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Article

Preparation and Evaluation of a Coumarin Library Towards the Inhibitory Activity of the Enzyme gGAPDH from *Trypanosoma cruzi*

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A doença de Chagas, causada pelo *Trypanosoma cruzi*, é endêmica em 15 países na América Latina. Neste trabalho uma coleção de 38 cumarinas foi preparada em solução e testada frente à enzima gliceraldeído-3-fostafo-desidrogenase (gGAPDH) do *T. cruzi*. A rota sintética foi baseada na condensação de Knoevenagel de diferentes 2-hidroxibenzaldeídos com ácido de Meldrum ou malonato de etila, seguido de O-alquilação e/ou reação de transesterificação. Dentre as cumarinas preparadas, os melhores resultados obtidos para inibir 50% da atividade catalítica da enzima foram entre 80 e 130 μM.

Chagas' disease, caused by *Trypanosoma cruzi*, is endemic in 15 countries in Latin America. In this work a library of 38 coumarins was prepared in solution phase and evaluated against *T. cruzi* glycolytic enzyme glyceraldehyde-3-phosphate-dehydrogenase (gGAPDH). The synthetic route was based on the Knoevenagel condensation of different 2-hydroxybenzaldehydes with Meldrum's acid or diethyl malonate, followed by O-alkylation and/or transesterification reactions. Among the prepared coumarins, the best values obtained to inhibit 50% of the enzymatic activity range from 80 to 130 μM.

Keywords: *Trypanosoma cruzi*, coumarin library, chalepin, gGAPDH

Introduction

Chagas' disease is endemic in 15 countries of Latin America, where 40 million people are at risk and 200,000 new cases are registered each year.[1] Owing to toxicity and lack of efficacy, most of the currently used compounds are unsatisfactory[2] and the design of novel classes of trypanocidal drugs has become urgent. In fact, the medical and economic problems caused by Chagas' disease explain why it has been selected by the World Health Organization for development of new or more effective treatments.[1]

One promising approach to accomplish this task is through the selective inhibition of enzymes that participate in the glycolytic pathway of the parasite (*Trypanosoma cruzi*). Glyceraldehyde-3-phosphate dehydrogenase (gGAPDH), the sixth enzyme in the glycolytic pathway, plays an essential role in the control of glycolytic flux.[3,4] Once intracellular amastigotes probably depend on glycolisis for ATP production, the inhibition of gGAPDH would prevent *T. cruzi* from being infective.[2,5] For this reason it has been identified as a suitable target for the development of inhibitors. Another reason to select gGAPDH as a good target for inhibitors design comes from the fact that 95% deficiency of GAPDH in human erythrocytes does not cause any clinical symptoms.

In a continuous effort towards the development of specific, potent inhibitors of the *T. cruzi* gGAPDH, several natural products have been screened[6] and, among some other active coumarins, chalepin showed a promising IC_{50} value (64 μM).[7] After the structural characterization of the chalepin-gGAPDH complex by X-ray crystallography,[8] essential interaction sites could be identified and structural modifications in the coumarin ring proposed both by molecular modeling and *de novo* design (Figure 1).

Structure-based ligand design suggested a simplification of chalepin ring, through the substitution of 1,2-dimethyl-allyl moiety by a more polar group, thus replacing van der Waals contacts by hydrogen bonds, and additional moieties at R^2, aiming again the increased hydrogen bond interactions. Recently, Montanari and coworkers. reported 3D QSAR studies on binding

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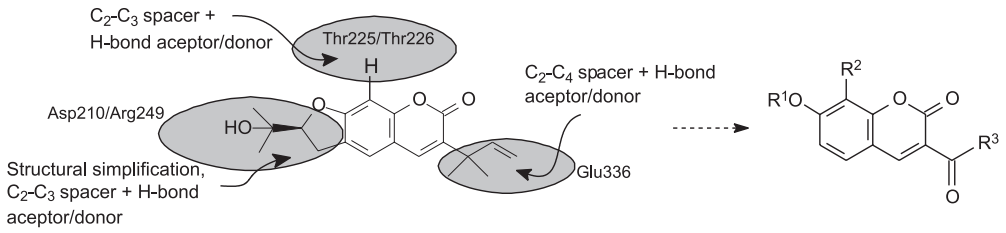


Figure 1. Proposed structural modifications in the chalepin structure.

affinities of some natural and synthetic coumarins for *T. cruzi* gGAPDH.[9,10]

Pharmaceutical companies have now been using combinatorial chemistry for drug discovery for about a decade. Some of the earlier libraries they synthesized have been discredited for being poorly designed, impractically large, and structurally simplistic.[11] That's why drug researchers are increasingly embracing natural-product-like libraries—moderate-size collections of complex compounds that are highly likely to exhibit interesting and useful types of biological activity.[12]

In order to contribute with the study of the structure-activity relationship, in this work a library containing 38 coumarin-3-carboxyesters was prepared in solution phase and evaluated against *T. cruzi* gGAPDH, following the structural modifications of chalepin shown in Figure 1. Coumarin-3-carboxamides and esters were reported as inhibitors of serine proteases, α-chymotrypsin (CT) and human leukocyte elastase (HLE). Serine proteases have been the focus of extensive study in terms of their vital roles in biological processes, their involvement in numerous diseases, and the development of suitable therapeutic inhibitors.[13]

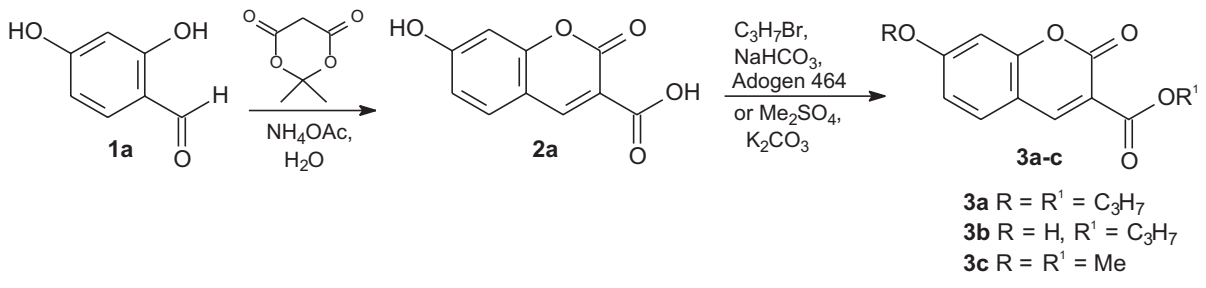
Results and Discussion

The synthetic route for the preparation of the coumarin derivatives was based on the Knoevenagel condensation of different 2-hydroxybenzaldehydes with Meldrum's acid[14] or diethyl malonate,[15] followed by *O*-alkylation and/or transesterification or bromination reactions. The condensation of the 2,4-di-hydroxybenzaldehyde (**1a**) with Meldrum´s acid using catalytic amount of NH_4OAc gave compound **2a** that was *O*-alkylated to obtain coumarins **3a-c** (Scheme 1).

