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Original article

Synthesis and evaluation of the antioxidant and antiinflammatory activities of some benzo[l]khellactone derivatives and analogues

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Abstract

Treatment of 3-hydroxy-β-lapachone **4** with ylide **5** gave the coumarin derivative **7a**, which was transformed to compounds **10–14**. Compound **14** was then transformed to benzo[f]seselin **15** as well as to benzo[l]khellactones **16, 18** from which the title compounds **17, 19_I, 19_{II}, 20, 21_I** and **21_{II}** were prepared. All the tested compounds were found to interact with DPPH in a concentration and time dependent manner. All the tested compounds highly inhibited the soybean lipoxygenase, whereas compounds **12, 17** and **19_{II}** highly compete with DMSO for $^{\bullet}$OH. Compounds **7a, 7b, 12** and **17** induced at 48.7–58.9% protection against carrageenin induced rat paw edema.
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Keywords: Benzo[l]khellactones; Benzo[f]seselin; Wittig reaction; Antioxidant activity; Antiinflammatory activity

1. Introduction

Recently, we reported the synthesis of some 3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromen-6-one derivatives **1** [1] (Scheme 1) possessing a benzene ring instead of the A pyran ring of calanolide A **2**. In continuation to our previous efforts in designing and synthesizing new coumarin derivatives with antiinflammatory/antioxidant activities [2–4], we report here our attempt to insert biologically active substituents in the pyran ring of compound **1a**, like in the active [5] *cis*-khellactone **3** and to study the biological response of the new derived compounds, as well as of the "mother compounds" **1a** and **7b**.

2. Chemistry

The title new compounds prepared are depicted in Schemes 2–4. The parent skeleton of 2,2-dimethyl-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromene-6-one was constructed according to the method reported previously for the synthesis of coumarins via the reaction of the appropriate *o*-quinones with alkoxycarbonylmethylene(triphenyl)-phosphoranes [1,6–10] (Scheme 2).

Treatment of 3-hydroxy-β-lapachone (stenocarpoquinone-A) [11] **4** with excess of ethoxycarbonylmethylene(triphenyl)phosphorane **5** at room temperature for 1 h in dry DCM and separation of the reaction mixture by column chromatography gave ethyl 3-hydroxy-2,2-dimethyl-6-oxo-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromene-8-carboxylate **7a** in 74% yield. When the reaction was carried out under reflux compound **7a** was obtained in 60% yield. Wittig monoolefination of the 6-CO of **4** with ylide **5** followed by a Michael addition of a second ylide **5** to the initially formed *o*-quinone methanide and further Hofmann elimination of triphenylphosphine from the adduct can afford the intermediate **6**. δ-Lactonization of **6** gave compound **7a**. The also possible [7,8] γ-lactonization's product **8**, as well as the possible isomers of compounds **7a** and **8** coming from an initial monoolefination of 5-CO of **4** were not detected or separated from the reaction mixture. The spectral data of compound **7a** resemble well with the structure proposed and are very similar to those of ethyl 2,2-dimethyl-6-oxo-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromene-8-carboxylate **7b** [1]. Furthermore the product in question shows strong fluorescence, like the 7-alkoxy-substituted coumarins [12].

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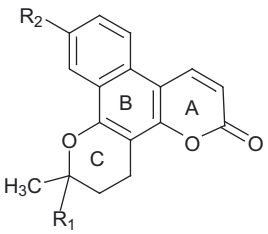
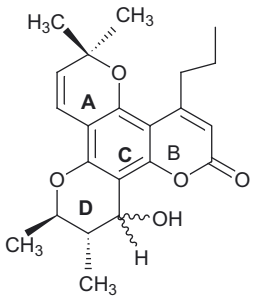


1a-c

1a: R_1=CH_3, R_2=H
b: R_1=CH_3, R_2=CH_3
c: R_1=H, R_2=H



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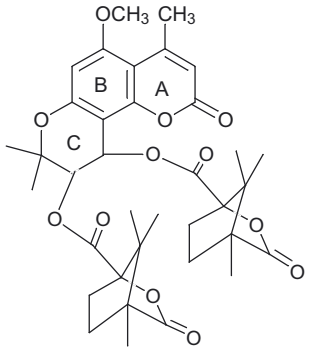

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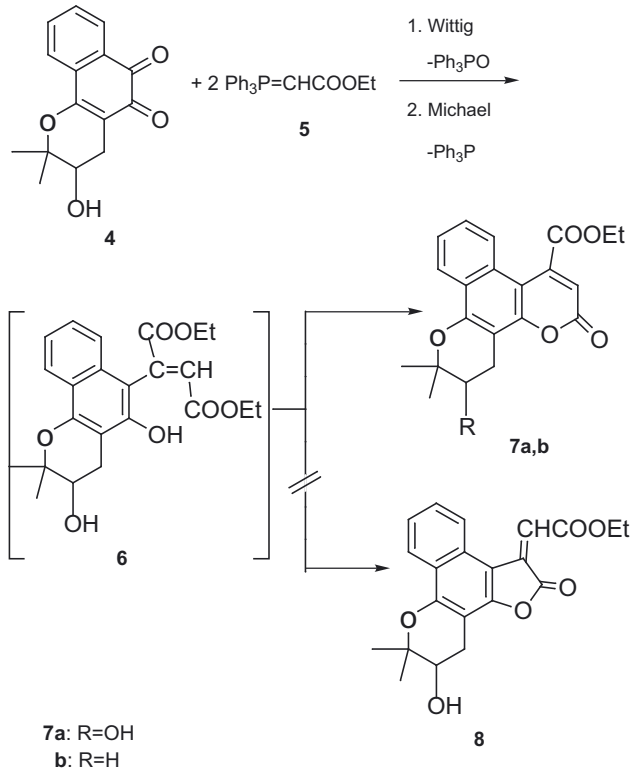
Scheme 1.



Scheme 2.

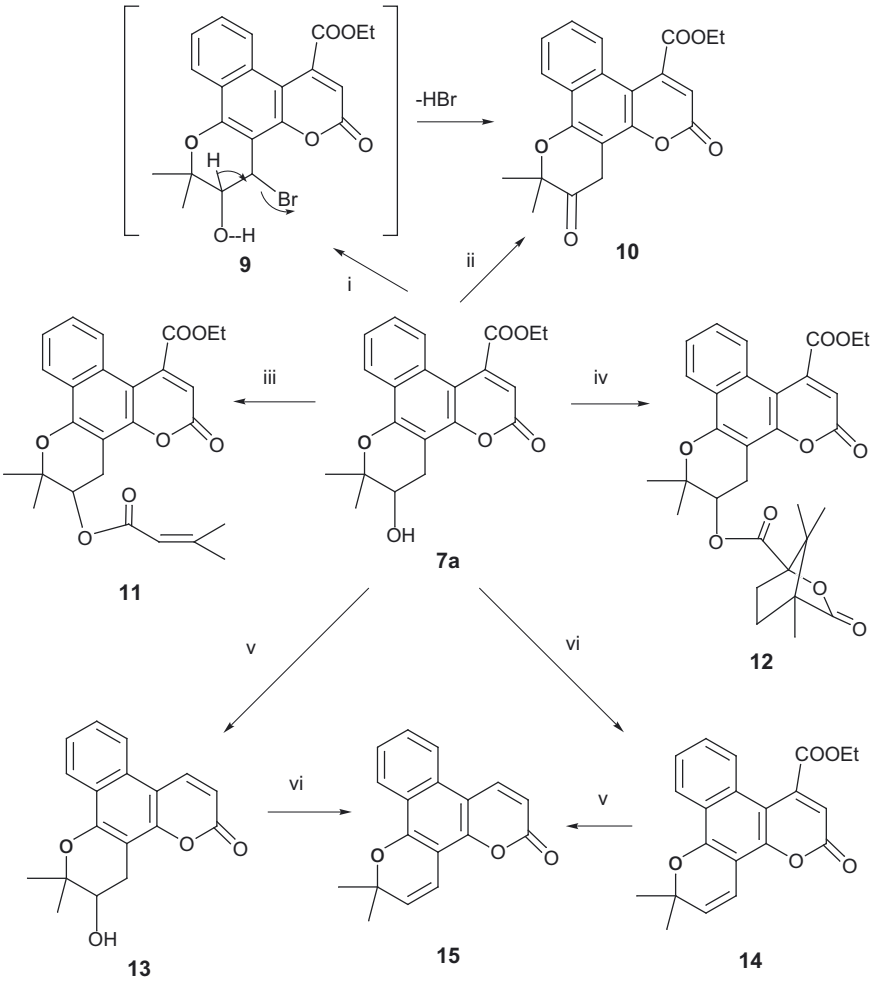
Jone's oxidation of compound **7a** at ~5 °C gave ethyl 2,2-dimethyl-3,6-dioxo-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano [2,3-h]chromene-8-carboxylate **10** in 25% yield. When the reaction was carried out at ~10 °C the yield improved to 38% (Scheme 3). Compound **10** was also obtained in 37% yield by treating compound **7a** with NBS–benzoylperoxide, obviously via the initially formed bromoderivative **9** and further hydrobromide elimination and enol–ketone tautomerization. The oxidation of **7a** was unsuccessful by its treatment with chromium trioxide/pyridine or with MnO_2/acetonitrile or by the Swern oxidation [13].

