Synthesis and Biological Evaluation of Benzo[7,8]chromeno[5,6-*b*][1,4]oxazin-3-ones[#]

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The *O*-methylmonoximes **2**, **3** of stenocarpoquinone-A and β-lapachone reacted with methyl phenylacetate to give 1,4-benzoxazine derivatives **8a, 8b** and oxazole **11a**. Compound **8a** was transformed to compounds $\mathbf{13_{I}}$, $\mathbf{13_{II}}$, **14**. Treatment of compound **14** with osmium tetroxide afforded compounds **15**, **16** and esterification of the latter gave the bis- and mono- esters $\mathbf{17_{I}}$, $\mathbf{17_{II}}$, **18**. All products are strongly fluorescent. Compounds **8a,b, 11a, 13-18** (azabenzo analogues of khellactones) were tested for their ability to interact with DPPH, to compete with dimethylsulfoxide for hydroxyl radicals, to inhibit soybean lipoxygenase and trypsin activities *in vitro*. Compounds **16** and $\mathbf{17_{II}}$ were found to compete significantly with dimethylsulfoxide for hydroxyl radicals, whereas compounds **8a, 11a, 14** and $\mathbf{17_{I}}$ were found to inhibit strongly soybean lipoxygenase.

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Introduction.

The 2*H*-1,4-benzoxazine [1] structure is incorporated in many naturally occurring substances and biologically active compounds with anticancer [2], antibiotic [3], antitumor [4] and antirheumatic [5] activities. 2*H*-1,4-Benzoxazin-2-ones [6] disclosed also some interesting biological activities. These compounds were prepared by the reaction of *o*-aminophenols with α-ketoesters [6] or with dimethylacetylene dicarboxylate and triphenylphosphine [7]. In 1996, we reported the preparation of the corresponding 2*H*-1,4-benzoxazin-2-ones by the treatment of *O*-methyl-*o*-quinonemonoximes with arylacetates [8], while we recently prepared 2*H*-1,4-benzoxazines by the reaction of *O*-methyl-*o*-quinonemonoximes with α-benzylethoxycarbonylmethylene(triphenyl)phosphorane [9].

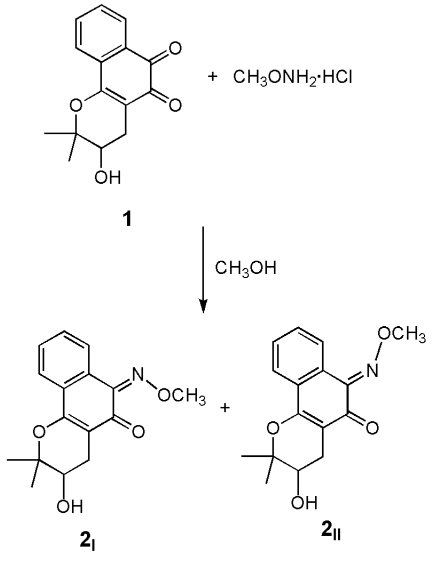
On the other hand, substituted khellactones (3',4'-dihydroxypyranocoumarins) [10] are natural products [11] exhibiting a broad range of biological activities, including antifungal, antitumor, antiviral effects and activity against HIV-1 replication [12-15]. The naturally occurring *o*-quinones [16-17] stenocarpoquinone-A **1** and the lead compound β-lapachone correlate interesting chemistry with important pharmacological properties such as anticancer activity, cytotoxic activity [18] and inhibition of DNA topoisomerase I and reverse transcriptase [17].

In continuation of our previous efforts in the synthesis of new coumarin derivatives [19] with potent antiinflammatory activity [20], we tried to combine the 2*H*-1,4-benzoxazine, khellactone and benzene (in lapachone) moieties in order to prepare the title compounds and study their chemistry and biological activities as possible antiinflammatory and antioxidant agents.

Results and Discussion.

Treatment of 3-hydroxy-β-lapachone [18] (stenocarpoquinone-A) **1** with methoxylamine hydrochloride in methanol at room temperature and separation of the reaction mixture by column chromatography gave a mixture of (*Z*)- and (*E*)-3-hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione-6-(*O*-methyloximes) $\mathbf{2_{I}}$ and $\mathbf{2_{II}}$ in 69% total yield (Scheme 1). The analytical data and the recorded mass spectrum of the mixture are in good agreement with the proposed structure of *O*-methylmonoximes **2**. The ^{1}H-nmr spectrum of the mixture showed the presence of both isomers $\mathbf{2_{I}}$, $\mathbf{2_{II}}$ in 1:2 ratio, since it exhibited a pair of doublets at δ 8.02 (H-7) and 8.62 (H-7) and a pair of singlets at δ 4.26 (CH_3O-) and 4.30 (CH_3O-), which is a strong indication for the 1:2 proportion of the isomers. The signals at δ 8.02 and 4.26 are attributed to the (*Z*)- isomer $\mathbf{2_{I}}$ and the absorptions at δ 8.62 and 4.30 to the (*E*)- isomer $\mathbf{2_{II}}$, since these absorptions are similar to the corresponding of the H-7 and OCH_3

Scheme 1



protons of (*Z*)- and (*E*)- isomers $\mathbf{3_{I}}$, $\mathbf{3_{II}}$ [9], which were gradually transformed into their mixture, quick after their separation as individual isomers.

