 100 μM; Inhibition of lipid peroxidation at 100 μM (LP %); In vitro inhibition of soybean lipoxygenase (LO) % at 100 μM.

No	DPPH % 20 min at 100 μM	DPPH % 60 min at 100 μM	HO· (%) at 100 μM	LP % inh. at 100 μM	LO % inh. at 100 μM	Clog P[36]
6a	49	71	90	86	No	1.91
6b	5	20	85	87	28	2.41
7a	18	19	No	1	No	2.73
7b	14	14	9	46	12	3.23
11a	9	10	77	91	60	2.41
11b	5	7	41	24	52.5	2.41
11c	5	5	No	57	No	2.41
12a	14	14	No	No	No	3.23
12b	13	13	88	No	No	3.23
12c	7	7	91	71	55.5	3.23
17	8	27	83	100	43	2.08
Coumarin	5	21	78	Nt	15	1.41
7-CH_3-coumarin	2	2	44	No	89	1.91
NDGA	81	83			84	
Trolox			73	63		

NDGA, nordihydroguaeretic acid; 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.

calculated values, is not well correlated with the results [36]. Thus, the presence of a methyl group in **6b** compared to **6a** (calculated log P value **6a** = 1.91, **6b** = 2.41) does not elevate the activity. The Δ^3-pyrroline derivatives **6a**, **6b** and **11a** are more potent than the corresponding pyrrolyl derivatives **7a**, **7b** and **12a**. This concept is not followed by Δ^3-pyrroline derivative **11b** (41%), which presents lower competition than the corresponding pyrrole derivative **12b** (88%), as well as Δ^3-pyrroline derivative **11c** (no) compaired to pyrrole derivative **12c** (91%). Control experiments showed that free coumarin presents significant scavenging behavior followed by 7-methylcoumarin. The later with CLOG P value similar to **6a** presents lower activity. Thus, the specific structural characteristics of the new synthesized derivatives are responsible for the increase of the % competition with DMSO for hydroxyl radicals.

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 [31,34]. In our experiments compounds **17**, **11a**, **6a**, **6b** and **12c** showed excellent inhibition on lipid peroxidation (LPO) at 100 μM (Table 1) compared to trolox, used as a standard (73%), whereas compound **11c** and **7b** followed with lower inhibition (57% and 46% respectively) and **11b** was found to present much lower activity (24%). Compounds **7a**, **12a**, **12b** do not seem to influence lipid peroxidation under our experimental conditions. No differences were observed between the **6a** and **6b** analogues. Thus, the presence of a methyl group, as well as the increase in lipophilicity (2.41 from 1.91), does not seem to influence the LPO. On the contrary significant differences are observed among **11a**, **11b** and **11c**. Although all present similar lipophilicity, the differences in the substitution's position led to different responses. Comparing **6a** to **17**, it is better the Δ^3-pyrroline ring to be located at position 4 instead of 7. The small increase in lipophilicity (2.08) enhances the % inhibition. Comparing **11a** to **12a** as well as **11b** to **12b** it seems that the Δ^3-pyrroline derivatives present enhanced anti-LPO activity. Among the 5-, 6- or 7-Δ^3-pyrroline derivatives the 6-N-substituted is more potent followed by 7-N and 5-N-substituted. The 6-N (**12a**) and 7-N (**12b**) pyrrolyl substituted derivatives did not show any anti-LPO activity with the exception of **12c** (**12c** > **11c** 71% > 57%).

Non-enzymatic superoxide anion radicals were generated. The superoxide producing system was set up by mixing phenazine methosulfate (PMS), nicotinamide–adenine–dinucleotide NADH and air–oxygen. The production of superoxide was estimated by the nitroblue tetrazolium method [34]. The compounds did not present scavenging activity at 100 μM.

The synthesized derivatives were evaluated for inhibition of soybean lipoxygenase by the UV-absorbance-based enzyme assay [34] and the results are presented in Table 1. As far as these derivatives are concerned, their majority did not show significant LO inhibitory activity at 100 μM. Among them compounds, **11a**, **11b** and **12c** exhibit better LO inhibitory activity. Lipophilicity seems to be an important physicochemical parameter for the increase of LO inhibition of compound **11b** (2.41) compared to **6a** (1.91). It is interesting that 7-methylcoumarin inhibits LO, whereas **6a** does not. Both present similar lipophilicity (1.91) regardless the specific moiety of substitution at position 7. Comparing **11a** to **12a** and **11b** to **12b** the biological response of Δ^3-pyrroline derivatives (**11a,b**) is better than the corresponding of the pyrrole derivatives (**12a,b**), with the exception of **12c** (55.5%), whereas **11c** does not present any result.

