Synthesis and Biological Evaluation of Several 3-(Coumarin-4-yl)tetrahydroisoxazole and 3-(Coumarin-4-yl)dihydropyrazole Derivatives

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A series of novel 3-(coumarin-4-yl)tetrahydroisoxazoles **5a,b, 7, 9** and 3-(coumarin-4-yl)dihydropyrazoles **13a-d, 14, 15a,b** were synthesized from coumarin-4-carboxaldehyde **1** *via* the intermediate *N*-methyl nitrone **3** and *N*-phenyl or *N*-methyl hydrazones **11a,b.** These coumarin derivatives were isolated, characterized and evaluated *in vitro* for their ability to inhibit trypsin, β-glucuronidase, soybean lipoxygenase and to interact with the stable radical 1,1-diphenyl-2-picrylhydrazyl. The compounds were tested *in vivo* as anti-inflammatory agents in the rat carrageenin paw edema assay. Compound **15a** seems to be a lead molecule to be modified in order to improve the lipoxygenase inhibition. The results are discussed in terms of structural characteristics.

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Coumarins have been reported to have multiple biological activities [1]. It is to be expected that coumarins might affect the formation and scavenging of reactive substances derived from oxygen (Reactive Oxygen Species, ROS) and influence processes involving free radical-mediated injury, as can some other plant phenolics and flavonoids [2,3]. There is evidence that the naturally occuring prototypical compound, coumarin can reduce tissue oedema and inflammation [4]. Coumarin and 7-hydroxycoumarin inhibit prostaglandin biosynthesis, which involves fatty acid hydroperoxy intermediates [5]. Various coumarin related derivatives are recognised as inhibitors not only of the lipoxygenase and cyclooxygenase pathways of arachidonate metabolism [6,7,8], but also of neutrophile dependent superoxide anion generation [9].

In connection to our previous work on the synthesis of coumarin derivatives [10-14] recent studies on the synthesis and biological activities of 4-(5'-isoxazolinyl)-, 4-(5'-1,2,4-oxadiazolinyl)- [15] and 4-(3'-isoxazolinyl)coumarins [16], as well as 4-(3'-1,2,4-oxadiazolinonyl)- and 4-(3'-1,2,4-oxadiazolyl)coumarins [17], demonstrated that these derivatives possess significant antiinflammatory and antioxidant activities as well as inhibitory activity on Soybean Lipoxygenase.

In continuation to these studies we tried to design and synthesize novel coumarins like the new 4-(3'-tetrahydroisoxazolyl)- and 4-(3'-dihydropyrazolyl)coumarin derivatives and to define structure features for active compounds and to discuss our results in terms of structure-activity relationships. The reactions studied and the products (new compounds) obtained are depicted in schemes 1-2.

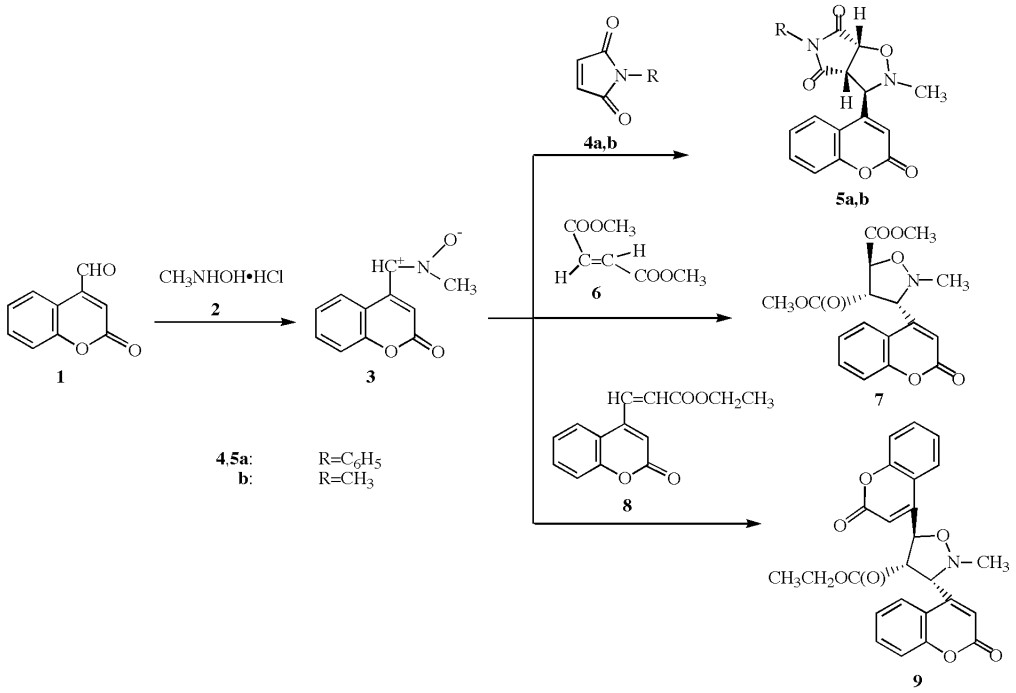
We prepared previously 4-(3'-isoxazolinyl)coumarins through 1,3-cycloaddition reactions of 2-oxo-2*H*-[1]benzopyran-4-carbonitrile *N*-oxide with different dipolarophiles [16]. In this work we try to extend those 1,3-cycloaddition reactions by preparing the new dipoles **3** and **12a,b** and studying their reactions with dipolarophiles **4a,b, 6, 8**.

