# New Tetracyclic Analogues of Photochemotherapeutic Drugs 5-MOP and 8-MOP: Synthesis, DNA Interaction, and Antiproliferative Activity

Lisa Dalla Via,*[,§] Ornella Gia,[§] Sebastiano Marciani Magno,[§] Lourdes Santana,[‡] Marta Teijeira,[‡] and Eugenio Uriarte[‡]

*Department of Pharmaceutical Sciences, University of Padua, and "Centro di Studio sulla Chimica del Farmaco e dei Prodotti Biologicamente Attivi del CNR" (associated with the National Institute for the Chemistry of Biological Systems - CNR), via Marzolo 5, 35131 Padova, Italy, and Department of Organic Chemistry, University of Santiago de Compostela, Spain*

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The synthesis of new tetrahydrobenzo- and benzopsoralen derivatives carrying at position 5 or 8 of the furocoumarin moiety a methoxy, hydroxy, or dimethylaminopropoxy side chain is reported. The study of their photoantiproliferative activity and ability to induce erythema on guinea pig skin allows us to state that the derivatives carrying the dimethylaminopropoxy side chain exhibit a very interesting photobiological pattern. Indeed, if compared with the lead compounds 5-MOP and 8-MOP, they exert a higher cytotoxic activity devoid of significant skin phototoxicity. Between them, the more interesting appears to be **16**, a nonphototoxic compound whose antiproliferative activity on HeLa cells is 2 orders of magnitude higher than that of the reference drug 8-MOP. Photoreaction experiments have revealed that, like classic furocoumarins, A-T is the preferred nucleic base pair in its photobinding. Moreover, the extent of covalent photoaddition to DNA correlates well with the photobiological activity. For this compound a certain effect was also observed in the dark. Evaluation of the ability to induce DNA cleavage in the presence of human topoisomerase II has suggested that this enzyme is probably the target accountable for this effect.

## Introduction

Natural and synthetic psoralens have been used in PUVA therapy (psoralen plus ultraviolet-A radiation) for at least 20 years, and nowadays numerous skin diseases are treated with 5-methoxypsoralen (5-MOP) or 8-methoxypsoralen (8-MOP).[1−3] Their therapeutic application is by topical or oral administration followed by ultraviolet (320−400 nm) exposure. A more recent development in this field has been photopheresis, an extracorporeal photochemotherapy, employed in the treatment of cutaneous T-cell lymphoma and autoimmune disorders such as pemphigus vulgaris and scleroderma.[4,5] Notwithstanding the unquestionable benefits obtained from the therapeutic use of these photosensitizers, it has to be underlined that photochemotherapy with psoralen provokes some serious side effects, such as skin photosensitization, genotoxicity, and the induction of cutaneous tumors.[6−8]

The biological effects have been mainly related to the combination with cell DNA. The intercalative complex formed in the dark between two base pairs of the macromolecule gives a photoaddition, upon irradiation with UVA light, that involves the 5,6 double bond of the pyrimidine bases and the 4′,5′ and/or 3,4 psoralen double bonds, forming mono- and diadducts in DNA.[9−11] Although both mono- and bifunctional adducts may be responsible for inhibition of cellular DNA synthesis, the compounds which cause bifunctional damage to the macromolecule are usually more phototoxic.[6−8]

Several approaches have been followed in order to obtain new psoralen analogues with good photoreactivity along with diminished undesirable side effects. One of the more interesting is the inactivation of one of the two photoreactive double bonds by means of the condensation of a benzenic or cyclohexenyl ring at the furan side of the psoralen molecule to achieve the inactivation of the 4′,5′ reactive site.[12−14] Indeed the 3,4 position represents the unique photoreactive site for benzopsoralens, while the tetrahydro congeners are still able to form interstrand cross-links because the cyclohexenyl ring does not prevent reactivity of the furan side. The derivatives obtained—benzo- and tetrahydrobenzopsoralens—showed a good capacity to photoreact with calf thymus DNA in vitro. Furthermore, the skin photosensitizing potency, evaluated as erythema induction on guinea pig skin, appears totally absent for all the benzo derivatives and is greatly reduced for the tetrahydrobenzo derivatives.[12−14]

On the basis of these results, we have synthesized a new series of tetrahydrobenzo- and benzopsoralens, closely related to the photochemotherapeutic drugs 5-MOP and 8-MOP. The new derivatives present a methoxy group in the same position as the lead compounds. Furthermore, the presence of a dimethylaminopropoxy side chain in position 5 or 8 of the psoralen moiety was set with a view to increasing the low solubility of the tetracyclic derivatives in aqueous media. The latter have been synthesized from the hydroxy derivative analogues which were also taken into account.

In this paper we describe the synthesis of six derivatives of 5-MOP (compounds **5**−**10)** and six of 8-MOP (compounds **11**−**16**), tetrahydrobenzo and benzo con-

* To whom correspondence should be addressed. Phone: +39 049 827 5712. Fax: +39 049 827 5366. E-mail: dallavia@dsfarm.unipd.it.
§ Italy.
‡ Spain.

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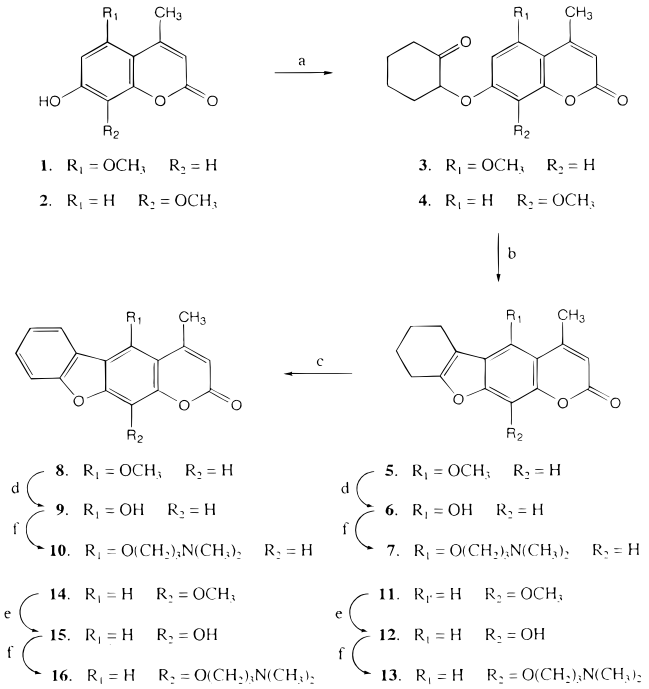
**Scheme 1**[a]




[a] Reagents: (a) 2-chlorocyclohexanone, $K_2CO_3$, acetone; (b) NaOH; (c) DDQ, toluene; (d) HI, AcOH, $Ac_2O$; (e) $AlCl_3$, $CH_2Cl_2$; (f) 3-chloro-*N*,*N*-dimethylpropylamine, NaH, NaI, DMF.

geners; their DNA interaction and photobiological properties were also studied. The availability of the 11-dimethylaminopropoxy derivative **16** as a tritiated compound allowed us to examine its photocombination ability in depth.

## Results and Discussion

**Chemistry.** The compounds studied were obtained as shown in Scheme 1. The preparation of **3**, **5**, and **8** in accordance with a general approach to the psoralen skeleton[15] has been described elsewhere;[16] compounds **4**, **11**, and **14** were prepared analogously, as follows. Williamson reaction of **2** with 2-chlorocyclohexanone for 24 h in refluxing acetone/$K_2CO_3$ gave the oxo ether **4** in 47% yield. Cyclization of **4** by heating in strong alkaline solution afforded the tetrahydrobenzofurocoumarin **11** in 82% yield. Finally, the unsubstituted terminal ring of **11** was aromatized by heating with DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) in refluxing toluene, which afforded the benzofurocoumarin **14** in 63.5% yield.

The 5-methoxypsoralen derivatives **5** and **8** were transformed to **6** and **9**, respectively, in almost quantitative yield by hydrolysis of their methoxy groups to hydroxyl groups with hydroiodic acid in a refluxing mixture of acetic acid and acetic anhydride. The 8-methoxypsoralen derivatives **11** and **14** were hydrolyzed to compounds **12** and **15** in 97% and 85.5% yields, respectively, by treatment with aluminum trichloride in refluxing methylene chloride.

