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Original article

Synthesis and anti-inflammatory evaluation of novel angularly or linearly fused coumarins

Theodoros Symeonidis [a], Konstantina C. Fylaktakidou [b], Dimitra J. Hadjipavlou-Litina [c,**], Konstantinos E. Litinas [a,*]

[a] Laboratory of Organic Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece
[b] Molecular Biology and Genetics Department, Democritus University of Thrace, Alexandroupolis 68100, Greece
[c] Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece

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ABSTRACT

Angular [7,8]-fused coumarins were obtained from the reaction of [2,3]-fused phenols with DMAD and PPh_3, while linear [6,7]-fused coumarins were formed from the analogous reaction of [3,4]-fused phenols with DMAD and PPh_3. These compounds were tested *in vitro* for antioxidant activity and they found to present significant scavenging activity. In parallel, these new compounds were evaluated *in vivo* for anti-inflammatory activity and they found to inhibit the carrageenin-induced paw edema (34–65%). Although their interaction with the free stable radical DPPH was low, the methyl 2,2-dimethyl-8-oxo-3,8-dihydro-2*H*-furo[2,3-h]chromene-6-carboxylate was the most potent (65%) in the *in vivo* experiment. The later seems to be a potent soybean Lipoxygenase inhibitor and does not acquire gastrointestinal toxicity.

1. Introduction

Coumarin derivatives are an interesting class of heterocyclic systems, since coumarin is the basis of a great variety of natural and synthetic biologically active compounds [1–3]. In particular among fused coumarins, furocoumarins are important as photochemotherapeutic [4] agents and exhibit antitumorial [5], antioxidant [3] and anti-inflammatory [3] activities. Pyranocoumarins are used also as photoactive drugs for skin disorders [6] and possess [7] antifungal, insecticidal, anticancer, anti-HIV [4c,8], anti-inflammatory [3,9] and antioxidant [3,9] activities. 1,3-Dioxolocoumarins show anti-inflammatory activity [10].

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain, fever and inflammation. All of the NSAIDs are approximately equivalent in terms of anti-inflammatory efficacy and cause untoward side effects (such as gastrointestinal ulcers, hemorrhages) in a significant fraction of treated patients and this fact frequently limits therapy.

The preparations of furocoumarins [4b,d,e,11a] or pyranocoumarins [4b,11] are achieved mainly by formation of furan or pyran ring starting from hydroxycoumarins. An alternative way have been used by our group starting from o-quinones for the construction of the fused heterocyclic [10,12] or pyranone [9,10,13,14] ring. Very recently we prepared pyranocoumarins [14a] and pyridocoumarins [14b] starting from [3,4]- and [2,3]-fused phenols in an effort to apply the earlier [15] reported by Yavari synthesis of coumarins from the reactions of phenols with dimethylacetylenedicarboxylate (DMAD) and triphenylphosphine (PPh_3). In continuation of our work we are reporting here the extension of this method to the synthesis of angular [7,8]- and linear [6,7]-coumarins fused to dihydrofuro-, 1,3-dioxolo-, 5-membered- or 6-membered alicyclic ring. Many non-steroidal anti-inflammatory drugs have been reported [16,17] to act either as inhibitors of free radical production or as radical scavengers. Compounds with antioxidant properties could be expected to offer protection in rheumatoid arthritis and inflammation and to lead to potentially effective drugs. Thus, we tested our new derivatives with regard to their antioxidant ability and in comparison to well known antioxidant agents e.g. nordihydroguaiaretic acid (NDGA), trolox, caffeic acid.

* Corresponding author. Tel.: +30 2310997864; fax: +30 2310997679.
** Corresponding author.
E-mail addresses: hadjipav@pharm.auth.gr (D.J. Hadjipavlou-Litina), klitinas@chem.auth.gr (K.E. Litinas).

