Angular Furoquinolinones, Psoralen Analogs: Novel Antiproliferative Agents for Skin Diseases. Synthesis, Biological Activity, Mechanism of Action, and Computer-Aided Studies

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With the aim of obtaining new potential photochemotherapeutic agents, having increased antiproliferative activity and decreased undesired effects, we have prepared some new furoquinolinones. Two of them have been studied in detail: 1,4,6,8-tetramethyl-2*H*-furo[2,3-*h*]-quinolin-2-one (**8**), and 4,6,8,9-tetramethyl-2*H*-furo[2,3-*h*]quinolin-2-one (**10**). These compounds form a molecular complex with DNA, undergoing intercalation inside the duplex macromolecule, as shown by linear flow dichroism. The complexed ligands, by subsequent irradiation with UV-A light, photobind with the macromolecule forming only monocycloadducts with thymine with *cis-syn* configuration. In order to evaluate the electronic effects induced by the nitrogen atom in position 1 of **8**, semiempirical calculations have been performed on both 4,6,4′-trimethylangelicin (TMA) and **8**. The results obtained do not clearly differentiate between the two molecules which, at this level of approximation, show the possibility of photoreaction with both the 3,4- and 8,9-olefinic bonds for **8** and the 3,4- and 4′,5′-bonds for TMA. In the lower energy conformation of intercalated **8**, the furan ring is turned toward the minor groove of the polynucleotide, in such a way that photoreaction of this ring with thymine is favored. These compounds unexpectedly inhibit DNA and RNA synthesis in Ehrlich cells, in the dark. They also show a strong photoantiproliferative activity, 2 orders of magnitude higher than 8-methoxypsoralen (8-MOP), the most used drug for photochemotherapy. Their mutagenic activity on *Escherichia coli* is similar to that of TMA and 8-MOP. On the basis of these results, the compounds should deserve evaluation of their activity in the treatment of hyperproliferative skin diseases.

Psoralens, called also furocoumarins, are naturally occurring or synthetic compounds exhibiting strong photobiological and phototherapeutic activities.[1,2] Psoralen photochemotherapy is carried out by topical application or oral administration of a psoralen and subsequent irradiation of the patient's skin with UV-A light (320−400 nm): A variety of diseases such as psoriasis, mycosis fungoides, lichen planus, vitiligo, and others may be treated. Some of these diseases are characterized by hyperproliferative conditions, e.g, psoriasis; vitiligo is manifested by the lack of pigmenting ability of the skin.[2]

Another type of psoralen photochemotherapy is carried out by photopheresis. This is a process by which peripheral blood is exposed in an extracorporeal system to photoactivated 8-methoxypsoralen (8-MOP) for the treatment of disorders caused by aberrant T-lymphocytes. Photopheresis is an effective therapy for cutaneous T-cell lymphoma and autoimmune disorders such as pemphigus vulgaris and scleroderma.[3]

However, the most widespread application of psoralen photochemotherapy (PUVA) is in the treatment of psoriasis. Many thousands of patients have been treated, and together with highly effective therapeutic efficacy, some side effects, such as skin phototoxicity, mutagenesis, risk of skin cancer and cataract, and aging of the skin, have been observed.[4] In this connection, the antiproliferative effects of psoralens are mainly due to interaction with cell DNA.[5,6] Psoralen first forms a molecular complex with the macromolecule undergoing intercalation; by subsequent irradiation with UV-A light, the intercalated psoralen induces selective damage to the DNA.[5−7] This photodamage is responsible for both antiproliferative effects and mutagenic and carcinogenic effects.[8]

With the aim of preparing and studying new potential photochemotherapeutic agents with increased antiproliferative activity and decreased undesired toxic effects, we recently synthesized some new angular furoquinolinones, angular analogs of psoralen, and isosters of angelicin. In this paper, we report the synthesis, study of mechanism of action, and evaluation of biological activity of some of these compounds, which show an activity much higher than that of psoralens and methylangelicins.

Results

Chemistry. The synthetic route followed to obtain the title compounds is summarized in Scheme 1. Starting material is 7-hydroxy-4,6-dimethylquinolin-2-one (**1**), condensed with allyl bromide to give the corresponding 7-*O*-allyl ether **2**. This compound was methylated with methyl sulfate to give the corresponding *N*-methyl derivative **3**; in this reaction a small amount of 2-methoxyquinoline **4** was also obtained. The 7-(al-

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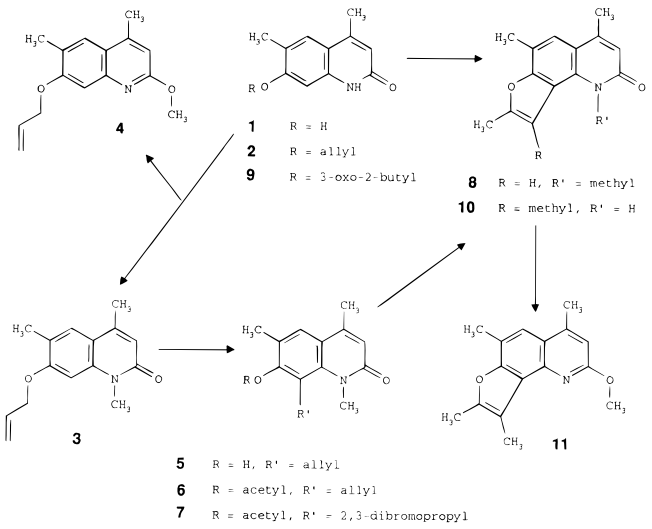
Scheme 1



lyloxy)-*N*-methyl-4,6-dimethylquinolin-2-one was submitted to Claisen rearrangement, yielding 8-allyl derivative **5**.

The pure 8-allyl compound was acetylated and brominated at room temperature to afford 8-dibromopropyl derivative **7**, which was submitted to cyclization in alkaline medium giving 1,4,6,8-tetramethyl-2*H*-furo[2,3-*h*]quinolin-2-one (**8**).

From 7-hydroxy-4,6-dimethylquinolin-2-one (**1**), 4,6,8,9-tetramethyl-2*H*-furo[2,3-*h*]quinolin-2-one (**10**) was also obtained by condensing it with 3-chlorobutan-2-one and cyclizing ether **9** in concentrated sulfuric acid. Methylation of **10** by either methyl sulfate or methyl iodide gave 2-methoxy-4,6,8,9-tetramethyl-2*H*-furo[2,3-*h*]quinoline (**11**) and not the desired *N*-methylfuroquinolin-2-one.

Interaction with DNA in the Ground State. It is well known that a preliminary complex in the ground state occurs between furocoumarins and DNA, in which the planar moiety of the furocoumarin undergoes intercalation inside duplex DNA.[9]

The formation of a molecular complex between compounds **8** and **10** is supported by the strong fluorescence quenching of the two small ligands ($\lambda_{exc} = 330$ nm; $\lambda_{fl} = 400$ nm) and by the decrease in their UV absorption ability, accompanied by a red shift of λ_{max} in the presence of DNA. This behavior, very similar to that shown by other furocoumarins,[9-11] reveals the formation of noncovalent complexes between **8** or **10** and DNA.

