Synthesis and Fluorescent Properties of some Heterobifunctional and Rigidized 7-Aminocoumarins

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Heterobifunctional fluorescent reagents of coumarin type are synthesized. They possess, in position 7, a rigidized or un-rigidized amino group and, in position 3 or 4, a carboxylic function. The fluorescence characteristics of these compounds are described and compared with the 7-amino-4-methylcoumarin. The influence of the relative freedom of rotation of the amino group or the position of the acid function on the fluorescence properties are also studied.

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Studies of organic fluorescence reagents have been undertaken to develop a series of fluorescence reagents using 7-amino-4-methylcoumarin (AMC) (**1**) as the key fluorophore. Thus several AMC amides of appropriate aminoacid derivatives have been successfully employed as optical brighteners for laboratory [1] and clinical testing of corresponding proteolytic enzymes: *e.g.*, leucine [2], trypsine [3], papaïn and cystine aminopeptidase [4], γ-glutamyltranspeptidase [5].

In the course of studying various biological systems it has become clear that the use of a conventional homobifunctional reagent is limited due to several inherent problems: random collisional cross-linking, long reaction times, difficulty in controlling the reaction and non selective cross-linking [6]. In the studies of biological systems [7], it is more desirable to use "heterobifunctional" reagents bearing two different reactive groups which are sufficiently different to permit well-controlled sequential reactions of each group in turn [8].

As described before [9], an electron-releasing substituent, like an amino group, in position 7 on the coumarin ring should induce large Stokes shifts between absorbance and fluorescence maxima.

In view of the above observations, we decided to prepare heterobifunctional coumarins which have, in position 7, an amino group capable of reacting with carboxylic ends of little peptides like enzymes substrates, and a carboxylic group capable of forming bridges between side chains of polymers or lateral chains of amino acids in carrier proteins. For studying the influence of the position of the carboxylic function on the wavelength shift of the derivatives, we decided to obtain heterobifunctional coumarins bearing the carboxylic function in position 3 or in position 4.

A common requirement for an ideal fluorophore is that the fluorogenic group should have a fluorescence emission maximum at longer wavelengths than 480 nm, in order to bypass the background fluorescence of many biological materials, which is "blue" (430-470 nm, like 7-aminocoumarin) in appearance. However it was reported that coumarins with rigidized 7-amino group would be expected to show a bathochromic shift, based on the values of the parent compounds [10,11]. We think that it could be interesting to synthesize coumarin derivatives with rigidized 7-amino group and a carboxylic function in position 4 and compare their fluorescence properties with rotating 7-amino group parents.

This paper describes the synthesis of several coumarin derivatives and the effects of the substituents on their fluorescence maxima and fluorescence quantum yields. The spectral properties of these derivatives were compared with the properties of the 7-amino-4-methylcoumarin (**1**) which is frequently used in fluorescent reagents as an optical brightener [10].

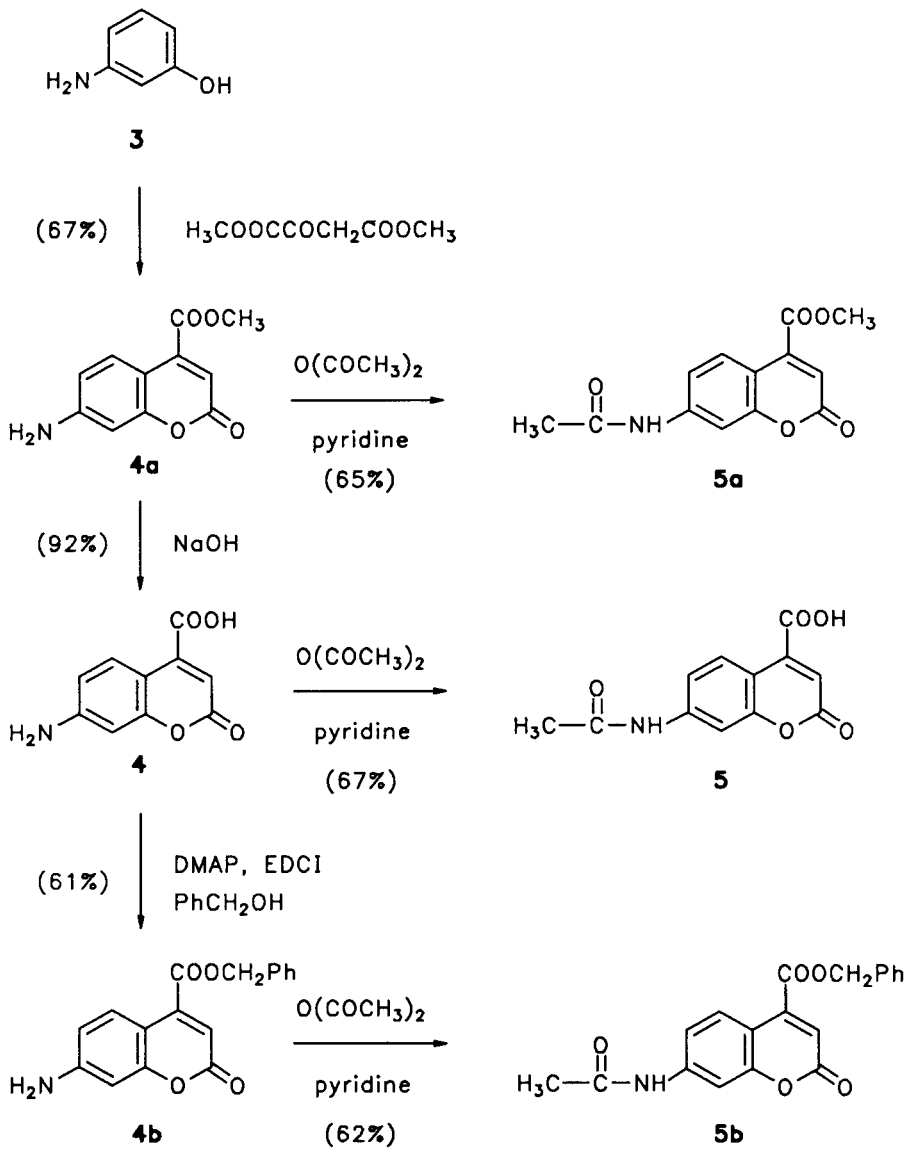
Results and Discussion.