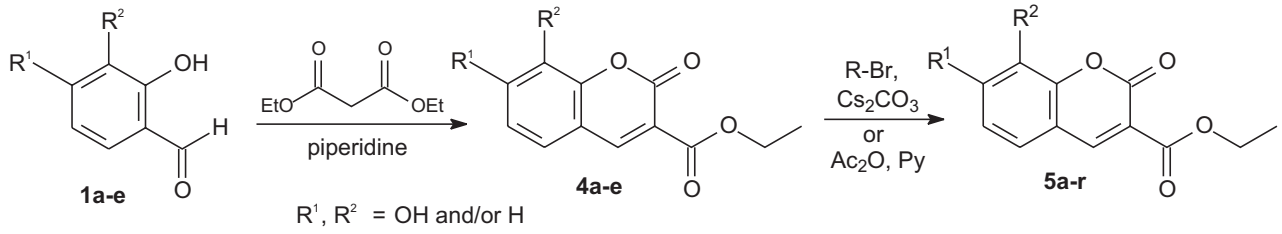
Aldehydes **1a**-**e** were reacted with diethyl malonate in the presence of piperidine to give ethyl coumarin-3-carboxylate derivatives **4a**-**e** that were *O*-alkylated with different alkyl bromides furnishing coumarins **5a**-**r** (Scheme 2). Treatment of **2a** and **4a** with bromine in acetic acid gave compounds **2b** and **6a**, that were *O*-alkylated to furnish compounds **6b** and **6c** (Scheme 3). Some of coumarin derivatives **4** were submitted to transesterification reactions to form coumarins **7a**-**h** (Scheme 4). In total, 38 coumarins were prepared (Table 1), submitted to inhibition assays and the observed inhibitory activities are shown in Table 2.

The prepared coumarins showed moderate inhibition profiles. The best values to inhibit 50% of the enzymatic activity range from 80 to 130 μM. One interesting point though was the unexpected behavior of compound **6b** when compared to compounds **4d**, **3c** and **6c**. According to inhibition assay results (compounds **4d** and **6c**) increased steric volume at R^3 or R^2 does not affect interaction profile significantly. On the other hand, when both substituents are present at the same time, deleterious effect on affinity is observed (compound **6b**).

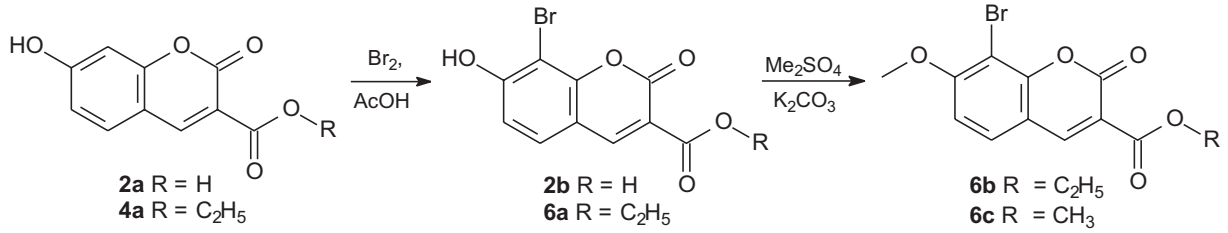
Another intriguing result comes from the comparison of compounds **5m**, **5n** and **5o**. Inhibition assay results suggest that bulky substituents at R^2 prevent inhibitors from binding unless R^1 position is also full-filled. This result is unexpected since gGAPDH-chalepin crystallographic structure shows that



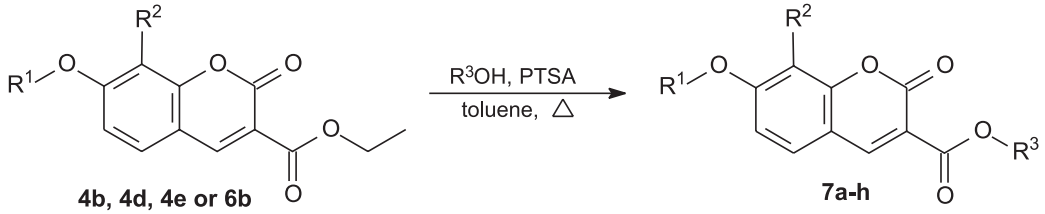
Scheme 1.



Scheme 2.



Scheme 3.



Scheme 4.

R^2 position should be directed to water molecule W348, which does not have an essential role in chalepin interaction profile.

Indeed, if compounds **1**-**7** bind in the same way as chalepin (Figure 1), explaining the structure-activity relationships becomes far from easy. In order to further investigate these results and thus generate useful data for the computer aided molecular design, modeling studies are being performed through docking protocol.

Experimental

Unless otherwise noted, all commercially available reagents were purchased from Aldrich Chemical Co. Reagents and solvents were purified when necessary according to the usual procedures described in the literature. ^1H and ^{13}C NMR spectra were recorded on a Bruker ARX-200 (200 and 50 MHz respectively). The IR spectra refer to films and were measured on a Bomem M102 spectrometer. Mass Spectra were recorded on a Shimadzu GCMS-QP5000 or Mass Spectrometer QuatroLC-Micromass. Elemental analyses were performed on a Fisons EA 1108 CHNS-O. Analytical thin-layer chromatography was performed on a 0.25 μm film of silica gel containing fluorescent indicator UV_{254} supported on an aluminum sheet (Sigma-Aldrich). Flash column chromatography was performed using silica gel (Kieselgel 60, 230-400 mesh, E. Merck). Gas chromatography was performed in a Shimadzu GC-17A with H_2 as carrier and using a DB-5 column. Melting points were performed in Microquimica MQAPF - 301.

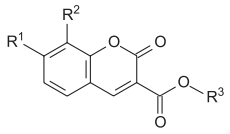
T. cruzi GAPDH activity

T. cruzi gGAPDH activity was determined according to a modification of a previously reported procedure.[7] Reduced NADH was measured spectrophotometrically at 340 nm at 30 s interval. The reaction medium contained 50 mmol L^{-1} Tris-HCl pH 8.6 buffer, 1 mmol L^{-1} β-mercapto-ethanol, 30 mmol L^{-1} Na_2HAsO_4 2.5 mmol L^{-1} NAD^+, 0.3 mmol L^{-1} glyceraldehyde-3-phosphate and 0.4-0.9 mg protein, in a total volume of 1000 μL. The reaction was initiated by addition of enzyme.

The specific activity (unit = U) of the enzyme was calculated as:

(U mg^{-1}) = {(Δ absorbance / Δt) x volume of cell} / 6.22 x volume of enzyme x [enzyme]

Table 1. Coumarins prepared



Compound	R^1	R^2	R^3
2a	OH	H	H
2b	OH	Br	H
3a	OC_3H_7	H	C_3H_7
3b	OH	H	C_3H_7
3c	OMe	H	CH_3
4a	OH	H	C_2H_5
4b	H	OH	C_2H_5
4c	OH	OH	C_2H_5
4d	OMe	H	C_2H_5
4e	H	H	C_2H_5
5a	OAc	H	C_2H_5
5b	H	OAc	C_2H_5
5c	OAc	OAc	C_2H_5
5d	O-Allyl	H	C_2H_5
5e	H	O-Allyl	C_2H_5
5f	O-Allyl	O-Allyl	C_2H_5
5g	OC_3H_6-OTHP	H	C_2H_5
5h	H	OC_3H_6-OTHP	C_2H_5
5i	OC_3H_6-OTHP	OC_3H_6-OTHP	C_2H_5
5j	OC_2H_4-OTHP	H	C_2H_5
5k	H	OC_2H_4-OTHP	C_2H_5
5l	OC_2H_4-OTHP	OC_2H_4-OTHP	C_2H_5
5m	OC_3H_6OH	H	C_2H_5
5n	H	OC_3H_6OH	C_2H_5
5o	OC_3H_6OH	OC_3H_6OH	C_2H_5
5p	OC_2H_4OH	H	C_2H_5
5q	H	OC_2H_4OH	C_2H_5
5r	OC_2H_4OH	OC_2H_4OH	C_2H_5
6a	OH	Br	C_2H_5
6b	OMe	Br	C_2H_5
6c	OMe	Br	CH_3
7a	H	OH	C_2H_4OH
7b	OMe	H	C_2H_4OH
7c	H	H	C_2H_4OH
7d	H	OH	$C_5H_{10}OH$
7e	OMe	H	$C_5H_{10}OH$
7f	H	H	$C_5H_{10}OH$
7g	OMe	H	*iso*-butyl
7h	OMe	Br	*iso*-butyl

where Δt = 0.5 min; volume of cell = 1.00 mL; NADH = 6.22 (mmol L^{-1}); volume of enzyme = 0.005 mL; [enzyme] concentration of enzyme in mg mL^{-1}.