Flow Dichroism Measurements. In order to show whether compounds **8** and **10** undergo intercalation inside duplex DNA when complexed with the macromolecule, as in the case of psoralen[9] or angelicin,[12] the flow linear dichroism (LD) of an aqueous solution of compound **8** or **10** in the presence of DNA was determined. This technique allows the long, stiff DNA molecule to be oriented in flow, and the chromophore of the macromolecule gives a peculiar negative dichroism.[13]

When a small planar ligand like **8** or **10** undergoes intercalation inside duplex DNA, it assumes an ordered position similar to that of purine and pyrimidine bases of the macromolecule. Negative dichroism of the ligand (at wavelengths different from those of DNA) is thus observed when the transition moment is polarized in parallel with the planar chromophore, as for purines and pyrimidines.[11,12] Assuming that in furoquinolinones the strong $\pi \rightarrow \pi^*$ transitions are polarized in the molecular plane, like in furocoumarins, the negative LD observed between 330 and 360 nm indicates that complexed **8** and **10** assume a position parallel to the DNA bases. This orientation is consistent with the intercalation of this ligand between two base pairs of DNA.

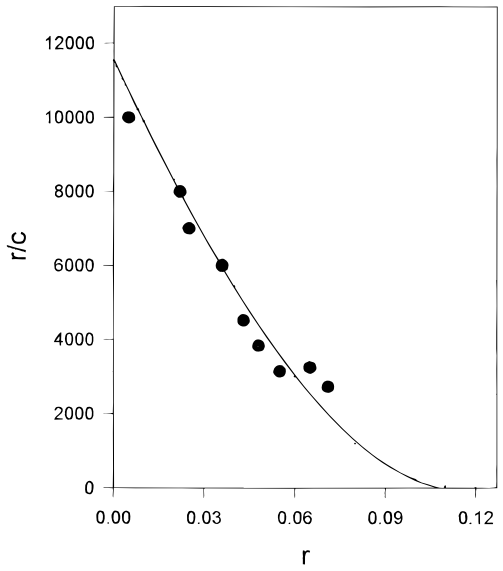


Figure 1. Scatchard plot for the binding in the dark of compound **8** to calf thymus DNA.

Table 1. Water Solubility and Partition Coefficient *n*-Octanol/Water of Furoquinolinone **8**, 8-MOP, and TMA and Binding Parameters of Their Complexes with Calf Thymus DNA Calculated According to Ref 15[a]

compd	solubility (mg mL^{-1})	partition coefficient	K (M^{-1})	n	1/n
8	4.7	163.5	11 700	8.44	0.12
8-MOP	23		736[b]	7.81	0.128
TMA	1.46	1897	10 100	10.99	0.091

[a] K = association constant to an isolated site; n = number of nucleotides occluded by a bound ligand; $1/n$ = frequency of binding sites. [b] Taken from ref 17.

Binding Parameters of the Complexes. To determine the binding parameters of the complexes, the binding process between **8** and the macromolecule was followed by equilibrium dialysis experiments.

The binding data allowed the values of r (molecules of ligand bound/nucleotide in various experimental conditions) and c (ligand free in the system, mol L^{-1}) to be calculated according to Peacocke and Skerrett[14] (Figure 1). The binding parameters of the complex, i.e., K (association constant to an isolated site), n (number of nucleotides occluded by a bound ligand at saturation of the system), and $1/n$ (frequency of binding sites, that is, number of ligand molecules bound to every nucleotide at saturation of the system), were calculated according to McGhee and von Hippel,[15] on the basis of the experimental data of r and c.

Compound **8** exhibits affinity toward DNA (K = 11 700; see Table 1) similar to that shown by TMA (K = 10 100), taken as reference compound, but much higher than that of 8-MOP (K = 736), the most frequently used drug for photochemotherapy.[17] Table 1 also shows data on water solubility and partition coefficient *n*-octanol/water. Compound **8,** in spite of the

presence of three methyl groups in the tricyclic moiety and a *N*-methyl group, has higher water solubility than TMA and lower lipophilicity.

Quantum Mechanics Results. Semiempirical calculations were performed to obtain frontier molecular orbital (MO) energies and atomic orbital (AO) coefficients,[8] both for the two furocoumarins and thymine which was experimentally found to be the principal counterpart for the photochemical reaction. When irradiated with UV light, furocoumarins can absorb a photon which causes an electron to leave one of the highest energy-occupied molecular orbitals and jump to a lowest unoccupied one. It is plausible to think that one of the singly occupied molecular orbitals which, using the terminology proposed by Flemming,[18] we can call HOMO and LUMO, or more generally HOMO $- n$ and LUMO $+ n$, interacts with one of the thymine molecular orbitals of proper size and symmetry. The assumption was that the [2 + 2] photoactivated reaction can be described by MO theory, which basically states that interactions between reactive species are proportional to the molecular orbital overlap and in inverse proportion to the energy gap between the corresponding molecular orbitals. AM1 calculations show that thymine presents the HOMO which is bonding in character and LUMO which is antibonding, strongly present on the 5,6-double bond. For TMA and **8,** HOMO is mostly present on the 4′,5′- and 8,9-double bonds, respectively, while LUMO is principally present on the 3,4-bond. According to molecular orbital theory, in terms of energetic gaps, the closest is that between thymine$_{LUMO}$ and **8**$_{LUMO}$ ($\Delta E = 0.074$ eV) followed by thymine$_{LUMO}$ and TMA$_{LUMO+1}$ ($\Delta E = -0.084$ eV), thymine$_{HOMO}$ and TMA$_{HOMO-1}$ ($\Delta E = -0.088$ eV), and thymine$_{LUMO}$ and **8**$_{LUMO+1}$ ($\Delta E = -0.241$ eV). These results do not show a clear difference between the two molecules which in terms of atomic orbital overlap and energy could photoreact with both the 3,4- and 4′,5′(or 8,9)-double bonds.

Monte Carlo/Energy Minimization Results. MC/EM (2000) steps were employed to yield 100 low-energy complexes, sorted in terms of lowest interaction energy between the oligonuclotide and the furoquinolinone. Compound **8** shows better interaction with the oligonucleotide with respect to TMA, in agreement with the slightly higher DNA binding shown in Table 1.

As concerns TMA, in spite of the *cis-syn* configuration of the isolated furan-side cycloadduct,[19] the studies of Demaret et al.[20] on the lowest energy conformation for the TMA−oligonucleotide shows that, besides the intercalation geometry leading to the *cis-syn* adduct, the *cis-anti* orientation appears possible as well, from energetic and geometric points of view. The lowest energy conformation observed for the **8**−oligonucleotide complex (Figures 2 and 3) is of *cis-syn* type and shows good overlap of the furan ring with thymine on the left side. On the contrary, neither the angle between the 3,4-double bond of **8** and the 5,6-double bond of thymine on the right side nor their distance is compatible with the cycloaddition at the quinolone moiety. This may explain the experimental evidence of an almost unique photoreaction of **8** through its furan ring and, consequently, its inability to form interstrand cross-links (see below).

DNA Photobinding. The events occurring in the photoreaction between furoquinolinones and DNA are similar to those of furocoumarins. Indeed, the latter

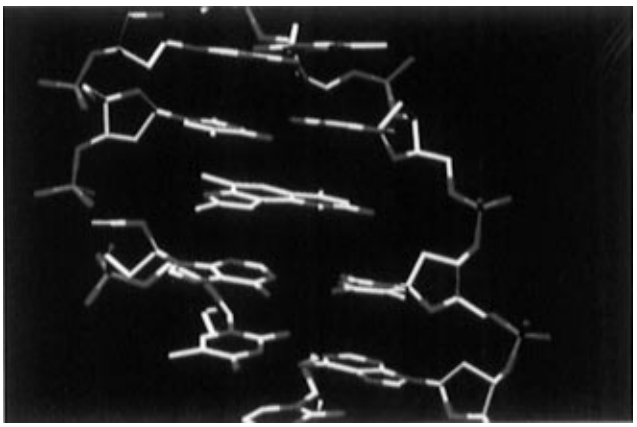


Figure 2. Intercalation site of **8**−oligonucleotide complex viewed in a projection plane parallel to the helix axis. The left thymine and the right adenine are above the furocoumarin. The minor groove is on the top of the figure.