Our study indicates that LPO and LO inhibitory activity is not accompanied by DPPH radical scavenging activity. In our case the hydroxyl radical scavenging activity is followed by efficient LPO inhibitory activity.

4. Conclusion

In summary, the broad spectrum of the observed antioxidant activity of the majority of the examined (2,5-dihydro-1*H*-pyrrol-1-yl)-2*H*-chromen-2-ones 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 hydroxyl radical scavengers and inhibit in vitro lipid peroxidation. Antioxidant power might be important in the inhibition of lipid peroxidation. Compounds **11a** and **12c** presenting higher LO inhibitory activity among the tested derivatives, as well as compound **17** were found to present a promising antioxidant and LO inhibitory profile. Attempts have been made to develop some preliminary structure activity conclusions based on the compounds synthesized and tested.

5. Experimental protocol

5.1. Chemistry

For the experiments under MW irradiation a Biotage (Initiator 2.0) scientific microwave oven has been used. Melting points were

determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were obtained with a Perkin–Elmer 1310 spectrophotometer as Nujol mulls. NMR spectra were recorded on a Bruker AM 300 (300 MHz and 75 MHz for ^{1}H and ^{13}C, respectively) 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 Shimadzu LCMS-2010 EV system under Electrospray Ionization (ESI) conditions. Microanalyses were performed on a Perkin–Elmer 2400-II Element analyzer (Table 2). Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values. Silica gel No. 60, Merck A.G. was used for column chromatography.

*5.1.1. General procedure for the allylation of aminocoumarins. Synthesis of 6-(diallylamino)-2H-chromene-2-one (**4a**) and 6-(allylamino)-2H-chromene-2-one (**3a**)*

Anhydrous K_2CO_3 (1.846 g, 13.3 mmol) and allyl bromide (**2**) (0.6 ml, 0.858 g, 7 mmol) were added to the solution of compound **1a** [28] (0.536 g, 3.33 mmol) in dry acetone (70 ml) and the mixture was refluxed for 5 days. The mixture was filtered and the filtrate was evaporated and separated by column chromatography (silica gel no. 60, DCM) to give **4a** (0.513 g, 64% yield) and **3a** (0.12 g, 18% yield).

*5.1.1.1. 6-(Diallylamino)-2H-chromene-2-one (**4a**).* Yellow crystals, m.p. 53–55 °C (ethyl acetate–hexane); IR (Nujol): 3060, 1710, 1555 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 3.94 (d, 4H, $J = 2.6$ Hz), 5.17 (d, 2H, $J = 11.1$ Hz), 5.18 (d, 2H, $J = 16.3$ Hz), 5.70–5.92 (m, 2H), 6.35 (d, 1H, $J = 9.5$ Hz), 6.64 (d, 1H, $J = 2.6$ Hz), 6.90 (dd, 1H, $J_1 = 2.6$ Hz, $J_2 = 9.4$ Hz), 7.17 (d, 1H, $J = 9.4$ Hz), 7.61 (d, 1H, $J = 9.5$ Hz); ^{13}C-NMR ($CDCl_3$) δ 53.2, 109.1, 116.4, 116.5, 117.1, 117.8, 119.2, 132.3, 133.2, 142.5, 143.8, 161.4; MS (EI) *m/z*: 241 ($M^{+\bullet}$, 32%), 215 (100), 201 (14), 173 (71), 145 (13), 117 (51), 89 (58); Anal. $C_{15}H_{15}NO_2$ (C, H, N).

*5.1.1.2. 6-(Allylamino)-2H-chromene-2-one (**3a**).* Yellow crystals, m.p. 88–90 °C (DCM-hexane); IR (Nujol): 3335, 3050, 1725, 1550 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 3.80 (d, 2H, $J = 5.0$ Hz), 4.50 (brs, 1H), 5.19 (d, 1H, $J = 9.5$ Hz), 5.30 (d, 1H, $J = 17.2$ Hz), 5.90–5.91 (m, 1H), 6.37 (d, 1H, $J = 9.8$ Hz), 6.59 (d, 1H, $J = 2.8$ Hz), 6.83 (dd, 1H, $J_1 = 2.8$ Hz, $J_2 = 7.3$ Hz), 7.16 (d, 1H, $J = 7.3$ Hz), 7.60 (d, 1H, $J = 9.8$ Hz); ^{13}C-NMR ($CDCl_3$) δ 46.8, 108.6, 116.7, 116.8, 117.5, 118.3, 119.4, 134.7, 143.4, 144.8, 146.9, 161.3; MS (EI) *m/z*: 201 ($M^{+\bullet}$, 100%), 175 (61), 161 (67), 144 (16), 133 (49), 118 (27), 104 (34), 91 (25); Anal. $C_{12}H_{11}NO_2$ (C, H, N).