T. cruzi GAPDH inhibitory activity

The inhibitory activity was recorded using the same reaction medium described above. Absorbance was also read at 340 nm at 30 s interval. In each case, a control experiment was performed with 10% DMSO in the reaction medium. Inhibitory activity was calculated as follows, and the data presented in Table 2 are the means of 3 repetitions.

% inhibitory activity = {(U mg^{-1} control – U mg^{-1} compound) / U mg^{-1} control} x 100

*7-hydroxy-2-oxo-2H-chromene-3-carboxylic acid (**2a**)*

To a suspension of 2,4-dihydroxybenzaldehyde (1.3 g, 9.5 mmol) in water (21 mL) were added the Meldrum´s acid (1.55 g, 10.4 mmol) and ammonium acetate (150 mg, 1.9 mmol). The suspension was stirred at room temperature for 1 h. The precipitate formed was filtered, washed with cold water (2 x 10 mL) and dried in vacuum to give **2a** as a yellow solid (1.88 g, 96% yield). mp 283-284°C; IR ν_{max}/ cm^{-1}: 3129, 3037, 1712, 1684, 1617; ^{1}H NMR (200 MHz, DMSO-d_6) d 6.74 (s, 1H), 6.85 (d, *J* 8.2 Hz, 1H), 7.75 (d, *J* 8.2 Hz, 1H), 8.70 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) d 102.0, 110.8, 112.8, 114.3, 132.1, 149.4, 157.2, 158.0, 164.2, 164.5.

*Coumarin-3-carboxylate derivatives (**3a** and **3b**)*

To a solution of coumarin **2a** (50 mg, 0.24 mmol) in a saturated solution of $NaHCO_3$ (250 μL) were added Adogen 464® (100 mg, 0.24 mmol) and a solution of 1-bromopropane (27 μL, 0.29 mmol) in dichloromethane (250 μL). The mixture was stirred for 72 h at 50°C and, after cooling to room temperature dichloromethane (1 mL) and water (1 mL) were added. The organic layer was extracted with dichloromethane (4 x 2 mL), washed with water (3 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The material was purified by flash chromatography using 33% hexane in ethyl acetate as eluent, giving coumarins **3a** (8.0 mg, 13% yield) and **3b** (5.8 mg, 10% yield).

*Propyl 7-propoxy-2-oxo-2H-chromene-3-carboxylate (**3a**).*

mp 104-105 °C; IR ν_{max}/cm^{-1}: 2964, 2928, 1777, 1603; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.03 (t, *J* 7.0 Hz, 3H), 1.06 (t, *J* 7.0 Hz, 3H), 1.71-1.98 (m, 4H), 4.01 (t, *J* 6.5 Hz, 2H), 4.29 (t, *J* 6.7 Hz, 2H), 6.80 (d, *J* 2.4 Hz, 1H), 6.88 (dd, *J* 8.6 and 2.4 Hz, 1H), 7.49 (d, *J* 8.6 Hz, 1H), 8.49 (s, 1H); MS (IE): *m/z* 290 (M^+), 248, 231, 206,189, 162 (100), 134, 105, 51.

*Propyl 7-hydroxy-2-oxo-2H-chromene-3-carboxylate (**3b**)*

mp 174-175 °C; IR ν_{max}/cm^{-1}: 3443, 3273, 1741, 1709; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.97 (t, *J* 7.4 Hz, 3H), 1.61-1.78 (m, 2H), 4.17 (t, *J* 6.5 Hz, 2H), 6.73 (s, 1H), 6.84 (d, *J* 8.3 Hz, 1H), 7.77 (d, *J* 8.3 Hz, 1H), 8.66 (s, 1H); MS (IE): *m/z* 248 (M^+), 206, 189, 162 (100), 134, 105, 51.

Table 2. Effect of coumarins on gGAPDH activity

Compound	Concentration (μmol L^{-1})	Absorbance	Specific Activity(U mg^{-1})	% InhibitoryActivity
3a	86	0.229	49.17	23
	138	0.149	32.09	50
	155	0.112	24.13	62
	172	0.054	11.72	82
3b	201	0.283	60.83	5
Control		0.300	64.30	
3c	128	0.162	34.77	55
Control		0.358	76.85	
4a	427	0.261	57.90	0
4b	213	0.100	21.43	62
4c	403	0.312	67.05	0
4d	80	0.132	28.40	50
5a	181	0.218	46.71	18
5b	362	0.221	47.31	17
5c	150	0.300	64.35	0
Control		0.264	56.76	
5d	109	0.193	41.48	31
Control		0.282	60.54	
5e	365	0.428	91.76	0
Control		0.284	56.76	
5f	121	0.211	45.36	41
Control		0.358	76.85	
5g	106	0.184	39.57	35
Control		0.282	60.54	
5h	265	0.284	60.92	0
5i	187	0.303	65.08	0
Control		0.264	56.76	
5m	102	0.187	40.09	34
Control		0.282	60.54	
5m	136	0.071	15.21	80
Control		0.358	76.85	
5n	342	0.263	56.57	0
Control		0.264	56.76	
5o	109	0.175	37.57	51
Control		0.358	76.85	
5j	138	0.177	38.11	44
Control		0.317	67.96	
5k	138	0.360	77.24	0
Control		0.358	76.85	
5l	99	0.244	52.47	13
Control		0.282	60.54	
5p	143	0.192	41.25	46
Control		0.358	76.85	
5q	359	0.347	74.53	0
Control		0.317	67.96	
5r	148	0.155	33.35	51
Control		0.317	67.96	
6a	319	0.307	65.10	0
6b	306	0.129	27.73	50
Control		0.256	54.93	
6c	128	0.150	32.32	52
Control		0.317	67.95	
6c	160	0.045	9.68	82
Control		0.256	54.93	
7a	200	0.253	61.15	20
7b	113	0.236	50.65	34
Control		0.358	76.85	
7c	213	0.274	58.87	14
Control		0.321	68.85	
7d	141	0.274	58.73	15
Control		0.321	68.85	
7e	130	0.159	34.21	43
Control		0.282	60.54	
7f	180	0.199	42.71	4
Control		0.208	44.68	
7g	145	0.201	43.19	36
Control		0.317	67.95	
7g	181	0.088	18.90	65
	362	0.006	1.26	98
7h	281	0.267	57.30	0
Control		0.256	54.93	

*Methyl 7-methoxy-2-oxo-2H-chromene-3-carboxylate (**3c**)*

To a solution of coumarin **2a** (50 mg, 0.24 mmol) in dry acetone (2 mL) were added K_2CO_3 (200 mg, 1.45 mmol), Me_2SO_4 (183 mg, 1.45 mmol), and the mixture was stirred under reflux for 5 h. After cooling to room temperature a saturated solution of NH_4Cl (4 mL) was added and the product was extracted with ethyl acetate (4 x 4 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The product was purified by flash chromatography using 5% ethyl acetate in dichloromethane as eluent to give **3c** (18 mg, 32% yield). mp 123-124 °C; IR ν_{max}/cm^{-1}: 3057, 2953, 1746, 1699; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.91 (s, 3H), 3.94 (s, 3H), 6.82 (d, *J* 2.3 Hz, 1H), 6.90 (dd, *J* 8.6 and 2.3 Hz, 1H), 7.51 (d, *J* 8.6 Hz, 1H), 8.55 (s, 1H); MS (IE): *m/z* 234 (M^+), 203 (100), 176, 119, 76.

