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**Short communication**

# Synthesis and biological evaluation of several coumarin-4-carboxamidoxime and 3-(coumarin-4-yl)-1,2,4-oxadiazole derivatives

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**Abstract** – A series of novel coumarin-4-carboxamidoximes **6a–g** and 3-(coumarin-4-yl)-1,2,4-oxadiazoles **9a–e** were synthesized from coumarin-4-carboxaldehyde **1** via the intermediate coumarin-4-nitriloxide **4**. These coumarin derivatives were isolated and characterized, and evaluated for their ability to inhibit trypsin, glucuronidase, and soybean lipoxygenase. The compounds were also tested for antioxidant activity, and as antiiflammatory agents in the rat carrageenin paw edema assay. © Elsevier, Paris

**coumarin-4-nitriloxides / coumarin-4-carboxamidoximes / 3-(coumarin-4-yl)-1,2,4-oxadiazoles / antioxidants / antiinflammatory**

## 1. Introduction

Coumarin compounds [1] have been used to treat such diverse ailments as cancer, burns, brucellosis and rheumatic disease. The coumarin nucleus incorporates the styryl carbonyl moiety into a rigid framework. It has been reported that styryl carbonyl derivatives possess appreciable anti-inflammatory activity [2, 3]. It is to be expected that they might affect the formation and scavenging of reactive substances derived from oxygen and influence processes involving free radical-mediated injury [4–6]. Further more coumarin and related derivatives are recognized as inhibitors not only of the lipoxygenase (LO) and cyclo-oxygenase (CO) pathways of arachidonic acid metabolism [7, 8] but also of neutrophil-dependent superoxide generation [9]. Several novel 4-styryl-coumarin [3, 10] derivatives have been studied for their anti-human immunodeficiency virus ability (HIV) and as anticancer agents [11]. The presence of one at least 4-hydroxy-coumarin ring, has been recently [11] correlated with the inhibition of HIV-1 integrase and protease.

In connection to our previous work on the synthesis of coumarin derivatives [12–16], in a recent work we studied the activity in the inflammatory process of 4-imino-, 4-vinyl-, 4-(5′-isoxazolinyl)- and 4-(5′-1,2,4-oxadiazolinyl)-coumarins [17]. These facts prompted us to design and synthesize a series of novel coumarins like the new 4-carboxamidoxime-, 4-(3′-1,2,4-oxadiazolinonyl)- and 4-(3′-1,2,4-oxadiazolyl)-coumarin derivatives and to study the activities of relevance to the inflammatory process, as well as to study preliminary their effects on β-glucuronidase, on trypsin, on soybean LO, on superoxide anion and their interaction with the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). The results are discussed in terms of structure–activity relationships and an attempt is made to define the structural features for active compounds.

## 2. Chemistry

The reactions studied and the products (new compounds) obtained are depicted in figures 1–4.

In a parallel work [18] we prepared the nitriloxide **4** by treating oxime **2** with bleach **3** and $Et_3N$ and we studied 1,3-dipolar cycloaddition reactions of **4** towards different dipolarophiles. An attempt to prepare **4** by treating oxime

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*Abbreviations*: DPPH: 1,1-diphenyl-2-picrylhydrazyl; LO: lipoxygenase; CO: cyclooxygenase

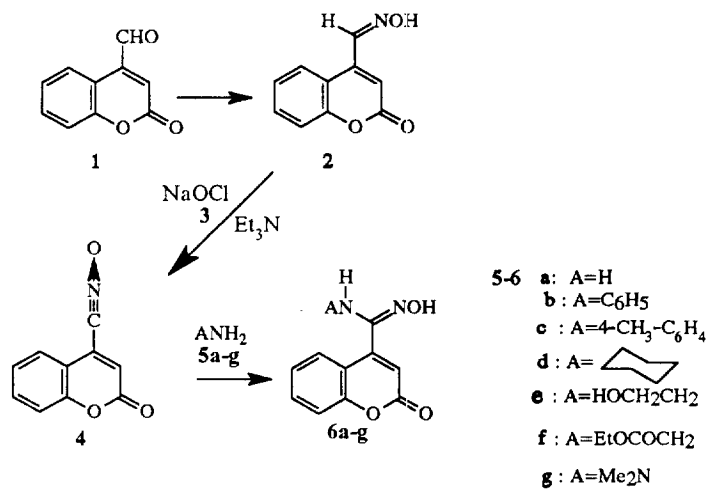


**Figure 1**. Synthesis of **6a–g**.

**2** with NCS resulted to a mixture of **4** with 3-chloro-substituted nitriloxide. The chemistry of this new 3-chlorosubstituted nitriloxide for the preparation of new amidoximes and substituted heterocyclic derivatives are under further investigation [19].

Previous studies [20] have demonstrated that reactions of nitriloxides with amines resulted to amidoximes. In this work we try to prepare coumarin amidoximes from the reaction of nitriloxide **4** with amines, besides the possibility for coumarin ring opening [21] or transformation to quinolin-2-ones [22]. Treatment of nitriloxide **4** with ammonia (gas) for 1 h resulted to the precipitation of coumarin amidoxime **6a** (53% yield). Analogous reactions (*figure 1*) of ethereal dispersion of nitriloxide **4** with the amines **5b–g** gave after separation of the reaction mixture by column chromatography the coumarin amidoximes **6b–g** in good to moderate yields (40–76%). In all these reactions no dimerization product [18] of **4** was detected in the reaction mixtures. The analytical and spectral data of the products **6a–g** are in good agreement with the proposed structures.

We tried also here to prepare new compounds containing together the coumarin ring and the 1,2,4-oxadiazole moiety. For this reason we studied reactions of coumarin-4-amidoximes **6a–c** with acid chlorides [23], anhydrides [24] and esters [25] in basic media, which could lead to 1,2,4-oxadiazole derivatives through the intermediate O-acyl derivatives. We performed also reactions of **6a–c** with ethyl orthoformate [25] and aldehydes [26], phosgene [27] and thiophosgene [28] for the preparation of 1,2,4-oxadiazole derivatives and 4,4-dihydro-1,2,4-oxadiazole derivatives.

Reactions of amidoxime **6a** with acetyl-**7a** or benzoyl-chloride **7b** in the presence of $Et_3N$ resulted to O-acylderivatives **8a** (44%) and **8b** (83%) respectively along with the condensation product **9a** (5%) in the first case (*figure 2*). Heating of the toluene solution of compounds **8a,b** resulted to the condensation products **9a** (56%) and **9b** (74%) respectively as indicated from the IR spectra (no $\nu$-$NH_2$ absorptions) and the other spectral data.

From the reactions of toluene dispersion of amidoxime **6a** with trifluoroacetic anhydride or ethyl acetoacetate (*figure 3*) the trifluoromethyl compound **9c** (51%) or the acetonyl compound **9d** (26%) formed respectively after separation by column chromatography. Analogous reaction of compound **6a** with excess of ethyl orthoformate **10** in the presence of $BF_3 \bullet Et_2O$ gave 1,2,4-oxadiazole **9e** (41%). The analytical and spectral data are in good agreement with the proposed structures. Especially in the $^1H$ NMR spectra of compounds **9a–e** there is a doublet peak in the region of $\delta$ 8.5–8.7 corresponding to 5-H of

