Synthesis and Biological Evaluation of some 4-(Isoxazolinyl or 1,2,4-Oxadiazolyl) Coumarins

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Received February 20, 1996

Dedicated to the memory of Professor Nicholas Alexandrou

Reaction of compound **3** with nitrile oxide **4a** affords compounds **5a** and **6** in 73% and 3% yield respectively, while reaction of **3** with **4b** affords only compound **5b** (85%). Reactions of compound **8** with the nitrile oxides **4a,b** result in compounds **9a,b**. The compound **10**, prepared from **1** and *O*-methylhydroxylamine, reacts with nitrile oxide **4b** to give the oxadiazole derivative **12**. The above referred coumarins are screened for antiinflammatory activity *in vivo* using the carrageenin rat paw edema and *in vitro* through their antiproteolytic activity and their ability to inhibit β-glucuronidase and 12-Lipoxygenase.

J. Heterocyclic Chem., **33**, 967 (1996).

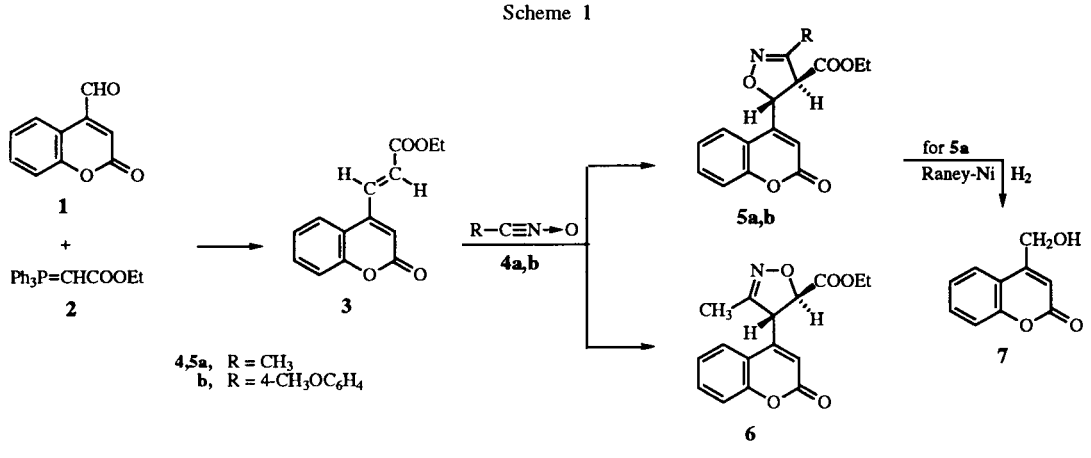
Considerable interest has been shown in coumarinyl-heterocycles on account of their pharmacological activity [1-3]. 5-Aryl-2-(coumarin-3-yl)-1,3,4-oxadiazoles exhibited antidepressant activity [4], while 5-aryl-3-(7-hydroxycoumarin-8-yl)isoxazoles reveal notable biological activities [5,6]. In connection with our previous work on the synthesis of coumarin derivatives [7-11], in the present paper we describe the preparation of the title 4-substituted coumarins, as well as their antiinflammatory activity. The reactions studied and the products obtained are depicted in Schemes 1-3.

Treatment of 4-formylcoumarin **1** [12] with ethoxycarbonylmethylene(triphenyl)phosphorane **2** in benzene solution, under reflux, gave ethyl (*E*-)-3-(2-oxo-2*H*-[1]benzopyran-4-yl)propenoate **3** in 72% yield. The also possible [13] olefination product from the Wittig reaction of the lactone carbonyl was not isolated or detected in the reaction products. The analytical and spectral data of the product are in good agreement with structure **3**.