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2. Chemistry

The reactions studied and the products obtained are depicted in Schemes 1–4. Treatment of sesamol (**1a**) with DMAD (**2**) in the presence of PPh_3 (**3**) in dichloromethane (DCM) at 0 °C for 15 min and then reflux for 24 h and separation of the reaction mixture by column chromatography afforded methyl 6-oxo-6*H*-[1,3]dioxolo[4,5-g]chromene-8-carboxylate (**7a**), a derivative of ayapin, in 82% yield, along with methyl (2E)-(6-oxofuro[2,3-f][1,3]benzodioxol-7(6*H*)-ylidene)acetate (**8a**) (3%) (Scheme 1). These products are produced through δ- or γ-lactonization of the intermediate o-hydroxybutenedioate **6a**, initially formed via an electrophilic attack [14,15] of the vinyltriphenylphosphonium cation **5** on the aromatic ring **4** ortho to the –OH substituent. From the reaction of 5-indanol (**1b**) with **2** and **3** under reflux for 3 days methyl 2-oxo-2,6,7,8-tetrahydrocyclopenta[g]chromene-4-carboxylate (**7b**) (43%) and methyl (2E)-(2-oxo-6,7-dihydro-2*H*-indeno[5,6-b]furan-3(5*H*)-ylidene)acetate (**8b**) (3%) were received, while 23% of the starting material was recovered. The results remained practically the same, when we repeated this reaction with reflux for 10 days.

The analogous reaction (Scheme 2) of 5,6,7,8-tetrahydro-2-naphthol (**9**) (reflux for 2 days) gave the regio-isomers methyl 2-oxo-6,7,8,9-tetrahydro-2*H*-benzo[g]chromene-4-carboxylate (**10**) (29%) and methyl 3-oxo-7,8,9,10-tetrahydro-3*H*-benzo[f]-chromene-1-carboxylate (**11**) (8%) (47% of the starting phenol was isolated by column chromatography). When we repeated the same procedure in boiling dioxan for 14 days no reaction was observed.

We obtained the methyl 2-oxo-7,8,9,10-tetrahydro-2*H*-benzo[h]chromene-4-carboxylate (**13a**) (34%) from the reaction of 5,6,7,8-tetrahydro-1-naphthol (**12a**) under reflux in DCM for 14 days (52% of the starting naphthol was remained unchanged), while the methyl 2,2-dimethyl-8-oxo-3,8-dihydro-2*H*-furo[2,3-h]chromene-6-carboxylate (**13b**) (33%) was isolated from the reaction (reflux in DCM for 24 h) of 2,2-dimethyl-2,3-dihydro-7-benzo[b]-furanol (**12b**) (40% of **12b** was recovered) (Scheme 3).

From the above experiments it is observed that sesamol (**1a**) is the most reactive probably because of the more nucleophilic character of **4a** (Scheme 1) due to the electron-releasing effect of O-atoms.

The Pechmann style reaction of naphthol **12a** with malic acid (**14**) in the presence of conc. H_2SO_4 under MW irradiation resulted to unsubstituted 7,8,9,10-tetrahydro-2*H*-benzo[h]chromene-2-one (**15**) (45%) (Scheme 4).

3. Biology

3.1. Antioxidant activity

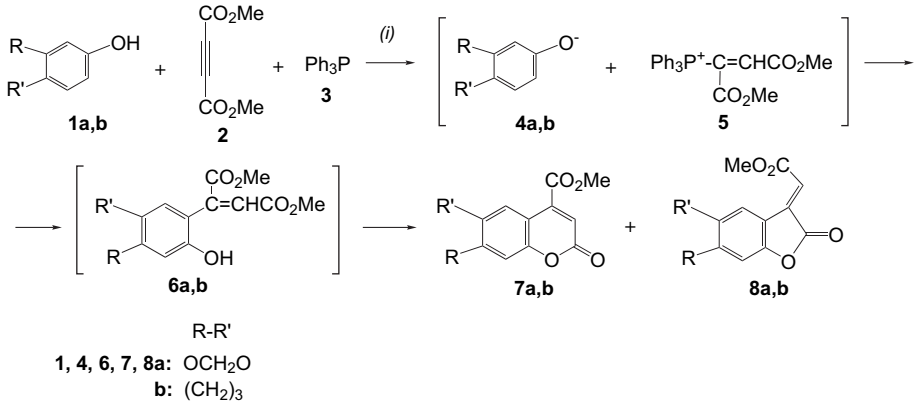
It is well known that free radicals play an important role in inflammatory process [17]. Consequently compounds with antioxidant properties could be expected to offer protection in rheumatoid arthritis and inflammation and to lead to potentially effective drugs. In fact, many non-steroidal anti-inflammatory drugs have been reported to act either as inhibitors of free radical production or as radical scavengers [17]. Herein the antioxidant activity was evaluated in three *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.