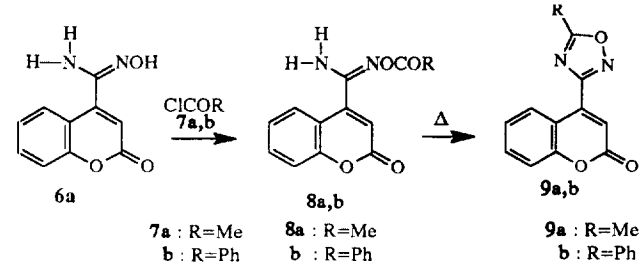


**Figure 2**. Synthesis of **8a,b** and **9a,b**.

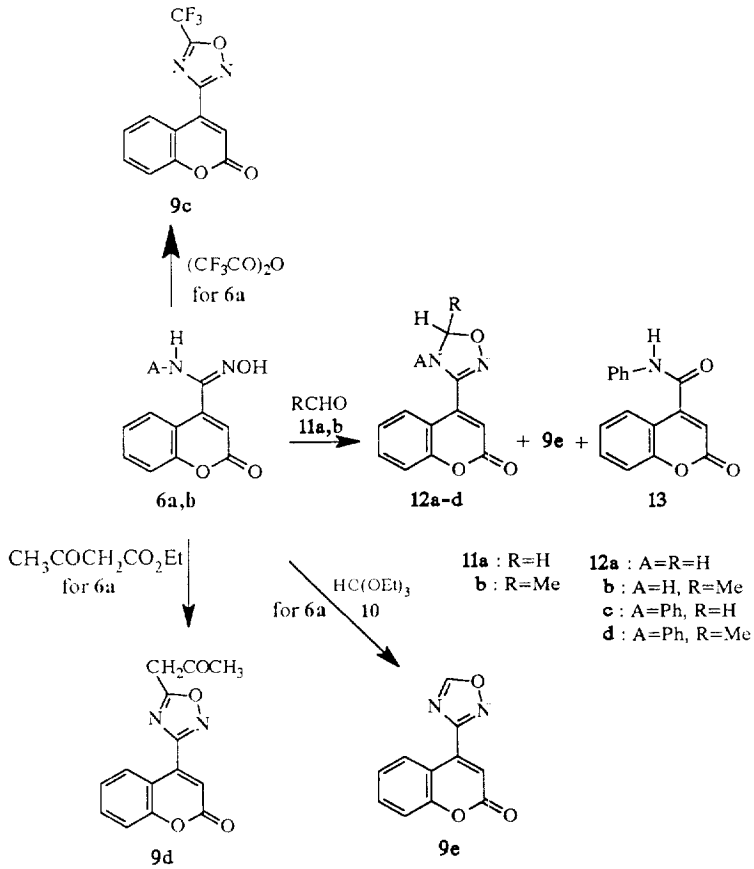


**Figure 3**. Synthesis of **9c–e** and **12a–d**.

coumarin ring, due to downfield deshielding by the aromatic 1,2,4-oxadiazole ring.

Treatment of amidoxime **6a** with aqueous solution of formaldehyde **11a** or acetic solution of acetaldehyde **11b** resulted to 4,5-dihydro-1,2,4-oxadiazole derivatives **12a** (22%) or **12b** (37%) respectively along with the oxidation product **9e** (5%) for the first case. Analogous treatment of compound **6b** with formaldehyde **11a** or acetaldehyde **11b** gave the expected compounds **12c** (45%) and **12d** (29%). The new amide **13** (16%) was also formed in the first case.

A dispersion of amidoximes **6a–c** in $CH_2Cl_2$ was treated with thionyl chloride to give 1,2,3,5-oxathiadiazoles **14a–c** in good yields (56–79%) (*figure 4*). A toluene solution of N-aryl amidoximes **6b,c** gave by treatment with phosgene or thiophosgene the 1,2,4-oxadiazolin-5-ones **15a,b** or 1,2,4-oxadiazolin-5-thiones **16a,b** respectively in high yields (80–92%). A chloroform solution of amidoxime **6c** was reacted with ethyl chloroformate **7c** to give compound **15b** (37%).

## 3. Results and discussion

*Table I* summarizes the effect of a number of coumarin derivatives on in vitro β-glucuronidase activity, trypsin induced proteolysis, superoxide anion scavenging activity, soybean lipoxygenase activity, and in vivo carrageenin induced paw edema (CPE). As pointed out anti-inflammatory agents have been reported to exhibit antiproteolytic [29] activity. In our case, we observed that compounds **6a–8b** exert on some inhibitory activity on trypsin induced proteolysis (78.4–19%), whereas only compound **6a** (*table I*) slightly inhibits β-glucuronidase. Under the reported experimental conditions none of the examined compounds scavenged superoxide anion (slightly compound **12a**, 3%: *table I*).

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

The anti-inflammatory activity of the examined compounds at 0.15 mmoles/kg body weight, is shown in

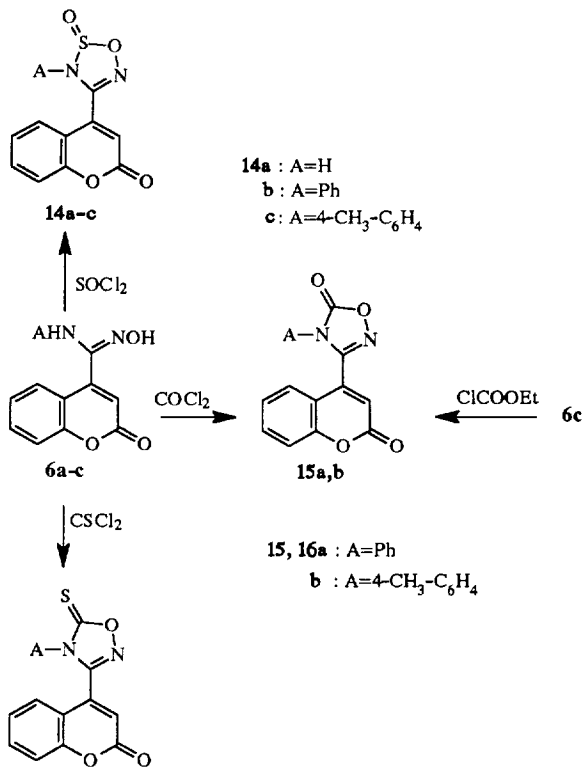


**Figure 4**. Synthesis of **14a–c**, **15a,b** and **16a,b**.

comparison with that of the parent compound **6a** (*table I*) and indometacin (0.11 mmoles/kg) a potent CO inhibitor. A significant increase of the biological effect was observed when R=H, was replaced by alkyl or phenyl moieties. Substituents on the amine moiety with positive hydrophobicity (in terms of $\pi$), increase the inhibition of carrageenin induced rat paw edema (*table I*, compounds **6a=6d, 6c**). In most of the cases the tested compounds were shown to protect against edema formation higher than the reference standard drug (e.g. indometacin 53.6% inhibition). The prostaglandin phase, 3.5 h, of carrageenin rat paw edema [30], was affected, suggesting a positive anti-CO activity. As pointed out by our experimental procedure, the examined coumarins interact with DPPH. This interaction expresses the reducing activity of the compounds. In general, compounds with reducing ability and superoxide anion scavenging effect would be good canditates as CO inhibitors. But in our case the examined compounds did not work as radical scavengers. Bulky and hydrophobic substituents like $C_6H_5$- on NH-R decrease the reducing ability (compound **8b**, *table I*). The presence of a sulfur atom (C=S, S=O) in the ring (comps. **14b**, **14c**, **16a**, **16b**) was found to have a positive effect on the antioxidant activity.

Some selected compounds were evaluated for inhibition of soybean lipoxygenase (LO) by the UV absorbance based enzyme assay [31]. While one may not extrapolate the quantitative results of this assay to the inhibition of mammalian 5-LO, it has been shown that inhibition of plant LO activity by NSAID's is qualitatively similar to their inhibition of the rat mast cell LO and may be used as a simple qualitative screen for such activity. Under our experimental conditions no LO inhibition was found for the tested compounds, with the exception of compounds **8a** and **9a** (*table I*) which inhibited both the enzyme, 22.6% at 0.1 mM. No sign of inhibition was shown at 1 mM and 0.3 mM (results not shown in *table I*). With these results no correlation between the in vivo anti-inflammatory activity and the in vitro soybean LO activity exists. This result strongly supports the view that these compounds are endowed with a different mechanism of action from LO inhibition.

Regression analyses were performed to find out the correlations between anti inflammatory ability (CPE %), reducing ability (RA %) and lipophilicity as $\pi$ values and other physicochemical parameters. The derived equations were not significant (few data points, variation). There is no much evidence on QSAR studies of anti inflammatory agents in general, mainly because inflammation is a complex phenomenon involving different mechanisms through a number of inflammatory mediators.

Poor correlations ($r < 0.5$) were also obtained between (1) reducing ability and antiinflammatory activity, (2) lipophilicity and antiproteolytic activity, (3) lipophilicity and lipoxygenase inhibition.

## 4. Conclusions

In conclusion, compound **8a**, on the basis of our results would be a good candidate, a lead molecule to be modified in order to improve the LO inhibition. Twelve from thirteen compounds tested with the carrageenin induced rat paw edema were found to be potent anti inflammatory agents (59.1–87.6%). These compounds were interacted with DPPH. For most of the above activities the presence of a double bond in the $C_4$-substituent (C=NOH, or as a ring 1, 2, 4-oxadiazolyl) and the replacement of the N-*OH* by a OCOR (R=$CH_3$- or $C_6H_5$-) function (compounds **8a,b**, *table I*) seemed to be essential and generate new derivatives with higher anti-inflammatory activity. The double bond in the $C_4$-substituent leads to a rigid conjugated system of a styryl-carbonyl type. Lipophilicity was found to play a role too.