Finally, treatment of compounds **6**, **9**, **12**, and **15** with 3-chloro-*N*,*N*-dimethylpropylamine and NaH in the presence of NaI in refluxing dimethylformamide, so as to replace their hydroxyl groups, afforded compounds **7**, **10**, **13**, and **16** in 50%, 30.5%, 83%, and 55.5% yields, respectively. Note that the yields of the 8-methoxypsoralen derivatives were significantly better than those of the corresponding 5-methoxypsoralen derivatives and that the yields of the tetrahydrobenzo compounds were likewise better than those of the corresponding benzo compounds.

**Table 1.** Cell Growth Inhibition in the Presence of Examined Compounds and 5-MOP and 8-MOP as Reference Drugs

| | cell lines $IC_{50}$ ($\mu$M) | | | |
|---|---|---|---|---|
| | HeLa | | HL-60 | |
| compd | dark | UVA | dark | UVA |
| **5** | >20 | 4.6 ± 0.5 | >20 | 4.9 ± 1.5 |
| **6** | >20 | >20 | >20 | 7.3 ± 0.5 |
| **7** | >20 | 3.7 ± 0.2 | 13.8 ± 3.6 | 3.4 ± 0.4 |
| **8** | >20 | >20 | >20 | 4.5 ± 1.1 |
| **9** | >20 | >20 | >20 | >20 |
| **10** | >20 | 2.8 ± 0.3 | 9.2 ± 1.1 | 0.73 ± 0.16 |
| **11** | >20 | >20 | >20 | 3.5 ± 0.3 |
| **12** | >20 | >20 | >20 | >20 |
| **13** | >20 | 1.2 ± 0.7 | 12.4 ± 1.5 | 0.60 ± 0.12 |
| **14** | >20 | >20 | >20 | 6.4 ± 1.1 |
| **15** | >20 | >20 | >20 | >20 |
| **16** | 9.6 ± 1.4 | 0.08 ± 0.02 | 4.2 ± 0.4 | 0.60 ± 0.09 |
| 5-MOP | >20 | 16.3 ± 0.8 | >20 | 3.35 ± 0.35 |
| 8-MOP | >20 | 10 ± 3 | >20 | 5.4 ± 0.7 |

**Photobiological Activity.** The antiproliferative activity of the new derivatives **5**−**16** was evaluated by means of an in vitro test carried out on human cervix adenocarcinoma (HeLa) and on human promyelocytic leukemia (HL-60) cell lines, determining the concentration of compound able to cause the death of 50% of the initial cell concentration. The results of the experiments, performed as indicated in the Experimental Section, are shown in Table 1; as reference we took both 5-MOP and 8-MOP.

Toward HeLa cells after exposure to UVA light, the derivatives carrying the dimethylaminopropoxy side chain (**7**, **10**, **13**, **16**) exert the higher activity. Furthermore, the 11-derivatives are more reactive than the 5-congeners and the benzo more than the tetrahydro analogues. It is worth noting the effect of compound **16** which is 100 times more efficient in inhibiting cell growth than 8-MOP and compound **13** which is about 8 times more efficient.

The four above-mentioned hydrophilic compounds appear to be more active on HL-60 cells as well as on HeLa. Moreover, the methoxy derivatives, both tetrahydrobenzo and benzo, also exert a certain cytotoxic activity; indeed the $IC_{50}$ values are similar to those of 8-MOP.

In some cases a certain activity in the dark was also observed, but it appears significantly less than that exerted upon UVA irradiation.

Experiments were carried out to determine the skin photosensitizing potency of the new tetracyclic psoralen, tested on guinea pigs. The results reported in Table 2 indicate that all the benzo derivatives are nonphototoxic. Regarding the tetrahydrobenzo compounds, only the very active **7** and **13** show a photosensitization capacity even if between them there is a significant difference in behavior. Indeed, the derivative carrying the dimethylaminopropoxy side chain in the 5 position of the tricyclic psoralen structure is clearly weaker in its ability to induce skin erythema with respect to the corresponding 11-derivative. Moreover it is noteworthy that, in both cases, the phototoxic effect is considerably

**Table 2.** Skin Phototoxicity in Guinea Pigs After Exposure to the Tested Compounds as Indicated in Experimental Section and 20 kJ $m^{-2}$ of UVA

| compd | formation of erythema[a] |
|---|---|
| **5** | − − − |
| **6** | − − − |
| **7** | + − − |
| **8** | − − − |
| **9** | − − − |
| **10** | − − − |
| **11** | − − − |
| **12** | − − − |
| **13** | + + + (without edema) |
| **14** | − − − |
| **15** | − − − |
| **16** | − − − |
| 5-MOP | + + + (without edema) |
| 8-MOP | + + + (with edema) |

[a] + + +, strong; + − −, mild; − − −, absent.

lower than that of the reference drugs because the new compounds induce a detectable effect only when used at a molar concentration about 3 times higher than that of the 5-MOP and 8-MOP.

**Noncovalent Binding to DNA.** The formation of a molecular complex between the new psoralen analogues and DNA was observed by means of flow linear dichroism experiments (LD). The spectra of DNA solutions in the presence of increasing amounts of compounds with the dimethylaminopropoxy side chain (**7**, **10**, **13**, **16**) are reported (Figure 1).

The DNA spectra are negative at the lowest wavelength region (260 nm), as expected for this macromolecule (traces a). In the region at longer wavelengths (320−380 nm), where only the added drug exhibits absorption, a dichroic signal was also observed. Since these small molecules cannot themselves become oriented in the flow field, the existence of a dichroic signal in this region allows us to state that these compounds form a complex with DNA. Moreover the sign of this signal is negative such as that observed for the strong LD band around 260 nm due to the DNA base pairs. In the case of planar aromatic molecules, all strong absorptions are $\pi \rightarrow \pi^*$ transitions, which are polarized in the plane of the chromophore. Thus, the negative LD sign is in qualitative agreement with an orientation of the molecular plane preferentially parallel to the plane of the DNA bases. This orientation is to be expected if the drug is intercalated between two adjacent base pairs.

Specific information on the geometry of the binding process has been obtained by the $LD_r$ calculations and using the equation reported in the Experimental Section. These values, determined at the wavelength region where the added drug absorbs, were close to those observed for the DNA absorption band (260 nm). The average angle α is 73° for the two tetrahydrobenzo analogues (**7** and **13**). This value is consistent with a geometry in which the molecular residues are in close proximity to the nucleic bases in a pseudo-intercalative conformation; this means that they are not in perfect parallel alignment with the base pairs of DNA. On the other hand, for the two benzo derivatives (**16** and **10**) the value is 90° as for typical intercalators. This difference in the complexation ability is probably attributable to the hydrogenated ring of the tetrahy-

