1,3-Cycloaddition Reactions of 2-Oxo-2*H*-[1]benzopyran-4-carbonitrile *N*-Oxide. Synthesis of Several New 4-Substituted Coumarins

Demetrios N. Nicolaides* [a], Konstantina C. Fylaktakidou [a], Konstantinos E. Litinas [a], George K. Papageorgiou [b] and Dimitra J. Hadjipavlou-Litina [c]

[a] Laboratory of Organic Chemistry, University of Thessaloniki, 54006 Thessaloniki, Greece
[b] Laboratory of Chemistry, Department of Primary School Education, Democritus University of Thrace, 68100 Alexandroupolis, Greece
[c] Department of Pharmaceutical Chemistry, School of Pharmacy, University of Thessaloniki, 54006 Thessaloniki, Greece

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1,3-Cycloaddition reactions of new 2-oxo-2*H*-[1]benzopyran-4-carbonitrile *N*-oxide **2** with dipolarophiles, *o*-aminophenols and *o*-phenylenediamine resulted in 4-heterocyclic substituted coumarin derivatives. These derivatives are screened for antiinflammatory activity *in vitro* through their antiproteolytic activity, the interaction with 1,1-diphenyl-2-picrylhydrazyl and the ability to affect superoxide anion and to inhibit β-glucuronidase and soybean lipoxygenase.

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The biological importance of coumarin derivatives has resulted in much interest in their synthesis. Recently we reported [1] the synthesis of some 4-isoxazolinyl or 1,2,4-oxadiazolylcoumarins *via* 1,3-cycloaddition reaction of simple nitrile oxides to 4-alkenyl or 4-iminomethylcoumarins and their further biological evaluation. In continuation of our previous studies on the synthesis of coumarin derivatives [2-6] and as part of our program directed towards developing new approaches to a variety of heterocycles incorporating 4-substituents on a coumarin moiety with expected potential biological activity, we report here the utility of the title nitrile oxide **2** as a building block for these syntheses.

Scheme 1

1 **2**

The reactions studied and the products obtained are depicted in Schemes 1-7. The title nitrile oxide **2** was prepared from the known [7] oxime **1** by treatment with sodium hypochloride/triethylamine. A solution of sodium hypochloride was added to a cold and stirred mixture of oxime **1** in dichloromethane during 1 hour followed by addition of triethylamine and the mixture was stirred for an additional 20 minutes. Addition of water, concentration of the organic layer and trituration of the residue with ether gave sufficiently pure **2** (60%) as indicated by thin layer chromatography and the spectral data of the precipitated solid. Efforts for further purification of **2** resulted to its transformation into the furoxan **3** (Scheme 2).

Furoxan **3** was obtained (70%) by refluxing a solution of **2** in chloroform for 12 hours. By refluxing a solution of **3** in triethylphosphite **4**, 3,4-bis(2-oxo-2*H*-[1]benzopyran-4-yl)-1,2,5-oxadiazole **5** was obtained (50%). When compound **2** was added to a mixture of ethanol/pyridine at room temperature, 3,6-bis(2-oxo-2*H*-[1]benzopyran-4-yl)-1,4,2,5-dioxadiazine **6** was formed in 66% yield (Scheme 2). The analytical and spectral data of compounds **3**, **5**, **6** are consistent with the structures proposed.

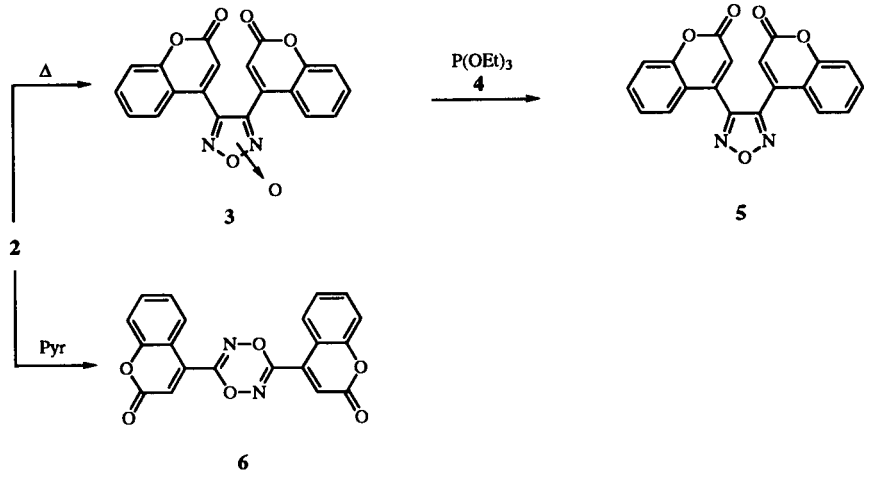
We then studied 1,3-dipolar cycloaddition reactions of **2** with some dipolarophiles as well as the reaction of **2** with some *o*-aminophenols and *o*-phenylenediamine.

Treatment of *trans*-stilbene **7a** with nitrile oxide **2**, prepared *in situ* from oxime **1**, for 4 days at 0° afforded the diphenyl derivative **8a** (43%). By similar treatment of ethyl (*E*)-3-(2-oxo-2*H*-[1]benzopyran-4-yl)propenoate **7b** [1] with **2** for 24 hours ethyl *trans*-3,5-bis-(2-oxo-2*H*-[1]benzopyran-4-yl)-4,5-dihydroisoxazole-4-carboxylate **8b** (45%) was obtained. The ^{1}H nmr spectrum of this product exhibited two doublets at δ 4.51 and 6.37 similar to the ^{1}H nmr spectra of other 4-ethoxycarbonyl-5-(coumarin-4-yl)isoxazolines studied previously [1] which are in good agreement with the suggested regio-form **8b**.