**Table I.** Reducing ability (RA, %), inhibition in vitro of trypsin induced proteolysis (Ipr, %), inhibition in vitro of β-glucuronidase (Gl, %), in vitro superoxide scavenging activity (S. SA, %), inhibition in vitro of soybean lipoxygenase (LO, %), in vivo inhibition of carrageenin rat paw edema (CPE, %).

| Compound | RA (%) (0.1 mM) [a] | Ipr (%) (0.1 mM) [a] | Gl (%) (1 mM) [a] | S. SA (%) (0.1 mM) [a] | LO (%) (0.1 mM) [a] | CPE [b] (%) (SEM) [c] |
|---|---|---|---|---|---|---|
| **1** | 97.7 | no | nt | nt | nt | nt |
| **2** | nt | no | nt | nt | nt | 68.7 (1.6) |
| **6a** | 96.2 | 52.3 | 1.4 | no | no | 54.7 (3.5) |
| **6b** | 86.2 | no | no | no | no | nt |
| **6c** | 84.8 | 30.5 | no | no | no | 73.6 (1.7) |
| **6d** | 17.0 | 28.8 | nt | nt | nt | 59.1 (4.3) |
| **6e** | 17.6 | no | no | nt | nt | 62.3 (4.4) |
| **6f** | 29.1 | no | no | nt | nt | nt |
| **6g** | 23.6 | no | nt | nt | nt | 81.1 (1.3) |
| **8a** | 69.2 | 78.4 | no | < 1 | 22.6 | 63.2 (3.9) |
| **8b** | 87.6 | no | no | no | no | 87.6 (1.5) |
| **9a** | 18.4 | 69.4 | no | < 1 | 22.6 | 68.4 (1.7) |
| **9b** | 56.4 | no | nt | no | nt | 75.9 (1.1) |
| **9c** | 28 | 69.4 | no | nt | nt | nt |
| **9d** | no | no | nt | nt | nt | nt |
| **9e** | 17.9 | 15.3 | nt | nt | nt | 65.0 (2.9) |
| **12a** | 97.6 | 57.7 | nt | 3 | nt | 67.7 (1.1) |
| **14a** | no | 98.2 | 1.65 | no | no | nt |
| **14b** | 20.7 | nt | nt | nt | nt | 76.1 (1.6) |
| **14c** | 29.8 | no | no | nt | nt | nt |
| **15a** | 13.5 | 65.4 | no | nt | nt | nt |
| **15b** | 10.5 | 59.8 | nt | nt | no | nt |
| **16a** | 21.6 | 46.2 | nt | nt | nt | 76.9 (3.1) |
| **16b** | 14 | 57.6 | nt | nt | nt | nt |
| SA | | 53.6 | 2.32 | | | |
| ASA | 80.5 | | | | | |
| CA | | | | 10 | | |
| NDGA | | | | | 83.7 | |
| IMA | | | | | | 53.6 (1.9) |

nt: not tested; no: 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%; [b] each value represents the mean ± of two independent experiments with 6 animals in each group; [c] (SEM: standard error of the mean) statistical significance of results was established using the Student's *t*-test ($p < 0.001$).

The anti-cyclooxygenase and lipoxygenase activities were not further investigated in this study. In recent times efforts could be made to clarify the above-mentioned activities.

## 5. Experimental protocols

### *5.1. Chemistry*

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 300 MHz on a Bruker AM 300 spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard unless otherwise stated. *J* values are reported in Hertz (Hz). $^{13}$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 and were within ±0.4% of theory. Nitriloxide **4** was prepared [18] by treating oxime **2** with bleach **3** in the presence of triethylamine.

#### *5.1.1. 2-Oxo-2H-[1]benzopyran-4-carboxamide oxime* ***6a***

$NH_3$ (gas) (**5a**) was passed for 1 h through a dispersion of nitriloxide **4** [18] (0.5 g, 2.7 mmol) in $Et_2O$ (50 mL). Stirring was continued for an additional 2 h and the precipitated **6a** was filtered: 0.291 g (53%), m.p. 224–226 °C (methanol); IR 3480, 3360, 3200, 1705, 1655 cm$^{-1}$; $^1$H NMR ($CDCl_3$ + DMSO-$d_6$): δ 5.83 (s, 2 H),

6.60 (s, 1 H), 7.27–7.36 (m, 2 H), 7.56 (t, $J$ = 7.7, 1 H), 8.26 (d, $J$ = 7.8, 1 H), 10.27 (s, 1 H); $^{13}$C NMR ($CDCl_3$ + DMSO-$d_6$): δ 114.6, 116.2, 116.9, 123.6, 127.5, 131.4, 146.3, 148.1, 153.4, 159.8 ppm; MS (*m/e*): 204 ($M^+$, 100), 186 (13), 173 (51), 158 (28), 145 (48), 131 (11). Anal. ($C_{10}H_8N_2O_3$) C, H, N.

*5.1.2. General procedure for the synthesis of N-substituted 2-oxo-2H-[1]benzopyran-4-carboxamide oximes* ***6b–g***

Amines **5b–g** (3 mmol) were added to a dispersion of nitriloxide **4** (0.561 g, 3 mmol) in $Et_2O$ (50 mL), and the mixture was stirred for 2 h at room temperature. The solvent was evaporated and the residue was separated by column chromatography [silica gel, $CH_2Cl_2$/hexane/EtOAc (10:1:1)] to give as first fraction the coumarin-4-amidoximes **6b–g**.

*5.1.2.1. N-Phenyl-2-oxo-2H-[1]benzopyran-4-carboxamide oxime* ***6b***. Reaction with aniline (**5b**) (0.279 g, 3 mmol) gave compound **6b** (0.614 g, 73%), m.p. 160–162 °C (hexane/$CHCl_3$); IR 3380, 3320, 1690, 1620 cm$^{-1}$; $^1$H NMR: δ 6.62 (s, 1H), 6.81 (d, $J$ = 7.7, 2H), 6.94 (m, 1H), 7.06–7.34 (m, 4H), 7.48 (m, 2H), 7.79 (dd, $J_{d1}$ = 7.9, $J_{d2}$ = 1, 1H), 9.02 (brs, 1H); $^{13}$C NMR: δ 117.0, 117.1, 117.9, 121.2, 124.3, 124.5, 126.4, 129.3, 132.3, 138.0, 145.3, 147.5, 153.7, 160.3 ppm; MS (*m/e*): 280 ($M^+$, 20), 263 (22), 252 (100), 235 (37), 220 (99), 205 (11), 145 (16). Anal. ($C_{16}H_{12}N_2O_4$) C, H, N.

*5.1.2.2. N-(4′-Methyl-phenyl)-2-oxo-2H-[1]benzopyran-4-carboxamide oxime* ***6c***. Reaction with p-toluidine (**5c**) (0.321 g, 3 mmol) for 3 h gave compound **6c** (0.671 g, 76%), m.p. 170–172 °C (hexane/EtOAc); IR 3380, 3310, 1685, 1625 cm$^{-1}$; $^1$H NMR: δ 2.17 (s, 3H), 6.58 (s, 1H), 6.71 (d, $J$ = 8, 2H), 6.89 (d, $J$ = 8, 2H), 7.12–7.53 (m, 5H), 7.79 (d, $J$ = 8, 1H); $^{13}$C NMR: δ 20.6, 117.1, 117.2, 117.9, 121.7, 124.5, 126.5, 129.8, 132.2, 134.3, 135.4, 145.4, 147.9, 153.7, 160.3 ppm; MS (*m/e*): 294 ($M^+$, 40), 277 (49), 266 (76), 249 (61), 234 (100), 145 (65), 116 (57), 91 (64). Anal. ($C_{17}H_{14}N_2O_3$) C, H, N.

*5.1.2.3. N-Cyclohexyl-2-oxo-2H-[1]benzopyran-4-carboxamide oxime* ***6d***. Reaction with cyclohexylamine (**5d**) (0.297 g, 3 mmol) gave compound **6d** (0.488 g, 57%), m.p. 161–163 °C (hexane/EtOAc); IR 3340, 3280, 1690, 1605 cm$^{-1}$; $^1$H NMR: δ 0.98–1.34 (m, 5H), 1.41–1.92 (m, 5H), 2.79 (m, 1H), 5.36 (d, $J$ = 10.4, 1H), 6.53 (s, 1H), 7.21–7.45 (m, 2H), 7.57 (m, 1H), 7.78 (d, $J$ = 8, 1H), 9.10 (brs, 1H); $^{13}$C NMR: δ 24.6, 25.0, 34.8, 52.3, 117.1, 117.3, 117.8, 124.7, 126.2, 132.4, 145.6, 150.4, 153.6, 160.2 ppm; MS (*m/e*): 286 ($M^+$, 11), 269 (8), 241 (28), 180 (100), 116 (15). Anal. ($C_{16}H_{18}N_2O_3$) C, H, N.