3.1.1. Determination of the reducing activity of the stable radical 1, 1-diphenyl-picrylhydrazyl (DPPH)

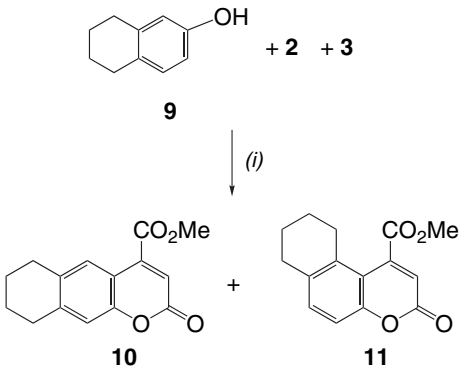
Among the plethora of methods used for the evaluation of the antioxidant activity, the DPPH test is very useful in the micromolar range demanding minutes to hours for both lipophilic and hydrophilic samples. In cases where the structure of the electron donor is not known this method can afford data on the reduction potential of the sample, and hence can be helpful in comparing the reduction potential of unknown materials. 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 [18] at 0.05 mM and 0.1 mM after 20 and 60 min (Table 1). This interaction indicates their reducing ability in an iron-free system. The results show that this interaction is only concentration independent but very low compared to the reference compound NDGA (nordihydroguaiaretic acid) (Table 1). Lipophilicity is not well correlated with the results. There is no evidence for any structural characteristic of the tested compounds which is correlated to the antioxidant activity. The presence of the coumarin nucleus is implicated by itself in the reducing procedure.

3.1.2. Competition of the tested compounds with DMSO for hydroxyl radicals

It is consistent that rates of reactive oxygen species (ROS) production are increased in most diseases [19]. Cytotoxicity of $O_2^{-\cdot}$ and H_2O_2 in living organisms is mainly due to their transformation into $^{\cdot}OH$, reactive radical metal complexes and $^1O_2$. During the inflammatory process, phagocytes generate the superoxide anion radical at the inflamed site and this is connected to other oxidizing species as $^{\cdot}OH$. Hydroxyl radicals are among the most reactive



Scheme 1. Reagents and conditions: (i) DCM, 0 °C (45 min) and then reflux (24 h).



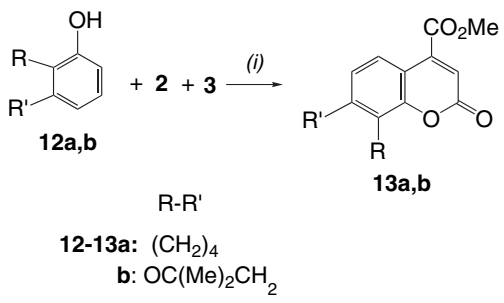
Scheme 2. Reagents and conditions: (i) DCM, 0 °C (45 min) and then reflux (48 h).

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, thus increasing prostaglandin synthesis.

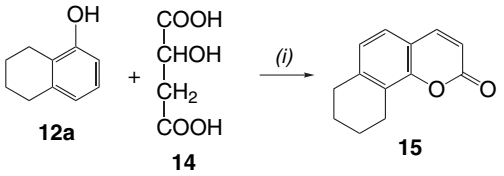
The competition of compounds with dimethyl sulfoxide (DMSO) for $^{\cdot}$OH radicals [18], generated by the Fe^{3+}/ascorbic acid system, expressed as the inhibition of formaldehyde production, was used for the evaluation of their hydroxyl radical scavenging activity. All the tested derivatives highly compete with DMSO (33 mM) at 0.1 mM in comparison to trolox (Table 2). Perusal of the % results shows that the linear derivatives **7a**, **7b** and **10** are more potent than the angular **11** and **13a**. The magnitude of the ring (5 or 6 membered) **7b** and **10** does not seem to influence the competition. Small differences are observed between the dioxo- and alicyclic-analogues (**7a** and **7b**). Lipophilicity is not well correlated with the results. 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.