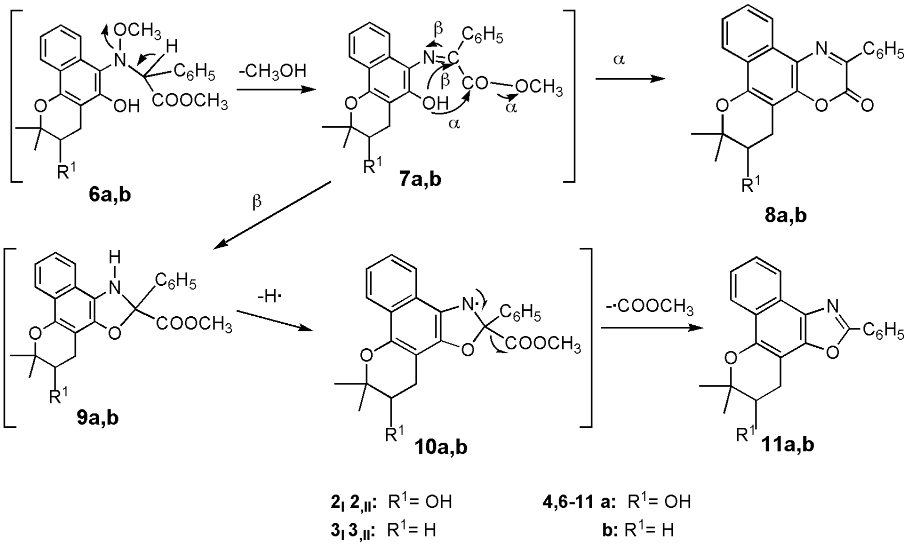
Treatment of $\mathbf{2_{(I+II)}}$ with methyl phenylacetate at 180 °C for 30 minutes and separation of the reaction mixture by column chromatography afforded 6,6-dimethyl-2-phenyl-5,6-dihydro-4*H*-benzo[7,8]chromeno[6,5-*d*][1,3]oxazol-5-ol **11a** (11%) and 6-hydroxy-7,7-dimethyl-2-phenyl-6,7-dihydro-3*H*,5*H*-benzo[7,8]chromeno[5,6-*b*][1,4]oxazin-3-one **8a** (37%). When the reaction was repeated in a higher scale compounds **11a, 8a** were obtained in 9% and 44% yields respectively.

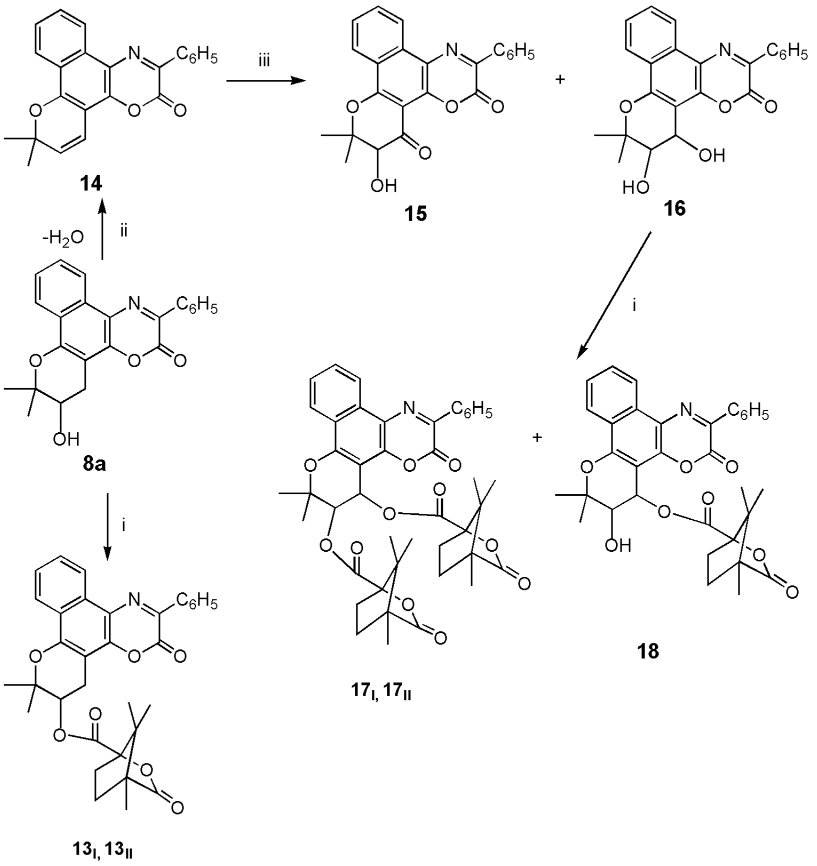
By a similar treatment of $\mathbf{3_{(I+II)}}$ with methyl phenylacetate for 1 hour and separation of the complicated reaction mixture by column chromatography, only compound **8b** was obtained in 21% yield. Compound **11b** was not detected or separated from the reaction mixture. Compounds **8, 11** can be obviously formed through the mechanism suggested in Scheme 2. The reaction between the initially formed radicals **4** and **5** through **6** followed by methanol elimination [21] can lead to the (*Z*)- and/or (*E*)-intermediate **7**. Lactonization of the (*Z*)- isomer only provides [8] compounds **8a,b**. Intramolecular nucleophilic attack of hydroxyl to the imine moiety [8,21-24] can lead to the intermediate **9**. Hydrogen abstraction by the former radicals **4, 5** present [25] lead to the radical **10** and finally to oxazole derivatives **11** by further methyl formate radical abstraction. In a recent paper, the high yield transformation of benzoxazinones to the corresponding benzoxazoles have been reported by the treatment with 10% potassium hydroxide in refluxing methanol [26]. The analytical and spectral data of the products resemble well the structures **8a, 8b, 11a** suggested for them.