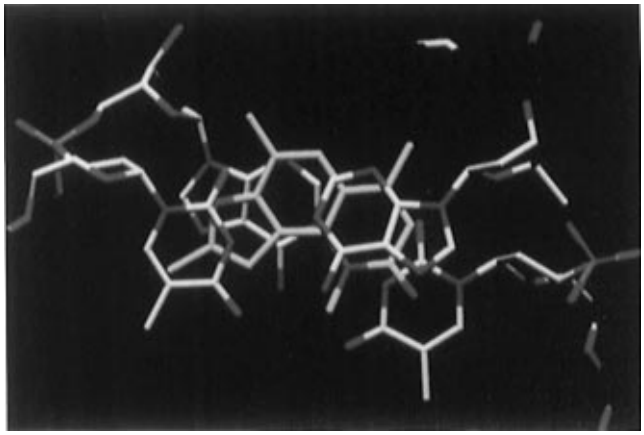


Figure 3. Intercalation site of **8**−oligonucleotide complex viewed in a projection plane orthogonal to the helix axis.

form a preliminary complex with the macromolecule undergoing intercalation;[6,7] after irradiation, the intercalated ligand photobinds covalently to DNA, forming mono- and diadducts having *cis-syn* stereochemistry.[6,7,21,22] One factor which controls the photobinding to DNA is the extent of ligand intercalation inside duplex DNA: The intercalated ligand is in equilibrium with a certain amount of free ligand dissolved in water, and the latter may undergo photomodification during irradiation.[23] In this way, photolysis of the free ligand implies a decrease in its concentration in the system, thus affecting the amount of intercalated ligand. However, this effect is small, as the extent of photolysis is lower than that of DNA photobinding.

The amount of ligand covalently photobound to the macromolecule as a function of time of irradiation was determined by radiochemical measurements on DNA irradiated in the presence of tritiated ligand. This photoreaction between furoquinolinone **8** and DNA behaves as a pseudo-first-order reaction with respect to the complexed ligand, showing behavior similar to that of psoralens[24] and methylangelicins.[12]

Table 2 gives the initial rate constant values of the photoreaction between **8** and DNA and, for comparison, those of TMA and 8-MOP. Compound **8** shows strong DNA photobinding, even higher than that of TMA and much higher than that of 8-MOP.

Figure 4 shows the HPLC profile of a DNA sample irradiated in the presence of compound **8** and hydro-

Table 2. Rate Constants of Photoreaction between Furoquinolinone **8**, 8-MOP, and TMA and Calf Thymus DNA

compd	k (min^{-1})
8	0.22
8-MOP	0.031
TMA	0.12

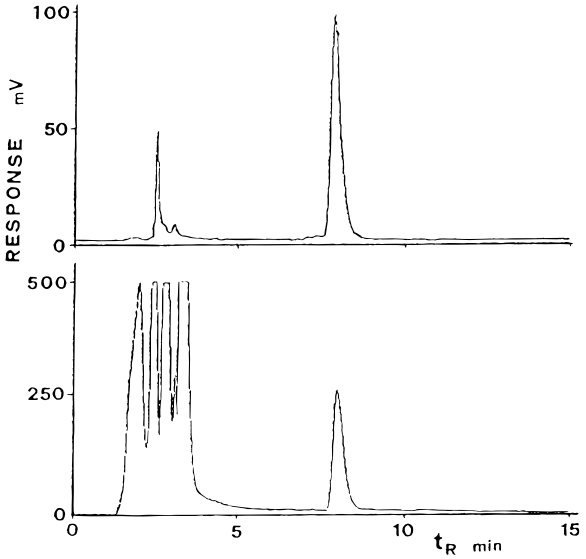


Figure 4. HPLC profiles of a DNA sample irradiated in the presence of compound **8** and hydrolyzed. UV detection at 320 (top) and 250 (bottom) nm. Chromatographic conditions were as in the Experimental Section.

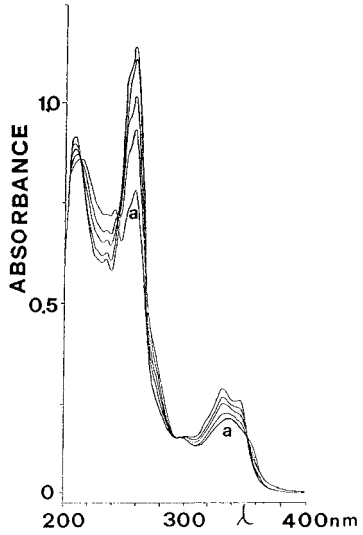


Figure 5. UV absorption spectrum of an ethanol solution of **8**−thymine adduct before (line a) and after irradiation at 254 nm (3, 6, 10, and 15 min, respectively).

lyzed, the upper part at 320 nm, the lower at 250 nm. Besides the large peaks eluting before 4 min, mainly belonging to the DNA bases, a single peak appears at 7.8 min which absorbs at both wavelengths (some remnants of unreacted **8** do not elute in these conditions and were washed out with 100% MeOH after each run). TLC also showed that only one photoproduct formed, appearing as a bright violet fluorescent band on the plates ($R_f = 0.30$). Its UV absorption spectrum is shown in Figure 5, line a, the others being recorded after increasing periods of irradiation at 254 nm. They show gradual photosplitting, after which both HPLC and TLC indicated that **8** and thymine formed. This behavior

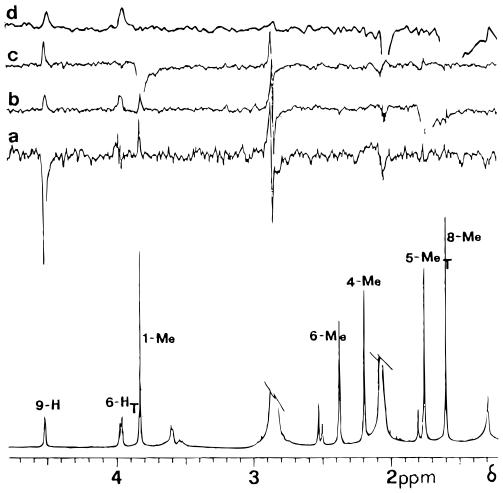


Figure 6. Relevant region of the ^{1}H NMR spectrum of the **8**−thymine adduct and the NOE difference spectra obtained by saturation of 9-H (a), 5-Me$_T$ (b), 1-Me (c), and 8-Me (d).

