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SYNTHESIS AND PHOTOBIOLOGICAL PROPERTIES OF 4-HYDROXYMETHYL-4′-METHYLPSORALEN DERIVATIVES

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Abstract—The synthesis and the photobiological activity of two new hydroxymethyl derivatives of psoralen namely 4-hydroxymethyl-4′-methyl- and 4-hydroxymethyl-4′-methyl-8-methoxypsoralen are described. Both compounds exhibited efficient photobinding to DNA and RNA. The DNA-photobinding process was investigated using different nucleic acid structures such as double-helical DNA, ribosomal RNA, bacterial DNA and DNA organized in the nucleosomal arrangement. The test derivatives were able to induce cross-links to a similar extent as 8-methoxypsoralen (8-MOP), used as a reference photochemotherapeutic drug. In contrast to 8-MOP, they produced relatively high levels of $^1O_2$. Most photobiological effects (DNA synthesis inhibition, T_2 phage sensitization, inhibition of tumor transmitting capacity) showed a good correlation with the extent of covalent photoaddition. On the other hand, the new 4-hydroxymethylpsoralens were unable to induce skin erythema, in striking contrast with 8-MOP. Thus, neither cross-linking of the nucleic acid nor $^1O_2$ production were coupled with skin phototoxicity in this class of compounds. The new derivatives appear to represent an important beginning to development of new active photochemotherapeutic agents devoid of undesired phototoxic side effects.

INTRODUCTION

Psoralens are a class of naturally occurring potent skin photosensitizers that are used in the photochemotherapy of various skin diseases.[1] The clinical treatment consists of an oral or topical administration; subsequently, the skin of the patient is irradiated with long-wavelength light (UV-A).† The basic molecular event underlying the biological effects is covalent photoaddition of the drug to the pyrimidine bases of DNA. Both monoadducts and di-adducts can be formed, involving one or both strands of DNA. In addition to nucleic acids, other possible biological targets are represented by membrane lipids and proteins.[2,3] Notwithstanding their wide use, psoralens exhibit a number of undesired side effects, including erythema and genotoxicity and the induction of cutaneous tumors.[1] Skin phototoxicity has been associated with covalent binding to DNA, in particular the formation of cross-linkages,[4] or with the production of singlet-oxygen species.[5–7] Only very few linear furocoumarins were found to be nonphototoxic.[8] All of them, however, were able to cross-link DNA to a very limited extent or were metabolized *in vivo* to inactive compounds.[1] A correlation between singlet oxygen production and skin phototoxicity was also proposed for a number of linear furocoumarins.[5,6] However, the same does not hold for angular furocoumarins.[8] Some examples do exist for very efficient singlet oxygen-producing psoralens that are not phototoxic.[8] To gain further insight into the relationship between DNA damage and erythema, we report here data obtained on two new derivatives of psoralen bearing hydroxymethyl and methyl substituents at position 4 and 4′, respectively (compounds **9** and **10** in Scheme 1). The initial aim of our work was to obtain very reactive psoralens, exhibiting reasonably high solubility in aqueous media. In addition the new compounds could represent interesting analogues of hydroxymethyl-benzopsoralens, which were devoid of skin phototoxicity.[9,10]

MATERIALS AND METHODS

Chemical synthesis

Melting points, uncorrected, were determined using a Buchi 510 apparatus. Infrared spectra were recorded on a Perkin-Elmer 781 and a 1640 spectrometer (KBr discs). The NMR spectra were recorded on a Varian CFT-20 (80 MHz) using tetramethylsilane (TMS) as internal standard (chemical shifts in δ values, J in Hz).

Ultraviolet spectra were recorded with a Perkin-Elmer 554 instrument. Elemental analyses were obtained on a Perkin-Elmer 240 B instrument (chlorine content: Schoeniger method). Preparative column chromatography (CC) was performed with silica gel (Merck 60, 70–230 mesh) and analytical thin-layer chromatography (TLC) was performed on precoated silica gel plates Merck (60 F254, 0.25 mm).

*4-Chloromethyl-7-hydroxycoumarin (**3**).* To 1,3-dihydroxybenzene (resorcinol, **1**) (10 g, 90.9 mmol) in concentrated H_2SO_4 (100 mL) at 0°C, ethyl 4-chloroacetoacetate (10 mL, 73.7 mmol) was added. The mixture was stirred 10 h at room temperature, then poured into water (500 mL) and left overnight. A yellow solid precipitated, which was recovered by filtration, washed with water and crystallized from ethyl alcohol–ligroin (4:1). Yield 10.5 g. Melting point 181°C. ^{1}H-NMR (D6-DMSO): 4.95 (s, 2H, $-CH_2-$); 6.42 (s, 1H, H3); 6.76 (d, 1H, J = 2.4, H8); 6.82 (dd, 1H (J = 2.4, 8.5), H6); 7.68 (d, 1H, J = 8.5, H5); 10.62 (s, 1H, OH). IR: 3300(OH); 3070; 1700(CO); 1607 and 1560 (C=C); 1319; 1137 cm^{-1}. $C_{10}H_7O_3Cl$. Calculated C 57.02, H 3.34, Cl 16.62; found C 56.65, H 3.37, Cl 16.83.