*5.1.2.4. N-(2′-Hydroxyethyl)-2-oxo-2H-[1]benzopyran-4-carboxamide oxime* ***6e***. Reaction of ethanolamine (**5e**) (0.183 g, 3 mmol) in $Et_2O$ (30 mL)/$CH_2Cl_2$ (10 mL) afforded compound **6e** (0.427 g, 57%), m.p. 189–190 °C (EtOAc/methanol); IR 3360, 3180 (br), 1715, 1635 cm$^{-1}$; $^1$H NMR($CDCl_3$ + DMSO-$d_6$): δ 3.02 (m, 2H), 3.54 (m, 2H), 4.42 (brs, 1H, exchangeable with $D_2O$), 5,94 (brs, 1H, exchangeable with $D_2O$), 6.51 (s, 1H), 7.22–7.75 (m, 3H), 7.84 (d, $J$ = 8, 1H), 9.96 (brs, 1H, exchangeable with $D_2O$); $^{13}$C NMR($CDCl_3$ + DMSO-$d_6$): δ 45.1, 61.2, 116.4, 116.7, 117.5, 124.1, 126.1, 131.7, 145.9, 150.2, 153.1, 159.8 ppm; MS (*m/e*): 248 ($M^+$, 100), 230 (24), 215 (26), 185 (45), 156 (47), 142 (70), 114 (43). Anal. ($C_{12}H_{12}N_2O_4$) C, H, N.

*5.1.2.5. N-(Ethoxycarbonylmethyl)-2-oxo-2H-[1]benzopyran-4-carboxamide oxime* ***6f***. Reaction of ethyl glycinate (**5f**) (0.309 g, 3 mmol) in $CH_2Cl_2$ (30 mL) in the presence of $Et_3N$ (0.303 g, 3 mmol) for 24 h gave after separation by column chromatography [silica gel, hexane/EtOAc (2:1)] compound **6f** (0.48 g, 55%), m.p. 115–117 °C ($CHCl_3$/hexane); IR 3340, 3260, 1740, 1685, 1630 cm$^{-1}$; $^1$H NMR: δ 1.19 (t, $J$ = 7.2, 3H), 3.67 (d, $J$ = 6.4, 2H), 4.13 (q, $J$ = 7.2, 2H), 6.02 (t, $J$ = 6.4, 1H), 6.55 (s, 1H), 7.27–7.39 (m, 2H), 7.58 (t, $J$ = 7.7, 1H), 7.74 (d, $J$ = 8.1, 1H); $^{13}$C NMR: δ 14.0, 44.8, 61.7, 117.2, 117.3, 118.1, 124.9, 126.1, 132.6, 144.3, 150.0, 153.7, 160.0, 169.8 ppm; MS (*m/e*): 290 ($M^+$, 100), 262 (24), 217 (84), 187 (88), 115 (40). Anal. ($C_{14}H_{14}N_2O_5$).

*5.1.2.6. N-(Dimethylamino)-2-oxo-2H-[1]benzopyran-4-carboxamide oxime* ***6g***. Reaction of N,N-dimethylhydrazine (**5g**) (0.18 g, 3 mmol) for 4 h gave compound **6g** (0.296 g, 40%), m.p. 136–138 °C (EtOAc); IR 3250 (br), 1680, 1620 cm$^{-1}$; $^1$H NMR: δ 2.40 (s, 6 H), 6.05 (brs, 1 H), 6.52 (s, 1 H), 7.27–7.40 (m, 2 H), 7.54 (t, $J$ = 7.7, 1 H), 7.78 (d, $J$ = 7.9, 1 H), 8.92 (brs, 1 H); $^{13}$C NMR: δ 48.7, 116.0, 117.0, 118.3, 124.2, 126.3, 131.8, 146.7, 151.7, 153.4, 160.7 ppm; MS (*m/e*): 247 ($M^+$, 100), 230 (37), 218 (33), 199 (37), 143 (54), 115 (20). Anal. ($C_{12}H_{13}N_3O_3$) C, H, N.

*5.1.3. Reaction of amidoxime* ***6a*** *with acetylchloride* ***7a****; synthesis of N-acetoxy-2-oxo-2H-[1]benzopyran-4-carboxamide oxime* ***8a*** *and 5-methyl-3-(2-oxo-2H-[1]benzopyran-4-yl)-1,2,4-oxadiazole* ***9a***

A solution of acetylchloride (**7a**) (78.5 mg, 1 mmol) in acetone (10 mL) was added dropwise during 30 min in a dispersion of amidoxime **6a** (0.204 g, 1 mmol) and $Et_3N$ (0.101 g, 1 mmol) in acetone (20 mL). The resulting mixture was stirred at room temperature for 30 min more and the solvent was evaporated. $H_2O$ (25 mL) was added in the residue and it was exctracted with $CH_2Cl_2$ (2 × 30 mL). The organic layer was dried over $Na_2SO_4$ and separated by column chromatography [silica gel, hexane/EtOAc (2:1)]. Compound **9a** was eluted first (11 mg, 5%), m.p. 143–144 °C (EtOAc); IR 1720, 1600 cm$^{-1}$; $^1$H NMR: δ 2.73 (s, 3 H), 7.23 (s, 1 H), 7.31–7.42 (m, 2 H), 7.60 (t, $J$ = 7.4, 1 H), 8.59 (d, $J$ = 8.1, 1 H); $^{13}$C NMR: δ 12.3, 115.9, 117.2, 118.6, 124.7, 127.6, 128.6, 132.4, 154.2, 159.9, 165.5, 176.8 ppm; MS (*m/e*): 228 ($M^+$, 97), 200 (15), 186 (100), 159 (54), 143 (41). Anal. ($C_{12}H_8N_2O_3$) C, H, N.

Compound **8a** was then eluted (0.107 g, 44%), m.p. 177–179 °C (EtOAc); IR 3430, 3300, 1740, 1705, 1630 cm$^{-1}$; $^1$H NMR: δ 2.26 (s, 3 H), 5.35 (brs, 2 H), 6.62 (s, 1 H), 7.27–7.40 (m, 2 H), 7.57 (t, $J$ = 7.0, 1 H), 7.98 (d, $J$ = 7.8, 1 H); $^{13}$C NMR: δ 19.7, 116.5, 117.1, 117.2, 124.8, 126.7, 132.8, 145.0, 151.6, 154.0, 156.7, 168.0 ppm; MS (*m/e*): 246 ($M^+$, 25), 228 (51), 204 (100), 188 (43), 174 (91), 159 (77), 143 (52). Anal. ($C_{12}H_{10}N_2O_4$) C, H, N.

*5.1.4. Reaction of amidoxime* ***6a*** *with benzoylchloride* ***7b****; synthesis of N-benzoyloxy-2-oxo-2H-[1]benzopyran-4-carboxamide oxime* ***8b***

Benzoylchloride (**7b**) (0.14 g, 1 mmol) was added dropwise during 1 h to a stirred dispersion of compound **6a** (0.204 g, 1 mmol) and $Et_3N$ (0.101 g, 1 mmol) in $CHCl_3$ (15 mL) and the mixture was stirred vigorously at room temperature for 2 h. The filtrate after removing of the $Et_3N$•HCl was concentrated under reduced pressure and the residue was treated with $Et_2O$/$CH_2Cl_2$ to give as precipitate compound **8b** (0.255 g, 83%), m.p. 154–156 °C

(hexane/EtOAc); IR 3420, 3305, 1720, 1640 cm $^{-1}$; $^1$H NMR: δ 5.43 (brs, 2H, exchanged by $D_2O$), 6.70 (s, 1H), 7.29–7.38 (m, 2H), 7.46–7.68 (m, 4H), 8.06–8.15 (m, 3H); $^{13}$C NMR: δ 116.5, 117.1, 117.2, 124.9, 125.6, 126.9, 128.7, 129.6, 132.8, 133.5, 145.1, 152.7, 154.0, 159.7, 163.5 ppm; MS (*m/e*): 308 ($M^+$, 7), 290 (69), 262 (21), 188 (6), 159 (41), 105 (77), 77 (100). Anal. ($C_{17}H_{12}N_2O_4$) C, H, N.