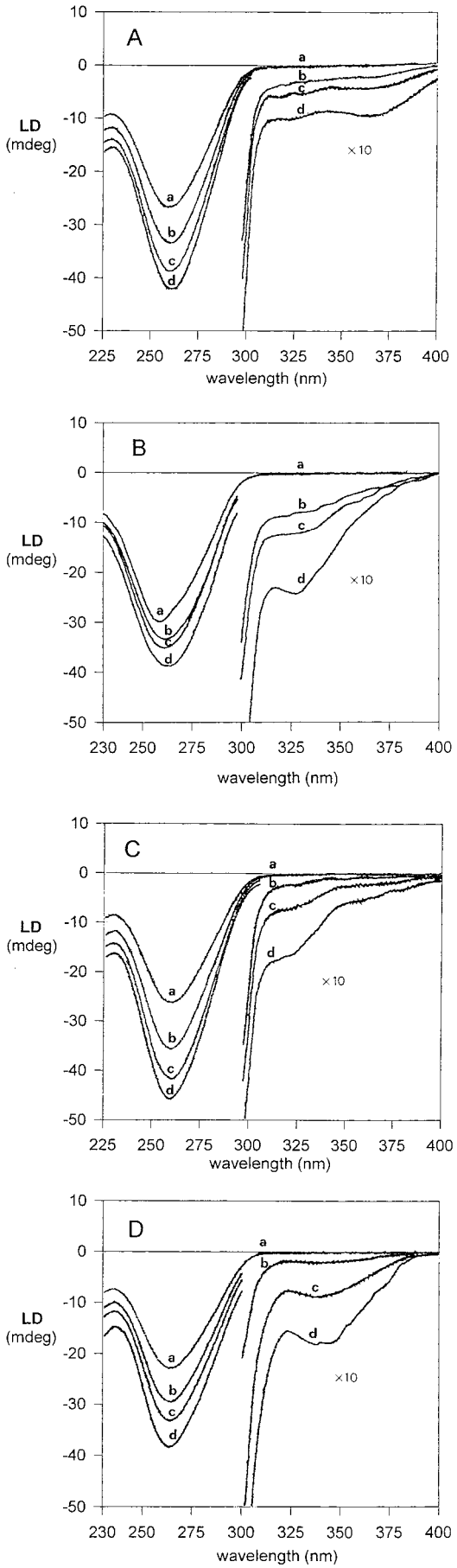



**Figure 1.** Linear flow dichroism spectra for compounds **7** (A), **10** (B), **13** (C), and **16** (D) at different [drug]/[DNA] ratios: a = 0; b = 0.02; c = 0.04; d = 0.08; [DNA] = $1.89 \times 10^{-3}$ M.

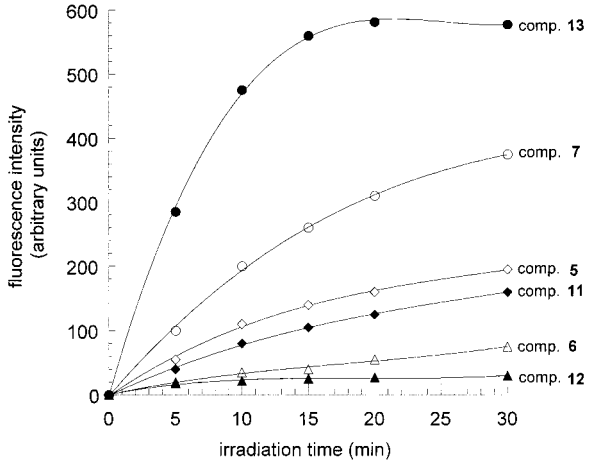



**Figure 2.** Fluorescence intensity of 4′,5′-cycloadducts obtained by hydrolysis of DNA irradiated in the presence of tetrahydrobenzo derivatives as a function of irradiation time.

drobenzopsoralen which is not planar with respect to the tricyclic moiety.

**Photobinding to DNA.** The tetrahydrobenzo- and benzopsoralen DNA photoaddition process, consequent to the irradiation of the intercalated furocoumarin moiety into the double helix, was studied evaluating the photoadduct formation. Regarding the tetrahydrobenzopsoralens, it has already been demonstrated that the cyclohexenyl ring does not inhibit the photoreactivity of the furan side of the furocumarinic moiety,[13] so that the possibility of forming adducts at both reactive positions of the furocoumarinic nucleus is substantially maintained. The presence of 4′,5′-cycloadducts, obtained after acid hydrolysis as described in the Experimental Section, can be verified from their strong fluorescence, the intensity of which increases with the amount of compound photobound to DNA. Fluorescence was detected after various increasing periods of irradiation at 365 nm, and the results are reported in Figure 2. Intensity is greater for the derivatives carrying the hydrophilic side chain. In particular the derivative with the substituent in the 11 position (**13**) appears more reactive than the 5-analogue (**7**). Among the others, the methoxy derivatives seem to be more photoreactive than the hydroxyl, whose fluorescence intensity is somewhat low.

In the case of benzo derivatives, which can only form pyrone monoadducts because of the 4′,5′ double bond's delocalization in a tetracyclic aromatic system, a rough indication of the covalent binding can be obtained by the absorption spectrum of irradiated DNA−compound solution after appropriate treatment, as described in the Experimental Section. The presence of UV absorption around 300 nm after extraction is attributable to the benzopsoralen-pyrimidine cycloadduct.[12,17] The absorption data are correlated to the extent of the photoproduct. Figure 3 reports the UV absorption spectrum obtained by irradiating the macromolecule in the presence of **16**. A similar trend was also observed for **10**. The absorption values for the compounds bearing the methoxy or hydroxy side chain are very much lower.

**Cross-Linking.** The photoreactive behavior of tetrahydrobenzo and benzo derivatives toward the double helix of DNA was also assessed by denaturation−renaturation experiments. The benzopsoralen derivatives are totally unable to cross-link the double helix of DNA (data not shown) according to previous findings on other benzopsoralen compounds.[13] Regarding the cyclohexenyl congeners, Figure 4 depicts the cross-linking ability of the compounds with the dimethylaminopropoxy side chain in comparison with the two drugs 5-MOP and 8-MOP.

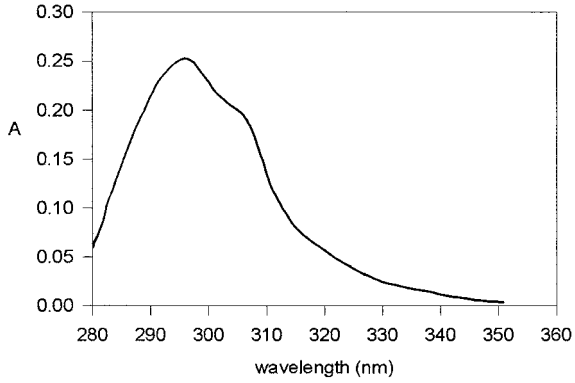



**Figure 3.** Absorption spectrum of pyrone cycloadduct obtained from calf thymus DNA and compound **16**, as reported in the Experimental Section.

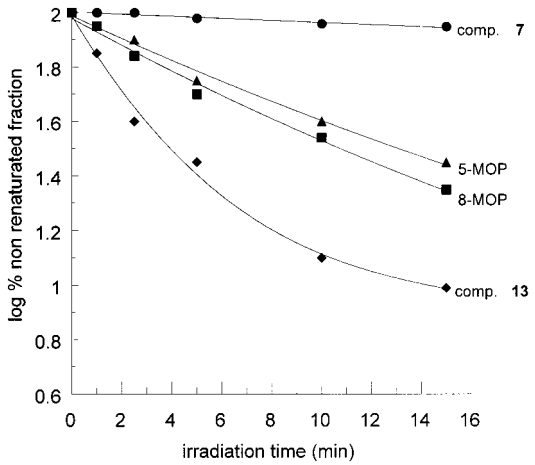



**Figure 4.** Cross-linking of compounds **7**, **13**, 5-MOP, and 8-MOP to double-stranded DNA from calf thymus (nucleotide−drug ratio = 78) as a function of irradiation time.

Compound **13** shows a higher capacity to form cross-links than the reference drug 8-MOP, whereas for compound **7** this ability appears to be practically absent. The presence of a substituent in the 5 or 8 position of the psoralen moiety plays a crucial role in the DNA photoaddition. This fact is slightly evident if we compare 5-MOP and 8-MOP, while it is clearly evident for compounds **7** and **13** bearing a dimethylaminopropoxy side chain. These results are in fair agreement with the photobiological effects and confirm previous studies on water-soluble compounds of furocoumarins.[18]

**Quantitative Evaluation of Photobinding to Macromolecules.** The availability of compound **16** as a tritiated sample allowed determination of the amount covalently linked to DNA from calf thymus. Figure 5 reports the nmol of drug photobound/mg of macromolecule as a function of the irradiation time: derivative **16** exerts remarkable reactivity, significantly higher than that of 8-MOP.

It is known that in the initial period of irradiation the photoreactions of furocoumarins toward DNA be-

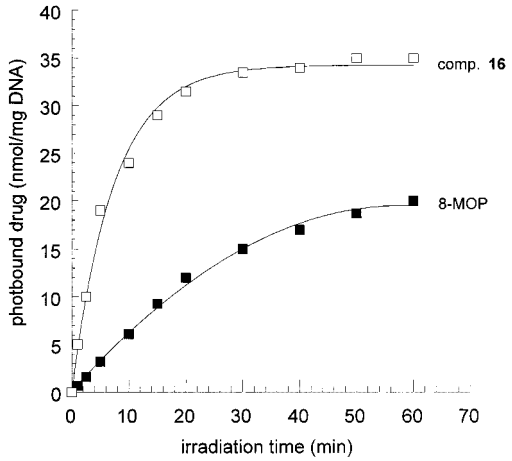



**Figure 5.** Photobinding of compound **16** and 8-MOP to double-stranded DNA from calf thymus (nucleotide−drug ratio = 80) as a function of irradiation time.

