Synthesis of Benzopsoralenquinone Derivatives

A. Chilin,[a] G. Pastorini,[b] A. Castellin,[a] F. Bordin,[a,b] P. Rodighiero,*[b] A. Guiotto[a,b]

[a] Dipartimento di Scienze Farmaceutiche, Università di Padova, Via Marzolo 5, I-35131 Padova, Italy
[b] Centro di Studio sulla Chimica del Farmaco e dei Composti Biologicamente Attivi del CNR, Via Marzolo 5, I-35131 Padova, Italy
Fax +39(49)8275366; E-mail Rodighiero@pdfarm.dsfarm.unipd.it

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Benzopsoralenquinone derivatives have been synthesized by linear annulation of a benzofuran moiety to the coumarin system and by selective oxidation to obtain the *p*-quinone function.

Psoralens have various photobiological effects, including antiproliferative activity, due to their capacity to photobind to DNA.[1] This property is successfully exploited in PUVA therapy for various skin diseases.[2] With the aim of eliminating adverse effects attributed to the capacity to form cross-links,[3] a number of derivatives or analogues have been synthesized,[4-6] including benzopsoralens.[7] These compounds retain their photosensitizing properties and behave as pure monofunctional reagents[8] but it is noteworthy that those carrying a 4-hydroxymethyl group effectively block DNA and RNA synthesis in the dark[9] through topoisomerase II inhibition.[10] As this result opens up new possibilities for their use as antitumoral agents, we studied the synthesis of benzopsoralen derivatives showing antiproliferative activity in the dark but lacking any photoreactivity. Accordingly and bearing in mind that the quinone function removes photodynamic activity from furocoumarin,[11] we report the synthesis of benzopsoralenquinones carrying different substituents on the 4-position.

In planning the synthetic route, our attention was directed to the substituent groups, which are differently affected by the oxidizing agents commonly used for quinone preparation. The synthetic pathway generally consists of building the tetracyclic nucleus already carrying the methyl or hydroxymethyl group in the 4-position and an appropriate oxygenated function on the benzene ring, suitable for oxidation to the *p*-quinone system by various methods. The starting material for the synthesis of 4-methylbenzopsoralenquinone is 4-methyl-7-hydroxy-8-methoxycoumarin (**1**, Scheme 1),[12] which was condensed with 2-chlorocyclohexanone to give 7-*O*-(2′-oxocyclohexyl) ether **2**. After cyclization in alkaline medium,[7] this compound gave 4-methyl-11-methoxy-6,7,8,9-tetrahydrobenzopsoralen **(3)**, which was first demethylated to **4** and then oxidized with chromium(III) oxide to the desired 4-methyl-6,7,8,9-tetrahydro-2*H*-benzofuro[3,2-*g*]-1-benzopyran-2,5,11-trione **(5)**. All attempts to aromatize this compound failed, so that intermediate **4** was first aromatized to compound **6**, which was then oxidized in the same manner to 4-methyl-2*H*-benzofuro[3,2-*g*]-1-benzopyran-2,5,11-trione **(7)**.