*4-Chloromethyl-7-hydroxy-8-methoxycoumarin (**4**).* This was prepared from 1,3-dihydroxy-2-methoxybenzene (**2**) in the same manner as described above. The product was purified by CC using toluene–ethylacetate (9:1). It was recrystallized from toluene. Yield 78%. Melting point 176°C. ^{1}H-NMR (D6-DMSO): 3.83 (s, 3H, CH_3O); 4.94 (s, 2H, $-CH_2-$); 6.44 (s, 1H, H3), 6.92 (d, 1H, J = 8.9, H6);

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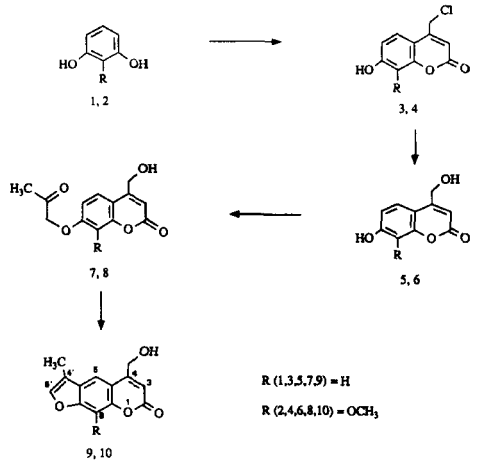
† *Abbreviations:* CC, column chromatography; 8-MOP, 8-methoxypsoralen; RNO, p-nitrosodimethylaniline; TLC, thin-layer chromatography; TMS, tetramethylsilane; UV-A, long-wavelength ultraviolet radiation.



Scheme 1

7.42 (d, 1H, J = 8.9, H5); 10.44 (s, 1H, OH). IR: 3388 (OH); 3120; 3095; 2951; 1718 (CO); 1608 and 1515 (C=C); 1438; 1219 cm^{-1}. $C_{11}H_9O_4Cl$. Calculated C 54.90, H 3.77, Cl 14.76; found C 54.92, H 3.72, Cl 14.92.

7-Hydroxy-3-hydroxymethylcoumarin (5). Compound **3** (1 g, 4.75 mmol) in H_2O (400 mL) was heated 5 h under reflux. The solution was concentrated to 150 mL and then was left overnight at 0°C. A solid precipitated, which was recrystallized from toluene. Yield 840 mg. Melting point 227°C. ^{1}H-NMR (D6-DMSO): 4.70 (d, 2H, J = 1.2, $-CH_2-$); 6.24 (d, 1H, J = 1.2, H3); 6.73 (d, 1H, J = 1.3, H8); 6.78 (dd, 1H (J = 2.4, 7.7), H6); 7.52 (dd, 1H (J = 1.3, 7.7), H5); 10.48 (bs, 1H, HO). IR: 3435 (OH); 3150, 1700 (CO); 1625, 1610 and 1570 (C=C); 1320; 1140; 1095; 840 cm^{-1}. $C_{10}H_8O_4$. Calculated C 62.50, H 4.19; found C 62.35, H 4.23.

7-Hydroxy-3-hydroxymethyl-8-methoxycoumarin (6). This was prepared from compound **4** in the same manner as described above. Product **6** was purified by CC using toluene–ethyl acetate (1:1) and recrystallized from toluene. Yield 95%. Melting point 197°C. ^{1}H-NMR (D6-DMSO): 3.83 (s, 3H, CH_3O); 4.70 (s, 2H, $-CH_2-$); 6.27 (s, 1H, H3); 6.86 (d, 1H, J = 8.8, H6); 7.26 (d, 1H, J = 8.8, H5); 10.29 (s, 1H, HO). IR: 3280 (OH); 3110; 2940; 1690 (CO); 1605 and 1580 (C=C); 1435; 1325; 1070; 860 cm^{-1}. $C_{11}H_{10}O_5$. Calculated C 59.46, H 4.53; found C 59.49, H 4.50.