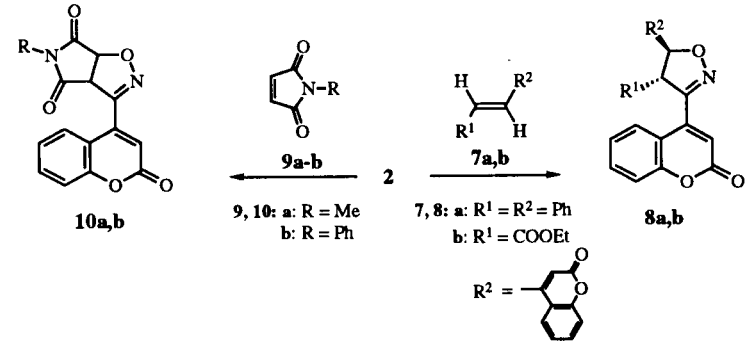
The reaction of **2** with *N*-methyl **9a** or *N*-phenylmaleimide **9b** for 7 or 5 hours respectively, under the same conditions, afforded compounds **10a** (57%) or **10b** (61%) (Scheme 3). The reaction of **2** with alkynes **11a**, **11b** for 4 days, under similar conditions, gave the substituted isoxazoles **12a** (9%) and **12b** (16%) (Scheme 4).

When a solution of compound **11a** and pure nitrile oxide **2** in chloroform was refluxed for 8 hours, isoxazole **12a** was obtained in 22% yield, along with furoxan **3** (14%). The suggested structure of ethyl 3-(2-oxo-2*H*-[1]benzopyran-4-yl)-5-phenylisoxazole-4-carboxylate for the product obtained from the reaction between **2** and **11b**

Scheme 2



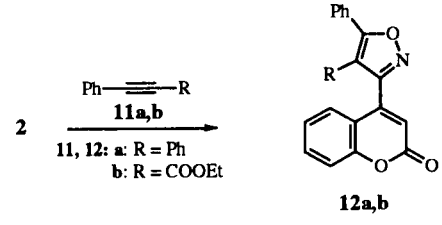
Scheme 3



instead of the also possible 4-phenyl-5-ethoxycarbonyl regioisomer is based on the reported [8,9] previously regioselectivity of the 1,3-cycloaddition reactions of other nitrile oxides to **11b**.

Treatment of 4-[(phenylimino)methyl]-2*H*-[1]benzopyran-2-one **13** with **2** in chloroform under reflux for 8.5 hours gave 3,5-bis(2-oxo-2*H*-[1]benzopyran-4-yl)-4-phenyl-4,5-dihydro-1,2,4-oxadiazole **14** (17%). By similar treatment of the *N*-methoxy imine **15** for 24 hours at room temperature, compound **16** (30%) was obtained, which was confirmed by the ^{1}H nmr spectrum of the product. Efforts for further purification of **16** by recrystallization from methanol/tetrahydrofuran resulted in its transformation *via* methanol elimination into the stable 5-(4-methoxyphenyl)-3-(2-oxo-2*H*-[1]benzopyran-4-yl)-1,2,4-oxadiazole **17** (85%) (Scheme 5).

Scheme 4



Treatment of the *o*-aminophenols **18a-c** in refluxing chloroform with equimolar amount of nitrile oxide **2,** for 18-62 hours, gave 2-(2-oxo-2*H*-[1]benzopyran-4-yl)benzo[*d*]oxazole derivatives **19a** (20%), **19b** (34%) and **19c** (23%) respectively (Scheme 6). Similarly, treatment of the *o*-phenylene diamine **18d** with **2** in refluxing chloroform for 72 hours afforded 2-(2-oxo-2*H*-[1]benzopyran-4-yl)benzimidazole **19d** (28%).

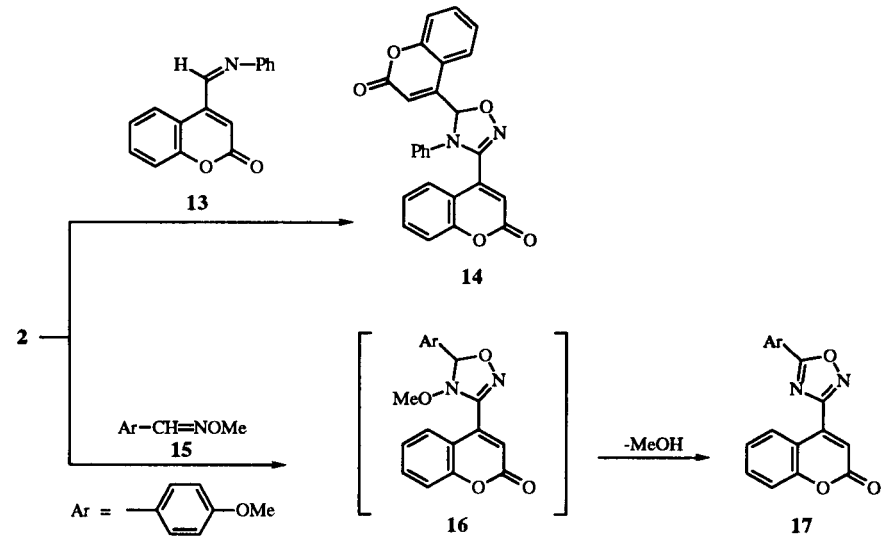
Finally, the reaction of coumarin-4-carboxaldehyde **20** with freshly prepared *p*-methoxybenzonitrile oxide **21** for 24 hours gave 4-(4-methoxyphenyl)-2-(2-oxo-2*H*-[1]benzopyran-4-yl)-1,3,5-dioxazole **22** in 83% yield (Scheme 7).