Treatment of ethanol solution of aldehyde **1** with *N*-methyl hydroxylamine hydrochloride **2** and sodium acetate under reflux (Scheme 1) gave as a precipitate, after ice/water work up, the new nitrone **3** (54% yield). Reactions of compound **3** with maleimides **4a,b** resulted to new isoxazolidines **5a** (47%), **5b** (83%) respectively as the sole 1,3-cycloadducts. The chemical shifts for 5-H (5.02, d, J = 7.6 Hz), 3-H (4.82, d, J = 2.5 Hz), 4-H (3.94, dd, J_1 = 2.5 Hz, J_2 = 7.6 Hz) of **5a** and 5-H (4.92, d, J = 7.4 Hz), 3-H (4.66, d, J = 2.7 Hz), 4-H (3.79, dd, J_1 = 2.7 Hz, J = 7.4 Hz) of **5b** resemble well with the proposed structures in analogy to 3-(3'-indolyl)- substituted isoxazolidines [18]. The coupling constants, $J_{4/5}$ for both compounds pointed to a quasi-axial position for 5-H and 4-H, while $J_{3/4}$ pointed to a quasi-equatorial position for 3-H.

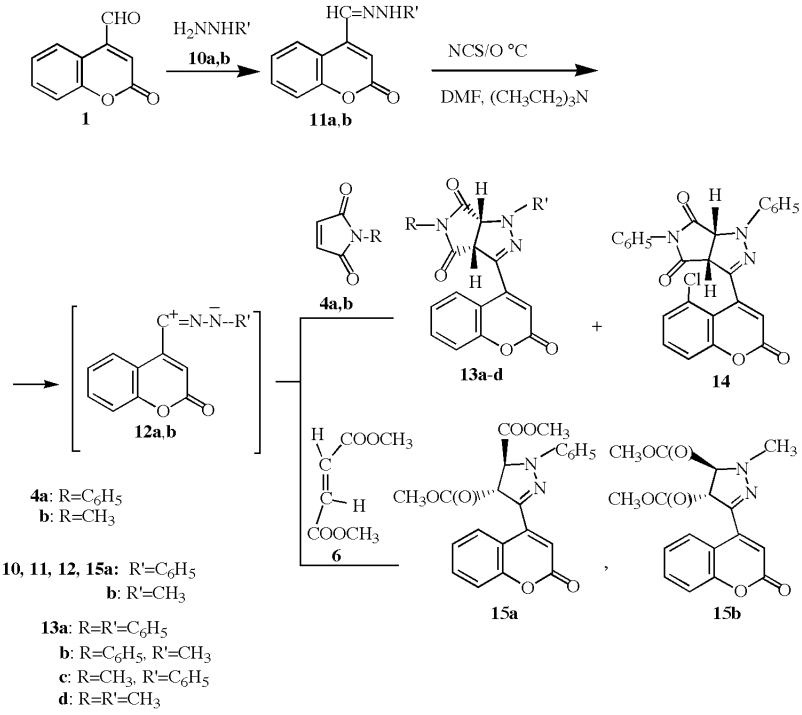
Isoxazolidine **7** (37%) was isolated from the reaction of nitrone **3** with excess (2 equivalents) of dimethyl fumarate **6**, after separation by column chromatography. The chemical shifts for 5-H (4.96, d, J = 3.7 Hz), 3-H (4.33, d, J = 7.0 Hz), 4-H (3.98, dd, J_1 = 3.7 Hz, J_2 = 7.0 Hz) are analogous to compounds **5a,b.** In this case $J_{3/4}$ revealed a quasi-axial position for 3-H and 4-H, while $J_{4/5}$ revealed a quasi-equatorial position for 5-H.

Reaction of nitrone **3** with the coumarin dipolarophile **8** resulted in cycloadduct **9** (22%), after separation by column chromatography and elution of unreacted compound **8** (69%). Isoxazolidine **9** has stereochemistry similar to compound **7** with 3-H, 4-H in quasi-axial positions ($J_{3/4}$ = 7.8 Hz, $J_{4/5}$ = 4.8 Hz) and 3-, 5- coumarinyl-substituents in quasi-equatorial, quasi-axial position respectively.