*Ethyl coumarin-3-carboxylate derivatives (**4a-e**)*

To a solution of 2-hydroxybenzaldehydes **1a-e** (3 mmol) in diethyl malonate (3 mmol) was added piperidine (10 drops), and the resulting solution was stirred for 30 minutes at room temperature. Then it was acidified with a solution of HCl 10%. The precipitate material was filtrated and washed with cold water. The desired products were purified by recrystallization from ethyl acetate or flash chromatography.

*Ethyl 7-hydroxy-2-oxo-2H-chromene-3-carboxylate (**4a**)*

(1.94 g, 76% yield), mp 168-169 °C (lit.[16] mp 166-167 °C); IR ν_{max}/cm^{-1}: 3550, 3470, 1739, 1617; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.29 (t, *J* 7.1 Hz, 3H), 4.26 (q, *J* 7.1 Hz, 2H), 6.73 (s, 1H), 6.84 (d, *J* 8.3 Hz, 1H), 7.77 (d, *J* 8.3 Hz, 1H), 8.67 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 14.0, 60.7, 110.3, 112.0, 132.0, 149.3, 156.3, 162.8, 163.9; MS (IE): *m/z* 234 (M^+), 206, 189, 178, 162 (100), 134, 105, 89, 77, 51.

*Ethyl 8-hydroxy-2-oxo-2H-chromene-3-carboxylate (**4b**)*

(350 mg, 45% yield), mp 174-175 °C; IR ν_{max}/cm^{-1}: 3303, 3045, 2984, 1746, 1696, 1611; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.32 (t, *J* 7.0 Hz, 3H), 4.30 (q, *J* 7.0 Hz, 2H), 7.17-7.26 (m, 2H), 7.28-7.39 (m, 1H), 8.69 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0, 61.1, 117.7, 118.6, 120.0, 120.5, 124.7, 143.1, 144.3, 149.0, 155.9, 162.6; MS (IE): *m/z* 234 (M^+), 206, 189, 162 (100), 134, 105, 89, 77, 51.

*Ethyl 7,8-dihydroxy-2-oxo-2H-chromene-3-carboxylate (**4c**)*

(560 mg, 70% yield), mp 233-234 °C; IR ν_{max}/cm^{-1}: 3476, 3214, 2973, 1738, 1693, 1618, 1588; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30 (t, *J* 7.1 Hz, 3H), 4.26 (q, *J* 7.1 Hz, 2H), 6.87 (d, *J* 8.5 Hz, 1H), 7.27 (d, *J* 8.5 Hz, 1H), 8.63 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.3, 61.0, 111.4, 112.1, 113.5, 121.8, 132.0, 145.1, 150.2, 152.8, 156.6, 163.2; APCI/MS: *m/z* 249 ($[M-H]^-$).

*Ethyl 7-methoxy-2-oxo-2H-chromene-3-carboxylate (**4d**)*

(406 mg, 68% yield), mp 132-133 °C (lit.[17] mp 120-125 °C); IR ν_{max}/cm^{-1}: 3052, 2986, 1619, 1381, 1216; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (t, *J* 7.1 Hz, 3H), 3.90 (s, 3H), 4.40 (q, *J* 7.1 Hz, 2H), 6.82 (d, *J* 2.4 Hz, 1H), 6.90 (dd, *J* 8.6 and 2.4 Hz, 1H), 7.50 (d, *J* 8.6 Hz, 1H), 8.51 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 55.8, 61.5, 100.2, 111.4, 113.4, 113.9, 130.6, 148.7, 157.0, 157.4, 163.2, 165.0; MS (IE): *m/z* 248 (M^+), 203, 176 (100), 148, 119, 76.

*Ethyl 2-oxo-2H-chromene-3-carboxylate (**4e**)*

(890 mg, 65% yield), mp 72-73 °C (lit.[16] mp 93-93 °C); IR ν_{max}/cm^{-1}: 1779, 1617, 1607, 1567; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (t, *J* 7.1 Hz, 3H), 4.42 (q, *J* 7.1 Hz, 2H), 7.27-7.41 (m, 2H), 7.57-7.72 (m, 2H), 8.53 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2, 61.9, 116.7, 117.8, 118.3, 124.8, 129.5, 134.3, 148.5, 155.1, 156.6, 163.0.; MS (IE): *m/z* 218 (M^+) 190, 173, 146 (100), 118, 101, 89, 63.

*Acetylcoumarin derivatives (**5a-c**)*

To a solution of hydroxycoumarin **4a-c** (0.13 mmol) in pyridine (0.5 mL) was added acetic anhydride (3 equiv. for each free hydroxyl group) and the mixture was stirred for 3 h at room temperature. Ethyl acetate (4 mL) was added, the organic layer was washed with a solution of HCl 10% (3 x 3 mL), NaOH 10% (2 x 3 mL) and water (3 x 3 mL). The solvent was dried over Na_2SO_4 and evaporated under reduced pressure. The product was purified by successive recrystallization from hot ethyl acetate, then hexane was added.

*Ethyl 7-acetyloxy-2-oxo-2H-chromene-3-carboxylate (**5a**)*

(27 mg, 76% yield): mp 154-155 °C; IR ν_{max}/cm^{-1}: 3087, 3060, 2981, 1756, 1700; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (t, *J* 7.1 Hz, 3H), 2.35 (s, 3H), 4.42 (q, *J* 7.1 Hz, 2H), 7.07-7.20 (m, 2H), 7.62 (d, *J* 8.3 Hz, 1H), 8.52 (s, 1H); MS (IE): *m/z* 276 (M^+), 234, 189, 162 (100), 134, 105, 89, 76, 51.

*Ethyl 8-acetyloxy-2-oxo-2H-chromene-3-carboxylate (**5b**)*

(16 mg, 47% yield), mp 150-151 °C; IR ν_{max}/cm^{-1}: 3045,

2984, 1760, 1695, 1620, 1577; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.40 (t, *J* 7.1 Hz, 3H), 2.42 (s, 3H), 4.41 (q, *J* 7.1 Hz, 2H), 7.25-7.55 (m, 3H), 8.51 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 20.6, 62.1, 119.1, 124.5, 126.8, 127.6, 137.6, 148.0, 168.4; MS (IE): *m/z* 276 (M^+), 234 (100), 188, 162, 134, 105, 76, 51.

*Ethyl 7,8-bis(acetyloxy)-2-oxo-2H-chromene-3-carboxylate (**5c**)*

(41 mg, 79% yield), mp 127-128 °C; IR ν_{max}/cm^{-1}: 2998, 2940, 1774, 1723, 1578; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (t, *J* 7.1 Hz, 3H), 2.34 (s, 3H), 2.41 (s, 3H), 4.40 (q, *J* 7.1 Hz, 2H), 7.19 (d, *J* 8.6 Hz, 1H), 7.50 (d, *J* 8.6 Hz, 1H), 8.50 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 20.2, 20.6, 62.1, 116.6, 118.0, 119.5, 126.5, 130.1, 147.3, 147.8, 148.4, 155.0, 162.6, 167.1, 167.4; MS (IE): *m/z* 334 (M^+), 292, 250, 204 (100), 178, 120, 92, 79, 63.