7-Acetonyloxy-4-hydroxymethylcoumarin (7). To a solution of **5** (1 g, 5.20 mmol) in anhydrous acetone (300 mL), chloroacetone (0.5 mL, 6.28 mmol) and K_2CO_3 (4 g) were added. The reaction mixture was heated 10 h to reflux. The precipitate was filtered out and the acetone solution was then evaporated under vacuum. The solid residue was purified by CC using ethyl acetate–hexane (9:1). Subsequent recrystallization from ethyl alcohol gave 880 mg. Melting point 168–169°C. ^{1}H-NMR (D6-DMSO): 2.17 (s, 3H, CH_3); 4.73 (s, 2H, $-CH_2OH$); 4.98 (s, 2H, $-CH_2-CO$); 5.59 (s, 1H, HO); 6.31 (s, 1H, H3); 6.91 (dd, 1H (J = 2.3, 7.7), H6); 6.96 (d, 1H, J = 2.3, H8); 7.60 (d, 1H, J = 7.7, H5). IR: 3421 (OH); 3110; 2921; 1716 (CO); 1615 (C=C); 1286; 1088; 852 cm^{-1}. $C_{13}H_{12}O_5$. Calculated C 62.90, H 4.87; found C 62.61, H 5.06.

7-Acetonyloxy-4-hydroxymethyl-8-methoxycoumarin (8). This compound was prepared from **6** in the same manner as described for compound **7**. Product **8** was recrystallized from ethyl alcohol–ligroin. Yield 72.5%. Melting point 164–165°C. ^{1}H-NMR (D6-DMSO): 2.18 (s, 3H, CH_3CO); 3.88 (s, 3H, CH_3O); 4.70 (d, 2H, J = 1.2, $-CH_2-OH$); 5.02 (s, 2H, $-CH_2CO$); 5.59 (s, 1H, HO); 6.33 (d, 1H, J = 1.2, H3); 6.96 (d, 1H, J = 9.0, H6); 7.34 (d, 1H, J = 9.0, H5). IR: 3363 (OH); 3115; 2940; 2904; 1716 (CO); 1676 and 1605 (C=C); 1302; 1110 cm^{-1}. $C_{14}H_{14}O_6$. Calculated C 60.43, H 5.07; found C 60.19, H 5.07.

4-Hydroxymethyl-4'-methylpsoralen (9). To a solution of **7** (1 g, 4.03 mmol) in ethyl alcohol (200 mL), 0.1 *N* NaOH (200 mL) was added. The mixture was heated 3 h to reflux. It was then acidified with HCl, evaporated to half volume and extracted with chloroform. The extract was washed with water, dried (Na_2SO_4), and the solvent was removed under reduced pressure to leave a residue that was purified by CC using toluene–ethyl acetate (4:1). Recrystallization was performed in toluene. Yield 206 mg. Melting point 228°C. ^{1}H-NMR (D6-DMSO): 2.26 (d, 3H, J = 1.2, CH_3); 4.85 (d, 2H, J = 1.3, $-CH_2-$); 5.66 (s, 1H, HO); 6.44 (d, 1H, J = 1.3, H3); 7.64 (s, 1H, H8); 7.86 (d, 1H, J = 1.2, H5'); 7.92 (s, 1H, H5). IR: 3380 (OH); 3110; 2950; 2920; 1700 (CO); 1630, 1615, 1595 and 1580 (C=C); 1330; 1310; 1135; 880 cm^{-1}. $C_{13}H_{10}O_4$. Calculated C 67.82, H 4.38; found C 67.90, H 4.29.

4-Hydroxymethyl-4'-methyl-8-methoxypsoralen (10). This compound was prepared from **8** in the same manner as described for compound **9**. The desired compound **10** was purified by CC using toluene–ethyl acetate (1:1); recrystallized from ethyl alcohol–ligroin. Yield 71%. Melting point 241°C. ^{1}H-NMR (D6-DMSO): 2.24 (d, 3H, J = 1.3, CH_3-); 4.16 (s, 3H, CH_3O); 4.85 (d, 2H, J = 1.5, $-CH_2-$); 5.67 (s, 1H, HO); 6.45 (d, 1H, J = 1.5, H3); 7.58 (s, 1H, H5); 7.89 (d, 1H, J = 1.3, H5'). IR: 3400 (OH); 3130; 2950; 1700 (CO); 1625, 1615 and 1590 (C=C); 1400; 1090; 870 cm^{-1}. $C_{14}H_{12}O_5$. Calculated C 64.61, H 4.65; found C 64.42, H 4.66.

Table 1. Physicochemical properties of derivatives **9** and **10** and of the reference drug 8-MOP

Compound	Water solubility (mg/L)	Molar extinction at 365 nm (in ethanol)	Singlet oxygen generation (% RNO bleaching)*
9	25	1150	1.4
10	15	980	0.4
8-MOP	23	1150	0.85

*After 4 min irradiation.