Biological Evaluation.

Several coumarins with a variation in the substitution pattern at the 4-position of the pyranone ring, have been screened *in vitro* for antiproteolytic effect, soybean lipoxygenase inhibition, reducing ability, β-glucuronidase inhibition and for their scavenging activity (with the xanthine/xanthine oxidase system for $O_2^{\cdot-}$.

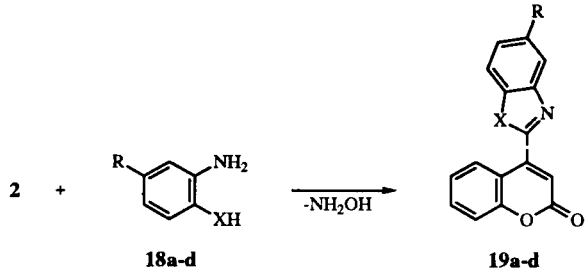
As far as their antiproteolytic activity is concerned compounds exhibited significant responses to this hydrolysis (39-64%). Compound **13** (Table 1) had a maximum activity whereas compound **8a** was found inactive under the experimental conditions. Sodium salicylate used as a stan-

Scheme 5



dard drug for comparison reasons, showed 54% inhibition. The compounds examined did not inhibit β-glucuronidase, an enzyme which is present in the lysosomes of polymorphonuclear leucocytes and is mentioned among the enzymes inhibited by nonsteroidal antiinflammatory drugs. Only compound **8b** shows a very weak effect.

Scheme 6



18, 19 a: R = H, X = O
b. R = Cl, X = O
c: R = NO_2, X = O
d: R = H, X = NH

In the soybean lipoxygenase assay compound **17** showed significant inhibition (41%) and compound **22**, 24%. The inhibition produced by the nordihydroguaiaretic acid as a standard was under the same experimental conditions. The interaction of the compounds examined with the stable free radical 1,1-diphenyl-2-picrylhydrazyl expresses the reducing ability of the compounds and indicates if they are able to scavenge free radicals [10]. The role of active oxygen species in the development of inflammation and of various kinds of diseases is known. Reducing activity of compounds **13, 22, 10a,b, 8b** and **12b** ranged from 25 to 40% at 0.1 m*M* after 20 minutes incubation (Table 1) while acetylsalicylic acid indicated 81%. Compound **8a** was completely inactive at 0.1 m*M*.

As shown by our experimental procedure, the derivatives tested did not affect superoxide anion (2-15%, caffeic acid 10% as a standard). The compounds were found to interact with 1,1-diphenyl-2-picrylhydrazyl, but the order of this activity was not parallel neither with that in the two *in vitro* series, lipoxygenase and superoxide anion scavenging activity, nor with the proteolytic assay.

Table 1

Reducing Ability (RA%), Inhibition *in vitro* of Trypsin Proteolysis (Ipr%).

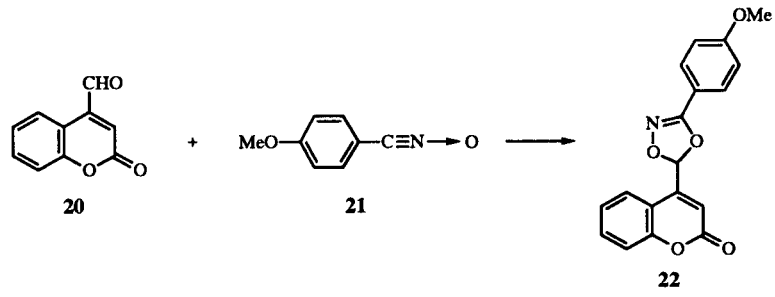
No.	RA%	Ipr%
7b	65 [1]	[a]
13	85	64
22	[a]	39
10a	12	40
10b	58	57
8b	33	60
8a	73	[a]
12b	30	59
17	26	[b]

[a] No activity. [b] Not tested.

Compound **17** on the basis of our results could be a good candidate for lipoxygenase inhibition whereas compound **22** would be a lead molecule to be modified in order to improve the lipoxygenase inhibition. Coumarins, in general are recognized as inhibitors of the pro-inflammatory lipoxygenase and cycloxygenase pathways of arachidonate metabolism [11,12].

In so far as the structure activity relationships are concerned, among the points that they have identified to produce significant changes in the *in vitro* examined activities of the derivatives are: 1) The presence of a double bond, alone or in a ring, in the C_4-substituent, in order to

Scheme 7



20 21 22

have a rigid conjugated system which can be stabilized *via* resonance with the coumarin phenyl ring. 2) In the case of compound **8a** there are some steric requirements for the substituent in position 4' of the oxazole ring. Lipophilicity seems to be a significant physicochemical parameter for compounds **10a,b**.