Table 2
Elemental analyses.

		Elemental analyses	
		Calculated	Found
3a	($C_{12}H_{11}NO_2$)	C% 71.62, H% 5.51, N% 6.96	C% 71.58, H% 5.67, N% 6.59
4a	($C_{15}H_{15}NO_2$)	C% 74.67, H% 6.27, N% 5.81	C% 74.46, H% 6.41, N% 5.65
4b	($C_{16}H_{17}NO_2$)	C% 75.26, H% 6.79, N% 5.49	C% 75.54, H% 6.63, N% 5.32
6a	($C_{13}H_{11}NO_2$)	C% 73.21, H% 5.20, N% 6.57	C% 73.07, H% 5.32, N% 6.50
6b	($C_{14}H_{13}NO_2$)	C% 73.98, H% 5.77, N% 6.17	C% 73.69, H% 5.49, N% 6.36
7a	($C_{13}H_9NO_2$)	C% 73.91, H% 4.30, N% 6.63	C% 74.02, H% 4.51, N% 6.49
7b	($C_{14}H_{11}NO_2$)	C% 74.65, H% 4.92, N% 6.22	C% 74.52, H% 4.75, N% 6.38
10a	($C_{16}H_{17}NO_2$)	C% 75.26, H% 6.72, N% 5.49	C% 74.94, H% 6.65, N% 5.48
10b	($C_{15}H_{15}NO_2$)	C% 74.67, H% 6.27, N% 5.81	C% 74.48, H% 6.43, N% 5.62
10c	($C_{16}H_{17}NO_2$)	C% 75.26, H% 6.72, N% 5.49	C% 75.20, H% 6.60, N% 5.29
11a	($C_{14}H_{13}NO_2$)	C% 73.98, H% 5.77, N% 6.17	C% 74.05, H% 5.51, N% 6.06
11b	($C_{14}H_{13}NO_2$)	C% 73.98, H% 5.77, N% 6.17	C% 73.76, H% 5.89, N% 6.32
11c	($C_{14}H_{13}NO_2$)	C% 73.98, H% 5.77, N% 6.17	C% 74.16, H% 5.58, N% 6.02
12a	($C_{14}H_{11}NO_2$)	C% 74.65, H% 4.92, N% 6.22	C% 74.43, H% 4.86, N% 6.13
12b	($C_{14}H_{11}NO_2$)	C% 74.65, H% 4.92, N% 6.22	C% 74.76, H% 4.69, N% 6.45
12c	($C_{14}H_{11}NO_2$)	C% 74.65, H% 4.92, N% 6.22	C% 74.71, H% 4.71, N% 6.29
16	($C_{15}H_{15}NO_2$)	C% 74.67, H% 6.27, N% 5.81	C% 74.62, H% 6.50, N% 5.89
17	($C_{13}H_{11}NO_2$)	C% 73.21, H% 5.20, N% 6.57	C% 73.02, H% 5.34, N% 6.34

*5.1.2. Synthesis of 7-(diallylamino)-4-methyl-2H-chromene-2-one (**4b**) and 7-(allylamino)-4-methyl-2H-chromene-2-one (**3b**)*

From the reaction of 7-amino-4-methylcoumarin (**1b**) with allyl bromide (**2**) the 7-allylamino-4-methylcoumarin (**3b**) (41%) and 7-diallylamino-4-methylcoumarin (**4b**) (56%) were received.