Chemicals

8-Methoxypsoralen (8-MOP) was purchased from Chinoin SpA (Milan, Italy). 4-Hydroxymethyl-4'-methylpsoralen (compound **9**) and 4-hydroxymethyl-4'-methyl-8-methoxypsoralen (compound **10**) were prepared as described above. Radioactive samples of the furocoumarins were prepared by Amersham Radiochemical Centre (Amersham, U.K.) and purified on TLC plates as described.[11] DNA from calf thymus, was purchased from Sigma Chemical Company (St. Louis, MO). Its hypochromicity was over 35%. RNA from baker's yeast, DNA from *Micrococcus lysodeikticus* and DNA from *Clostridium perfringens* were purchased from Sigma. Calf thymus nucleosomes (175 bp) were obtained according to the literature.[12]

Photochemical and photobiological methods.

UV light source. Irradiations were performed by means of Philips HPW 125 lamps equipped with a filter emitting over 90% at 365 nm; the irradiation intensities determined by a potassium ferrioxalate chemical actinometer were 5.5 and 9.3 J s^{-1} m^{-2} for the photochemical and the photobiological experiments, respectively.

Interaction with DNA in vitro: *photobinding experiments.* The experiments with DNA from calf thymus and with RNA as well as cross-links evaluation were performed as previously reported.[13] Following irradiation in the presence of test compounds, nucleosomes and DNA from *M. lysodeikticus* and from *C. perfringens* were exhaustively dialyzed using a 10000 Da cut-off membrane (Thomas Scientific, Philadelphia, PA) to remove unreacted material and low molecular weight by-products. Negligible dilution effects were observed in the DNA-containing compartment. Appropriate control experiments showed complete removal of tritiated drug in the absence of irradiation without loss of macromolecular species.

Photobiological effects. DNA synthesis inhibition in Ehrlich ascites cells and T2 phage sensitization were determined as previously described.[14] Drug concentrations were 2×10^{-5} *M*.

Skin phototoxicity. The ability to induce skin erythema was studied in albino guinea pigs (outbred Dunkin-Hartley strain); the com-

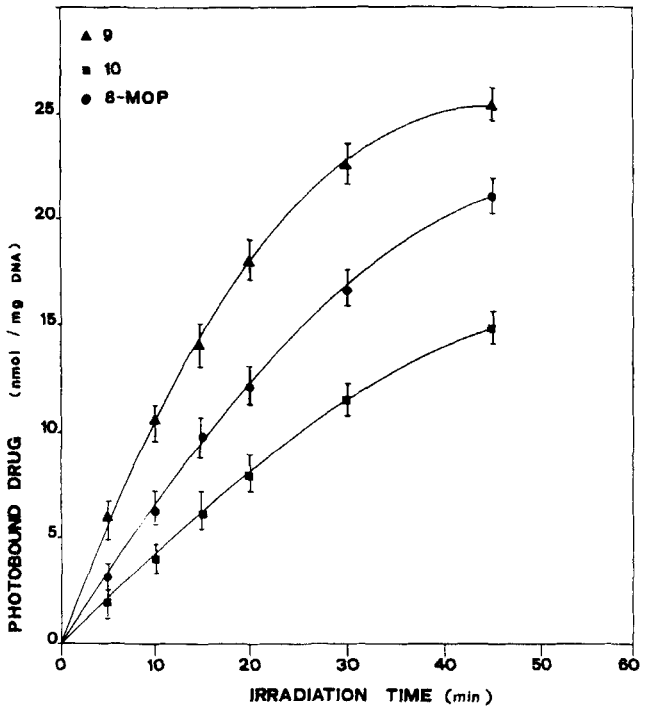


Figure 1. Photobinding of test compounds **9** and **10** and 8-MOP to double-stranded DNA from calf thymus (drug concentration = 2×10^{-5} *M*; nucleotide/drug ratio = 75) as a function of irradiation time.

pounds were applied topically on the depilated skin as a 0.1% solution in methanol. The animals were kept for 45 min in a dark room and then the treated skin was irradiated; erythema was scored after 48 h.

