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Photobiological studies of new cyclopentene–psoralens☆

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Abstract

Psoralen analogues bearing a cyclopentane ring fused to either the 4′,5′ double bond (compound **4**) or the 3,4 double bond (compound **7**) of the tricyclic furocoumarin structure were prepared. AM1 theoretical calculations performed for these compounds indicated that the electronic properties of their reactive double bonds were very similar to those of psoralen and its derivative 8-methoxypsoralen (8-MOP), though the overall molecular geometries were clearly different, particularly as regards the change in molecular curvature produced by the introduction of the cyclopentane ring. Compound **4** showed a capacity similar to that of 8-MOP to inhibit the growth of human cervix adenocarcinoma cells (HeLa) and to induce mutagenic effects, but it was definitely less phototoxic to skin than 8-MOP. Its ability to photoadd to DNA and to cross-link DNA strands was also demonstrated. Instead, compound **7** was practically devoid of biological activity and no interaction with the macromolecule could be detected. These differences in behaviour between **4** and **7** are probably due to the molecular curvature resulting from the introduction of the cyclopentane ring.

Keywords: Synthesis; Cyclopentene–psoralens; Photobiological properties; Mutagenesis; Phototoxicity

1. Introduction

Psoralens are a group of natural or synthetic linear furocoumarins with interesting photobiological properties. Some of them are used clinically in psoralen plus ultraviolet-A radiation therapy (PUVA) for hyperproliferative skin diseases [1] and for extracorporeal treatment of cutaneous T-cell lymphoma (photopheresis) [2].

The biological activity of psoralens is mainly due to their ability to photoreact with DNA. Upon irradiation with UVA light, photoaddition of the 4′,5′ double bond of their furane ring and/or of the 3,4 double bond of their pyrone ring with the 5,6 double bond of pyrimidine bases (usually thymine) occurs, leading to the formation of mono- or di-adducts, the latter involving both strands of the macromolecule [3,4]. Although both mono- and bifunctional photoaddition can inhibit the synthesis of cellular DNA, the compounds which cause bifunctional damage to the macromolecule are usually more mutagenic and provoke skin phototoxicity [5–7]. For this reason, several research groups have been working to develop analogue psoralen derivatives that retain high affinity towards the macromolecule while giving only monoaddition. This result may be reached by deactivating one of the two photoreactive sites, either changing electronic properties or by means of steric hindrance [8,9]. In the present work we synthesized two new analogues bearing a cyclopentane ring fused to either the 4′,5′ double bond (compound **4**) or the 3,4 double bond (compound **7**) of the tricyclic furocoumarin structure, aiming at selective prevention of the photoaddition of the affected double bond without altering the planarity of the molecule. The effects of these modifications on the photobiological activity of **4** and **7** were also evaluated.

☆ Dedicated to Professor Antonio Maccioni.

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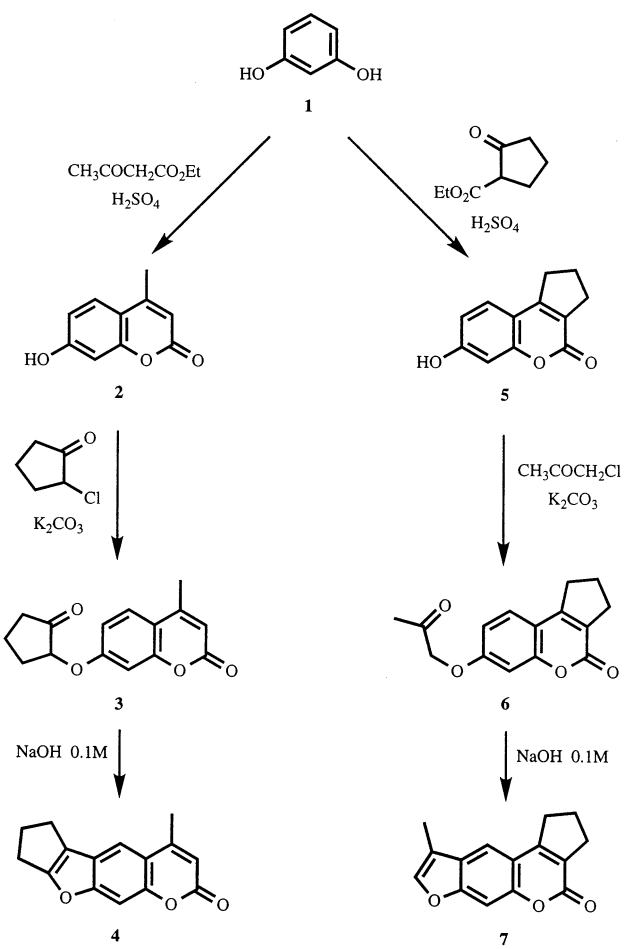
2. Chemistry

