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Synthesis and biological evaluation of novel coumarin derivatives with a 7-azomethine linkage☆

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Abstract—The synthesis of several novel coumarin derivatives with a 7-azomethine linkage was carried out starting from 7-formyl-coumarin. The compounds were tested in vivo for their anti-inflammatory activity and in vitro for their antioxidant ability. Compounds **3a** and **3e** possess significant protection against carrageenin induced rat paw edema.

Many coumarin derivatives have special ability to scavenge reactive oxygen species (ROS)—free radicals, such as hydroxyl radicals, superoxide radicals or hypochlorous acid, and to influence processes involving free radical-injury.[1,2] They have also been found to inhibit lipid peroxidation[3] and to possess vasorelaxant,[3] anti-inflammatory and anticoagulant activities.[4] Moreover they have been used as inhibitors of lipoxygenase and cyclooxygenase in the arachidonic acid cascade[5–7] of serine proteases[8–12] and of tyrosine kinase.[3]

Coumarins have been evaluated in vitro for their inhibitory activity toward bovine α-chymotrypsin, human leukocyte elastase[12–14] and thrombin, plasmin and tissue plasminogen activator.

In our previous work the synthesis of several coumarin derivatives with significant anti-inflammatory/antioxidant activity has been reported.[14–18]

The reported derivatives were tested for their antioxidant and anti-inflammatory activities and their ability to inhibit various enzymes involved in the complex phenomenon of inflammation. Non Steroidal Anti-inflammatory Drugs (NSAIDs) have a broad spectrum of effects and it has been suggested that the variations in both efficacy and their tolerability are partly due to differences in their physicochemical properties, which determine their distribution in the body and their ability to pass through and to enter the interior of membranes.[17,19] Thus, partition coefficients such as R_M values are performed and compared with the corresponding theoretically calculated (log *P*) values of *n*-octanol–water.

The synthesis of compound **2** is described in the Figure 1. 7-formyl-coumarin **2** was synthesized using powdered

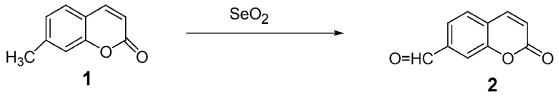

Figure 1. SeO_2, 180–190 °C.

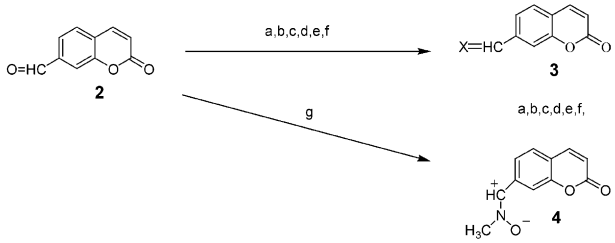

Figure 2. (a) $NH_2OH{\cdot}HCl$, sodium acetate, ethanol/water; (b) $NH_2OCH_3{\cdot}HCl$, sodium acetate, ethanol/water; (c) $PhNHNH_2{\cdot}HCl$, ethanol; (d) $NH_2NH_2{\cdot}2HCl$, ethanol; (e) CH_3NHNH_2, ethanol; (f) $PHNH_2$, benzene; (g) $CH_3NHOH{\cdot}HCl$, sodium acetate, ethanol/water.

Keywords: Coumarin; Anti-inflammatory; Antioxidant; LOX inhibitors; Trypsin; Chymotrypsin.
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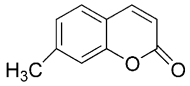
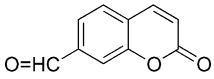
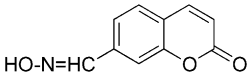
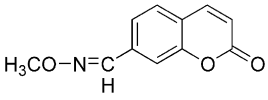
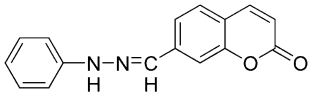
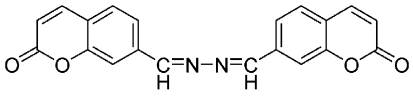
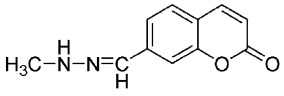
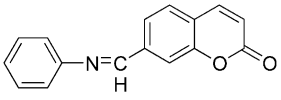
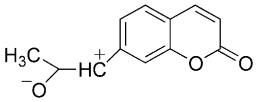
SeO_2 without solvent at high temperature.[20–22] We are particularly interested in extending the rearrangement to molecules in which the aldehydic group has been replaced by C=N. The effective role of azomethine linkage in certain biological reactions[23,24] is well documented. Coumarin-4-oximes and carboxamidoximes,[25] simple stable molecules containing the hydroxamic functionality,[26,27] as well as Schiff bases[27] were found to be inhibitors of soybean lipoxygenase and potent anti-inflammatories. Thus, we extended our study in the synthesis of oximes **3a**,**b**, hydrazones **3c**–**e**, imine **3f** and in addition we also synthesized an oxyimine **4** (Fig. 2).