Scheme 1



Scheme 2



Treatment of ethanol solution of aldehyde **1** with *N*-methyl hydrazine **10b** gave the new hydrazone **11b** (70%). Cooled (0° C) solutions of hydrazones **11a,b** in DMF were treated with NCS, followed by the addition of dienophiles **4a,b** or **6** and triethylamine to give after separation by column chromatography the new pyrazoline derivatives **13a-d** and **15a,b** in moderate to good yields (28-81%).

Compounds **13a-d** and **14** have the suggested structures, since each of them possesses two doublets in the region

4.6-5.7 ppm for 4-H and 5-H of pyrazoline ring. These protons possess the quasi-axial position with coupling constants 11.0-12.1 Hz. Compound **14** contains a Cl atom possibly in the 5- position of the coumarin ring, because of the disappearence of the doublet in the region 8.8-9.1 ppm (compounds **13a-d**). This compound seems to come from substitution of one H by Cl during the treatment of reaction mixture with NCS. Compounds **15a** and **15b** have the proposed structures, where 4-H and 5-H are in quasi-equatorial ($J_{4/5}$ = 4.7 Hz) and quasi-axial ($J_{4/5}$ = 9.0 Hz) positions respectively like in a former case of isoxazolidine derivatives produced from dimethyl fumarate [19].

Biological Evaluation.

Table I summarizes the effect of a number of coumarin derivatives on *in vitro* trypsin induced proteolysis, β-glucuronidase activity, soybean lipoxygenase activity, interaction with 1,1-diphenyl-2-picrylhydrazyl.

As pointed out antiinflammatory agents have been reported to exhibit antiproteolytic activity [20]. The antiproteolytic activity was measured by determining the ability of the compounds to inhibit trypsin induced hydrolysis of bovine serum albumin as a substrate. In our case, we observed that compounds **13a** (52.6), **13c** (61), **14** (75.1) (Table I) exert significant inhibitory activity on trypsin induced proteolysis, whereas compounds **5b** (16.1), **13b** (30.1) and **15a** (20.5) possess some inhibition. Compounds **3**, **5a** and **11b** do not show any antiproteolytic activity.

β-Glucuronidase most widely is used as marker for lysosomes in biochemical studies. Under our experimental conditions none of the examined compounds (1mmol) inhibits β-glucuronidase [**13b** (1.2%) and **13c** (1%) very slightly <2%, Table I] [21]. None of the tested compounds scavenged superoxide anion (data not shown in Table I) (10^{-4} *M*) [22]. Compounds **5a**, **5b**, **5c**, **8a**, **8b**, **9a**, **12a**, **14a** were studied for their superoxide scavenging capacity by the nitroblue tetrazolium reduction method [23,24]. The examined coumarins interact with 1,1-diphenyl-2-picrylhydrazyl; compound **11a** shows the highest interaction (70%), whereas **13c** is almost inactive (2.8%).

In acute toxicity experiments, the *in vivo* examined compounds were endowed with a 50% lethal dose of >0.3 mmoles/kg body weight.

The antiinflammatory activity of compounds **13b** and **15a** at 0.15 mmoles/kg body weight is shown in Table II. The antiinflammatory efficacy was examined by using the functional model of carrageenin induced edema (0.1 ml 2% carrageenin) [25] in rats. Compound **13b** showed very low effect (11%), whereas compound **15a** exhibited mild activity (43.6%). Concerning the structures of the tested compounds the antiinflammatory efficacy decreases by the closure of the second ring.

Table I

Inhibition *in vitro* of Trypsin induced Proteolysis (Ipr %), Inhibition *in vitro* of β-Glucuronidase (Gl %), Reducing Ability (RA %), Inhibition *in vitro* of Soybean Lipoxygenase (LO %), Clog *P* Values

Compound	Ipr % (0.1 m*M*)[a]	Gl % (1 m*M*)[a]	RA % (0.1 m*M*)[a]	LO % (0.3 m*M*)[a]	Clog *P*
3	ns	ns	35	ns	2.02
5a	ns	ns	5.4	ns	1.33
5b	16.1	ns	15.5	17.8	0.57
11a	72.5	ns	70	19.6	2.98
11b	ns	ns	30.4	ns	1.94
13a	52.6	ns	35	13.7	2.17
13b	30.1	1.2	13.6	ns	1.28
13c	61	1	2.8	ns	1.3
14	75.1	ns	29.1	ns	2.75
15a	20.5	ns	17.2	24	2.47
SA	53.6	2.32	nt	nt	
ASA	nt	nt	80.5	nt	
NDGA	nt	nt	nt	91.5	

SA: salicylic acid, ASA: acetylsalicylic acid, NDGA: nor-dihydroguaieritic acid as reference drugs, nt: not tested; ns: no action under the experimental conditions; [a] Data are means of two independent determinations at least and the deviation in absorbance values was less than 10%.

Table II

In vivo Inhibition of Carrageenin Rat Paw Edema (CPE %)

Compound	CPE %[a] (SEM)[b]
13b	11 (1)
15a	43.6 (3.8)
IMA	53.6 (1.9)

IMA: Indometacin; [a] Each value represent the mean of two independent experiments with 6 animals in each group; [b] (SEM standard error of the mean) Statistical significance of results was established using the student's T-test ($p<0.001$).