3.1.3. Soybean lipoxygenase inhibition study in vitro

Our compounds were further evaluated for inhibition of soybean lipoxygenase LO by the UV absorbance based enzyme assay [20]. While one may not extrapolate the quantitative results of this assay to the inhibition of mammalian 5-LOX, it has been shown that inhibition of plant LOX activity by NSAIDs is qualitatively similar to their inhibition of the rat mast cell LOX and may be used as a simple qualitative screen for such activity. Many flavonoids and other phenolics as well as coumarin derivatives inhibit soybean lipoxygenase. This inhibition is related to their ability to reduce from the iron species in the active site to the catalytically inactive ferrous form [21]. Lipoxygenases oxidize certain fatty acids at specific positions to hydroperoxides that are the precursors of leukotrienes, which contain a conjugated triene structure. Most of the LO inhibitors are antioxidants or free radical scavengers, since



Scheme 3. Reagents and conditions: (i) DCM, 0 °C (45 min) and then reflux (4 days).



Scheme 4. Reagents and conditions: (i) Conc. H_2SO_4, MW, 80 W (60 s).

lipoxygenation occurs via a carbon-centered radical. It is known that soybean lipoxygenase, is inhibited by NSAIDs in a qualitatively similar way to that of the rat mast cell lipoxygenase and may be used in a reliable screen for such activity. Perusal of % inhibition values (Table 2) shows that compound **13a** ($IC_{50} = 100$ μM) is the most active within the set compared to the reference compound caffeic acid. The majority of the LO inhibitors act as: a) antioxidants or free radical scavengers [21], since lipoxygenation occurs via a carbon-centered radical, b) 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) and c) excellent ligands for Fe^{+3}.

Although lipophilicity is referred [22a,b] as an important physicochemical property for LO inhibitors, all the above tested derivatives do not follow this concept.

3.1.4. Non enzymatic assay of superoxide radicals – measurement of superoxide radical scavenging activity

Non enzymatic superoxide anion radicals [18] 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. The majority of the compounds does not present scavenging activity at 0.1 mM (Table 2), with the exception of compound **10** (100%) which is highly potent.

3.2. Inhibition of the carrageenin-induced edema

The *in vivo* anti-inflammatory effects of the tested coumarins were assessed by using the functional model of carrageenin-induced rat paw edema and are presented in Table 2, as percentage of weight increase at the right hind paw in comparison to the uninjected left hind paw. Carrageenin-induced edema is a non-specific inflammation resulting from a complex of diverse mediators [23]. Since edemas of this type are highly sensitive to non-steroidal anti-inflammatory drugs (NSAIDs), carrageenin has been accepted as a useful agent for studying new anti-inflammatory drugs [24]. This model reliably predicts the anti-inflammatory efficacy of the NSAIDs. Representative compounds (**13a**,

Table 1
Theoretically calculated *C* log *P* values; Interaction % with DPPH – reducing ability (RA %).

Compd	*C log P*	RA %	0.05 mM	RA %	0.1 mM
		20 min	60 min	20 min	60 min
7a	1.09	12	9	18	21
7b	2.39	19	14	22	27
10	3.11	12	12	21	25
11	3.11	14	18	17	20
13a	2.95	27	no	31	no
13b	2.75	11	no	12	no
15	2.98	25	no	15	no
NDGA		63	65	81	83

no: no action under the reported conditions.

Table 2
Competition % of compounds with DMSO for hydroxyl radical ($HO^{\cdot}$ %); *In vitro* inhibition of soybean lipoxygenase (LO) (IC_{50}); % Superoxide radical scavenging activity ($O_2^{-\cdot}$); Inhibition % of induced carrageenin rat paw edema (CPE %) at 0.01 mmol/kg.