In vivo experiments are in progress concerning the anti-inflammatory activity of these new compounds using the carrageenin rat paw edema model and their mechanism of action.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. The ir spectra were obtained using a Perkin-Elmer 1310 spectrophotometer as Nujol mulls. The ^1H nmr spectra were recorded in deuteriochloroform using a Bruker AW 80 (80 MHz) or on a Bruker AM-300 (300 MHz) spectrometer, with tetramethylsilane as the internal standard. The ^{13}C nmr spectra were obtained at 75 MHz as deuteriochloroform solutions 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. Earlier reported methods were used for the preparation of known compounds **1** [7], **7b** [1], **13** [7] and **20** [13].

Preparation of 2-Oxo-2*H*-[1]benzopyran-4-carbonitrile *N*-Oxide **2**.

A solution of aldoxime **1** (0.189 g, 1 mmole) in dichloromethane (30 ml) was cooled at -5°. To the vigorously stirred solution commercial bleach (5.2 ml) and triethylamine (6 drops) were added. The reaction mixture was allowed stirred for 20 minutes, then diluted with water (30 ml) and extracted with dichloromethane (30 ml). The extracts were washed with water, dried over anhydrous sodium sulfate, concentrated *in vacuo* and the residue was triturated with ether to give crude compound **2** (0.112 g, 60%), mp 125-128°; ir: 2290, 1720, 1595, 1545 cm^{-1}; ^1H nmr (300 MHz): δ 6.71 (s, 1H), 7.20-8.0 (m, 4H); ms: m/z 187 [M+] (100), 158 (12), 129 (23), 101 (54), 75 (23).

Anal. Calcd. for $C_{10}H_5NO_3$: C, 64.18; H, 2.69; N, 7.48. Found: C, 64.17; H, 2.94; N, 7.19.

Efforts for further purification of **2** *via* recrystalization caused dimerization into furoxan **3**.

3,4-Bis(2-oxo-2*H*-[1]benzopyran-4-yl)-1,2,5-oxadiazole 2-(*N*-oxide) **3**.

A stirred solution of nitrile oxide **2** (30 mg, 0.16 mmole) in chloroform (10 ml) was refluxed for 12 hours. After cooling the mixture was concentrated *in vacuo* to give compound **3** (21 mg, 70%), mp 241-243° (methanol); ir: 1718, 1595 cm^{-1}; ^1H nmr (80 MHz, deuteriochloroform/trifluoroacetic acid): δ 6.70 (s, 1H), 6.79 (s, 1H), 7.05-8.10 (m, 8H); ms: m/z 374 [M+] (30), 357 (8), 338 (43), 320 (45), 292 (25), 272 (49), 255 (98), 238 (39), 223 (28), 213 (38), 202 (23), 187 (54), 159 (44), 145 (39), 133 (23), 113 (34), 103 (29), 91 (100), 81 (64).

Anal. Calcd. for $C_{20}H_{10}N_2O_6$: C, 64.18; H, 2.69; N, 7.48. Found: C, 64.28; H, 2.84; N, 7.48.

3,4-Bis(2-oxo-2*H*-[1]benzopyran-4-yl)-1,2,5-oxadiazole **5**.

A solution of compound **3** (0.187 g, 0.5 mmole) in triethylphosphite **4** (5 ml) was heated at reflux for 1 hour to give a precipitate of furazan **5** (90 mg, 50%), mp 294-296° (methanol); ir: 1700, 1590 cm^{-1}; ^1H nmr (80 MHz, deuteriochloroform/trifluoroacetic acid): δ 6.71 (s, 2H), 7.15-8.05 (m, 8H); ms: m/z 358 [M+] (8), 330 (10), 291 (74), 263 (33), 250 (13), 181 (12), 143 (22), 132 (23), 121 (32), 115 (29), 105 (44), 91 (100), 77 (31).

Anal. Calcd. for $C_{20}H_{10}N_2O_5$: C, 67.04; H, 2.81; N, 7.82. Found: C, 67.00; H, 2.68; N, 7.50.

3,6-Bis(2-oxo-2*H*-[1]benzopyran-4-yl)-1,4,2,5-dioxadiazine **6**.

To a stirred mixture of pyridine (10 ml) and ethanol (15 ml) nitrile oxide **2** (0.374 g, 2 mmoles) is added and the mixture is allowed to stir for a further 15 minutes at room temperature. A precipitate is formed and separated by filtration to give compound **6** (0.245 g, 65.5%), mp 249-251° (ethanol); ir: 1720, 1590, 1550 cm^{-1}; ^1H nmr (80 MHz, deuteriochloroform/trifluoroacetic acid): δ 7.15-8.05 (m, 8H), 8.35 (d, 2H, J = 8 Hz); ms: m/z 374 [M+] (7), 358 (8), 330 (7), 292 (6), 258 (23), 205 (27), 187 (13), 177 (30), 171 (39), 159 (18), 143 (100), 115 (42), 101 (43), 89 (70), 77 (36).

Anal. Calcd. for $C_{20}H_{10}N_2O_6$: C, 64.18; H, 2.69; N, 7.48. Found: C, 64.00; H, 2.74; N, 7.38.

Trans 3-(2-Oxo-2*H*-[1]benzopyran-4-yl)-4,5-diphenyl-4,5-dihydroisoxazole **8a**.