*Ethyl 2-oxo-2H-chromene-3-carboxylate derivatives (**5d-l**)*

To a stirred solution of coumarin **4a-c** in dry DMF (4 mL mmol^{-1}) were added Cs_2CO_3 (2 equiv. for each free hydroxyl group) and the corresponding bromide (2 equiv. for each free hydroxyl). The mixture was stirred at 50 °C for 16 h. After cooling to room temperature, ethyl acetate was added and the organic layer was washed with saturated solution of NH_4Cl. The solvent was dried over Na_2SO_4 and evaporated; the resulting products were purified by recrystallization from ethyl acetate or flash chromatography.

*Ethyl 7-(allyloxy)-2-oxo-2H-chromene-3-carboxylate (**5d**)*

(42 mg, 72% yield), mp 103-104 °C; IR ν_{max}/cm^{-1}: 3055, 2985, 1756, 1618; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (t, *J* 7.1 Hz, 3H), 4.40 (q, *J* 7.1 Hz, 2H), 4.56-4.74 (m, 2H), 5.26-5.55 (m, 2H), 6.05 (ddt, *J* 17.1, 15.7 and 5.2 Hz, 1H), 6.82 (d, *J* 2.2 Hz, 1H), 6.91 (dd, *J* 8.7 and 2.2 Hz, 1H), 7.50 (d, *J* 8.7 Hz, 1H), 8.50 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2, 61.7, 69.5, 101.20, 111.7, 114.11, 118.9, 130.7, 131.7, 148.9, 157.1, 157.4, 163.4, 164.0; MS (IE): *m/z* 274 (M^+), 246, 229, 202 (100), 188, 160, 132, 105, 76, 50.

*Ethyl 8-(allyloxy)-2-oxo-2H-chromene-3-carboxylate (**5e**)*

(50 mg, 86% yield), mp 89-90 °C; IR ν_{max}/cm^{-1} : 1759, 1703, 1608, 1572, 1473; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (t, *J* 7.1 Hz, 3H), 4.41 (q, *J* 7.1 Hz, 2H), 4.71 (ddd, *J* 10.5, 3.0 and 1.5 Hz, 2H), 5.33 (ddd, *J* 10.5, 3.0 and 1.5 Hz, 2H), 5.45 (ddd, *J* 17.2, 3.0 and 1.5 Hz, 1H), 6.08 (ddt, *J* 17.2, 10.5 and 5.3 Hz, 1H), 7.11-7.30 (m, 3H), 8.49 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2, 61.9, 70.2, 117.8, 118.5, 120.9, 124.5, 132.4, 145.2, 145.9, 148.7, 156.2, 163.0.; MS (IE): *m/z* 274 (M^+), 246, 233, 205, 188, 177, 160, 133, 115, 105 (100), 76, 50; Anal. Calc. for $C_{15}H_{14}O_5$: C, 65.69; H, 5.14, Found: C, 65.42; H, 4.82%.

*Ethyl 7,8-bis(allyloxy)-2-oxo-2H-chromene-3-carboxylate (**5f**)*

(43 mg, 69% yield), mp 91-92 °C; IR ν_{max}/cm^{-1}: 3092, 2994, 1760, 1607; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (t, *J* 7.1 Hz, 3H), 4.39 (q, *J* 7.1 Hz, 2H), 4.61-4.80 (m, 4H), 5.21 (ddt, *J* 10.2, 2.0 and 1.7 Hz, 1H), 5.30 (ddd, *J* 6.6, 2.7 and 1.4 Hz, 1H), 5.37 (dd, *J* 2.8 and 1.4 Hz, 1H), 5.45 (ddt, *J* 17.2, 3.0 and 1.6 Hz, 1H), 5.95-6.25 (m, 2H), 6.90 (d, *J* 8.7 Hz, 1H), 7.29 (d, *J* 8.7 Hz, 1H), 8.46 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2, 61.7, 70.0, 74.6, 110.4, 112.8, 114.7, 118.4, 118.8, 124.9, 132.0, 133.6, 149.0, 156.4, 157.0, 163.4; MS (IE): *m/z* 306, 302, 288, 257, 229, 210, 185, 170, 158, 129, 115, 103, 91, 77, 55 (100).

*Ethyl 2-oxo-7-[3-(tetrahydro-2H-pyran-2-yloxy)propoxy]-2H-chromene-3-carboxylate (**5g**)*

(62 mg, 77% yield), mp 83-84 °C; IR ν_{max}/cm^{-1}: 2948, 2866, 1748, 1698, 1603; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (t, *J* 7.1 Hz, 3H), 1.50-1.90 (m, 6H), 2.03-2.20 (m, 2H), 3.44-3.65 (m, 2H), 3.75-4.03 (m, 2H), 4.18 (t, *J* 6.4 Hz, 2H), 4.40 (q, *J* 7.1 Hz, 2H), 4.56-4.67 (m, 1H), 6.83 (d, *J* 2.4 Hz, 2H), 6.89 (dd, *J* 8.6 and 2.4 Hz, 1H), 7.50 (d, *J* 8.6 Hz, 1H), 8.51 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2, 19.5, 25.3, 29.3, 30.6, 61.6, 62.3, 63.5, 65.9, 98.9, 100.8, 111.5, 113.9, 130.6, 148.9, 157.1, 157.5, 163.4, 164.5; MS (IE): *m/z* 292, 234, 220, 206, 189, 162 (100), 134, 105, 76, 51; Anal. Calc. for $C_{20}H_{24}O_7$: C, 63.82; H, 6.43, Found: C, 63.92; H, 6.36%.

*Ethyl 2-oxo-8-[3-(tetrahydro-2H-pyran-2-yloxy)propoxy]-2H-chromene-3-carboxylate (**5h**)*

(71 mg, 88% yield), viscous liquid, IR (film) ν_{max}/cm^{-1}: 2941, 2872, 1762, 1609; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (t, *J* 7.1 Hz, 3H), 1.45-1.65 (m, 4H), 1.66-1.90 (m, 2H), 2.10-2.25 (m, 2H), 3.42-3.57 (m, 1H), 3.58-3.72 (m, 1H), 3.77-3.89 (m, 1H), 3.90-4.02 (m, 1H), 4.19-4.30 (m, 2H), 4.41 (q, *J* 7.1 Hz, 2H), 4.56-4.65 (m, 1H), 7.10-7.30 (m, 3H), 8.49 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 19.7, 25.3, 29.4, 30.6, 61.8, 62.5, 63.8, 66.6, 99.1, 117.2, 118.4, 118.5, 120.5, 124.6, 145.0, 146.5, 148.7, 156.2, 163.0;

MS (IE): *m/z* 316, 278, 247, 234, 188, 162, 134, 129, 105, 85, 73 (100), 55.