Regression analysis was performed to find out whether any correlation exists between the interaction with 1,1-diphenyl-2-picrylhydrazyl (DPPH)% and lipophilicity. Theoretical calculations of lipophilicity as clog *P* using the Hansch and Leo method were performed [26].

$$\log \text{DPPH\%} = 0.693 \log P - 0.027 \quad \text{eq. 1}$$

$n = 7$, $r = 0.898$, $r^2 = 0.806$, $s = 0.242$, $F_{1,7} = 20.75$, $\alpha = 0.01$

The correlation is poor. In this equation compounds **5b**, **14**, **15a** are outliers.

Some selected compounds were evaluated for inhibition of soybean lipoxygenase *in vitro*. The conversion of sodium linoleate to 13-hydroperoxy-linoleic acid with appropriate standard, in each case, at 234 nm was compared [27]. Compounds **5b**, **11a**, **13a**, **15a** were found to inhibit the enzyme mild (13.7-24%) at concentration 0.3 m*M* comparing to nor-dihydroguaieritic acid (91.5%), which has been used extensively as a standard to compare lipoxygenase inhibitors. No sign of inhibition was found for compounds **3, 5a**, **11b**, **13b**, **13c, 14**. The compounds were tested in several concentrations (0.05-0.3 m*M*, data not shown) and inhibition was found to be concentration dependent.

Compound **15a**, on the basis of our results would be a good candidate, a lead molecule to be modified in order to improve the lipoxygenase inhibition. For most of the tested compounds the presence of a double bond in the C_4 substituent (-C=N-NHR, or as a ring) generates new derivatives with potential activity. Lipophilicity was found to be significant too.

Further investigation is in progress concerning: 1) structural requirements, 2) elucidation of the 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 ^{1}H NMR spectra were recorded at 80 MHz on a Bruker AW 80 or at 300 MHz on a Bruker AM 300 spectrometer in $CDCl_3$, using tetramethylsilane as an internal standard unless otherwise stated. Coupling constants (*J* values) are reported in Hertz (Hz). The ^{13}C NMR spectra were obtained at 75.5 MHz on a Bruker AM 300 spectrometer in $CDCl_3$ solutions with tetramethylsilane as internal reference unless otherwise stated. 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 analyzer. 4-(2-Oxo-2*H*-[1]benzopyran)carboxaldehyde **1** [28] and 4-(2-oxo-2*H*-[1]benzopyranH)carboxaldehyde *N*-phenyl hydrazone **11a** [29] were prepared according to the literature. Albumin used was Rinderblut (Fluka) fraction V; trypsin (pancreasprotease) 200 Fip U/g, salicylic acid, acetyl-salicylic acid, β-glucuronidase/arylsulfatase, *p*-nitrophenyl-β-glucopyranosiduronic acid, Tween-80 were from Merck AG, Darmstaadt; protein determination kit (biuret method) was obtained from Elitech Diagnostics, France. Xanthine, xanthine oxidase, nitroblue tetrazolium (NBT), soybean lipoxidase (Lipoxygenase E.C 1.13.11.12 Type I-B), linoleic acid sodium salt were obtained from Sigma Chemical Co (St. Louis, MO USA). 1,1-diphenyl-2-picrylhydrazyl, nor-dihydroguairetic acid, caffeic acid were from Aldrich.

Synthesis of 4-[(*N*-methyl-*N*-oxyimino)methyl]-2*H*-[1]benzopyran-2-one (**3**).

Compound **2** (0.251 g, 3 mmol) was added to a solution of compound **1** (0.522 g, 3 mmol) in aqueous ethanol (5.5 ml, ethanol/water: 10/1). Sodium acetate (0.123 g, 1.5 mmol) was then added and the mixture was refluxed for 7 hours. After cooling the mixture was poured to an ice/water mixture (30 ml) and the precipitate formed was filtered and washed with water to give nitrone **3** (0.326 g, 54%), mp 240-242° C (methanol/water); ir: 1700, 1660, 1600 cm^{-1}; ^{1}H nmr (80 MHz): δ 4.03 (s, 3H), 7.20-7.70 (m, 4H), 7.86 (s, 1H), 8.46 (s, 1H); ms: m/z 203 [M$^+$] (17), 187 (16), 175 (100), 158 (46), 146 (46), 130 (58), 118 (60), 102 (62).

Anal. Calcd. for $C_{11}H_9NO_3$: C, 65.02; H, 4.46; N, 6.89. Found: C, 65.11; H, 4.60; N, 7.11.

General Procedure for the Reactions of Nitrone **3** with Dienophiles **4a,b, 6, 8**.