A mixture of aldoxime **1** (0.189 g, 1 mmole), *trans*-stilbene **7a** (0.198 g, 1.1 mmoles) and dichloromethane (13 ml) was cooled at 0°. Commercial bleach (3.16 ml) and triethylamine (6 drops) were added to the vigorously stirred mixture. The reaction mixture was allowed to stir at room temperature for 4 days, then diluted with water (30 ml) and extracted with dichloromethane (2 x 20 ml). The extracts were washed with

water, dried over sodium sulfate and then concentrated *in vacuo* up to 1/6 of its volume to give compound **8a** (0.13 g) as a precipitate. The filtrate was chromatographed on silica gel column (4:1 *n*-hexane/ethyl acetate as eluent) to give first unreacted stilbene (0.198 g, 53%) and then an additional amount of compound **8a** (26 mg, total yield 43%), mp 213-215° (methanol/tetrahydrofuran); ir 1720, 1605, 1550 cm^{-1}; ^1H nmr (80 MHz): δ 4.70 (d, 1H, J = 6.4 Hz), 5.60 (d, 1H, J = 6.4 Hz), 6.24 (s, 1H), 7.20-7.90 (m, 13H), 8.74 (d, 1H, J = 8 Hz); ms: m/z 367 [M+] (13), 261 (86), 233 (18), 217 (10), 204 (30), 180 (100), 165 (59), 145 (28), 102 (32).

Anal. Calcd. for $C_{24}H_{17}NO_3$: C, 78.46; H, 4.66; N, 3.81. Found: C, 78.28; H, 4.39; N, 3.60.

Ethyl *trans*-3,5-Bis(2-oxo-2*H*-[1]benzopyran-4-yl)-4,5-dihydroisoxazole-4-carboxylate **8b**.

A mixture of aldoxime **1** (0.102 g, 0.54 mmole), ester **7b** (0.144 g, 0.59 mmole) and dichloromethane (7 ml) was treated with commercial bleach (1.7 ml) and triethylamine (3 drops) for 24 hours and then worked up as in the preparation of **8a**, to give, after elution of the unreacted ester **7b** (44 mg, 31%), compound **8b** (0.103 g, 45%), mp 187-189° (ethyl acetate); ir 1720 (br), 1600, 1560 cm^{-1}; ^1H nmr (80 MHz): δ 1.19 (t, 3H, J = 8 Hz), 4.29 (q, 2H, J = 8 Hz), 4.51 (d, 1H, J = 5.6 Hz), 6.37 (d, 1H, J = 5.6 Hz), 6.46 (s, 1H), 6.55 (s, 1H), 7.15-7.90 (m, 7H), 8.50 (d, 1H, J = 8 Hz); ms: m/z 431 [M+] (22), 358 (27), 330 (10), 288 (8), 244 (11), 214 (21), 187 (16), 171 (36), 145 (49), 131 (24), 121 (46), 101 (71), 89 (100).

Anal. Calcd. for $C_{24}H_{17}NO_7$: C, 66.82; H, 3.97; N, 3.25. Found: C, 66.70; H, 3.80; N, 3.04.

3-(2-Oxo-2*H*-[1]benzopyran-4-yl)-4,5-dihydroisoxazole-4,5-dicarboxylic Acid *N*-Methylimide **10a**.

A mixture of oxime **1** (0.189 g, 1 mmole), *N*-methylmaleimide **9a** (0.121 g, 1.1 mmoles) and dichloromethane (13 ml) was treated with commercial bleach (3.16 ml) and triethylamine (6 drops) for 7 hours and then worked up as in the preparation of **8a** to give after the concentration of the extracts directly compound **10a** (0.169 g, 57%), mp 243-245° (tetrahydrofuran): ir 1720, 1700, 1600 cm^{-1}; ^1H nmr (80 MHz, deuteriochloroform/trifluoroacetic acid): δ 3.10 (s, 3H), 5.05 (d, 1H, J = 10.4 Hz), 5.80 (d, 1H, J = 10.4 Hz), 7.20-7.80 (m, 4H), 8.67 (d, 1H, J = 8 Hz); ms: m/z 298 [M+] (100), 270 (18), 185 (95), 157 (11), 143 (19), 129 (7), 115 (7), 101 (10).

Anal. Calcd. for $C_{15}H_{10}N_2O_5$: C, 60.41; H, 3.38; N, 9.39. Found: C, 60.28; H, 3.36; N, 9.13.

3-(2-Oxo-2*H*-[1]benzopyran-4-yl)-4,5-dihydroisoxazole-4,5-dicarboxylic Acid *N*-Phenylimide **10b**.

A mixture of oxime **1** (0.189 g, 1 mmole), *N*-phenylmaleimide **9b** (0.191 g, 1.1 mmoles) and dichloromethane (13 ml) was treated with commercial bleach (3.16 ml) and triethylamine (6 drops) for 5 hours and then worked up like in the case of preparation of compound **8a** to give after the concentration of the extracts compound **10b** (0.22 g, 61%), mp 240-242° (tetrahydrofuran); ir: 1710, 1600, 1490 cm^{-1}; ^1H nmr (80 MHz, hexadeuteriodimethylsulfoxide): δ 5.55 (d, 1H, J = 10.4 Hz), 5.89 (d, 1H, J = 10.4 Hz), 7.15-7.80 (m, 9H), 8.51 (d, 1H, J = 8 Hz); ms: m/z 360 [M+] (43), 332 (51), 288 (7), 260 (5), 212 (44), 184 (55), 173 (100), 157 (79), 143 (77), 129 (62), 115 (37), 101 (38).