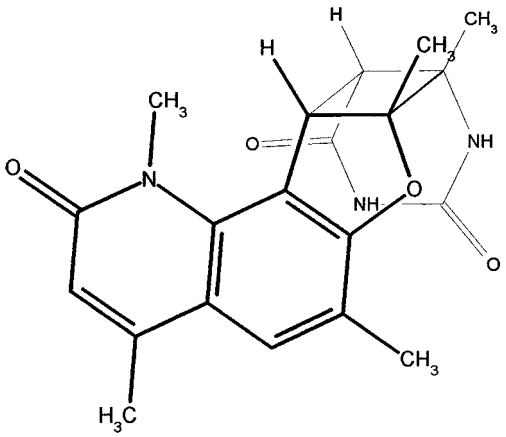


Figure 7. Molecular structure of the **8**−thymine cycloadduct.

suggests that the photoproduct is a cyclobutane adduct between the 5,6-double bond of the pyrimidine base and one of the double bonds of **8**, while the fluorescence data (λ_{exc} = 337 nm, λ_{fl} = 374 nm) are in favor of the involvement of the furan-side double bond in the cycloaddition. This was confirmed by mass spectroscopy, which revealed the molecular ion and confirmed the easy cleavage of the cyclobutane ring, yielding the parent compounds. MS/MS (both MIKES and B^2/E experiments) also confirmed this assumption. NMR data were also in agreement with the structure, and nuclear Overhauser effect measurements allowed the configuration to be assigned to the adduct. Figure 6 shows that saturation of each of the cyclobutane methyls causes equal enhancement of both cyclobutane protons (spectra b and d), while the mutual effect between the latter is much lower (lines a and c). These data indicate a *cis-syn* configuration, as shown in Figure 7. Moreover, the enhancement of the 1-Me signal at 3.83 ppm when the signal at 1.76 ppm is saturated (c) allowed the assignment of the latter to 5-Me$_T$. Very similar results were obtained with compound **10** (HPLC, t_R = 8.8 min; TLC, R_f = 0.36; λ_{exc} = 335 nm, λ_{fl} = 371 nm). As mentioned before, psoralen−pyrimidine[21] and angelicin−pyrimidine[19] monoadducts involving the furan ring also have *cis-syn* configuration.

Monofunctional Photoaddition to DNA. It is well known that psoralen, when intercalated inside duplex

Table 3. T2 Phage Inactivation in the Dark

compd	surviving fraction
none	1
8	0.69
10	0.7
8-MOP	0.8
TMA	0.6

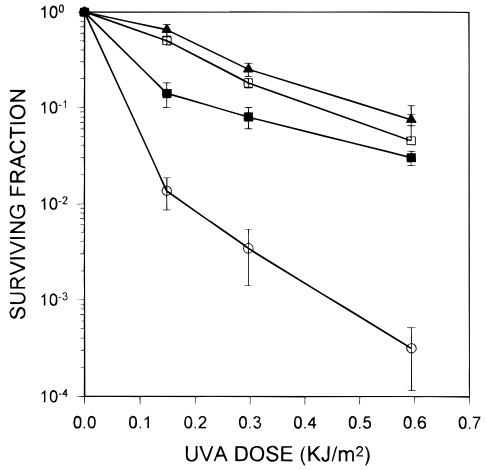


Figure 8. T2 phage inactivation. Virus particles were exposed to increasing UV-A light doses in the presence of furoquinolinones, and then the number of plaque-forming units was determined. 8-MOP and TMA were used as reference compounds. The symbols and drug concentrations are **8**, ○, 2 μM; **10**, ■, 4 μM; 8-MOP, ▲, 23 μM; and TMA, □, 13 μM.

DNA by subsequent irradiation, forms mono- and di-adducts (cross-links) with the pyrimidine bases of the macromolecule. In particular, the monoadducts may be furan-side or pyrone-side. Furan-side cycloadducts may absorb UV-A radiation and further photoreact with a pyrimidine of the complementary strand of DNA, thus forming interstrand cross-links.[23] It has also been shown that angular furocoumarins only form monoadducts owing to their intercalation geometry.[25]

As **8** and **10** have angular structure, in order to verify whether they photobind monofunctionally to DNA, unlike psoralens but like angelicins, the behavior of DNA samples irradiated in the presence of furoquinolinones was studied by hydroxylapatite column chromatography. This technique separates single-strand (heat-denatured) DNA from double-strand (native or renatured) DNA.[6]

The elution profiles of DNA irradiated in the presence of **8** or **10** are identical with that obtained with untreated DNA, showing complete denaturation of the macromolecule, while 8-MOP induced an almost complete renaturation due to the presence of cross-links.

T2 Phage Inactivation. The activity of furoquinolinones on T2 phage was first assayed by incubation in the dark; the virus particles were incubated for 15 min in the presence of the drugs (5 μM). The results are summarized in Table 3. All compounds caused a small reduction of the survival fraction, with values very similar for all compounds tested. Therefore, in experiments with UV-A irradiation, furoquinolinones were employed at lower concentrations; TMA and 8-MOP were assayed at higher concentrations without further increase in their dark inhibition of T2 infectivity.

Figure 8 shows the survival curves obtained after UV-A irradiation of T2 phage particles in the presence of furoquinolinones. The most effective derivative appears to be **8,** which reduced the survival fraction to very low values in very mild experimental conditions (2 μM). The reference compounds 8-MOP and TMA employed at much higher concentrations (23 and 13 μM, respectively) still showed noticeable activity, although much lower in comparison with **8**.

Table 4. Inhibition of DNA and RNA Synthesis in the Dark

compd	DNA synthesis ID_{50} (μM)	relative activity	RNA synthesis ID_{50} (μM)	relative activity
8	2.33	1	1.35	1
10	25.15	9.26×10^{-2}	5.27	0.256

Table 5. Inhibition of DNA and RNA Synthesis by UV-A Irradiation

compd	DNA synthesis (D_U)	relative activity	RNA synthesis (D_U)	relative activity
8	0.90	77.6	0.803	461
10	1.19	102.6	1.048	582
8-MOP	1.16×10^{-2}	1.00	1.8×10^{-3}	1.00
TMA	4.81×10^{-2}	4.14	3.03×10^{-2}	16.83

DNA and RNA Synthesis in Ehrlich Cells. The effect of furoquinolinones on DNA and RNA synthesis in Ehrlich cells by incubation in the dark is shown in Table 4. Unlike 8-MOP and TMA, which were entirely inactive in such experimental conditions (data not shown), furoquinolinones inhibited both RNA and RNA synthesis. This effect is evident in **8,** while in **10** it was still significant but clearly lower. Furoquinolinones also appeared to be more effective on RNA than on DNA synthesis.

Data on experiments with UV-A irradiation are reported in Table 5. Both furoquinolinones tested induced very strong inhibition of macromolecular synthesis. **8** and **10** were several times more effective than reference compounds 8-MOP and TMA. In particular, the activity of **10** was 100 times higher than that of 8-MOP. Both **8** and **10** induced more intense inhibition of RNA synthesis.

Mutagenic Activity. Mutagenic activity was determined in *Escherichia coli* TM9, a strain defective in DNA repair (*uvrA*) and carrying a well-known plasmid (R46) which codifies efficient error-prone DNA repair. **8** and **10** were assayed in very mild experimental conditions: 1−2 μM concentration and UV-A doses in the range of 0−0.12 kJ m^{-2}. For comparison, 8-MOP and TMA were also assayed at the same UV-A doses but at a higher concentration, 20 μM. This difference was selected in order to have comparable amounts of surviving fractions. Figure 9 shows the results; the data are reported as number of revertants/million survivors as a function of the log of the surviving fraction, thus comparing the mutagenicity of the compounds at the same level of antiproliferative activity.

Compounds **8** and **10** showed reduced mutagenic activity, very similar to that of TMA, with modest differences between the responses of the two drugs. Instead, 8-MOP induced higher numbers of revertants. In general, defective strains containing plasmids are regarded as poorly sensitive to bifunctional mutagens.[26,27] However, plotting the number of revertants/million survivors as a function of the surviving fraction allows comparison of the mutagenic activity of the drugs tested at the same level as their cytotoxicity, independently of their different antiproliferative activity.