*5.1.5. Synthesis of 5-methyl-3-(2-oxo-2H-[1]benzopyran-4-yl)-1,2,4-oxadiazole* ***9a***

A solution of compound **8a** (46 mg, 0.19 mmol) in toluene (5 mL) was refluxed for 48 h. The solvent was evaporated and the residue was treated with $Et_2O/CH_2Cl_2$ to give compound **9a** (24 mg, 56%) mentioned above.

*5.1.6. Synthesis of 5-phenyl-3-(2-oxo-2H-[1]benzopyran-4-yl)-1,2,4-oxadiazole* ***9b***

A solution of compound **8b** (0.16 g, 0.52 mmol) in toluene (5 mL) was refluxed for 48 h. The solvent was evaporated and the residue was treated with $Et_2O/CH_2Cl_2$ to give as a precipitate compound **9b** (0.112 g, 74%), m.p. 165–166 °C (EtOAc); IR 1725, 1600 cm $^{-1}$; $^1$H NMR: δ 7.40 (s, 1H), 7.37–7.46 (m, 2H), 7.57–7.71 (m, 4H), 8.24 (d, $J$ = 7.5, 2H), 8.70 (d, $J$ = 8.5, 1H); $^{13}$C NMR: δ 116.0, 117.3, 118.8, 123.3, 124.8, 127.7, 128.4, 129.3, 132.4, 133.5, 139.4, 154.2. 160.0, 166.0, 175.8 ppm; MS (*m/e*): 290 ($M^+$, 22), 262 (11), 159 (32), 105 (100), 77 (67). Anal. ($C_{17}H_{10}N_2O_3$) C, H, N.

*5.1.7. Reaction of amidoxime* ***6a*** *with trifluoroacetic anhydride; synthesis of 5-trifluoromethyl-3-(2-oxo-2H-[1]benzopyran-4-yl)-1,2,4-oxadiazole* ***9c***

A solution of trifluoroacetic anhydride (0.63 g, 3 mmol) in toluene (15 mL) was added to a toluene (15 mL) dispersion of amidoxime **6a** (0.204 g, 1 mmol) and the mixture was refluxed for 10 min. The solvent was evaporated and the residue was separated by column chromatography [silica gel, hexane/EtOAc (9:1)] to give compound **9c** (0.143 g, 51%), m.p. 78–80 °C (hexane/EtOAc); IR 1720, 1590 cm $^{-1}$; $^1$H NMR: δ 7.33 (s, 1 H), 7.39–7.5 (m, 2 H), 7.66 (t, $J$ = 8.0, 1 H), 8.53 (d, $J$ = 7.5, 1 H); $^{13}$C NMR: δ 110.3, 113.9, 115.1, 117.4, 117.5, 120.2. 121.2, 125.1, 127.2, 132.9, 137.4, 154.2, 159.2, 165.1, 165.7, 166.25, 166.3, 166.9 ppm; MS (*m/e*): 282 ($M^+$, 100), 254 (99), 213 (11), 185 (8), 157 (27). Anal. ($C_{12}F_3H_5N_2O_3$) C, H, N.

*5.1.8. Reaction of compound* ***6a*** *with ethyl acetoacetate; synthesis of 5-acetonyl-3-(2-oxo-2H-[1]benzopyran-4-yl)-1,2,4-oxadiazole* ***9d***

A dispersion of compound **6a** (0.204 g, 1 mmol) and ethyl acetoacetate (0.3 g, 2.3 mmol) in toluene (15 mL) was refluxed for 35 h. The solvent was evaporated and the residue was chromatographed [column, silica gel, hexane/EtOAc (7:2)] to give first compound **9d** (70 mg, 26%), m.p. 175–177 °C (EtOAc); IR 1720, 1600 cm $^{-1}$; $^1$H NMR: δ 2.41 (s, 3 H), 4.23 (s, 2 H), 7.27 (s, 1 H), 7.32–7.48 (m, 2 H), 7.62 (t, $J$ = 7.1, 1 H), 8.61 (d, $J$ = 7.9, 1 H); $^{13}$C NMR: δ 30.0, 41.3, 115.7, 117.3, 118.9, 124.8, 127.6, 132.5, 139.0, 154.2, 159.9, 165.7, 173.3, 206.1 ppm; MS (*m/e*): 270 ($M^+$, 100), 255 (13), 228 (67), 200 (10), 186 (58), 158 (98), 143 (93), 115 (49), 103 (84). Anal. ($C_{14}H_{10}N_2O_4$) C, H, N. Unreacted compound **6a** (62 mg, 30%) was then eluted.

*5.1.9. Reaction of amidoxime* ***6a*** *with ethyl orthoformate* ***10****; synthesis of 3-(2-oxo-2H-[1]benzopyran-4-yl)-1,2,4-oxadiazole* ***9e***

Compound **6a** (0.204 g, 1 mmol) was added to excess **10** (2.5 g). Borontrifluoride ethyletherate (1 mL) was then added and the mixture was refluxed for 30 min. After cooling the mixture was filtered and the filtrate was purified by column chromatography [silica gel, hexane/EtOAc (6:1)] to give oxadiazole **9e** (88 mg, 41%), m.p. 172–173 °C (hexane/EtOAc); IR 1705, 1600 cm $^{-1}$; $^1$H NMR: δ 7.33 (s, 1H), 7.35–7.47 (m, 2H), 7.63 (t, $J$ = 7.9, 1H), 8.63 (d, $J$ = 8.5, 1H), 8.96 (s, 1H); $^{13}$C NMR: δ 115.7, 117.3, 119.1, 124.8, 127.5, 132.6, 138.8, 154.2, 159.8, 164.8, 164.9 ppm; MS (*m/e*): 214 ($M^+$, 94), 186 (100), 159 (11), 143 (19), 103 (67). Anal. ($C_{11}H_6N_2O_3$) C, H, N.

*5.1.10. Reaction of amidoxime* ***6a*** *with formaldehyde* ***11a****; synthesis of 4,5-dihydro-3-(2-oxo-2H-[1]benzopyran-4-yl)-1,2,4-oxadiazole* ***12a*** *and oxadiazole* ***9e***

Formaldehyde (**11a**) (8 mL, 39% w/v) was added to a stirred dispersion of compound **6a** in $H_2O$ (15 mL) and the mixture was refluxed for 7 h and then exctracted with $CH_2Cl_2$ (2 × 30 mL). The organic layer was dried over $Na_2SO_4$, concentrated under reduced pressure and chromatographed [column, silica gel, hexane/EtOAc (4:1)] to give first compound **9e** (10 mg, 5%), followed by the compound **12a** (46 mg, 22%), m.p. 188–190 °C (EtOAc); IR 3350, 1710, 1600 cm $^{-1}$; $^1$H NMR ($CDCl_3$ + DMSO-$d_6$): δ 5.45 (d, $J$ = 3.3, 2 H), 6.82 (s, 1 H), 7.30–7.42 (m, 3 H), 7.60 (t, $J$ = 7.1, 1 H), 8.58 (d, $J$ = 7.9, 1 H); $^{13}$C NMR ($CDCl_3$ + DMSO-$d_6$): δ 81.9, 115.4, 115.9, 116.3, 124.0, 127.6, 131.8, 138.0 153.2, 153.7, 159.2 ppm; MS (*m/e*): 216 ($M^+$, 96), 188 (33), 158 (100), 143 (57), 131 (53), 103 (87). Anal. ($C_{11}H_8N_2O_3$) C, H, N. Unreacted amidoxime **6a** (27 mg, 13%) was then eluted.

*5.1.11. Reaction of amidoxime* ***6a*** *with acetaldehyde* ***11b****; synthesis of 5-methyl-4,5-dihydro-3-(2-oxo-2H-[1]benzopyran-4-yl)-1,2,4-oxadiazole* ***12b***

Acetaldehyde (**11b**) (0.14 g, 3.18 mmol) was added to a solution of amidoxime **6a** (0.204 g, 1 mmol) in acetic acid (1 mL) and the mixture was heated at 90 °C for 30 min. The mixture was evaporated to dryness and separated by column chromatography [silica gel, hexane/EtOAc (4:1)] to give compound **12b** (86 mg, 37%), m.p. 189–190 °C (hexane/EtOAc); IR 3290, 1705, 1600 cm $^{-1}$; $^1$H NMR: δ 1.60 (d, $J$ = 5.0, 3H), 4.78 (brs, 1H), 5.90 (m, 1H), 6.71 (s, 1H), 7.29–7.38 (m, 2H), 7.58 (t, $J$ = 7.9, 1H), 8.59 (d, $J$ = 8.0, 1 H); $^{13}$C NMR: δ 22.0, 90.1, 115.7, 116.0, 117.1, 124.8, 128.1, 132.5, 138.9, 152.8, 153.9, 160.2 ppm; MS (*m/e*): 230 ($M^+$, 93), 215 (100), 187 (90), 172 (34), 159 (72), 145 (40), 115 (42), 101 (91). Anal. ($C_{12}H_{10}N_2O_3$) C, H, N.