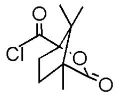
Treatment of compound **8a** with (1*S*)-(-)-camphanic chloride **12** in dry pyridine/dichloromethane at 0 °C under nitrogen atmosphere gave (Scheme 3) a mixture of diastereoisomers 7,7-dimethyl-3-oxo-2-phenyl-6,7-dihydro-3*H*,5*H*-benzo[7,8]chromeno[5,6-*b*][1,4]oxazin-6-yl 4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxylates $\mathbf{13_{I}}$, $\mathbf{13_{II}}$ in 60% yield followed by the pure diastereomer $\mathbf{13_{II}}$ (11%), which exhibited signals in its ^{1}H-nmr spectrum for camphanic CH_3 at δ 0.88, 0.93, 1.06, and finally unreacted compound **8a** (23%). Efforts for the separation of the pure stereoisomer $\mathbf{13_{I}}$ by repeated chromatographic separations were not successful. The ^{1}H-nmr spectrum of the above mixture exhibited absorptions for the camphanic CH_3 at δ 0.92, 1.01, 1.07 and 0.88, 0.93, 1.06 in 1:2 ratio. It means that the mixture constituted from $\mathbf{13_{I}}$ and $\mathbf{13_{II}}$ in 1:2 ratio and the total yield of $\mathbf{13_{II}}$ was 51%.

Treatment of compound **8a** with triphenylphosphine in dry carbon tetrachloride/acetonitrile solution [27] under reflux gave 7,7-dimethyl-2-phenyl-3*H*,7*H*-benzo[7,8]-

Scheme 2





Scheme 3. *Reagents and conditions:* i,  **12**, Dichloromethane, Pyridine, **ii**, $(C_6H_5)_3P/CCl_4$, CH_3CN, iii, OsO_4, *N*-methylmorpholine *N*-oxide, Acetone.

chromeno[5,6-*b*][1,4]oxazin-3-one **14** in 88% yield. Treatment of compound **14** with osmium tetroxide/*N*-methylmorpholine *N*-oxide [28] in acetone-water at room temperature and separation of the reaction mixture by column chromatography afforded 6-hydroxy-7,7-dimethyl-2-phenyl-6,7-dihydro-3*H*,5*H*-benzo[7,8]-chromeno[5,6-*b*][1,4]oxazine-3,5-dione **15** (23%) and 5,6-dihydroxy-7,7-dimethyl-2-phenyl-6,7-dihydro-3*H*,5*H*-benzo[7,8]chromeno[5,6-*b*][1,4]oxazine-3-one **16** (54%) together with 6% unreacted starting compound **14**. The 6-hydroxy-structure **15** instead of the 5-hydroxy structure was suggested on the basis of the comparison of the ^{1}H-nmr spectrum of the compound and especially of the signals of its 7,7-dimethyl protons at δ 1.40 and 1.86 with those of compounds **8a** (at δ 1.43 and 1.49), **11a** (1.43 and 1.58), $\mathbf{2_I}$ (1.47), $\mathbf{2_{II}}$ (1.41), **16** (1.57) and other compounds [29], since the corresponding two methyl proton signals of ethyl 2,2-dimethyl-3,6-dioxo-3,4-dihydro-2*H*,6*H*-benzo[*f*]pyrano[2,3-*h*]chromene-8-carboxylate [30] exhibited signals at δ 1.59 (6H). The formation of compound **15** can be explained by partial oxidation of the dihydroxy derivative **16** with osmium tetroxide [30,31].

Treatment of compound **16** with chloride **12** in dry pyridine/carbon tetrachloride at room temperature and separation of the reaction mixture by column chromatography gave the two isomers (5*R*, 6*R*)- and (5*S*, 6*S*)-di-*O*-(-)camphanoyl diastereoisomers (*cis*)-7,7-dimethyl-3-oxo-2-phenyl-5-{[(4,7,7-trimethyl-3-oxo-2-oxabicyclo-[2.2.1]hept-1-yl)carbonyl]oxy}-6,7-dihydro-3*H*,5*H*-benzo[7,8]chromeno[5,6-*b*][1,4]oxazin-6-yl 4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxylates $\mathbf{17_I}$ (9%) and $\mathbf{17_{II}}$ (12.5%) and 6-hydroxy-7,7-dimethyl-3-oxo-2-phenyl-6,7-dihydro-3*H*,5*H*-benzo-[7,8]chromeno[5,6-*b*][1,4]oxazin-5-yl 4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxylate **18**, in 22% yield. It was not able to assign the absolute *R,R* or *S,S* configuration at the C-5 and C-6 of compounds $\mathbf{17_I}$, $\mathbf{17_{II}}$ on the basis of the data available. We suggest the 6-hydroxy-structure **18** for the monoester in question instead of the also possible 5-hydroxy peri-isomer structure on the basis of the comparison of its recorded signals at δ 4.27 (d, 6-H) and 6.62 (d, 5-H), since the signals for the compounds **16, 17_I, 17_{II}** were observed at δ 4.00, 5.63, 5.54 for 6-H and at 5.33, 6.84, 6.78 for 5-H respectively.