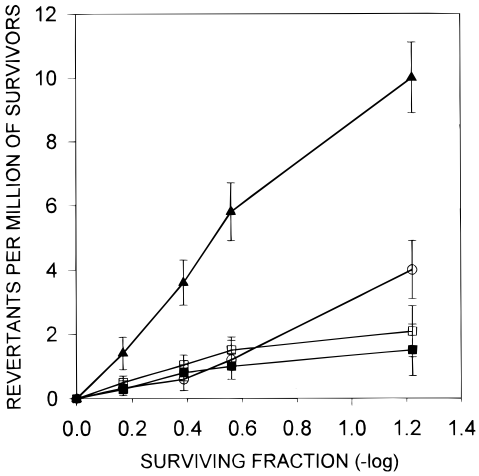


Figure 9. Mutagenicity in *E. coli* TM9 reported as a function of the log of survival fraction. Bacteria were exposed to increasing UV-A light doses in the presence of furoquinolinones. The number of survivors and the number of revertants/million survivors were determined. 8-MOP and TMA were used as reference compounds. The symbols are **8**, ○; **10**, ■; 8-MOP, ▲; and TMA, □.

Conclusions

A new series of psoralen analogs was prepared. Of them, compounds **8** and **10** were studied in terms of mechanism of action and antiproliferative and mutagenic activity. Taking into account the molecular similarity of these new compounds with psoralens and angelicins, their interactions with DNA were studied.

In particular, **8** and **10** form molecular complexes with DNA, undergoing intercalation between two base pairs of the macromolecule, as shown by linear dichroism experiments. The binding parameters of the ligand−DNA complex were determined by equilibrium dialysis experiments on **8**, which was previously tritiated. It exhibited strong affinity toward DNA, much stronger than that shown by 8-MOP and very similar to that of TMA. Moreover, although showing similar affinity toward the macromolecule, **8** was more water soluble and had lower value of the partition coefficient *n*-octanol/water than TMA.

It is well known that psoralen intercalated inside duplex DNA, when irradiated with UV-A light, photoreacts with pyrimidine bases of the macromolecule, forming C_4-cycloadducts. In this connection, labeled compound **8** was studied. It showed strong DNA photobinding capacity, much higher than that of 8-MOP and 2 times as high as that of TMA, as shown by the initial rate constant of photoreaction. These compounds have angular structure, and according to the intercalation model shown in Figures 2 and 3, they should not be able to form interstrand cross-links in DNA, owing to the lack of alignment of the 3,4-double bond with the 5,6-double bond of the thymine belonging to the complementary strand of duplex DNA. We were able to confirm this monofunctional addition of compounds **8** and **10** since they could not induce renaturation after heat denaturation in the treated DNA.

Further support for the monofunctional character of photoreaction was obtained by isolation from DNA and characterization of the monocycloadducts between either **8** or **10** and thymine. They involve the 8,9-double bond of the furoquinolinone moiety and the 5,6-double bond of thymine. The regio- and stereochemistry of these furan-side monoadducts was *cis-syn*, further confirming intercalation of the ligand inside duplex DNA during photoreaction.

Although a nitrogen atom in position 1 changes the electronic properties, as shown by the absorption spectra, quantum mechanics calculations indicate that both TMA and **8** should photoreact through both their double bonds.

The experimental evidence was, at variance, that the main adduct involves the furan ring of both compounds. The energy calculations on the complexes with the oligonucleotide, however, show that the orientation leading to the *cis-syn* furan-side adduct is preferred for **8**, while for TMA both *cis-syn* and *cis-anti* configurations are possible.[20] The small differences between **8** and TMA (for example, the little amount of pyrone-side adduct that the latter forms) may be explained by the interference of the thymine methyl group, which clashes with the 4′-methyl of TMA, while giving favorable hydrophobic interaction with the 8-methyl of **8**.

Bearing in mind that the photobiological activity of psoralens and angelicins generally depends on light activation, while in the dark they have poor or no activity, we studied the photobiological activity of the new compounds after UV-A light excitation. As recently, however, some tetracyclic derivatives of psoralen proved to affect various biological substrates even in the dark,[28] we decided to study this aspect too in the new compounds.

Unexpectedly, DNA and RNA synthesis in Ehrlich cells was inhibited in the dark. The extent of inhibition was quite marked in compound **8** but about one-tenth lower in **10**. This new property may make a sinergistic contribution to total antiproliferative activity (dark and photo) of the compounds.

Photoantiproliferative activity was tested on both T2 phage and Ehrlich cells. Compounds **8** and **10** showed stronger effects than 8-MOP and TMA in the virus. In Ehrlich ascites tumor cells, inhibition of DNA and RNA synthesis induced by both furoquinolinones was very strong, about 2 orders of magnitude higher than that of 8-MOP and about 20 times higher than that of TMA.

Mutagenic activity was determined on *E. coli* TM9, the drug concentration and light dose being chosen to have the same antiproliferative activity. Compounds **8** and **10** proved as much mutagenic as TMA, while bifunctional 8-MOP induced higher number of revertants. These results, in particular the relevant dark antiproliferative and photoantiproliferative activities, candidate the new furoquinolinones as potential agents for hyperproliferative skin diseases.

Experimental Section

Melting points were recorded using a Gallenkamp melting point apparatus and are uncorrected. Analytical TLC was performed on precoated 60 F_{254} silica gel plates (0.25 mm; Merck), developing with EtOAc/cyclohexane mixture (35:65). Preparative column chromatography was performed using silica gel 60 (0.063−0.100 mm; Merck). ^{1}H NMR spectra were recorded on a Varian Gemini-200 spectrometer with TMS as internal standard. Elemental analyses were obtained on all intermediates and are within ±0.4% of theoretical values.

7-(Allyloxy)-4,6-dimethylquinolin-2-one (2). A mixture of 7-hydroxy-4,6-dimethylquinolin-2-one (**1**)[29] (2.5 g, 13.2 mmol), allyl bromide (1.9 g, 15.8 mmol), and anhydrous K_2CO_3 (30.0 g) in acetone (200 mL) was heated to reflux for 10 h. After cooling, K_2CO_3 was filtered off and washed with fresh

acetone. The solvent was removed under reduced pressure, and the residue was crystallized from MeOH to give **2** (1.8 g, 60%): mp 229 °C; ^{1}H NMR ($CDCl_3$) δ 7.36 (q, 1H, J = 1.3 Hz, 5-H), 6.75 (s, 1H, 8-H), 6.41 (q, 1H, J = 1.0 Hz, 3-H), 6.15−5.99 (m, 1H, 2′-H), 5.52−5.26 (m, 2H, 3′-H), 4.01 (dt, 2H, J = 5.1, 1.5 Hz, 1′-H), 2.43 (d, 3H, J = 1.0 Hz, 4-Me), 2.27 (d, 3H, J = 1.3 Hz, 6-Me).

7-(Allyloxy)-1,4,6-trimethylquinolin-2-one (3). A mixture of **2** (1.8 g, 8.3 mmol), dimethyl sulfate (1.2 g, 9.9 mmol), and anhydrous K_2CO_3 (30.0 g) in acetone (500 mL) was heated to reflux for 30 h. The reaction mixture was worked up as previously described, and the residue was purified by column chromatography, eluting with EtOAc to give 7-(allyloxy)-2-methoxy-4,6-dimethylquinoline (**4**) (0.1 g, 6%) followed by the desired product **3** (1.6 g, 78%). **4**: mp 95−6 °C (MeOH); ^{1}H NMR ($CDCl_3$) δ 7.59 (br s, 1H, 5-H), 7.16 (s, 1H, 8-H), 6.60 (q, 1H, J = 1.1 Hz, 3-H), 6.25−6.06 (m, 1H, 2′-H), 5.55−5.25 (m, 2H, 3′-H), 4.69 (dt, 2H, J = 5.0, 1.5 Hz, 1′-H), 4.03 (s, 3H, -OMe), 2.56 (d, 3H, J = 1.1 Hz, 4-Me), 2.40 (d, 3H, J = 0.8 Hz, 6-Me).