Compounds **4** and **7** were prepared from resorcinol (**1**) in parallel sequences, each comprising three steps (Scheme 1). The first step was Pechmann reaction of **1** with ethyl acetoacetate or ethyl 2-oxocyclopentanocarboxylate in sulfuric acid, which gave hydroxycoumarins **2** (also available commercially from Aldrich) and **5** in 44 and 70% yields, respectively. Next, the Williamson reaction of **2** with 2-chlorocyclopentanone, and the corresponding reaction of **5** with chloroacetone, gave ketoethers **3** and **6** in 58 and 97% yield, respectively. Compounds **3** and **6** were cyclized by heating in a strongly alkaline solution, affording cyclopentene–furocoumarins **4** and **7** in 43 and 66% yields, respectively.

3. Experimental

3.1. Chemistry

Melting points were determined on a Koffler hot-stage microscope and are uncorrected. IR spectra (KBr discs) were recorded on a Perkin–Elmer 1640 FT spectrometer (υ in cm^{-1}). ^1H NMR spectra were recorded on a Brüker WM (250 MHz) spectrometer using TMS as the internal standard (chemical shifts in δ values and J in Hz). Elemental analyses were determined by a Perkin–Elmer 240B microanalyser; all were within $\pm$ 0.4% of calculated values. Flash chromatography (FC) was performed on silica gel (Merck 60, 230–4000 mesh) and analytical thin-layer chromatography (TLC) on precoated silica gel plates (Merck 60 F254, 0.25 mm).



Scheme 1.

3.2. 4-Methyl-7-(2′-oxocyclopentyloxy)coumarin (3)

To a solution of 7-hydroxycoumarin **2** (5 g, 28.41 mmol) in anhydrous acetone (30 ml) 2-chlorocyclopentanone (6.50 g, 55.10 mmol) and potassium carbonate (9.5 g) were added and the mixture was heated at reflux for 20 h. The precipitate was filtered out, the acetone was evaporated under vacuum, and the solid residue was purified by FC using 3:2 toluene–ethyl acetate as the eluent. Compound **3** (4.2 g, 58%): m.p. 141–143°C. ^1H NMR ($CDCl_3$): 7.50 (d, 1H, H5, $J = 8.72$), 6.92 (m, 2H, H8 + H6), 6.16 (d, 1H, H3, $J = 1.12$), 4.69 (dd, 1H, CH–O, $J = 8.90$ and 7.75), 2.55 (m, 1H, *H*CH–CO), 2.42 (m, 2H, HC*H*–CO + *H*CH–CH–O), 2.40 (d, 3H, CH_3, $J = 1.12$), 2.25–1.95 (m, 3H, HC*H*–CH–O + CH_2–CH_2–CO). IR: 3073, 2975, 2944, 2883, 1752, 1712, 1608, 1557, 1269, 1206, 1157, 1139, 848. *Anal.* ($C_{15}H_{14}O_4$) C, H.