Treatment of compound **7a** in dry pyridine-carbon tetrachloride solution with 3,3-dimethylacryloyl chloride or with (*S*)-(–)-camphanic chloride gave the esters **11** and **12** in 50% and 75% yield, respectively, (Scheme 3).

Treatment of compound **7a** with copper powder/quinoline for 18 h at 170–175 °C under N_2 atmosphere gave 3-hydroxy-2,2-dimethyl-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano [2,3-h]chromene-6-one **13** in 32% yield (Scheme 3). When the mixture was heated at 220–230 °C for 12 h, decomposition of the starting compound was observed, while it remained unchanged when heated at ~120 °C for 4 h. Compound **13** was also obtained in 26% yield by heating compound **7a** in DMF-water solution, in the presence of sodium chloride [14], at first at ~120 °C for 4 h (no reaction) and then at 170–175 °C for 48 h.

Treatment of compound **7a** with triphenylphosphine in dry carbon tetrachloride/acetonitrile solution [15] under reflux for 3 h gave ethyl 2,2-dimethyl-6-oxo-2*H*,6*H*-benzo [f]pyrano[2,3-h]chromene-8-carboxylate **14** in 97% yield (two efforts), identical in all respects to that obtained previously in 23% yield by treatment of **7b** with NBS [1].

By a similar treatment of compound **13** with triphenylphospine 2,2-dimethyl-2*H*,6*H*-benzo[f]pyrano[2,3-h] chromen-6-one **15** was obtained, but at lower yield (60%) (Scheme 3). Benzo[f]seselin **15** was also obtained in 18% yield by treatment of compound **14** with copper powder/quinoline at ~150 °C for 22 h. Both compounds **14** and **15** are interesting for further modifications to 3,4-dihydroxy-derivatives, with possible biological interest, but compound **15** was prepared in low total yield even via the route **7a** → **13** → **15** or via the route **7a** → **14** → **15**.



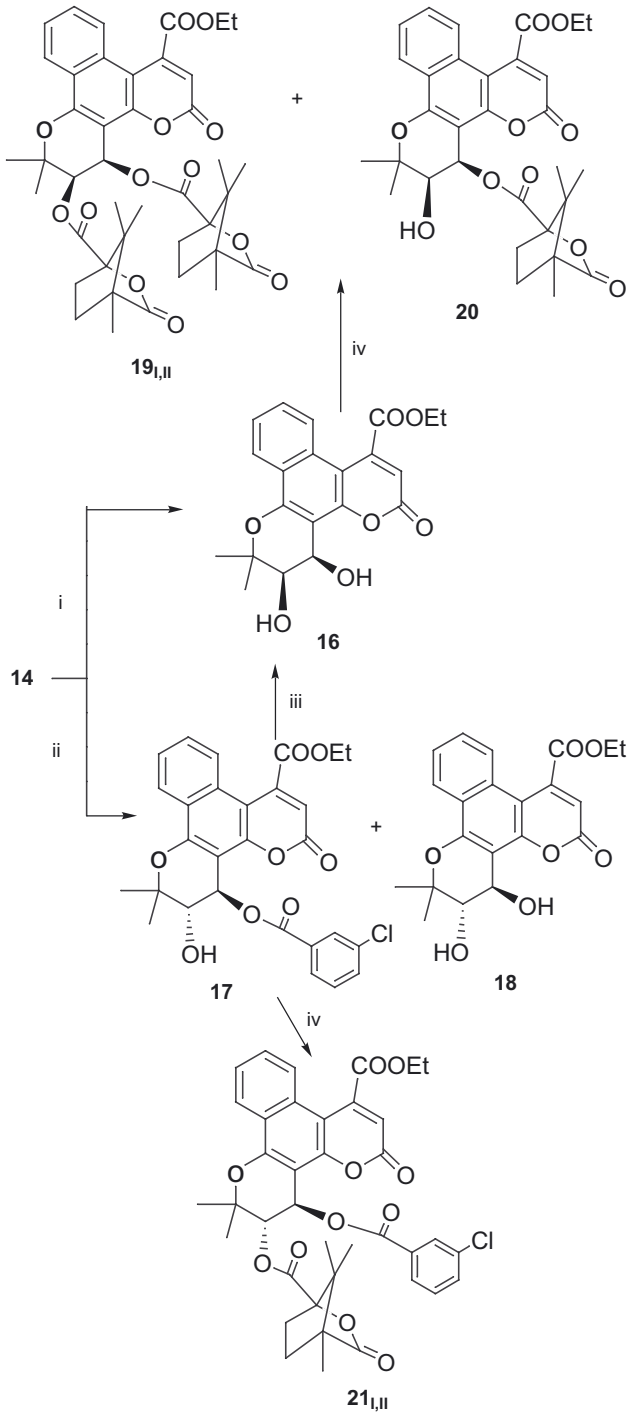
Scheme 3. Reagents and conditions: i, NBS, CCl_4, $(PhCO)_2O_2$, reflux, 6h; ii, CrO_3, H_2SO_4, MeCOMe, N_2, 5°C; iii, Me_2C=CHCOCl, dry Pyridine, 0–24°C; iv, (1S)-(-)-camphanic chloride, dry pyridine, 0–24°C; v, Cu, dry quinoline, 170–175^{0}C, 18 h, N_2 or DMF, water, NaCl, 170–175^{0}C; 48 h, for **13**; vi, Ph_3P, CCl_4, MeCN, reflux, 3–8 h.

The further transformations of compound **14** are depicted in Scheme 4. In an effort for the preparation of its *cis*-dihydroxy derivative, compound **14** was treated, according to the literature [16] with osmium tetroxide/*N*-methyl morpholine-*N*-oxide, at room temperature for 18 h, to give ethyl *cis*-3,4-dihydroxy-2,2-dimethyl-6-oxo-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromene-8-carboxylate **16** in 66% yield (obviously as the racemic mixture of its [3*R*,4*R*]- and [3*S*,4*S*]- stereoisomers). The ^{1}H NMR spectrum of the product in question showed two absorptions at δ 5.29 (d, 1H, J = 5.2 Hz) and 3.96 (d, 1H, J = 5.2 Hz) for the pyran ring protons.

