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Synthesis, Photobiological Activity and Photoreactivity of Methyl-thieno-8-azacoumarins, Novel Bioisosters of Psoralen

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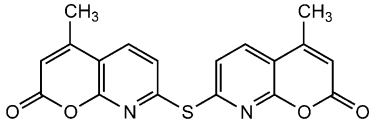
Abstract—4,4′-dimethyl-thieno-8-azacoumarin (**6**) and 4,4′,5′-trimethyl-thieno-8-azacoumarin (**8**) were synthesised. Their photobiological activity was tested on human tumour cell lines. Interestingly, for **6**, a photocytotoxic ability higher in HL-60, comparable in HeLa cells, with respect to that of the well-known drug 8-methoxypsoralen (8-MOP), was demonstrated. The covalent photoaddition to DNA occurs by means of the molecular mechanism already demonstrated for furocoumarins. However, it is noteworthy that no skin phototoxicity appears. © 2002 Elsevier Science Ltd. All rights reserved.

Oral administration of psoralens (P) followed by irradiation of the cutaneous area by long wave ultraviolet (UVA) radiation (PUVA therapy) has been successfully used for the treatment of numerous skin diseases, such as psoriasis, mycosis fungoides, lichen planus and vitiligo, and for the management of disorders related to the immune system, such as cutaneous T-cell lymphoma.[1,2]

The therapeutic effectiveness of this photochemotherapy in the treatment of skin diseases is well established, however its use is limited by some undesired side effects such as skin phototoxicity (induction of erythema and blisters).[3,4] One of the main goals of the research in this field is to synthesise new derivatives which retain the therapeutic benefits but lack the adverse effects. In this regard, the synthesis of new psoralen isosters is one of the more important efforts. Among the various chemical approaches, replacement of the central benzene ring of the furocoumarin structure with the pyridine ring leads to 8-azapsoralens.[5] In particular, a clinical evaluation of 4,4′,5′-trimethyl-8-azapsoralen by topical treatment of psoriatic plaques has revealed a therapeutic effect and clearing ability lower than that of the well-known drug 8-methoxypsoralen (8-MOP), but no appearance of erythematous reaction.[6–8] Among the furocoumarin isosters, interesting results have also been obtained with thienocoumarins, that is coumarin derivatives where the oxygen atom of the furane ring has been replaced with a sulfur.[9] It has been demonstrated that these compounds are able to exert a higher antiproliferative activity on human cell lines than that of 8-MOP.[9,10]

This paper reports the synthesis of two new furocoumarin isosters, 3,5-dimethyl-7*H*-pyrano[2,3-*b*]thieno[3,2-*e*]pyridin-7-one (4,4′-dimethyl-thieno-8-azacoumarin, compound **6**) and 2,3,5-trimethyl-7*H*-pyrano[2,3-*b*]thieno[3,2-*e*]pyridin-7-one (4,4′,5′-dimethyl-thieno-8-azacoumarin, compound **8**), characterised by having a pyridine nucleus instead of a central benzene ring and a sulfur atom which substitutes the oxygen of the furane ring. The photobiological behaviour of these new thieno-8-azacoumarins has also been investigated.

The synthesis of new thieno-8-azacoumarins started with 4-methyl-7-hydroxypyrano[2,3-*b*]pyridin-2-one (**1**), which was condensed with *N*,*N*-dimethylthiocarbamoyl chloride in acetone solution in the presence of potassium carbonate (Scheme 1). Later, heating in a thermostatic bath at 220 °C under a constant flux of nitrogen produced isomerisation of *N*,*N*-dimethylthiocarbamate (**2**) to *N*,*N*-dimethylcarbamate (**3**). Heating was limited to 2 min to avoid the fast formation of dimer (I).



I

The hydrolysis of **3** in alkaline medium yielded the key compound 7-mercapto-4-methyl-8-azacoumarin (**4**).