Anal. Calcd. for $C_{20}H_{12}N_2O_5$: C, 66.67; H, 3.36; N, 7.77. Found: C, 66.41; H, 3.20; N, 7.58.

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

Method A.

A mixture of oxime **1** (0.102 g, 0.54 mmole), diphenylacetylene **11a** (0.196 g, 1.1 mmoles) and dichloromethane (13 ml) was treated with commercial bleach (3.16 ml) and triethylamine (6 drops) for 4 days and then worked up like in the case of preparation of **8a**, to give, after the concentration of the extracts and trituration of the residue with dichloromethane, compound **12a** (33 mg, 9%), mp >300° (methanol); ir: 1720, 1585 cm^{-1}; ^1H nmr (80 MHz): δ 7.15-7.95 (m); ms: m/z 365 [M+] (11), 337 (9), 221 (47), 165 (32), 158 (26), 149 (9), 137 (61), 114 (33), 105 (86), 89 (23), 77 (100).

Anal. Calcd. for $C_{24}H_{15}NO_3$: C, 78.89; H, 4.14; N, 3.83. Found: C, 78.75; H, 4.02; N, 3.97.

Separation of the filtrate by preparative thin layer chromatography (silica gel, 4:1 *n*-hexane/ethyl acetate) gave only the unreacted **11a** (0.127 g, 65%).

Method B.

A solution of freshly prepared nitrile oxide **2** (0.211 g, 1.1 mmoles) and **11a** (0.178 g, 1 mmole) in chloroform (10 ml) was heated under reflux for 8 hours. Column chromatography of the reaction mixture on silica gel, using 5:1 *n*-hexane:ethyl acetate as eluent, gave first the unreacted **11a** (0.116 g, 65%) and then compound **12a** (79 mg, 22%) and next furoxan **4** (28 mg, 14%).

Ethyl 3-(2-Oxo-2*H*-[1]benzopyran-4-yl)-5-phenylisoxazole-4-carboxylate **12b**.

A mixture of oxime **1** (0.102 g, 0.54 mmole), ethyl phenylpropiolate **11b** (0.192 g, 1.1 mmoles) and dichloromethane (13 ml) was treated with commercial bleach (3.16 ml) and triethylamine (6 drops) for 4 days and then worked up as in preparation of **8a**. The extracts were concentrated and the residue was subjected to column chromatography (silica gel, 4:1 *n*-hexane-ethyl acetate as eluent) to give first the unreacted **11b** (0.15 g, 68%) and next compound **12b** (57 mg, 16%), mp 82-84° (ether/dichloromethane); ir: 1730, 1605, 1590, 1570, 1490 cm^{-1}; ^1H nmr (300 MHz): δ 0.81 (t, 3H, J = 7 Hz), 4.02 (q, 2H, J = 7 Hz), 6.61 (s, 1H), 7.23-7.68 (m, 8H), 8.11 (dd, 1H, J_o = 8 Hz, J_p = 1.5 Hz); ^{13}C nmr: δ 13.1, 61.4, 108.8, 117.2, 117.6, 118.3, 124.5, 125.7, 126.0, 128.6, 129.2, 132.1, 132.3, 143.9, 153.5, 158.5, 159.6, 160.5, 173.8; ms: m/z 361 [M+] (100), 333 (14), 316 (21), 288 (11), 260 (7), 105 (62), 89 (27), 77 (82).

Anal. Calcd. for $C_{21}H_{15}NO_5$: C, 69.80; H, 4.18; N, 3.88. Found: C, 70.01; H, 4.28; N, 3.99.

Ethyl 3,5-Bis(2-oxo-2*H*-[1]benzopyran-4-yl)-4-phenyl-4,5-dihydro-1,2,4-oxadiazole **14**.

A solution of nitrile oxide **2** (0.178 g, 0.95 mmole) and imine **13** (0.20 g, 0.8 mmole) in chloroform (15 ml) was refluxed for 8.5 hours. The solvent was evaporated and the residue was subjected to column chromatography (silica gel, 4:1 *n*-hexane/ethyl acetate) to give first the unreacted imine **13** (33 mg, 17%) and next compound **14** (60 mg, 17%), mp 172-174° (methanol/ethyl acetate); ir: 1720, 1600, 1565, 1540, 1490 cm^{-1}; ^1H nmr (80 MHz): δ 6.63 (s, 1H), 6.67 (s, 1H), 6.80-7.95 (m, 13H), 8.40 (d, 1H, J = 8 Hz); ms: m/z 436 [M+] (57), 408 (27), 291 (69), 263 (78), 248 (24), 237 (70), 220 (65), 205 (36), 174 (75), 145 (100), 118 (87), 93 (99), 77 (83).

Anal. Calcd. for $C_{26}H_{16}N_2O_5$: C, 71.56; H, 3.70; N, 6.42. Found: C, 71.38; H, 3.71; N, 6.21.

5-(4-Methoxyphenyl)-3-(2-oxo-2*H*-[1]benzopyran-4-yl)-1,2,4-oxadiazole **17**.