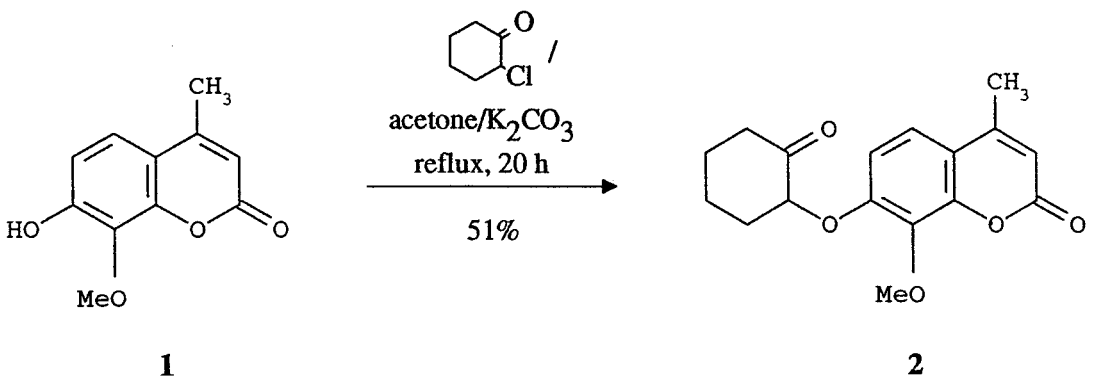
The strategy for the synthesis of 4-hydroxymethylbenzopsoralenquinone was slightly different (Scheme 2). The starting material was 2,5-dimethoxyresorcin **(8)**,[13] which was condensed under Pechmann conditions with ethyl 4-chloroacetoacetate to give 4-chloromethyl-5,8-dimethoxy-7-hydroxycoumarin **(9)**. This was first acetylated to obtain the acetoxymethyl group in the 4-position and then condensed with 2-chlorocyclohexanone before hydrolysis to take advantage of the better solubility of diacetyl ester **10**, the hydrolyzed compound being poorly soluble. 7-*O*-(2′-Oxocyclohexyl)ether **11** was then hydrolyzed under acidic conditions and cyclized with *p*-toluenesulfonic acid to **13**. Both desacetylation of **11** to **12** and cyclization of **12** to **13** require an acidic medium because of the high lability of the lactone ring, even in weakly alkaline conditions, particularly enhanced by the hydroxymethyl group in the 4-position: in alkaline medium, in fact, ring contraction forming a furan ring carrying a carboxymethyl group easily occurs.[14] Previous desacetylation of **11** to **12** by methanol/hydrochloric acid was preferred to simultaneous desacetylation and cyclization by *p*-toluenesulfonic acid, because the cumulative reaction yields secondary products. Oxidative demethylation by dilute nitric acid[15] of **13** furnished the desired 4-hydroxymethyl-6,7,8,9-tetrahydro-2*H*-benzofuro[3,2-*g*]-1-benzopyran-2,5,11-trione **(14)**. In this case too, aromatization of **14** failed under various conditions; consequently 4-hydroxymethyl-2*H*-benzofuro[3,2-*g*]-1-benzopyran-2,5,11-trione **(16)** was obtained by the same synthetic procedure described above, first aromatizing **13** and then oxidizing **15** to **16** with dilute nitric acid. Testing of the biological properties of these new compounds is currently in progress.

Analytical TLC was performed on precoated 60-F-254 silica gel plates (Merck) with an EtOAc/cyclohexane mixture (1:1). Column chromatography was performed using silica gel (0.040–0.063 mm, Merck) eluting with CH_2Cl_2. Melting points were determined on an open-capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian-Gemini 200 MHz spectrometer and refer to the deuterium lock signal from the sample solvent. Microanalyses were performed by the Microanalytical Laboratory of the Department of Pharmaceutical Sciences of University of Padova under the direction of A. Pietrogrande. All reagents and solvents were of commercial quality and were used without further purification.

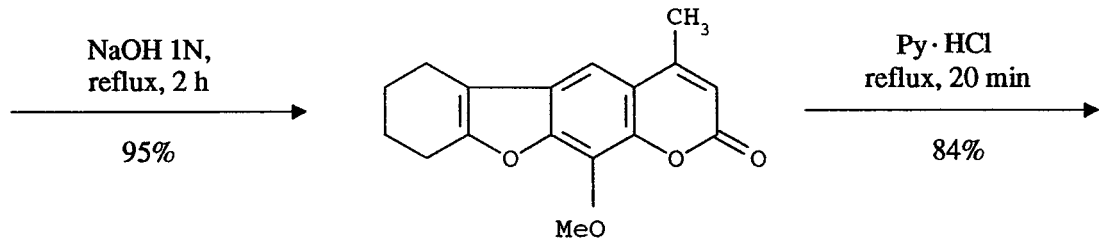
4-Methyl-8-methoxy-7-(2′-oxocyclohexyloxy)coumarin (2):
To a solution of **1** (4.0 g, 19.4 mmol) in acetone (600 mL) were added 2-chlorocyclohexanone (3.8 g, 29.1 mmol) and anhydr. K_2CO_3 (30 g). The mixture was refluxed until **1** had disappeared (20 h, TLC). After cooling, the solid was filtered and washed with acetone. The solvent was evaporated from the pooled filtrate and washings and the residue was crystallized from MeOH to give **2** (3.0 g, 51%); mp 170°C.