3: mp 132−3 °C (EtOAc/*n*-hexane); ^{1}H NMR ($CDCl_3$) δ 7.44 (br s, 1H, 5-H), 6.71 (s, 1H, 6-H), 6.45 (q, 1H, J = 1.1 Hz, 3-H), 6.21−6.02 (m, 2H, 2′-H), 5.56−5.32 (m, 2H, 3′-H), 4.68 (dt, 2H, J = 5.1, 1.6 Hz, 1′-H), 3.68 (s, 3H, 1-Me), 2.42 (br s, 3H, 4-Me), 2.31 (s, 3H, 6-Me).

8-Allyl-7-hydroxy-1,4,6-trimethylquinolin-2-one (5). A solution of **3** (1.5 g, 6.2 mmol) in *N*,*N*-diethylaniline (20 mL) was refluxed for 3 h. The reaction mixture was cooled, and *n*-hexane was added. The precipitate obtained was collected, washed with cyclohexane, and crystallized from EtOAc/*n*-hexane to give **5** (1.3 g, 85%): mp 215−6 °C; ^{1}H NMR ($CDCl_3$) δ 7.36 (br s, 1H, 5-H), 6.41 (q, 1H, J = 1.1 Hz, 3-H), 6.38−6.22 (m, 1H, 2′-H), 5.54 (s, 1H, -OH), 5.45−5.21 (m, 2H, 3′-H), 3.64 (s, 3H, 1-Me), 3.61 (dt, 2H, J = 4.4, 2.2 Hz, 1′-H), 2.38 (d, 3H, J = 1.1 Hz, 4-Me), 2.30 (d, 3H, J = 0.8 Hz, 6-Me).

7-Acetoxy-8-allyl-1,4,6-trimethylquinolin-2-one (6). A mixture of **5** (1.2 g, 4.9 mmol) and anhydrous sodium acetate (1.0 g) in acetic anhydride (25 mL) was refluxed for 45 min. The reaction mixture was carefully diluted with water (40 mL), refluxed for 10 min, and poured into water (400 mL). The precipitate was collected, washed with water, and crystallized from MeOH to give **6** (1.1 g, 82%): mp 137−8 °C; ^{1}H NMR ($CDCl_3$) δ 7.46 (br s, 1H, 5-H), 6.54 (d, 1H, J = 1.1 Hz, 3-H), 6.17−5.98 (m, 1H, 2′-H), 5.27−5.02 (m, 2H, 3′-H), 3.71 (s, 3H, 1-Me), 3.54 (br d, 2H, J = 5.0 Hz, 1′-H), 2.43 (d, 3H, J = 1.1 Hz, 4-Me), 2.33 (s, 3H, Ac), 2.23 (d, 3H, J = 0.7 Hz, 6-Me).

7-Acetoxy-8-(2′,3′-dibromopropyl)-1,4,6-trimethylquinolin-2-one (7). An acetic acid solution (20 mL) containing a stoichiometric amount of bromine was dropped at room temperature over the period of 20 min into an acetic acid solution (25 mL) of **6** (1.1 g, 3.8 mmol). After the addition was completed, the solution was further stirred for 10 min and the solvent was removed under reduced pressure to give **7** (1.5 g, 90%): mp 153−5 °C; ^{1}H NMR ($CDCl_3$) δ 7.47 (br s, 1H, 5-H), 6.53 (d, 1H, J = 1.1 Hz, 3-H), 4.27-4.14 (m, 1H, 2′-H), 3.94−3.34 (m, 4H, 1′-H, 3′-H), 3.67 (s, 3H, 1-Me), 2.43 (s, 3H, Ac), 2.42 (d, 3H, J = 1.1 Hz, 4-Me), 2.23 (br s, 3H, 6-Me).

1,4,6,8-Tetramethyl-2*H*-furo[2,3-*h*]quinolin-2-one (8). To an ethanol solution (50 mL) of **7** (1.5 g, 3.4 mmol) was added an ethanolic 4% KOH solution (50 mL), and the mixture was refluxed for 1.5 h. After cooling, the mixture was acidified with 2 N HCl and diluted with water. The obtained precipitate was collected and purified by column cromatography, eluting with $CHCl_3$ to give **8** (0.8 g, 73%): mp 213−24 °C; ^{1}H NMR ($CDCl_3$) δ 7.32 (q, 1H, J = 0.8 Hz, 5-H), 6.90 (q, 1H, J = 1.1 Hz, 9-H), 6.55 (q, 1H, J = 1.0 Hz, 3-H), 3.99 (s, 3H, 1-Me), 2.53 (d, 3H, J = 0.8 Hz, 6-Me), 2.50 (d, 3H, J = 1.1 Hz, 8-Me), 2.48 (d, 3H, J = 1.0 Hz, 4-Me).

Compound **8** was tritium-labeled by Amersham International plc, Amersham, U.K. Its specific activity was 8.5 Ci mol^{-1}.

4,6-Dimethyl-7-(3′-oxo-2′-butyl)quinolin-2-one (9). A mixture of **1** (1.0 g, 5.3 mmol), 3-chlorobutan-2-one (0.7 g, 6.3 mmol), and anhydrous K_2CO_3 (10.0 g) in acetone (200 mL) was heated to reflux for 24 h. The reaction mixture was worked up as previously described for compound **2,** and the residue was purified by column chromatography eluting with $CHCl_3$ to give **9** (1.1 g, 80%): mp 238 °C; ^{1}H NMR ($CDCl_3$) δ 11.63 (s, 1H, 1-H), 7.44 (s, 1H, 5-H), 6.58 (s, 1H, 8-H), 6.42 (q, 1H, J = 1.1 Hz, 3-H), 4.84 (q, 1H, J = 6.8 Hz, 2′-H), 2.45 (d, 3H, J = 1.1 Hz, 4-Me), 2.34 (s, 3H, 6-Me), 2.25 (s, 3H, 4′-H), 1.57 (d, 3H, J = 6.8 Hz, 1′-H).

4,6,8,9-Tetramethyl-2*H*-furo[2,3-*h*]quinolin-2-one (10). Compound **3** (0.5 g, 1.9 mmol) was dissolved in H_2SO_4 (20 mL), and the solution was kept at room temperature for 0.5 h. The mixture was poured into cold water (200 mL), and the obtained precipitate was collected. The residue was purified by column chromatography, eluting with $CHCl_3$ to give **10** (0.4 g, 77%): mp 231 °C (MeOH); ^{1}H NMR (CD_3OD) δ 7.19 (q, 1H, J = 0.8 Hz, 5-H), 6.32 (q, 1H, J = 1.0 Hz, 3-H), 2.44 (d, 3H, J = 1.0 Hz, 4-Me), 2.41 (d, 3H, J = 0.8 Hz, 6-Me), 2.37 (q, 3H, J = 0.8 Hz, 8-Me or 9-Me), 2.34 (q, 3H, J = 0.8 Hz, 8-Me or 9-Me).

2-Methoxy-4,6,8,9-tetramethyl-2*H*-furo[2,3-*h*]quinoline (11). A mixture of **10** (0.3 g, 1.4 mmol), dimethyl sulfate (0.2 g, 1.4 mmol), and anhydrous K_2CO_3 (10.0 g) in acetone (200 mL) was heated to reflux for 2 h. The reaction mixture was worked up as previously described, and the residue was crystallized from MeOH to give **11** (0.3 g, 75%): mp 162 °C; ^{1}H NMR ($CDCl_3$) δ 7.40 (q, 1H, J = 1.0 Hz, 5-H), 6.69 (q, 1H, J = 1.0 Hz, 3-H), 4.08 (s, 3H, -OMe), 2.64 (q, 3H, J = 0.8 Hz, 8-Me or 9-Me), 2.63 (d, 3H, J = 1.0 Hz, 4-Me), 2.60 (d, 3H, J = 0.8 Hz, 6-Me), 2.47 (q, 3H, J = 0.8 Hz, 8-Me or 9-Me).