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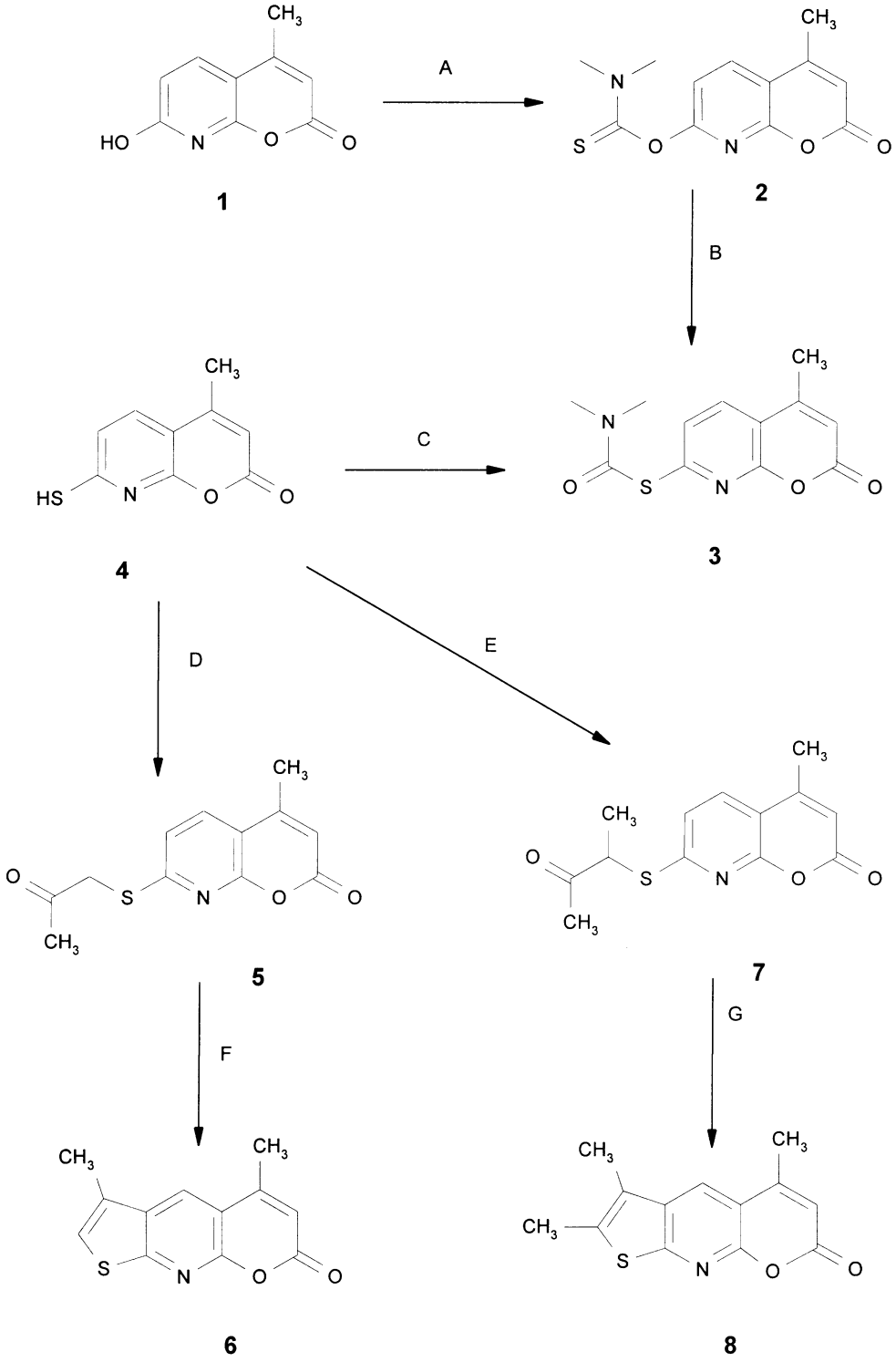

A: N,N-dimethylthiocarbamoyl chloride, K_2CO_3, acetone, 20 h, reflux.
B: 220 °C, N_2, 2'.
C: KOH, MeOH, 3 h, reflux.
D: chloroacetone, K_2CO_3, acetone, 3 h, reflux.
E: 3-chlorobutan-2-one, K_2CO_3, acetone, 4 h, reflux.
F: KOH, MeOH, 3,5 h, reflux.
G: KOH, MeOH, 45', reflux.

Scheme 1.