A chloroform solution of compound **3** was treated with acetonitrile oxide **4a**, prepared *in situ* [14] from nitroethane, benzenesulfonyl chloride and triethylamine, to give ethyl 5-(2-oxo-2*H*-[1]benzopyran-4-yl)-3-methyl-4,5-dihydroisoxazole-4-carboxylate **5a** and ethyl 4-(2-oxo-2*H*-[1]benzopyran-4-yl)-3-methyl-4,5-dihydroisoxazole-5-carboxylate **6** in 73% and 3% yield respectively. Although it has been reported recently [15] that nitrile oxides react easily with the ethylenic bond of the coumarin system to give through 1,3-dipolar cycloaddition 3a,9b-dihydro-4-oxo[1]benzopyrano[3,4-*d*]isoxazoles, the analytical and spectral data of the products resemble well to the structures **5a** and **6** suggested for them. Both regioisomers show the expected carbonyl absorptions for ester group and mainly for coumarin ring at about 1700-1725 cm^{-1}, while the carbonyl of the dihydrobenzopyran ring absorbs at 1750-1770 cm^{-1} [16-17]. Furthermore catalytic hydrogenation of the main product **5a** in aqueous methanol over Raney Ni, in the presence of boric acid at rt afforded 4-hydroxymethylcoumarin **7** [12], a fact that supports beyond any doubt the suggested regio form for it.

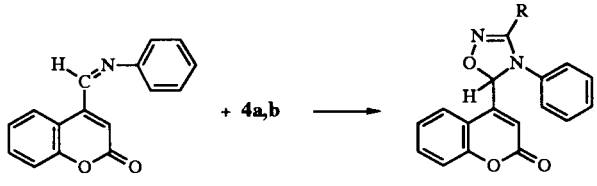
An ethereal solution of 4-methoxybenzonitrile oxide **4b** soon after its preparation from 4-methoxybenzhydroximoyl

Scheme 1



chloride and triethylamine at 0°, was added to a chloroform solution of compound **3** at rt. Separation of the reaction mixture by column chromatography gave only regioisomer **5b** in 85% yield. All efforts to isolate the regio-isomer **6b** remained unsuccessful, although it was previously reported [18] that reaction of nitrile oxides with methyl cinnamate gives both regioisomers in a ratio ~70:30. The chemical shifts of the protons of the isoxazoline ring of the product are similar to those of the protons of compound **5a**, in agreement with the regio-form **5b** proposed for it.

Scheme 2



Then we studied the reaction of nitrile oxides **4a,b** with the known [19] 4-[(phenylimino)methyl]-2*H*-[1]benzopyran-2-one **8** under the conditions described above. Treatment of compound **8** with compound **4a** for 20 hours at rt afforded 5-(2-oxo-2*H*-[1]benzopyran-4-yl)-3-methyl-4-phenyl-4,5-dihydro-1,2,4-oxadiazole **9a** (Scheme 2) in 47% yield, along with unreacted starting imine **8** (24%). By a similar treatment of **8** with excess of nitrile oxide **4b** 5-(2-oxo-2*H*-[1]benzopyran-4-yl)-3-(4-methoxyphenyl)-4-phenyl-4,5-dihydro-1,2,4-oxadiazole **9b** was obtained in 80% yield along with compound **8** (4%). Compounds **9a, 9b** exhibit carbonyl absorptions at 1720 and 1710 cm^{-1} respectively like compounds **5a,b, 6** in agreement again with the 4-substituted coumarin structure proposed for them.

Finally we studied the reactions depicted in Scheme 3. A solution of equimolar amounts of aldehyde **1** and *O*-methylhydroxylamine hydrochloride in aqueous ethanol solution was heated under reflux for 4 hours in the presence of sodium acetate to give compound **10** in 84% yield. Treatment of oxime **10** with nitrile oxide **4b** at rt for 3 days and separation of the reaction mixture with column chromatography gave 5-(2-oxo-2*H*-[1]benzopyran-4-yl)-3-(4-methoxyphenyl)-1,2,4-oxadiazole **12** (22%), obviously *via* an initial intermediate **11** and further methanol elimination. No efforts were made to optimize the yield.

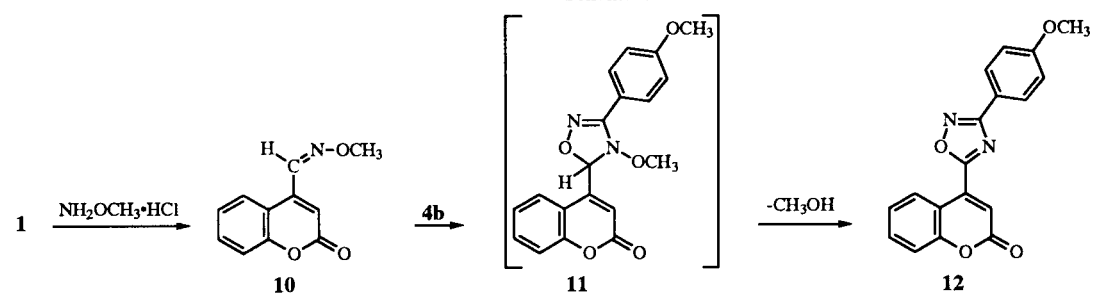
Biological Evaluation.