^{1}H NMR ($CDCl_3$): δ = 1.74–2.14 (m, 4H, 4′-H_2 and 5′-H_2), 2.38 (d, J = 1.2 Hz, 3H, 4-CH_3), 2.41–2.68 (m, 4H, 3′-H_2 and 6′-H_2), 4.00 (s, 3H, 8-OCH_3), 4.83 (dd, J = 9.9 Hz, 5.6 Hz, 1H, 1′-H), 6.17 (q, J = 1.2 Hz, 1H, 3-H), 6.78 (d, J = 8.8 Hz, 1H, 6-H), 7.22 (d, J = 8.8 Hz, 1H, 5-H).

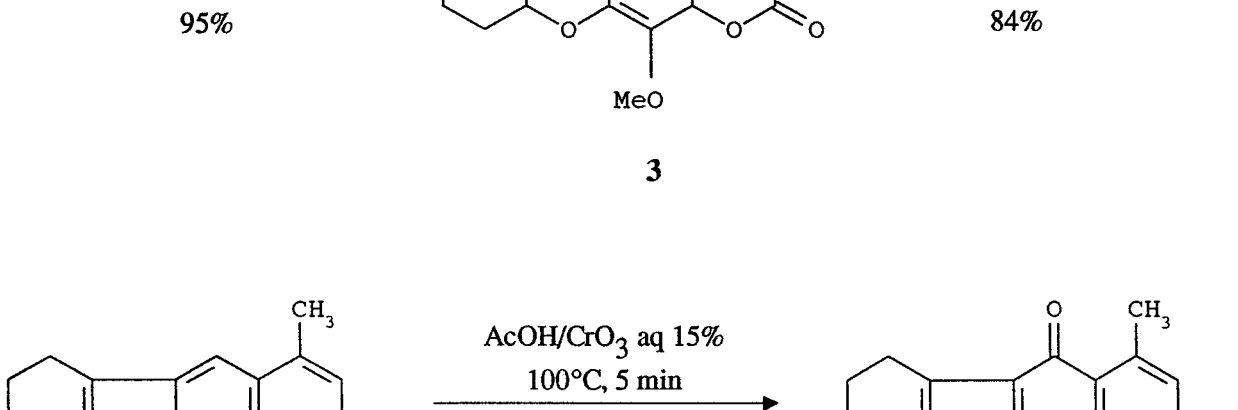
6,7,8,9-Tetrahydro-11-methoxy-4-methyl-2*H*-benzofuro[3,2-*g*]-1-benzopyran-2-one (3):
A solution of **2** (3.0 g, 9.9 mmol) in 1 N NaOH (1 L) was refluxed under N_2 in the dark for 2 h. After cooling the mixture was acidified with 2 N HCl and diluted with H_2O. The obtained precipitate was