7-Mercapto-4-methyl-8-azacoumarin (**4**) was condensed with chloroacetone in acetone solution in the presence of K_2CO_3. Thioether **5**, submitted to cyclisation in alkaline medium, yielded 3,5-dimethyl-7*H*-pyrano[2,3-*b*]thieno[3,2-*e*]pyridin-7-one (**6**). Condensing 7-mercapto-4-metil-8-azacoumarin (**4**) with 2-chlorobutanone in the same way led to the corresponding ether **7**, which was cyclised in alkaline conditions obtaining 2,3,5-trimethyl-7*H*-pyrano[2,3-*b*]thieno[3,2-*e*]pyridin-7-one (**8**).

The antiproliferative activity of **6** and **8** after UVA irradiation (365 nm, 0.793 J cm^{-2}) has been evaluated on two human tumour cell lines, HeLa and HL-60, using previously established procedures.[11] The results obtained, reported as IC_{50} values, are shown in Table 1. 8-MOP was used as reference drug. As already found for 8-MOP,[11] the new test compounds also appear to be more effective toward HL-60 cells compared with HeLa.

In detail, **6** appears to be more active than the reference drug in HL-60. In fact, its IC_{50} value is half compared with 8-MOP. In contrast, no significant differences emerge from the results obtained for HeLa.

The IC_{50} of **8** is higher than that of the drug in HL-60 cells, while the value found in HeLa indicates that it is practically inactive in these cells.

In general, it was observed that the introduction of methyl groups enhances the photobiological potency of the furocoumarin moiety.[12,13] Nevertheless, a comparison between the antiproliferative activity of **6** and **8** indicates that the presence of the third methyl in the 5′ position of the thiophene ring causes a considerable decrease in antiproliferative effect. It is known that the biological activity of psoralens is mainly due to their ability to photoadd to the double helix of DNA. In detail, after the formation of an intercalation complex between two base pairs, upon irradiation with UVA light, the 4′,5′ double bond of the furane ring and/or the 3,4 double bond of the pyrone ring can react with the 5,6 double bond of pyrimidine bases, mainly thymine, leading to monoadducts or diadducts linking both strands of DNA. The lower photobiological activity observed for **8**, with respect to **6** could be ascribed to physico-chemical properties derived from the methyl group inserted in 5′ position which may be responsible for greater difficulty in establishing a correct overlapping of the photoreactive sites as well as a decrease in solubility in aqueous media.

Table 1. Cell-growth inhibition and skin phototoxicity after UVA irradiation

Compd	IC_{50} (μM)		Skin phototoxicity	
	HeLa	HL-60	Dose (μmol cm^{-2})	Formation of erythema[a]
6	8.9±0.6	2.2±0.1	0.2	–
8	>20	7.3±0.3	0.2	–
8-MOP	10±3	5.4±0.7	0.05	+ + + (with edema)

[a] + + +, strong; –, absent.

Furthermore, both **6** and **8** are totally ineffective in the dark, like the reference compound (data not shown).

The appearance of erythema (a marker of cutaneous photosensitisation) is one of the most commonly reported undesired short-term side effects which follow phototherapeutic treatment with 8-MOP in PUVA therapy. To verify skin photosensitisation, experiments on depilated skin of Dunkin–Hartley guinea pigs have been performed as previously described.[10] The results obtained, reported in Table 1, reveal that both new thienoazacoumarins are clearly not phototoxic. Indeed, they do not induce any erythematous reaction even though at a dose 4 times higher than that of 8-MOP.

Significant antiproliferative activity together with a lack of skin photosensitisation, has stimulated further studies to investigate the molecular mechanism which could account for the effects of the new thienoazacoumarin derivative **6**. The biological events which take place after irradiation in the presence of a furocoumarin have been mainly attributed to its ability to photoreact with DNA giving rise to a covalent cycloadduct. Nevertheless, as reported above, the preliminary step, essential for a subsequent effective photoaddition to the macromolecule, is that the furocoumarin molecule undergoes intercalation between two base pairs prior to irradiation. Linear flow dichroism experiments have allowed us to analyse the interaction process between **6** and DNA. In Figure 1, the spectra of a solution of DNA alone (trace a), in the presence of 8-MOP or **6** (traces b and c, respectively), are depicted. For **6**, like 8-MOP, it is possible to observe a dichroic signal in the long-wavelength (310–400) region of the spectra where DNA has no absorption. The existence of this signal immediately confirms the ability of the compound to interact with the macromolecule. Indeed, this small molecule cannot undergo an orientation under flow field. Furthermore, according to previous results obtained for 8-MOP,[14] for the new thienoazacoumarin,