Efforts also were made for the preparation of the isomeric *trans*- diol [5]. Compound **14** treated with *m*-chloroperoxybenzoic acid (*m*-CPBA) at room temperature and afforded ethyl (±)-(*trans*)-4[(3-chlorobenzoyl)oxy]-2,2-dimethyl-**6**-oxo-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromene-8-carboxylate **17** in 69% yield in addition to the expected *trans*- diol **18** in low yield (26%). A similar monoester is also formed by treating seselin with *m*-CPBA under similar conditions [5]. Hydrolysis of compound **17** by treating with 0.5 N potassium hydroxide gave again the *cis*-diol **16**, but in very low yield (15%), probably due to hydrolysis of the pyranone ring.

Treatment of compound **16** with (1*S*)-(–)-camphanic chloride/pyridine at rt gave the two [3*R*,4*R]* and [3*S*,4*S*]-di-*O*-(–)-camphanoyl diastereomers ethyl (*cis*)-2,2-dimethyl-6-oxo-3,4-bis{[(4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]hept-1-yl)carbonyl]oxy}-3,4-dihydro-2*H*,6*H*-benzo-[f]pyrano[2,3-h]chromene-8-carboxylates $\mathbf{19_I}$ (15%), $\mathbf{19_{II}}$ (16%) along with (–)-camphanoyl ethyl 3-hydroxy-4{[(4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]hept-1-yl)carbonyl]oxy}-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromene-8-carboxylate **20** in 67% yield (Scheme 4). Treatment of the monoester **17** with (1*S*)-(–)-camphanic chloride/pyridine at rt afforded the stereoisomers ethyl (*trans*)-4-[(chlorobenzoyl)oxy]-2,2-dimethyl-6-oxo-3-{[(4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]hept-1-yl)carbonyl]oxy}-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromene-8-carboxylates $\mathbf{21_I}$ and $\mathbf{21_{II}}$ in 31% and 26% yield, respectively.

The analytical and spectral data of compounds **18–21** resemble well with the structures suggested for them. The recorded ^{1}H NMR spectra of the products in question exhibited the expected absorptions for their pyran protons at δ 4.16



Scheme 4. Reagents and conditions: i, OsO_4, N-methylmorpholine N-Oxide; ii, m-CPBA, dry $CHCl_3$, N_2; iii, 0.5 N KOH, dioxane; iv, (1S)-(-)-camphanic chloride, dry DCM, pyridine, 0°C.

(d, 1H, J = 3.8 Hz, H-3) and 6.49 (d, 1H, J = 3.8 Hz, H-4) for compound **18**, at δ 5.59 (d, 1H, J = 5.09 Hz, H-3) and 6.84 (d, 1H, J = 5.09 Hz, H-4) for compound $\mathbf{19_I}$ (mp 241–243 °C), at δ 5.50 (d, 1H, J = 5.09 Hz, H-3) and 6.75 (d, 1H, J = 5.09 Hz, H-4) for compound $\mathbf{19_{II}}$ (mp 188–189 °C), at δ 4.25 (d, 1H, J = 5.08 Hz, H-3) and 6.64 (d, 1H, J = 5.08 Hz, H-4) for compound **20**, at δ 5.65 (d, 1H, J = 3.81 Hz, H-3) and 6.56 (d, 1H, J = 3.81 Hz, H-4) for compound $\mathbf{21_I}$ (mp 106–108 °C) and at δ 5.63 (d, 1H, J = 3.81 Hz, H-3) and 6.58 (d, 1H, J = 3.81 Hz, H-4) for compound $\mathbf{21_{II}}$ (mp 88–90 °C), in good agreement with those of similar products reported in the literature [5]. We were not able to assign the absolute *R,R* or *S,S* configuration at their C-3 and C-4 on the basis of the data available.

3. Biological results

The biological activities of the compounds were screened by in vivo and in vitro assays. Inhibitory activities were measured against isolated enzymes (soybean lipoxygenase and trypsin).

The reducing abilities of the examined compounds were determined by their interaction with the free stable radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) at three different concentrations at 20–60 min. Antioxidants can react with DPPH and produce 1,1-diphenyl-2-picryl-hydrazine [17]. Due to its odd electron DPPH gives a strong absorption band at 517 nm. As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes and the resulting decolorization is stoichiometric with respect to the number of electrons taken up. The change of absorbance produced in this reaction is assessed to evaluate the antioxidant potential of test samples and this assay is useful as a primary screening system. All the tested compounds, compared with acetylsalicylic acid (80.5%) or nordihydroguaeretic acid (94.4%) used as standard drugs, were found in the most of the cases (compounds **7a, 10, 14, 16** and **17**, Table 1), to interact with DPPH in a concentration and time dependent manner (the interaction increases with the increase of the concentration of the tested compounds and the time). Compound **13** was found inactive in 0.1 mM, whereas compound **16** was found to highly interact with DPPH in a concentration and a time dependent manner. For compound **12** the interaction seems to decrease and to disappear after 60 min. For compound **17** the percent interaction does not change from 0.1 to 0.2 mM and from 20 to 60 min. Compounds **15, $\mathbf{19_I}$, $\mathbf{19_{II}}$** and **20** were found to slightly interact to DPPH in 0.1 mM, but they interact highly as the concentration increases.

Because $HO^{\cdot}$ is one of the most potent oxidizing agents, which under certain conditions might be implicated in lipid peroxidation and because hydrogen peroxide as a source of $HO^{\cdot}$ has been implicated in inflammation [18], we attempted to investigate the ability of the synthesized compounds to compete with DMSO for hydroxyl radicals. Compounds **10, 11, 13, 14, 15, 16, $\mathbf{19_I}$, 20** could not be possible to be studied due to solubility problems. Under the reported experimental conditions only compounds **1a, 12, 17** and $\mathbf{19_{II}}$ seem to compete with DMSO for hydroxyl radicals. These compounds highly compete with DMSO (Table 2, 63.8–100%).

All compounds inhibited the soybean lipoxygenase (58.5–100%). It is important that compounds **14, 15, 16, 17** under our experimental conditions are highly active. No attempt

Table 1
Inhibition % of soybean lipoxygenase (LOX%) [a,b]. Reduction ability % (RA%) [a,c]

Compounds	LOX% 1 mM *	RA% 0.1 mM * 20 min	RA% 0.1 mM * 60 min	RA% 0.2 mM * 20 min	RA% 0.2 mM * 60 min	RA% 0.5 mM * 20 min	RA% 0.5 mM * 60 min
1a	No	21.0	21.0	Nt	Nt	Nt	Nt
7a	89.8	27.8	18.9	21.2	17.9	33.5	34.5
7b	No	24.3	24.3	Nt	Nt	Nt	Nt
10	58.5	81.2	67.8	86.3	78.0	100	100
11	97.3	No	23.5	10.9	8.3	11.4	13.0
12	98.8	30.3	14.5	No	No	No	No
13	98.3	No	No	15.0	5.0	40.6	7.2
14	100	21.9	18.2	27.8	26.3	44.5	51.5
15	94.4	13.7	33.9	44.1	69.3	20.0	50.2
16	100	24.5	26.7	54.0	62.0	62.2	80.4
17	100	24.5	25.0	10.4	10.4	32.8	39.8
19_I	3.9	19.6	38.8	82.8	31.0	28.2	29.8
19_{II}	No	10.0	17.8	51.2	61.2	22.0	36.1
20	0.5	9.0	18.4	24.9	43.9	21.2	28.4

* Final concentrations of the tested compounds; No, no action under the experimental conditions; Nt, not tested.
[a] Data are means of two or three independent experiments and the deviation in absorbance values were less than 10%.
[b] Nor-dihydroguaeretic acid 84.7% (1 mM).
[c] Acetylsalicylic acid as a standard 80.5% (0.1 mM) and nor-dihydroguaeretic acid 94.4% (0.1 mM).