All the title compounds prepared show a very strong fluorescence.

Biological Evaluation.

The compounds were screened for biological activities (Table 1) by *in vitro* assays.

Inhibitory activities were measured against isolated enzymes (soybean lipoxygenase and trypsin). A qualitative similarity between the inhibition of plant lipoxygenase activity by non-steroid anti-inflammatory drugs and the corresponding inhibition of the mammalian mast cells lipoxygenase has been reported [32]. Monohydroxy coumarins *e.g.* 7-OH, inhibited the formation of 5-lipoxygenase. Compounds **8b, 11a, 14** and **17_I** were found to highly inhibit soybean lipoxygenase. The role played by proteases in the early stage of inflammatory process is well documented. Some anti-inflammatory compounds have been reported to inhibit trypsin. Only the **17_{II}** isomer exhibits significant inhibition.

The reducing abilities of the examined compounds were determined by their interaction with the stable free radical 1,1-diphenylpicrylhydrazyl (DPPH). Antioxidants can react with DPPH producing 1,1-diphenylpicrylhydrazine [33]. 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 compounds, comparing to acetylsalicylic acid (80.5 %) or nor-dihydroguaeretic acid (94.4 %) used as standard drugs, were found to interact (16.7-80.8 %) with DPPH. For compounds **8b, 14, 15** and **17_I** the interaction increases with the increase of the concentration of the tested compounds, whereas compound **17_{II}** highly interacts (100%) in both concentrations.

Table 1

Reduction Ability[a] (RA %), Competition with Dimethylsulfoxide for Hydroxyl Radical [b] (HO· %), Soybean Lipoxygenase Inhibition [c] (LOX %), Trypsin Inhibition [d] (Itr %)

Compound	RA % 0.1mM	0.5mM	HO · % 1mM	(LOX %) 0.1mM	(Itr %) 0.1mM
8a	24.8	24.1	No	No	No
8b	16.7	36.5	No	71.4	No
11a	25	25	6.7	79.2	Nt
13_{II}	40.4	40.4	No	Nt	Nt
14	24.8	26	No	80.2	No
15	12.3	36	23.6	No	2
16	30.1	31	40.3	Nt	Nt
17_I	80.8	100	No	51.7	38.2
17_{II}	100	100	46.6	No	6
18	20	21	Nt	No	No

Nt: not tested; No: no action under the reported experimental conditions-dissolution problems; Data are means of two or three independent experiments and the deviation in absorbance values were less than 10 %; [a] Acetyl-salicylic acid as a standard 80.5 % (0.1 mM) and nor-dihydroguaeretic acid 94.4 %; [b] tocopherol acetate as a standard 83.4 %, dimethylsulfoxide as a standard 78.5 %; [c] nor–dihydroguaeretic acid 83.7 % (0.1 mM); [d] salicylic acid 18.1 % (0.1 mM).

Because hydroxyl radicals 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 hydroxyl radicals has been implicated in inflammation [34], we attempted to investigate the ability of the synthesized compounds to compete with dimethylsulfoxide for hydroxyl radicals. The competition of compound **11a** for hydroxyl radicals was very low (6.7 %), whereas for compounds **15, 16** and **17_{II}** was higher (23.6 %, 40.3 % and 46.6%). Compounds **8a-b**, **13_{II}**, and 14 did not show any effect under the reported experimental conditions for dissolution reasons.

Compounds **8a, 14** and **16** seemed to be highly cytotoxic in an anti-HIV screening test (results not shown).

Lipophilicity in our case does not seem to affect the biological activity. On the contrary sterimol parameters, expressing steric requirements must be more important. Certain similarities in the biological screening exist among the **8a, 11a, 14** and **16** structures, whereas the **17_I** isomer combines good antioxidant properties in correlation to its significant inhibition of lipoxygenase. It should be possible that no specificity exists in the presence of an 1,4-oxazine or 1,3-oxazole ring fused to naphthopyran skeleton, on the contrary the presence of the naphthopyran moiety seems to be crucial for the biological activity.

EXPERIMENTAL

Melting points are uncorrected and were measured on a Kofler hot-stage apparatus. 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 deuteriochloroform as solvent and tetramethylsilane 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 Electrospray 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. Analyses indicated by the symbols of the elements were within ±0.4% of the theoretical values. Earlier reported procedures were used for the preparation of compounds **1** [18] and **3_I, 3_{II}** [9].

Preparation of (*Z*)- and (*E*)-3-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione-6-(*O*-methyloximes) $\mathbf{2_I}$ + $\mathbf{2_{II}}$.

