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Synthesis and biological activity of linear and angular 4-methoxymethylthienocoumarins and 4-acetoxymethylthienocoumarins

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Abstract

This paper reports the synthesis of 4-methoxymethyl and 4-acetoxymethyl-6,9-dimethyl-2*H*-thieno[3,2-g]-1-benzopyran-2-one as well as 4-methoxymethyl- and 4-acetoxymethyl-6,9-dimethyl-2*H*-thieno[2,3-h]-1-benzopyran-2-one. The synthesized derivatives were tested on human cells in UVA irradiation conditions. Skin phototoxicity and cross-link formation in DNA were also studied. Results indicate that the new thienocoumarins have good antiproliferative activity, greater than that of the well-known photochemotherapeutic drug 8-methoxypsoralen, but they are practically devoid of skin photosensitization effects.

Keywords: Methoxymethylthienocoumarins; Acetoxymethylthienocoumarins; Antiproliferative activity; Phototoxicity

1. Introduction

Psoralens, especially 8-methoxypsoralen (8-MOP), are photoactive drugs used in the treatment of numerous skin diseases such as psoriasis, mycosis fungoides, lichen planus and vitiligo. Their therapeutic application is by topical or oral administration in combination with ultraviolet (UVA, 320–400 nm) exposure [1–4]. A more recent application of psoralen photochemotherapy, for cutaneous T-cell lymphoma and other autoimmune diseases, is photopheresis, extracorporeal treatment in which drug-containing lymphocytes are collected by leukapheresis, exposed to UVA light extracorporeally, and then reinfused [5,6]. The mechanism of the antiproliferative action of psoralens has been extensively studied: after intercalation between two base pairs of DNA and upon exposure to UVA light, psoralens covalently bind to the 5,6 double bond of pyrimidine bases (mainly thymine). Due to the presence of two reactive sites in the psoralen moiety, the 3,4 and 4′,5′ double bonds, both monoadducts and diadducts may form, the latter involving both strands of DNA [7,8].

Notwithstanding their extensive use, psoralens have a number of side-effects, both short- (erythema, hyperpigmentation, genotoxicity) and long-term (risk of skin cancer, cataract). In most of the compounds described by various authors, skin photosensitization appears when cross-links with DNA are formed [9–12].

Various approaches have been followed to obtain new furocoumarin derivatives with better DNA photobinding ability and lower toxicity [13,14]. One of these involves the synthesis of heteroanalogues, in which nitrogen substitutes one of the two intracyclic atoms of oxygen, leading to the formation of pyrrolocoumarins [15,16] and furoquinolinones [17].

More recently, new heteroanalogues replacing one or both of the two intracyclic oxygen atoms with sulfur and/or selenium have been synthesized [18–20]. In certain cases, considerable enhancement of the photobinding ability of the furocoumarin system to the target macromolecules has been observed [19,21]. In particular, most of the heteroanalogues containing sulfur and selenium have been shown to inactivate viral DNA much more efficiently than psoralen and 8-MOP, thus revealing great promise for photochemotherapy [22]. In this connection, new derivatives replacing the oxygen atom in the furane ring with a sulfur have been prepared (Scheme