3.3. 4-Methylcyclopentenefuro[3,2-g]coumarin (4)

A mixture of coumarin **3** (1.5 g, 5.81 mmol) and 0.1 N NaOH (500 ml) was heated at reflux for 24 h. It was then acidified with 1 N HCl, and the precipitating solid was filtered out, washed with water and purified by FC using 9:1 hexane–ethyl acetate as the eluent. Compound **4** (600 mg, 43%): m.p. 222°C. ^1H NMR ($CDCl_3$): 7.55 (s, 1H, H5), 7.38 (s, 1H, H8), 6.25 (d, 1H, H3, $J = 1.10$), 2.89, 2.77 and 2.61 (3m, $3 \times 2H$, $(CH_2)_3$), 2.49 (d, 3H, CH_3, $J = 1.10$). IR: 3067, 2930, 2861, 1701, 1639, 1568, 1445, 1126. *Anal.* ($C_{15}H_{12}O_3$) C, H.

3.4. 7-Hydroxy-3,4-cyclopentenecoumarin (5)

A mixture of resorcinol (**1**, 1 g, 9.08 mmol), ethyl 2-oxocyclopentanecarboxylate (1.6 g, 10.21 mmol) in concentrated H_2SO_4 (5 ml) was stirred at room temperature for 8 h and then poured into cold water (50 ml). The resulting precipitate was filtered out, washed with water, and purified by FC with 1:1 hexane–ethyl acetate as the eluent. Compound **5** (1.36 g, 70%): m.p. 250–252°C. ^1H NMR (DMSO-d_6): 10.42 (s, 1H, OH), 7.41 (d, 1H, $J = 8.40$, H5), 6.76 (m, 2H, H6 and H8), 3.01 (t, 2H, allylic CH_2, $J = 7.50$), 2.70 (t, 2H, allylic CH_2, $J = 7.50$), 2.07 (quintet, 2H, CH_2–CH_2–CH_2, $J =$

7.50). IR: 3208, 1675, 1621, 1559, 1393, 1307, 1133, 1075. *Anal.* ($C_{12}H_{10}O_3$) C, H.

3.5. 7-Acetonyloxy-3,4-cyclopentenecoumarin (6)

To a solution of 7-hydroxycoumarin **5** (500 mg, 2.47 mmol) in anhydrous acetone (100 ml) were added chloroacetone (0.60 ml, 7.53 mmol) and potassium carbonate (2 g) and the mixture was heated at reflux for 15 h. The precipitate was filtered out, the acetone was evaporated under vacuum, and the solid residue was purified by FC using 1:1 hexane–ethyl acetate as the eluent. Compound **6** (620 mg, 97%): m.p. 139°C. ^{1}H NMR ($CDCl_3$): 7.38 (d, 1H, H5, $J = 8.60$), 6.88 (dd, 1H, H6, $J = 8.60$ and 2.40), 6.81 (d, 1H, H8, $J = 2.40$), 4.63 (s, 2H, CH_2–O), 3.05 (t, 2H, allylic CH_2, $J = 7.50$), 2.91 (t, 2H, allylic CH_2, $J = 7.50$), 2.31 (s, 3H, CH_3), 2.21 (quintet, 2H, CH_2–CH_2–CH_2, $J = 7.50$). IR: 3065, 2910, 1719, 1611, 1420, 1396, 1073. *Anal.* ($C_{15}H_{14}O_4$) C, H.

3.6. 3,4-Cyclopenten-4′-methylfuro[3,2-g]coumarin (7)

A mixture of 7-acetonyloxycoumarin **6** (420 mg, 1.63 mmol) and 0.1 N NaOH (200 ml) was heated at reflux for 20 h. The mixture was acidified with 1 N HCl, and the precipitating solid was filtered out, washed with water and purified by FC using 9:1 hexane–ethyl acetate as the eluent. Compound **7** (260 mg, 66%): m.p. 237°C. ^{1}H NMR ($CDCl_3$): 7.51 (s, 1H, H5), 7.46 (d, 1H, HC–O, $J = 1.30$), 7.45 (s, 1H, H8), 3.17 (t, 2H, CH_2, $J = 7.70$), 2.96 (t, 2H, CH_2, $J = 7.70$), 2.29 (s, 3H, CH_3, $J = 1.30$), 2.27 (quintet, 2H, CH_2–CH_2–CH_2, $J = 7.70$). IR: 3070, 2921, 1718, 1637, 1128, 1067, 1052. *Anal.* ($C_{15}H_{12}O_3$) C, H.