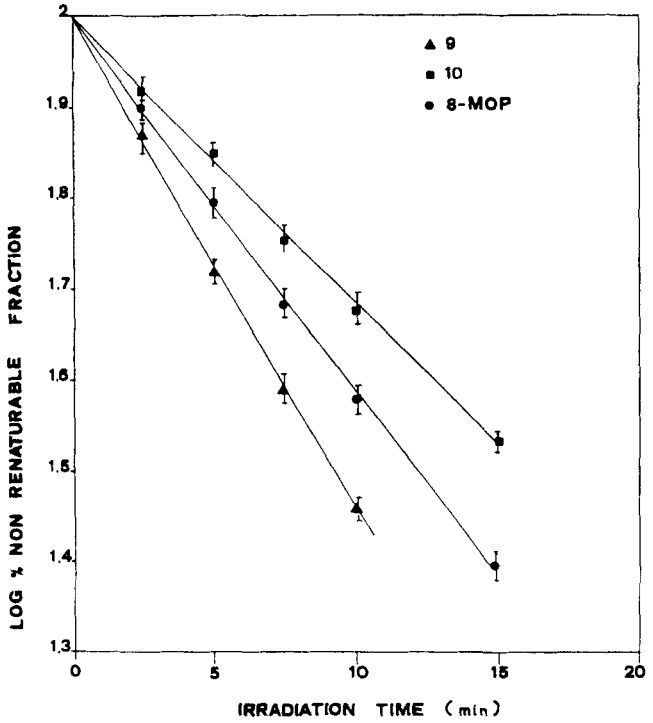


Figure 2. DNA cross-linking of the test compounds **9** and **10** and 8-MOP to calf thymus DNA (drug concentration = 2×10^{-5} *M*; nucleotide/drug ratio = 75) as a function of irradiation time.

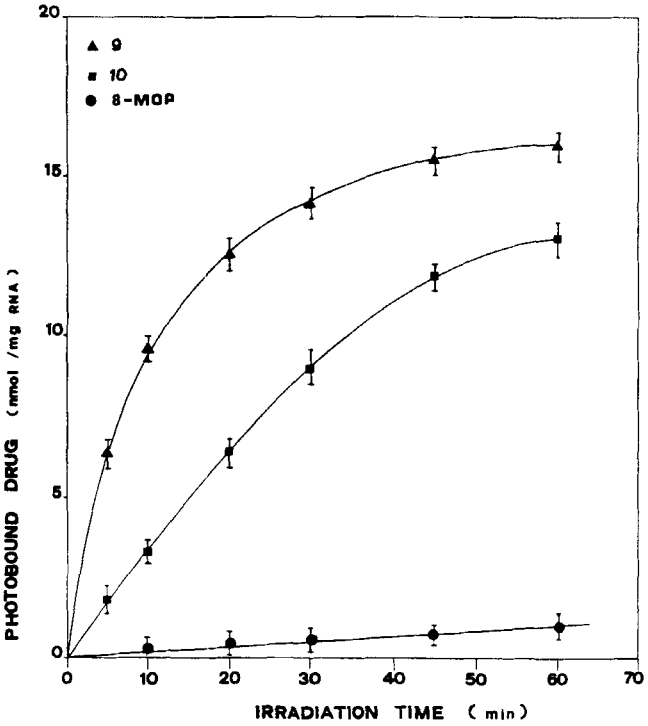


Figure 3. Photobinding of test compounds **9**, **10** and 8-MOP to ribosomal RNA from baker's yeast (drug concentration = 2×10^{-5} *M*; nucleotide/drug ratio = 75) as a function of irradiation time.

RESULTS

Chemical synthesis

The synthesis of 4-hydroxymethylpsoralens (**9** and **10**) was performed starting from resorcinol (**1**) or 2-methoxyresorcinol (**2**), respectively, according to the pathways shown in Scheme 1 and described in the previous section.

Treatment of compounds **1**, **2** with ethyl 4-chloroacetoacetate led to the related 4-chloromethylcoumarins **3**, **4** involving a Pechmann condensation and further hydrolysis of halogen derivatives to yield the 4-hydroxymethyl-7-hydroxycoumarins **5** and **6**. The synthesis of the furane ring was performed by reaction of 7-hydroxycoumarins and chloroacetone in acetone solution in the presence of potassium carbonate, followed by treatment of the corresponding acetonyl derivatives in alkaline solution, and acidification to obtain the final psoralen derivatives **9** and **10**. Selected physicochemical properties of these compounds are reported in Table 1.

Photobinding to nucleic acids

Photobinding to DNA from calf thymus. Irradiation of an aqueous solution of DNA in the presence of ^{3}H-hydroxymethyl derivatives allowed a determination of the amount of furocoumarin covalently linked to nucleic acid. 8-Methoxypsoralen was also tested as a reference. The extent of photobinding of these compounds as a function of irradiation time is reported in Fig. 1.