Nucleic Acid. DNA from calf thymus (cat. D1501) from Sigma Chemical Co, St. Louis, MO, was employed.

Linear Flow Dichroism Measurements. A DNA solution (3.8 mM) containing 2 mM NaCl and 1 mM EDTA was used, either in the absence or presence of compound **8** or **10** (0.17 mM). LD = $A_{\parallel} - A_{\perp}$ was measured on a Jasco J500 circular dichroism spectrometer converted for LD. The measuring device was designed by Wada and Kozawa.[13] A constant shear gradient of 1000 s^{-1} was used for recording the LD spectra, and the base line was taken at zero gradient.

Equilibrium Dialysis Experiments. Cylindrical containers, 4 cm in diameter, 1.6 cm deep, divided into two parts by a Visking cellophane membrane (Serva, Heidelberg, Germany), were used. One part of the cell contained the aqueous solution of labeled compound containing NaCl (0.02 M) and EDTA (1 mM), at a concentration lower than its water solubility; the other part contained aqueous DNA solution in the presence of labeled compound **8**, at the same ionic strength, DNA having decreasing concentrations in the range 3−0.3 mM. The cells were mechanically shaken for 12 h in a thermostat at 25 ± 0.05 °C in the dark. After shaking, small (0.2 mL) volumes of the two phases were used for radiochemical measurements, to determine the furoquinolinone concentrations in the two phases and calculate r and c values.[15]

Computation of Interaction Parameters. The method of computation involved an iterative procedure designed to satisfy the following equation of McGhee and von Hippel:

$$\frac{r}{c} = K(1 - nr)\left[\frac{1 - nr}{1 - (n - 1)r}\right]^{n-1}$$

given the experimentally determined values of r and c and the initial values of K (intrinsic binding constant to an isolated site) and n (number of nucleotides occluded by a furoquinolinone molecule). A program based on the least-squares method of the Taylor series expansion of the above equation was recycled until K and n changed by <1% and then, to give final values, with a calculated binding isotherm at 5% saturation increments.

Computational Methods. The molecular mechanics calculations were performed on IBM RISC 6000/530 and IBM PowerPC 7011 workstations using the Macromodel/Batchmin[30] software package, employing the AMBER* force field.[31] For the quantum mechanics calculations, the MOPAC 6.0[32] program was used.

The model polynucleotide d(CGCGATATCGCG)$_2$ was built using the standard coordinate for the B-form[33] using the polymer builder utility within the Macromodel software. Furocoumarins moieties were built from a 2D sketch and afterwards minimized using the full matrix Newton Raphson

algorithm until the rms energy gradient was inferior to 0.01 kJ $Å^{-1}$ mol^{-1}. The intercalation site of the polynucleotide was produced by unwindig of 28°, rising of 3.6 Å, and C3′-endo (3′−5′) C2′-endo sugar repuckering between base pairs 7 and 6 using as reference the intercalation geometry observed in the proflavine−cytidylyl−(3′,5′)-guanosine complex.[34] The structure so obtained was minimized using 1000 steepest descents steps in order to relax the structure in the new conformation which represented the initial structure where the furocoumarin was intercalated.

Since the calculations were carried out in vacuum, special treatment of the electrostatics was needed in order to simulate the solvent environment. According to the Debye−Manning theory, a net charge of −0.25 e was assigned to the phosphate groups in order to simulate the counterion effects; moreover, a distance-dependent dielectric constant ($\epsilon = 4r_{ij}$) was also used in order to simulate the shielding effects of the solvent.[35] Electrostatic potential-derived charges were calculated for the furocoumarin derivatives inside the MOPAC program using the MNDO approximation (keywords: ESP MNDO GNORM = 0.01). For the Monte Carlo energy minimization (MC/EM) calculations, the furocoumarins were manually intercalated between the sixth and seventh base pairs. A harmonic restrain of 50 kJ $Å^{-2}$ was applied on the first and last base pairs of the oligonucelotide in order to simulate an "infinite" chain. The nonbonded cut-off protocol employed was 15.0 Å for the van der Waals and electrostatic interactions and 4.0 Å for the hydrogen bonding. A "usage directed" conformational search was employed for the MC/EM procedure[36] in which the conformation used in each step is taken from a set of previously saved low-energy structures with preference given to the one that has been less used in the previous calculations. Each conformation is then submitted to a Monte Carlo cycle in which the furocoumarin is randomly rotated around the molecular center of mass and randomly translated along the *x*,*y*,*z* axes by a distance between 0.0 and 3.0 Å. It follows a full minimizazion protocol using the conjugate gradients procedure as long as the energy gradient rms was <0.01 kJ $Å^{-1}$ mol^{-1}. The best 100 structures were saved, and for these, the interaction energies between the oligonucleotide and the furocoumarins were computed from the equation

$$E_{\text{interaction}} = E_{\text{total}} - E_{\text{oligonucleotide}} - E_{\text{furocoumarin}}$$

Quantum mechanics calculations were executed at the AM1 semiempirical level of approximation with the aim of obtaining information on the electronic effects that play a fundamental part in the photocycloaddiction between the thymine and the furocoumarin. Atomic orbital coefficients and energies were calculated from the MOPAC output file as described by Clark[37] on fully optimized structure (keywords: GNORM = 0.01 AM1).

UV-A Irradiation. UV-A exposures were performed with Philips HPW 125 lamps, provided with a built-in Philips filter. Emission was in the 320-400 nm range, with a maximum, over 90% of the total, at 365 nm; irradiation intensity, determined by a UV-X radiometer (Ultraviolet Products plc, Cambridge, U.K.), was 5.5 J s^{-1} m^{-2}.

DNA Photobinding in Vitro. An aqueous solution of DNA (2.3 mM) containing 2 mM NaCl and 1 mM EDTA was added to 30 μM compound **8**; its concentration was always checked by radiochemical measurements and if necessary corrected.

Irradiation was carried out in a test tube immersed in a thermostatically controlled cell by a Philips HPW 125 lamp; irradiation intensity, determined by means of a chemical actinometer,[38] was 29.3 J s^{-1} m^{-2}. After irradiation, solid NaCl was added up to 2 M followed by 2 vol of ethanol. The precipitated DNA, collected by centrifugation, was washed with 80% ethanol and redissolved in the initial volume of water.

The same procedure was repeated using nonlabeled compound **8** or **10**. The final solution was made 0.5 N with HCl, heated at 100 °C for 1 h, and neutralized. Fifty microliters was then injected into a liquid chromatograph (LDC Analytical constaMetric 3000) equipped with a Lichrosorb RP18 column, 250 × 4 mm (Merck, Darmstadt, Germany), mean particle size 7 μm, eluted with a 1:1 mixture of MeOH and H_2O. A Milton Roy Spectromonitor 3100 UV detector was used, set at either 250 or 320 nm, and the data were collected on a PE-Nelson 1020 data station.