1 2



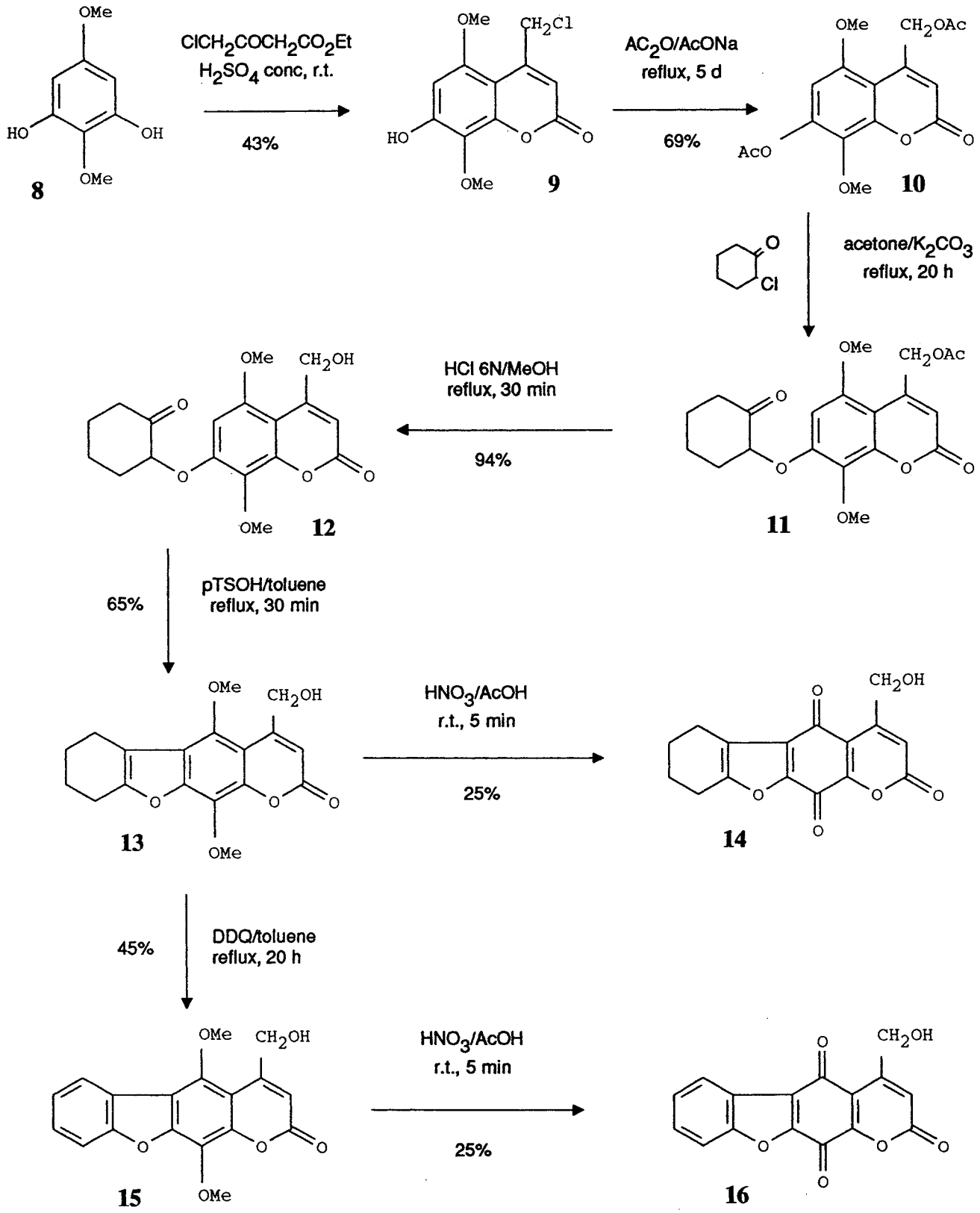
3



4 5

6 7

Scheme 1



Scheme 2

collected and crystallized from MeOH to give **3** (2.7 g, 95%); mp 208°C.

^{1}H NMR ($CDCl_3$): δ = 1.86–1.98 (m, 4H, 7-H_2 and 8-H_2), 2.49 (d, J = 1.2 Hz, 3H, 4-CH_3), 2.61–2.66 (m, 2H, 6-H_2), 2.75–2.81 (m, 2H, 9-H_2), 4.26 (s, 3H, 11-OCH_3), 6.24 (q, J = 1.2 Hz, 1H, 3-H), 7.24 (s, 1H, 5-H).

