A. Chilin [a], P. Manzini [a,b], S. Caffieri [a,b],
P. Rodighiero [b] and A. Guiotto [a,b]*

[a] Department of Pharmaceutical Sciences, University of Padua, Via Francesco Marzolo 5, I-35131 Padova, Italy
[b] Centro di Studio sulla Chimica del Farmaco e dei Prodotti Biologicamente Attivi del C.N.R.
(associated to the National Institute for the Chemistry of Biological Systems)
Department of Pharmaceutical Sciences, University of Padua, Via Francesco Marzolo 5, I-35131 Padova, Italy
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New tetracyclic psoralen-like compounds have been synthesized, which are characterized by two furan rings angularly condensed on the coumarin nucleus and are therefore called difurocoumarins. This structural feature may favour the intercalation into the DNA helix and then monofunctionally photoreaction with DNA bases. Their synthesis started from 5,7-dihydroxy-4-methylcoumarin, which was condensed with α-halo-ketones; the resulting ketoethers were then cyclized to give the title compounds.

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Furocoumarins, currently called psoralens, are natural or synthetic photoactive drugs used in PUVA (Psoralen plus UVA irradiation) therapy to cure several skin diseases [1] and in photopheresis to prevent rejection in organ transplants and to treat T-cell lymphoma and various autoimmune diseases [2]. PUVA therapy, performed by using linearly annulated psoralens such as 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP) and 4,8,5'-trimethylpsoralen (TMP) (Figure 1), is highly effective, but some severe side effects are to be expected, such as skin erythema [1], genotoxicity [3-5], and carcinogenicity [6], mostly attributed to the lesions induced in DNA by psoralen sensitization. Indeed, different kinds of DNA lesions can be recognized, *i.e.*, covalent mono- and di-adducts with pyrimidine bases [7], and covalent DNA-protein cross-links (DPC) [8].

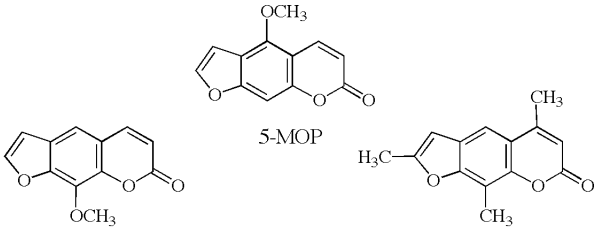


Figure 1

Since many authors considered inter-strand cross-links (ISC) responsible for furocoumarin genotoxicity, several monofunctional derivatives have been prepared and studied, such as angularly annulated furocoumarins as well as angular furoquinolinones [9,10]. Thus, difurocoumarin (DFC) derivatives, in which two furan rings are condensed on the 5,6 and 7,8 bonds of the coumarin nucleus (Figure 2), were designed and prepared in order to obtain drugs lacking the adverse effects shown by psoralens in PUVA therapy, while maintaining the same therapeutic effectiveness. In fact, the DFC structure is reminiscent of that of angelicin and allopsoralen (Figure 2), angular isomers of psoralen, whose methylated derivatives are effective as antiproliferative agents, but unable to give ISC [11,12].

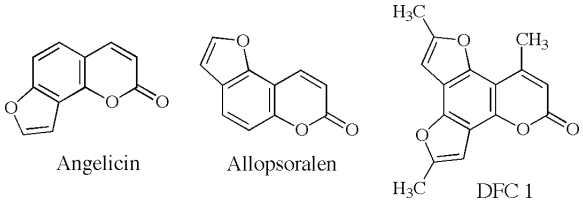


Figure 2

A trimethyl derivative of DFC, *i.e.*, 2,5,10-trimethyl-8*H*-difuro[2,3-*f*:2',3'-*h*][1]benzopyran-8-one (DFC **1**, Figure 2), has previously been synthesized and studied [13]. It proved to be able to photobind DNA in a monofunctional way.