For preparative purposes, the neutralized solution was extracted three times with chloroform, and the organic phases, collected and dried over Na_2SO_4, were submitted to TLC on silica gel plates. The bands appearing on the plates were scraped, extracted with ethanol, and used for spectrophotometric, fluorimetric, NMR (Varian Gemini 200 spectrometer, 200 MHz), and MS (VG ZAB 2F double-focused, reverse-geometry spectrometer, operating in FAB conditions, the sample being dissolved in glycerol) measurements. Photosplitting experiments were also performed, irradiating ethanol solutions of the products in quartz cuvettes with a mineral lamp (254 nm).

Compound 8−thymine adduct: ^{1}H NMR (acetone-d_6) δ 8.61 (br, 1H, 3-H_T), 7.43 (q, 1H, J = 0.8 Hz, 5-H), 6.94 (br, 1H, 1-H_T), 6.27 (q, 1H, J = 1.2 Hz, 3-H), 4.51 (d, 1H, J = 1.3 Hz, 10-H), 3.97 (dd, 1H, J = 3.3 Hz, 6-H_T), 3.83 (s, 3H, 1-Me), 2.37 (d, 3H, 4-Me), 2.19 (d, 3H, 6-Me), 1.76 (s, 3H, 5-Me_T), 1.60 (s, 3H, 9-Me); MS (FAB) *m*/*z* 346 (24, $[M + H]^+$), 242 (100, [**8** + $H]^+$), 127 (19, [thymine + $H]^+$).

Compound 10−thymine adduct: ^{1}H NMR (acetone-d_6) δ 8.76 and 8.53 (br, 1H each, 1-H, 3-H_T), 7.41 (q, 1H, J = 0.9 Hz, 5-H), 6.89 (br, 1H, 1-H_T), 6.15 (q, 1H, J = 1.2 Hz, 3-H), 3.79 (d, 1H, J = 2.7 Hz, 6-H_T), 2.38 (d, 3H, 4-Me), 2.21 (d, 3H, 6-Me), 1.69, 1.64, 1.62 (3s, 3H each, 8-Me, 9-Me, 5-Me_T).

Radioactivity Measurements. A Packard Model 4430 liquid scintillation spectrometer was used. Emulsifier scintillator 299 was purchased from Packard, Downers Grove, IL.

Evaluation of Cross-Links. Evaluation of cross-links formed in DNA was carried out by measuring the renaturation capacity of cross-linked DNA after heat denaturation;[39] 1.5 mL of an aqueous solution (2 mM NaCl, 1 mM EDTA) of DNA (2.3 mM) in the presence of furoquinolinone (30 μM) was irradiated for increasing periods of time. This solution was heated for 10 min in a boiling water bath, immersed in ice for 15 min, chromatographed on a column (0.6 × 4 cm) of hydroxylapatite Biogel type (BioRad Laboratories, Richmond, CA), and developed using a linear gradient of 0.05−0.3 M phosphate buffer (pH 6.98) with a flow rate of 15 drops/min. Fractions of 3.5 mL were collected, and absorbance at 260 nm was determined in each fraction. On the basis of the single-strand (non-cross-linked) and double-strand (renatured, cross-linked) fractions of DNA separated and determined in this way, the presence of cross-links formed in DNA could be evaluated.

Experiments with T2 Phage. All biological experiments were always carried out sheltered from light or in red light. Most of the dark treatments were carried out in an incubator, which guarantees suitable dark conditions.

The host bacteria (*E. coli* B48) were grown in brain−heart infusion at 37 °C, collected in log phase, suspended in $MgSO_4$ (2 mM) at a density of 10^9 cells mL^{-1}, and then infected with T2 phage at a multiplicity of 1. The culture was then incubated at 37 °C for 2 h. Phage titers were determined using the standard bilayer method[40] and the same *E. coli* strain. Phage suspensions were diluted to 10^{10} viral particles/mL with $MgSO_4$ (2 mM) containing the compound to be studied. Aliquots (5 mL) of these virus suspensions were poured into Petri dishes (5 cm in diameter), incubated at 37 °C for 15 min in the dark, and then exposed to UV-A light. After irradiation, the viral suspensions were further diluted with the same medium and the numbers of plaque-forming units/mL were scored.

In experiments carried out in the dark, the phage suspensions containing the drug, prepared as above in $MgSO_4$ (2 mM), were incubated at 37 °C for 15 min in the dark, and then the numbers of infective virus particles were scored.

DNA and RNA Synthesis in Ehrlich Cells. Just before testing, a calculated amount of a 1 mg mL^{-1} solution of the drug in dimethyl sulfoxide (DMSO, which concentration never exceeded 0.5%) was added in the dark to suspensions (PBS) containing Ehrlich cells (Lettré strain). Samples of these suspensions containing 2 × 10^7 Ehrlich ascites cells/mL were

kept in the dark at room temperature for 15 min and then irradiated with increasing UV-A doses in Petri dishes (5 cm in diameter). The cells were incubated for 30 min at 37 °C in the presence of 40 kBq mL^{-1} [^{3}H]thymidine or [^{3}H]uridine (4.77 and 1.1 TBq $mmol^{-1}$, respectively; Amersham International plc, U.K.). The acid-insoluble fraction was precipitated by 5% ice-cold trichloroacetic acid and collected on Whatman GF/C filters (2.5 cm in diameter). After several washings with cold 1% trichloroacetic acid, the filters were dried and counted. The results were calculated as the percent of radioactivity incorporated into the DNA of untreated control cells (ca. 3-6 kBq); filtrations were carried out with a Sample Manifold apparatus (Millipore Corp., Bedford). For the experiments carried out in the dark, the ID_{50}, i.e., the drug concentration inducing 50% inhibition of the macromolecular synthesis, was calculated by means of probit analysis. For the experiments with UV-A irradiation, the dose unit (D_U) was computed, defined as follows:

$$D_U = \frac{1}{ID_{50} \cdot \mu M}$$

where the ID_{50} was the UV-A dose inducing 50% inhibition of macromolecular synthesis when delivered in the presence of the drug concentration expressed in μmol mL^{-1} (μM).

Mutagenesis Tests. The strain used was *E. coli* TM9 (*WP2, uvrA, R46*) carrying a nonsense mutation in the *trpE* gene which is reverted by UV light and most base pair substitution mutagens.[41] Bacteria were grown overnight in a minimal Davis−Mingioli salts glucose medium supplemented with tryptophan (20 mg L^{-1}). *E. coli* cells were washed and then suspended in phosphate-buffered saline (pH 7.0) containing the compound to be studied (20 μM) at the density of 10^8 cells mL^{-1}. Nitrogen or air (sterilized by filtration) was bubbled at room temperature, and then the bacteria were irradiated with UV-A as described for DNA solutions. For the mutagenesis test, 0.1 mL aliquots of the irradiated suspensions were added to 2 mL of molten 0.6% top agar and poured onto plates containing 20 mL of SEM agar (MMA fortified with 0.1 mg mL^{-1} Difco nutrient broth). For the determination of the surviving fraction, the irradiated cells (0.1 mL) were diluted with phosphate buffer, added to 2 mL of molten 0.6% agar, and plated on Davis−Mingioli minimal medium supplemented with tryptophan. The plates were incubated for 48 h at 37 °C in the dark, and then the colonies were counted. The mutation frequency was expressed as mutants/10^6 survivors,[42] which had been computed by dividing the number of revertants observed/plate by the number of surviving bacteria at the same treatment and subtracting from the result the number of revertant colonies/million survivors observed in the controls. In this test all manipulations were done under red light.

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Supporting Information Available: LD spectrum of the **8**−DNA complex, photobinding to DNA, NMR spectrum of the **8**−thymine adduct, interaction energy profile, atomic orbital energies and p_z coefficients of compound **8**, TMA, and thymine, and elemental analysis data (5 pages). Ordering information is given on any current masthead page.

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