6,7,8,9-Tetrahydro-11-hydroxy-4-methyl-2*H*-benzofuro[3,2-*g*]-1-benzopyran-2-one (4):

A mixture of **3** (1.0 g, 3.5 mmol) and pyridine hydrochloride (5 g) was heated to melt and heated at 190°C for 20 min. After cooling the mixture was diluted with 2 N HCl (50 mL) and extracted with Et_2O (3 × 50 mL). The combined extracts were dried (Na_2SO_4),

concentrated under reduced pressure and the residue was crystallized from MeOH to give **4** (0.8 g, 84%); mp > 300 °C.

^{1}H NMR ($CDCl_3$): δ = 1.84–1.99 (m, 4H, 7-H and 8-H), 2.52 (d, J = 1.2 Hz, 3H, 4-CH_3), 2.59–2.69 (m, 2H, 6-H), 2.74–2.82 (m, 2H, 9-H), 6.25 (q, J = 1.2 Hz, 1H, 3-H), 7.26 (s, 1H, 5-H).

6,7,8,9-Tetrahydro-4-methyl-2*H*-benzofuro[3,2-*g*]-1-benzopyran-2,5,11-trione (5):
To a hot solution of **4** (0.1 g, 0.37 mmol) in AcOH (20 mL) was added 15% aq CrO_3 (3 mL). After 5 min the mixture was poured into H_2O (50 mL) and the precipitate was collected and crystallized from MeOH to give **5** (42 mg, 40%); mp 217 °C.

^{1}H NMR ($CDCl_3$): δ = 1.81–1.92 (m, 4H, 7-H_2 and 8-H_2), 2.61 (d, J = 1.3 Hz, 3H, 4-CH_3), 2.74–2.80 (m, 4H, 6-H_2 and 9-H_2), 6.35 (q, J = 1.3 Hz, 1H, 3-H).

11-Hydroxy-4-methyl-2*H*-benzofuro[3,2-*g*]-1-benzopyran-2-one (6):
A solution of **4** (0.5 g, 1.8 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.8 g, 3.6 mmol) in toluene (300 mL) was refluxed until **4** had completely reacted (20 h, TLC). After cooling, the solid was filtered and the solvent was evaporated under reduced pressure. The residue was purified on a silica gel column and then crystallized from MeOH to give **6** (0.2 g, 45%); mp > 300 °C.

^{1}H NMR (acetone-d_6): δ = 2.62 (d, J = 1.2 Hz, 3H, 4-CH_3), 6.33 (q, J = 1.2 Hz, 1H, 3-H), 7.41 (ddd, J = 7.6, 7.2, 1.3 Hz, 1H, 8-H), 7.53 (ddd, J = 8.2, 7.2, 1.5 Hz, 1H, 7-H), 7.65 (ddd, J = 8.2, 1.3, 0.7 Hz, 1H, 6-H), 7.94 (s, 1H, 5-H), 8.10 (ddd, J = 7.6, 1.5, 0.7 Hz, 1H, 9-H).

4-Methyl-2*H*-benzofuro[3,2-*g*]-1-benzopyran-2,5,11-trione (7):
This compound was prepared from **6** (0.1 g, 0.4 mmol) in an analogous manner to **5**. The solid was crystallized from MeOH to give **7** (40 mg, 38%); mp > 300 °C.

^{1}H NMR (DMSO-d_6): δ = 2.57 (d, J = 1.3 Hz, 3H, 4-CH_3), 6.49 (q, J = 1.3 Hz, 1H, 3-H), 7.50 (ddd, J = 7.6, 1.2 Hz, 1H, 8-H), 7.62 (ddd, J = 8.2, 7.1, 1.5 Hz, 1H, 7-H), 7.79 (ddd, J = 8.2, 1.2, 0.6 Hz, 1H, 6-H), 8.15 (ddd, J = 7.6, 1.5, 0.6 Hz, 1H, 9-H).

4-Chloromethyl-7-hydroxy-5,8-dimethoxycoumarin (9):
To a mixture of **8**[13] (11.0 g, 64.6 mmol) and ethyl 4-chloroacetoacetate (10.6 g, 64.6 mmol) was added cautiously conc. H_2SO_4 (100 mL). After 15 min the brownish solution was poured into ice and H_2O (1 L) and the precipitate was collected and washed with H_2O. The solid was crystallized from MeOH to give **9** (7.5 g, 43%); mp 204 °C.