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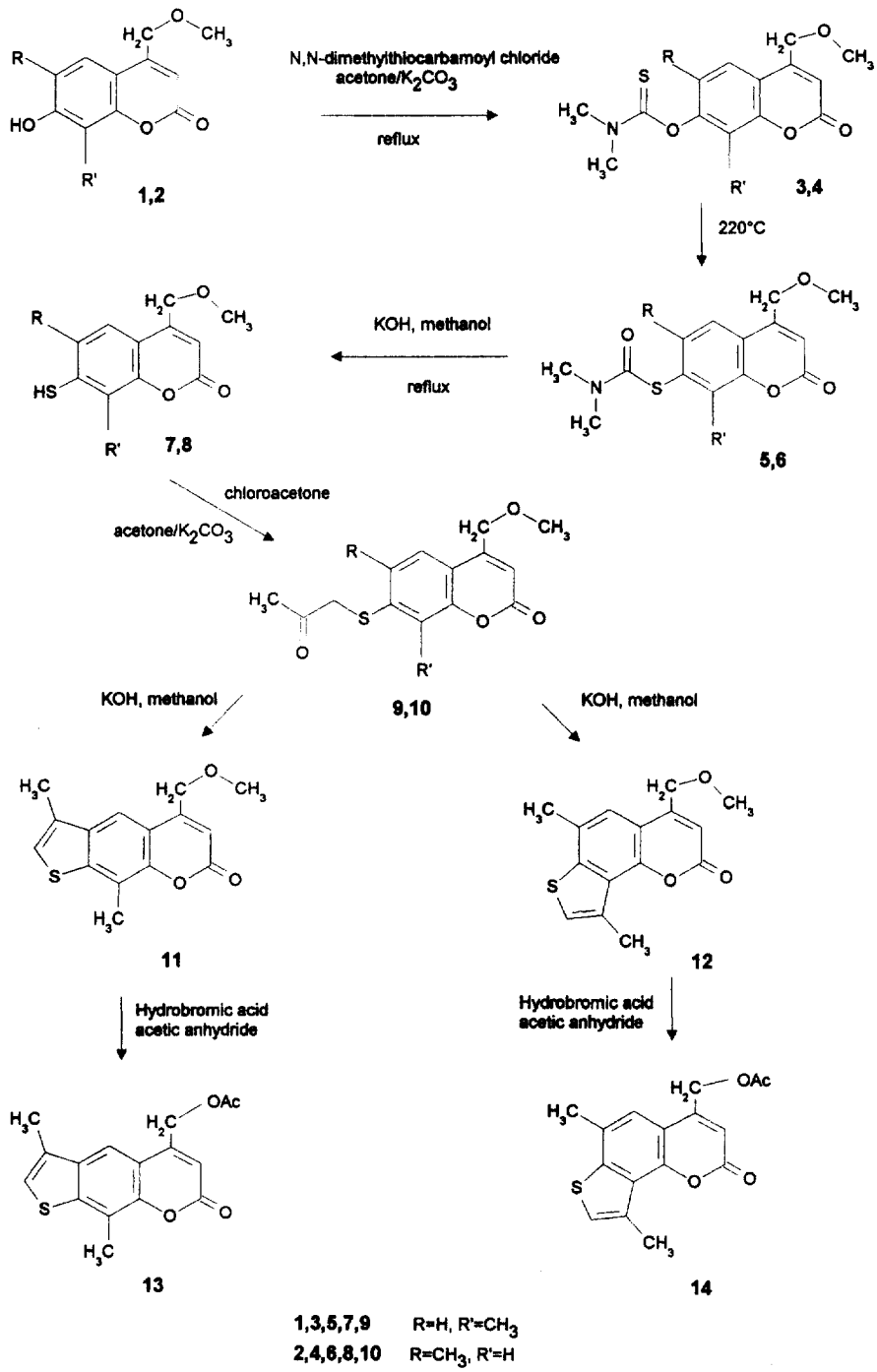

Scheme 1.

1). Their activity on human cell line, their skin phototoxicity and cross-link formation in DNA are reported here.

2. Chemistry

Synthesis of the new thienocoumarins started from 7-hydroxy-4-methoxymethyl-8-methylcoumarin **1** or 7-hydroxy-4-methoxymethyl-6-methylcoumarin **2**; a methyl group was inserted in the 8 and 6 positions of the coumarin nucleus to avoid the formation of linear isomers in angular derivatives and of angular isomers in linear ones.

In synthesizing thienocoumarins, it is essential to introduce the thiol group into the coumarin in position 7. In order to substitute the hydroxy group with the thiol group, suitable 7-hydroxycoumarins (**1**, **2**) were condensed with *N,N*-dimethylthiocarbamoyl chloride in acetone solution in the presence of potassium carbonate (Scheme 1). Later heating in a thermostatic bath (10°C up to melting point) under a constant flux of nitrogen produced isomerization of *N,N*-dimethylthio-

carbamates **3** and **4** to *N,N*-dimethylcarbamates **5** and **6** [23]. The hydrolysis of **5** and **6** in alkaline medium yielded the key compounds 7-mercapto-4-methoxymethyl-8-methyl- (**7**) and 7-mercapto-4-methoxymethyl-6-methylcoumarin (**8**).

7-Mercaptocoumarin **7** and **8** were condensed with chloroacetone in acetone solution in the presence of K_2CO_3. Thioethers **9** and **10**, submitted to cyclization in alkaline medium, yielded 6,9-dimethyl-4-methoxymethyl-2*H*-thieno-[3,2-g]-1-benzopyran-2-one **11** and 6,9-dimethyl-4-methoxymethyl-2*H*-thieno[2,3-h]-1-benzopyran-2-one **12**, respectively. Compounds **11** and **12** were submitted to bromination by boiling in an acetic solution of hydrobromic acid. In this reaction, partial substitution of bromine with the acetoxy group was obtained. The crude mixture was therefore heated in acetic anhydride, yielding the desired 4-acetoxymethyl-6,9-dimethyl-2*H*-thieno[3,2-g]-1-benzopyran-2-one **13** and 4-acetoxymethyl-6,9-dimethyl-2*H*-thieno[2,3-h]-1-benzopyran-2-one **14**.