A solution of nitrile oxide **2** (0.178 g, 0.95 mmole) and *N*-methoxyimine **15** (0.248 g, 1.5 mmoles) in chloroform (7 ml) was allowed to stir at room temperature for 24 hours and then evaporated in a rotary. Trituration of the residue with a mixture of *n*-hexane/dichloromethane gave as a precipitate furoxan **4** (43 mg, 24%). Concentration of the filtrate and column chromatography of the residue on silica gel using 7:1 *n*-hexane/ethyl acetate as eluent gave first unreacted imine **15** (96 mg, 39%) and next an unstable product (0.10 g, 30%) with recorded ^{1}H nmr spectrum [(80 MHz): δ 3.64 (s, 3H), 3.78 (s, 3H), 6.32 (s, 1H), 6.93 (d, 2H, J = 6.4 Hz), 7.15-7.90 (m, 6H), 8.50 (d, 1H, J = 8 Hz)] resembling closely the expected cycloproduct 4-methoxy-5-(4-methoxyphenyl)-3-(2-oxo-2*H*-[1]benzopyran-4-yl)-4,5-dihydro-1,2,4-oxadiazole **16**. Efforts to recrystallize the product afforded compound **17** (0.077 g, 85%), mp 235-237° (methanol/tetrahydrofuran); ir: 1720, 1595 cm^{-1}; ^{1}H nmr (300 MHz, deuteriochloroform/hexadeuteriodimethyl sulfoxide): δ 3.94 (s, 3H), 7.13 (d, 2H, J = 8.8 Hz), 7.31 (s, 1H), 7.42 (m, 2H), 7.70 (m, 1H), 8.19 (d, 2H, J = 8.8 Hz), 8.69 (d, 1H, J = 6.7 Hz); ms: m/z 320 [M$^+$] (56), 292 (8), 159 (19), 135 (100), 103 (4).

Anal. Calcd. for $C_{18}H_{12}N_2O_4$: C, 67.50; H, 3.78; N, 8.75. Found: C, 67.38; H, 3.52; N, 8.68.

2-(2-Oxo-2*H*-[1]benzopyran-4-yl)benzoxazole **19a**.

A solution of nitrile oxide **2** (0.187 g, 1 mmole) and *o*-aminophenol **18a** (0.109 g, 1 mmole) in chloroform (5 ml) was refluxed for 36 hours and then was subjected to column chromatography on silica gel using 20:1 up to 10:1 *n*-hexane/ethyl acetate to give first compound **19a** (53 mg, 20%), mp 172-174° (chloroform); ir: 1760, 1725 cm^{-1}; ^{1}H nmr (80 MHz): δ 7.25-7.95 (m, 8H), 9.18 (d, J = 8 Hz, 1H); ms: m/z 263 [M$^+$] (87), 262 (100), 235 (92), 234 (95), 218 (7), 206 (25), 205 (14), 178 (37), 177 (24), 151 (12).

Anal. Calcd. for $C_{16}H_9NO_3$: C, 73.0; H, 3.45; N, 5.32. Found: C, 72.88; H, 3.30; N, 5.20.

5-Chloro-2-(2-oxo-2*H*-[1]benzopyran-4-yl)benzoxazole **19b**.

A solution of nitrile oxide **2** (0.187 g, 1 mmole) and 4-chloro-*o*-aminophenol **18b** (0.143 g, 1 mmole) in chloroform (5 ml) was refluxed for 13 hours and then was separated by column chromatography (silica gel, 5:1 *n*-hexane/ethyl acetate) to give as the first fraction compound **19b** (101 mg, 34%), mp 165-166° (chloroform); ir 1750, 1720 cm^{-1}; ^{1}H nmr (300 MHz) 7.34 (s, 1H), 7.38-7.52 (m, 3H), 7.58-7.70 (m, 2H), 7.9 (s, 1H), 9.16 (d, J = 7.4 Hz, 1H); ms: m/z 299 (72), 298 (51), 297 [M$^+$] (67), 272 (15), 270 (100), 241 (26), 213 (27), 206 (39), 178 (36), 151 (17).

Anal. Calcd. for $C_{16}H_8ClNO_3$: C, 64.55; H, 2.71; N, 4.71. Found: C, 64.30; H, 2.88; N, 4.67.

5-Nitro-2-(2-oxo-2*H*-[12]benzopyran-4-yl)benzoxazole **19c**.

A solution of nitrile oxide **2** (0.187 g, 1 mmole) and 4-nitro-*o*-aminophenol **18c** (0.154 g, 1 mmole) in chloroform (5 ml) was refluxed for 72 hours and the reaction mixture was then subjected to column chromatography on silica gel using 3:1 *n*-hexane/ethyl acetate as eluent to give first compound **19c** (71 mg, 23%), mp 227-228° (chloroform); ir: 3100, 1760, 1725, 1710 cm^{-1}; ^{1}H nmr (80 MHz): δ 7.25-7.90 (m, 5H), 8.49 (d, J = 7.6 Hz, 1H), 8.80 (s, 1H), 9.11 (d, J = 8 Hz, 1H); ms: m/z 308 [M$^+$] (80), 280 (100), 234 (46), 206 (19), 177 (14), 151 (12).

Anal. Calcd. for $C_{16}H_8N_2O_5$: C, 62.34; H, 2.62; N, 9.09. Found: C, 62.31; H, 2.82; N, 8.98.

2-(2-Oxo-2*H*-[1]benzopyran-4-yl)benzimidazole **19d**.