^{1}H NMR (acetone-d_6): δ = 3.84 (s, 3H, 8-OCH_3), 3.94 (s, 3H, 5-OCH_3), 5.01 (d, J = 1.1 Hz, 2H, 4-CH_2), 5.62 (s, 1H, OH), 6.34 (t, J = 1.1 Hz, 1H, 3-H), 6.52 (s, 1H, 6-H).

7-Acetoxy-4-acetoxymethyl-5,8-dimethoxycoumarin (10):
A mixture of **9** (7.5 g, 27.7 mmol), anhyd. NaOAc (2.0 g) and Ac_2O (150 mL) was refluxed for 5 d. The mixture was cautiously diluted with H_2O (100 mL), refluxed for 10 min and poured into H_2O (1 L). The precipitate was collected, washed with H_2O and crystallized from MeOH to give **10** (6.4 g, 69%); mp 174 °C.

^{1}H NMR ($CDCl_3$): δ = 2.22 (s, 3H, $OCOCH_3$), 2.38 (s, 3H, $OCOCH_3$), 3.87 (s, 3H, 5-OCH_3, or 8-OCH_3), 3.92 (s, 3H, 5-OCH_3 or 8-OCH_3), 5.43 (d, J = 1.8 Hz, 2H, 4-CH_2), 6.42 (t, J = 1.8 Hz, 1H, 3-H), 6.49 (s, 1H, 6-H).

4-Acetoxymethyl-5,8-dimethoxy-7-(2′-oxocyclohexyloxy)coumarin (11):
This compound was prepared from **10** (6.4 g, 19.0 mmol) in an analogous manner to **2**. The crude product was crystallized from MeOH to give **11** (3.6 g, 48%); mp 220 °C.

^{1}H NMR ($CDCl_3$): δ = 2.20 (s, 3H, $OCOCH_3$), 1.73–2.63 (m, 8H, 3′-H_2, 4′-H_2, 5′-H_2 and 6′-H_2), 3.84 (s, 3H, 5-OCH_3 or 8-OCH_3), 3.89 (s, 3H, 5-OCH_3 or 8-OCH_3), 4.87 (dd, J = 9.4, 5.4 Hz, 1H, 1′-H), 5.40 (d, J = 1.7 Hz, 2H, 4-CH_2), 6.29 (t, J = 1.7 Hz, 1H, 3-H), 6.36 (s, 1H, 6-H).

4-Hydroxymethyl-5,8-dimethoxy-7-(2′-oxocyclohexyloxy)coumarin (12):
To a solution of **11** (3.5 g, 8.9 mmol) in MeOH (300 mL) was added 6 N HCl (50 mL) and the mixture was refluxed until **11** had disappeared (30 min, TLC). The solvent was evaporated from the mixture and the residue was crystallized from MeOH to give **12** (2.9 g, 4%); mp 186 °C.

^{1}H NMR (DMSO-d_6): δ = 1.81–1.96 (m, 4H, 4′-H_2 and 5′-H_2), 2.31–2.75 (m, 4H, 3′-H_2 and 6′-H_2), 3.79 (s, 3H, 5-OCH_3 or 8-OCH_3), 3.82 (s, 3H, 5-OCH_3 or 8-OCH_3), 4.76 (d, J = 5.4 Hz, 2H, 4-CH_2), 5.40 (dd, J = 9.6, 5.5 Hz, 1H, 1′-H), 5.53 (t, J = 5.4 Hz, 1H, OH), 6.37 (s, 1H, 3-H), 6.51 (s, 1H, 6-H).

6,7,8,9-Tetrahydro-4-hydroxymethyl-5,11-dimethoxy-2*H*-benzofuro[3,2-*g*]-1-benzopyran-2-one (13):
To a solution of **12** (2.9 g, 8.3 mmol) in anhydrous toluene (300 mL) was added TsOH (1.6 g, 8.3 mmol). The mixture was refluxed for 30 min then was extracted with H_2O (2 × 200 mL). The organic phase was dried (Na_2SO_4), the solvent evaporated under reduced pressure and the residue was crystallized from EtOAc to give **13** (1.8 g, 65%); mp 196 °C.