7-Amino-4-carboxylic Acid Derivatives.

In the strategy developed for these derivatives, the synthesis of the methyl ester of 7-aminocoumarin-4-carboxylic acid (**4a**) appears to be easily obtained by the reaction of Von Pechmann [12] with 3-aminophenol (**3**) and dimethyloxalacetate (prepared from dimethylacetylene dicarboxylate using the Sucrow *et al* method [13]). In comparison with the synthesis described by Kanaoka [8] after which the ester crystallizes by trituration in ethyl ether, our method yielded after solubilization in a minimum of ethanol and coprecipitation with methylene chloride, the methyl ester in a very satisfying yield (67% instead of 27%). In a second time, it was treated in the presence of potassium hydroxide and resulted in a very good yield (92%) of the corresponding aminoacid coumarin **4**. The amine function was transformed into an acetamido group with good yields (65%) using acetic anhydride in pyridine.

In order to synthesize directly the benzyl ester of **4**, we tried to obtain dibenzyl oxalacetate by the same previous method from dibenzyl acetylenedicarboxylate. Unfortunately this compound was not easily available, and moreover the synthesis of the dibenzyl oxalacetate using the

Scheme 1



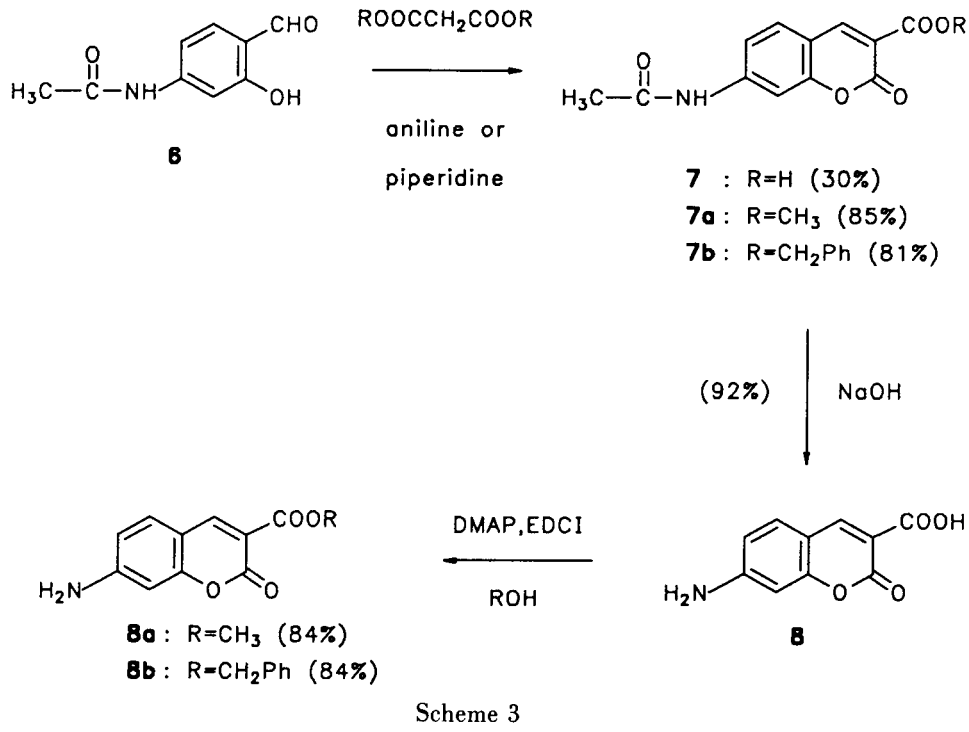
Sucrow method failed. Considering these findings we decided to protect the acid function by a convenient method using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride and 4-dimethylaminopyridine in the presence of benzyl alcohol as describes for some amino-acids [14].

7-Amino-3-carboxylic Acid Derivatives.