A solution of nitrile oxide **2** (0.280 g, 1.5 mmoles) and *o*-phenylenediamine **18d** (0.162 g, 1.5 mmoles) in chloroform (5 ml) was refluxed for 72 hours and the reaction mixture was then separated by column chromatography (silica gel, 3:1 *n*-hexane/ethyl acetate) to give first furoxan **4** (18 mg, 6%) and next compound **19d** (110 mg, 28%), mp 275-276° (chloroform/methanol); ir: 3275, 1725, 1708, 1680 cm^{-1}; ^{1}H nmr (80 MHz, deuteriochloroform/hexadeuteriodimethyl sulfoxide): δ 7.01 (s, 1H), 7.26-7.80 (m, 8H), 9.10 (d, J = 8 Hz, 1H); ms: m/z 262 [M$^+$] (50), 234 (100), 205 (69), 179 (17), 103 (20).

Anal. Calcd. for $C_{16}H_{10}N_2O_2$: C, 73.27; H, 3.84; N, 10.68. Found: C, 73.51; H, 3.55; N, 10.42.

4-(4-Methoxyphenyl)-2-(2-oxo-2*H*-[1]benzopyran-4-yl)-1,3,5-dioxazole **22**.

A solution of 4-methoxybenzhydroximoyl chloride [14] (0.204 g, 1.1 mmoles) in ether (20 ml) was cooled at 0° and a solution of triethylamine (0.111 g, 1.1 mmoles) in ether (5 ml) was then added to it. The resulting mixture was filtered and the filtrate was then added to a solution of coumarin-4-carboxaldehyde **20** (0.174 g, 1 mmole) and stirred for 24 hours. The solvent was then evaporated under reduced pressure and the residue was subjected to column chromatography (silica gel, 3:1 *n*-hexane/dichloromethane, then dichloromethane and finally ethyl acetate) to give compound **22** (0.268 g, 83%), mp 157-159° (ethyl acetate); ir: 1728, 1620, 1600 cm^{-1}; ^{1}H nmr (300 MHz): δ 3.87 (s, 3H), 6.73 (s, 1H), 6.96 (d, 2H, J = 9 Hz), 7.14 (s, 1H), 7.31-7.43 (m, 2H), 7.52-7.65 (m, 1H), 7.73-7.83 (m, 3H); ^{13}C nmr: δ 55.5, 103.2, 113.7, 114.0, 114.4, 116.2, 117.5, 124.6, 124.8, 128.9, 132.3, 147.1, 154.2, 158.7, 160.1, 162.8; ms: m/z 323 [M$^+$] (37), 174 (9), 149 (66), 134 (100).

Anal. Calcd. for $C_{18}H_{13}NO_5$: C, 66.87; H, 4.05; N, 4.33. Found: C, 66.70; H, 3.89; N, 4.18.

Biological Testing.

Materials and Methods.

Albumin used was Rinderblut (Fluka) Fraction V. Trypsin (pancreasprotease) 200FIP U/g, salicylic acid, acetyl-salicylic acid, β-glucuronidase/arylsulfatase, *p*-nitrophenyl-β-glucopyranosiduronic acid were from Merck A.G. Darmstaadt. Soybean lipoxygenase (LO), linoleic acid sodium salt, xanthine, xanthine oxidase and nitroblue tetrazolium chloride were from Sigma Chemical Co. St Louis, MO USA. Nordihydroguaiaretic acid, caffeic acid and 1,1-diphenyl-2-picrylhydrazyl were from Aldrich chemical Co St. Louis, MO USA.

A protein determination kit using the biuret method was obtained from Elitech diagnostics, France.

A Perkin-Elmer 554 UV-Vis spectrophotometer was used for the *in vitro* experiments.

In vitro Assays.

Each experiment was performed at least in triplicate and the standard deviation of absorbance was less than 10%.

Inhibition of Proteolysis (Ipr%) [1].

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

Inhibition of β-Glucuronidase [1].

Compounds 1 m*M* in acetate buffer 0.1 m*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-glucopyranosiduronic acid.

The compounds examined in the above two experiments were dissolved in the buffer by addition of dimethylformamide, approximately 1%.

Determination of the Reducing Ability of the Stable Radical 1,1-Diphenyl-2-picrylhydrazyl [1].

Compounds in 10^{-4} *M* ethanolic solution were added to an equal molar ethanolic solution of 1,1-diphenylpicrylhydrazyl (10^{-4} *M*). The mixture was kept at room temperature. After 20 minutes the absorbance at 517 nm was measured and the percent reduction according to reference [1] was estimated. Ethanol was of analytical grade, iron content less than 10^{-5}%.

Soybean-LO Inhibition Study [1].

The compounds tested dissolved in 60% aqueous ethanol (final concentration 0.1 m*M*) were incubated at room temperature with sodium linoleate (0.1 m*M*) 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 the appropriate standard. Only compounds **17** (41.3%) and **22** (23.7%) showed significant inhibition, whereas the rest of the compounds tested had no activity under the experimental conditions.

Superoxide Scavenging Activity [15].

The superoxide anion was generated by the xanthine/xanthine oxidase system and measured by nitroblue tetrazolium chloride. To the reaction mixture in phosphate buffer 7.4 (0.1 *M*) containing xanthine, nitroblue tetrazolium chloride and test compounds (0.1m*M*, in dimethylformamide <1%) was added xanthine oxidase (0.07 U/ml). After incubating for 10 minutes at room temperature the absorbance was read at 560 nm.

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