A solution of 3-hydroxy-β-lapachone **1** (1.0 g, 3.876 mmoles) and methoxylamine hydrochloride (324 mg, 3.879 mmoles) in methanol (75 mL) was stirred at room temperature for 2 hours, until full consumption of quinone. The solvent was evaporated in a rotary evaporator and the residue was subjected to a column chromatography (silica gel, hexane/ethyl acetate 2:1) to give a yellow solid, (1:2) mixture of isomers $\mathbf{2_I}$ and $\mathbf{2_{II}}$ (0.77 g, 69%) m.p. 180-183°C (from ether); ir: 3430, 3390, 3070, 1630, 1605 cm^{-1}; ^{1}H-nmr: δ 1.41 (s, 12H), 1.47 (s, 6H), 2.04 (brs, 3H), 2.56-2.66 (m, 3H), 2.78-2.86 (m, 3H), 3.87 (t, *J*=5.09, 3H), 4.26 (s, 3H), 4.30 (s, 6H), 7.42-7.47 (m, 6H), 7.83 (d, *J*=8.9, 1H), 7.92 (d, *J*=6.4, 2H), 8.02 (d, *J*=6.4, 1H), 8.62 (d, *J*=6.4, 2H); ^{13}C-nmr: δ 21.8, 21.9, 24.9, 25.5, 25.7, 64.9, 68.7, 80.1, 80.3, 108.8, 113.4, 123.3, 123.7, 123.8, 126.4, 128.1, 128.8, 129.1, 129.8, 130.0, 130.2, 130.6, 130.8, 144.4, 182.7; ms (ESI): m/z 288 (M+H)$^+$, 310 (M+Na)$^+$.

Anal. Calcd for $C_{16}H_{17}NO_4$: C, 66.88; H, 5.96; N, 4.88. Found: C, 67.13; H, 5.80; N, 4.81.

Reaction of Monoximes $\mathbf{2_{(I+II)}}$ with Methyl Phenylacetate.

A: A mixture of compounds $\mathbf{2_{(I+II)}}$ (150 mg, 0.52 mmole) and methyl phenylacetate (0.9 mL, excess) was heated in an oil bath at ~175-180 °C for 30 minutes and then it was concentrated in a rotary evaporator. The residue was subjected to a column chromatography (silica gel, hexane/ethyl acetate 12:1). The fractions eluted first gave compound **11a** (19 mg, 11%) as colorless crystals, m.p. 271-272 °C (from ethyl acetate/hexane); ir: 3390, 3050, 1570 cm^{-1}; ^{1}H-nmr: δ 1.43 (s, 3H), 1.58 (s, 3H), 1.79 (brs, 1H, exchanged with deuterium oxide), 3.17 (dd, 1H, J_1=3.81, J_2=17.8), 3.35 (dd, 1H, J_1=5.1, J_2=17.8), 4.04 (dd, 1H, J_1=3.81, J_2=5.1), 7.46-7.55 (m, 4H), 7.65 (t, 1H, *J*=7.6), 8.20 (d, 1H, *J*=7.6), 8.24-8.27 (m, 2H), 8.50 (d, 1H, *J*=7.6); ^{13}C-nmr: δ 22.8, 24.4, 26.6, 68.7, 78.0, 106.6, 122.0, 122.8, 123.6, 124.7, 127.0, 127.1, 127.5, 128.8, 130.5, 130.6, 155.9, 158.5, 159.6, 160.9; ms (ESI): m/z 346 (M+H)$^+$.

Anal. Calcd for $C_{22}H_{19}NO_3$: C, 76.50; H, 5.55; N, 4.06. Found: C, 76.68; H, 5.42; N, 4.07.

The next fractions gave compound **8a** (72 mg, 37%), as yellow crystals (green fluoresence), m.p. 271-273°C (from ethyl acetate/hexane); ir: 3450, 3060, 1730, 1580 cm^{-1}; ^{1}H-nmr: δ 1.45 (s, 3H), 1.51 (s, 3H), 1.60 (brs, 1H, exchanged with deuterium oxide), 3.07 (dd, 1H, J_1=5.1, J_2=17.8), 3.24 (dd, 1H, J_1=5.1, J_2=17.8), 4.03 (t, 1H, *J*=5.1), 7.52-7.62 (m, 4H), 7.72 (t, 1H, *J*=7.6), 8.25 (d, 1H, *J*=7.6), 8.47-8.51 (m, 2H), 8.82 (d, 1H, *J*=7.6); ^{13}C-nmr: δ 22.3, 24.7, 25.8, 68.5, 79.0, 102.6, 122.0, 122.6, 123.3, 126.1, 128.2, 128.3, 129.1, 130.5, 130.6, 135.0, 139.8, 153.8, 155.2, 160.0, 161.3; ms (ESI): m/z 374 (M+H)$^+$, 396 (M+Na)$^+$.

Anal. Calcd for $C_{23}H_{19}NO_4$: C, 73.98; H, 5.13; 3.75. Found: C, 73.88; H, 4.89; N, 3.74.

B: When the reaction was repeated between compounds $\mathbf{2_{(I+II)}}$ (550 mg, 1.916 mmoles) and methyl phenylacetate (2.75 mL, excess) compounds **11a** (9%) and **8a** (44%) were obtained again.

Reaction of Monoximes $\mathbf{3_{(I+II)}}$ with Methyl Phenylacetate.