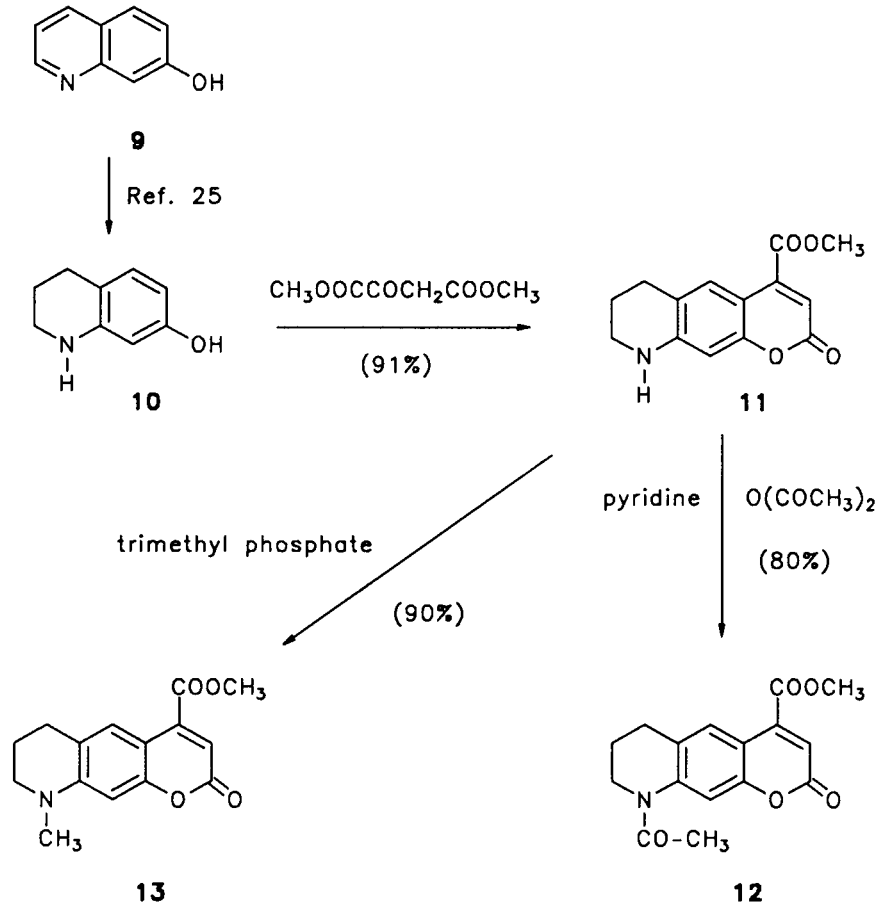
The methyl ester **7a** and the benzyl ester **7b** were prepared with good yields (85% and 81%) by the Knoevenagel condensation method [15] using respectively dimethyl and dibenzyl malonate and 4-acetamidosalicylaldehyde (**6**) in the presence of piperidine [16]. The aldehyde **6** was prepared by the Giesecke *et al* method [17] in which 3-acetamidophenol was treated with 1,1',3,3'-tetraphenyl-2,2'-biimidazolidinylidene. As previously described, we should protect the amine function of the 3-aminophenol, because the direct synthesis of the 4-aminosalicylaldehyde does not occur. The 7-acetamidocoumarin-3-carboxylic acid (**7**) was synthesized in a 30% yield by a method using malonic acid in aniline, instead of piperidine, as it was described for some other 3-carboxylic acid coumarins [15].

The heating of the *N*-protected methyl ester **5a** with 10 equivalents of sodium hydroxide provided 7-aminocoumarin-3-carboxylic acid (**8**) (92%) which can react with methanol or benzyl alcohol in the presence of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride and 4-dimethylaminopyridine, to give respectively the 3-methyl ester **8a** and the 3-benzyl ester **8b** of the free amino derivatives with the same yield (84%).

Scheme 2



Scheme 3



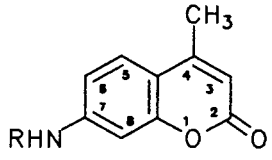
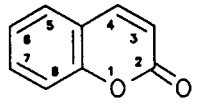
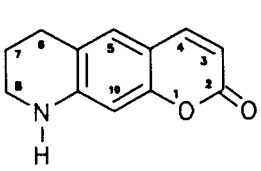


Figure 1: **1** R = H:7-amino-4-methylcoumarin (AMC)
2 R = $COCH_3$:7-acetamido-4-methylcoumarin





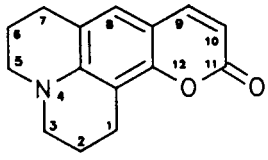


Figure 2: Chemical Abstract Numbering of coumarins

Table : Spectral data of the compounds (in ethanol)

Compound	UV max (nm)	log ε	fluorescence max (nm)	Quantum yield [a]
1 [b]	354	4.07	435	0.88 [c]
2 [d]	328	3.66	385	0.23
4	388	3.71	494	0.40
4a	383	3.84	540	0.04
4b	392	3.78	541	0.025
5	333	3.83	427	<0.01
5a	348	3.94	472	0.52
5b	348	3.76	474	0.56
7	350	4.18	410	<0.01
7a	355	3.52	420	1.26
7b	356	3.85	421	1.00
8	404	4.03	445	0.195
8a	399	4.03	442	0.58
8b	401	4.21	442	0.97
11	420	3.89	551	<0.01
12	353	3.86	500	0.10
13	422	3.48	560	<0.01
15	434	3.96	575	<0.01

[a] calculated by the method described in [20] and [21], based on a value of 0.55 for quinine sulfate [22].

[b] synthesized as describe in [10].

[c] In accordance with the values given in references [23] and [24].

[d] obtained by treatment of 1 with acetic anhydride in presence of pyridine.