3.7. Theoretical calculations

Theoretical optimization of molecular geometries was calculated by the AM1 semi-empirical quantum mechanical method [10], using the AMPAC program [11] run on an SGI work station.

3.8. Spectrophotometric measurements

UV absorption spectra were recorded at 25°C on a Perkin–Elmer UV–Vis spectrophotometer (model Lambda 12).

3.9. Photobiological studies

3.9.1. Irradiation procedure

Irradiation was performed by means of Philips HPW 125 lamps, equipped with Philips filters, so that they emitted over 90% of their radiation at 365 nm. Irradiation intensity, determined by a Cole–Parmer radiometer (model 97503-00, Cole–Parmer Instrument, IL), was 4.24 mW/cm^2.

3.9.2. Assay of inhibition of human cell growth

Human cervix adenocarcinoma cells (HeLa) were grown in nutrient mixture F12 [HAM] medium (Sigma, N 6760), supplemented with 10% heat-inactivated fetal calf serum (Seromed), 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B (Sigma). The cells were cultured at 37°C in a humidified atmosphere with 5% carbon dioxide in air.

A total of 1×10^5 cells were seeded into each well of a 24-well microtitex plate. After incubation for 24 h, the medium was replaced with an equal volume of Dulbecco's modified Eagle's medium without phenol red (DMEM, Sigma, D2902), and various concentrations of the test compound were added.

The cells were kept in the dark for 1 h and then irradiated with a UVA dose of 0.79 J/cm^2. After irradiation, the medium containing the compounds was removed and the cells were incubated in complete F12 medium for 24 h. Cell viability was determined by means of the trypan blue exclusion method. Cytotoxicity was expressed as IC_{50}, i.e. the concentration of test compound inducing a 50% reduction in cell number with respect to control cultures.

3.9.3. Nucleic acid

Salmon testes DNA was purchased from Sigma Chemical (D 1626, St. Louis, MO); its hypochromicity was over 35%. The DNA concentration was determined using extinction coefficient 6600 M^{-1}/cm at 260 nm.

3.9.4. Flow linear dichroism

Flow linear dichroism was performed on a Jasco J500A spectropolarimeter equipped with a Jasco interface linked to an IBM PC.

The test compound and nucleic acid were mixed in ETN buffer (10 mM Tris, 1 mM EDTA, 10 mM NaCl, pH 7.0) in order to reach a [compound]/[DNA] ratio of 0.04. The irradiated solutions (21.2 J/cm^2) were also submitted to exhaustive extraction with chloroform to remove the free compound. A DNA solution treated in the same way was used as reference.

Samples were oriented by a device designed by Wada and Kosawa [12] at a shear gradient of 800 rpm; each spectrum was accumulated four times. Reduced linear dichroism (LD_r) was determined by dividing the linear dichroism values (LD) by the relative isotropic absorbance ($LD_r = LD/A$). Orientation angle α was determined using the following equation [13]:

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

where $(LD_r)_L$ is the reduced linear dichroism for the ligand, calculated at the chromophore absorption band;

$(LD_r)_{DNA}$ is that of DNA, measured at 260 nm, and α_L defines the relative drug–DNA orientation.

3.9.5. Cross-linking evaluation

Solutions of DNA and the tested cyclopentene–furocoumarin in ETN buffer ([DNA]/[compound] ratio = 75) were irradiated at 365 nm for various times. After irradiation, the DNA was denatured thermally (100°C for 10 min) and quickly cooled in ice. The renaturation capacity of DNA, due to cross-link formation, was investigated recording absorbance at 260 nm. Data are expressed in terms of log % of non-renaturated fraction of irradiated psoralen–DNA complex relative to that of irradiated DNA, as suggested by Blais et al. [9].