We now report the synthesis of new DFC methylated derivatives, carrying methyl groups in the 3 and 6 positions, according to Scheme 1, or in both the 3,6 and 2,5 positions, according to Scheme 2. The synthesis of DFC **1** was also revised by setting up a different and more convenient method (Scheme 3).

5,7-Dihydroxy-4-methylcoumarin (**2**) [14] was used as starting material for all the syntheses. This compound was condensed with chloroacetone or 2-chloro-3-butanone to give the diethers **3** (Scheme 1) and **7** (Scheme 2) respectively. Due to the high reactivity of the dihydroxycoumarin nucleus, during the etherification reaction a variety of compounds were formed, in addition to the

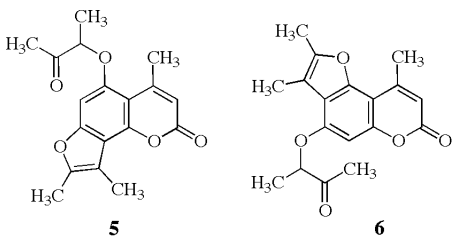
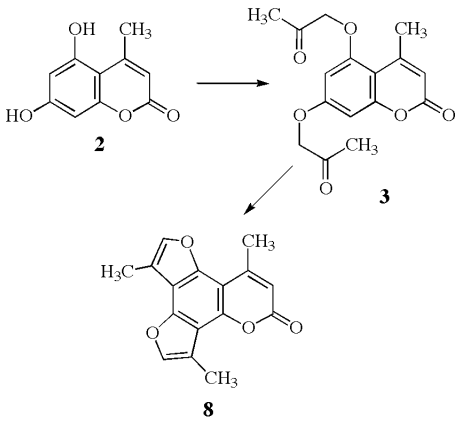