Rigidized 7-Amino Group Derivatives.

The free rotation of the 7-amino group can be restricted by insertion of the N atom in a non-aromatic cycle as exemplified by compound **10**. In the synthesis of these compounds, we used the same Von Pechmann method as for the 4-carboxylic acid derivatives. The reaction occurs without solvent and dimethyloxalacetate was condensed with **10** to obtain a secondary amino group which can be transformed in an amido group using acetic anhydride in the presence of pyridine (80%).

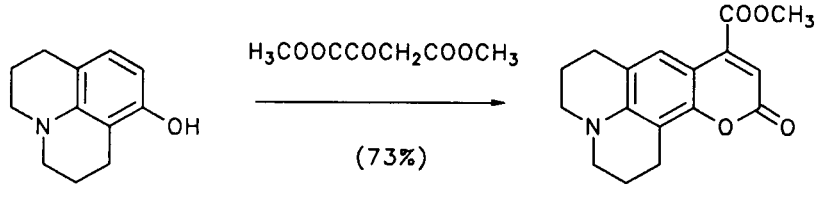
For the methylation of the functional amino group of the 1,2,3,4-tetrahydroquinoline, we used a convenient procedure developed for similar compounds where classical procedures failed [10]. A simple heating of the precursor in the presence of trimethyl phosphate in excess gives a very good yield of the desired product (90%).

A good yield (74%) of a strongly rigidized amino group is obtained by the reaction of 8-hydroxyjulolidine (**14**) with dimethyloxalacetate. In the compound obtained, the totally substituted amino group is part of a quinolizidine system which constitutes a strong inversion barrier for the nitrogen.

Fluorescent Properties.

The 7-aminocoumarin-3-carboxylic acid derivatives (compounds **7** and **8**) have a relatively good quantum yield, but a bathochromic effect of 50 nm was observed in the absorption and emission spectra, and the overlap between the absorption spectra and the emission spectra is large.

Scheme 4



14 **15**

In contrast, the 7-aminocoumarin-4-carboxylic acid derivatives (compounds **4** and **5**) have an excellent bathochromic effect and a very small overlapping of the absorption and emission spectra. They show a red shift of 70 nm with the fluorescence emission maximum of the AMC, but their low quantum yield shows that the position of the carboxylic function could influence the fluorescence wavelengths and quantum yields.

As expected compounds **11, 12, 13** and **15** show fluorescence emission maximum at longer wavelengths than 480 nm and a very small overlapping between the absorption and emission spectra, but their quantum yields fall below 1%.

The heterobifunctional amine **11** and its amide **12** have a large difference (70 and 50 nm) between their absorption and emission spectra but the most interesting is that their emission show a very large red shift (maximum wavelength near 500 nm). Their lasing properties can be compared with the properties of the benzyl 3-(3-amino-9-carbazolyl)-propionate, an heterobifunctional "blue" probe used for the detection of enzymes [18].

As described for their 4-methyl coumarin parents [10, 11], rigidized 7-amino group induce a bypass of the common "blue" fluorescence but as observed for the 4-carboxylic acid derivatives compared with AMC, the presence of carboxylic function directly on the coumarin ring, results in a sharp fall in the quantum yields.

In order to complete our study about the influence of the carboxylic function on the coumarin fluorescence, we observed that the presence of one carbon between the coumarin ring and the carboxylic function is sufficient to dramaticallyl reduce the influence of the acid function thus inducing good lasing properties of this compound. This was showed for the alkyl or aralkyl 7-aminocoumarin-4-acetate which presents fluorescence properties which are nearly the same as the 7-amino-4-methylcoumarin (AMC) [8,19].

Some of these dyes are going to be used for studying some cellular and biological systems.

EXPERIMENTAL

Melting points are uncorrected. The ir spectra were obtained using a Perkin-Elmer 196 and the ^{1}H nmr spectra were recorded at 300 MHz on a Bruker AM 300WB spectrometer. Chemical shifts are reported in parts per million downfield from tetramethylsilane (δ units). Analytical thin layer chromatography (tlc) was performed on Merck 60F-254 silica gel plates. Preparative column chromatography was performed using Merck silica gel 60F (70-230 mesh). Mass spectra were measured on a Nermag R-10-10C spectrometer, absorption spectra were run on a Hitachi 2000 spectrometer and fluorescence spectra at room temperature on a Shimadzu RF-500 fluorimeter.

Methyl 7-Aminocoumarin-4-carboxylate (**4a**).

A mixture of dimethyl oxalacetate [13] (5 g, 31.2 mmoles) and 3-aminophenol (**3**) (3 g, 27.5 mmoles) was stirred in an oil bath at 120° for 2 hours. The hot syrup obtained was solubilized with a minimum of ethanol. After cooling, methylene chloride was added on the solution to give a voluminous precipitate which was collected by filtration washed and dried to provide 4.51 g (67%) of **4a** as orange cubes, mp 190° dec; ir (potassium bromide): 3440, 3340, 3240 (NH_2), 1690 (C=O); ^{1}H nmr (DMSO-d_6): 3.89 (s, CH_3, 3H), 6.28 (s, H_3, 1H), 6.32 (s, NH_2, 2H), 6.43 (d, $J_{6,8}$ = 2.0 Hz, H_8 1H), 6.56 (dd, $J_{5,6}$ = 8.7 Hz and $J_{6,8}$ = 2.0 Hz, H_6, 1H), 7.65 (d, $J_{5,6}$ = 8.7 Hz, H_5, 1H); ms: m/e 220 (M^+).