3.9.6. Phototoxicity evaluation

Skin phototoxicity was tested on depilated albino guinea pigs (Dunkin–Hartley) as described by Carlassare et al. [14]. An ethanol solution of each new compound was applied topically on the skin up to 50 μg/cm^2. For 8-MOP the concentration used was 10 μg/cm^2. The animals were kept in the dark for 45 min and then the treated skin was irradiated with 20 kJ/m^2 of UVA. The degree of erythema was estimated 72 h after irradiation.

3.9.7. Growth conditions and cell survival assay

Salmonella typhimurium strain TA100 was grown in brain heart infusion (Difco) and cultivated at 37°C in aerobic conditions.

Cells from broth cultures in the logarithmic growth phase were harvested by centrifugation at $2000 \times g$ for 15 min, washed twice with 10 mM phosphate-buffered saline (PBS), pH 7.2, containing 0.15 M NaCl and 2.7 mM KCl, and then diluted in the same buffer in order to obtain a final $A_{650} = 0.700$, corresponding to 8×10^7 cells per ml. Small measured volumes of concentrated solutions in DMSO of the tested compounds were added to the cell suspensions to reach a final concentration of 2×10^{-6} M. The cells were incubated in the dark for 1 h and then irradiated with a UVA dose of 0.79 J/cm^2.

Control experiments in the presence and absence of an equal volume of DMSO alone were also carried out.

Unirradiated and irradiated cell samples were tenfold serially diluted in the above-mentioned buffer and each dilution was plated in duplicate on brain heart agar (Difco). After incubation of the plates at 37°C for 24 h, the number of colony-forming units per ml (c.f.u./ml) was counted.

3.9.8. Mutagenesis

Compounds were tested for mutagenic activity on *Salmonella typhimurium* strain TA100 according to the plate incorporation test of Maron and Ames [15]. Experimental conditions were the same as those of the cell survival assay.

Table 1
Spectroscopic properties of test compounds and 8-MOP in UV region

Compound	λ (nm)	ε (M^{-1}/cm)[a]
4	292	9500
	338	5480
	365	2800
7		
	299	11 200
	328	8800
	365	850
8-MOP		
	300	12 300
	365	970

[a] Calculated in ethanol solution.

4. Results and discussion

4.1. Spectroscopic properties

Table 1 shows the major UV-absorption peaks of cyclopentene–furocoumarins **4** and **7** in comparison with those of the tricyclic compound 8-MOP. Both **4** and **7** show λ_{max} at around 300 nm, like 8-MOP, but with a shoulder at around 325–340 nm. Note that at 365 nm (the irradiation wavelength used in the photobiological experiments; vide infra), the molar extinction coefficient of compound **7** is very similar to that of 8-MOP, while compound **4** shows higher absorption.

4.2. Theoretical calculations

Theoretical calculations performed for compounds **4** and **7** by the semi-empirical method AM1 (see Section 3) indicated that both these tetracycles were practically planar. The molecular curvatures of the compounds, as measured by the angle between the line joining the centres of the three rings of psoralen and that joining the centres of the cyclopentane and furane or pyrone rings were 32° for compound **4** and 58° for compound **7** (Fig. 1). The energies of the frontier molecular orbitals of compounds **4** and **7** were very similar (HOMOs = −8.73 and −8.74 eV, LUMOs = −0.94 and −1.02 eV, respectively), and closely agreed with those of 8-MOP (−9.01 and −1.02 eV). In theory, these compounds should thus show similar reactivity to 8-MOP in 2 + 2 cycloaddition reactions. Moreover, the HOMO and LUMO orbital coefficients of **4**, **7**, 8-MOP and psoralen are similar (Fig. 1), suggesting that these compounds should also show similar regioselectivity in their 2 + 2 cycloaddition reactions.