A mixture of compounds $\mathbf{3_{(I+II)}}$ (121 mg, 0.446 mmole) and methyl phenylacetate (4 mL, excess) was heated in an oil bath at ~180 °C for 1 hour and then was concentrated in a rotary evaporator. The residue was separated by column chromatography (silica gel, hexane/ethyl acetate 100:1) to give yellow crystals of compound **8b** (34 mg, 21%), m.p. 220-222°C (from ether/hexane); ir: 3050, 1725, 1595 cm^{-1}; ^{1}H-nmr: δ 1.50 (s, 6H), 1.99 (t, 2H, *J*=6.4), 3.00 (t, 2H, *J*=6.4), 7.50-7.58 (m, 4H), 7.68 (t, 1H, *J*=7.6), 8.23 (d, 1H, *J*=7.6), 8.49-8.52 (m, 2H), 8.79 (d, 1H, *J*=7.6); ^{13}C-nmr: δ 16.5, 26.7, 31.5, 76.5, 104.5, 121.8, 122.4, 123.6, 125.8, 128.0, 128.2, 128.9, 129.7, 130.2, 134.9, 135.1, 143.1, 145.3, 153.3, 159.3; ms (ESI): m/z 358 (M+H)$^+$, 380 (M+Na)$^+$; For $C_{23}H_{20}NO_3$ hrms Calcd. 358.1443 (MH$^+$); Found 358.1426.

Anal. Calcd for $C_{23}H_{19}NO_3$: C, 77.29; H, 5.36; N, 3.92. Found: C, 77.51; H, 5.43; N, 3.91.

Reaction of Compound **8a** with (1*S*)-(-)-Camphanic Chloride **12**.

A solution of compound **8a** (51 mg, 0.136 mmole) and compound **12** (122 mg, 0.563 mmole) in a dry mixture of dichloromethane (4 mL) and pyridine (1 mL) was stirred at room temperature for 16 hours. The mixture was then concentrated in a rotary evaporator and the residue was treated with water (10 mL) and the mixture was extracted with chloroform (10 mL). The organic layer was washed with water (5 mL) and brine (5 mL), dried (sodium sulfate) and concentrated in a rotary evaporator. The residue was subjected to column chromatography (silica gel, dichloromethane/ethyl acetate 50:1). The fractions eluted first gave a yellow solid of a mixture of diastereoisomers $\mathbf{13_{I,}}$ $\mathbf{13_{II}}$ (45 mg, 60%), with ^{1}H-nmr: δ 0.88 (s, 6H), 0.92 (s, 3H), 0.93 (s, 6H), 1.01 (s, 3H), 1.06 (s, 6H), 1.07 (s, 3H), 1.51 (s, 6H), 1.53 (s, 12H), 1.56-1.74 (m, 3H), 1.81-1.95 (m, 3H), 1.96-2.14 (m, 3H), 2.30-2.45 (m, 3H), 3.06 (dd, 1H, J_1=5.5, J_2=17.3), 3.12 (dd, 2H, J_1=5.5, J_2=17.3), 3.43 (dd, 3H, J_1=5.5, J_2=17.3), 5.35 (t, 3H, *J*=5.5), 7.42 (t, 1H, *J*=7.6), 7.51-7.55 (m, 9H), 7.60 (t, 2H, *J*=8.2), 7.74 (t, 2H, *J*=8.2), 7.89 (t, 1H, *J*=7.6), 8.26 (d, 2H, *J*=8.2), 8.33 (d, 1H, *J*=7.6), 8.48-8.51 (m, 6H), 8.85 (d, 3H, *J*=8.2). The fractions eluted next gave yellow crystals of compound $\mathbf{13_{II}}$ (8 mg, 11%, total 38 mg, 51%), m.p. 268-270°C (from ether/hexane); ir: 1785, 1730, 1690, 1580 cm^{-1}; ^{1}H-nmr: δ 0.88 (s, 3H), 0.93 (s, 3H), 1.06 (s, 3H) 1.51 (s, 3H), 1.53 (s, 3H), 1.56-1.74 (m, 1H), 1.81-1.95 (m, 1H), 1.96-2.14 (m, 1H), 2.30-2.45 (m, 1H), 3.12 (dd, 1H, J_1=5.5, J_2=17.3), 3.43 (dd, 1H, J_1=5.5, J_2=17.3), 5.35 (t, 1H, *J*=5.5), 7.51-7.55 (m, 3H), 7.60 (t, 1H, *J*=8.2), 7.74 (t, 1H, *J*=8.2), 8.26 (d, 1H, *J*=8.2), 8.48-8.51 (m, 2H), 8.85 (d, 1H, *J*=8.2); ^{13}C-nmr: δ 9.6, 16.7, 22.7, 23.1, 24.9, 28.9, 30.8, 54.1, 54.7, 71.2, 90.7, 101.7, 122.0, 122.6, 123.2, 126.2, 126.9, 128.3, 128.5, 129.0, 129.9, 130.4, 130.6, 132.8, 140.6, 144.2, 148.0, 157.5, 159.0, 177.7, 180.3; ms (EI): m/z 553 (35, M$^+$), 525 (10), 355 (20), 312 (75), 273 (70), 105 (100), 77 (65); For $C_{33}H_{32}NO_7$ hrms Calcd. 554.2173 (MH$^+$); Found 554.2155. Unreacted compound **8a** was eluted next (12 mg, 23%).

Dehydration of Compound **8a**. Preparation of Compound **14**.