Anal. Calcd. for $C_{11}H_9NO_4$: C, 60.28; H, 4.14; N, 6.39. Found: C, 60.33; H, 4.12; N, 6.42.

7-Aminocoumarin-4-carboxylic Acid (**4**).

A solution of **4a** (2.5 g, 11.4 mmoles) in ethanol (50 ml) was treated with 2*N* potassium hydroxide (5 ml). The mixture was refluxed for 3 hours, then allowed to cool and water was added. The *p*H of the resulting solution was adjusted to 2-3 with hydrochloric acid until the 7-aminocoumarin-4-carboxylic acid had precipitated in a voluminous brown precipitate. The water was evaporated to dryness to give a brown mass which was solubilized with hot ethanol. After cooling the precipitated salt was eliminated by filtration, the solvent was removed *in vacuo* and the product purified by column chromatography with methanol/methylene chloride as the eluent, to give red cubes which was dried to provide 1.28 g (92%) of **4**, mp 250°; ir (potassium bromide): 3400-2500 (NH_2 and COOH), 1680 (C=O); ^{1}H nmr (DMSO-d_6): 6.27 (s, H_3, 1H), 6.44 (d, $J_{6,8}$ = 1.8 Hz, H_8, 1H), 6.58 (dd, $J_{5,6}$ = 8.9 Hz and $J_{6,8}$ = 1.8 Hz, H_6, 1H), 7.73 (d, $J_{5,6}$ = 8.9 Hz, H_5, 1H); ms: m/e 206 (M^+).

Anal. Calcd. for $C_{10}H_7NO_4$: C, 58.54; H, 3.44; N, 6.82. Found: C, 58.50; H, 3.40; N, 6.75.

Benzyl 7-Aminocoumarin-4-carboxylate (**4b**).

An emulsion of **4** (0.5 g, 2.44 mmoles), 4-dimethylaminopyridine (DMAP, 0.15 g, 1.22 mmoles) and benzyl alcohol (0.3 ml, 2.7 mmoles) in 7 ml of methylene chloride was cooled by stirring in an ice bath. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCI, 0.52 g, 2.7 mmoles) was added and the reaction mixture was stirred at 0° for 2 hours and at room temperature for 48 hours. The grey precipitate formed was eliminated by filtration and the solution was concentrated to dryness *in vacuo*. The resulting solid was triturated in ethyl ether to give orange-yellow cubes which was filtered, washed and dried to provide 0.44 g (61%) of **4b**, mp 120°; ir (potassium bromide): 3440, 3340, 3220 (NH_2), 1690 (C=O); ^{1}H nmr (DMSO-d_6): 5.37 (s, CH_2, 2H), 6.32 (s, H_3, 1H), 6.36 (s, NH_2, 2H), 6.45 (d, $J_{6,8}$ = 2.0 Hz, H_8, 1H), 6.55 (dd, $J_{5,6}$ = 8.3 Hz and $J_{6,8}$ = 2.0 Hz, H_6, 1H), 7.35-7.57 (m, ArH, 5H), 7.67 (d, $J_{5,6}$ = 8.3 Hz, H_5, 1H); ms: m/e 296 (M^+).

Anal. Calcd. for $C_{17}H_{13}NO_4$: C, 69.14; H, 4.43; N, 4.74. Found: C, 69.29; H, 4.54; N, 4.70.

7-Acetamidocoumarin-4-carboxylic Acid (**5**).

A solution of **4** (0.1 g, 0.49 mmole) and acetic anhydride (2.5 ml, 9.8 mmoles) in 5 ml of pyridine was stirred overnight at room temperature. The precipitate obtained was collected by filtration, washed twice with ethyl ether and dried to provide 0.08 g (67%) of **5** as pale yellow cubes, mp 180°; ir (potassium bromide): 3340

(NH), 1690 (C=O); ^{1}H nmr (DMSO-d_6): 2.1 (s, $COCH_3$, 3H), 6.63 (s, H_3, 1H), 7.43 (dd, $J_{6,8}$ = 2.0 Hz and $J_{5,6}$ = 9.9 Hz, H_6, 1H), 7.83 (d, $J_{6,8}$ = 2.0 Hz, H_8, 1H), 8.1 (d, $J_{5,6}$ = 9.9 Hz, H_5, 1H), 10.45 (s, NH, 1H); ms: m/e 248 (M^+).

Anal. Calcd. for $C_{12}H_9NO_5$: C, 58.30; H, 3.67; N, 5.66. Found: C, 58.36; H, 3.70; N, 5.58.

Methyl 7-Acetamidocoumarin-4-carboxylate (**5a**).