Eight coumarins with varying degrees of substitution, were screened for their reducing ability, antiproteolytic activity, their ability to inhibit β-glucuronidase and 12-Lipoxygenase.

The antiinflammatory activity of compound **9b** was evaluated in terms of effect on carrageenin induced paw edema in rats. In particular, compound **9b** (64.6%) could be verified to exert an effect significant higher than that of indomethacin (53.3%). Compounds **3, 8, 9a, 9b** and **10**, were found to interact significantly with the stable radical 1,1-diphenyl-2-picrylhydrazyl (98.2-45.1%), while acetylsalicylic acid showed 80.5% (standard drug). Compounds **12** and **5b** slightly interact (12.2-17.6%). The interaction of the examined compounds with 1,1-diphenyl-2-picrylhydrazyl, indicates that they are able to scavenge free radicals [20].

As far as proteolytic activity is concerned, compounds **5a, 8, 9a** and **10** exhibited good responses to this hydrolysis (64.0-43.0). Compounds **3** and **12** can be regarded as inactive, while sodium salicylate showed 53.6% (standard drug). This activity did not proceed in parallel with that of reducing ability (with the exception of compounds **5a, 8, 9a** and **10**). Under experimental conditions, none of the examined compounds inhibit β-glucuronidase which plays an important role in the progress of inflammation. In the soybean 12-Lipoxygenase assay the tested compounds **3, 8, 9a, 9b** and **10** were found inactive. However the significant inhibition in the carrageenin paw edema [21] of compounds **3, 8, 9a, 9b** and **10** (54-64.6%) suggest a possible anticyclooxygenase activity. In general the compounds which act as antioxidants and superoxide anion scavengers would be good candidates as cyclooxygenase inhibitors. This could be possible for these compounds which showed inactive in the 12-Lipoxygenase assay. Furthermore, coumarins are recognized as inhibitors of the pro-inflammatory lipoxygenase and cyclooxygenase pathways of arachidonate metabolism [22, 23]. Inhibition of superoxide generation [24],

Scheme 3



especially by those coumarins possessing *ortho*-dihydroxyl functions, has also been reported [23]. Further investigation is in progress for elucidating the influence on cyclooxygenase and their mechanism of action.

Lipophilicity, in conjunction with inflammation seems to be an important physicochemical parameter for this group of compounds, but we can not insist on the idea that only an ideal lipophilic character is necessary for higher activity. In so far as the structure-activity relationships are concerned, some more derivatives are needed to be studied in order to define the effect that is important for the biological response.

In conclusion, the obtained results indicate that the synthesized coumarins interacted with 1,1-diphenyl-2-picrylhydrazyl and four of them can inhibit *in vitro* proteolysis. Evidently compound **9b**, a non acidic derivative, showed a good overall profile of biological activity. Further investigations are in progress.

Table 1

Reducing ability (RA %), inhibition *in vitro* of trypsin induced proteolysis (Ipr %). In vivo inhibition of carrageenin rat paw edema (CPE%±SD).

No	RA %	Ipr %	CPE
3	64.7	no [a]	54.0 ± 2.7 [b]
5a	29.2	43.0	nt [c]
5b	17.6	nt [c]	nt [c]
8	85.0	64.0	55.4 ± 4.8 [b]
9a	45.2	56.3	58.5 ± 4.5 [b]
9b	98.2	14.2	64.6± 3.1 [b]
10	45.1	56.8 [d]	59.8 ± 4.7 [b]
12	12.2	no [a, d]	nt [c]

[a]: No activity. [b]: Significant level $p < 0.001$ compared to controls (student's T-test). [c]: Not tested. [d]: Tested in 1m*M*.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. The ir spectra were obtained using a Perkin-Elmer 297 spectrophotometer as Nujol mulls. The ^{1}H nmr spectra were recorded with deuteriochloroform as solvent on a Bruker Model AW 80 (80 MHz) or on a Bruker AM 300 (300 MHz) spectrometer, with tetramethylsilane as the internal standard. Mass spectra were determined on a VG-250 spectrometer with ionization energy maintained at 70 eV. Microanalyses were performed on a Perkin-Elmer 240B CHN analyser.