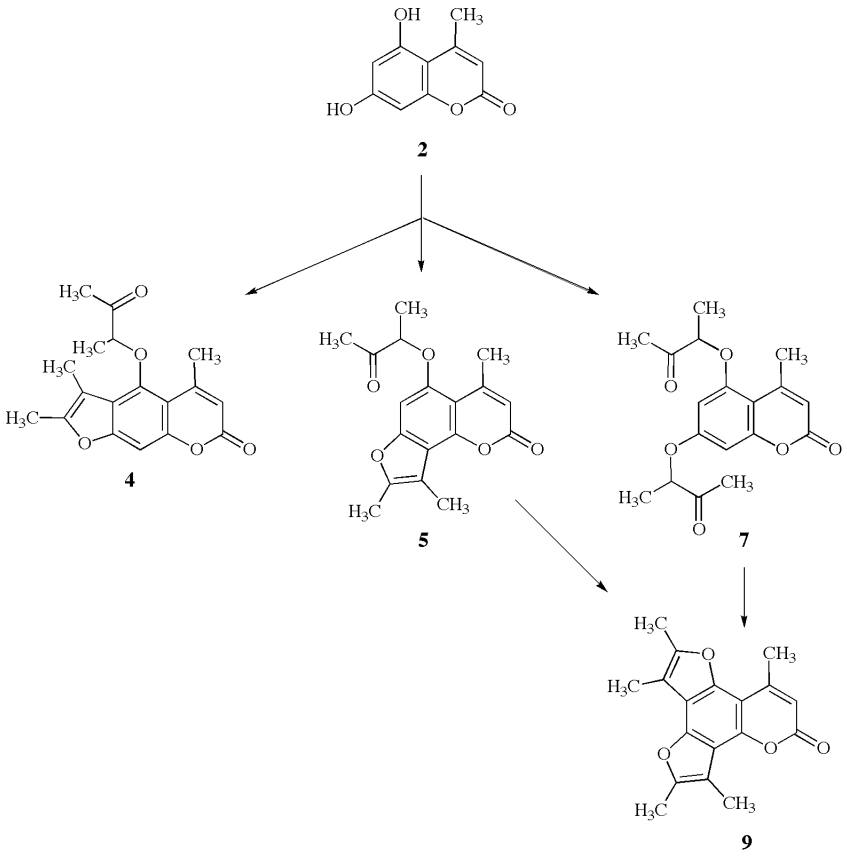
Figure 3

Scheme 1



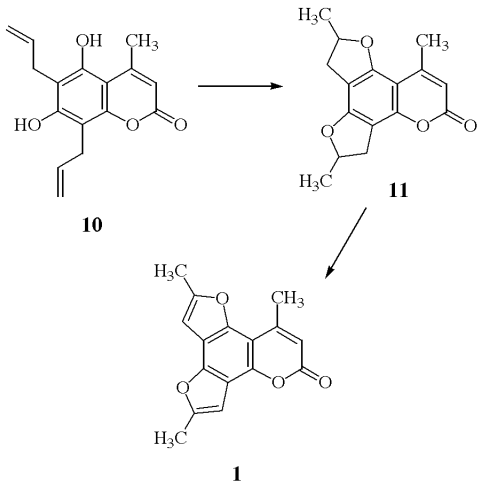
cyclization occuring during etherification could give three possible products: in addition to linear compound **4** and compound **5** with an angelicin-like geometry (Scheme 2), the angular annulation of the furan ring could theoretically afford compound **6** with the allopsoralen-like structure (Figure 3). To establish whether the unique angular reaction product presented the angelicin-like structure **5** or the allopsoralen-like structure **6**, further spectroscopic investigations were carried out. The very similar molecular geometry of isomers **5** and **6** did not allow the unambigous assignment of the product structure by ^{1}H-^{13}C hetero-correlated 2D-spectroscopy, neither by HMQC neither by HMBC experiments. However, a ^{1}H-^{1}H NOESY interaction was observed between the vinylic methyl protons of the lactone (δ 2.69) and the chetonic (δ 2.18) and aliphatic (δ 1.62) methyl protons. This contact

Scheme 2



desired diethers. In fact, as shown in Scheme 2, condensation of 2-chloro-3-butanone with **2** yielded ether **7**, which was accompanied by two partially cyclized products, **4** and **5**. The angular annulation of the furan ring for compound **5** was confirmed on the basis that subsequent cyclization of this compound afforded DFC **9**; on the other hand, no cyclization occurred for monoether **4**, confirming the linear annulation of the furan ring. Actually, the partial was possible only in structure **5**; therefore, this structure was suggested as the most probable for the molecule, although a high degree of free rotation around the benzene-oxygen of the β-cheto-ether moiety was required to allow both NOESY interactions. Since no NOESY contact was observed between the unique aromatic proton and the protons of the cheto-ether group, a significant presence of structure **6** was excluded.

Scheme 3



Ethers **3** and **7** were submitted to cyclization in alkaline (KOH) or acidic (methanesulfonic acid) medium: both conditions afforded generally a complex mixture of products, which gave, after chromatographic separation, 3,6,10-trimethyl-8*H*-difuro[2,3-*f*:2',3'-*h*]-[1]benzopyran-8-one (**8**) and 2,3,5,6,10-pentamethyl-8*H*-difuro[2,3-*f*:2',3'-*h*][1]benzopyran-8-one (**9**) respectively. Overall, the acidic conditions worked better, giving higher yield.

A new synthetic pathway to DFC **1** was also explored, according to Scheme 3. The 6,8-diallyl-4,5-dimethylcoumarin (**10**), previously reported [13], was cyclized in concentrated sulfuric acid to give tetrahydrodifurocoumarin **11**, which was then submitted to aromatization affording 2,5,10-trimethyl-8*H*-difuro[2,3-*f*:2',3'-*h*][1]-benzopyran-8-one (**1**), in yield higher than those obtained in the previous synthesis [13].