A solution of compound **8a** (314 mg, 0.84 mmole) and triphenylphosphine (655 mg, 2.49 mmoles) in a mixture of carbon tetrachloride (11 mL) and acetonitrile (11 mL) was heated under reflux for 2.5 hours. The mixture was concentrated in a rotary evaporator and the residue was subjected to column chromatography (silica gel, hexane/ethyl acetate 25:1) to give yellow crystals of compound **14** (0.261 g, 88%), m.p. 213-215°C (from ethyl acetate/hexane); ir: 1710, 1585 cm^{-1}; ^{1}H-nmr: δ 1.61 (s, 6H), 5.82 (d, 1H, *J*=10.2), 6.95 (d, 1H, *J*=10.2), 7.52-7.59 (m, 4H), 7.69 (t, 1H, *J*=7.6), 8.22 (d, 1H, *J*=8.9), 8.49-8.53 (m, 2H), 8.79 (d, 1H, *J*=7.6); ^{13}C-nmr: δ 28.2, 78.7, 105.0, 115.0, 121.1, 122.1, 122.7, 122.9, 126.0, 128.3, 128.6, 128.9, 129.7, 130.4, 134.9, 142.1, 143.8, 152.3, 153.0, 159.0; ms (ESI): m/z 356 (M+H)$^+$, 378 (M+Na)$^+$.

Anal. Calcd for $C_{23}H_{17}NO_3$: C, 77.73; H, 4.82; N, 3.94. Found: C, 77.55; H, 4.72; N, 3.88.

Reaction of Compound **14** with Osmium Tetroxide. Preparation of Compounds **15** and **16**.

To a solution of compound **14** (200 mg, 0.56 mmole) and *N*-methylmorpholine *N*-oxide (133 mg, 1.13 mmoles) in 80% aqueous acetone (28 mL, free from ethanol) an 4% aqueous solution of osmium tetroxide (0.46 mL) was added and the mixture was stirred at room temperature for 18 hours. Water (15 mL) was then added and the mixture was concentrated in a rotary evaporator to an aqueous residue, which was extracted with ethyl acetate (3x15 mL). The organic layer was dried over anhydrous sodium sulfate, the solvent was evaporated and the residue was separated by column chromatography (silica gel, hexane/ethyl acetate 6:1). The fractions eluted first gave yellow crystals of compound **15** (50 mg, 23%), m.p. 214-216 °C (from ethyl acetate/hexane); ir: 3460, 3040, 1685, 1675, 1625, 1590 cm^{-1}; ^{1}H-nmr: δ 1.40 (s, 3H), 1.86 (s, 3H), 3.94 (s, 1H, exchanged with deuterium oxide), 4.63 (s, 1H), 7.53-7.63 (m, 4H), 7.82 (t, 1H, *J*=7.6), 8.33-8.36 (m, 2H), 8.39 (d, 1H, *J*=8.9), 8.51 (d, 1H, *J*=8.9); ^{13}C-nmr: δ 17.1, 26.9, 76.4, 85.6, 102.8, 110.4, 122.5, 123.2, 124.7, 125.8, 127.2, 128.9, 130.0, 130.9, 131.1, 132.3, 143.4, 157.2, 162.5, 191.1; ms (ESI): m/z 388 (M+H)$^+$.

Anal. Calcd for $C_{23}H_{17}NO_5$: C, 71.31; H, 4.42; N, 3.62. Found: C, 71.38; H, 4.46; N, 3.69.

The fractions eluted next gave yellow crystals of compound **16** (117 mg, 54%), m.p. 238-240 °C (from ethanol); ir: 3430, 1710, 1570 cm^{-1}; ^{1}H-nmr: δ 1.57 (s, 6H), 3.23 (s, 2H, exchanged with deuterium oxide), 4.00 (d, 1H, *J*=5.08), 5.33 (d, 1H, *J*=5.08), 7.51-7.62 (m, 4H), 7.74 (t, 1H, *J*=7.6), 8.28 (d, 1H, *J*=7.6), 8.45-8.48 (m, 2H), 8.81 (d, 1H, *J*=7.6); ^{13}C-nmr: δ 21.5, 25.3, 61.4, 71.2, 80.0, 105.8, 109.6, 121.4, 122.6, 122.9, 123.3, 126.3, 128.4, 129.0, 129.1, 129.6, 130.7, 145.6, 151.5, 152.8, 160.4; ms (ESI): m/z 390 (M+H)$^+$, 412 (M+Na)$^+$.

Anal. Calcd for $C_{23}H_{19}NO_5$: C, 70.94; H, 4.92; N, 3.60. Found: C, 70.59; H, 4.92; N, 3.83.

Reaction of Compound **16** with Compound **12**. Preparation of Compounds **17**$_{\mathbf{I}}$, **17**$_{\mathbf{II}}$, **18**.