Ethyl (*E*-)-3-(2-oxo-2*H*-[1]benzopyran-4-yl)propenoate (**3**).

A solution of compounds **1** (0.522 g, 3 mmoles) and **2** (1.253 g, 3.6 mmoles) in benzene (25 ml) was heated at reflux for 8 hours. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel column (3:1 *n*-hexane/ethyl acetate as eluent) to give pale yellow crystals of compound **3** (0.527 g, 72%), mp 69-70° (*n*-hexane/ethyl acetate); ir: 1720, 1705 cm^{-1}; ^{1}H nmr (80 MHz): δ 1.36 (t, J = 8 Hz, 3H), 4.31 (q, J = 8 Hz, 2H), 6.52 (s, 1H), 6.56 (d, J = 16.8 Hz, 1H), 7.10-7.75 (m, 4H), 7.91 (d, J = 16.8 Hz, 1H); ms: m/z 244 [M$^+$] (30), 199 (9), 171 (100), 115 (32), 89 (10), 63 (10).

Anal. Calcd. for $C_{14}H_{12}O_4$: C, 68.85; H, 4.95. Found: C, 69.11; H, 4.90.

Reaction of Acetonitrile Oxide **4a** with Compound **3**. Preparation of Ethyl 5-(2-Oxo-2*H*-[1]benzopyran-4-yl)-3-methyl-4,5-dihydroisoxazole-4-carboxylate (**5a**) and Ethyl 4-(2-oxo-2*H*-[1]benzopyran-4-yl)-3-methyl-4,5-dihydroisoxazole-5-carboxylate (**6**).

Benzenesulfonyl chloride (0.353 g, 2 mmoles) was added dropwise to a solution of nitroethane (75 mg, 1 mmole), compound **3** (0.244 g, 1 mmole) and triethylamine (0.202 g, 2 mmoles) in chloroform (20 ml) and the mixture was stirred at room temperature for 24 hours. Tlc examination of the reaction mixture showed that compound **3** was not consumed; an equal amount of the chloride, nitromethane and triethylamine was added again to the reaction mixture and stirring was continued for further 24 hours. After partial evaporation of the solvent, compound **5a** was precipitated as white crystals (0.172 g, 58%), mp 139-140° (dichloromethane/ethyl ether); ir: 1725, 1685, 1615, 1600 cm^{-1}; ^{1}H nmr (80 MHz): δ 1.36 (t, J = 8 Hz, 3H), 2.10 (s, 3H), 3.91 (d, J = 6.4 Hz, 1H), 4.35 (q, J = 8 Hz, 2H), 6.20 (d, J = 6.4 Hz, 1H), 6.56 (s, 1H), 7.10-8.10 (m, 4H); ms: m/z 301 [M$^+$] (21), 273 (7), 255 (17), 217 (6), 193 (8), 147 (20), 133 (22), 102 (79), 73 (100).

Anal. Calcd. for $C_{16}H_{15}NO_5$: C, 63.78; H, 5.02; N, 4.65. Found: C, 63.48; H, 5.09; N, 4.70.

Filtrates were evaporated under reduced pressure and the residue was chromatographed on silica gel column (5:1 *n*-hexane/ethyl acetate as eluent) to give an additional amount of compound **5a** (44 mg, total yield 73%). Compound **6** was eluted second as white crystals (10 mg, 3%), mp 111-113° (dichloromethane/ethyl ether); ir: 1745, 1705, 1600 cm^{-1}; ^{1}H nmr (80 MHz): δ 1.33 (t, J = 7 Hz, 3H), 2.02 (s, 3H),4.31 (q, J = 7 Hz, 2H), 4.89 (br s, 2H), 6.21 (s, 1H), 7.20-7.85 (m, 4H); ^{1}H nmr (80 MHz) (hexadeuteriobenzene): δ 0.89 (t, J = 7 Hz, 3H), 1.33 (s, 3H), 3.90 (q, J = 7 Hz, 2H), 4.48 (d, J = 4 Hz, 1H), 4.52 (d, J = 4 Hz, 1H), 5.98 (s, 1H), 6.60-7.0 (m, 3H), 7.51 (d, J = 8 Hz, 1H); ms: m/z 301 [M$^+$] (12), 228 (62), 200 (80), 187 (15), 171 (16), 143 (24), 131 (50), 115 (100), 102 (34).