A solution of nitrone **3** (0.5 mmol) and dienophile **4a,b, 6, 8** (0.5 mmol) in toluene (10 ml) was refluxed for 4-24 hours. The solvent was evaporated and the residue was treated with hexane/dichloromethane or ether to give as precipitate compound **5a** or **5b** respectively; while separation of the other residues by column chromatography [silica gel, hexane/ethyl acetate (4:1)] gave compounds **7** and **9**.

Synthesis of 2-Methyl-3-(2-oxo-2*H*-[1]benzopyran-4-yl)tetrahydroisoxazole-4,5-dicarboxylic Acid *N*-Phenyl Imide (**5a**).

Reaction of nitrone **3** (0.102 g, 0.5 mmol) and *N*-phenylmaleimide **4a** (88 mg, 0.5 mmol) under reflux for 4 hours gave compound **5a** (88 mg, 47%), mp 129-131°C (ethyl acetate); ir: 1805, 1725, 1715, 1600 cm^{-1}; ^{1}H nmr (80 MHz): δ 2.88 (s, 3H), 3.94 (dd, J_{d1} = 7.6, J_{d2} = 2.5, 1H), 4.82 (d, J = 2.5, 1H), 5.02 (d, J = 7.6, 1H), 6.76 (s, 1H), 7.30-7.70 (m, 8H), 8.07 (d, J = 8, 1H); ms: m/z 376 [M$^+$] (20), 203 (6), 187 (6), 173 (100), 158 (27), 146 (13), 130 (30), 119 (23).

Anal. Calcd. for $C_{21}H_{16}N_2O_5$: C, 67.02; H, 4.28; N, 7.44. Found: C,67.27; H, 4.32; N, 7.35.

Synthesis of 2-Methyl-3-(2-oxo-2*H*-[1]benzopyran-4-yl)tetrahydroisoxazole-4,5-dicarboxylic Acid *N*-Methyl Imide (**5b**).

Reaction of nitrone **3** (0.102 g, 0.5 mmol) with *N*-methylmaleimide **4b** (55.5 mg, 0.5 mmol) under reflux for 24 hours resulted to compound **5b** (0.13 g, 83%), mp 208-210 °C (hexane/ethyl acetate); ir: 1790, 1720, 1710, 1600 cm^{-1}; ^{1}H nmr (300 MHz): δ 2.76 (s, 3H), 3.11 (s, 3H), 3.79 (dd, J_{d1} = 7.4, J_{d2} = 2.7, 1H), 4.66 (d, J = 2.7, 1H), 4.92 (d, J = 7.4, 1H), 6.72 (s, 1H), 7.38-7.43 (m, 2H), 7.60 (t, J = 7.6, 1H), 8.0 (d, J = 8, 1H); ^{13}C nmr: δ 25.5, 44.1, 56.1, 67.9, 74.4, 114.9, 117.2, 117.7, 125.1, 124.7, 132.3, 150.1, 154.0, 160.2, 173.1, 175.0 ppm; ms: m/z 314 [M$^+$] (69), 228 (11), 200 (13), 184 (15), 175 (12), 169 (100), 158 (41), 147 (19).

Anal. Calcd. for $C_{16}H_{14}N_2O_5$: C, 61.14; H, 4.49; N, 8.91. Found: C, 61.01; H, 4.44; N, 8.86.

Synthesis of Dimethyl 2-Methyl-3-(2-oxo-2*H*-[1]benzopyran-4-yl)-tetrahydroisoxazol-4,5-dicarboxylate (**7**).

A solution of nitrone **3** (0.102 g, 0.5 mmol) and dimethylfumarate **6** (72 mg, 0.5 mmol) was refluxed for 6 hours. An additional amount of **6** (72 mg, 0.5 mmol) was added and the reflux was continued for 18 hours more to give compound **7** (0.128 g, 37%), mp 95-96 °C (hexane/ethyl acetate); ir: 1750, 1710, 1600 cm^{-1}; ^{1}H nmr (300 MHz): δ 2.77 (s, 3H), 3.77 (s, 3H), 3.86 (s, 3H), 3.98 (dd, J_{d1} = 3.7, J_{d2} = 7.0, 1H), 4.33 (d, J = 7.0, 1H), 4.96 (d, J = 3.7, 1H), 6.69 (s, 1H), 7.27-7.39 (m, 2H), 7.57 (t, J = 7.4, 1H), 7.85 (d, J = 8.1, 1H); ^{13}C nmr: δ 29.6, 43.5, 53.0, 57.8, 70.2, 78.1, 115.3, 117.5, 117.6, 124.3, 124.5, 132.1, 151.2, 153.9, 160.3, 170.4, 171.0 ppm; ms: m/z: 347 [M$^+$] (77), 260 (25), 238 (12), 228 (83), 219 (78), 186 (65), 170 (42), 131 (78), 119 (16), 69 (100).

Anal. Calcd. for $C_{17}H_{17}NO_7$: C, 58.79; H, 4.93, N, 4.03. Found: C, 59.07; H, 4.86; N, 4.12.