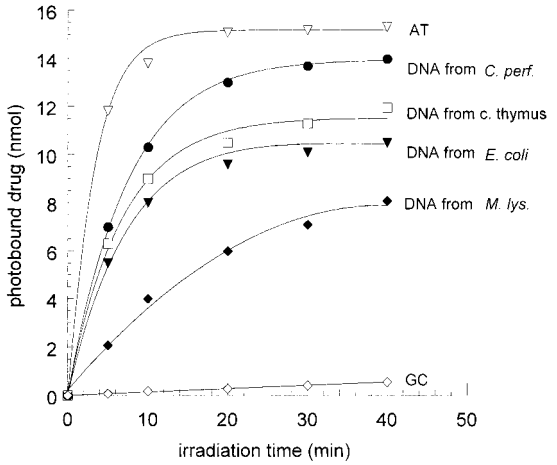



**Figure 6.** Photobinding of compound **16** to synthetic polydeoxyribonucleotides and DNA with various A-T contents (nucleotide−drug ratio = 8) as a function of irradiation time.

have like pseudo-first-order reactions with respect to the furocoumarins.[19] The photoreactions between **16** and DNA also show this behavior, and the calculated initial rate constant was $1.7 \times 10^{-1}$ min$^{-1}$, about 6 times higher than that of 8-MOP ($3.1 \times 10^{-2}$ min$^{-1}$), thus confirming the good activity of this benzo derivative.

With the aim of highlighting whether the photobinding process is driven by the presence of particular base sequences along the double helix, we also studied the photoreactivity of compound **16** toward bacterial DNA characterized by a different base pair composition (see Experimental Section). The results are reported in Figure 6 in terms of a photobound drug as a function of irradiation time. It is evident that the amount of covalent product increases as A-T content increases. In Figure 6 the photobinding to poly[dA-dT]−poly[dA-dT] and poly[dG-dC]−poly[dG-dC] is also reported. The results are in agreement with the previous ones; indeed the amount of compound **16** covalently bound to the polynucleotides strictly depends on the presence of A-T pairs.

To obtain a clearer evaluation of this behavior, the extent of covalent photobinding, at the same irradiation time, has been plotted against the percent of A-T content (Figure 7). The pattern obtained is quite similar to that of classical furocoumarins, which generally exert a

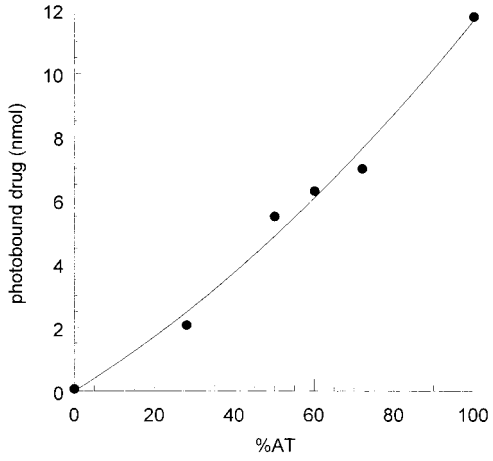



**Figure 7.** Photobinding of compound **16** as a function of % A-T content in nucleic acids (irradiation time 5 min).

preference toward the pyrimidine base thymine as the target of photoreaction.

**Topoisomerase II-Mediated Cleavable Complex.** The biological effects evoked by the furocoumarin derivatives generally require UVA light activation; indeed the well-known drugs 5-MOP and 8-MOP are completely ineffective in the dark. However, for some photobiological active compounds, a certain activity in the dark was also observed so that two different mechanisms, one light-mediated and the other in the dark, had to be taken into account to explain their antiproliferative effects.[20,21] Because of the interaction with DNA, the proposed mechanism was the involvement of topoisomerase I and/or II.[20]

DNA topoisomerases are a unique class of enzymes that change the topological state of DNA by breaking and re-uniting the phosphodiester backbone of the nucleic acid. Some antitumor drugs interfere with the activity of mammalian DNA topoisomerase II by trapping a key covalent reaction intermediate, called the "cleavable complex". The treatment of this complex with a strong protein denaturant such as SDS results in DNA breakage. In particular for a benzopsoralen derivative the capacity to inhibit the activity of mammalian topoisomerase II in vitro in both the catenation and decatenation assay was detected.[22]

Among the new tetracyclic compounds, **16** also shows higher antiproliferative activity in the dark. Its dark effect is remarkably lower than that exerted upon UVA irradiation; moreover it may be responsible for some molecular events which could contribute to the overall antiproliferative effect. Figure 8, lanes C−E, shows the effect on human topoisomerase II activity of compound **16** at three different concentrations, as indicated. The presence of DNA cleavage site at the higher concentration taken into account (100 $\mu$M) is evident, while there is no significant effect at lower concentrations, even though at 50 $\mu$M a weak inhibition of the enzymatic activity appears.

## Conclusions

A new series of 5- or 11-methoxytetrahydrobenzo- and benzopsoralen derivatives whose chemical structure is related to the well-known photochemotherapeutic drugs 5-MOP and 8-MOP was synthesized. With the aim of increasing the low solubility in aqueous media, the


**Figure 8.** Topoisomerase II-mediated cleavage of pBR322 DNA. Cleavage reactions were carried out as described in the Experimental Section: lane A, pBR322 DNA control (no enzyme); lane B, pBR322 DNA and topoisomerase II (no drug); lanes C−E, same as lane B with 10, 50, and 100 $\mu$M **16**, respectively.

methoxy group was replaced with a dimethylaminopropoxy side chain. The synthetic pathway followed to obtain these latter compounds provides for the replacement of a hydroxyl group with the dimethylaminopropoxy side chain so that the hydroxy derivatives were also taken into account. Nevertheless, neither 5- nor 11-hydroxytetrahydrobenzo- and benzopsoralens reveal any interesting photobiological pattern.

Regarding the methoxy derivatives, in most cases they exert an antiproliferative activity comparable with that of the tricyclic reference drug. However, unlike 5-MOP and 8-MOP, they do not induce skin erythema in guinea pigs. Keeping in mind that skin phototoxicity is a short-term side effect which has constituted a severe limit to the development of photochemotherapy with psoralens, the lack of cutaneous photosensitivity appears to be a noteworthy feature.

The new compounds bearing the dimethylaminopropoxy side chain have shown remarkable antiproliferative activity upon UVA irradiation, clearly higher than that induced by reference drugs. Regarding skin photosensitizing potency, only **13** induces erythema, although to a substantially lower extent than the two reference compounds 5-MOP and 8-MOP. The events occurring in the photoreaction between the new tetracyclic compound and DNA are similar to those of furocoumarins. Indeed they form a preliminary complex with the macromolecule undergoing intercalation. After irradiation, the intercalated ligand photobinds covalently to DNA giving rise to monoadducts and also diadducts in the case of tetrahydrobenzo derivatives. Photobiological activity is well-correlated with photoaddition to the macromolecule.

The most antiproliferative activity is exerted by compound **16**, the benzo derivative carrying the dimethylaminopropoxy side chain in the 11 position. The availability of labeled **16** allowed us to examine its photobiological pattern in depth. The ability to photoadd with mammalian DNA is higher than that for 8-MOP, thus confirming that the macromolecule may be considered a crucial target for photobiological events. The investigation of photoaddition toward bacterial nucleic acids and synthetic polydeoxyribonucleotides highlights the fact that, like the majority of furocoumarins, A-T is by far the preferred base pair in its photobinding. Generally, for the classical furocoumarins increasing A-T content causes a linear increase in the amount of drug covalently bound to the polynucleotide. The behavior shown by the new benzo derivative confirms these previous findings and indicates a leading preference toward A-T residues independently of the flanking sequences.

