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A New Benzoangelicin with Strong Photobiological Activity

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Abstract—Benzoangelicins **4**–**6** were synthesized in good yields from 7-hydroxy-5-methoxy-4-methylcoumarin (**1**). In the absence of UVA radiation, compounds **5** and **6** were only weakly active against HL60 and HeLa tumour cells; in its presence, compound **6** was 10 times more active than the reference compound 8-methoxypsoralen. None of **4**–**6** exhibited cutaneous phototoxicity.

The furocoumarin family includes members that are widely used in the photochemotherapy of various skin diseases.[1] The utility of linear furocoumarins (psoralens) is limited by the side effects deriving from their ability to crosslink the two strands of DNA by binding to the thymine residues.[2–4] In previous work we pursued two of the most important strategies put forward to avoid this effect: fusing the furan ring to the coumarin moiety in [2,3-*h*] instead of [3,2-*g*] configuration so as to create an 'angular' furocoumarin, or angelicin,[5] and fusing a benzene ring to the furan ring.[6,7] Although the first attempts to combine these two strategies afforded compounds with little activity,[8–10] we have now found that compound **6**, a benzoangelicin with a dimethylaminopropyloxy group at position 5, is not only 10 times as effective as 8-methoxypsoralen (8-MOP) against HL60 and HeLa cells, but also appears to lack the cutaneous phototoxicity of 8-MOP.

Compound **6** was synthesized via angelicins **4** and **5** starting from 7-hydroxy-5-methoxy-4-methylcoumarin (**1**), as follows. Compound **4** was obtained from **1** as described previously[6] in an overall yield of 38% (the step from **2** to **3** gives a 3:2 mixture of **3** and its linear isomer 6,7,8,9-tetrahydro-5-methoxy-4-methylbenzofuro[3,2-*g*]coumarin). The methoxy group of **4** was hydrolized for 4 h with a refluxing mixture of hydriodic acid, acetic acid and acetic anhydride to give compound **5** in 97% yield after chromatography with methylene chloride as eluant.[11] The hydroxyl hydrogen was then replaced with a dimethylaminopropyl group by Williamson's reaction using either 3-chloro-*N*,*N*-dimethylpropylamine hydrochloride, sodium hydride and sodium iodide in refluxing DMF (30 min; yield 58% after chromatography with chloroform as eluant) or potassium carbonate and HMPA in refluxing acetone (2 h; yield 77%)[12] (Scheme 1).

The antiproliferative activity of compounds **4**–**6** was evaluated in vitro against human cervix adenocarcinoma (HeLa) cells and human promyelocytic (HL60) cells in parallel with that of the reference drug 8-MOP. Experiments were performed with and without irradiation at 365 nm (0.793 J cm^{-2}) using previously established procedures.[13] Compound **4** was inactive, and compounds **5** and **6** were only weakly active in the dark. Under irradiation, compound **5** was roughly as active as 8-MOP against HL60, and compound **6**, which has a protonable dimethylaminopropyloxy side-chain, was 10 times more active in both cell lines (Table 1). Tests of skin photosensitizing potency on Dunkin–Hartley depilated albino guinea pigs, performed as previously reported,[13] showed **4**–**6** to be completely devoid of phototoxicity at concentrations below 50 mg cm^{-2}, whereas 8-MOP is highly toxic at 10 mg cm^{-2} (Table 1).

To evaluate the binding of the new benzofurocoumarin **6** to double-stranded DNA, linear flow dichroism (LD) measurements were performed at a [DNA]/[drug] ratio of 12.5. A negative band in the chromophore absorption region of the resulting spectrum (310–380 nm) shows that **6** adopts a position parallel to the plane of the DNA bases (not shown). The reduced linear dichroism value ($LDr = LD/A_{iso}$) determined at the wavelength of

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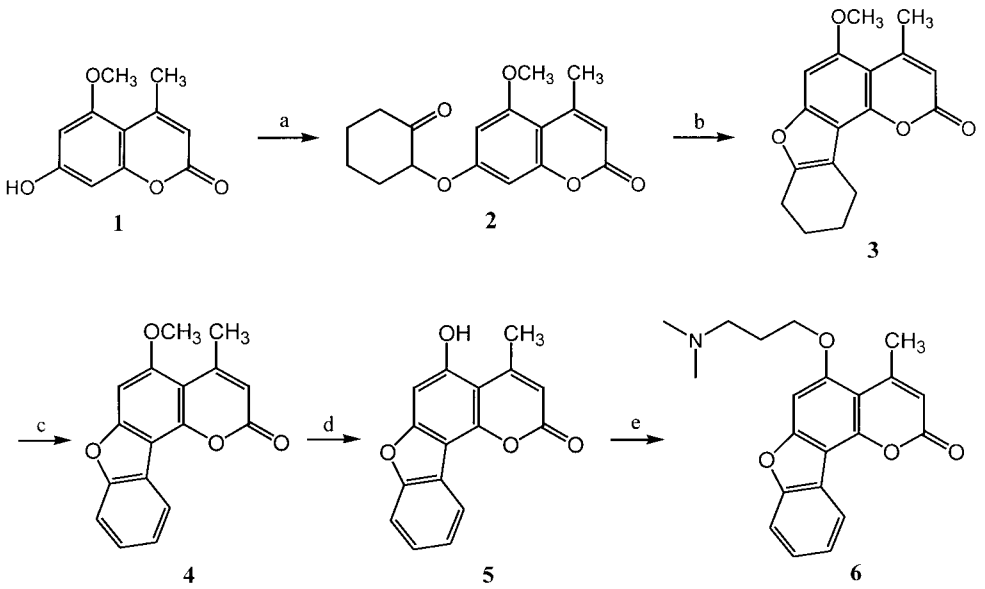