Compd	$HO^{\cdot}$ % 0.1 mM	LO % (0.1 mM) IC_{50} (μM)	$O_2^{-\cdot}$ (%) 0.1 mM	CPE (%)[a] 0.01 mmol/kg body weight
7a	78	0%	nd	45.5*
7b	64	10%	nd	nd
10	74	78%	100	43.4*
11	53	31%	No	34*
13a	No	100 μM	No	62*
13b	72	4%	No	65**
15	No	4%	16	54*
Indomethacin				47*
Caffeic acid		600	46	
Trolox	88			

no: no action under the reported conditions; nd: not determined; [a]$p^* < 0.05$, $p^{**} < 0.01$.

13b and **15**) were tested in order to delineate the role of the nature of the ring at 7, 8 positions (dose ip 0.01 mmol/ml/Kg body weight) and after 3.5 h [18] they induced protection (ranged from 54 to 65%) against carrageenin-induced paw edema, while the reference drug indomethacin (IMA) induced 47% protection at an equivalent dose. Compounds **13a** and **13b** present almost equipotent effect although the nature of their ring (R′, R) is different. No significant differences are also observed between compounds **7a** and **10**. Thus, neither lipophilicity nor the nature of the angular ring seems to influence the *in vivo* effect. Comparing **13a** to **15** the presence of 4-$COOCH_3$ group seems to affect the anti-inflammatory potency.

The antiradical activity of the tested compounds supports at least in part, the *in vivo* anti-inflammatory activity.

3.3. Ulcerogenic activity

Since the most of the non-steroidal anti-inflammatory agents currently in use have serious gastrointestinal toxicity, intimately connected to their molecular mode of action, we found it necessary to examine the most potent compound on a model protocol which we have used earlier [25]. We gave the compound **13b** and indomethacin intraperitoneally to rats at 0.1 mmol/kg body weight a dose at which indomethacin caused 50% mortality. Compound **13b** does not cause any mortality or any of the signs of gastrointestinal toxicity observed after indomethacin administration. This greatly reduced gastrointestinal toxicity may be the result of the entire molecular structure integrating hydroxyl scavenging activity and the "covering" of a free acidic group through the ester group (Table 3).

4. Conclusion

In summary, we synthesized a series of novel angularly or linearly fused coumarins. Most of them are potent $^{\cdot}OH$ scavengers. One of them (**13a**) presented significant IC_{50} LO inhibition value, whereas **7b**, **10**, **11**, **13a**, **13b** and **15** showed anti-inflammatory activity *in vivo*. The most potent *in vivo* analogue **13b** does not present any ulcerogenic activity. The results of this investigation prompt us to continue the development and testing of novel angularly or linearly fused coumarins as new therapeutic tools against inflammation and to carry out further studies to investigate SAR and their mechanism of action.

5. Experimental protocol

5.1. Chemistry

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 ^1H 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. 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 reactions of phenols with DMAD and PPh_3. Synthesis of methyl 6-oxo-6H-[1,3]dioxolo[4,5-g] chromene-8-carboxylate (*7a*)

Sesamol (**1a**) (1.382 g, 10 mmol) and Ph_3P (2.62 g, 10 mmol) were dissolved in DCM (25 ml). A solution of DMAD (1.23 ml, 1.42 g, 10 mmol) in DCM (10 ml) was added dropwise over 15 min period at 0 °C and the solution heated under reflux for 24 h. Evaporation of the solvent and separation by column chromatography (hexane/ethyl acetate 3:1) gave **8a** (77 mg, 3%) and **7a** (2.034 g, 82%).

*5.1.1.1. Methyl 6-oxo-6H-[1,3]dioxolo[4,5-g]chromene-8-carboxylate (**7a**).* Yellow solid; mp 151–153 °C (ethyl acetate/hexane); IR (Nujol): 1725, 1710, 1620 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.99 (s, 3H), 6.10 (s, 2H), 6.83 (s, 1H), 6.85 (s, 1H), 7.72 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 53.2, 98.4, 102.3, 102.5, 104.3, 116.1, 142.1, 145.4, 151.5, 151.9, 161.1, 164.5; MS (EI) *m/z*: 248 (M^+, 94), 220 (100), 189 (59), 161 (43), 133 (11). Anal. $C_{12}H_8O_6$ (C, H).