It is well-known that the photobiological activity of furocoumarins generally requires light activation, while in the dark there is only negligible or no effect. Compound **16**, besides the high photoantiproliferative activity, also shows modest, but detectable, cytotoxicity in the dark. Experiments performed to gain further insight into this unusual behavior demonstrate the involvement of the nuclear enzyme topoisomerase II. The inhibition ability of **16** toward the catalytic activity of this enzyme only appears at higher concentrations, and this is well-correlated with the extent of the antiproliferative effect in the dark.

Taking into account that furocoumarins have been successfully used in combination with UVA to manage hyperproliferative skin diseases such as psoriasis and mycosis fungoides, the presence of a notable antiproliferative activity besides the reduction in ability to induce skin erythema renders this new family of compounds a very promising tool for the development of new photochemotherapeutic agents.

## Experimental Section

Melting points are uncorrected and were determined in a Reichert Kofler thermopan or capillary tubes in a Büchi 510 apparatus. IR spectra were recorded in a Perkin-Elmer 1640FT spectrometer (KBr disks, $\upsilon$ in $cm^{-1}$). $^1H$ NMR (300 MHz) and $^{13}C$ NMR (75.4 MHz) spectra were recorded in a Bruker AMX spectrometer, using TMS as internal standard (chemical shifts in $\delta$ values, $J$ in Hz). Mass spectrometry was carried out on a Kratos MS-50 or Varian MAT-711 spectrometer. Elemental analyses were performed by a Perkin-Elmer 240B microanalyzer and were within ±0.4% of calculated values in all cases. Flash chromatography (FC) was performed on silica gel (Merck 60, 230−400 mesh); analytical TLC was performed on precoated silica gel plates (Merck 60 $F_{254}$, 0.25 mm).

**8-Methoxy-4-methyl-7-(2-oxocyclohexanyloxy)coumarin (4).** A mixture of 7-hydroxy-8-methoxycoumarin (5 g, 24.2 mmol), 2-chlorocyclohexanone (6.4 g, 36.4 mmol), and $K_2CO_3$ (5 g, 36.2 mmol) in dry acetone (100 mL) was refluxed for 48 h. The mixture was cooled, the precipitate collected, and the solvent evaporated under reduced pressure. The crude product was purified by FC with 8:2 hexane/ethyl acetate as eluent, which gave pure **2** (3.40 g, 47%): mp 142−144 °C. $^1H$ NMR ($CDCl_3$): 7.28 (d, 1H, H5, $J$ = 8.80), 6.73 (d, 1H, H6, $J$ = 8.80), 6.13 (d, 1H, H3, $J$ = 1.15), 4.82 (dd, 1H, H1′, $J$ = 10.10 and 5.50), 3.96 (s, 3H, $OCH_3$), 2.58 (m, 1H, 1H3′), 2.40 (m, 2H, 1H3′ and 1H6′), 2.35 (d, 3H, $CH_3$, $J$ = 1.15), 2.10−1.78 (m, 5H, H4′, H5′ and 1H6′). $^{13}C$ NMR ($CDCl_3$): 207.0 (C-2′), 160.6 (C-2), 153.5 (C-4), 152.7 (C-8a), 148.0 (C-7), 137.1 (C-8), 119.2 (C-5), 115.5 (C-4a), 112.6 (C-6), 111.9 (C-3), 81.8 (C-1′), 61.5 ($OCH_3$), 40.6 (C-3′), 34.5 (C-6′), 27.6 (C-4′), 23.1 (C-5′), 18.8 ($CH_3$). IR: 2937, 1717, 1602, 1292, 1104. Anal. ($C_{17}H_{18}O_4$) C, H.

**6,7,8,9-Tetrahydro-11-methoxy-4-methylbenzofuro[3,2-*g*]coumarin (11).** Oxo ether **4** (2.4 g, 7.9 mmol) was refluxed in 1 M NaOH (500 mL) for 5 h. The mixture was cooled and acidified with 1 M HCl, and the precipitate formed was collected, washed with water, and purified by FC with 8:2 hexane/ethyl acetate as eluent, to give **11** (1.85 g, 82%): mp 196−197 °C. $^1H$ NMR ($CDCl_3$): 7.23 (s, 1H, H5), 6.24 (d, 1H, H3, $J$ = 1.05), 4.25 (s, 3H, $OCH_3$), 2.79 (m, 2H, H9), 2.63 (m, 2H, H6), 2.48 (d, 3H, $CH_3$, $J$ = 1.05), 2.00−1.87 (m, 4H, H7 and H8). $^{13}C$ NMR ($CDCl_3$): 161.2 (C-2), 156.5 (C-4), 153.6 (C-10a), 147.3 (C-11a), 143.0 (C-9a), 132.8 (C-11), 127.7 (C-5a),

117.3 (C-4a), 113.5 (C-3), 113.4 (C-5b), 107.1 (C-5), 61.8 ($OCH_3$), 23.9, 23.1, 22.8, 20.7, 19.9 (C-9, C-6, C-8, C-7, $CH_3$). IR: 2949, 2841, 1716, 1582, 1441, 1063, 865. MS: *m/z* 285 ($M^+$, 18), 284 ($M^+$, 100), 256 ($M^+ - CO$, 24), 228 (256 − $C_2H_4$, 15), 128 (10). Anal. ($C_{17}H_{16}O_4$) C, H.

**11-Methoxy-4-methylbenzofuro[3,2-*g*]coumarin (14).** A solution of tetrahydrobenzopsoralen **11** (5 g, 17.6 mmol) and DDQ (8 g, 35.2 mmol) in toluene (500 mL) was heated at reflux for 6 h. The mixture was cooled, the precipitate collected, and the solvent evaporated under reduced pressure. The residue was purified by FC with 8:2 hexane/EtOAc as eluent, which gave benzopsoralen **14** (4.5 g, 91%): mp 191−193 °C. $^1$H NMR ($CDCl_3$): 7.96 (dd, 1H, H6, $J$ = 7.65 and 1.15), 7.82 (s, 1H, H5), 7.62 (dd, 1H, H9, $J$ = 8.25 and 0.80), 7.51 (m, 1H, H8, $J$ = 8.25 and 1.15), 7.40 (m, 1H, H7, $J$ = 7.65 and 0.80), 6.31 (d, 1H, H3, $J$ = 1.20), 4.33 (s, 3H, $OCH_3$), 2.57 (d, 3H, $CH_3$, $J$ = 1.20). $^{13}$C NMR ($CDCl_3$): 160.3 (C-2), 156.9 (C-4), 152.8 (C-9a), 148.9 (C-10a), 145.1 (C-11a), 132.9 (C-11), 127.9 (C-8), 123.6 (C-5a), 123.3 (C-7), 122.4 (C-5b), 120.7 (C-6), 117.2 (C-4a), 113.5 (C-9), 112.0 (C-3), 109.3 (C-5), 61.6 ($OCH_3$), 19.4 ($CH_3$). IR: 3418, 1718, 1684, 1654, 1636, 1584, 1508, 1457, 1397, 1188, 1021. MS: *m/z* 281 ($M^+$, 9), 280 ($M^+$, 100), 252 ($M^+ - CO$, 18), 237 (252 − $CH_3$, 29), 209 (237 − $C_2H_4$, 13), 181 (209 − CO, 24), 152 (181 − CHO, 29). Anal. ($C_{17}H_{12}O_4$) C, H.

**6,7,8,9-Tetrahydro-5-hydroxy-4-methylbenzofuro[3,2-*g*]coumarin (6).** A mixture of **5** (150 mg, 0.528 mmol), acetic acid (2 mL), acetic anhydride (2 mL), and HI (4 mL) was refluxed for 2 h. The solvent was then eliminated under vacuum and the residue purified by FC using 8:2 hexane/EtOAc as eluent to yield **6** (140 mg, 98%): mp 270−272 °C. $^1$H NMR (MeOD): 6.53 (s, 1H, H11), 6.06 (d, 1H, H3, $J$ = 0.95), 2.85 (m, 2H, H9), 2.75 (m, 2H, H6), 2.70 (d, 3H, $CH_3$, $J$ = 0.95), 1.96 (m, 2H, H8), 1.86 (m, 2H, H7). $^{13}$C NMR (MeOD): 164.5 (C-2), 157.2, 155.6, 154.8, 154.4, 153.0 (C-10a, C-11a, C-5, C-4, C-9a), 116.8 (C-5a), 114.3 (C-5b), 110.6 (C-3), 102.4 (C-4a), 97.9 (C-11), 24.6, 24.3, 24.2, 23.3, 22.5 (C-9, C-6, C-8, C-7, $CH_3$). IR: 3496, 3125, 2944, 1676, 1594, 1419, 1206, 843. MS: *m/z* 271 ($M^+$, 18), 270 ($M^+$, 100), 242 ($M^+ - CO$, 51), 214 (242 − $C_2H_4$, 39), 185 (214 − CHO, 4), 115 (12). Anal. ($C_{16}H_{14}O_4$) C, H.