Oximes **3a**,**b** were both synthesized according to the general procedure. To an aqueous ethanol solution of compound **2**, hydroxylamine hydrochloride or *O*-methylhydroxylamine hydrochloride and sodium acetate were added and heated under reflux.[14,21] For the synthesis of hydrazones **3c**–**e**, an ethanolic solution of compound **2** was added to an ethanolic solution of

Table 1. Inhibition % of induced carrageenin rat paw edema CPE (%),[a,29] Lipophilicity values: Theoretically calculated clog P[b,30] values and experimentally determined R_M[c,31] Interaction % with DPPH (RA%),[d,29] Competition % with DMSO for hydroxyl radical (HO·%)[e,29]

Compounds		CPE (%)[a]	clog P[b]	R_M[c] (±SD)	RA (%)[d] 20 min			RA (%)[d] 60 min			HO· (%)[e]	
					0.1 mM	0.2 mM	0.5 mM	0.1 mM	0.2 mM	0.5 mM	0.01 mM	0.1 mM
1		55.1	1.13	0.124	0.29	1.61	2.05	2.5	5.4	3.5	30.2	43.8
2		29.3	1.13	−0.173	1.47	2.49	16.2	3.5	7.1	12.8	33.9	35.2
3a		58.6	1.62	0.589	0.29	3.52	9.7	4.8	7.1	12.8	43.4	50.8
3b		31.1	1.51	0.284	12.7	14.5	13.9	9.8	11.6	11.9	85.6	90.2
3c		35.2	2.91	1.067	36.2	48.9	78.7	56.2	64.4	79.1	15.4	65.5
3d		54.0[g]	0.39	0.714	7.04	6.30	64.4	9.9	8.4	67.5	44.5	45.3
3e		54.7	1.00	−0.470	69.7	84.8	92.4	80.7	86.7	87.3	97.3	nR[f]
3f		42.5	2.12	−0.146	12.9	13.5	14.3	10.0	10.6	11.6	72.2	89.6
4		18.0	−0.41	−0.078	14.5	18.4	22.9	13.3	16.8	25.6	80.7	81.8
Coumarin		30.2**			4.9	nt	5.8	21	nt	6.7	nt	78.0
Warfarin		41.0*			9.2	nt	10.3	24	nt	12.8	nt	95.8
Indomethacin		47.0										

[a] Each value represents the mean of two independent experiments with 5 animals in each group, statistical studies were done with student's T-test, $*p<0.01$, $**p<0.05$; The dose was 0.01 mmoles/kg body weight.
[b] Theoretically calculated clog P values.
[c] R_M values are the average of at least 10 measurements, SD < 10%.
[d] NDGA (Nor-dihydro-guaretic acid) used as standard 96.5% (0.5 mM) at 60 min. The % interaction is not changed by the concentration and time increase.
[e] Trolox as standard 73.4% (0.01 mM) and 88.2% (0.1 mM).
[f] nR: No Results due to solubility problems; nt: not tested; no: no action under the experimental conditions; Percent inhibiton is based on absorbance values of samples with the tested compounds against controls containing equal volume of the solvent: Standard deviation of absorbance values was less than ±10%, $n=3$–5.
[g] % CPE at 0.005 mmol/kg 55.1%.

Table 2. In vitro inhibition of trypsin induced proteolysis (Iptr%)[a,29] in vitro inhibition of chymotrypsin induced proteolysis (Ipch%),[b] inhibition in vitro of β-glucuronidase (GI,%)[c,33] in vitro inhibition of soybean lipoxygenase (LOX%)[d,29] in vitro % inhibition of trypsin acting as esterase (IEtr%)[e,29] in vitro % inhibition of chymotrypsin acting as esterase (IEch%)[f,29,32]