The photobinding efficiency of **9** is more or less twice as high as **10**, whereas the reference drug 8-MOP exhibits an intermediate photoreactivity. Both new derivatives are able

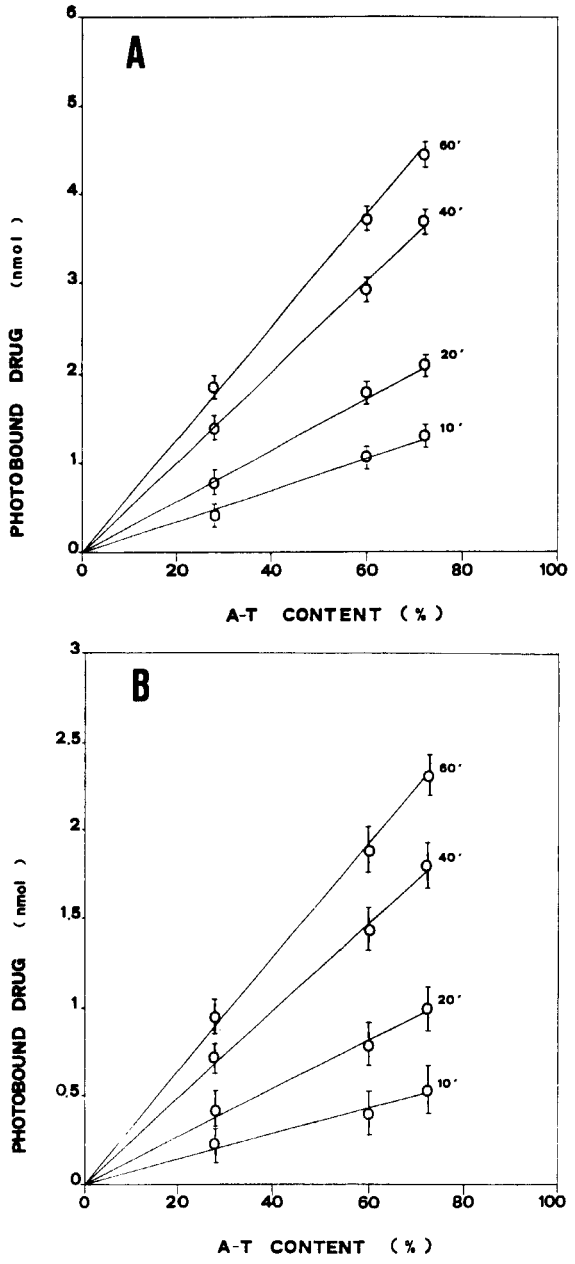


Figure 4. Photobinding of test compounds to DNA (drug concentration = $2 \times 10^{-5} M$; nucleotide/drug ratio = 10) as a function of % A-T content (A: compound **9** B: compound **10**).

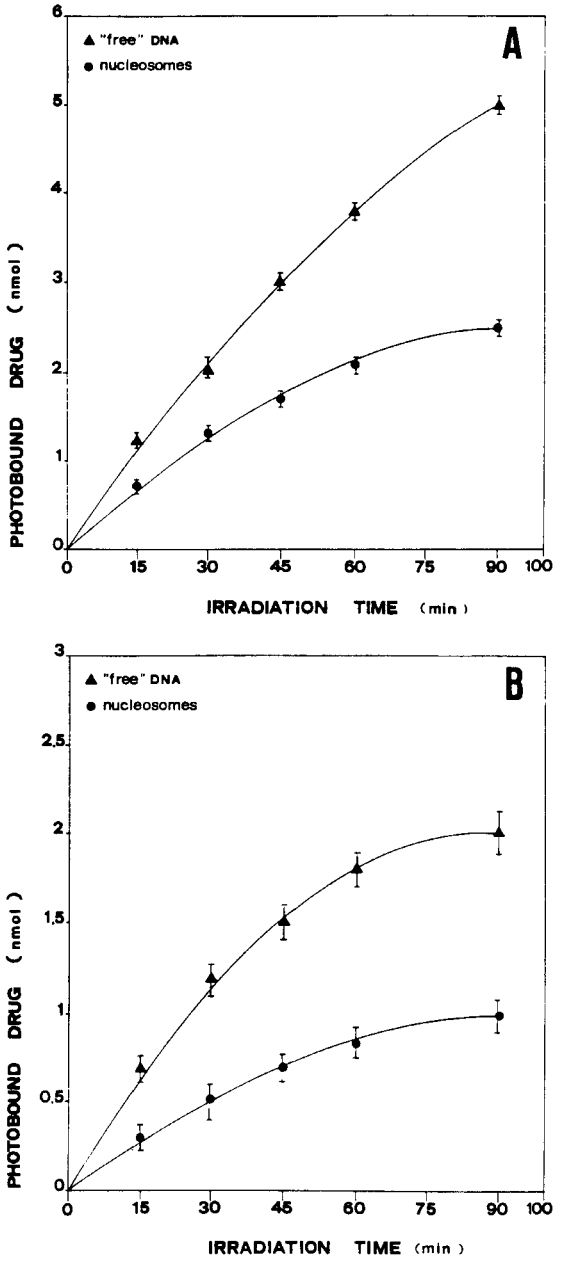


Figure 5. Photobinding of test compounds to 175 bp nucleosomes (drug concentration = $2 \times 10^{-5} M$; nucleotide/drug ratio = 10) as a function of irradiation time (A: compound **9**; B: compound **10**).

to induce cross-links to an extent that parallels the photoreactivity exhibited by each drug. The results are reported in Fig. 2.