Table 2
Inhibition % of carrageenin induced rat paw edema (CPE%) [a] at 0.1 mmoles/kg. Competition% to DMSO for ·OH radicals (·OH%) [b]. Inhibition % of trypsin (Itr%) [c]

Compounds	CPE%	·OH%	Itr%
1a	Nt	100	33.3
7a	48.7	No	No
7b	54.0	No	No
12	58.9	96.5	15.0
17	53.1	60.1	No
19_{II}	Nt	63.8	96.3

No, no result under experimental conditions; Nt, not tested.
[a] Indomethacin 57% (0.1 mmoles).
[b] Tocopherol acetate as a standard 83.4%, DMSO as a standard 78.5%.
[c] Salicylic acid 18.1% (0.1 mM).

was made to find the concentration of substrate-compound which produces maximal inhibition. No specific requirements for the LOX inhibition were found, since all the tested compounds highly inhibit the soybean LOX. The presence or absence of the 4-COOEt group does not seem to be crucial for the enzyme's inhibition. The strong inhibitory ability would be correlated with the overall benzodihydroseselin active ring system. The tested compounds will be used in the future as templates for the design of potent LOX inhibitors as candidates against psoriasis or asthma.

Proteases are intimately involved in the initiating events, cellular recruitment and degenerative aspects of inflammation [19]. Antiinflammatory agents have been reported to inhibit trypsin [20]. Only compounds **12, 19_{II}** and **3a** seem to inhibit trypsin (Table 2, 15–96.3%). For the rest of the compounds (not tested) there were stability problems under the reported experimental conditions.

Compounds **7a, 7b, 12** and **17** have been chosen to be screened in vivo (0.1 mmol/kg) for their antiinflammatory activity using the fractional model of carrageenin induced rat paw edema. The basic idea was to examine as much as we could the effect of the changes caused on the "mother compound" **7a**. Thus, compound **7a** the "mother compound" in the synthetic procedure for compounds **11** and **12** has been tested in vivo as well as compound **7b**, in which the OH group has been replaced by hydrogen atom. In order to delineate the role of the camphanoyl group on the in vivo biological response, we studied compound **12**, which is the corresponding ester of compound **7a**. Compound **17** is the representative monobenzoylester of diol **18**. The results are given as percentage of weight increase at the right hind paw in comparison to the uninjected left hind paw. Compounds induced a 48.7–58.9% protection against carrageenin induced paw edema, while the reference drug indomethacin induced 57% protection at equivalent concentration. Compound **7b**, with the basic skeleton of dihydrobenzo[f]seselin, significantly protects (54%) against carrageenin induced rat paw edema. It seems that esterification of the hydroxyl group (**7a**, 48.7%) by benzoyl or camphanoyl groups leads to the potent antiinflammatory agents (Table 2, **12** and **17**, 58.9% and 53.1%).

Our attempt to correlate our biological results with some physicochemical parameters was unsuccessful. However, from our preliminary results seems that the newly synthesized compounds are biologically potent. Furthermore, lipophilicity does not seem to affect predominantly the biological activity. On the contrary steric requirements are more important.

4. Conclusion

In conclusion compounds **7a, 12, 16** and **19_{II}** on the basis of our results would be good candidates and lead molecules for antiinflammatory/antioxidant activity, as well as new lead

molecules for trypsin and lipoxygenase inhibitors. In all the examined compounds a common structural moiety is the basic skeleton of dihydrobenzo[f]seselin, which seems to be correlated with significant biological activity.

5. Experimental

5.1. Chemistry

Mps 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 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 Perkin Elmer API 100 Sciex Simple quadrupole under Electronspray Ionization (ESI) conditions. High resolution mass spectra (HRMS) were recorded on an Ionspec mass spectrometer under Matrix-Assisted Laser Desorption–Ionization Fourier Transform Mass Spectrometer (MALDI–FTMS) conditions with 2,5-dihydroxybenzoic acid (DHB) as the matrix. Microanalyses were performed on a Perkin-Elmer 2400-II Element analyzer. Silica gel No. 60, Merck A.G. has been used for column chromatographies. Compound **4** was prepared with literature method [11].

5.1.1. Ethyl 3-hydroxy-2,2-dimethyl-6-oxo-3,4-dihydro-2H,6H-benzo[f]pyrano[2,3-h]chromene-8-carboxylate *7a*

(A) A solution of quinone **4** (0.167 g, 0.65 mmol) and ylide **5** (0.789 g, 2.27 mmol) in dry DCM (3 ml) was stirred at room temperature for 1 h. The solvent was removed in a rotary evaporator and the residue was separated by column chromatography (hexane–ethyl acetate 2:1) to give compound **7a** (0.178 g, 74%), mp 170–171 °C (ether–hexane); IR (cm^{-1}) 3400, 1730, 1690; ^{1}H NMR: δ 1.39 (t, 3H, J = 7.0), 1.45 (s, 3H), 1.52 (2, 3H), 2.15 (brs, 1H), 3.06 (dd, 1H, J_1 = 5.2, J_2 = 17.8), 3.25 (dd, 1H, J_1 = 4.9, J_2 = 17.8), 3.98-4.04 (m, 1H), 4.51 (q, 2H, J = 7.0), 6.35 (s, 1H), 7.48–7.58 (m, 2H), 7.75 (d, 1H, J = 8.2), 8.33 (d, 1H, J = 9.0); ^{13}C NMR: δ 13.9, 22.1, 24.8, 26.4, 62.9, 68.5, 79.1, 103.9, 104.2, 111.0, 122.9, 123.2, 123.4, 125.4, 127.4, 127.9, 146.7, 153.3, 155.2, 160.1, 167.8; EIMS: *m/z* 368 (M^+, 76%), 350 (7), 335 (47), 298 (73), 297 (73), 268 (26), 139 (57), 43 (100). Anal. $C_{21}H_{20}O_6$ (CH).

(B) A solution of **4** (1.259 g, 4.88 mmol) and **5** (5.095 g, 14.64 mmol) in dry DCM (20 ml) was heated under reflux for 1 h and the mixture was worked up as above to give compound **7a** (1.07 g, 60%).

5.1.2. Ethyl 2,2-dimethyl-3,6-dioxo-3,4-dihydro-2H,6H-benzo[f]pyrano[2,3-h]chromene-8-carboxylate *10*

(A) A solution of chromium (III) oxide (19 mg) in a mixture of concentrated sulfuric acid (0.2 ml) and water (0.06 ml) was added slowly with a syringe to a solution of compound **7a** (100 mg, 0.271 mmol) in acetone (1 ml, free from ethanol) under N_2 atmosphere and the mixture was stirred for 5 h at 5 °C and left overnight at rt. The mixture was then extracted with ether (4 × 10 ml), the organic layer washed with 5% $NaHCO_3$ (2 × 10 ml) and then with water (2 × 5 ml) and dried with Na_2SO_4. The solvent was evaporated in vacuum and the residue was separated by column chromatography (hexane–ethyl acetate 4:1 up to 2:1) to give compound **10** (24 mg, 25%), mp 149–150 °C (ether); IR (cm^{-1}) 3085, 1720, 1675, 1622, 1580; ^{1}H NMR: δ 1.42 (t, 3H, J = 7.2), 1.59 (s, 6H), 3.88 (s, 2H), 4.55 (q, 2H, J = 7.2), 6.45 (s, 1H), 7.58–7.63 (m, 2H), 7.81 (d, 1H, J = 8.0), 8.35 (d, 1H, J = 7.3); ^{13}C NMR: δ 13.9, 23.6, 32.4, 63.0, 83.2, 105.2, 105.9, 112.2, 122.8, 123.4, 123.6, 125.9, 127.7, 128.4, 146.5, 152.5, 153.3, 159.3, 167.4, 205.2; EIMS: *m/z* 366 (M^+, 100%), 351 (7), 338 (10), 297 (47), 268 (33), 243 (35), 195 (33). Anal. $C_{21}H_{18}O_6$ (CH).

The unreacted compound **7a** (53 mg, 53%) was eluted then. When the reaction was carried out at –10 °C compound **10** was obtained in 38% yield.