Compd	Iptr%[a]		Ipch%[b]		GI%[c]		LOX%[d]		IEtr%[e]		IEch%[f]	
	0.1 mM	1 mM	0.1 mM	1 mM	0.01 mM	0.1 mM	0.01 mM	0.1 mM	0.1 mM	1 mM	0.1 mM	1 mM
1	90.4	100	48.1	97.4	no	95.9	73.5	88.9	13.6	88.7	16.4	97.7
2	83.4	100	32.8	96.6	no	98.9	8.6	89.7	12.6	99.1	22.7	98.9
3a	70.7	77.7	37.4	98.7	no	100	37.2	98.8	20.3	98.5	28.5	100
3b	100	100	no	56.9	21.9	94.1	no	100	25.5	99.9	16.6	100
3c	no	64.5	no	80.1	5.9	96.8	77.4	71.2	31.4	99.9	30.6	88.7
3d[b]	99.9	100	24.5	100	2.3	100	8.6	10.2	9.4	65.4	29.9	65.2
3e	no	100	no	93.9	6.9	93.3	70.8	100	22.6	97.6	10.0	83.4
3f	35.3	95.9	90.5	73.9	26.1	95.4	54.3	100	22.2	99.6	40.6	99.3
4	41.1	95.4	no	63.1	7.5	92.3	no	100	29.3	99.4	31.3	100
Coumarin	12.7	95.9	no	89.3	nt	nt	nt	15.1	5.0	98.0	28.5	63.9
Warfarin	92.5	94.8	62.4	86.7	nt	nt	nt	No	21.2	97.5	18.4	98.2

nt: not tested; no: no action under the experimental conditions
[a] Salicylic acid used as a standard drug 53.6% (0.1 mM) and 96.1% (1 mM) and NDGA 100% (in both 0.1 mM and 1 mM).
[b] NDGA was used as standard 94.5% (0.1 mM) and 100% (1 mM).
[c] Acetylsalicylic acid as standard drug 2.32% (1 mM), phenylbutazone 4.6% (0.1 mM).
[d] Nordihydroguaretic acid (NDGA) 83.7% (0.1 mM).
[e] Salicylic acid was used as a standard 18.1% (0.1 mM) and NDGA no (0.1 mM) and 18.3% (1 mM).
[f] Acetylsalicylic acid was used as a standard 34.63% (0.01 mM) and 91.5% (0.1 mM).

phenylhydrazine or hydrazine or methylhydrazine. The mixture was refluxed and then pured properly. In the case of hydrazine a dimer product was isolated (Table 1).[21,25]

A mixture of compound **2** and aniline were dissolved in benzene and refluxed in a Dean–Stark apparatus. An imine-Base Schiff-compound **3f** was produced.[21] For the synthesis of compound **4** the same procedure was followed. An ethanolic solution of compound **2** and sodium acetate was added to an ethanolic solution of $CH_3NHOH{\cdot}HCl$ and the mixture was refluxed.[25]

All the products gave satisfactory analytical and spectroscopic data in full accord with their assigned structures.[28]

In acute toxicity experiments, the in vivo examined compounds were endowed with a 50% lethal dose of >0.5 mmoles/kg body weight. To access the anti-inflammatory activity of the coumarin derivatives the rat carrageenin induced paw edema assay was employed as a model for acute inflammation. Indomethacin was included as a reference drug. The development of the edema induced by carrageenin has been described as a biphasic event. The first phase of the inflammatory response is mediated by histamine and serotonin, the second phase is mediated by kinins and presumably by prostaglandins. Since edemas of this type are highly sensitive to NSAIDs, carrageenin has been accepted as a useful agent for studying new NSAIDs. This model reliably predicts the anti-inflammatory efficacy of the NSAIDs during the second phase. It detects compounds that are anti-inflammatory agents, as a result of inhibition of prostaglandin amplification. It is of interest that from the tested compounds especially oxime **3a** (58.6%) and methylhydrazone **3e** (55.1%) possess significant protection.

The protection ranges from 18 to 58.6%. Compound **4** has the lowest effect (29.3%). Lipophilicity does not seem to affect the biological responses, on the contrary low lipophilicity is combined with higher inhibition values (compounds **1**, **3a**,**d**,**e**). All compounds were tested for their interaction with the stable free radical DPPH. This interaction indicates their radical scavenging activity in an iron free system and expresses their reducing activity. Compound **1**, **2**, **3a**,**b**,**f**, **4**, were found to have very low activity, whereas compounds **3c**, **3d** and **3e** showed the highest interactions. In general the results are not proceeded in parallel to the increase of time and concentration (Table 2).

The competition of coumarinic derivatives with DMSO for $^{\bullet}OH$, generated by the Fe^{+3}/ascorbic acid system expressed as the inhibition of formaldehyde production was used for the evaluation of their hydroxyl radical scavenging activity. Compounds **1** and **2** did not show any inhibition, whereas compounds **3a**, **3e** inhibited significantly the DMSO oxidation in two different concentrations. Compound **3e** was found to be the most active. The order of decreasing $^{\bullet}OH$ activity was **3e** > **4** > **3d** > **3f** (0.01 mM) and **3b** > **3f** > **4** > **3e** (0.1 mM). Low lipophilicity seems to play an important role.