*Ethyl 2-oxo-7,8-bis[3-(tetrahydro-2H-pyran-2-yloxy)-propoxy]-2H-chromene-3-carboxylate (**5i**)*

(87 mg, 81% yield), IR (film) ν_{max}/cm^{-1}: 2942, 2873, 1763, 1605; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.39 (t, *J* 7.1 Hz, 3H), 1.46-1.90 (m, 12H), 2.00-2.27 (m, 4H), 3.41-3.76 (m, 4H), 3.77-4.05 (m, 4H), 4.24 (t, *J* 6.3 Hz, 4H), 4.39 (q, *J* 7.1 Hz, 2H), 4.55-4.60 (m, 1H), 4.61-4.69 (m, 1H), 6.93 (d, *J* 8.7 Hz, 1H), 7.29 (d, *J* 8.7 Hz, 1H), 8.46 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2, 19.7, 25.3, 25.4, 29.5, 30.5, 30.6, 30.7, 61.6, 62.4, 62.5, 63.6, 64.2, 66.5. 71.3, 99.0, 99.1, 109.9, 112.6, 114.5, 124.8, 149.0, 149.4, 156.4, 157.5, 163.4; MS (IE): *m/z* 308, 281, 250, 204, 175, 143, 85 (100), 69, 57.

*Ethyl 2-oxo-7-[2-(tetrahydro-2H-pyran-2-yloxy)ethoxy]-2H-chromene-3-carboxylate (**5j**)*

(63 mg, 80% yield), mp 93-94 °C; IR ν_{max}/cm^{-1}: 2939, 2871, 1788, 1760, 1604; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (t, *J* 7.1 Hz, 3H), 1.50-1.90 (m, 6H), 3.49-3.60 (m, 1H), 3.79-3.94 (m, 2H), 4.05-4.15 (m, 1H), 4.23-4.28 (m, 2H), 4.39 (q, *J* 7.1 Hz, 2H), 4.69-4.72 (m, 1H), 6.85 (d, *J* 2.4 Hz, 1H), 6.93 (dd, *J* 8.6 and 2.4 Hz, 1H), 7.50 (d, *J* 8.6 Hz, 1H), 8.51 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2, 19.3, 25.3, 30.4, 61.6, 62.2, 65.3, 68.2, 99.1, 101.1, 111.6, 114.0, 114.1, 130.6, 148.9, 157.4, 163.4, 164.4; MS (IE): *m/z* 362 (M^+) 278, 248, 234, 206, 189, 162, 129, 105, 85 (100), 73, 55.

*Ethyl 2-oxo-8-[2-(tetrahydro-2H-pyran-2-yloxy)ethoxy]-2H-chromene-3-carboxylate (**5k**)*

(67 mg, 86% yield), IR (film) ν_{max}/cm^{-1}: 2942, 2872, 1761, 1609; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (t, *J* 7.1 Hz, 3H), 1.47-1.90 (m, 6H), 3.45-3.64 (m, 1H), 3.81-4.04 (m, 2H), 4.05-4.22 (m, 1H), 4.25-4.38 (m, 2H), 4.41 (q, *J* 7.1 Hz, 2H), 4.69-4.82 (m, 1H), 7.12-7.35 (m, 3H), 8.49 (s, 1H).; ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 19.3, 25.3, 30.4, 61.8, 62.2, 65.5, 69.2, 99.0, 117.9, 118.4, 118.5, 120.0, 124.5, 145.2, 146.4, 148.6, 156.1, 163.0; MS (IE): *m/z* 316, 278, 247, 188, 162, 129, 105, 85, 73 (100), 55.

*Ethyl 2-oxo-7,8-bis-[2-(tetrahydro-2H-pyran-2-yloxy)-ethoxy]-2H-chromene-3-carboxylate (**5l**)*

(18 mg, 18% yield), viscous liquid, ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.39 (t, *J* 7.1 Hz, 3H), 1.45-1.92 (m, 12H), 3.42-3.61 (m, 2H), 3.76-3.95 (m, 4H), 3.96-4.19 (m, 2H), 4.25-4.47 (m, 6H), 4.64-4.80 (m, 2H), 6.95 (d, *J* 8.7 Hz, 1H), 7.29 (d, *J* 8.7 Hz, 1H), 8.46 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2, 19.2, 19.3, 25.3, 25.4, 30.4, 30.5, 61.6, 62.1, 62.1, 65.6, 66.6, 68.9, 72.8, 98.8, 99.0, 110.6, 112.8, 114.7, 124.7, 135.5, 148.9, 149.4, 156.4, 157.1, 163.4. MS (IE): *m/z* 281, 251, 223, 179 (100), 151, 135, 121, 109, 95, 85, 67, 55.

*Ethyl coumarin-3-carboxilates (**5m-r**)*

To a solution of coumarin **5d-l** (0.11 mmol) in methanol (2 mL) was added Amberlyst® 15 (15% m/m) and the resulting mixture was stirred for 12 h at room temperature, then it was filtrated and washed with methanol (1 mL). The solvent was evaporated under reduced pressure and the product was purified by successive recrystallization. The product was dissolved in hot ethyl acetate, than hexane was added.

*Ethyl 7-(3-hydroxypropoxy)-2-oxo-2H-chromene-3-carboxylate (**5m**)*

(30 mg, 90% yield), mp 134-135 °C; IR ν_{max}/cm^{-1}: 3524, 2956, 1757, 1620, 1608; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (t, *J* 7.1 Hz, 3H), 2.01-2.17 (m, 2H), 3.88 (t, *J* 5.9 Hz, 2H), 4.21 (t, *J* 6.0 Hz, 2H), 4.39 (q, *J* 7.1 Hz, 2H), 6.81 (d, *J* 2.4 Hz, 1H), 6.88 (dd, *J* 8.6 and 2.4 Hz, 1H), 7.48 (d, *J* 8.6 Hz, 1H), 8.48 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2, 31.7, 59.2, 61.6, 65.9, 100.9, 111.5, 113.8, 116.6, 130.6, 148.9, 157.1, 157.5, 163.4, 164.5. MS (IE): *m/z* 292 (M^+), 247, 234, 206, 189, 162 (100), 134, 105, 77, 51; Anal. Calc. for $C_{15}H_{16}O_6$: C, 61.64; H, 5.52, Found: C, 61.20; H, 5.17%.

*Ethyl 8-(3-hydroxypropoxy)-2-oxo-2H-chromene-3-carboxylate (**5n**)*

(18 mg, 55% yield), mp 98-99°C; IR n_{max}/cm^{-1}: 3463, 2964, 1758, 1699, 1608, 1572; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (t, *J* 7.1 Hz, 3H), 2.05-2.21 (m, 2H), 2.26 (s, 1H), 3.93 (t, *J* 5.9 Hz, 2H), 4.27 (t, *J* 5.9 Hz, 2H), 4.40 (q, *J* 7.1 Hz, 2H), 7.12-7.30 (m, 3H), 8.46 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 31.8, 59.4, 61.9, 67.0, 117.5, 118.3, 118.5, 120.8, 124.7, 145.0, 146.3, 148.7, 156.3, 163.0; MS (IE): *m/z* 292 (M^+) 247, 234, 206, 188 (100), 162, 134, 105, 77, 51.

*Ethyl 7,8-bis-(3-hydroxypropoxy)-2-oxo-2H-chromene-3-carboxylate (**5o**)*

(33 mg, 80% yield), mp 100-101 °C; IR ν_{max}/cm^{-1}: 3435, 3295, 2953, 2881, 1746, 1609. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.39 (t, *J* 7.1 Hz, 3H), 2.02 (quint, *J* 5.7 Hz, 2H), 2.12 (quint, *J* 5.9 Hz, 2H), 2.92 (s, 2H), 3.88 (t, *J* 5.9 Hz, 2H),

3.94 (t, *J* 5.7 Hz, 2H), 4.27 (t, *J* 5.7 Hz, 2H), 4.29 (t, *J* 5.9 Hz, 2H), 4.39 (q, *J* 7.1 Hz, 2H), 6.94 (d, *J* 8.8 Hz, 1H), 7.30 (d, *J* 8.8 Hz, 1H), 8.44 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2, 31.7, 32.5, 59.4, 59.9, 61.7, 67.0, 72.1, 109.9, 112.7, 114.4, 125.17, 134.8, 149.1, 156.5, 157.0, 163.1. APCI/MS: *m/z* 365 ([M-H]$^-$). Anal. Calc. for $C_{18}H_{22}O_8$: C, 59.01; H, 6.05, Found: C, 59.19; H, 5.84%.