(B) A mixture of compound **7a** (56 mg, 0.152 mmol), NBS (27 mg, 0.152 mmol) and benzoylperoxide (a few crystals) in carbon tetrachloride (3 ml) was heated under reflux for 6 h. The precipitated succinimide formed was filtered and washed with carbon tetrachloride (2 ml). The filtrate was concentrated in a rotary evaporator and the residue was separated like in method A to give compound **10** (10 mg, 37% on the basis of the compound **7a** consumed). Compound **7a** (24 mg, 43%) was eluted then.

5.1.3. Ethyl 2,2-dimethyl-3-[(3-methyl-2-butenoyl)oxy]-6-oxo-3,4-dihydro-2H,6H-benzo[f]pyrano[2,3-h]chromene-8-carboxylate *11*

To a solution of compound **7a** (74 mg, 0.2 mmol) in a mixture of dry pyridine (0.5 ml) and dry DCM (4.5 ml), 3.3-dimethylacryloyl chloride (36 mg, 0.3 mmol) was added at 0 °C and the mixture was stirred for 3 h and gradually was heated to rt. The solvent was removed in a rotary evaporator, the residue was dissolved in chloroform (10 ml), the solution was washed with water (2 × 5 ml) and brine (2 × 5 ml) and dried with Na_2SO_4. The solvent was evaporated in vacuum and the residue was separated by column chromatography (hexane–ethyl acetate 12:1 up to 10:1) to give compound **11** (45 mg, 50%), mp 177–179 °C (ethyl acetate/hexane); IR (cm^{-1}) 3050, 1735, 1725, 1680, 1635, 1580; ^{1}H NMR: δ 1.41 (t, 3H, J = 7.6), 1.46 (s, 3H), 1.51 (s, 3H), 1.86 (s, 3H), 2.16 (s, 3H), 3.10 (dd, 1H, J_1 = 5.1, J_2 = 17.8), 3.30 (dd, 1H, J_1 = 5.1, J_2 = 17.8), 4.53 (q, 2H, J = 7.6), 5.26 (t, 1H, J = 5.1), 5.64 (s, 1H), 6.38 (s, 1H), 7.50–7.59 (m, 2H), 7.78 (d, 1H, J = 7.6), 8.34 (d, 1H, J = 8.9); ^{13}C NMR: δ 13.9, 20.3, 23.0, 23.7, 24.7, 27.4, 62.8, 68.2, 77.6, 103.8, 103.9, 111.1, 115.4, 122.9, 123.2, 123.3, 125.4, 127.4, 127.9, 146.6, 153.2, 155.0, 158.5, 159.9, 165.5, 167.8; ESIMS: *m/z* 451 $[M + H]^+$, 473 $[M + Na]^+$. Anal. $C_{26}H_{26}O_7$ (CH).

*5.1.4. Ethyl 2,2-dimethyl-6-oxo-3-{[(4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]hept-1-yl)carbonyl]oxy}-3,4-dihydro-2H,6H-benzo[f]pyrano[2,3-h]chromene-8-carboxylate **12***

To a solution of compound **7a** (92 mg, 0.25 mmol) in a mixture of dry pyridine (0.5 ml) and dry DCM (4.5 ml) (1*S*)-(–)-camphanic chloride (82 mg, 0.375 mmol) was added at 0 °C and the mixture was then stirred for 24 h at rt. Additional amount of the acid chloride (14 mg, 0.064 mmol) was added and the reaction mixture was stirred for further 96 h. The solvent was evaporated under vacuum, the residue was dissolved in chloroform (10 ml) and the solution was washed with water (2 × 5 ml), then with brine and dried with Na_2SO_4. The solvent was removed in a rotary evaporator and the residue was separated by column chromatography (hexane–ethyl acetate 2:1) to give compound **12** (103 mg, 75%), mp 158–160 °C (ether–hexane); IR (cm^{-1}) 3050, 1785, 1735, 1710, 1690, 1575; ^{1}H NMR: δ 0.89 (s, 3H), 0.93 (s, 3H), 1.07 (s, 3H), 1.41 (t, 3H, J = 7.3), 1.49 (s, 3H), 1.51 (s, 3H), 1.60–1.74 (m, 1H), 1.82–1.95 (m, 1H), 1.98–2.16 (m, 1H), 2.31–2.46 (m, 1H), 3.11 (dd, 1H, J_1 = 5.8, J_2 = 17.4), 3.40 (dd, 1H, J_1 = 4.4, J_2 = 17.4), 4.54 (q, 2H, J = 7.2), 5.32–5.36 (m, 1H), 6.39 (s, 1H), 7.51–7.58 (m, 2H), 7.79 (d, 1H, J = 8.7), 8.32 (d, 1H,J = 7.3); ^{13}C NMR: δ 9.6, 13.9, 16.6, 22.4, 22.6, 23.7, 28.9, 30.7, 54.2, 54.8, 62.9, 71.1, 71.2, 78.4, 90.8, 103.3, 111.4, 122.5, 122.9, 123.2, 123.5, 125.6, 127.5, 128.1, 146.7, 150.1, 154.6, 163.5, 166.7, 173.0, 175.1; EIMS: *m/z* 548 (M^+, 57%), 350 (42), 335 (56), 307 (10), 296 (28), 268 (18), 235 (18), 83 (100). Anal. $C_{31}H_{32}O_9$ (CH).

Compound **7a** was eluted then (9 mg, 10%).

*5.1.5. 3-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromen-6-one **13***

(A) A mixture of compound **7a** (0.1 g, 0.27 mmol), copper powder (0.119 g) in dry quinoline (5 ml) was heated for 18 h at 170–175 °C, under N_2 atmosphere. Ethyl acetate (50 ml) and ether (50 ml) was then added and the mixture was filtered from the copper. The filtrate was treated with 5% hydrochloric acid (2 × 25 ml) and the organic layer was washed with water (2 × 25 ml) and dried with Na_2SO_4. The solvent was removed in a rotary evaporator and the residue was separated by column chromatography (hexane–ethyl acetate 3:1) to give compound **13** (26 mg, 32%), mp 231–233 °C (ethyl acetate–hexane); IR (cm^{-1}) 3450, 3050, 1700, 1580; ^{1}H NMR: δ 1.45 (s, 3H), 1.53 (s, 3H) 1.74 (brs, 1H), 3.07 (dd, 1H, J_1 = 5.1, J_2 = 17.8), 3.26 (dd, 1H, J_1 = 5.1, J_2 = 17.8), 4.02 (t, 1H, J = 5.1), 6.37 (d, 1H, J = 10.2), 7.53 (t, 1H, J = 7.6), 7.65 (t, 1H, J = 7.6), 8.13 (d, 1H, J = 7.6), 8.31 (d, 1H, J = 7.6), 8.40 (d, 1H, J = 10.2); ^{13}C NMR: δ 22.1, 24.8, 26.2, 68.5, 78.8, 103.9, 106.9, 111.2, 120.9, 122.8, 125.3, 128.2, 128.4, 139.5, 142.5, 152.3, 154.1, 161.6; EIMS: *m/z* 296 (M^+, 36%), 225 (63), 196 (26), 172 (31), 43 (100). Anal. $C_{18}H_{16}O_4$ (CH).

(B) To a solution of compound **7a** in a mixture of DMF-water (10:1, 2.2 ml), sodium chloride (16 mg) was added and the mixture was heated at 170–175 °C for 48 h. The mixture was concentrated to dryness in a rotary evaporator and water (5 ml) was added to the residue. The mixture was extracted with DCM (3 × 6 ml) and the organic layer was concentrated. The residue was then separated by column chromatography (hexane–ethyl acetate 3:1) to give compound **13** (18 mg, 26%).