^{1}H NMR ($CDCl_3$): δ = 1.83–1.96 (m, 4H, 7-H_2 and 8-H_2), 2.72–2.84 (m, 4H, 6-H_2 and 9-H_2), 3.88 (s, 3H, 5-OCH_3 or 8-OCH_3), 4.12 (s, 3H, 5-OCH_3 or 8-OCH_3), 4.94 (dd, J = 5.4 Hz, 1.3 Hz, 2H, 4-CH_2), 5.53 (t, J = 5.4 Hz, 1H, OH), 6.55 (t, J = 1.3 Hz, 1H, 3-H).

6,7,8,9-Tetrahydro-4-hydroxymethyl-2*H*-benzofuro[3,2-*g*]-1-benzopyran-2,5,11-trione (14):
To a solution of **13** (0.5 g, 1.5 mmol) in AcOH (50 mL) was added HNO_3 (d = 1.14, 1 mL) dropwise. After 5 min the red solution was poured into H_2O (100 mL) and the precipitate was collected. The orange solid was crystallized from EtOAc to give **14** (0.12 g, 25%); mp 231 °C.

^{1}H NMR (DMSO-d_6): δ = 1.72–1.88 (m, 4H, 7-H_2 and 8-H_2), 2.57–2.81 (m, 4H, 6-H_2 and 9-H_2), 4.83 (dd, J = 5.3 Hz, 1.4 Hz, 2H, 4-CH_2), 5.72 (t, J = 5.3 Hz, 1H, OH), 6.73 (t, J = 1.4 Hz, 1H, 3-H).

4-Hydroxymethyl-5,11-dimethoxy-2*H*-benzofuro[3,2-*g*]-1-benzopyran-2-one (15):
This compound was prepared from **13** (0.3 g, 0.9 mmol) in an analogous manner to **6**. The crude product was purified on silica gel column and crystallized from MeOH to give **15** (0.13 g, 45%); mp 241 °C.

^{1}H NMR (DMSO-d_6): δ = 4.02 (s, 3H, 5-OCH_3 or 11-OCH_3), 4.15 (s, 3H, 5-OCH_3 or 11-OCH_3), 5.01 (dd, J = 5.4 Hz, 1.8 Hz, 2H, 4-CH_2), 5.73 (t, J = 5.4 Hz, 1H, OH), 6.65 (t, J = 1.8 Hz, 1H, 3-H), 7.53 (ddd, J = 7.6, 7.4, 1.3 Hz, 1H, 8-H), 7.63 (ddd, J = 8.1, 7.4, 1.6 Hz, 1H, 7-H), 7.84 (ddd, J = 8.1, 1.3, 0.8 Hz, 1H, 6-H), 8.11 (ddd, J = 7.6, 1.6, 0.8 Hz, 1H, 9-H).

4-Hydroxymethyl-2*H*-benzofuro[3,2-*g*]-1-benzopyran-2,5,11-trione (16):
This compound was prepared from **15** (0.1 g, 0.3 mmol) in an analogous manner to **14**. The orange solid was crystallized from EtOAc to give **16** (90 mg, 25%); mp 268 °C.

^{1}H NMR (DMSO-d_6): δ = 4.92 (dd, J = 5.4, 1.3 Hz, 2H, CH_2OH), 5.80 (t, J = 5.4 Hz, 1H, OH), 6.81 (t, J = 1.3 Hz, 1H, 3-H), 7.64 (ddd, J = 7.5, 7.2, 1.3 Hz, 1H, 8-H), 7.73 (ddd, J = 8.1, 7.2, 1.5 Hz, 1H, 7-H), 7.97 (ddd, J = 8.1, 1.3, 0.7 Hz, 1H, 6-H), 8.15 (ddd, J = 7.5, 1.5, 0.7 Hz, 1H, 9-H).

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