*5.1.12. Reaction of amidoxime* ***6b*** *with formaldehyde* ***11a****; synthesis of 4-phenyl-4,5-dihydro-3-(2-oxo-2H-[1]benzopyran-4-yl)-1,2,4-oxadiazole* ***12c*** *and N-phenyl-2-oxo-2H-[1]benzopyran-4-carboxamide* ***13***

A dispersion of amidoxime **6b** (0.25 g, 0.89 mmol) in formaldehyde (**11a**) (25 mL, 39% w/v) was refluxed for 24 h. After cooling compound **12c** (77 mg) was precipitated and the filtrate was exctracted with $CH_2Cl_2$ (2 × 20 mL). The organic layer was dried over $Na_2SO_4$, evaporated and the residue was chromatographed [column, silica gel, hexane/EtOAc (6:1)] to give **12c** (41 mg, total 0.118 g, 45%), m.p. 166–168 °C (hexane/EtOAc); IR 1715, 1600 cm $^{-1}$; $^1$H NMR: δ 5.83 (s, 2H), 6.60 (s, 1H), 6.89 (d,

$J = 8$, 2 H), 7.08–7.45 (m, 5 H), 7.59 (t, $J = 7$, 1 H), 8.22 (dd, $J_{d1} = 7$, $J_{d2} = 1.1$, 1 H); $^{13}C$ NMR: δ 89.0, 116.0, 117.3, 118.0, 122.4, 124.8, 126.1, 127.1, 129.9, 132.7, 138.8, 140.0, 151.4, 154.0, 159.6 ppm; MS (*m/e*): 292 ($M^+$, 60), 264 (77), 236 (14), 219 (33), 171 (72), 143 (100), 115 (71), 105 (99), 93 (94), 77 (80). Anal. ($C_{17}H_{12}N_2O_3$) C, H, N, followed by compound **13** (38 mg, 16%), m.p. 195–197 °C (hexane/EtOAc); IR 3240, 1720, 1650, 1600 $cm^{-1}$; $^1H$ NMR: δ 6.58 (s, 1 H), 7.20–7.34 (m, 3 H), 7.41 (t, $J = 7.8$, 2 H), 7.54 (t, $J = 7.9$, 1 H), 7.74 (d, $J = 7.8$, 2 H), 7.90 (d, $J = 7.9$, 1 H), 8.65 (brs, 1 H); $^{13}C$ NMR: δ 113.9, 115.9, 117.6, 120.3, 125.0, 125.6, 126.7, 129.2, 132.8, 137.1, 149.4, 154.1, 160.7, 162.3 ppm; MS (*m/e*): 265 ($M^+$, 5), 237 (48), 220 (38), 173 (32), 145 (39), 101 (100). Anal. ($C_{16}H_{11}NO_3$) C, H, N.

*5.1.13. Reaction of amidoxime **6b** with acetaldehyde **11b**; synthesis of 4-phenyl-5-methyl-4,5-dihydro-3-(2-oxo-2H-[1]benzopyran-4-yl)-1,2,4-oxadiazole **12d***

To a solution of compound **6b** (0.24 g, 0.86 mmol) in acetic acid (1 mL) acetaldehyde (**11b**) (0.14 g, 3.18 mmol) was added and the resulting mixture was heated at 90 °C for 6 h. $H_2O$ (30 mL) was then added and the mixture exctracted with $CH_2Cl_2$ (2 × 30 mL). The organic layer was dried over $Na_2SO_4$ and evaporated to dryness. The residue was separated by column chromatography [silica gel, hexane/EtOAc (4:1)] to give compound **12d** (76 mg, 29%), m.p. 94–97 °C (hexane/$CH_2Cl_2$); IR 1710, 1590 $cm^{-1}$; $^1H$ NMR: δ 1.68 (d, $J = 5.3$, 3 H), 5.91 (q, $J = 5.3$, 1 H), 6.53 (s, 1 H), 6.96 (d, $J = 7$, 2 H), 7.12–7.45 (m, 4 H), 7.57 (t, $J = 8$, 2 H), 8.28 (dd, $J_{d1} = 8$, $J_{d2} = 1.7$, 1 H); $^{13}C$ NMR: δ 21.5, 97.2, 116.2, 117.2, 117.7, 124.2, 124.7, 127.0, 127.1, 129.9, 132.6, 139.3, 139.9, 151.6, 153.9, 159.7 ppm; MS (*m/e*): 306 ($M^+$, 50), 291 (57), 278 (28), 263 (63), 207 (81), 179 (77), 145 (54), 121 (100). Anal. ($C_{18}H_{14}N_2O_3$) C, H, N. Unreacted compound **6b** (90 mg, 37%) was then eluted.

*5.1.14. General procedure for reactions of amidoxime **6a–c** with thionylchloride*

A solution of pyridin (0.124 g, 1.57 mmol) in $CH_2Cl_2$ (1 mL) was added at once to an ice-cooled dispersion of amidoxime **6a–c** (0.5 mmol) in $CH_2Cl_2$ (20 mL). Thionylchloride (0.146 g, 1.22 mmol) in $CH_2Cl_2$ (7 mL) was then added dropwise during 30 min. The mixture was stirred for 1 h and $H_2O$ (20 mL) was added. The organic layer was washed with $H_2O$ (2 × 20 mL), dried over $Na_2SO_4$ and evaporated. The residue was treated with $Et_2O$ giving as precipitate the corresponding 1,2,3,5-oxathiadiazol-2-oxides **14a–c**.

*5.1.15. 4-(2-Oxo-2H-[1]benzopyran-4-yl)1,2,3,5-oxathiadiazol-2-oxide **14a***

Amidoxime **6a** (0.102 g, 0.5 mmol) gave compound **14a** (70 mg, 56%), m.p. 182–184 °C ($CH_2Cl_2/Et_2O$); IR 3120, 1700, 1635, 1600 $cm^{-1}$; $^1H$ NMR ($CDCl_3$ + DMSO-$d_6$): δ 6.07 (brs, 1 H), 6.61 (s, 1 H), 7.22–7.48 (m, 2 H), 7.58 (t, $J = 7.3$, 1 H), 8.21 (d, $J = 7.6$, 1 H); $^{13}C$ NMR ($CDCl_3$ + DMSO-$d_6$): δ 115.2, 116.6, 117.0, 124.0, 127.7, 131.9, 142.2, 148.9, 153.8, 160.0 ppm; MS (*m/e*): 250 ($M^+$, 74), 220 (36), 192 (21), 172 (99), 143 (100), 128 (14), 115 (44). Anal. ($C_{10}H_6N_2O_4S$) C, H, N.

*5.1.16. 3-Phenyl-4-(2-oxo-2H-[1]benzopyran-4-yl)-1,2,3,5-oxathiadiazol-2-oxide **14b***

Amidoxime **6b** (0.14 g, 0.5 mmol) gave compound **14b** (0.13 g, 79%), m.p. 161–163 °C (hexane/EtOAc); IR 1725, 1595 $cm^{-1}$; $^1H$ NMR: δ 6.54 (s, 1 H), 7.21–7.44 (m, 7 H), 7.57 (t, $J = 7.9$, 1 H), 7.75 (dd, $J_{d1} = 7.5$, $J_{d2} = 1.6$, 1 H); $^{13}C$ NMR: δ 116.3, 117.3, 120.1, 125.0, 126.0, 127.6, 130.15, 130.2, 131.1, 133.2, 137.4, 148.0, 153.6, 158.3 ppm; MS (*m/e*): 326 ($M^+$, 8), 298 (7), 262 (63), 234 (100), 219 (12), 205 (33), 145 (72). Anal. ($C_{16}H_{10}N_2O_4S$) C, H, N.