**5-Hydroxy-4-methylbenzofuro[3,2-*g*]coumarin (9).** This compound was prepared from **8** (150 mg) in an analogous manner to **6**. The crude product was purified by FC with 8:2 hexane/EtOAc as eluent which gave pure **9** (140 mg, 98%): mp 260 °C. $^1$H NMR (DMSO-$d_6$): 10.75 (bs, 1H, OH), 8.26 (dd, 1H, H6, $J$ = 7.40 and 1.35), 7.67 (dd, 1H, H9, $J$ = 8.10 and 1.05), 7.47 (m, H8, $J$ = 8.10, 7.40 and 1.35), 7.41 (m, 1H, H7, $J$ = 7.40 and 1.05), 7.33 (s, 1H, H11), 6.17 (d, 1H, H3, $J$ = 1.15), 2.73 (d, 3H, $CH_3$, $J$ = 1.15). $^{13}$C NMR (DMSO-$d_6$): 159.9 (C-2), 157.8 (C-10a), 155.6, 155.3, 155.0 (C-9a, C-11a, C-5), 151.6 (C-4), 127.2 (C-8), 123.8, 122.9 (C-7, C-6), 122.6 (C-5b), 112.3, 112.0, 111.5 (C-5a, C-3, C-9), 108.1 (C-4a), 93.5 (C-11), 24.3 ($CH_3$). IR: 3378, 1684, 1639, 1601, 1573, 1438, 747. MS: *m/z* 267 ($M^+$, 11), 266 ($M^+$, 100), 238 ($M^+ - CO$, 71), 209 (238 − CHO, 6), 181 (209 − CO, 28), 152 (181 − CHO, 15), 119 (5). Anal. ($C_{16}H_{10}O_4$) C, H.

**6,7,8,9-Tetrahydro-11-hydroxy-4-methylbenzofuro[3,2-*g*]coumarin (12).** A mixture of $AlCl_3$ (141 mg, 0.105 mmol) and anhydrous $CH_2Cl_2$ (1.5 mL) was stirred 2 h at room temperature. The compound **11** (100 mg, 0.352 mmol) in anhydrous $CH_2Cl_2$ (1 mL) was added and the mixture stirred other 2 h. The reaction was then acidified with HCl and extracted with $CH_2Cl_2$ (100 mL × 3). The extract was dried ($Na_2SO_4$) and the solvent evaporated under reduced pressure to leave a residue which was purified by FC with 8:2 hexane/EtOAc as eluent to give pure **12** (260 mg, 97%): mp 285−286 °C. $^1$H NMR ($CDCl_3$): 7.14 (s, 1H, H5), 6.24 (d, 1H, H3, $J$ = 1.15), 2.79 (m, 2H, H9), 2.63 (m, 2H, H6), 2.51 (d, 3H, $CH_3$, $J$ = 1.15), 1.98−1.85 (m, 4H, H7 and H8). $^{13}$C NMR ($CDCl_3$): 160.8 (C-2), 157.0 (C-4), 154.5 (C-10a), 143.7 (C-11a), 138.8 (C-9a), 129.2 (C-11), 127.7 (C-5a), 116.6 (C-4a), 113.4 (C-5b), 113.0 (C-3), 104.8 (C-5), 23.9, 23.1, 22.8, 20.8, 19.8 (C-9, C-6, C-8, C-7, $CH_3$). IR: 3346, 2949, 1707, 1593, 1457, 1421, 1051, 849. MS: *m/z* 271 ($M^+$, 18), 270 ($M^+$, 100), 242 ($M^+ - CO$, 39), 214 (242 − $C_2H_4$, 31), 185 (214 − CHO, 3), 115 (14). Anal. ($C_{16}H_{14}O_4$) C, H.

**11-Hydroxy-4-methylbenzofuro[3,2-*g*]coumarin (15).** This compound was prepared from **14** (100 mg) in an analogous manner to **12**. The crude product was purified by FC with 8:2 hexane/EtOAc as eluent which gave pure **15** (80 mg, 85.5%): mp 263−265 °C. $^1$H NMR (DMSO-$d_6$): 8.29 (s, 1H, OH), 8.20 (d, 1H, H6, $J$ = 7.30), 8.06 (s, 1H, H5), 7.73 (d, 1H, H9, $J$ = 8.15), 7.54 (m, 1H, H8, $J$ = 8.15, 7.30 and 1.30), 7.44 (t, 1H, H7, $J$ = 7.30), 6.38 (s, 1H, H3), 2.55 (s, 3H, $CH_3$). $^{13}$C NMR (DMSO-$d_6$): 158.0 (C-2), 154.5 (C-4), 152.5 (C-9a), 144.4 (C-10a), 140.2 (C-11a), 128.5 (C-11), 126.2 (C-8), 121.9 (C-5a), 121.8 (C-7), 119.8 (C-5b), 119.4 (C-6), 115.3 (C-4a), 110.9 (C-9), 110.1 (C-3), 105.6 (C-5), 17.2 ($CH_3$). IR: 3277, 1700, 1653, 1597, 1056. MS: *m/z* 267 ($M^+$, 18), 266 ($M^+$, 100), 238 ($M^+ - CO$, 38), 210 (238 − $C_2H_4$, 5), 181 (210 − CHO, 15), 152 (181 − CHO, 16). Anal. ($C_{16}H_{10}O_4$) C, H.

**6,7,8,9-Tetrahydro-5-dimethylaminopropoxy-4-methylbenzofuro[3,2-*g*]coumarin (7).** A mixture of hydroxytetrahydrobenzofurocoumarin **6** (110 mg, 0.407 mmol), 3-chloro-*N*,*N*-dimethylpropylamine hydrochloride (77 mg, 0.488 mmol), 60% NaH (48 mg, 1.22 mmol), NaI (73 mg, 0.488 mmol), and dimethylformamide (12 mL) was heated 2 h at 100 °C. It was then concentrated under reduced pressure and the residue purified by FC with $CHCl_3$ as eluent to give pure **7**·HCl (80 mg, 50%): mp 238−240 °C. $^1$H NMR (MeOD): 6.70 (s, 1H, H11), 6.10 (d, 1H, H3, $J$ = 1.15), 4.26 (t, 2H, H1′, $J$ = 5.90), 3.24 (m, 2H, H3′), 2.84 (s, 6H, H4′), 2.83 (m, 4H, H6 and H9), 2.66 (d, 3H, $CH_3$, $J$ = 1.15), 2.28 (m, 2H, H2′), 2.00−1.87 (m, 4H, H7 and H8). $^{13}$C NMR (MeOD): 163.9 (C-2), 157.2, 155.4, 155.0, 154.4, 152.3 (C-10a, C-11a, C-5, C-4, C-9a), 117.1, 114.1, 111.9 (C-5a, C-5b, C-3), 103.5 (C-4a), 95.7 (C-11), 67.4 (C′), 57.1 (C-3′), 44.6 (2C, C-4′), 26.7, 24.6, 24.3, 24.1, 23.6, 22.4 (C-2, C-9, C-6, C-8, C-7, $CH_3$). IR: 3452, 2926, 1718, 1604, 1386, 1106. MS: *m/z* 356 ($M^+$, 2), 355 ($M^+$, 8), 269 ($M^+ - (CH_3)_2N(CH_2)_3$, 5), 86 ($(CH_3)_2N(CH_2)_3^+$, 71), 58 ($(CH_3)_2NCH_2^+$, 100). Anal. ($C_{21}H_{26}Cl\,NO_4$) C, H, Cl, N.