EXPERIMENTAL

Analytical tlc was performed on silica gel plates (Merck pre-coated 60 F_{254}, 0.25 mm), eluting with chloroform, unless otherwise indicated. Column chromatography was performed using silica gel (Merck; 0.063-0.100 mm), eluting with dichloromethane, unless otherwise indicated. Melting points were determined on a Gallenkamp MFB-595-010M apparatus and are uncorrected. Ultraviolet (UV) spectra were recorded on a Perkin-Elmer Lambda 20 UV-VIS spectrophotometer. Infrared (ir) spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrometer. ^{1}H NMR spectra were recorded on Varian Gemini-200 and Bruker Avance-400 spectrometers with TMS as internal standard and deuteriochloroform as solvent. Electron-impact (EI) mass spectra were recorded on a Varian MAT112 spectrometer. Elemental analyses were carried out on all intermediates and are within ±0.4%.

5,7-Di-(2'-oxopropyloxy)-4-methylcoumarin (**3**).

To a solution of 5,7-dihydroxy-4-methylcoumarin (**2**) [14] (5.0 g, 26.0 mmoles) in acetone (500 mL), were added chloroacetone (7.2 g, 78.0 mmoles) and anhydrous potassium carbonate (40.0 g). The mixture was refluxed until **2** disappeared (17 hours). After cooling, the solid was isolated by filtration and washed with fresh acetone. The solvent was evaporated from the pooled filtrate and washings and the residue was crystallized from methanol to give **3** (5.2 g, 66%), mp 186-187°; ^{1}H nmr: δ 2.28 (s, 6 H, 3'-H), 2.64 (d, 3 H, 4-Me, J = 1.2 Hz), 4.69 and 4.61 (2s, 2 H each, 1'-H), 6.03 (q, 1 H, 3-H, J = 1.2 Hz), 6.22 and 6.35 (2d, 1 H each, 6-H and 8-H, J = 2.4 Hz both).

Anal. Calcd. for $C_{16}H_{16}O_6$: C, 63.15; H, 5.30. Found: C, 65.25; H, 5.28.

5,7-Di-[2'-(3'-oxo)butyloxy]-4-methylcoumarin (**7**), 2,3,5-trimethyl-4-[2'-(3'-oxo)butyloxy]furo[3,2-*g*]benzopyran-7-one (**4**) and 4,8,9-trimethyl-5-[2'-(3'-oxo)butyloxy]furo[2,3-*h*]-benzopyran-2-one (**5**).

To a solution of 5,7-dihydroxy-4-methylcoumarin (**2**) (10.0 g, 52.0 mmoles) in acetone (1000 mL), were added 2-chlorobutan-3-one (12.7 g, 119.7 mmoles) and anhydrous potassium carbonate (40.0 g). The mixture was refluxed until **2** disappeared (22 hours). After this period the reaction mixture showed by tlc (ethyl acetate/cyclohexane, 1:1) two minor spots with blue and yellow fluorescence respectively and a main spot with blue fluorescence. After cooling, the solid was filtered off and washed with fresh acetone. The solvent was evaporated from the pooled filtrate and washings and the residue was crystallized from methanol to give **7** (3.5 g, 21%), mp 115-116°; ^{1}H nmr: δ 1.52 and 1.61 (2d, 3 H each, 1'-H, J = 6.9 Hz both), 2.17 and 2.19 (2s, 3 H each, 4'-H), 2.62 (d, 3 H, 4-Me, J = 1.2 Hz), 4.64 and 4.74 (2q, 1 H each, 2'- H, J = 6.9 Hz both), 6.02 (q, 1 H, J = 1.2 Hz, 3-H), 6.04 and 6.30 (2d, 1 H each, 6-H and 8-H, J = 2.3 Hz both).

Anal. Calcd. for $C_{18}H_{20}O_6$: C, 65.05; H, 6.07. Found: C, 65.17; H, 6.20.

The residue from the mother liquors was purified by column chromatography, eluting with ethyl acetate/cyclohexane (1:1) mixture. Compounds **4** and **5** first eluted together, followed by further crop of pure **7** (7.6 g, total 70%). From the fractions containing the first two products the solvent was evaporated and the residue (1.1 g) after fractional cristallization from methanol gave pure **5** at first and finally **4** containing trace amount of **5**.