No inhibition was observed on soybean lipoxygenase under the reported experimental conditions for compounds **3d**, **4** (0.01 mM). Compounds **2**, **3a** and **3d** possess low inhibition in low concentration, while high results are given in the 0.1 mM concentration. Compounds **1**, **3f**, **3e** highly inhibit the enzyme. It seems that in the most of the cases the inhibition is concentration dependent. No role for the lipophilicity was found.

These derivatives were also tested for their ability to inhibit β-glycuronidase and to affect trypsin and chymotrypsin enzymatic activities. The role played by proteases in the early stage of inflammatory process is well documented. It was found that all the tested coumarins highly inhibit the above-referred enzymes. The inhibition seems to be concentration dependent.

Compound **1** was tested in vivo/in vitro in an attempt to elucidate the structural characteristics responsible for the anti-inflammatory activity. The in vivo anti-inflammatory activity of compounds **3a** and **3e** can be attributed: (a) to their antioxidant activity, (b) to their inhibition against LOX and (c) to their inhibition against the proteolytic enzymes implicated in inflammation. The minimal structural requirements for in vivo/in vitro activities in this type of compounds are: the coumarin nucleus and the 7-substitution. The nature of the substituent next to the nitrogen of the C=N group does not have a marked effect on the in vivo/in vitro activities.

The in vitro/in vivo activities have not been able to provide a clear correlation among all the physicochemical parameters in a QSAR analysis. Poor correlation ($r < 0.6$) was obtained between clog P and R_M.

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28. **Compound 2:** (28.5%); mp 188–192°C; IR(Nujol) cm^{-1}: 1710–1730; ^1H NMR ($CDCl_3$): δ 6.56–6.59 (d, $J=9.8$ Hz, 1H), 7.75–7.78 (d, $J=9.8$, 1H), 7.64–7.67 (d, $J=7.9$, 1H), 7.8–7.83 (d, $J=7.9$ Hz, 1H), 7.8 (s, 1H), 10.09 (s, 1H); ^{13}C NMR ($CDCl_3$): 118.15, 119.48, 124.46, 124.75, 126.79, 128.70, 138.40, 142.25, 154.5, 162.50. Anal. ($C_{10}H_6O_3$) C,H.
Compound 3a: (65.4%); mp 200–202 °C; IR (Nujol) cm^{-1}: 1710–1730, 3200–3250; ^1H NMR ($CDCl_3$): δ 6.44–6.47 (d, $J=10.17$, 1H), 7.52–7.54 (m, $J=6.36$, 2H), 7.66 (s, 1H), 7.8–7.83 (d, $J=10.17$, 1H), 8.14 (s, 1H), 11.4 (s, 1H); ^{13}C NMR ($CDCl_3$): 113,13 115.63, 118.41, 121.80, 127.40, 135.97, 142.41, 146.23, 153.12, 159.46; MS: 189 [M+], 175 (62.8), 172 (61.7), 161 (93.2), 145 (61.6). Anal. ($C_{10}H_7NO_3$) C, H, N.
Compound 3e: (11.9%); mp 198–201 °C; IR (Nujol) cm^{-1}: 1720–1730, 3050; ^1H NMR ($CDCl_3$): δ 3.0 (s, 3H), 5.05 (s, 1H), 7.16 (s, 1H), 6.34–6.37 (d, $J=8.85$, 1H), 7.39–7.42 (d, $J=7.89$, 1H), 7.48 (s, 1H), 7.48–7.51 (d, $J=8.85$, 1H), 7.65–7.68 (d, $J=8.46$, 1H); ^{13}C NMR ($CDCl_3$): 35.36, 80.95, 112.97, 115.46, 117.76, 121.55, 128.60, 141.02, 143.19, 154.67, 161.08; MS 202 (100) [M+], 172 (16.8), 158 (7.5), 145 (31.4), 89 (68.4). Anal. ($C_{11}H_{10}N_2O_2$) C, H, N.
Compound 4: (29.2%), mp 121–124 °C; IR (Nujol) cm^{-1}: 1720–1730, 3050; ^1H NMR ($CDCl_3$): δ 3.9 (s, 3H), 6.40–6.43 (d, $J=9.39$, 1H), 7.53 (s, 1H), 7.57–7.61 (d, $J=9.39$, 1H), 7.74–7.77 (d, $J=9.2$, 1H), 8.12–8.15 (d, $J=8.7$, 1H), 8.33 (s, 1H); ^{13}C NMR ($CDCl_3$): 54.88, 115.89, 117.05, 117.58, 124.05, 125.56, 127.81, 133.65, 142.47, 151.62, 160.20; MS 203 (26) [M+], 145 (8.5). Anal. ($C_{11}H_{19}NO_3$) C, H, N.
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