A solution of **4** (0.4 g, 1.83 mmoles) and acetic anhydride (2.5 ml, 36.6 mmoles) in 5 ml of pyridine was stirred overnight at room temperature. To the orange solution obtained, 2 ml of methanol was added at 0° to hydrolyse the excess of acetic anhydride. After 15 minutes of stirring, ethyl ether was added and the organic solution washed twice with water and dried over magnesium sulfate. The solvent was removed by evaporation *in vacuo* and 0.3 g (65%) of **5a** was obtained as yellow cubes, mp 245°; ir (potassium bromide): 3320 (NH), 1690 (C=O); ^{1}H nmr (DMSO-d_6): 2.1 (s, $COCH_3$, 3H), 3.92 (s, $COOCH_3$, 3H), 6.74 (s, H_3, 1H), 7.45 (dd, $J_{6,8}$ = 1.4 Hz and $J_{5,6}$ = 9.2 Hz, H_6, 1H), 7.73 (d, $J_{6,8}$ = 1.4 Hz, H_8, 1H), 8.1 (d, $J_{5,6}$ = 9.2 Hz, H_5, 1H), 10.45 (s, NH, 1H); ms: m/e 262 (M^+).

Anal. Calcd. for $C_{13}H_{11}NO_5$: C, 59.77; H, 4.24; N, 5.36. Found: C, 59.86; H, 4.37; N, 5.37.

Benzyl 7-Acetamidocoumarin-4-carboxylate (**5b**).

As described for **5**, **4b** (0.4 g, 1.35 mmoles) and acetic anhydride (2.5 ml, 36.6 mmoles) were treated in 5 ml of pyridine. The product crystallized after evaporation of the organic phase to give 0.29 g (62%) of **5b** as yellow cubes, mp 195°; ir (potassium bromide): 3320 (NH), 1690 (C=O); ^{1}H nmr (DMSO-d_6): 2.1 (s, $COCH_3$, 3H), 5.42 (s, CH_2, 2H), 6.74 (s, H_3, 1H), 7.36-7.53 (m, H_6 + ArH, 6H), 7.73 (d, $J_{6,8}$ = 1.2 Hz, H_8, 1H), 8.1 (d, $J_{5,6}$ = 8.9 Hz, H_5, 1H), 10.45 (s, NH, 1H); ms: m/e 338 (M^+).

Anal. Calcd. for $C_{19}H_{15}NO_5$: C, 67.65; H, 4.48; N, 4.15. Found: C, 67.74; H, 4.53; N, 4.09.

Methyl 7-Acetamidocoumarin-3-carboxylate (**7a**).

To a mixture of 4-acetamidosalicaldehyde (**6**) (0.3 g, 1.67 mmoles) and dimethyl malonate (0.21 ml, 1.83 mmoles) in methanol (10 ml), 0.1 ml of piperidine was added. The solution was heated at 90-100° for 1 hour and the product crystallized readily as the solution cooled. The crystalline product was collected by filtration, washed with methanol and dried to give 0.472 g (86%) of **7a** as pale yellow cubes, mp 265° dec; ir (potassium bromide): 3320 (NH), 1740-1700 (C=O); ^{1}H nmr (DMSO-d_6): 2.1 (s, $COCH_3$, 3H), 3.82 (s, $COOCH_3$, 3H), 7.43 (dd, $J_{6,8}$ = 1.6 Hz and $J_{5,6}$ = 9.0 Hz, H_6, 1H), 7.78 (d, $J_{6,8}$ = 1.6 Hz, H_8, 1H), 7.93 (d, $J_{5,6}$ = 9.0 Hz. H_5, 1H), 8.68 (s, H_4, 1H), 10.52 (s, NH, 1H); ms: m/e 262 (M^+).

Anal. Calcd. for $C_{13}H_{11}NO_5$: C, 59.77; H, 4.24; N, 5.36. Found: C, 59.69; H, 4.31; N, 5.42.

Benzyl 7-Acetamidocoumarin-3-carboxylate (**7b**).

A solution of 4-acetamidosalicaldehyde (**6**) (0.3 g, 1.67 mmoles) with dibenzyl malonate (0.46 ml, 1.83 mmoles) and 0.1 ml of piperidine in propionitrile was stirred at 90-100° for 1 hour. After cooling, the yellow precipitate formed was collected by filtration, washed and dried to provide 0.455 g (81%) of **7b**, mp 240°; ir (potassium bromide): 3320 (NH), 1740-1700 (C=O); ^{1}H nmr (DMSO-d_6): 2.1 (s, $COCH_3$, 3H), 5.3 (s, CH_2, 2H), 7.32-7.5 (m, H_6 + ArH, 6H), 7.78 (d, $J_{6,8}$ = 1.6 Hz, H_8, 1H), 7.84 (d, $J_{5,6}$ = 8.7 Hz, H_5, 1H), 8.71 (s, H_4, 1H), 10.56 (s, NH, 1H); ms: m/e 338 (M^+).

Anal. Calcd. for $C_{19}H_{15}NO_5$: C, 67.65; H, 4.48; N, 4.15. Found: C, 67.72; H, 4.43; N, 4.18.

7-Acetamido-3-carboxylic Acid (**7**).

A mixture of 4-acetamidosalicaldehyde (**6**) (1 g, 5.58 mmoles) malonic acid (0.581 g, 5.58 mmoles) and 0.1 ml of aniline in ethanol (20 ml) was heated and stirred 3 hours at 70-80°. Ethanol was evaporated *in vacuo* and the yellow-grey mass obtained was washed 3 times with 150 ml of water. The cubes filtered were taken in water (50 ml) with 1 ml of concentrated hydrochloric acid. The emulsion was heated 2 hours at 70° and the precipitate obtained was filtered, washed and dried to give 0.4 g (30%) of **7**, mp 310-320°; ir (potassium bromide): 3490 (NH), 1720 (C=O); ^{1}H nmr (DMSO-d_6): 2.1 (s, $COCH_3$, 3H), 7.45 (dd, $J_{6,8}$ = 2.0 Hz and $J_{5,6}$ = 8.9 Hz, H_6, 1H), 7.78 (d, $J_{6,8}$ = 2.0 Hz, H_8, 1H), 7.83 (d, $J_{5,6}$ = 8.9 Hz, H_5, 1H), 8.67 (s, H_4, 1H), 10.5 (s, NH, 1H); ms: m/e 248 (M^+).