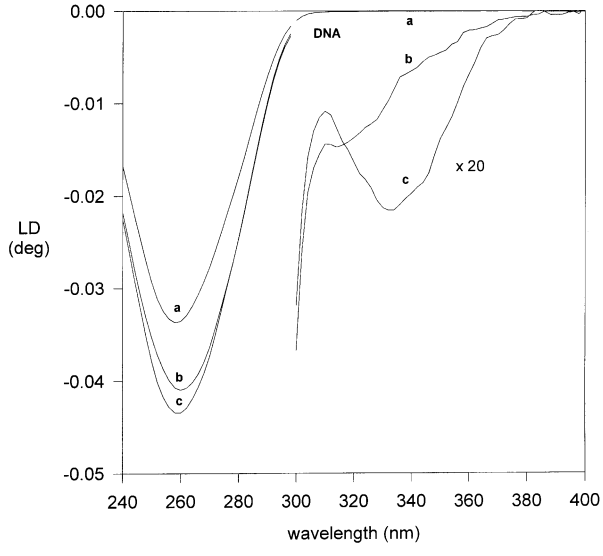

Figure 1. Linear flow dichroism spectra of DNA, 8-MOP and compound **6** (traces a–c, respectively). [DNA] = 1.89×10^{-3} M, [DNA]/[drug] = 25.

the negative sign of this signal can also be retained in qualitative agreement with an orientation of the tricyclic plane preferentially parallel to the plane of the DNA bases. This would be expected if the compound undergoes an intercalative mode of binding with the macromolecule.

The photoreaction of the linear furocoumarin moiety (psoralen) can give rise to the formation of covalent diadducts between two pyrimidine bases of opposite strands of the DNA helix (interstrand cross-links). This lesion plays an important role in the photobiological effects of the psoralens. However, in some cases it has been indicated as related to undesired side effects which follow PUVA therapy such as skin phototoxicity[15] and genotoxicity.[16,17] It therefore appears interesting to evaluate the ability of **6** to form cross-links. Figure 2 shows the results of denaturation-renaturation experiments, performed as previously indicated.[10] The number of cross-links induced by **6** is clearly higher than the reference drug. These results indicate that the thienoazacoumarin moiety is an interesting photobiological lead to be developed further.

Isolation of the adducts which derive from photoreaction with DNA could be of interest to elucidate in greater depth the mechanism of the action. Following an established experimental procedure,[11] the irradiation of a DNA solution (365 nm) in the presence of **6**, followed by precipitation, acid hydrolysis and extraction with $CHCl_3$, allowed us to separate the DNA bases from photoadducts. Subsequent TLC purification ($CHCl_3$/ethanol, 9:1) allowed a violet fluorescent band to be highlighted. This is usually considered to be consistent with the monoadduct resulting from the C_4-photocycloaddition, which involves the 4′,5′ double bond of the furocoumarin moiety and the 5,6 double bond of thymine.[18] This assumption has been further supported by photoreversion experiments. When irradiated at 254 nm, C_4-cycloadducts between furocoumarins and pyrimidine bases are broken yielding the parent compounds. In our experiments too, the UV spectrum of an ethanol solution containing the fluorescent product showed a gradual modification during irradiation at 254 nm. In detail, the peak around 340 nm decreases, increasing irradiation time from 0 to 120 min, and a parallel increase in the absorption band at 323 nm, characteristic of **6**, is observed (data not shown). Finally, to confirm the above-mentioned hypothesis, the fluorescent product extracted from the silica has been characterised by means of ^{1}H NMR experiments. Selected NMR data (acetone-d_6; δ, ppm and J, Hz): 8.78 (br, 1H, 3-H_T), 7.68 (s, 1H, 5-H), 6.80 (br, 1H, 1-H_T), 6.27 (q, 1H, 3-H, $J=1.4$), 4.59 (d, 1H, 5′-H_T, $J=6.6$), 4.21 (dd, 1H, 6-H_T, $J=6.6$ and 2.9), 2.42 (d, 3H, 4-H, 4-Me, $J=1.4$), 1.67 (s, 3H, 4′-Me), 1.63 (s, 3H, 5-Me_T). They confirm that the isolated product is a C_4-cycloadduct between the 4′,5′ double bond of the 4,4′-dimethyl-thieno-8-azacoumarin and the 5,6 double bond of thymine. Indeed, the disappearance of the signal of the proton in 5′ position of the thienoazacoumarin from the aromatic region, and the shift to highfield, highlights the thiophene ring's loss of aromaticity. The coupling pattern of protons of the newly formed cyclobutane ring shows the proximity between 6-H of the base and 5′-H of the thiophene ring of thienoazacoumarin. Moreover, coupling constants values are in agreement with those found in several furan side adducts with *cis* stereochemistry. The proposed molecular structure for the furan side adduct obtained between **6** and thymine is shown in Figure 3.