*5.1.1.2. Methyl (2E)-(6-oxofuro[2,3-f][1,3]benzodioxol-7(6H)-ylidene)acetate (**8a**).* Orange-red solid; mp 196–198 °C (DCM/hexane); IR (Nujol): 1780, 1700, 1660, 1595 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 3.87 (s, 3H), 6.06 (s, 2H), 6.66 (s, 1H), 6.72 (s, 1H), 8.14 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 52.2, 94.0, 102.3, 107.8, 115.9, 121.1, 144.2, 146.5, 152.4, 154.2, 159.1, 165.1; MS (EI) *m/z*: 248 (M+, 82), 220 (100), 189 (64), 161 (52), 133 (13). Anal. $C_{12}H_8O_6$ (C, H).

5.1.2. Synthesis of methyl 2-oxo-2,6,7,8-tetrahydrocyclopenta-[g]chromene-4-carboxylate (*7b*)

From the reaction of 5-indanol (**1b**) with **2** and **3** (3 days reflux) **8b** (73 mg, 3%) and **7b** (1.049 g, 43%) were isolated [0.308 g (23%) of **1b** was received unchanged].

*5.1.2.1. Methyl 2-oxo-2,6,7,8-tetrahydrocyclopenta[g]chromene-4-carboxylate (**7b**).* Yellow solid; mp 120–122 °C (DCM/hexane); IR (Nujol): 1715, 1705, 1600 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.03–2.19 (m, 2H), 2.87–3.07 (m, 4H), 4.00 (s, 3H), 6.83 (s, 1H), 7.21 (s, 1H), 8.01 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 25.7, 32.2, 33.3, 53.1, 112.9, 113.9, 117.5, 121.4, 141.3, 142.9, 150.4, 153.6, 160.6, 164.7; MS (ESI): *m/z* for $[M + Na]^+$ 267. Anal. $C_{14}H_{12}O_4$ (C, H).

*5.1.2.2. Methyl (2E)-(2-oxo-6,7-dihydro-2H-indeno [5,6-b]furan-3(5H)-ylidene)acetate (**8b**).* Orange solid; mp 115–117 °C (MeOH); IR (Nujol): 1790, 1715, 1610 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.06–2.18 (m, 2H), 2.88–2.99 (m, 4H), 3.89 (s, 3H), 6.82 (s, 1H), 6.99 (s, 1H), 8.43 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ MS (ESI): *m/z* for $[M + Na]^+$ 267. Anal. $C_{14}H_{12}O_4$ (C, H).

*5.1.3. Synthesis of methyl 2-oxo-6,7,8,9-tetrahydro-2H-benzo[g] chromene-4-carboxylate (**10**) and methyl 3-oxo-7,8,9,10-tetrahydro -3H-benzo[f]chromene-1-carboxylate (**11**)*

The reaction of 5,6,7,8-tetrahydro-2-naphthol (**9**) with **2** and **3**, under reflux for 2 days, provided **10** (0.748 g, 29%) and **11** (0.206 g, 8%), while 47% of the starting material was recovered. No reaction was observed, when dioxan was used as solvent under reflux for 14 days.

*5.1.3.1. Methyl 2-oxo-6,7,8,9-tetrahydro-2H-benzo[g]chromene-4-carboxylate (**10**).* Yellow solid; mp 96–98 °C (ethyl acetate/hexane); IR (Nujol): 1710, 1690, 1600 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.74–1.93 (m, 4H), 2.78–2.98 (m, 4H), 4.00 (s, 3H), 6.83 (s, 1H), 7.06 (s, 1H), 7.90 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.4, 22.5, 22.9, 23.1, 53.1, 113.5, 116.7, 118.1, 126.5, 134.2, 142.4, 143.2, 156.2, 160.5, 164.5; MS (ESI): *m/z* for $[M+Na]^+$ 281. Anal. $C_{15}H_{14}O_4$ (C, H).