**5-Dimethylaminopropoxy-4-methylbenzofuro[3,2-*g*]-coumarin (10).** This compound was prepared from **9** (100 mg) in an analogous manner to **7**. The crude product was purified by FC with $CHCl_3$ as eluent and treated with ion-exchange resin IRA-4000 to give pure **10** (40 mg, 30.5%): mp 103−105 °C. $^1$H NMR ($CDCl_3$): 8.02 (d, 1H, H6, $J$ = 7.50), 7.57 (d, 1H, H9, $J$ = 8.10), 7.49 (m, 1H, H8), 7.40 (m, 1H, H7), 7.31 (s, 1H, H11), 6.16 (s, 1H, H3), 4.16 (t, 2H, H1′, $J$ = 6.65), 2.72 (s, 3H, $CH_3$), 2.67 (m, 2H, H3′), 2.38 (s, 6H, H4′), 2.25 (m, 2H, H2′). $^{13}$C NMR (MeOD): 162.5 (C-2), 159.8 (C-10a), 157.9 (C-9a), 156.0 (C-11a), 155.6 (C-5), 153.7 (C-4), 129.3 (C-8), 125.5 (C-7), 123.9 (C-6), 123.0 (C-5b), 116.9 (C-5a), 115.1 (C-3), 113.0 (C-9), 111.8 (C-4a), 98.3 (C-11), 79.9 (C-1′), 56.9 (C-3′), 45.2 (C-4′), 31.0 (C-4′), 28.4 (C-2′), 24.0 ($CH_3$). IR: 3452, 2923, 1725, 1602, 1383, 747. MS: *m/z* 352 ($M^+$, 3), 351 ($M^+$, 9), 279 ($M^+ - (CH_3)_2N(CH_2)_2$, 4), 266 ($M^+ - (CH_3)_2N(CH_2)_3$, 30), 128 (20), 58 (100). Anal. ($C_{21}H_{21}O_4N$) C, H, N.

**6,7,8,9-Tetrahydro-11-dimethylaminopropoxy-4-methylbenzofuro[3,2-*g*]coumarin (13).** This compound was prepared from **12** (100 mg) in an analogous manner to **7**. The crude product was purified by FC with 9:1 $CHCl_3$/MeOH as eluent to give pure **13**·HCl (120 mg, 83%): mp 198−201 °C. $^1$H NMR ($CDCl_3$): 7.17 (s, 1H, H5), 6.12 (d, 1H, H3, $J$ = 1.00), 4.50 (t, 2H, H1′, $J$ = 5.50), 3.52 (t, 2H, H3′, $J$ = 7.50), 2.87 (s, 6H, H4′), 2.71 (t, 2H, H9, $J$ = 5.70), 2.55 (t, 2H, H6, $J$ = 5.70), 2.40 (d, 3H, $CH_3$, $J$ = 1.00), 2.33 (m, 2H, H2′), 1.81 (m, 4H, H7 and H8). $^{13}$C NMR ($CDCl_3$): 161.1 (C-2), 156.8 (C-4), 154.1 (C-10a), 147.4 (C-11a), 143.0 (C-9a), 130.8 (C-11), 127.8 (C-5a), 117.1 (C-4a), 113.5 (C-3), 113.1 (C-5b), 108.1 (C-5), 71.6 (C-1′), 56.6 (C-3′), 44.2 (C-4′), 30.0 (C-4′), 26.1 (C-2′), 23.9 (C-9), 23.0 (C-6), 22.7 (C-8), 20.7 (C-7), 19.9 ($CH_3$). IR: 3445, 2923, 2852, 2693, 1716, 1585, 1456, 1352, 1070. MS: *m/z* 356 ($M^+$, 1), 355 ($M^+$, 5), 296 ($M^+ - (CH_3)_2NHCH_2$, 1), 270 ($M^+ - (CH_3)_2N(CH_2)_3$, 2), 128 (6), 115 (5), 86 ($(CH_3)_2NH(CH_2)_3^+$, 8), 58 ($(CH_3)_2NCH_2^+$, 100). Anal. ($C_{21}H_{26}ClNO_4$) C, H, Cl, N.

**11-Dimethylaminopropoxy-4-methylbenzofuro[3,2-*g*]-coumarin (16).** This compound was prepared from **15** (100 mg) in an analogous manner to **7**. The crude product was purified by FC with 9:1 $CHCl_3$/MeOH as eluent to give pure **16**·HCl (80 mg, 55.5%): mp 227−229 °C. $^1$H NMR (MeOD): 8.16 (s, 1H, H5), 8.13 (m, 1H, H6), 7.65 (m, 1H, H9), 7.58 (m, 1H, H8), 7.46 (m, 1H, H7), 6.36 (d, 1H, H3, $J$ = 1.10), 4.64 (t, 2H, H1′, $J$ = 5.55), 3.61 (t, 2H, H3′, $J$ = 7.35), 3.04 (s, 6H, H4′), 2.60 (d, 3H, $CH_3$, $J$ = 1.10), 2.34 (m, 2H, H2′). $^{13}$C NMR (MeOD): 162.7 (C-2), 158.8 (C-4), 156.6 (C-9a), 150.7 (C-10a), 146.8 (C-11a), 132.6 (C-11), 129.8 (C-8), 125.5 (C-7), 124.9 (C-5a), 124.5 (C-5b), 122.8 (C-6), 119.0 (C-4a), 114.1 (C-9), 113.2 (C-3), 112.7 (C-5), 73.3 (C-1′), 57.9 (C-3′), 44.5 (C-4′), 26.9 (C-2′), 19.9 ($CH_3$). IR: 3153, 2923, 1702, 1654, 1636, 1583, 1508, 1458, 1437, 1403, 1340, 1258, 1195, 1155, 1075, 1031, 753. MS: *m/z* 352 ($M^+$, 3), 351 ($M^+$, 9), 266 ($M^+ - (CH_3)_2N(CH_2)_3$, 25), 237 (3), 181 (5), 128 (17), 58 (100). Anal. ($C_{21}H_{22}ClNO_4$) C, H, Cl, N.

**Photobiological Methods. Cell Cultures.** HL-60 and HeLa cells were grown in RPMI 1640 (Sigma Chemical Co.) supplemented with 15% heat-inactivated fetal calf serum (Seromed) and Nutrient Mixture F-12 (HAM) (Sigma Chemical Co.) supplemented with 10% heat-inactivated fetal calf serum (Seromed), respectively; 100 U/mL penicillin, 100 $\mu$g/mL streptomycin, and 0.25 $\mu$g/mL amphotericin B (Sigma Chemical Co.) were added to both media. The cells were cultured at 37 °C in a moist atmosphere of 5% carbon dioxide in air.

**Irradiation Procedure.** Irradiations were performed by means of Philips HPW 125 lamps equipped with a Philips filter emitting over 90% at 365 nm. Irradiation intensity was checked on a UV-X radiometer (Ultraviolet Products Inc., Cambridge, U.K.) for each experimental procedure.

**Inhibition Growth Assays.** HeLa cells ($10^5$) were seeded into each well of a 24-well cell culture plate. After incubation for 24 h, the medium was replaced with an equal volume of Dulbecco's modified Eagle medium (DMEM; Sigma Chemical Co.) without phenol red, and various concentrations of the test agent were added. One hour later the cells were irradiated with a UVA dose of 0.793 J cm$^{-2}$. After irradiation, the medium containing the compounds was removed, and the cells were incubated in complete F-12 medium for 24 h. For the experiments carried out in the dark, the replacement of the incubation medium was omitted and the cells were incubated in complete F-12 medium in the presence of the test compound for 24 h.

HL-60 cells ($10^5$) were seeded into each well of a 24-well cell culture plate. After incubation for 24 h, various concentrations of the test agents were added in complete medium. The cells were kept in the dark for 1 h, irradiated with a UVA dose of 0.793 J cm$^{-2}$, and then incubated for a further 24 h.

A trypan blue assay was performed to determine cell viability. Cytotoxicity data were expressed as $IC_{50}$ values, i.e., the concentrations of the test agent inducing 50% reduction in cell numbers compared with control cultures.