Synthesis of Ethyl 2-Methyl-3,5-bis(2-oxo-2*H*-[1]benzopyran-4-yl)tetrahydroisoxazol-4-carboxylate (**9**).

The reaction mixture of nitrone **3** (0.102 g, 0.5 mmol) and dipolarophile **8** (0.122 g, 0.5 mmol) was refluxed for 24 hours and chromatographed to give at first unreacted compound **8** (84 mg, 69%), followed by the compound **9** (49 mg, 22%), mp 172-175 °C (hexane/ethyl acetate); ir: 1715, 1600 cm^{-1}; ^{1}H nmr (300 MHz): δ 1.18 (t, J = 7.1, 3H), 2.89 (s, 3H), 3.52 (dd, J_{d1} = 4.8, J_{d2} = 7.8, 1H), 4.27 (dq, J_d = 2.7, Jq = 7.1, 2H), 4.39 (d, J = 7.8, 1H), 5.84 (d, J = 4.8, 1H), 6.65 (s, 1H), 6.93 (s, 1H), 7.23-7.43 (m, 4H), 7.49-7.63 (m, 3H), 7.79 (d, J = 7.9, 1H); ^{13}C nmr:

δ 13.7, 29.5, 43.1, 62.4, 71.8, 76.7, 112.8, 115.4, 117.1 117.2, 117.3, 117.4, 123.8, 124.1, 124.2, 124.5. 131.9, 132.2., 150.5, 153.3, 153.4, 153.8, 159.9, 160.4, 170.4 ppm; ms: m/z: 447 [M^+] (7), 303 (5), 277 (6), 260 (5), 244 (80), 216 (31), 199 (61), 187 (50), 171 (100), 115 (87).

Anal. Calcd. for $C_{25}H_{21}NO_7$: C, 67.11; H, 4.73; N, 3.13. Found: C, 67.27; H, 4.73; N, 3.18.

Unreacted nitrone **3** (21 mg, 21%) was then eluted.

Reaction of Aldehyde **1** with *N*-Methylhydrazine **10b**. Synthesis of 2-Oxo-2*H*-[1]benzopyran-4-carboxaldehyde *N*-Methylhydrazone (**11b**).

A solution of aldehyde **1** (0.522 g, 3 mmol) and *N*-methylhydrazine **10b** (0.138 g, 3 mmol) in absolute ethanol (25 ml) was stirred vigorously for 1 hour. The precipitate formed was filtered to give compound **11b** (0.427 g, 70%), mp 139-140 °C (hexane/ethyl acetate); ir: 1715, 1595 cm^{-1}; ^1H nmr (300 MHz): δ 3.12 (s, 3H), 6.49 (s, 1H), 7.20-7.60 (m, 5H), 8.38 (d, *J* = 8, 1H); ms: m/z: 202 [M^+] (89), 187 (32), 174 (30), 158 (21), 146 (8), 131 (100), 115 (20).

Anal. Calcd. for $C_{11}H_{10}N_2O_2$: C, 65.34; H, 4.98; N, 13.85. Found: C, 65.20; H, 5.02; N, 14.00.

General Procedure for the Reactions of *N*-Substituted Hydrazones **11a,b** with *N*-Chlorosuccinimide in the Presence of Dienophiles **4a,b, 6**. Synthesis of Dihydropyrazoles **13a-d, 15a,b**.

N-Chlorosuccinimide (0.21 g, 1.57 mmol) was added portionwise during 1 hour period to a cooled (0 °C) solution of hydrazones **11a,b** (1 mmol) in DMF (50 ml). The dienophiles **4a,b, 6** (1 mmol) were then added followed by addition of triethylamine (0.101 g, 1 mmol). The mixture was well stirred for 15 minutes to 2 days and was then poured in water (50 ml) and extracted with dichloromethane (3x50 ml). The organic layer was washed with water (6x100 ml) and dried over anhydrous sodium sulfate. The solvent was evaporated and the residue was separated by column chromatography [silica gel, hexane/ethyl acetate (3:1)] to give as first fraction the dihydropyrazole derivatives **13b-d, 15a,b**.

Synthesis of 1-Phenyl-3-(2-oxo-2*H*-[1]benzopyran-4-yl)-4,5-dihydropyrazole-4,5-dicarboxylic *N*-phenylimide **13a** and 1-Phenyl-3-(5-chloro-2-oxo-2*H*-[1]benzopyran-4-yl)-4,5-dihydropyrazole-4,5-dicarbocyclic *N*-Phenylimide **14**.