To a solution of compound **16** (91 mg, 0.23 mmole) in a mixture of dry dichloromethane (4 mL) and dry pyridine (1 mL) compound **12** (203 mg, 0.94 mmole) was added at 0 °C and the mixture was stirred at room temperature for 18 hours under nitrogen. The mixture was concentrated in a rotary evaporator and the residue was treated with water (10 mL) and extracted with chloroform (10 mL). The organic layer was washed with water (5 mL), then with brine (5 mL), was dried over anhydrous sodium sulfate and was concentrated. The residue was subjected to column chromatography (silica gel, dichloromethane/ethyl acetate 30:1). The fractions eluted first gave yellow crystals of compound **17**$_{\mathbf{I}}$ (16 mg, 9%), m.p. 254-256 °C (from ethanol); ir (dichloromethane): 3035, 1780, 1740, 1730, 1690, 1670, 1600 cm^{-1}; ^{1}H-nmr: δ 0.99 (s, 3H), 1.06 (s, 3H), 1.09 (s, 3H), 1.12 (s, 3H), 1.15 (s, 3H), 1.55 (s, 3H), 1.61 (s, 3H), 1.69 (s, 3H), 1.84-1.99 (m, 3H), 2.04-2.22 (m, 3H), 2.27-2.44 (m, 1H), 2.51-2.64 (m, 1H), 5.63 (d, 1H, *J*=3.8), 6.84 (d, 1H, *J*=3.8), 7.50-7.53 (m, 3H), 7.63-7.80 (m, 2H), 8.28 (d, 1H, *J*=8.9), 8.42-8.50 (m, 2H), 8.86 (d, 1H, *J*=7.6); ms (EI): m/z 749 (5, M$^+$), 170 (40), 152 (25), 139 (80), 125 (40), 109 (99), 83 (85), 55 (100); For $C_{43}H_{43}NO_{11}Na$ hrms Calcd. 772.2728 (MNa$^+$); Found 772.2742.

The fractions eluted next gave yellow crystals of compound **17**$_{\mathbf{II}}$ (22 mg, 12.5%), m.p. 212-214 °C (from ether/hexane); ir (dichloromethane): 3035, 1780, 1745, 1725, 1690, 1675, 1570 cm^{-1}; ^{1}H-nmr: δ 1.00 (s, 3H), 1.01 (s, 3H), 1.08 (s, 3H), 1.11 (s, 3H), 1.13 (s, 3H), 1.57 (s, 6H), 1.62 (s, 3H), 1.60-1.78 (m, 2H), 1.85-2.00 (m, 2H), 2.21-2.33 (m, 2H), 2.45-2.59 (m, 2H), 5.54 (d, 1H, *J*=5.08), 6.78 (d, 1H, *J*=5.08), 7.45-7.58 (m, 3H), 7.63 (t, 1H, *J*=7.6), 7.80 (t, 1H, *J*=7.6), 8.28 (d, 1H, *J*=8.9), 8.42-8.50 (m, 2H), 8.87 (d, 1H, *J*=7.6); ms (EI): m/z 749 (25, M$^+$), 326 (10), 170 (28), 139 (60), 125 (30), 109 (100), 83 (65); For $C_{43}H_{43}NO_{11}Na$ hrms Calcd. 772.2728 (MNa$^+$); Found 772.2738.

The following fractions afforded yellow crystals of compound **18** (29 mg, 22%), m.p. 226-228 °C (from ether/hexane); ir: 3430, 3040, 1780, 1730, 1690, 1600, 1580 cm^{-1}; ^{1}H-nmr: δ 1.02 (s, 3H), 1.05 (s, 3H), 1.09 (s, 3H), 1.66 (s, 6H), 1.85-1.97 (m, 2H), 2.08-2.21 (m, 1H), 2.41-2.58 (m, 1H), 2.72 (brs, 1H), 4.25 (d, 1H, *J*=5.1), 6.62 (d, 1H, *J*=5.1), 7.48-7.52 (m, 3H), 7.60 (t, 1H, *J*=7.6), 7.76 (t, 1H, *J*=7.6), 8.28 (d, 1H, *J*=7.6), 8.43-8.47 (m, 2H), 8.83 (d, 1H, *J*=7.6); ms (EI): m/z 569 (5, M$^+$), 372 (60), 344 (45), 330 (30), 316 (35), 274 (53), 200 (50), 172 (50), 153 (60), 139 (100); For $C_{33}H_{31}NO_8Na$ hrms Calcd. 592.1942 (MNa$^+$); Found 592.1937.

Competition of the Tested Compounds with Dimethylsulfoxide for Hydroxyl Radicals.

The hydroxyl radicals generated by the Fe^{3+}/ascorbic acid system were detected by the determination of formaldehyde produced from the oxidation of dimethylsulfoxide [26]. The reaction mixture contained EDTA (0.1mM), Fe^{3+} (167 μM, as a 1:2 mixture with EDTA), dimethylsulfoxide (33mM), in phosphate buffer (50 mM, pH 7.4), the tested compounds (final concentration 1mM-final volume of the samples 1 mL). 150 μL of ascorbic acid (10 mM in phosphate buffer was added at the end in order to have the reaction started). The mixture was incubated at 37 °C for 30 min. The reaction was stopped by the addition of 250 μL trichloroacetic acid (17.5 % w/v) and the formaldehyde formed was detected spectrophotometrically at 412 nm by the method of Nash [35].

Interaction of the Synthesized Compounds with DPPH [34,36].

To a solution of DPPH (0.1- 0.5 mM) in absolute ethanol, an equal volume of the compounds dissolved in ethanol was added (0.1 mM). A control solution containing ethanol was also used. After 20 and 60 min at room temperature, absorbance was recorded at 517 nm (Table 1).

Soybean Lipoxygenase Inhibition [36].

The tested compounds, dissolved in 60% aqueous ethanol (final concentration 0.1 mM), were incubated at room temperature 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 (nordihydroguaretic acid 0.1mM, 83.7%).

Trypsin Inhibition [37].

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). The test compounds (0.1 mM) dissolved in 50% methanol was added. 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 over the next 4 min.

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