*5.1.2.1. 7-(Diallylamino)-4-methyl-2H-chromene-2-one (**4b**).* Yellow crystals, m.p. 50–52 °C (DCM-hexane), IR (Nujol): 3080, 1725, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.34 (s, 3H), 3.98 (d, 4H, $J = 4.1$ Hz), 5.17 (d, 2H, $J = 15.3$ Hz), 5.21 (d, 2H, $J = 8.9$ Hz), 5.78–5.91 (m, 2H), 5.97 (s, 1H), 6.56 (s, 1H), 6.61 (d, 1H, $J = 8.9$ Hz), 7.38 (d, 1H, $J = 8.9$ Hz); ^{13}C-NMR ($CDCl_3$) δ 18.4, 53.0, 109.1, 109.5, 119.9, 125.5, 126.5, 128.4, 132.4, 138.1, 144.0, 150.3, 161.4; MS (EI): 255 ($M^{+\bullet}$, 100%), 254 (63), 227 (68), 213 (34), 186 (86), 184 (41), 159 (64), 158 (47),103 (55); Anal. $C_{16}H_{17}NO_2$ (C, H, N).

*5.1.2.2. 7-(Allylamino)-4-methyl-2H-chromene-2-one (**3b**), m.p. 107–109 °C (DCM/hexane) (lit.[37] m.p. 110 °C).*

(d) *6-(Diallylamino)-7-methyl-2H-chromene-2-one* (**10a**) (52%) (the yield is only 25% with equimolar amount of K_2CO_3), m.p. 123–125 °C (DCM-hexane), IR (Nujol): 3045, 1730, 1570 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.40 (s, 3H), 3.57 (d, 4H, $J = 5.9$ Hz), 5.10 (d, 2H, $J = 9.8$ Hz), 5.15 (d, 2H, $J = 17.7$ Hz), 5.69–5.83 (m, 2H), 6.32 (d, 1H, $J = 9.8$ Hz), 7.02 (s, 1H), 7.15 (s, 1H), 7.60 (d, 1H, $J = 9.8$ Hz); ^{13}C-NMR ($CDCl_3$) δ 18.7, 55.9, 115.5, 116.7, 117.6, 118.7, 120.3, 134.6, 140.1, 144.3, 146.6, 150.2, 161.2; MS (EI): 255 ($M^{+\bullet}$, 100%), 254 (65), 228 (39), 214 (49), 199 (35), 187 (76), 158 (38),130 (62); Anal. $C_{16}H_{17}NO_2$ (C, H, N).

(e) *7-(Diallylamino)-6-methyl-2H-chromene-2-one* (**10b**) (51%), m.p. 57–60 °C (DCM-hexane), IR (Nujol): 3040, 1725, 1600 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.26 (s, 3H), 3.60 (d, 4H, $J = 5.9$ Hz), 5.10 (d, 2H, $J = 9.9$ Hz), 5.14 (d, 2H, $J = 16.7$ Hz), 5.63–5.77 (m, 2H), 6.16 (d, 1H, $J = 9.8$ Hz), 6.81 (s, 1H), 7.15 (s, 1H), 7.51 (d, 1H, $J = 9.8$ Hz); ^{13}C-NMR ($CDCl_3$) δ 18.4, 54.9, 108.7, 113.6, 118.0, 118.2, 129.0, 129.6, 133.9, 143.2, 153.1, 153.7, 161.5; MS (EI): 255 ($M^{+\bullet}$, 100%), 254 (65), 228 (39), 214 (49), 199 (35), 187 (76), 158 (38),130 (62); Anal. $C_{15}H_{15}NO_2$ (C, H, N).

(f) *5-(Diallylamino)-6-methyl-2H-chromene-2-one* (**10c**) (51%), m.p. 74–76 °C (DCM-hexane), IR (Nujol): 3060, 1710, 1578 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.35 (s, 3H), 3.72 (d, 4H, $J = 5.9$ Hz), 5.08 (d, 2H, $J = 9.9$ Hz), 5.13 (d, 2H, $J = 17.7$ Hz), 5.68–5.90 (m, 2H), 6.37 (d, 1H, $J = 9.8$ Hz), 7.06 (d, 1H, $J = 8.9$ Hz), 7.29 (d, 1H, $J = 8.9$ Hz), 8.14 (d, 1H, $J = 9.8$ Hz); ^{13}C-NMR ($CDCl_3$) δ 19.0, 56.5, 113.8, 115.3, 117.5, 118.7, 132.9, 134.5, 135.1, 141.7, 146.4, 153.2, 161.0; MS (EI): 255 ($M^{+\bullet}$, 11%), 254 (10), 240 (33), 228 (21), 214 (76), 198 (81), 186 (85), 170 (95), 158 (69), 130 (100); Anal. $C_{16}H_{17}NO_2$ (C, H, N).