Reaction of *N*-phenylhydrazone **11a** (0.264 g, 1 mmol) with *N*-phenylmaleimide **4a** (0.173 g, 1 mmol) according to the above general procedure under stirring for 15 minutes gave at first compound **14** (58 mg, 12%), mp 277-279 °C (hexane/ethyl acetate); ir: 1730, 1715, 1595 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$ + $DMSO_{-d6}$): δ 5.51 (d, *J* = 11.0, 1H), 5.64 (d, *J* = 11.0, 1H), 7.05-7.17 (m, 1H), 7.25-7.30 (m, 2H), 7.32-7.50 (m, 7H), 7.55-7.80 (m, 4H); ^{13}C nmr ($CDCl_3$ + $DMSO_{-d6}$): δ 55.1, 64.9, 114.4, 116.8, 121.6, 122.2, 124.9, 125.7, 128.7, 128.8, 128.9, 130.8, 132.0, 135.7, 140.6, 142.8, 151.6, 156.1, 160.3, 161.5, 169.9, 170.2 ppm; ms: m/z 471 [M^+] (39), 469 [M^+] (100), 350 (6), 348 (17), 322 (16), 294 (18), 231 (10), 219 (36), 131 (17).

Anal. Calcd. for $C_{26}H_{16}ClN_3O_4$: C, 66.46; H, 3.43; N, 8.94. Found: C, 66.38; H, 3.28; N, 8.78.

Compound **13a** was then eluted (0.133 g, 31%), mp >300 °C (ethyl acetate/methanol); ir: 1790, 1720, 1705, 1590 cm^{-1}; ^1H nmr (300 MHz, $CDCl_3$+$DMSO_{-d6}$): δ 5.42 (d, *J* = 11.4, 1H), 5.69 (d, *J* = 11.4, 1H), 7.06-7.13 (m, 1H), 7.16 (s, 1H), 7.30-7.50 (m, 9H), 7.58-7.70 (m, 3H), 9.03 (d, *J* = 8.0, 1H); ^{13}C nmr ($CDCl_3$ + $DMSO_{-d6}$): δ 52.9, 63.9, 114.0, 115.2, 115.5, 116.0, 121.7, 123.5, 125.5, 127.2, 127.9, 128.0, 128.2, 130.6, 140.7, 146.3, 152.7, 156.9, 158.0, 159.1, 167.6, 169.9 ppm; ms: m/z: 435 [M^+] (94), 315 (18), 288 (8), 260 (80), 231 (11), 155 (11), 119 (29), 104 (13), 77 (100).

Anal. Calcd. for $C_{26}H_{17}N_3O_4$ C, 71.72; H, 3.94; N, 9.65. Found: C, 71.56; H, 4.00; N, 9.53.

Synthesis of 1-Methyl-3-(2-oxo-2*H*-[1]benzopyran-4-yl)-4,5-dihydropyrazole-4,5-dicarbocyclic *N*-Phenylimide (**13b**)

Reaction of *N*-methylhydrazone **11b** (0.202 g, 1 mmol) with *N*-phenylmaleimide **4a** (0.173 g, 1 mmol) according to the above procedure under stirring for 15 minutes gave compound **13b** (72 mg, 28%), mp 224-225 °C (hexane/ethyl acetate); ir: 1785, 1710, 1700, 1590 cm^{-1}; ^1H nmr (80 MHz): δ 3.47 (s, 3H), 4.67 (d, *J* = 12, 1H), 4.94 (d, *J* = 12, 1H), 6.96 (s, 1H), 7.20-7.85 (m, 8H), 8.87 (d, *J* = 8, 1H); ms: m/z 373 [M^+] (37), 253 (14), 226 (12), 198 (54), 156 (8), 127 (22), 119 (14), 91 (100).

Anal. Calcd. $C_{21}H_{15}N_3O_4$: C, 67.56; H, 4.05; N, 11.25. Found: C, 67.39; H, 4.18; N, 10.99.

Synthesis of 1-Phenyl-3-(2-oxo-2*H*-[1]benzopyran-4-yl)-4,5-dihydropyrazole-4,5-dicarboxylic *N*-Methylimide (**13c**).

From the reaction of *N*-phenylhydrazone **11a** (0.264 g, 1 mmol) with *N*-methylmaleimide **4b** (0.111 g, 1 mmol) as above after stirring for 3 hours, evaporation of the solvent, and addition of diethyl ether to the residue compound **13c** was precipitated (0.301 g, 81%), mp 278-280 °C (methanol); ir: 1780, 1720, 1690, 1590 cm^{-1}; ^1H nmr (80 MHz, $CDCl_3$+$DMSO_{-d6}$): δ 3.02 (s, 3H), 5.27 (d, *J* = 11.5, 1H), 5.54 (d, *J* = 11.5, 1H), 7.03-7.12 (m, 1H), 7.14 (s, 1H), 7.35-7.45 (m, 4H), 7.55-7.65 (m, 3H), 8.97 (d, *J* = 8, 1H); ms: m/z 373 [M^+] (100), 315 (11), 288 (13), 260 (45), 130 (12), 111 (14), 104 (10).

Anal. Calcd. for $C_{21}H_{15}N_3O_4$: C, 67.56; H, 4.05; N, 11.25. Found: C, 67.75; H, 4.14; N, 10.86.