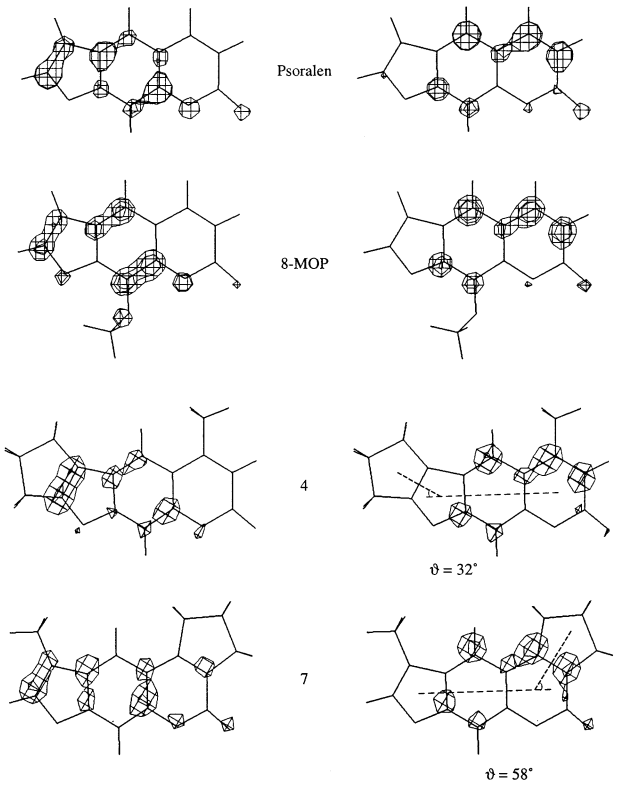


Fig. 1. HOMO (left) and LUMO (right) distributions of studied compounds.

The charge densities at the atoms of the reactive double bonds of compounds **4** and **7** were very slightly different from those at the corresponding atoms of the pyrone and furane rings of psoralen and 8-MOP, due to the inductive effect of the alkyl substituents. Thus, the introduction of cyclopentane rings into psoralens, as in **4** and **7**, is unlikely to change the course of reactions in which electronic factors play a determining role.

4.3. Antiproliferative activity

The new cyclopentene–furocoumarins were evaluated for cytotoxic potency in vitro by the inhibition growth assay on a cervix adenocarcinoma cell line (HeLa) under UVA irradiation. The results are shown in Table 2. Data are reported as IC_{50} values and 8-MOP was taken as reference drug. Compound **4** showed significant activity, whereas compound **7** was practically devoid of biological activity.

4.4. Interaction with DNA

It is well-known that psoralen derivatives intercalate inside the double helix of DNA when complexed with the macromolecule. Under UVA irradiation, these intercalated compounds undergo covalent photoconjugation to the pyrimidine bases of DNA.

Table 2
Biological activity on HeLa cells and skin phototoxicity evaluation (8-MOP as reference drug)

Compound	IC_{50} (μM)	Erythema intensity[a]
4	7.0 ± 1.9	+ − −
7	25 ± 4	− − −
8-MOP	10 ± 3	+ + + (with edema)

[a] Symbols: + + + = strong; + − − = mild; − − − = absent.

Linear dichroism (LD) measurements of a solution of the new cyclopentene–psoralens **4** and **7** in the presence of DNA show the typical strong LD negative band around 260 nm derived from the DNA base pairs, and a negative LD signal between 300 and 380 nm where DNA has no absorption (data not shown). This is the first evidence that these small molecules are complexed to DNA, because they cannot themselves become oriented in the flow field. In the case of psoralens, all strong absorption are $\pi \rightarrow \pi^*$ transitions, which are polarized in the molecular plane. Assuming that also for cyclopentene–psoralens $\pi \rightarrow \pi^*$ transitions are polarized in the molecular plane, the observed negative signals (at wavelengths different from those of DNA) indicate that the complexed **4** and **7** molecules assume a position parallel to the plane of the DNA bases. This orientation would be expected if the ligand was intercalated between two base pairs.