(g) *4-(Diallylamino)l-2H-chromene-2-one* (**16**) (from 1.3 equiv. of K_2CO_3, after refluxing for 12 days) (54%), m.p. 66–69 °C (DCM-hexane), IR (Nujol): 3020, 1725, 1583 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 3.97 (d, 4H, $J = 4.9$ Hz), 5.32–5.37 (m, 4H), 5.69 (s. 1H), 5.83–5.95(m, 2H), 7.23 (d, 1H, $J = 7.8$ Hz), 7.29–7.54 (m, 2H), 7.72 (d, 1H, $J = 8.6$ Hz); ^{13}C-NMR ($CDCl_3$) δ 53.9, 104.5, 106.7, 117.1, 118.1, 118.7, 123.2, 132.2, 133.9, 145.6, 150.6, 161.2; MS (EI): 241 ($M^{+\bullet}$, 100%), 240 (38), 213 (44), 200 (23), 172 (45), 115 (32), 91 (32); Anal. $C_{15}H_{15}NO_2$ (C, H, N).

(h) *General procedure. RCM reaction 6-(diallylamino)-2H-chromene-2-one* (**4a**).

(i) Under reflux. The catalyst **5** [38 mg (in seven parts after 9–10 h each), 0.046 mmol, 11 mol %] was added to

a solution of compound **4a** (0.104 g, 0.43 mmol) in 30 ml dry DCM after the removing of the air by a pump and filling with Argon (in three cycles) and the solution was refluxed under Argon atmosphere for 3 days. After the evaporation of the solvent the residue was separated by repeated (two times) PTLC [silica gel, hexane/ethyl acetate (2:1)] to give from the faster moving band the unreacted compound **4a** (4 mg, 4%) followed by the *6-(1H-pyrrol-1-yl)-2H-chromen-2-one* (**7a**) (25 mg, 27% yield), m.p. 128–130 °C (DCM-hexane), IR (Nujol): 3060, 1720, 1555 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 6.38 (t, 2H, $J = 2.2$ Hz), 6.49 (d, 1H, $J = 9.6$ Hz), 7.06 (t, 2H, $J = 2.2$ Hz), 7.40 (d, 1H, $J = 8.8$ Hz), 7.46 (d, 1H, $J = 2.6$ Hz), 7.57 (dd, 1H, $J_1 = 2.6$ Hz, $J_2 = 8.8$ Hz), 7.72 (d, 1H, $J = 9.6$ Hz); ^{13}C-NMR ($CDCl_3$) δ 107.0, 111.2, 118.2, 119.6, 121.7, 124.5, 134.7, 142.7, 150.1, 155.6, 161.1; MS (ESI): 212 [$M^{+\bullet}$ + H]; Anal. $C_{13}H_9NO_2$ (C, H, N).

(ii) Under MW irradiation. Dry CH_2Cl_2 (2 ml) was placed in a tube (5 ml) for the Biotage Initiator 2.0, MW oven, equipped with a Teflon-coated stirring bar; the compound **4a** (21 mg, 0.087 mmol) was added followed by the catalyst **5** (1.4 mg, 2 mol %), argon was flushed in the tube and the mixture was irradiated at 100 °C for 30 min (checked by TLC). Another portion of **5** (1.1 mg, 1.5 mol %, totally 2.5 mg, 3.5 mol %) was added and the solution was irradiated for further 30 min (totally 1 h). After cooling and evaporation of the solvent the residue was separated by column chromatography [silica gel No 60, hexane/EtOAc (3:2)] to give after the elution of unreacted starting material **4a** (1.9 mg, 9%) compounds **7a** (1.1 mg, 6% yield) and **6a** (15 mg, 81% yield).

(i) *6-(2,5-Dihydro-1H-pyrrol-1-yl)-2H-chromen-2-one* (**6a**) eluted next (61 mg, 66% yield), m.p. 149–151 °C (ethyl acetate–hexane), IR (Nujol): 3055, 1725, 1545 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 4.13 (s, 4H), 5.98 (s, 2H), 6.39 (d, 1H, $J = 8.9$ Hz), 6.46 (d, 1H, $J = 2.8$ Hz), 6.74 (dd, 1H, $J_1 = 2.8$ Hz, $J_2 = 8.9$ Hz), 7.23 (d, 1H, $J = 8.9$ Hz), 7.65 (d, 1H, $J = 8.9$ Hz); ^{13}C-NMR ($CDCl_3$) δ 54.7, 107.3, 111.1, 115.7, 116.9, 117.5, 126.3, 143.7, 150.9, 153.7, 160.6; MS (EI): 213 ($M^{+\bullet}$, 77%), 211 (90), 183 (100), 156 (70), 154 (83), 145 (79), 128 (90), 117 (90); Anal. $C_{13}H_{11}NO_2$ (C, H, N).