Compound **4** has ^{1}H nmr: δ 1.26 (d, 3 H, 1'-H, J = 7.5 Hz), 2.25 (broad s, 3 H, 3-Me), 2.37 (broad s, 3 H, 2-Me), 2.38 (s, 3 H, 4'-H), 2.66 (d, 3 H, 5-Me, J = 1.2 Hz), 4.60 (q, 1 H, 2'- H, J = 7.5 Hz), 6.14 (q, 1 H, 6-H, J = 1.2 Hz), 7.12 (s, 1 H, 9-H).

Anal. Calcd. for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.67; H, 5.80.

Compound **5** has ^{1}H nmr: δ 1.62 (d, 3 H, 1'-H, J = 6.8 Hz), 2.18 (s, 3 H, 4'-H), 2.34 (broad s, 3 H, 9-Me), 2.39 (broad s, 3 H, 8-Me), 2.69 (d, 3 H, 4-Me, J = 1.1 Hz), 4.73 (q, 1 H, 2'- H, J = 6.8 Hz), 6.11 (q, 1 H, 3-H, J = 1.1 Hz), 6.54 (s, 1 H, 6-H).

Anal. Calcd. for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.70; H, 5.75.

General Procedure for Alkaline Cyclization of ethers **3** and **7**.

To an ethanolic solution (500 mL) of ether **3** or **7** (10.0 mmoles) was added a 4% ethanolic potassium hydroxide solution (70 mL) and the mixture was refluxed in the dark for 30 minutes. The solution was cooled, diluted with water (200 mL) and acidified with diluted hydrochloric acid. The ethanol was

evaporated and the mixture was extracted with chloroform (3 x 100 mL). The organic phase was dried (sodium sulfate) and the solvent was evaporated. The residue was purified by column chromatography to give **8** or **9**.

3,6,10-Trimethyl-8*H*-difuro [2,3-*f*:2',3'-*h*][1]benzopyran-8-one (**8**).

This compound was obtained from **3** in 16% yield, mp 295° (methanol); UV (ethanol 95%): λ max nm 229, 233, 258, 263 (shoulder), 317, λ min nm 231, 242, 283; ir (KBr): cm^{-1} 3120 (aromatic C-H), 2930 (aliphatic C-H), 1720 (C=O), 1610 (C=C), 1380, 1210, 1110 (C-O), 940, 900, 840; ^{1}H nmr: δ 2.50 and 2.56 (2d, 3 H each, 3-Me and 6-Me, J = 1.4 Hz both), 2.77 (d, 3 H, 10-Me, J = 1.2 Hz), 6.22 (q, 1 H, 9-H, J = 1.2 Hz), 7.43 and 7.50 (2q, 1 H each, 2-H and 5-H, J = 1.4 Hz both); ms (EI): *m/z* (relative intensity) 268 (M^+, 55), 240 (100), 239 (32), 212 (32), 152 (34), 128 (38), 115 (41).

Anal. Calcd. for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.62; H, 4.56.

2,3,5,6,10-Pentamethyl-8*H*-difuro[2,3-*f*:2',3'-*h*][1]benzopyran-8-one (**9**).

This compound was prepared from **7** in 32% yield, mp >300° (methanol); UV (ethanol 95%): λ max nm 232, 237 (shoulder), 263, 267 (shoulder), λ min nm 247, 290, 323; ir (KBr): cm^{-1} 2920 (aliphatic C-H), 1710 (C=O), 1600 (C=C), 1390, 1210, 1080 (C-O), 950, 900, 840; ^{1}H nmr: δ 2.24 (s, 6 H, 2-Me and 3-Me or 5-Me and 6-Me), 2.36 and 2.43 (2q, 3 H each, 2-Me and 3-Me or 5-Me and 6-Me, J = 0.9 Hz both), 2.69 (d, 3 H, 10-Me, J = 1.2 Hz), 6.12 (d, 1 H, 9-H, J = 1.2 Hz); ms (EI): *m/z* (relative intensity) 296 (M^+, 85), 268 (100), 253 (25), 152 (30), 128 (33), 115 (47).