Scheme 1. Reagents: (a) 2-chlorocyclohexanone, K_2CO_3, Me_2CO, reflux; (b) NaOH, reflux; (c) DDQ, benzene, reflux; (d) HI, AcOH, Ac_2O, reflux; (e) 3-chloro-*N*,*N*-dimethylpropylamine, K_2CO_3, HMPA, Me_2CO, reflux.

Table 1. Antitumour activity of compounds **4**–**6** under irradiation at 365 nm

Compound	IC_{50} (mM)[a]		Erythema formation[b]
	HL60	HeLa	
4	>20	>20	−−−
5	6.95	>20	−−−
6	0.52	1.12	−−−
8-MOP	5.4	10	+ + +

[a]Concentration required to reduce proliferation of tumour cells by 50%.
[b]Symbols: + + +, severe erythema with edema; −−−, no erythema.

peak absorption by **6**, is close to that of the DNA absorption band at 260 nm, and implies[14] a value of 90° for the angle α defining the orientation of the ligand with respect to the axis of the double helix. The geometry of the DNA–drug complex is thus consistent with an intercalative binding mode similar to that of psoralens[15] and angelicins.[16]

Quantitative assays of photobinding by tritiated compound **6** to calf thymus DNA showed its photoreactivity to be significantly less than that of 8-MOP (Fig. 1). This lack of correlation between cytotoxicity and covalent interaction with DNA is unprecedented among furocoumarin derivatives. The inkling that other molecular mechanisms, apart from the covalent photoaddition to the DNA, are involved in the photobiological events evoked by the furocoumarins, is present in literature.[17,18] Nevertheless, their real contribution has not been evaluated yet. The peculiar behaviour of compound **6** can be considered an experimental confirmation of these alternative mechanisms and an evidence of their importance to determine the biological activity and, finally, the therapeutic effects of the furocoumarins. Studies to identify intracellular targets of compound **6** other than DNA are currently in progress.

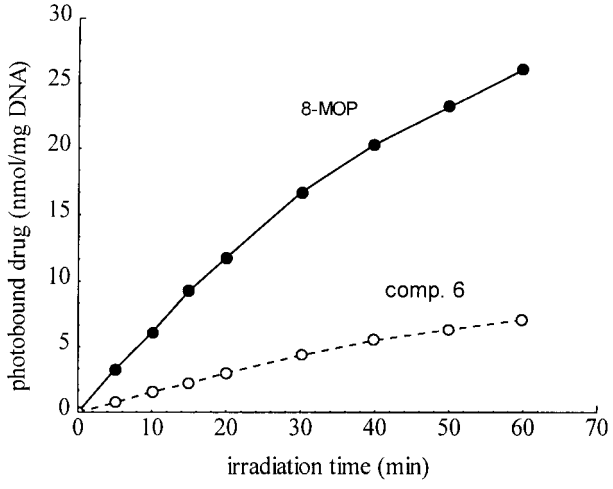

Figure 1. Photobinding of compound **6** and 8-MOP to double-stranded DNA from calf thymus (nucleotide–drug ratio = 75) as a function of irradiation time.

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11. 5-Hydroxy-4-methyl-2H-benzofuro[2,3-*h*]-1-benzopyran-2-one (**5**). Mp 332–334 °C. ^{1}H NMR (MeOD): 11.19 (s, 1H, OH), 8.02 (m, 1H, H-11), 7.65 (m, 1H, H-8), 7.44 (m, 2H, H-9 and H-10), 6.98 (s, 1H, H-6), 6.17 (d, 1H, H-3, $J=0.90$), 2.60 (d, 3H, CH_3, $J=0.90$) ppm. IR (KBr disc): 3148, 1689, 1639, 1612, 1444, 1366, 1342, 1103, 1086, 740 cm^{-1}. Anal. ($C_{16}H_{10}O_4$) C, H.
12. 5-(*N*,*N*-Dimethylaminopropyloxy)-4-methylbenzofuro[2,3-*h*]coumarin (**6**). Mp 140 °C (dec). ^{1}H NMR ($CDCl_3$): 8.31 (m, 1H, H-11), 7.54 (m, 1H, H-8), 7.41 (m, 2H, H-9 and H-10), 6.96 (s, 1H, H-6), 6.13 (d, 1H, H-3, $J=1.15$), 4.19 (t, 2H, O-CH_2, $J=6.35$), 2.66 (d, 3H, CH_3, $J=1.15$), 2.55 (t, 2H, N-CH_2, $J=7.10$), 2.31 (s, 6H, $N(CH_3)_2$), 2.11 (m, 2H, CH_2-CH_2-CH_2) ppm. IR (KBr disc): 2935, 1727, 1640, 1606, 1451, 1108, 1086 cm^{-1}. Anal. ($C_{21}H_{21}NO_4$) C, H, N.
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