The same reaction with the catalyst **5** (6 mol %) at r.t. for 3 days yielded compounds **6a** (12%), **7a** (2%) and the unreacted **4a** (78%).

(j) *7-(2,5-Dihydro-1H-pyrrol-1-yl)-4-methyl–2H-chromen-2-one* (**6b**) (70% yield) (at r.t. for 5 days), m.p. 144–146 °C (DCM-hexane), IR (Nujol): 3050, 1722, 1585 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.35 (s, 3H), 4.17 (s, 4H), 5.97 (s, 1H), 5.98 (s, 2H), 6.38 (d, 1H, $J = 1.7$ Hz), 6.47 (dd, 1H, $J_1 = 1.7$ Hz, $J_2 = 8.5$ Hz), 7.42 (d, 1H, $J = 8.5$ Hz); ^{13}C-NMR ($CDCl_3$) δ 22.6, 53.8, 98.7, 103.5, 109.2, 113.2, 125.4, 125.9, 146.5, 155.1, 157.5, 161.3; MS (ESI): 228 [$M^{+\bullet}$ + H], 250 [$M^{+\bullet}$ + Na]; Anal. $C_{14}H_{13}NO_2$ (C, H, N).

(k) *6-(2,5-Dihydro-1H-pyrrol-1-yl)-7-methyl–2H-chromen-2-one* (**11a**) [83% yield, after refluxing for 2 days with the catalyst **5** (7 mol%) or 75% yield under MW irradiation], m.p. 104–106 °C (DCM-hexane), IR (Nujol): 3020, 1710, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.46 (s, 3H), 4.16 (s, 4H), 5.94 (s, 2H), 6.43 (d, 1H, $J = 8.8$ Hz), 6.90 (s, 1H), 7.10 (s, 1H), 7.63 (d, 1H, $J = 8.8$ Hz); ^{13}C-NMR ($CDCl_3$) δ 18.2, 53.9, 109.4, 116.9, 117.8, 119.6, 122.2, 125.4, 142.5, 151.3, 157.1, 161.9; MS (EI): 227 ($M^{+\bullet}$, 47%), 197 (100), 180 (14), 168 (73), 154 (25), 141 (58), 115 (42), 102 (31); Anal. $C_{14}H_{13}NO_2$ (C, H, N).

(l) *7-(2,5-Dihydro-1H-pyrrol-1-yl)-6-methyl–2H-chromen-2-one* (**11b**) (64% yield) (at r.t. for 4 days), m.p. 171–173 °C (DCM-hexane), IR (Nujol): 3060, 1720, 1600 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.50 (s, 3H), 4.36 (s, 4H), 5.93 (s, 2H), 6.09 (d, 1H, $J = 9.3$ Hz), 6.53 (s, 1H), 7.08 (s, 1H), 7.52 (d, 1H, $J = 9.3$ Hz); ^{13}C-NMR ($CDCl_3$) δ 22.3, 57.4, 101.3, 110.7, 118.0, 121.2, 125.9, 131.1, 143.2, 144.8, 153.2, 159.7; MS (ESI): 228 [$M^{+\bullet}$ + H], 250 [$M^{+\bullet}$ + Na], 266 [$M^{+\bullet}$ + K]; Anal. $C_{14}H_{13}NO_2$ (C, H, N).

(m) *5-(2,5-Dihydro-1H-pyrrol-1-yl)-6-methyl–2H-chromen-2-one* (**11c**) (56% yield or 65% yield under MW irradiation or 46% yield with the catalyst **13** (12 mol %) under stirring at r.t. for 2 days), m.p. 105–107 °C (DCM-hexane), IR (Nujol): 3050, 1725, 1580 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.26 (s, 3H), 4.12 (s, 4H), 5.97 (s, 2H), 6.35 (d, 1H, $J = 9.7$ Hz), 7.10 (d, 1H, $J = 8.6$ Hz), 7.36 (d, 1H, $J = 8.6$ Hz), 7.97 (d, 1H, $J = 9.7$ Hz); ^{13}C-NMR ($CDCl_3$) δ 17.5, 60.7, 109.8, 114.3, 115.8, 119.5, 127.4, 134.4, 141.0, 145.6, 153.4, 161.0; MS (ESI): 228 [$M^{+\bullet}$ + H], 250 [$M^{+\bullet}$ + Na]; Anal. $C_{14}H_{13}NO_2$ (C, H, N).