Anal. Calcd. for $C_{16}H_{15}NO_5$: C, 63.78; H, 5.02; N, 4.65. Found: C, 63.70; H, 5.11; N, 4.72.

Reduction of compound **5a**.

To a solution of compound **5a** (126 mg, 0.4 mmole) in a mixture of 8:2:5 methanol/water/ethyl acetate (6 ml) boric acid (63.5 mg, 1 mmole) and a spatula tip (estimated 10 mg) of W-2 Raney Nickel (Fluke AG) were added. The mixture was placed under nitrogen by repeated (5 times) evacuation and flushing with hydrogen gas, by means of a balloon attached to a three-way stopcock. The mixture was stirred vigorously for 24 hours and then filtered through Celite into a separating funnel containing water/methylene chloride (1:1) (35 ml). After separation the aqueous layer was extracted with methylene chloride (3 x 20 ml) and the combined organic layers were washed with brine (2 x 15 ml), dried over anhydrous sodium sulfate and the solvent was concentrated to a small volume to give white crystals of compound **7** (12 mg, 17 %), mp 137-139° (lit [12] 137-138°).

Ethyl 4-(2-Oxo-2*H*-[1]benzopyran-4-yl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazole-5-carboxylate (**5b**).

To a stirred solution of 4-methoxybenzhydroximoyl chloride [25] (0.204 g, 1.1 mmoles) in ethyl ether (20 ml) a solution of triethylamine (0.111 g, 1.1 mmoles) in ether (5 ml) at 0° was added and the reaction mixture was filtered. The filtrate was then added to a stirred solution of compound **3** (0.244 g, 1 mmole) in chloroform (10 ml) and the mixture was left under stirring at rt for 21 hours. Additional amounts of the hydroximoyl chloride (0.051 g, 0.275 mmoles) and triethylamine (0.028 g, 0.0275 mmoles) were then added to the reaction mixture and the stirring was continued for 3 hours. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel column (4:1 *n*-hexane/ethyl acetate as eluant) to give compound **5b** (0.334 g, 85%), mp 168-170° (ethyl acetate); ir: 1725, 1600 cm^{-1}; ^1H nmr (80 MHz): δ 1.23 (t, J = 8 Hz, 3H), 3.79 (s, 3H), 4.28 (q, J = 8 Hz, 2H), 4.37 (d, J = 4.8 Hz, 1H), 6.24 (d, J = 4.8 Hz, 1H), 6.61 (s, 1H), 6.88 (d, J = 8.8 Hz, 2H), 7.10-7.90 (m, 6H); ms: m/z 393 [M^+] (100), 320 (80), 292 (58), 276 (18), 215 (55), 186 (98), 175 (67), 146 (79), 145 (89), 133 (83), 118 (53).

Anal. Calcd. for $C_{22}H_{19}NO_6$: C, 67.17; H, 4.87; N, 3.56. Found: C, 67.24; H, 5.00; N, 3.65.

5-(2-Oxo-2*H*-[1]benzopyran-4-yl)-3-methyl-4-phenyl-4,5-dihydro-1,2,4-oxadiazole (**9a**).

Benzenesulfonyl chloride (0.353 g, 2 mmoles) was added dropwise to a solution of nitroethane (75 mg, 1 mmole), compound **8** (0.249 g, 1 mmole) and triethylamine (0.202 g, 2 mmoles) in chloroform (10 ml) and the mixture was stirred at rt for 20 hours. Water (15 ml) was added and the mixture was extracted with methylene chloride (3 x 10 ml). The combined extract was washed with water (3 x 10 ml), dried over anhydrous sodium sulfate and the solvent was evaporated *in vacuo*. The residue was chromatographed on silica gel column (4:1 *n*-hexane/ethyl acetate as eluent) to give first the unreacted imine **8** (60 mg, 29%) and second compound **9a** (0.143 g, 47%), mp 169-171° (methylene chloride/*n*-hexane); ir: 1710, 1590 cm^{-1}; ^1H nmr (80 MHz): δ 2.02 (s, 3H), 6.52 (s, 1H), 6.67 (s, 1H), 7.02-7.65 (m, 8H), 7.80 (d, J = 8 Hz, 1H); ms: m/z 306 [M^+] (48), 278 (9), 249 (8), 220 (17), 161 (100), 145 (9), 133 (24), 118 (27), 77 (22).