*5.1.6. Ethyl 2,2-dimethyl-6-oxo-2*H*,6*H*-benzo-[f]pyrano[2,3-h]chromene-8-carboxylate **14***

To a solution of compound **7a** (0.111 g, 0.301 mmol) and triphenylphosphine (0.145 g, 0.553 mmol) in carbon tetrachloride (4 ml), acetonitrile (4 ml) was added and the mixture was heated under reflux for 2 h. Triphenylphosphine (45 mg, 0.17 mmol) was added again and reflux was continued for 1 h. The solvent was removed in a rotary evaporator and the residue was separated by column chromatography (hexane–ethyl acetate 10:1) to give compound **14** (0.102 g, 97%), mp 112–114 °C (hexane) (lit.[1] mp 112–113 °C).

*5.1.7. 2,2-Dimethyl-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromen-6-one **15***

(A) A solution of compound **13** (41 mg, 0.138 mmol) and triphenylphosphine (44 mg, 0.166 mmol) in a mixture of carbon tetrachloride–acetonitrile (1:1, 3.5 ml) was heated under reflux for 2 h and triphenylphosphine (62 mg, 0.23 mmol) was added again. The mixture was refluxed for further 10 h, the solvents were removed in a rotary evaporator and the residue was separated by column chromatography (hexane–ethyl acetate 4:1) to give compound **15** (23 mg, 60%), mp 150–151 °C (ether–hexane); IR (cm^{-1}) 3050, 1725, 1560; ^{1}H NMR: δ 1.58 (s, 6H), 5.78 (d, 1H, J = 10.1), 6.38 (d, 1H, J = 9.2), 7.01 (d, 1H, J = 10.1), 7.52 (t, 1H, J = 8.4), 7.64 (t, 1H, J = 8.4), 8.11 (d, 1H, J = 8,4), 8.27 (d, 1H, J = 8.4), 8.39 (d, 1H, J = 9.2); ^{13}C NMR: δ 28.2, 78.5, 106.2, 106.8, 111.4, 115.6, 121.2, 122.5, 123.0, 125.4, 128.6, 129.1, 129.4, 139.6, 151.1, 152.6, 161.3; EIMS: *m/z* 278 (M^+, 71%), 264 (27), 263 (100), 135 (25), 189 (7), 178 (7), 55 (16). Anal. $C_{18}H_{14}O_3$ (CH).

(B) A mixture of compound **14** (0.105 g, 0.3 mmol) and copper powder (0.127 g) in dry quinoline (5 ml) was heated for 22 h at ~150 °C, under N_2 atmosphere. The mixture was cooled at rt and ethyl acetate (100 ml) and then ether (50 ml) were added. The copper powder was filtered and the filtrate was treated with 5% hydrochloric acid (2 × 20 ml) and then was washed with water (2 × 20 ml). The organic layer dried with Na_2SO_4 and concentrated in a rotary evaporator. The residue was separated by column chromatography (hexane–ethyl acetate 12:1) to give compound **15** (15 mg, 18%), identical to that obtained by method A.

5.1.8. Ethyl cis*-3,4-dihydroxy-2,2-dimethyl-6-oxo-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromene-8-carboxylate **16***

(A) To a solution of compound **14** (0.3 g, 0.857 mmol) and *N*-methylmorpholine *N*-oxide (0.201 g, 1.714 mmol) in 80% aqueous acetone (4.5 ml, free from ethanol, distilled over

potassium permanganate) 4% aqueous OsO_4 solution (0.5 ml) was added and the mixture was stirred at rt for 18 h. During the addition of the OsO_4 the original yellow solution turned to yellow–violet in 5 min. The mixture was diluted with water (20 ml) and concentrated in a rotary evaporator to an aqueous solution, which was extracted with ethyl acetate (3 × 20 ml). The organic layer dried with Na_2SO_4, the solvent was removed in a rotary evaporator and the residue was separated by column chromatography (hexane–ethyl acetate 2:1) to give compound **16** (0.217 g, 66%), mp 146–148 °C (ethyl acetate–hexane); IR (cm^{-1}) 3440, 3310, 3050, 1720, 1680, 1615, 1570; ^{1}H NMR: δ 1.39 (t, 3H, J = 7.2), 1.53 (s, 3H), 1.57 (s, 3H), 3.64 (brs, 1H, exchanged by D_2O), 3.96 (d, 1H, J = 5.2), 4.45 (brs, 1H, exchanged by D_2O), 4.51 (q, 2H, J = 7.2), 5.29 (d, 1H, J = 5.2), 6.38 (s, 1H), 7.48–7.61 (m, 2H), 7.75 (d, 1H, J = 7.7), 8.35 (d, 1H, J = 7.7); ^{13}C NMR: δ 13.9, 21.1, 25.6, 61.2, 62.9, 71.3, 80.1, 104.1, 107.4, 110.8, 123.2, 123.4, 123.8, 125.7, 128.3, 128.7, 147.0, 153.7, 155.7, 160.0, 167.5; EIMS: *m/z* 384 (M^+, 100%), 355 (22), 313 (83), 312 (99), 284 (98), 267 (23), 256 (44), 183 (30). Anal. $C_{21}H_{20}O_7$ (CH).

(B) To a solution of compound **17** (0.18 g, 0.345 mmol) in dioxane (2.5 ml) 0.5 N potassium hydroxide solution (3.3 ml) was added. The color of the mixture turned to red. The mixture was stirred for 40 min at rt and then it was acidified with addition of 20% hydrochloric acid up to pH ~3–4, while the red color of the mixture turned to yellow. The mixture was stirred for 1 h at rt, the solvent was removed in a rotary evaporator, water (20 ml) was added to the residue and the mixture was extracted with ethyl acetate (3 × 20 ml). The organic layer was dried over Na_2SO_4, the solvent was evaporated in vacuum and the residue was separated by column chromatography (hexane–ethyl acetate 3.5:1) to give compound **16** (14 mg, 15%).

*5.1.9. Ethyl 4-[(3-chlorobenzoyl)oxy]-3-hydroxy-2,2-dimethyl-6-oxo-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]-chromene-8-carboxylate **17***

A mixture of compound **14** (0.45 g, 1.285 mmol) and *m*-CPBA (0.606 g, 1.93 mmol) in dry chloroform (9 ml) was stirred at rt under N_2 atmosphere for 18 h. The mixture was diluted by addition of ether (10 ml) and was washed with saturated $NaHCO_3$ solution (10 ml), then with water (90 ml) and then with brine (10 ml). The organic layer was dried with anhydrous Na_2SO_4, concentrated in a rotary evaporator and the residue was separated by column chromatography (hexane–ethyl acetate 3:1) to give first compound **17** (0.458 g, 69%), mp 213–215 °C (ethanol); IR (cm^{-1}) 3420, 3050, 1725, 1720, 1690, 1580; ^{1}H NMR: δ 1.40 (t, 3H, J = 7.6), 1.61 (s, 3H), 1.62 (s, 3H), 3.36 (brs, 1H), 4.16 (d, 1H, J = 3.8), 4.52 (q, 2H, J = 7.6), 6.33 (s, 1H), 6.49 (d, 1H, J = 3.8), 7.33 (t, 1H, J = 7.6), 7.49-7.65 (m, 3H), 7.77 (d, 1H, J = 7.6), 7.86 (d, 1H, J = 7.6), 7.92 (s, 1H), 8.41 (d, 1H, J = 8.9); ^{13}C NMR: δ 13.9, 22.7, 24.2, 62.9, 68.5, 72.2, 79.7, 102.9, 104.3, 111.9, 123.2, 123.3, 123.7, 125.6, 125.7, 128.0, 128.7, 129.0, 129.8, 131.1, 133.4, 134.6, 146.2, 154.6, 155.1, 158.8, 165.4, 167.5; ESIMS: *m/z* 545 $[M + Na]^+$, 557 $[M + Cl]^+$. MALDIHRMS (DHB): *m/z* 545.0964 $[M + Na]^+$. $C_{28}H_{23}ClNaO_8$ requires *m/z* 545.0978. Anal. $C_{28}H_{23}ClO_8$ (CH).