(n) *4-(2,5-Dihydro-1H-pyrrol-1-yl)-2H-chromen-2-one* (**17**) (75% yield) (at r.t. for 3 days), m.p. 145–147 °C (DCM-hexane), IR (Nujol): 3085, 1705, 1580 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 4.58 (s, 4H), 5.24 (s, 1H), 6.01 (s, 2H), 7.23 (t, 1H, $J = 8.3$ Hz), 7.38 (d, 1H, $J = 8.3$ Hz), 7.52 (t, 1H, $J = 8.3$ Hz), 8.07 (d, 1H, $J = 8.3$ Hz); ^{13}C-NMR ($CDCl_3$) δ 58.4, 87.3, 118.6, 122.9, 125.1, 125.2, 131.3, 137.8, 143.4, 158.1, 160.5; MS (EI): 213 ($M^{+\bullet}$, 100%), 185 (33), 184 (32), 170 (20), 118 (39), 90 (29); Anal. $C_{13}H_{11}NO_2$ (C, H, N).

(o) *4-Methyl-7-(1H-pyrrol-1-yl)-2H-chromen-2-one* (**7b**) (6% yield) (at r.t. for 5 days), m.p. 169–171 °C (DCM-hexane), IR (Nujol): 3050, 1705, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.44 (s, 3H), 6.25 (s, 1H), 6.40 (t, 2H, $J = 2.0$ Hz), 7.16 (t, 2H, $J = 2.0$ Hz), 7.30–7.36 (m, 2H), 7.63 (d, 1H, $J = 9.3$ Hz); ^{13}C-NMR ($CDCl_3$) δ 18.8, 107.9, 111.9, 114.2, 115.8, 119.1, 126.1, 134.4, 142.3, 151.5, 155.6, 160.6; MS (ESI): 226 [$M^{+\bullet}$ + H], 248 [$M^{+\bullet}$ + Na]; Anal. $C_{14}H_{11}NO_2$ (C, H, N).

(p) *7-Methyl-6-(1H-pyrrol-1-yl)-2H-chromen-2-one* (**12a**) [7% yield, after refluxing for 2 days with the catalyst **5** (7 mol%) or 9% yield under MW irradiation], light yellow crystals, m.p. 135–137 °C (DCM-hexane), IR (Nujol): 3040, 1720, 1595 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.27 (s, 3H), 6.34 (t, 2H, $J = 1.8$ Hz), 6.42 (d, 1H, $J = 9.5$ Hz), 6.76 (t, 2H, $J = 1.8$ Hz), 7.26 (s, 1H), 7.37 (s, 1H), 7.65 (d, 1H, $J = 9.5$ Hz); ^{13}C-NMR ($CDCl_3$) δ 18.2, 106.1, 109.4, 115.1, 116.8, 118.8, 122.2, 125.4, 137.7, 142.5, 155.3, 160.5; MS (ESI): 226 [$M^{+\bullet}$ + H]; Anal. $C_{14}H_{11}NO_2$ (C, H, N).

(q) *6-Methyl-7-(1H-pyrrol-1-yl)-2H-chromen-2-one* (**12b**) (21% yield) (at r.t. for 4 days), m.p. 202–204 °C (dec.) (DCM-hexane), IR (Nujol): 3020, 1708, 1580 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.30 (s, 3H), 6.36 (t, 2H, $J = 2.1$ Hz), 6.43 (d, 1H, $J = 9.3$ Hz), 6.83 (t, 2H, $J = 2.1$ Hz), 7.24 (s, 1H), 7.40 (s, 1H), 7.69 (d, 1H, $J = 9.3$ Hz); ^{13}C-NMR ($CDCl_3$) δ 18.1, 108.5, 110.2, 114.1, 116.8, 123.0, 124.8, 129.2, 137.7, 142.5, 157.0, 161.5; MS (ESI): 226 [$M^{+\bullet}$ + H], 248 [$M^{+\bullet}$ + Na]; Anal. $C_{14}H_{11}NO_2$ (C, H, N).