Synthesis of 1-Methyl-3-(2-oxo-2*H*-[1]benzopyran-4-yl)-4,5-dihydropyrazole-4,5-dicarboxylic *N*-Methylimide (**13d**).

The reaction mixture of *N*-methylhydrazone **11b** (0.202 g, 1 mmol) and *N*-methylmaleimide **4b** (0.111 g, 1 mmol) treated as above was stirred for 6 hours. After evaporation of the solvent, and addition of diethyl ether to the residue precipitated compound **13d** (0.202 g, 65%), mp 253-255 °C (methanol/tetrahydrofuran); ir: 1780, 1700, 1680, 1590 cm^{-1}; ^1H nmr (80 MHz): δ 3.05 (s, 3H), 3.46 (s, 3H), 4.59 (d, *J* = 11, 1H), 4.76 (d, *J* = 11, 1H), 6.96 (s, 1H), 7.24-7.36 (m, 2H), 7.52 (t, *J* = 7.8, 1H), 8.84 (d, *J* = 8.6, 1H); ms: m/z 311 [M^+] (100), 253 (17), 242 (11), 226 (22), 198 (99), 169 (14), 155 (28), 143 (13), 127 (15), 115 (13).

Anal. Calcd. for $C_{16}H_{13}N_3O_4$: C, 61.73; H, 4.21; N, 13.50. Found: C, 61.72; H, 4.05; N, 13.54.

Synthesis of Dimethyl 1-Phenyl-3-(2-oxo-2*H*-[1]benzopyran-4-yl)-4,5-dihydropyrazol-4,5-dicarboxylate (**15a**).

The reaction mixture of *N*-phenylhydrazone **11a** (0.264 g, 1 mmol) and dimethylfumarate **6** (0.144 g, 1 mmol) was stirred as above for 15 minutes and treated with ether like above to give compound **15a** (0.136 g). The filtrate was separated by column chromatography [siliga gel, hexane/ethyl acetate (4:1)] to give compound **15a** (46 mg, total 0.182 g, 44%), mp 197-200 °C (hexane/ethyl acetate); ir: 1745, 1730, 1695, 1590 cm^{-1}; ^1H nmr (300 MHz): δ 3.79 (s, 3H), 3.80 (s, 3H), 4.61 (d, *J* = 4.7, 1H),

5.31 (d, *J* = 4.7, 1H), 6.50 (s, 1H), 7.06 (t, *J* = 7.3, 1H), 7.20-7.30 (m, 3H), 7.36-7.45 (m, 3H), 7.55-7.62 (m, 1H), 8.99 (d, *J* = 8, 1H); ^{13}C nmr: δ 53.4, 53.6, 55.2, 64.9, 114.1, 114.3, 116.7, 117.3, 122.4, 124.3, 124.5, 128.5, 129.5, 131.8, 139.2, 141.8, 153.9, 163.9, 168.5, 169.4 ppm; ms: m/z 406 [M$^+$] (11), 343 (56), 312 (31), 303 (20), 285 (71), 268 (29), 254 (19), 243 (28), 236 (39), 225 (59), 208 (100), 198 (22), 186 (37), 155 (83).

Anal. Calcd. for $C_{22}H_{18}N_2O_6$: C, 65.02; H, 4.46; N, 6.89. Found: C, 64.73; H, 4.35; N, 7.05.

Synthesis of Dimethyl 1-Methyl-3-(2-oxo-2*H*-[1]benzopyran-4-yl)-4,5-dihydropyrazole-4,5-dicarboxylate (**15b**).

The reaction mixture of *N*-methylhydrazone **11b** (0.202 g, 1 mmol) and dimethylfumarate **6** (0.144 g, 1 mmol) was stirred as above for 2 days to give at first compound **15b** (0.114 g, 33%), mp 80-82 °C; (dichloromethane/ethyl acetate); ir: 1740, 1730, 1710, 1605 cm^{-1}; ^{1}H nmr (300 MHz): δ 3.34 (s, 3H), 3.78 (s, 3H), 3.86 (s, 3H), 4.49 (d, *J* = 9, 1H), 4.65 (d, *J* = 9, 1H), 6.41 (s, 1H), 7.28-7.41 (m, 2H), 7.53 (t, *J* = 6.8, 1H), 8.71 (d, *J* = 8.6, 1H); ^{13}C nmr: δ 40.9, 53.1, 53.4, 55.4, 70.8, 113.3, 117.1, 117.2, 124.2, 128.4, 131.7, 137.4, 141.9, 153.7, 161.6, 166.0, 169.8 ppm; ms: m/z 344 [M$^+$] (100), 312 (16), 285 (53), 257 (85), 241 (56), 121 (12).

Anal. Calcd. for $C_{17}H_{16}N_2O_6$: C, 59.30; H, 4.68; N, 8.14. Found: C, 59.39; H, 4.54; N, 8.19.

Unreacted hydrazone **11a** was then eluted (30 mg, 15%).

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