Anal. Calcd. for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 73.02; H, 5.35.

General Procedure for Acid Cyclization of ethers **3-7**.

A toluene solution (200 mL) of ether **3-7** (5.0 mmoles) and methanesulfonic acid (25 mmoles) was refluxed for 4 hours. After cooling, the solution was diluted with ethyl acetate (100 mL) and washed with water. The organic phase was dried (sodium sulfate) and the solvent evaporated. The residue was purified by column chromatography. Cyclization of compounds **5** and **7** gave **9** in 49% and 83% yield respectively (mp and ^{1}H nmr as mentioned above). Cyclization of compound **3** gave **8** in 31% yield (mp and ^{1}H nmr as mentioned above).

2,5,10-Trimethyl-8*H*-2,3,5,6-tetrahydrodifuro[2,3-*f*:2',3'-*h*]-[1]benzopyran-8-one (**11**).

A solution of 4-methyl-5,7-dihydroxy-6,8-diallylcoumarin (**10**) [13] (0.70 g, 2.6 mmoles) in concentrated sulphuric acid (30 mL) was kept at room temperature until **10** disappeared - (30 minutes). The solution was diluted with ice and water (300 mL), yielding a solid, which was filtered, washed with water and dried. The solid was purified by column chromatography to give **11** (0.20 g, 28%): mp 178-180°; ^{1}H nmr: δ 1.48 and 1.50 (2d, 3 H each, 2-Me and 5-Me, J = 6.3 Hz both), 2.47 (d, 3 H, 10-Me, J = 1.1 Hz), 2.65-3.45 (m, 4 H, 3-H), 5.07 and 5.08 (2q, 1 H each, 2-H, J = 6.3 Hz both), 5.86 (q, 1 H, 9-H, J = 1.3 Hz).

Anal. Calcd. for $C_{16}H_{16}O_4$: C, 70.57; H, 5.92. Found: C, 70.66; H, 5.89.

2,5,10-Trimethyl-8*H*-difuro[2,3-*f*:2',3'-*h*] [1]benzopyran-8-one (**1**).

A mixture of 2,5,10-trimethyl-8*H*-2,3,4,5-tetrahydro-difuro[2,3-*f*:2',3'-*h*][1]benzopyran-8-one (**11**) (0.59 g, 2.2 mmoles) and 2,3-dichloro-5,6-dicyanobenzoquinone (1.06 g, 4.7 mmoles) in toluene (150 mL) was refluxed for 45 minutes. After cooling, the solid was isolated by filtration and the solution evaporated. The residue was purified by column chromatography to give **1** (0.28 g, 47%): mp 267-268° (methanol); UV (ethanol 95%): λ max nm 228, 234, 259, 265 (shoulder), 317, λ min nm 232, 242, 283; ir (KBr): cm^{-1} 3110 (aromatic C-H), 2920 (aliphatic C-H), 1720 (C=O), 1610 (C=C), 1390, 1210, 1110 (C-O), 890, 850; ^{1}H nmr: δ 2.52 and 2.54 (2d, 3 H each, 2-Me and 5-Me, J = 1.1 Hz both), 2.77 (d, 3H, 10-Me, J = 1.3 Hz), 6.02 (q, 1 H, 9-H, J = 1.3 Hz), 6.63 and 6.74 (2q, 1 H each, 3-H and 6-H, J = 1.1 Hz both); ms (EI): *m/z* (relative intensity) 268 (M^+, 60), 240 (100), 239 (64), 212 (30), 139 (16), 115 (34).

Anal. Calcd. for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.62; H, 4.56.

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[*] Correspondence should be addressed to: Adriano Guiotto, Dipartimento di Scienze Farmaceutiche, Università di Padova, Via Marzolo 5, 35131 - Padova, ITALY; Phone: +39-049-8275351; FAX: +39-049-8275366; E-mail: guiotto@dsfarm.unipd.it

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