In previous studies by Tjerned et al. [16] and Vigny et al. [17], the flow LD technique was used to investigate the properties of covalent adducts derived from the photobinding of 8-MOP and other psoralen derivatives to DNA. Non-covalent DNA-psoralen complexes were irradiated by UVA light and, after irradiation, the non-covalently bound compound was removed. Flow LD measurements allowed calculation of the angle between the transition moment of the furan-side photoproduct and the axis of the DNA helix. For 8-MOP and its isomer 5-MOP, the above authors found values of 76–79° and 72–76°, respectively, in agreement with a geometry in which the psoralen residues are in a pseudo-intercalative conformation, taking into account the fact that, for well-known intercalators, the value of this angle is 90°.

For indications on covalent photobinding, we also used the same LD technique. Fig. 2 shows the absorbance (top) and linear dichroic (bottom) spectra of a DNA solution irradiated in the presence of compound **4**. The negative linear dichroism signal in the furan-side photoadduct region (320–360 nm) indicates that photobinding occurs. Eq. (1) (see Section 3) estimates the value of orientation angle α, which ranges between 72 and 76°, according to the values reported in the abovementioned studies [16,17] and shows that the photoproduct has a pseudo-intercalative mode of binding. This is consistent with the assumption that the photo-

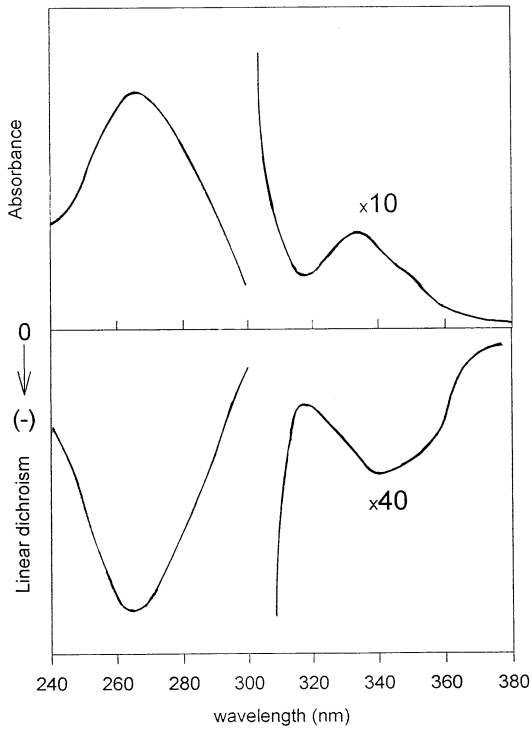


Fig. 2. Absorption (top) and linear dichroism (bottom) spectra of aqueous DNA solution irradiated in presence of compound **4**.

binding of compound **4** to DNA may induce distortion of the macromolecule structure, as hypothesized previously for 8-MOP [16,17].

With regard to compound **7**, only a very weak dichroic signal was detected, indicating that it practically does not photoreact with DNA.

Further information regarding the photoreactivity of compound **4** was obtained by investigating its ability to form interstrand cross-links in the macromolecule. A DNA solution was irradiated in the presence of test cyclopentene–furocoumarin and thermally denatured. After rapid cooling, the absorbance at 260 nm was recorded for all samples. The renaturation capacity of DNA, due to cross-link formation, was expressed as the log of the percentual fraction of denaturated DNA at various irradiation times (Fig. 3). It may be observed that **4** forms cross-links practically to the same extent as the reference drug 8-MOP. For compound **7,** no cross-link capacity was detected. From these results, it may be concluded that, for compound **4**, the steric hindrance of the fourth ring and the molecular curvature are not sufficient to hamper the photoreactivity of the 4′,5′ and 3,4 double bonds. Otherwise, the introduction of the cyclopentane ring at the pyrone side (compound **7**) would yield lower photoreactivity towards the pyrimidine bases, probably due to more difficult overlapping between the reactive sites.