*5.1.17. 3-(4'-Methylphenyl)-4-(2-oxo-2H-[1]benzopyran-4-yl)-1,2,3,5-oxathiadiazol-2-oxide **14c***

Amidoxime **6c** (0.147 g, 0.5 mmol) gave compound **14c** (0.113 g, 66%), m.p. 166–168 °C (hexane/EtOAc); IR 1720, 1600 $cm^{-1}$; $^1H$ NMR: δ 2.31 (s, 3 H) 6.51 (s, 1 H), 7.12 (s, 4 H), 7.27–7.40 (m, 2 H), 7.58 (t, $J = 7.2$, 1 H), 7.77 (d, $J = 7.8$, 1 H); $^{13}C$ NMR: δ 21.1, 116.5, 117.3, 120.1, 125.0, 126.1, 127.7, 128.2, 130.8, 133.2, 137.5, 140.7, 148.2, 153.6, 158.4 ppm; MS (*m/e*): 340 ($M^+$, 15), 313 (42), 279 (78), 263 (45), 247 (51), 206 (45), 171 (57), 145 (100), 104 (80). Anal. ($C_{17}H_{12}N_2O_4S$) C, H, N.

*5.1.18. General procedure for the reactions of amidoximes **6b,c** with phosgene*

Phosgene (75 mg, 0.76 mmol, 0.375 mL 20% in toluene) was added to a solution of amidoxime **6b,c** (0.5 mmol) and $Et_3N$ (0.15 g, 1.49 mmol) in dry toluene (15 mL) and the mixture was stirred for 15 min. The precipitated salt of $Et_3N^+H\ Cl^-$ was filtered and the filtrate was evaporated. The residue was separated by column chromatography [silica gel, hexane/ EtOAc (3:1)] to give 1,2,4-oxadiazolin-5-ones **15a,b**.

*5.1.19. 4-Phenyl-4,5-dihydro-3-(2-oxo-2H-[1]benzopyran-4-yl) 1,2,4-oxadiazol-5-one **15a***

Amidoxime **6b** (0.14 g, 0.5 mmol) gave compound **15a** (0.126 g, 82%), m.p. 172–174 °C (EtOAc); IR 1780, 1720, 1600 $cm^{-1}$; $^1H$ NMR: δ 6.41 (s, 1 H), 7.21–7.5 (m, 7 H), 7.61 (t, $J = 7.8$, 1 H), 7.70 (d, $J = 8.1$, 1 H); $^{13}C$ NMR: δ 115.3, 117.7, 119.9, 125.1, 125.6, 125.9, 130.1, 130.2, 130.7, 133.5, 137.0, 153.2, 153.9, 157.1, 158.2 ppm; MS (*m/e*): 306 ($M^+$, 40), 278 (71), 234 (29), 205 (46), 145 (39), 103 (68), 77 (100). Anal. ($C_{17}H_{10}N_2O_4$) C, H, N.

*5.1.20. 4-(4'-Methylphenyl)-4,5-dihydro-3-(2-oxo-2H-[1]benzopyran-4-yl)-1,2,4-oxadiazol-5-one **15b***

Amidoxime **6c** (0.147 g, 0.5 mmol) gave compound **15b** (0.147 g, 86%), m.p. 188–190 °C (EtOAc); IR 1780, 1720, 1600 $cm^{-1}$; $^1H$ NMR: δ 2.35 (s, 3 H), 6.39 (s, 1 H), 7.10 (d, $J = 8.1$, 2 H), 7.22 (d, $J = 8.1$, 2 H), 7.27–7.45 (m, 2 H), 7.62 (t, $J = 7.8$, 1 H), 7.74 (d, $J = 7.8$, 1 H); $^{13}C$ NMR: δ 21.2, 115.4, 117.6, 119.8, 125.1, 125.5, 126.0, 128.0, 130.8, 133.4, 137.0, 140.6, 153.3, 153.9, 157.2, 158.3 ppm; MS (*m/e*): 320 ($M^+$, 59), 292 (100), 276 (14), 248 (42), 145 (60). Anal. ($C_{18}H_{12}N_2O_4$) C, H, N.

Ethyl chloroformate (**7c**) (77 mg, 0.71 mmol) was added to a solution of amidoxime **6c** (0.147 g, 0.5 mmol) and $Et_3N$ (82 mg, 0.81 mmol) in $CHCl_3$ (25 mL) and the mixture was stirred for 12 h. The solvent was evaporated and the residue was separated by PTLC [silica gel, hexane/EtOAc (4:1)] to give compound **15b** (50 mg, 37%), identical to that described above.

*5.1.21. General procedure for the reactions of amidoximes **6b,c** with thiophosgene*

A solution of thiophosgene (75 mg, 0.65 mmol) in benzene (0.7 mL) was added dropwise to a solution of amidoximes **6b,c** (0.5 mmol) and dry $Et_3N$ (0.132 g, 1.3 mmol) in dry toluene

(15 mL) during 1 h. The precipitated salt of $Et_3N^+H\ Cl^-$ was filtered and the filtrate was evaporated. The residue was separated with column chromatography [silica gel, hexane/EtOAc (3:1)] to give 1,2,4-oxadiazolin-5-thiones **16a,b**.

*5.1.22. 4-Phenyl-4,5-dihydro-3-(2-oxo-2H-[1]benzopyran-4-yl)-1,2,4-oxadiazol-5-thione* ***16a***

Amidoxime **6b** (0.14 g, 0.5 mmol) gave compound **16a** (0.148 g, 92%), m.p. 208–210 °C (hexane/EtOAc); IR 1720, 1600 $cm^{-1}$; $^1H$ NMR: δ 6.38 (s, 1 H), 7.27–7.33 (m, 2 H), 7.34–7.40 (m, 2 H), 7.46–7.52 (m, 3 H), 7.60–7.69 (m, 2 H); $^{13}C$ NMR: δ 115.4, 117.7, 120.3, 125.2, 125.8, 126.9, 130.3, 130.9, 132.1, 133.7, 136.1, 153.8, 154.2, 158.0, 186.0 ppm; MS (*m/e*): 322 ($M^+$, 100), 294 (77), 266 (14), 151 (65), 143 (40). Anal. ($C_{17}H_{10}N_2O_3S$) C, H, N.

*5.1.23. 4-(4'-Methylphenyl)-4,5-dihydro-3-(2-oxo-2H-[1]benzopyran-4-yl)-1,2,4-oxadiazol-5-thione* ***16b***

Amidoxime **6c** (0.147 g, 0.5 mmol) gave compound **16b** (0.134 g, 80%), m.p. 169–171 °C (EtOAc); IR 1715, 1600 $cm^{-1}$; $^1H$ NMR: δ 2.37 (s, 3 H), 6.37 (s, 1 H), 7.15 (d, $J$ = 8.1, 2 H), 7.26 (d, $J$ = 8.1, 2 H), 7.32–7.41 (m, 2 H), 7.64 (t, $J$ = 7.8, 1 H), 7.68 (d, $J$ = 8.2, 1 H); $^{13}C$ NMR: δ 21.3, 115.5, 117.7, 120.3, 125.2, 125.9, 126.7, 129.4, 131.0, 133.6, 136.1, 141.5, 153.8, 154.3, 158.1, 186.2 ppm; MS (*m/e*): 336 ($M^+$, 58), 307 (51), 279 (23), 247 (36), 170 (43), 142 (79), 136 (100), 114 (30). Anal. ($C_{18}H_{12}N_2O_3S$) C, H, N.

*5.2. Pharmacology*

Albumin used was Rinderblut (Fluka) fraction V; trypsin (pancreas*protease*) 200 Fip U/g, *salicylic acid, acetyl-salicylic acid,* β-glucuronidase/arylsulfatase, p-nitrophenyl-β-glucopyranosiduronic *acid (p-NPG), Tween-80 were from Merck AG, Darmstadt;* 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 (DPPH), nor-dihydroguaeretic acid (NDGA), caffeic acid were from Aldrich.

*5.2.1. Experiments in vitro; determination of the reducing activity of the stable radical 1,1-diphenyl-picrylhydrazyl (DPPH) [32]*

Compounds $10^{-4}$ M in absolute ethanol were added to an equimolar ethanolic solution of DPPH. After 20 min, the absorbance at 517 nm was measured. The difference in absorbance between this and that of the control was taken as the reducing ability. The results are shown in *table I.* Acetyl-salicylic acid (ASA) was used as a standard drug (0.1 mM, 80.5%).

*5.2.2. Proteolytic activity of trypsin in vitro [33]*

The antiproteolytic activity was measured by determining the ability of the compounds to inhibit trypsin induced hydrolysis of bovine serum albumin as a substrate. The tested compounds at a final concentration of 0.1 mM in 0.1 M phosphate buffer (pH 7.6), were incubated with 0.075 mg/mL trypsin for 20 min before the addition of albumin. After this time 1 mL bovine serum albumin (6 g/100 mL of 0.1 M phosphate buffer) was added. The reaction was carried out for 20 min at 37 °C and then stopped by the addition of 3 mL 5% $CCl_3COOH$ solution. The acid soluble products of protein breakdown were determined spectroscopically at 550 nm. Each experiment was done in duplicate. Salicylic acid (0.1 mM and 1 mM) was used as a standard drug (53.6% and 96.1% respectively).