*Ethyl 7-(2-hydroxyethoxy)-2-oxo-2H-chromene-3-carboxylate (**5p**)*

(24 mg, 76% yield), mp 138-139 °C; IR ν_{max}/cm^{-1}: 3476, 2964, 1751, 1739, 1604; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (t, *J* 7.1 Hz, 3H), 2.18 (s, 1H), 4.03 (t, *J* 4.0 Hz, 2H), 4.19 (t, *J* 4.0 Hz, 2H), 4.39 (q, *J* 7.1 Hz, 2H), 6.83 (d, *J* 2.3 Hz, 1H), 6.92 (dd, *J* 8.6 and 2.3 Hz, 1H), 7.51 (d, *J* 8.6 Hz, 1H), 8.48 (s, 1H);. ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2, 60.9, 61.7, 70.1, 101.1, 111.8, 113.8, 114.2, 130.7, 148.8, 157.1, 157.3, 163.3, 164.2; MS (IE): *m/z* 278 (M$^+$) 232, 206, 189, 162 (100), 134, 105, 89, 77, 51.

*Ethyl 8-(2-hydroxyethoxy)-2-oxo-2H-chromene-3-carboxylate (**5q**)*

(20 mg, 47% yield), mp 115-116 °C; IR ν_{max}/cm^{-1}: 3480, 3448, 2945, 1740, 1698; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.41 (t, *J* 7.1 Hz, 3H), 2.74 (s, 1H), 4.03-4.14 (m, 2H), 4.19-4.28 (m, 2H), 4.41 (q, *J* 7.1 Hz, 2H), 7.13-7.32 (m, 3H), 8.48 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2, 61.0, 62.0, 71.0, 117.4, 118.4, 118.5, 121.0, 124.7, 145.0, 146.2, 148.7, 156.2, 163.0; MS (IE): *m/z* 278 (M$^+$), 247, 234, 206, 188 (100), 162, 134, 105, 89, 77, 51.

*Ethyl 7,8-bis-(2-hydroxyethoxy)-2-oxo-2H-chromene-3-carboxylate (**5r**)*

(10 mg, 82% yield), mp 131-132 °C; IR ν_{max}/cm^{-1}: 3325, 3211, 2932, 1748, 1700, 1606; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.30 (t, *J* 7.1 Hz, 3H), 3.70 (t, *J* 5.3 Hz, 2H), 3.78 (t, *J* 4.8 Hz, 2H), 4.09 (t, *J* 5.3 Hz, 2H), 4.19 (t, *J* 4.8 Hz, 2H), 4.28 (q, *J* 7.1 Hz, 2H), 5.74 (s, 2H), 7.18 (d, *J* 8.8 Hz, 1H), 7.63 (d, *J* 8.8 Hz, 1H), 8.69 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 14.0, 59.4, 60.2, 60.9, 70.9, 74.8, 110.7, 112.3, 113.6, 125.7, 134.1, 148.5, 149.3, 155.9, 156.9, 162.7; MS (IE): *m/z* 251, 223, 204 (100), 178, 150, 121, 92, 79, 65, 53.

*Ethyl 8-bromo-7-hydroxy-2-oxo-2H-chromene-3-carboxylate (**6a**)*

To a solution of coumarin **4a** (570 mg, 2.41 mmol) in glacial acetic acid (16 mL) at 60 °C was added slowly a solution of bromine (0.13 mL, 2.65 mmol) in acetic acid (1 mL). The mixture was stirred for 3 h, then the temperature was allowed to rise to room temperature and the precipitate was filtrated, washed with cool water and dried under vacuum giving coumarin **6a** (365 mg, 49% yield). mp 270-271 °C; IR ν_{max}/cm^{-1}: 3282, 1758, 1603, 1552; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.31 (t, *J* 7.1 Hz, 3H), 4.28 (q, *J* 7.1 Hz, 2H), 7.01 (d, *J* 8.6 Hz, 1H), 7.76 (d, *J* 8.6 Hz, 1H), 8.69 (s, 1H), 11.89 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 14.0, 60.8, 96.2, 111.3, 112.6, 113.3, 130.4, 149.2, 153.6, 155.7, 160.6, 162.5; APCI/MS: *m/z* 313 ([M-H]$^-$ + 2), 311 ([M-H]$^-$).

*Ethyl 8-bromo-7-methoxy-2-oxo-2H-chromene-3-carboxylate (**6b**)*

To a solution of coumarin **6a** (92 mg, 0.29 mmol) in dry acetone (2.0 mL), K_2CO_3 (49 mg, 0.35 mmol) and Me_2SO_4 (45 mg, 0.35 mmol) were added. The mixture was stirred under reflux for 5 h. After cooling to room temperature was added a saturated solution of NH_4Cl (2 mL) and the product was extracted with ethyl acetate (4 x 4mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The product was purified by flash chromatography eluting with 10% ethyl acetate in dichloromethane and gave **6b** (58 mg, 60% yield). mp 189-190 °C; IR ν_{max}/cm^{-1}: 1757, 1609, 1543; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40 (t, *J* 7.1 Hz, 3H), 4.03 (s, 3H), 4.38 (q, *J* 7.1 Hz, 2H), 6.93 (d, *J* 8.7 Hz, 1H), 7.55 (d, *J* 8.7 Hz, 1H), 8.46 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.2, 57.0, 61.8, 99.4, 108.5, 112.7, 115.1, 129.5, 148.3, 153.6, 155.9, 161.2, 162.9; MS (IE): *m/z* 328 (M$^+$ + 2), 326 (M$^+$), 282, 280, 254, 256, 228, 226,199, 197, 147, 103, 88, 75 (100), 53; Anal. Calc. for $C_{13}H_{11}BrO_5$: C, 47.73; H, 3.39, Found: C, 48.05; H, 3.63%.

*Methyl 8-bromo-7-methoxy-2-oxo-2H-chromene-3-carboxylate (**6c**)*

To a solution of coumarin **2a** (70 mg, 0.34 mmol) in glacial acetic acid (2.3 mL) at 60 °C was added slowly a solution of bromine (0.16 mL, 0.37 mmol) in acetic acid (0.15 mL) and the mixture was stirred for 3 h. The heating and the stirring were removed; the precipitate was filtrated, washed with cool water and dried under vacuum. The crude product **2b** (50 mg) was dissolved in dry acetone (1.5 mL) and K_2CO_3 (145 mg, 1.05 mmol) and Me_2SO_4 (132 mg, 1.05 mmol) were added. The mixture was stirred under reflux for 5 h. After cooling to room temperature was added a saturated solution of NH_4Cl (4 mL) and the product was extracted with ethyl acetate (4 x 4mL). The organic layer was dried

over Na_2SO_4 and the solvent was removed under reduced pressure. The product was purified by flash chromatography eluting with 10% ethyl acetate in dichloromethane, giving **6c** (24 mg, 45% yield). IR ν_{max}/cm^{-1}: 1753, 1710, 1612 971, 804; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.83 (s, 3H), 4.02 (s, 3H), 7.25 (d, *J* 8.8 Hz, 1H), 7.96 (d, *J* 8.8 Hz, 1H), 8.78 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 52.7, 57,0, 99.4, 108.6, 112.7, 114.7, 129.7, 148.9, 153.6, 155.8, 161.3, 163.6; MS (IE): *m/z* 314 (M^+ + 2), 312 (M^+), 282, 280, 256, 254, 197, 195, 184, 182, 147, 103, 75 (100), 59.