Anal. Calcd. for $C_{12}H_9NO_5$: C, 58.30; H, 3.67; N, 5.66. Found: C, 58.35; H, 3.64; N, 5.72.

7-Aminocoumarin-3-carboxylic Acid (**8**).

A mixture of **7a** (0.45 g, 2.15 mmoles) and sodium hydroxide (0.86 g, 21.5 mmoles) in 10 ml of water was heated under reflux for 3 hours. Water was evaporated and the yellow mass obtained was taken with methanol. A white precipitate was removed by filtration and the solution concentrated to dryness. The syrup was solubilized with water and the *p*H of the solution was adjusted to 3 with a solution of sulfuric acid (20%). The orange precipitate formed was collected, washed and dried to provide 0.41 g (93%) of **8** as orange cubes, mp 340-345°; ir (potassium bromide): 3420, 3320, 3220 (NH_2), 1730 (C=O); ^{1}H nmr (DMSO-d_6): 6.41 (s, NH_2, 2H), 6.59 (dd, $J_{6,8}$ = 1.7 Hz and $J_{5,6}$ = 8.9 Hz, H_6, 1H), 6.83 (d, $J_{6,8}$ = 1.7 Hz, H_8, 1H), 7.52 (d, $J_{5,6}$ = 8.9 Hz, H_5, 1H), 8.53 (s, H_4, 1H); ms: m/e 206 (M^+).

Anal. Calcd. for $C_{10}H_7NO_4$: C, 58.54; H, 3.44; N, 6.82. Found: C, 58.60; H, 3.57; N, 6.67.

Methyl 7-Aminocoumarin-3-carboxylate (**8a**).

An emulsion of **8** (0.1 g, 0.5 mmole), DMAP (0.03 g, 0.25 mmole) and methanol (0.13 ml, 0.55 mmole) in propionitrile (15 ml) was cooled by stirring in an ice bath. EDCI (0.105 g, 0.55 mmole) was added and the mixture was stirred at 0° for 2 hours and 48 hours at room temperature. The solvent was removed by evaporation *in vacuo* and the product was purified by column chromatography with methylene chloride-methanol (19:1) as the eluent. The solvent was removed by evaporation to give 0.09 g (84%) of **8a** as orange cubes, mp 254°; ir (potassium bromide): 3420, 3320, 3220 (NH_2), 1730 (C=O); ^{1}H nmr (DMSO-d_6): 3.74 (s, CH_3, 3H), 6.36 (d, $J_{6,8}$ = 2.0 Hz, H_8, 1H), 6.58 (dd, $J_{5,6}$ = 8.7 Hz and $J_{6,8}$ = 2.0 Hz, H_6, 1H), 6.79 (s, NH_2, 2H), 7.52 (d, $J_{6,5}$ = 8.7 Hz, H_5, 1H), 8.52 (s, H_4, 1H); ms: m/e 220 (M^+).

Anal. Calcd. for $C_{11}H_9NO_4$: C, 60.28; H, 4.14; N, 6.39. Found: C, 60.20; H, 4.10; N, 6.45.

Benzyl 7-Aminocoumarin-3-carboxylate (**8b**).

As describes for the methyl ester, **8** (0.1 g, 0.5 mmole) was treated with DMAP (0.03 g, 0.25 mmole), benzyl alcohol (0.057 ml, 0.55 mmole) and EDCI (0.105 g, 0.55 mmole) in methylene chloride. The product was purified by column chromatography with methylene chloride-methanol (19:1) as the eluent. The sol-

vent was evaporated and the mass dried to provide 0.12 g (84%) of **8b** as orange cubes, mp 232°; ir (potassium bromide): 3420, 3320, 3220 (NH_2), 1730 (C=O); ^1H nmr (DMSO-d_6): 5.26 (s, CH_2, 2H), 6.39 (d, $J_{6,8}$ = 1.8 Hz, H_8, 1H), 6.58 (dd, $J_{5,6}$ = 8.6 Hz and $J_{6,8}$ = 1.8 Hz, H_6, 1H), 6.84 (s, NH_2, 2H), 7.32-7.48 (m, ArH, 5H), 7.55 (d, $J_{5,6}$ = 8.6 Hz, H_5, 1H), 8.56 (s, H_4, 1H); ms: m/e 296 (M^+).

Anal. Calcd. for $C_{17}H_{13}NO_4$: C, 69.14; H, 4.43; N, 4.74. Found: C, 69.10; H, 4.50; N, 4.78.

7-Hydroxyquinoline (**9**).

This compound was prepared from aminophenol (**3**) *via* the Skraup quinoline synthesis using the Bradford *et al.* procedure [25].

Methyl 2-Keto-6,7,8,9-tetrahydro-2*H*-pyrano[3,2-*g*]quinoline-4-carboxylate (**11**).