Photobinding to RNA from baker's yeast. The extent of photoreaction with this macromolecule was determined using the same method described for DNA. The results are reported in Fig. 3. Clearly both derivatives are efficient in photobinding to RNA, whereas 8-MOP does not react to a significant level. The latter result is in agreement with previous experimental evidence.[15]

In particular, at comparable nucleic acid and drug concentration, half the amount of derivative **9** is bound to RNA with reference to DNA (Fig. 1), whereas comparable photoreactivity is observed using derivative **10.** Thus the presence of an hydroxymethyl moiety in the test drugs appears to play a role in stabilizing psoralen–ribonucleic acid intermediates.

Photobinding to DNA with different A-T content. Thymine is by far the preferred pyrimidine base in the photoreaction between furocoumarins and DNA. Although the 5'-TpA sequence is favored over the 5'-ApT one, recent investigations have pointed out modulating effects of flanking sequences too.[16,17] To examine possible specificity effects exhibited by compounds **9** and **10,** we studied the covalent binding to DNA with different base pair composition. The results are reported in Fig. 4 where the extent of covalent photobinding

Table 2. DNA synthesis inhibition by irradiation (365 nm) in the presence of test compounds and 8-MOP as the reference drug

Compound	ID_{50} (J/m²)*
9	3.25 ± 0.92
10	8.98 ± 0.90
8-MOP	7.56 ± 0.30

*UV-A dose that induces 50% inhibition of DNA synthesis in the presence of tested compounds at 2×10^{-5} *M* concentration.

is expressed as a function of A-T content. Increasing A-T content (28% in *M. lysodeikticus*, 60% in calf thymus, 72% in *C. perfringens*) causes a linear increase in the amount of drug covalently bound to the polynucleotide. The relative photobinding efficiency shown by the test drugs is again in favor of derivative **9**.

Photobinding to nucleosomes. Because the native structure of eukaryotic DNA is organized with basic proteins to give the nucleosomal arrangement, useful information on the activity *in vivo* can be obtained by investigating the extent of covalent photobinding to this structure. Both new compounds show a remarkable decrease in photobinding ability to calf thymus nucleosomes when compared to "free" DNA (Fig. 5).

Biological DNA effects synthesis inhibition in Ehrlich cells

The results of inhibition of DNA synthesis in Ehrlich ascites tumor cells, expressed in terms of ID_{50}, are reported in Table 2. Compound **9** is at least twice as active as the reference drug, whereas compound **10** induces inhibition of DNA synthesis practically to the same extent as 8-MOP. These data are reasonably correlated to those obtained in the photobinding experiments.

T2 phage sensitization

The sensitization of T2 phage suspension after UV-A irradiation in the presence of 4-hydroxymethylpsoralens is reported in Fig. 6. Both compounds appear to be effective in the inactivation of the phage. Their behavior is substantially similar to 8-MOP.

Inhibition of the tumor transmitting capacity

Table 3 shows the results obtained in such experiments. Compound **9** inhibits tumor transmission almost as much as 8-MOP, whereas compound **10** is less effective in this test.

Table 3. Inhibition of the tumor transmitting capacity of Ehrlich ascites tumor cells

Compound*	% Mortality after minutes of irradiation					
	0	2	4	8	16	32
None	100					100
9	100	70	50	10	0	0
10	100	100	100	90	80	0
8-MOP	100	100	40	20	0	0

*The concentration of test compounds was 2×10^{-5} *M*.