*5.2.3. B-glucuronidase [34]*

Compounds the most potent in the antiproteolytic and reducing activity tests and some representatives were preincubated at 37 °C in 0.1 M acetate buffer pH 7.4 (0.1 mL final concentration 1 mM) for 5 min with 0.8 mL of 2.5 mM p-nitrophenyl-β-D-glucopyranosiduronic acid (p-NPG). 0.1 mL of 1 U/mL β-glucuronidase was added and the mixture was incubated for 30 min at 37 °C. After that 2 mL NaOH 0.5 N was added and the absorbance at 410 nm was recorded. Results are expressed as the means of duplicate experiments. In the above mentioned tests, the tested compounds were dissolved in the buffer by addition of dimethylformamide DMF (1% approximately). Salicylic acid (SA) was used as a standard drug (1 mM, 2.32%).

*5.2.4. Soybean lipoxygenase inhibition study [31]*

The tested compounds, dissolved in 60% aqueous ethanol (final concentrations 0.1–0.3–1 mM) with sodium linoleate (0.1 mM), 0.15 mL of enzyme solution (1/$10^4$ w/v in saline) were evaluated in room temperature. The conversion of sodium linoleate to 13-hydroperoxy-linoleic acid with appropriate standard, in each case, at 234 nm was compared. Nor-dihydroguaeretic acid (NDGA) was used as a standard drug (0.1 mM, 83.7%).

*5.2.5. Superoxide scavenging activity*

The tested compounds (**6a, 6b, 6c, 8a, 8b, 9a, 12a, 14a**) were studied for their superoxide scavenging capacity by the NBT *(nitroblue tetrazolium) reduction method [35, 36]. Superoxide* anion was generated by xanthine–xanthine oxidase system. The reaction system contained 0.1 mM xanthine, 0.6 mM NBT in 0.1 M phosphate buffer pH 7.8. Compounds were dissolved in 1% DMF in Buffer and added to the reaction mixture (final concentration 0.1 mM). 0.05 U/mL xanthine oxidase was added for the reaction to start. The absorbance was read at 560 nm after incubation at 25 °C for 10 min. Each experiment was performed in duplicate and percent scavenging of superoxide was calculated by comparing the results of the tested compounds with those of the control experiments. Caffeic acid (CA, 0.1 mM) was used as a standard drug (10%).

*5.2.6. Experiments in vivo; carrageenin induced edema*

All compounds tested were dispersed in sterilized saline with a concentration of 0.15 mmoles/kg, stabilized by 0.05% Tween 80 and administered i.p. (intraperitoneally). Fisher 344 male and female rats (pregnant excluded) weighing 180–220 g, were used. The animals were housed under standard conditions. Indometacin, 0.11 mmoles/kg b.w. was administered as a standard drug (47 ± 1.96).

Acute anti inflammatory activity [33] (*table I*) was measured after 3.5 h by reduction of rat paw carrageenin edema, induced by injection of 0.1 mL carrageenin 2% (K100, commercially available) in sterilized saline, intradermally into the right foot pad. The examined compounds were administered simultaneously. Control animals accepted only vehicle.

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## References

[1] Murray R.D.H., Mendez I., Brawn S.A., The Natural Coumarins, Wiley, New York, 1982.

[2] Alka K., Vasant K.A., Rao M.N.A., Die Pharmazie 44 (1989) 870.

[3] Maddi V., Raghu K.S., Rao M.N.A., J. Pharmaceut. Sci. 81 (1992) 964–966.

[4] Larson R.A., Phytochemistry 27 (1988) 969–978.

[5] Mora A., Paya M., Rios J.L., Alcaraz M.J., Biochem. Pharmacol. 40 (1990) 793–797.

[6] Paya M., Halliwell B., Hoult J.R.S., Biochem. Pharmacol. 44 (1992) 205–214.

[7] Neichi T., Kashihara Y., Musota S.I., Biochim. Biophys. Acta 753 (1993) 130–132.

[8] Kimura Y., Okuda H., Arichi S., Baba K., Kozawa M., Biochim. Biophys. Acta 834 (1985) 224–229.

[9] Ozaki Y., Ohashi T., Niwa Y., Biochem. Pharmacol. 35 (1986) 3481–3488.

[10] Gharde A.D., Ghiya B.J., J. Indian Chem. Soc. 69 (1992) 397–398.

[11] Mazunder A., Wang S., Neamati N., Nicklams M., Sunder S., Chen J., Milne G.W.A., Rice W., Burke Jr. T., Pommier Y., J. Med. Chem. 39 (1996) 2472–2481.

[12] Litinas K.E., Nicolaides D.N., Varella E.A., J. Heterocycl. Chem. 27 (1990) 769–774.

[13] Nicolaides D.N., Bezergiannidou-Balouctsi C., Litinas K.E., Malamidou-Xenikaki E., Mentzafos D., Terzis A., J. Chem. Res. (S) (1993) 108–109; (M) (1993) 826–851.

[14] Nicolaides D.N., Bezergiannidou-Balouctsi C., Litinas K.E., Malamidou-Xenikaki E., Mentzafos D., Terzis A., Tetrahedron 49 (1993) 9127–9136.

[15] Bezergiannidou-Balouctsi C., Litinas K.E., Malamidou-Xenikaki E., Nicolaides D.N., Liebigs Ann. Chem. (1993) 1175–1177.

[16] Nicolaides D.N., Fylaktakidou K.C., Bezergiannidou-Balouctsi C., Litinas K.E., J. Heterocycl. Chem. 31 (1994) 173–176.

[17] Nicolaides D.N., Fylaktakidou K.C., Litinas K.E., Hadjipavlou-Litina D., J. Heterocycl. Chem. 33 (1996) 967–971.

[18] Nicolaides D.N., Fylaktakidou K.C., Litinas K.E., Papageorgiou G.K., Hadjipavlou-Litina D., J. Heterocycl. Chem. 35 (1998) 619–625.

[19] Nicolaides D.N., Fylaktakidou K.C., Litinas K.E., Hadjipavlou-Litina D., unpublished results.

[20] Nicolaides D.N., Varella E.A., The Chemistry of Amidoximes Suppl. B, In: Patai S. (Ed.), The Chemistry of Acid Derivatives, Vol. 2, Part 2, Wiley Interscience, New York, 1992, p. 875.

[21] Staunton J., In: Sammes P.G. (Ed.), Comprehensive Organic Chemistry, Vol. 4, Pergamon Press, Oxford, 1979, p. 648.

[22] Soliman A.Y., El-Nagdy S., Bakeer H.M., Moustafa M.M., Indian J. Chem. 29(B) (1990) 239–243.

[23] Bata I., Korbonits D., Kolonits P., Podanyi B., Takacsy-Eros T., Simon K., Chem. Ber. 126 (1993) 1835–1841.

[24] Berndt E.W., Fratzke H.A., Held B.G., J. Heterocycl. Chem. 9 (1972) 137–140.

[25] Kocevar M., Stanovnik B., Tisler M., J. Heterocycl. Chem. 19 (1982) 1397–1402.

[26] Yale H.L., Spitzmiller E.R., J. Heterocycl. Chem. 15 (1978) 1373–1378.

[27] Burakevich J.V., Butler R.S., Volpp G.P., J. Org. Chem. 37 (1972) 593–596.

[28] Sümengen D., Pelter A., J. Chem. Soc. Perkin Trans. I (1983) 687–691.

[29] Tandon M., Tandon P., Barthwhal J.P., Bhalla T.N., Bhagrava K.P., Arzneim. Forsch. Drug Res. 32 (1982) 1233–1235.

[30] Di Rosa M., Girroud J.P., Willougby D.A., J. Pathol. 104 (1971) 15–23.

[31] Taraborewala R.B., Kauffman J.M., J. Pharm. Sci. 79 (1990) 173–178.

[32] Blois M.S., Nature (London) 181 (1958) 1199.

[33] Hadjipavlou-Litina D., Res. Commun. Chem. Pathol. Pharmacol. 81 (1993) 1091–1102.

[34] Hadjipavlou-Litina D., Geronikaki A.A., Arzneim. Forsch. Drug. Res. 46 (1996) 805–808.

[35] Robak J., Gryglewski R.J., Biochem. Pharmacol. 37 (1988) 837–841.

[36] Rajakumar D.V., Rao M.N., Biochem. Pharmacol. 45 (1993) 2067–2072.