A mixture of the compound **10** (0.1 g, 0.67 mmole, synthesized by hydrogenation of **9** using the Atkins *et al* procedure [10]) and dimethyloxalacetate (0.11 g, 0.67 mmole) was stirred in an oil bath at 90°, and the hot syrup obtained was solubilized with a minimum of hot isopropanol. After cooling, the crystalline yellow product was filtered, washed and dried to give 0.16 g of **11** (91%) as red needles, mp 220-222°; ir (potassium bromide): 3360 (NH), 1690 (C=O); ^1H nmr (deuteriochloroform): 1.94 (m, H_7, 2H), 2.80 (t, J = 6.38 Hz, H_6, 2H), 3.39 (dt, J = 2.69 Hz and J = 5.37 Hz, H_8, 2H), 3.95 (s, $COOCH_3$, 3H), 6.34 (s, H_3, 1H), 6.49 (s, H_{10}, 1H), 7.73 (s, H_5, 1H); ms: m/e 260 (M^+).

Anal. Calcd. for $C_{14}H_{13}NO_4$: C, 64.86; H, 5.05; N, 5.40. Found: C, 64.80; H, 5.15; N, 5.46.

Methyl Acetamido-2-keto-6,7,8,9-tetrahydro-2*H*-pyrano[3,2-*g*]-quinoline-4-carboxylate (**12**).

A solution of **11** (0.07 g, 0.27 mmole) and acetic anhydride (2.1 ml, 5.4 mmoles) in 3 ml of pyridine was stirred overnight at room temperature. To the orange solution obtained, 2 ml of methanol was added at 0° to hydrolyse the excess of acetic anhydride. After 15 minutes of stirring, water was added and the yellow solution washed twice with diethyl ether. The organic phase was dried over magnesium sulfate and evaporated to dryness. The product was crystallized with isopropanol to give 0.064 g (80%) of **12** as yellow needles, mp 195°; ir (potassium bromide): 1710 (C=O); ^1H nmr (deuteriochloroform): 2.01 (m, H_7, 2H), 2.33 (s, $COCH_3$, 3H), 2.83 (t, J = 6.20 Hz, H_6, 2H), 3.83 (t, J = 5.91 Hz, H_8, 2H), 4.0 (s, $COOCH_3$, 3H), 6.86 (s, H_3, 1H), 7.27 (s, H_{10}, 1H), 8.07 (s, H_5, 1H); ms: m/e 302 (M^+).

Anal. Calcd. for $C_{16}H_{15}NO_5$: C, 63.79; H, 5.02; N, 4.65. Found: C, 63.75; H, 5.10; N, 4.68.

Methyl 2-Keto-9-methyl-6,7,8,9-tetrahydro-2*H*-pyrano[3,2-*g*]quinoline-4-carboxylate (**13**).

Trimethyl phosphate (0.81 g, 5.8 mmoles) and **9** (0.1 g, 0.38 mmole) were heated in an oil bath at 200° for 1 hour. After cooling, the resulting dark oil was solubilized in methylene chloride and the product purified by column chromatography with 5% methanol in methylene chloride as the eluent. The solvent was evaporated to give 0.095 g (90%) of **11** as red needles, mp 172°; ir (potassium bromide): 1700 (C=O); ^1H nmr (deuteriochloroform): 1.97 (m, H_7, 2H), 2.77 (t, J = 6.32 Hz, H_6, 2H), 2.97 (s, NCH_3, 3H), 3.38 (t, J = 5.53 Hz, H_8, 2H), 3.95 (s, $COOCH_3$, 3H), 6.39 (s, H_3, 1H), 6.50 (s, H_{10}, 1H), 7.70 (s, H_5, 1H); ms: m/e 274 (M^+).

Anal. Calcd. for $C_{15}H_{15}NO_4$: C, 65.94; H, 5.53; N, 5.12. Found: C, 65.97; H, 5.48; N, 5.16.

Methyl *N*-(2,3,6,7-Tetrahydro-11-oxo-1*H*,5*H*,11*H*-benzo[*b*]pyrano-6,7-*i*,*j*]quinolizin-4-carboxylate (**15**).

A mixture of dimethyloxalacetate (0.085 g, 0.53 mmole) and 8-hydroxyjulolidine (**14**) (0.1 g, 0.53 mmole) was stirred in oil bath at 110° for 20 minutes. After cooling, the red syrup obtained was solubilized in methylene chloride and the product purified by column chromatography with methylene chloride as the eluent. The solvent was evaporated to dryness to provide 0.115 g of **15** (73%) as orange needles; mp 156°; ir (potassium bromide): 1700 (C=O); ^1H nmr (deuteriochloroform): 1.90-2.02 (m, H_6 and H_2, 4H), 2.77 (t, J = 6.32 Hz, H_1, 2H), 2.88 (t, J = 6.32 Hz, H_7, 2H), 3.27 (q, J = 5.13 Hz, H_3 and H_5, 4H), 3.94 (s, $COOCH_4$, 3H), 6.42 (s, H_{10}, 1H), 7.54 (s, H_8, 1H); ms: m/e 301 (M^+).

Anal. Calcd. for $C_{17}H_{17}NO_4$: C, 68.22; H, 5.72; N, 4.68. Found: C, 68.28; H, 5.67; N, 4.71.

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