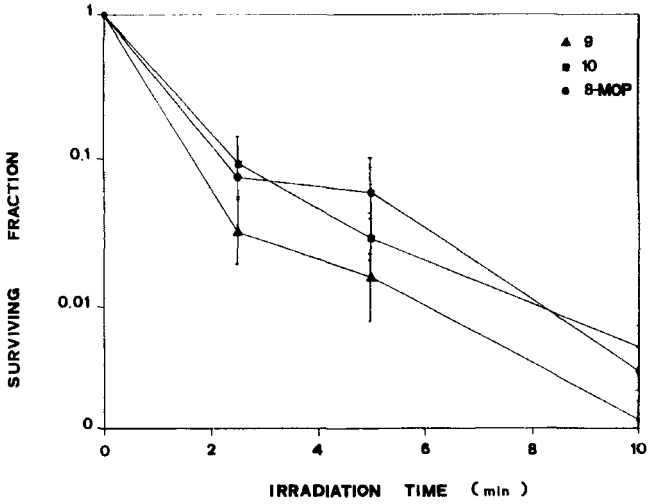


Figure 6. T2 phage sensitization by 4-hydroxymethyl-4′-methylpsoralens; virus particles were exposed to UV-A light in the presence of the compound to be studies ($2 \times 10^{-5}M$) and then their infectivity was assayed.

Skin phototoxicity

The results related to erythema formation in guinea pig skin are reported in Table 4. In striking contrast to 8-MOP, the new 4-hydroxymethylpsoralens are totally unable to induce skin phototoxicity in the entire range of concentrations and UV-A doses examined.

DISCUSSION AND CONCLUSIONS

Hydroxymethylpsoralens **9** and **10** exhibit prominent DNA-photobinding properties when compared to 8-MOP, a leading photochemotherapeutic agent, and efficiently cross-link the opposite strands of DNA. On the contrary, their benzo- and tetrahydrobenzo-psoralen analogues either do not produce or produce very limited cross-linking.[9,10] The test derivatives do not appear to show marked preference for specific sequences of the nucleic acid, as indicated by the almost linear dependence of the amount of photobound drug upon A-T content, T being, by far, the preferred base in photoaddition. Thus DNA lesions should be more randomly distributed among A-T sequences in comparison to other furocoumarins.[16,17] However, to assess specific neighboring effects of adjacent base pairs, footprinting studies are re-

Table 4. Skin phototoxicity in guinea pigs

Furocoumarin	Drug concentration ($\mu g\ cm^{-2}$)	UV-A doses ($kJ\ m^{-2}$)	Erythema formation*
8-MOP	5	5	− − −
8-MOP	5	6	+ − −
8-MOP	5	10	+ + +
9	5–50	5–20	− − −
10	5–50	5–20	− − −

*+ + +, Strong erythema with edema; + − −, barely perceptible erythema; − − −, no erythema.

quired. Reduced binding to nucleosomes is in line with previous studies on 8-MOP and congeners[18] and confirms that DNA structurally organized as it is in the cell is a poorer target than the purified nucleic acid, probably due to the reduced accessibility of the drugs to the highly compact and protein-bound regions of chromatin constituents. An interesting property shared by compounds **9** and **10**, but not by 8-MOP, is a relatively high photoreactivity toward RNA. This seems to indicate that RNA might represent an additional biological target for hydroxymethylpsoralens.

As far as photobiological properties are concerned, both derivatives **9** and **10** behave similarly to 8-MOP in DNA synthesis inhibition in Ehrlich cells, T_2 phage sensitization and, at least compound **9**, in tumor transmitting capacity.

Notwithstanding the close similarities with 8-MOP thus far discussed, an important exception is observed. In fact, compounds **9** and **10** are practically devoid of skin phototoxicity. Lack of penetration into the skin is probably not responsible for this fact as compounds **9** and **10** exhibit solubility (and, in general, physicochemical) properties at room temperature close to 8-MOP (Table 1). Light-filtering effects should also be ruled out by the similarity in absorption spectra of the test drug and of 8-MOP (Table 1). In addition compounds **9** and **10** are photochemically stable (O. Gia, unpublished results). As mentioned in the Introduction, the photosensitizing ability of psoralens is generally attributed to the formation of mono- and di-adducts to DNA. In addition, a role for singlet oxygen has been more recently proposed, as a correlation was found between $^1O_2$ production and erythema.[4–6] In the present case both mechanisms should be efficiently operating, at least for compound **9**. In fact it photobinds to DNA rather effectively and forms cross-linkages to a large extent. In addition it photosensitizes singlet oxygen formation almost twice as much as 8-MOP (Table 1).

Indeed, neither of the proposed mechanisms for psoralen-induced phototoxicity appears to be correlated to erythema formation in the present case.

To our knowledge this is the first example of efficient DNA cross-linking, singlet-oxygen-forming, linear furocoumarin derivatives unable to produce skin phototoxicity. This has double significance from a pharmacological point of view. First of all, compounds like **9** and **10** could represent the beginning to development of effective chemotherapeutic agents devoid of phototoxic effects. This would correspond to an important advance in this field. Secondly, the data reported here confirm that the mechanisms that finally lead to phototoxicity are rather complicated and possibly include pathways different from DNA damage. These need to be investigated in deeper detail to include and explain the present findings.

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