In summary, the onset of skin phototoxicity constitutes a severe limitation to PUVA therapy. On the basis of clinical evaluation, the phototherapeutic profile of 4,4′,5′-trimethyl-8-azapsoralen appears to be of interest due to the absence of an eritematous reaction. Nevertheless, a lower therapeutic effectiveness with respect to 8-MOP, was detected for this furocoumarin isoster.[6]

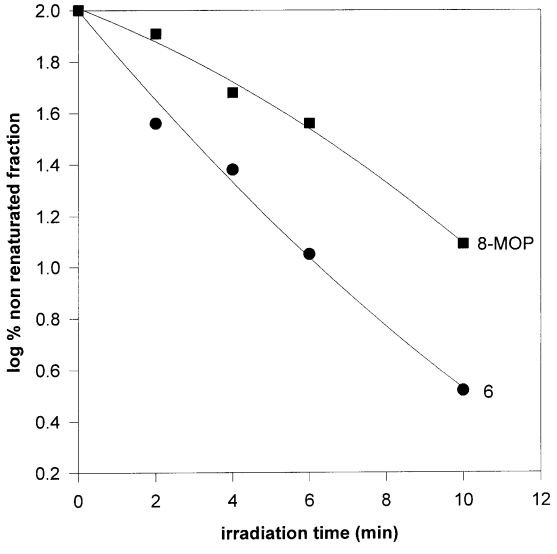

Figure 2. Cross-linking of compound **6** and 8-MOP to double-stranded DNA as a function of irradiation time. [DNA]/[drug] = 75.

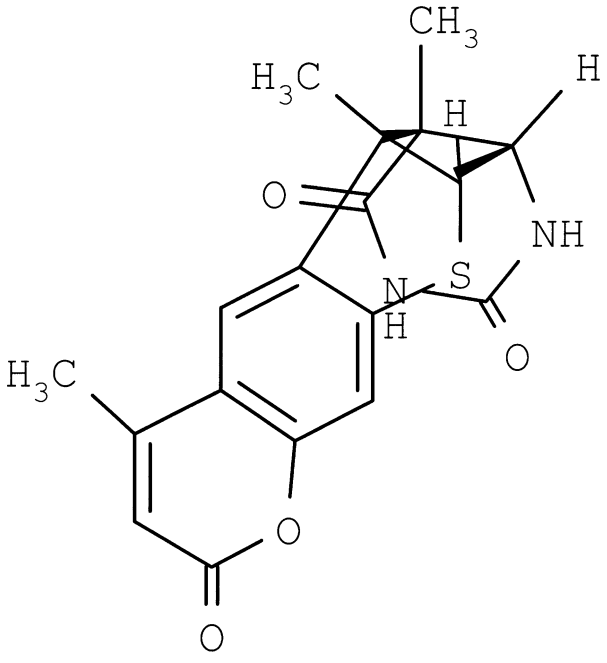

Figure 3. Molecular structure of furan side adduct between **6** and thymine.

Within the class of furocoumarin isosters, the thienocoumarins also displayed interesting photobiological properties. Indeed a noticeable antiproliferative activity on human tumour cell lines (up to one order of magnitude higher than 8-MOP) has been found.[10] In this paper, the synthesis of a thieno-8-azacoumarin nucleus, along with photobiological studies, have been reported. In particular, it was possible to highlight some interesting features of the 4,4′-dimethyl-thieno-8-azacoumarin (**6**). This furocoumarin isoster, as well as the absence of an eritematous effect, displays an antiproliferative activity on human tumour cell lines which is higher with respect to that of the reference drug. Therefore, the thieno-8-azacoumarin derivative **6** appears worth consideration as a new lead compound in the future development of photochemotherapeutic drugs suitable for PUVA therapy.

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