*5.1.3.2. Methyl 3-oxo-7,8,9,10-tetrahydro-3H-benzo[f]chromene-1-carboxylate (**11**).* White solid; mp 130–132 °C (ethyl acetate/hexane); IR (Nujol): 1720, 1705, 1630 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.72–1.89 (m, 4H), 2.70–2.88 (m, 4H), 3.99 (s, 3H), 6.40 (s, 1H), 7.15 (d, $J=8.5$ Hz, 1H), 7.29 (d, $J=8.5$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.0, 22.9, 27.0, 29.6, 53.3, 115.2, 115.8, 123.6, 134.1, 134.2, 138.4, 146.4, 153.4, 159.6, 165.0; MS (ESI): *m/z* for $[M+Na]^+$ 281. Anal. $C_{15}H_{14}O_4$ (C, H).

*5.1.4. Synthesis of methyl 2-oxo-7,8,9,10-tetrahydro-2H-benzo[h]chromene-4-carboxylate (**13a**)*

The reaction of 5,6,7,8-tetrahydro-1-naphthol (**12a**) with **2** and **3**, under reflux for 14 days [0.77 g (52%) of **12a** was received], resulted to **13a** (0.877 g, 34%). Yellow solid; mp 85–87 °C (ethyl acetate/hexane); IR (Nujol): 1735, 1720, 1595 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.72–1.91 (m, 4H), 2.82–2.97 (m, 4H), 3.99 (s, 3H), 6.84 (s, 1H), 7.05 (d, $J=8.2$ Hz, 1H), 7.93 (d, $J=8.2$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.4, 22.6, 23.1, 30.2, 53.4, 113.4, 117.8, 123.4, 125.9, 126.1, 143.4, 143.6, 152.8, 160.9, 165.0; MS (EI) *m/z*: 258 (M^+, 100), 230 (26), 199 (50), 171 (35). Anal. $C_{15}H_{14}O_4$ (C, H).

*5.1.5. Synthesis of methyl 2,2-dimethyl-8-oxo-3,8-dihydro-2H-furo[2,3-h]chromene-6-carboxylate (**13b**)*

From the reaction of 2,2-dimethyl-2,3-dihydro-7-benzo[b]furanol (**12b**) with **2** and **3** the **13b** (0.904 g, 33%) was separated [0.657 g (40%) of **12b** was isolated]. Bright yellow solid; mp 139–140 °C (ethyl acetate/hexane); IR (Nujol): 1740, 1715, 1620 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.56 (s, 6H), 3.14 (s, 2H), 3.99 (s, 3H), 6.84 (s, 1H), 7.09 (d, $J=8.2$ Hz, 1H), 7.66 (d, $J=8.2$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.2, 43.4, 53.1, 89.7, 115.8, 118.2, 118.4, 120.9, 132.6, 143.1, 145.8, 150.1, 160.0, 164.6; MS (EI) *m/z*: 274 (M^+, 100), 256 (13), 213 (18), 187 (18). Anal. $C_{15}H_{14}O_5$ (C, H).

*5.1.6. Synthesis of 7,8,9,10-tetrahydro-2H-benzo[h]chromene-2-one (**15**)*

A homogeneous mixture of 5,6,7,8-tetrahydro-1-naphthol (**12a**) (1.038 g, 7 mmol) and malic acid (**14**) (0.938 g, 7 mmol) was getting wet with 1 ml conc. H_2SO_4 and irradiated under MW at 80 W for 75 s in a commercial MW oven. The mixture was treated with 20 g ice to afford compound **15** (0.627 g, 45%). White solid; mp 145–147 °C (ethanol); IR (Nujol): 1715, 1605 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.78–1.92 (m, 4H), 2.86–2.95 (m, 2H), 2.96–3.01 (m, 2H), 6.35 (d, $J=9.8$ Hz, 1H), 7.01 (d, $J=7.9$ Hz, 1H), 7.21 (d, $J=7.9$ Hz, 1H), 7.66 (d, $J=9.8$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.0, 22.8, 30.3, 36.0, 111.4, 115.3, 124.7, 125.6, 134.4, 138.7, 144.3, 146.1, 152.7, 161.0; MS (ESI): *m/z* for $[M+H]^+$ 201, for $[M+Na]^+$ 223. Anal. $C_{13}H_{12}O_2$ (C, H).