**Skin Phototoxicity.** Skin phototoxicity was tested on depilated albino guinea pigs (outbred Dunkin-Hartley strain), as previously reported.[15] An ethanol solution of each new compound was applied topically to the skin up to 50 $\mu$g cm$^{-2}$. For 5-MOP and 8-MOP the concentration used was 10 $\mu$g cm$^{-2}$. The animals were then kept in the dark for 45 min, and the treated skin was irradiated with 20 kJ m$^{-2}$ of UVA; erythema was scored after 48 h.

**Nucleic Acids.** Calf thymus DNA was purchased from Sigma Chemical Co. Its hypochromicity, determined according to Marmur and Doty,[23] was over 35%. DNA from *Micrococcus lysodeikticus* (Cat. D-8259), *Escherichia coli* (Cat. D-2001), and *Clostridium perfringens* (Cat. D-1760), poly[dA-dT]·poly[dA-dT] (Cat. P-0883), and poly[dG-dC]·poly[dG-dC] (Cat. P-9389) also came from Sigma.

**Linear Flow Dichroism.** LD measurements were performed on a Jasco J500A circular dichroism spectropolarimeter converted for LD and equipped with an IBM PC and a Jasco J interface.

Linear dichroism is defined as:

$$LD_{(\lambda)} = A_{||(\lambda)} - A_{\perp(\lambda)}$$

where $A_{||}$ and $A_{\perp}$ correspond to the absorbances of the sample when polarized light is oriented parallel or perpendicular to the flow direction, respectively. The orientation is produced by a device designed by Wada and Kozawa[24] at a shear gradient of 500−700 rpm.

The reduced linear dichroism is defined as:

$$LD_r = LD_{(\lambda)}/A_{iso(\lambda)}$$

where $A_{iso(\lambda)}$ is the absorbance of the sample in the absence of flow. This quantity may be related to an orientation factor ($S$) and the angle between the active transition moment in the chromophore and the DNA helix axis, $\alpha$:[25,26]

$$LD_r = 3/2(3\cos^2\alpha - 1)S$$

Assuming a value of $\alpha = 90°$ for the DNA base pair chromophore with respect to a local helix axis, it is possible to evaluate $\alpha_L$ for a given ligand:

$$\alpha_L = \arccos[1/3 - (LD_r)_L/3(LD_r)_{DNA}]^{1/2}$$

where $(LD_r)_L$ is the reduced linear dichroism for the ligand, $(LD_r)_{DNA}$ is the reduced LD for DNA, and $\alpha_L$ defines the ligand−DNA relative orientation. For the intercalated system, $(LD_r)_L \approx (LD_r)_{DNA}$ and $\alpha_L \cong 90°$.

A solution of calf thymus DNA ($1.5 \times 10^{-3}$ M) in ETN buffer (containing 10 mM TRIS, 10 mM NaCl, and 10 mM EDTA, pH = 7) was used. Spectra were recorded at 25 °C at different [DNA]/[drug] ratios.

**Fluorimetric Determinations.** Fluorimetric determinations were essentially made following previous indications.[27] Volumes of concentrated solutions of the examined compound were added to calf thymus DNA in ETN solution to achieve a DNA/compound ratio of about 80. Aliquots of these solutions were introduced into calibrated glass tubes, immersed in a thermostatically controlled bath, and then irradiated for various periods of time. After irradiation the DNA was precipitated with NaCl and cool ethanol, washed with ethanol 80%, and collected by centrifugation. The pellet was then dissolved in a measured volume of buffer in order to reach an exact DNA concentration. The final solution was made 0.5 N with HCl, heated at 100 °C for 1 h, and neutralized; the fluorescence intensity was determined by means of a Perkin-Elmer LS50B luminescence spectrometer at $\lambda_{ex}$ = 320 nm and $\lambda_{em}$ = 420 nm.

**Spectrophotometric Determinations.** Spectrophotometric determinations were performed as described in ref 17 with some modifications. Briefly, volumes of concentrated solutions of the examined compound were added to calf thymus DNA in ETN solution to achieve a DNA/compound ratio of about 20. Aliquots of these solutions were introduced into calibrated glass tubes, immersed in a thermostatically controlled bath, and then irradiated for 90 min. After irradiation the DNA was precipitated with NaCl and cool ethanol, washed with ethanol 80%, and collected by centrifugation. The pellet was then dissolved in a measured volume of buffer in order to reach an exact DNA concentration. The final solution was made 0.5 N with HCl, heated at 100 °C for 1 h, neutralized, and extracted exhaustively with $CHCl_3$ for the hydroxy and methoxy derivatives and $CHCl_3$/methanol (2:1) for the dimethylaminopropoxy derivatives. After this procedure the organic layers were collected, dried under high vacuum, and dissolved in ethanol. UV spectra were recorded on a Perkin-Elmer model Lambda 5 spectrophotometer.

**Evaluation of Interstrand Cross-Links in Vitro.** After irradiation in the presence of the examined compound, the DNA solutions ([DNA]/[drug] = 78) were thermally denatured (95 °C for 15 min) and quickly cooled in ice. One milliliter of each of these solutions was chromatographed on a column of

hydroxylapatite (Bio-Gel HTP, 130-0420, Biorad) developing with a linear gradient of 0.005−0.5 M phosphate buffer, pH = 7; the flow rate was 15 drops/min. Fractions of 2 mL were collected, and the absorbance at 260 nm was recorded.

**Radiochemical Determinations.** Radioactivity measurements were made by means of a Packard model TRI-CARB 4000 liquid scintillation spectrometer. The efficiency of the apparatus for counting tritium, calculated by using an internal standard, was within 35−40%.

Compound **16** was tritium-labeled by Amersham International (Buckinghamshire, U.K.) and purified on thin-layer chromatography plates (silica gel, Merck, 2 mm). Plates were developed with $CHCl_3$/methanol, 8:2 (v/v); purified product showed a specific activity of 514 Ci/mol.

**Photobinding to DNA in Vitro.** Small measured volumes of concentrated ethanol solutions of the labeled compound were added to aqueous 0.05% calf thymus DNA (containing 10 mM TRIS, 10 mM NaCl, and 10 mM EDTA, pH = 7) to achieve a DNA/compound molar ratio of about 80. Aliquots of these solutions were introduced into calibrated glass tubes, immersed in a thermostatically controlled bath, and then irradiated for various periods of time. After irradiation, the DNA was precipitated with NaCl and ethanol and washed with ethanol 80%, and the pellets were dissolved in the initial volume of buffer before radiochemical measurements were performed.

In the experiments on bacterial DNA and synthetic polynucleotides, buffer solutions of nucleic acids ($1.5 \times 10^{-4}$ M) were added to the labeled furocoumarins ($1.85 \times 10^{-5}$ M). Irradiations were performed as above. After irradiation, the solutions were extracted with chloroform in order to remove the nonphotobound drug and other low-molecular-weight products. Afterward, the aqueous solutions were used for radiochemical measurements.

**Topoisomerase II-Mediated DNA Cleavage.** The topoisomerase II-mediated DNA cleavage assay was performed as reported by Tewey et al.[28] with some modifications. Briefly, pBR322 plasmid DNA (Sigma) was linearized with *Eco*RI (Sigma) and then end-labeled at the 3′ termini with the Klenow fragment of DNA polymerase I (Sigma) in the presence of [α-$^{32}$P]dATP (Amersham). Unincorporated triphosphates were removed by ethanol precipitation in the presence of 2 M ammonium acetate. The linear plasmid was further digested with *Hin*dIII restriction endonuclease (Sigma) to remove a 31-base pair small fragment.

The cleavage reaction was performed in a reaction mixture containing 40 mM TRIS-HCl (pH = 7.6), 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM EDTA, 30 μg/mL bovine serum albumin, 1.6 mM ATP, 50 ng of the uniquely end-labeled pBR322 DNA, 20 ng of human type II topoisomerase (TopoGEN, Inc.), and test compound as indicated. Reactions were incubated at 37 °C for 30 min and then terminated by the addition of 0.5% SDS and 75 μg/mL proteinase K (Sigma). DNA samples were analyzed on a 1% agarose gel in TBE buffer (89 mM Tris-borate (pH = 8.3) and 2 mM EDTA). Gels were dried and autoradiographed for 1 or 2 days.

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