Anal. Calcd. for $C_{18}H_{14}N_2O_3$: C, 70.58; H, 4.61; N, 9.14. Found: C, 70.68; H, 4.71; N, 9.30.

5-(2-Oxo-2*H*-[1]benzopyran-4-yl)-3-(4-methoxyphenyl)-4-phenyl-4,5-dihydro-1,2,4-oxadiazole (**9b**).

To a stirred solution of compound **8** (0.249 g, 1 mmole) in chloroform (10 ml) a solution of nitrile oxide **4b** [prepared from 4-methoxybenhydroximoyl chloride (0.204 g, 1.1 mmoles) as it is described in the preparation of compound **5b**] in ethyl ether (25 ml) was added. The mixture was stirred at rt for 24 hours. During this time an additional amount of nitrile oxide **8** (prepared from 0.408 g, 2.2 mmoles of 4-methoxybenzhydroximoyl chloride) was added in small doses. The reaction mixture was filtered, the filtrate was condensed by evaporation under reduced pressure and the residue was chromatographed on silica gel column (4:1 *n*-hexane/ethyl acetate as eluent) to give compound **9b** (0.320 g, 80%), mp 160-162° (methylene chloride/ethyl ether); ir: 1720, 1595 cm^{-1}; ^1H nmr (80 MHz): δ 3.79 (s, 3H), 6.70-7.90 (m, 15H); ms: m/z 398 [M^+] (100), 253 (75), 225 (75), 222 (27), 221 (46), 209 (43), 189 (34), 149 (73), 118 (54), 77 (76).

Anal. Calcd. for $C_{24}H_{18}N_2O_4$: C, 72.35; H, 4.55; N, 7.03. Found: C, 72.48; H, 4.70; N, 7.28.

O-Methyl-4-(2-oxo-2*H*-[1]benzopyran)carboxaldehyde Oxime (**10**).

To a solution of compound **1** (0.522 g, 3 mmoles) in aqueous ethanol (5.5 ml, ethanol/water= 5/0.5), *O*-methylhydroxylamine hydrochloride (0.251 g, 3 mmoles) and sodium acetate (0.123 g, 1.5 mmoles) were added and the mixture was heated under reflux for 4 hours. Ice-water (50 ml) was added, the precipitate was filtered and washed with water to give compound **10** (0.51 g, 84%), mp 109-110° (methanol); ir: 1725, 1610, 1600 cm^{-1}; ^1H nmr (80 MHz): δ 4.09 (s, 3H), 6.54 (s, 1H), 7.16-7.62 (m, 3H), 8.21 (s, 1H), 8.28 (d, J = 8 Hz, 1H); ms: m/z 203 [M^+] (62), 175 (8), 172 (13), 160 (13), 143 (16), 133 (33), 116 (36), 102 (19), 89 (100), 63 (92).

Anal. Calcd. $C_{11}H_9NO_3$: C, 65.02; H, 6.89; N, 4.46. Found: C, 64.90; H, 6.72; N, 4.46.

5-(2-Oxo-2*H*-[1]benzopyran-4-yl)-3-(4-methoxyphenyl)-1,2,4-oxadiazole (**12**).