(r) *6-Methyl-5-(1H-pyrrol-1-yl)-2H-chromen-2-one* (**12c**) (21% yield or 10% yield under MW irradiation or 20% yield with the catalyst **13** (12 mol %) under stirring at r.t. for 2 days), light yellow crystals m.p. 143–145 °C (DCM-hexane), IR (Nujol): 3080, 1705, 1580 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.14 (s, 3H), 6.34 (d, 1H, $J = 9.7$ Hz), 6.40 (t, 2H, $J = 2.1$ Hz), 6.69 (t, 2H, $J = 2.1$ Hz), 7.12 (d, 1H, $J = 9.7$ Hz), 7.30 (d, 1H, $J = 8.7$ Hz), 7.44 (d, 1H, $J = 8.7$ Hz); ^{13}C-NMR ($CDCl_3$) δ 16.7, 109.8, 116.7, 117.5, 122.7, 126.6, 127.5, 133.3, 139.3, 150.5, 153.6 160.3; MS (ESI): 226 [$M^{+\bullet}$ + H], 248 [$M^{+\bullet}$ + Na]; Anal. $C_{14}H_{11}NO_2$ (C, H, N).

5.2. Determination of lipophilicity as Clog P

Lipophilicity was theoretically calculated as Clog *P* values in *n*-octanol-buffer by CLOG *P* Programme of Biobyte Corp. [36]

5.3. Biological assays

Materials. All the reagents used were commercially available by Merck, 1,1-diphenyl-2-picrylhydrazyl (DPPH), nordihydroguairetic acid (NDGA) were purchased from the Aldrich Chemical Co. Milwaukee, WI, USA. Soybean Lipoxygenase, linoleic acid sodium salt, nicotinamido–adenine–dinucleotide NADH, Nitrotetrazolium Blue (NBT), porcine heme and indomethacin were obtained from Sigma Chemical, Co. (St. Louis, MO, USA) and carrageenin, type K, was commercially available. For the in vivo experiments, male and female Fischer-344 rats (180–240 g) were used. *N*-methyl-phenazonium-methyl sulfate and trolox were purchased by Fluka A.G. For in vitro determination a UV–Vis Perkin–Elmer Lambda Spectrophotometer was used.

5.3.1. In vitro

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

5.3.1.1. Determination of the reducing activity of the stable radical 1, 1-diphenyl-picrylhydrazyl (DPPH) [34]. To a solution of DPPH (100 μM) in absolute ethanol an equal volume of the compounds dissolved in ethanol was added. As control solution ethanol was used. The concentrations of the solutions of the compounds were 50 μM and 100 μM. After 20 and 60 min at room temperature the absorbance was recorded at 517 nm (Table 1). NDGA was used as a standard, [the percentage of DPPH reduction DPPH $\% = (A_{standard} - A_{sample}/A_{standard}) \times 100$].

5.3.1.2. Competition of the tested compounds with DMSO for hydroxyl radicals. The hydroxyl radicals generated by the Fe^{3+}/ascorbic acid system [35], 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 μM), DMSO (33 mM) in phosphate buffer (50 mM, pH 7.4), the tested compounds (concentration 0.01 mM and 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.

5.3.1.3. Inhibition of linoleic acid lipid peroxidation. Production of conjugated diene hydroperoxide by oxidation of linoleic acid sodium salt in an aqueous solution was monitored at 234 nm [34]. 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was used as a free radical initiator. 10 μl 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 the examined compounds. 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 (Table 1). Trolox was used as a standard.

5.3.1.4. Soybean lipoxygenase inhibition study in vitro. In vitro study was evaluated as reported previously [34]. The tested compounds dissolved in ethanol were incubated at room temperature with sodium linoleate (0.1 mM) and 0.2 ml of enzyme solution (1/9×10^{-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 μM) (Table 1).

5.3.1.5. Non-enzymatic assay of superoxide radicals – measurement of superoxide radical scavenging activity. The superoxide producing system was set up by mixing phenazine methosulfate (PMS), NADH and air–oxygen [34]. The production of superoxide was estimated by the nitroblue tetrazolium method. The reaction mixture containing 3 μM PMS, 78 μM NADH, and 25 μM NBT in 19 μM phosphate buffer pH 7.4 was incubated for 2 min at room temperature and the absorption measured at 560 nm against a blank containing PMS. The tested compounds were preincubated for 2 min before adding NADH.

Acknowledgments

The authors gratefully acknowledge Drs. C. Hansch, A. Leo and Biobyte Corp. 201 West 4th Street, Suite 204, Claremont, CA 91711, USA for free access to the C-QSAR program.

Part of this research (A. Vronteli) has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) – Research Funding Program: Heracleitus II. Investing in knowledge society through the European Social Fund.

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