5.2. Determination of lipophilicity as C log P

Lipophilicity was theoretically calculated as *C* log *P* values in n-octanol-buffer by CLOGP Programme of Biobyte Corp. [26].

5.3. Biological assays

Materials. All the reagents used were commercially available by Merck, 1,1-diphenyl-2-picrylhydrazyl (DPPH), nordihydroguaiaretic 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-methylphenazonium-methyl sulfate and trolox were purchased by Fluka A.G.

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) [18]. To a solution of DPPH (0.1 mM) 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 0.05 and 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.

5.3.1.2. Competition of the tested compounds with DMSO for hydroxyl radicals [18]. 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 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 2). Trolox was used as a standard.

5.3.1.3. Soybean lipoxygenase inhibition study in vitro [18]. In vitro study was evaluated as reported previously. The tested compounds dissolved in ethanol were incubated at room temperature with sodium linoleate (0.1 mM) and 0.2 ml of enzyme solution ($1/9\times10^{-4}$ 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 2).

5.3.1.4. Non enzymatic assay of superoxide radicals – measurement of superoxide radical scavenging activity [18]. The superoxide producing system was set up by mixing phenazine methosulfate (PMS), NADH and air–oxygen. 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 (Table 2).

5.3.2. In vivo assay

5.3.2.1. Inhibition of the carrageenin-induced edema [18]. Edema was induced in the right hind paw of Fisher 344 rats (150–200 g) by

Table 3
Gastrointestinal toxicity (0.1 mmol/kg, ip) of indomethacin and compound 1**3b** in rats.

Compound	Percent mortality	Percent GI ulcers	Body weight change (g/100 g)	Melena incidence
Indomethacin	50	78	−9.0	+
13b	0	0	+2.5	−

the intradermal injection of 0.1 ml 2% carrageenin in water. Both sexes were used. Females pregnant were excluded. Each group was composed of 6–15 animals. The animals, which have been bred in our laboratory, were housed under standard conditions and received a diet of commercial food pellets and water *ad libitum* during the maintenance but they were entirely fasted during the experiment period. Our studies were in accordance with recognised guidelines on animal experimentation.

The tested compounds 0.01 mmol/kg body weight, were suspended in water, with few drops of Tween 80 and ground in a mortar before use (bases) or dissolved in water (salts) and were given intraperitoneally simultaneously. The rats were euthanisized 3.5 h after carrageenin 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 2. Indomethacin used in 0.01 mmol/kg (47%). Values CPE % are the mean from two different experiments with a standard error of the mean less than 10%. Statistical studies were done with student's T-test.

5.3.2.2. Ulcerogenic activity [23]. Compound **13b** and indomethacin were administered ip to rats Fisher 344 once a day for 4 days. Perforating gastrointestinal ulcers, melena defecation, weight change and mortality were recorded 24 h after the last administration (Table 3).

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Elemental analyses

7a ($C_{12}H_8O_6$) Calcd.: C % 58.07, H % 3.25. Found: C % 58.05, H % 3.25.

8a ($C_{12}H_8O_6$) Calcd.: C % 58.07, H % 3.25. Found: C % 58.02, H % 3.08.

7b ($C_{14}H_{12}O_4$) Calcd.: C % 68.85, H % 4.95. Found: C % 68.49, H % 4.67.

8b ($C_{14}H_{12}O_4$) Calcd.: C % 68.85, H % 4.95. Found: C % 69.03, H % 4.72.

10 ($C_{15}H_{14}O_4$) Calcd.: C % 69.76, H % 5.46. Found: C % 69.97, H % 5.21.

11 ($C_{15}H_{14}O_4$) Calcd.: C % 69.76, H % 5.46. Found: C % 70.04, H % 5.37.

13a ($C_{15}H_{14}O_4$) Calcd.: C % 69.76, H % 5.46. Found: C % 69.97, H % 5.17.

13b ($C_{15}H_{14}O_5$) Calcd.: C % 65.69, H % 5.15. Found: C % 65.51, H % 5.13.

15 ($C_{13}H_{12}O_2$) Calcd.: C % 77.97, H % 6.04. Found: C % 78.15, H % 5.83.

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