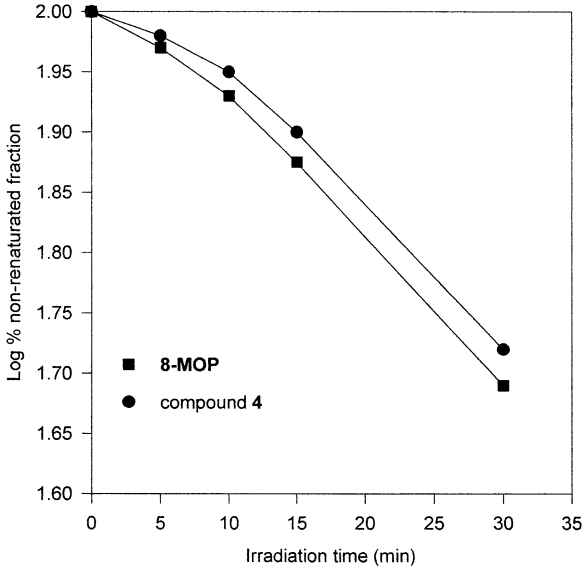


Fig. 3. Evaluation of cross-linking capacity of compound **4** and 8-MOP as reference drug.

4.5. Skin photosensitizing potency

Experiments were carried out to determine the potency of the new cyclopentene–furocoumarins in inducing skin erythema in guinea pigs. The results are shown in Table 2. Compound **7** does not show any skin phototoxicity, also confirming in this case its biological inactivity. Interestingly, the effect of compound **4** differs from that of the reference drug. In fact, only at concentrations five times higher than that of 8-MOP does the new cyclopentene–psoralen **4** cause a barely detectable erythematogenic effect.

4.6. Mutagenesis test

It is known that psoralens, under UVA irradiation, may produce genotoxic effects, and the important role played by cross-links in the production of such mutations has been suggested [18,19]. In this context, it appeared interesting to analyse the behaviour of compound **4**.

Salmonella typhimurium strain TA100 was used to test mutagenic activity. Cells were treated as reported in Section 3, and surviving and revertant numbers were scored. The results are shown in Table 3. The behaviour of compound **4** is consistent with that of the reference drug, as the number of revertants per survivor is comparable.

5. Conclusions

With the aim of modulating the photobiological activity of the tricyclic pharmacophoric nucleus, two cyclopentene–psoralen derivatives were synthesized.

Table 3
Reversion frequencies induced in *Salmonella typhimurium* strain TA100 (8-MOP as reference drug)

	Revertants per plate (dark)	Revertants per plate (UVA)	Revertants per survivor (%) (UVA)
Control	105(29)[a]	111(19)	0.0013
Solvent (DMSO)	97(24)	107(34)	0.0017
Compound **4**	111(25)	36(9)	0.0406
8-MOP	101(25)	39(10)	0.0304

[a] Standard deviations in brackets.

When the cyclopentane ring is fused to the 4′,5′ double bond, a derivative (**4**) which exerts antiproliferative activity comparable to that of the reference drug 8-MOP, is obtained. Compound **4** is also able to photobind with DNA forming cross-links, and damage to the macromolecule is responsible for the antiproliferative activity. Due to this peculiar mechanism of action, a mutagenic effect is also observed. Otherwise, the capacity to cause skin erythema is greatly reduced. It is to be noted that this side-effect represents a severe restriction for photochemotherapy.

When the cyclopentane ring is fused to the 3,4 double bond, a compound devoid of biological activity is obtained (**7**). Furthermore, in our experimental conditions, it did not show any interaction ability towards DNA.

The different behaviour between **4** and **7** may be explained by theoretical calculations which demonstrate that the introduction of the cyclopentane ring leads to different molecular curvatures.

Interestingly, it should be stressed that appropriate structural modifications can greatly reduce skin phototoxicity without altering either molecular events or in vitro photobiological activity.

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