To a stirred solution of compound **10** (0.203 g, 1 mmole) in chloroform (10 ml) a solution of nitrile oxide **4b** [prepared from 4-methoxybenzhydroximoyl chloride (0.204 g, 1.1 mmoles) as it is described in the preparation of compound **5b**] in ethyl ether (25 ml) was added and the reaction mixture was stirred at rt for 24 hours. Tlc examination of the reaction mixture showed no consumption of compound **10**; an equal amount of the nitrile oxide **4b** was added twice in the reaction mixture and stirring continued for further 48 hours. Then the solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel column (3:1 *n*-hexane/dichloromethane as eluent) to give the furoxan of the nitrile oxide **4b** [25] (0.168 g, 34%) followed by the unreacted compound **10** (0.126 g, 62%) and finally the compound **12** (70 mg, 22%), mp 192-193° (*n*-hexane/ethyl acetate); ir: 1715, 1605 cm^{-1}; ^1H nmr (300 MHz): δ 3.89 (s, 3H), 7.02 (d, J = 8 Hz, 2H), 7.28 (s, 1H), 7.40-7.46 (m, 2H), 7.62-7.70 (m, 1H), 8.10 (d, J = 7.6 Hz, 2H), 8.80 (d, J = 8 Hz, 1H); ms: m/z 320 [M^+] (100), 294 (71), 292 (63), 278 (97), 262 (11), 172 (70), 157 (83), 145 (10), 129 (74), 119 (23), 91 (71).

Anal. Calcd. for $C_{18}H_{12}N_2O_4$: C, 67.50; H, 3.78; N, 8.75. Found: C, 67.68; H, 3.80; N, 8.38.

Biological Testing.

Materials and Methods.

Albumin used was Rinderblut (Fluka) fraction V. Trypsin (pancreasprotease) 200fip U/g, salicylic acid, acetylsalicylic acid, β-glucuronidase/arylsulfatase, *p*-nitrophenyl-β-glucopyranosiduronic acid were from Merck, A.G. Darmstaadt. 12-Soybean lipoxidase (lipoxygenase), linoleic acid sodium salt from Sigma Chemical Co. St Louis, MO USA, and 1,1-diphenyl-2-picrylhydrazyl was from Aldrich Chemical Co. St Louis, MO USA. A protein determination kit (biuret method) was obtained from Elitech Diagnostics, France. A Perkin-Elmer 554 UV-Vis spectrophotometer was used for the *in vitro* experiments.

Interaction with 1,1-diphenyl-2-picrylhydrazyl [26,27].

Compounds **3, 5a, 5b, 8, 9a, 9b, 10** and **12**, 0.1 m*M* in absolute ethanol, were added to an equimolar ethanolic solution of 1,1-diphenyl-2-picrylhydrazyl. After 20 minutes the absorbance at 517 nm was measured, in order to determine their reducing ability (R.A %) by the difference in absorbance between this and that of the control.

Inhibition of Proteolysis (IPr %) [28].

The antiproteolyic activity was measured by determining the ability of the compounds **3, 5a, 8, 9a, 9b, 10, 12,** 0.1 m*M* to inhibit trypsin (0.075 mg/ml) induced hydrolysis of bovine serum albumin (6 g/100 ml), as a substrate, in 0.1*M* phosphate buffer (*p*H 7.6).

Inhibition of β-glucuronidase [29].

Compounds **3, 8, 9b** and **10**, 1 m*M* in acetate buffer 0.1*M* (*p*H 7.4), were tested against β-glucuronidase (0.1 ml of 1 U/ml), with 2.5 m*M* *p*-nitrophenyl-β-D-glucopyranosiduronic acid.

The examined compounds in the above two experiments were dissolved in the buffer by addition of dimethyl formamide (1% approximately).

12-Lipoxygenase soybean Lipoxygenase Inhibition Test [30].

The conversion of sodium linoleate (0.1 m*M*), by 12-Lipoxygenase (0.15 ml $1/10^4$ w/v in saline) to 13-hydroxy-peroxylinoleic acid in *p*H 9 Tris buffer, with the presence of the examined compounds **3, 8, 9b** and **10** (0.1, 0.3, 1 m*M* in 60% aqueous ethanol), was compared in each case with appropriate standard.

The results from all *in vitro* tests are the means of duplicate experiments. In Table 1 the biological results are given.

In vivo Carrageenin Induced Paw Edema [28,31].

Fisher 344 rats were used (180-220 g). Acute antiinflammatory activity was measured after 3.5 hours by reduction of rat paw carrageenin edema, induced by injection of 0.1 ml carrageenin 2% (K100), intradermally into the right foot pad. Compounds **3, 8, 9a, 9b, 10** were administered simultaneously (0.15 mmol/Kg). Statistical significance of results was established using the student's T-test. Indomethacin (4 mg/ml/0.1 Kg body weight) was administered as a standard drug (53.3% ±5.6, $p< 0.001$).

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