*5.1.10. Ethyl (*trans*)-3,4-dihydroxy-2,2-dimethyl-6-oxo-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromene-8-carboxylate **18***

From the previous reaction, after the elution of compound **17**, compound **18** (93 mg, 26%) was eluted, mp 188–190 °C (DCM–hexane); IR (cm^{-1}) 3370, 3330, 3050, 1700, 1680, 1610, 1575; ^{1}H NMR: δ 1.40 (t, 3H, J = 7.6), 1.43 (s, 3H), 1.65 (s, 3H), 2.54 (brs, 1H, exchanged by D_2O), 3.78 (brs, 1H, exchanged by D_2O), 3.98 (d, 1H, J = 6.4), 4.52 (q, 2H, J = 7.6), 5.11 (d, 1H, J = 6.4), 6.40 (s, 1H), 7.51-7.61 (m, 2H), 7.76 (d, 1H, J = 7.6), 8.34 (d, 1H, J = 7.6); ^{13}C NMR: δ 13.9, 20.5, 25.3, 63.0, 66.6, 74.8, 80.3, 104.4, 107.9, 110.8, 117.8, 123.2, 123.7, 125.7, 128.3, 128.6, 147.2, 150.5, 155.5, 160.0, 167.5; EIMS: *m/z* 384 (M^+, 78%), 313 (66), 312 (100), 285 (38), 284 (82), 256 (30), 183 (30). Anal. $C_{21}H_{20}O_7$ (CH).

*5.1.11. Ethyl (*cis*)-(3*R*,4*R *or 3S,4S)-2,2-dimethyl-6-oxo-3,4-bis{[(4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]hept-1-yl)carbonyl]oxy}-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromene-8-carboxylate **19$_I$** and ethyl (*cis*)-(3*S*,4*S *or 3*R*,4*R*)-2,2-dimethyl-6-oxo-3,4-bis{[(4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]hept-1-yl)carbonyl]oxy}-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromene-8-carboxylate **19$_{II}$***

To a solution of compound **16** (0.184 g, 0.479 mmol) in a mixture of dry DCM (4.5 ml) and dry pyridine (0.5 ml) (1*S*)-(–)-camphanic chloride (0.311 g, 1.437 mmol) was added at 0 °C and the mixture was stirred at rt for 12 h. The solvent was removed in a rotary evaporator, water (10 ml) was added to the residue and the mixture was extracted with chloroform (10 ml). The organic layer was washed with water (10 ml) and then with brine (10 ml) and was dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuum and the residue was separated by column chromatography (DCM–ethyl acetate 20:1) to give first compound **19$_I$** (50 mg, 15%), mp 241–243 °C (ether–hexane); IR (cm^{-1}) 3050, 1775, 1750, 1725, 1710, 1690, 1580; ^{1}H NMR: δ 0.92 (s, 3H), 1.07 (s, 6H), 1.13 (s, 6H), 1.15 (s, 3H), 1.41 (t, 3H, J = 7.6), 1.61 (s, 3H), 1.68 (s, 3H), 1.63–1.76 (m, 2H), 1.87–1.99 (m, 2H), 2.06-2.19 (m, 2H), 2.32–2.42 (m, 1H), 2.51–2.61 (m, 1H), 4.53 (q, 2H, J = 7.6), 5.59 (d, 1H, J = 5.09), 6.39 (s, 1H), 6.84 (d, 1H, J = 5.09), 7.54–7.66 (m, 2H), 7.79 (d, 1H, J = 7.6), 8.35 (d, 1H, J = 7.6); ^{13}C NMR: δ 9.5, 9.6, 13.9, 16.3, 16.4, 16.8, 16.9, 21.5, 26.5, 28.8, 28.9, 31.2, 31.7, 54.1, 54.4, 54.8, 54.9, 61.2, 63.0, 72.2, 78.2, 90.4, 91.0, 102.9, 104.4, 112.2, 122.9, 123.4, 123.5, 126.0, 128.9, 129.3, 146.3, 154.1, 154.2, 158.5, 166.8, 167.1, 167.3, 177.8, 178.6; EIMS: *m/z* 744 (M^+, 13%), 547 (5), 368 (5), 350 (14), 335 (33), 308 (12), 135 (75), 91 (100); $[\alpha]_D$ +29.3^0 (c 0.66, $CHCl_3$). Anal. $C_{41}H_{44}O_{13}$ (CH).

Compound **19$_{II}$** was eluted then (54 mg, 16%), mp 188–189 °C (ethanol); IR (cm^{-1}) 3050, 1780, 1750, 1725, 1710,

1690, 1580; ^{1}H NMR: δ 0.99 (s, 3H), 1.00 (s, 3H), 1.08 (s, 3H), 1.12 (s, 6H), 1.13 (s, 3H), 1.41 (t, 3H, J = 7.6), 1.60 (s, 3H), 1.61 (s, 3H), 1.60–1.73 (m, 2H), 1.82–2.04 (m, 2H), 2.13–2.33 (m, 2H), 2.42–2.58 (m, 2H), 4.53 (q, 2H, J = 7.6), 5.50 (d, 1H, J = 5.09), 6.40 (s, 1H), 6.75 (d, 1H, J = 5.09), 7.54-7.67 (m, 2H), 7.79 (d, 1H, J = 7.6), 8.35 (d, 1H, J = 7.6); ^{13}C NMR: δ 9.5, 9.6, 13.8, 16.3, 16.4, 16.6, 16.7, 21.1, 26.4, 28.8, 28.9, 30.8, 31.2, 54.2, 54.6, 54.9, 55.0, 61.6, 62.9, 72.2, 77.8, 90.7, 91.3, 102.9, 104.5, 112.3, 122.9, 123.4, 123.5, 125.9, 128.9, 129.3, 146.2, 154.0, 154.1, 158.4, 166.6, 166.9, 167.3, 177.9, 178.4; EIMS: *m/z* 744 (M^+, 51%), 743 (43), 547 (30), 546 (40), 350 (55), 335 (74), 312 (45), 83 (100); $[\alpha]_D$ –39.9° (c 0.78, $CHCl_3$). Anal. $C_{41}H_{44}O_{13}$ (CH).

*5.1.12. Ethyl (*cis*)-3-hydroxy-4{[(4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]hept-1-yl)carbonyl]oxy}-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromene-8-carboxylate* ***20***

It was obtained from the above reaction of compound **16**, after the elution of compound $\mathbf{19_{II}}$, (0.18 g, 67%), mp 189–191 °C (ether–hexane); IR (cm^{-1}) 3520, 1790, 1740, 1725, 1690, 1580; ^{1}H NMR: δ 0.98 (s, 3H), 1.08 (s, 3H), 1.10 (s, 3H), 1.41 (t, 3H, J = 7.6), 1.67 (s, 6H), 1.85–1.97 (m, 2H), 2.08–2.21 (m, 1H), 2.41–2.58 (m, 1H), 2.98 (brs, 1H), 4.25 (d, 1H, J = 5.08), 4.52 (q, 2H, J = 7.6), 6.36 (s, 1H), 6.64 (d, 1H, J = 5.08), 7.52–7.64 (m, 2H), 7.76 (d, 1H, J = 7.6), 8.35 (d, 1H, J = 7.6); ^{13}C NMR: δ 9.6, 13.9, 16.5, 16.7, 20.2, 26.5, 28.9, 31.2, 54.4, 54.9, 62.9, 64.5, 71.7, 79.5, 91.3, 102.9, 104.2, 111.8, 123.2, 123.4, 123.7, 125.8, 128.9, 129.2, 146.4, 154.6, 154.7, 158.8, 167.4, 168.8, 178.2; ESIMS: *m/z* 565 [M + H]$^+$, 587 [M + Na]$^+$, 563 [M – H]$^+$, 599 [M + Cl]$^+$. Anal. $C_{31}H_{32}O_{10}$ (CH).