*Coumarin-3-carboxylates (**7a-i**)*

To a solution of coumarin **4b**, **4d**, **4e** or **6b** (0.12 mmol) in toluene (0.5 mL) was added ethylene glycol, 1,5-pentanediol or *iso*-butyl alcohol (1.44 mmol) and PTSA (20% m/m). The reaction mixture was heated at 50 °C and stirred for 12 h. After cooling to room temperature the mixture was poured into water (2 mL) and the organic layer was extracted with ethyl acetate (3 x 4 mL). The combined organic layers were washed with saturated solution of NaCl (4 mL), dried over Na_2SO_4 and evaporated under reduced pressure.

*2-Hydroxyethyl 8-hydroxy-2-oxo-2H-chromene-3-carboxylate (**7a**)*

(21 mg, 65% yield), mp 199-200 °C; IR ν_{max}/cm^{-1}: 3532, 3453, 3103, 1746, 1609; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.70 (dd, *J* 5.5 and 4.9 Hz, 2H), 4.26 (t, *J* 4.9 Hz, 2H), 4.93 (t, *J* 5.5 Hz, 1H), 7.17-7.26 (m, 2H), 7.26-7.37 (m, 1H), 8.74 (s, 1H), 10.40 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 59.1, 67.0, 117.5, 118.8, 120.2, 120.8, 125.0, 143.4, 144.6, 149.3, 156.1, 162.7; MS (IE): *m/z* 250 (M^+), 220, 189 (100), 162, 134, 105, 77, 51.

*2-Hydroxyethyl 7-methoxy-2-oxo-2H-chromene-3-carboxylate (**7b**)*

(15 mg, 50% yield), mp 172-173 °C; IR ν_{max}/cm^{-1}: 3309, 1740, 1618, 1560; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.69 (t, *J* 5.2 Hz, 2H), 3.90 (s, 3H), 4.24 (t, *J* 5.2 Hz, 2H), 5.74 (s, 1H), 6.50-7.10 (m, 2H), 7.82 (d, *J* 9.0 Hz, 1H), 8.76 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 56.5, 59.2, 66.9, 100.6, 111.6, 113.4, 113.7, 131.9, 149.6, 156.6, 157.3, 162.9, 165.1; MS (IE): *m/z* 264 (M^+), 234, 203 (100), 176, 148, 133, 119, 76, 50.

*2-Hydroxyethyl 2-oxo-2H-chromene-3-carboxylate (**7c**)*

(16 mg, 59% yield), mp 135-136 °C; IR ν_{max}/cm^{-1}: 3505, 3304, 1752, 1609; ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.92-3.99 (m, 2H), 4.46-4.53 (m, 2H), 7.32-7.43 (m, 2H), 7.61-7.73 (m, 2H), 8.57 (s, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 60.7, 67.5, 116.8, 117.8, 125.0, 129.6, 134.5, 149.2, 155.1; MS (IE): *m/z* 204, 173 (100), 146, 118, 101, 89, 63.

*5-Hydroxypentyl 8-hydroxy-2-oxo-2H-chromene-3-carboxylate (**7d**)*

(18 mg, 53% yield), mp 121-123 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.35-1.55 (m, 4H), 1.62-1.68 (m, 2H), 2.08 (s, 1H), 4.24 (t, *J* 6.5 Hz, 2H), 4.40 (t, *J* 5.0 Hz, 1H), 7.15-7.27 (m, 2H), 7.28-7.40 (m, 1H), 8.68 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d_6) δ 21.8, 27.8, 31.9, 60.4, 65.0, 118.6, 120.0, 120.4, 124.7, 144.3, 144.7, 148.9; MS (IE): *m/z* 278, 247, 234, 188 (100), 162, 134, 105, 89, 77, 51.

*5-Hydroxypentyl 7-methoxy-2-oxo-2H-chromene-3-carboxylate (**7e**)*

(24 mg, 65% yield), mp 81-82 °C; IR ν_{max}/cm^{-1}: 3497, 2940, 2857, 1762; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.44-1.91 (m, 6H), 3.68 (t, *J* 6.3 Hz, 2H), 4.35 (t, *J* 6.5 Hz, 2H), 6.81 (d, *J* 2.3 Hz, 1H), 6.89 (dd, *J* 8.8 and 2.3 Hz, 1H), 7.51 (d, *J* 8.6 Hz, 1H), 8.50 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.2, 28.3, 32.2, 56.0, 62.6, 65.6, 100.3, 111.6, 113.6, 130.7, 149.0, 157.6, 163.6, 165.2; MS (IE): *m/z* 306 (M^+), 276, 220, 203 (100), 176, 148, 133, 119, 76, 55.

*5-Hydroxypentyl 2-oxo-2H-chromene-3-carboxylate (**7f**)*

(17 mg, 52% yield), mp 82-84 °C; IR ν_{max}/cm^{-1}: 3397, 2948, 2863, 1777, 1761; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.40-1.73 (m, 4H), 1.74-1.90 (m, 2H), 3.69 (t, *J* 6.1 Hz, 2H), 4.37 (t, *J* 6.5 Hz, 2H), 7.25-7.42 (m, 2H), 7.57-7.72 (m, 2H), 8.52 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.2, 28.3, 32.1, 62.6, 65.9, 116.7, 117.8, 118.4, 124.8, 129.5, 134.3, 148.6, 155.2, 163.0; MS (IE): *m/z* 246, 218, 191, 173 (100), 146, 118, 101, 89, 63; Anal. Calc. for $C_{15}H_{16}O_5$: C, 65.21; H, 5.84, Found: C, 64.46; H, 5.55%.

*Isobutyl 7-methoxy-2-oxo-2H-chromene-3-carboxylate (**7g**)*

(16 mg, 50% yield), mp 110-111 °C; IR ν_{max}/cm^{-1}: 2958, 2886, 1755, 1688; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.03 (d, *J* 6.7 Hz, 6H), 1.99-2.19 (m, 1H), 3.91 (s, 3H), 4.12 (d, *J* 6.7 Hz, 1H), 6.81 (d, *J* 2.3 Hz, 1H), 6.89 (dd, *J* 8.7 and 2.3 Hz, 1H), 7.50 (d, *J* 8.7 Hz, 1H), 8.48 (s, 1H); MS (IE): 276 (M^+), 220, 203 (100), 176, 148, 119, 76, 50.

*Isobutyl 8-bromo-7-methoxy-2-oxo-2H-chromene-3-carboxylate (**7h**)*

(17 mg, 40% yield), mp 167-168 °C; IR ν_{max}/cm^{-1}: 1749, 1611, 1543; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.04 (d, *J* 6.7 Hz, 6H), 2.00-2.20 (m, 1H), 4.05 (s, 3H), 4.13 (d, *J* 6.6 Hz, 1H), 6.94 (d, *J* 8.8 Hz, 1H), 7.56 (d, *J* 8.8 Hz, 1H), 8.46 (s, 1H); MS (IE): *m/z* 355 (M^++1), 353 (M^+-1), 299, 297, 282, 280 (100), 255, 253, 198, 196, 103, 88, 75, 57; Anal. Calc. for $C_{15}H_{15}BrO_5$: C, 50.72; H, 4.26, Found: C, 50.42; H, 3.93%.

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