*5.1.13. Ethyl (*trans*)-(3*R*,4*S *or 3*S*,4*R*)-4-[(3-chlorobenzoyl)oxy]2,2-dimethyl-6-oxo-3-{[(4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]hept-1-yl)carbonyl]oxy}-3,4-dihydro-2*H*,6*H*-benzo[f]pyrano[2,3-h]chromene-8-carboxylates* $\boldsymbol{21_I}$ *and ethyl (*trans*)-(3*S*,4*R *or 3*R*,4*S*)-4-[(3-chlorobenzoyl)-oxy]2,2-dimethyl-6-oxo-3-{[(4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]hept-1-yl)carbonyl]oxy}-3,4-dihydro-2*H*,6*H-*benzo[f]pyrano[2,3-h]chromene-8-carboxylates* $\boldsymbol{21_{II}}$

To a solution of compound **17** (0.105 g, 0.20 mmol) in a mixture of dry DCM (4.5 ml) and dry pyridine (0.5 ml) (1*S*)-(–)-camphanic chloride (65 mg, 0.30 mmol) was added at 0 °C. The mixture was stirred for 24 h at rt, an additional amount of the chloride (65 mg, 0.30 mmol) was added and the mixture was stirred for further 48 h. The mixture was concentrated in a rotary evaporator, water (10 ml) was added to the residue and the mixture was extracted with chloroform (20 ml). The organic layer was washed with water (10 ml), then with brine (10 ml) and it was dried over Na_2SO_4. The solvent was evaporated in vacuum and the residue was separated by column chromatography (DCM–ethyl acetate 20:1) to give mixture of compounds $\mathbf{21_I}$, $\mathbf{21_{II}}$ (81 mg, 56%). The unreacted starting compound **17** (40 mg, 39%) was eluted next. The mixture of compounds $\mathbf{21_{I,II}}$ was further separated by ptlc (silica gel, hexane–ethyl acetate 3:1) to give from the faster moving band compound $\mathbf{21_I}$ (43 mg, 31%), mp 106–108 °C (ether–hexane); IR (DCM, cm^{-1}) 3020, 1780, 1745, 1735, 1720, 1690, 1580; ^{1}H NMR: δ 0.98 (s, 3H), 1.03 (s, 3H), 1.08 (s, 3H), 1.41 (t, 3H, J = 7.6), 1.58 (s, 3H), 1.67 (s, 3H), 1.82–1.95 (m, 2H), 1.96–2.11 (m, 1H), 2.35–2.50 (m, 1H), 4.53 (q, 2H, J = 7.6), 5.65 (d, 1H, J = 3.81), 6.35 (s, 1H), 6.56 (d, 1H, J = 3.81), 7.36 (t, 1H, J = 7.6), 7.51–7.67 (m, 3H), 7.80 (d, 1H, J = 7.6), 7.91 (d, 1H, J = 7.6), 7.96 (s, 1H), 8.39 (d, 1H, J = 7.6); ^{13}C NMR: δ 9.6, 14.2, 16.6, 16.7, 21.0, 23.1, 28.7, 30.9, 54.4, 54.9, 60.3, 65.1, 73.4, 78.1, 89.9, 101.8, 102.9, 112.4, 123.1, 123.4, 123.6, 125.2, 125.9, 128.0, 128.6, 129.2, 129.8, 131.1, 133.4, 134.4, 146.1, 153.7, 154.2, 158.3, 164.1, 171.1, 172.4, 184.4; EIMS: *m/z* 702 (M^+, 58%), 548 (40), 505 (30), 490 (55), 366 (75), 350 (55), 335 (60), 156 (70),139 (100); MALDIHRMS (DHB): *m/z* 725.1760 [M + Na]$^+$. $C_{38}H_{35}ClNaO_{11}$ requires *m/z* 725.1760.

Compound $\mathbf{21_{II}}$ was eluted next (37 mg, 25%), mp 88–90 °C (ether–hexane); IR (DCM, cm^{-1}) 3020, 1780, 1750, 1730, 1715, 1690, 1575; ^{1}H NMR: δ 0.98 (s, 3H), 1.03 (s, 3H), 1.09 (s, 3H), 1.41 (t, 3H, J = 7.6), 1.58 (s, 3H), 1.65 (s, 3H), 1.78–1.98 (m, 2H), 2.01–2.20 (m, 1H), 2.29–2.46 (m, 1H), 4.53 (q, 2H, J = 7.6), 5.63 (d, 1H, J = 3.81), 6.36 (s, 1H), 6.58 (d, 1H, J = 3.81), 7.36 (t, 1H, J = 7.6), 7.50–7.67 (m, 3H), 7.80 (d, 1H, J = 7.6), 7.91 (d, 1H, J = 7.6), 7.96 (s, 1H), 8.40 (d, 1H, J = 7.6); ^{13}C NMR: δ 9.6, 14.2, 16.6, 16.7, 23.4, 24.1, 28.8, 31.0, 54.7, 54.9, 60.3, 64.9, 73.1, 77.9, 90.8, 102.6, 104.6, 112.4, 123.1, 123.4, 123.7, 125.9, 126.3, 128.0, 128.8, 129,.2, 129.7, 131.1, 133.4, 134.6, 146.1, 154.1, 154.5, 158.5, 164.0, 166.3, 171.1, 180.0; EIMS: *m/z* 702 (M^+, 5%), 702 (12), 548 (4), 489 (15), 350 (5), 335 (15), 156 (20), 139 (100); MALDIHRMS (DHB): *m/z* 725.1752 [M + Na]$^+$. $C_{38}H_{35}ClNaO_{11}$ requires *m/z* 725.1760.

5.2. Biology

5.2.1. Competition of the tested compounds with DMSO for hydroxyl radicals [21]

The hydroxyl radicals generated by the Fe^{3+}/ascorbic acid system, were detected according to Nash [21], by the determination of formaldehyde produced from the oxidation of DMSO. The reaction mixture contained EDTA (0.1 mM), Fe^{3+} (167 μM), DMSO (33 mM) in phosphate buffer (50 mM, pH 7.4), the tested compounds and ascorbic acid (10 mM). After 30 min of incubation (37 °C) the reaction was stopped with CCl_3COOH (17% w/v).

5.2.2. Interaction of the tested compounds with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical [18,22]

To a solution of DPPH (0.1 mM) in absolute ethanol an equal volume of the compounds (0.1, 0.2, 0.5 mM) dissolved in ethanol was added. As control solution ethanol was used. After 20 and 60 min at rt the absorbance was recorded at 517 nm.

5.2.3. Soybean lipoxygenase inhibition [22]

The tested compounds dissolved in 60% aqueous ethanol (final concentration 1 mM), were incubated at rt with sodium linoleate (0.1 mM) and 0.15 ml of enzyme solution ($1/10^4$ w/v in saline). The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with an appropriate standard inhibitor (nordihydroguaeretic acid 1 mM, 84.7%).

5.2.4. Trypsin inhibition [3]

Tosyl arginine methyl ester (TAME) was used as substrate for trypsin. The reaction mixture consisted of 1.5 ml buffer (0.1 M Tris–HCl, pH 7.8 in 50% v/v methanol) and 1.4 ml TAME (0.01 M in 50% v/v methanol). Compounds dissolved in 50% v/v methanol were added (final concentration 0.1 mM). The reaction was started by addition of 0.1 ml trypsin (1 mg/ml 0.001 N HCl). The increase in the absorbance at 256 nm was determined in the next 4 min.

5.2.5. Inhibition of the carrageenin-induced edema [3]

Edema was induced in the right hind paw of Fischer 344 rats (150–180 g, 2–3 months old) by the intradermal injection of 0.1 ml 2% carrageenin in water. Both sexes were used. Females pregnant were excluded. The tested compounds, 0.1 mmol/kg body weight, were suspended in water and with a few drops of Tween 80 were given intraperitoneally simultaneously. The mice were killed 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 in 0.1 mmol/kg (57%) was administered as a standard drug for comparison reasons. Values CPE% are the mean from two different experiments with a standard error of the mean less than 10% ($P < 0.05$ compared with control values).

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