3. Experimental

3.1. Chemistry

Melting points (uncorrected) were determined using a Gallenkamp MFB-595-010M melting point apparatus. Analytical thin-layer chromatography (TLC) was performed on precoated 60 F_{254} silica gel plates (Merck, 0.2 mm), developing with an ethyl acetate/cyclohexane mixture (3:7). Preparative column chromatography was performed using silica gel 60 (Merck, 0.063–0.100 mm), eluting with chloroform. ^{1}H NMR spectra were recorded on a Varian Gemini 200 MHz instrument, using $CDCl_3$ as solvent. Coupling constants are given in Hz. Elemental analyses, carried out by the Microanalytical Laboratory of the Department of Pharmaceutical Sciences of the University of Padua, gave satisfactory results ($\pm$0.4% of theoretical values of C, H, S) for all synthesized products.

3.1.1. 7-(4-Methoxymethyl-8-methylcoumarinyl)-N,N-dimethylthiocarbamate 3

N,N-Dimethylthiocarbamoyl chloride (8.5 g, 68.7 mmol) and anhydrous potassium carbonate (30.0 g) were added to a solution of 7-hydroxy-4-methoxymethyl-8-methylcoumarin **1** (7.6 g, 34.5 mmol) in acetone (500 ml) and the mixture was refluxed until the starting material disappeared (18 h, TLC). After cooling, potassium carbonate was filtered off and washed with fresh acetone. The solvent was evaporated from the pooled filtrate and washed, yielding a pure (TLC) solid which was crystallized from methanol to give 9.6 g (90%) of 7-(4-methoxymethyl-8-methylcoumarinyl)-*N,N*-dimethylthiocarbamate **3**, m.p. 211°C; ^{1}H NMR: δ (ppm) 7.41 (d, J=8.8 Hz, 1H, H-5), 6.99 (d, J=8.8 Hz, 1H, H-6), 6.53 (t, J=1.5 Hz, 1H, H-3), 4.62 (d, J=1.5 Hz, 2H, 4-CH_2–), 3.51 (s, 3H, Me–N), 3.48 (s, 3H, Me–N), 3.41 (s, 3H, –OMe), 2.30 (s, 3H, Me-8). *Anal.* ($C_{15}H_{17}NO_4S$) C, H, N, S.

3.1.2. 7-(4-Methoxymethyl-6-methylcoumarinyl)-N,N-dimethylthiocarbamate 4

This compound was prepared from 7-hydroxy-4-methoxymethyl-6-methylcoumarin **2** in an analogous manner to **3**, m.p. 214°C (methanol, 72%); ^{1}H NMR: δ (ppm) 7.38 (br s, 1H, H-5), 7.03 (s, 1H, H-8), 6.50 (t, J=1.4 Hz, 1H, H-3), 4.61 (d, J=1.4 Hz, 2H, 4-CH_2–), 3.51 (s, 3H, Me–N), 3.48 (s, 3H, Me–N), 3.39 (s, 3H, –OMe), 2.25 (s, 3H, Me-6). *Anal.* ($C_{15}H_{17}NO_4S$) C, H, N, S.

3.1.3. 7-(4-Methoxymethyl-8-methylcoumarinyl)mercapto-N,N-dimethylcarbamate 5

7-(4-Methoxymethyl-8-methylcoumarinyl)-*N,N*-dimethylthiocarbamate **3** (3.0 g, 9.8 mmol) was heated in a thermostatic bath at 220°C under a flux of nitrogen until the starting material disappeared (2 h). The resulting crude mixture was crystallized from ethanol, giving 2.3 g (77%) of 7-(4-methoxymethyl-8-methylcoumarinyl)mercapto-*N,N*-dimethylcarbamate **5**, m.p. 166°C; ^{1}H NMR: δ (ppm) 7.43 (d, J=8.3 Hz, 1 H, H-5 or H-6), 7.37 (d, J=8.3 Hz, 1H, H-6 or H-5), 6.58 (t, J=1.5 Hz, 1H, H-3), 4.61 (d, J=1.5 Hz, 2H, 4-CH_2–), 3.51 (s, 3H, –OMe), 3.15 (br s, 3H, Me–N), 3.04 (br s, 3H, Me–N), 2.56 (s, 3H, Me-8). *Anal.* ($C_{15}H_{17}NO_4S$) C, H, N, S.

3.1.4. 7-(4-Methoxymethyl-6-methylcoumarinyl)mercapto-N,N-dimethylcarbamate 6

This compound was prepared from 7-(4-methoxymethyl-6-methylcoumarinyl)-*N,N*-dimethylthiocarbamate **4** in an analogous manner to **5**, m.p. 160°C (methanol, 87%); ^{1}H NMR: δ (ppm) 7.52 (s, 1H, H-8), 7.42 (br s, 1H, H-5), 6.55 (t, J=1.4 Hz, 1H, H-3), 4.61 (d, J=1.4 Hz, 2H, 4-CH_2–), 3.51 (s, 3H, –OMe), 3.13 (br s, 3H, Me–N), 3.04 (br s, 3H, Me–N), 2.45 (s, 3H, Me-6). *Anal.* ($C_{15}H_{17}NO_4S$) C, H, N, S.

3.1.5. 7-Mercapto-4-methoxymethyl-8-methylcoumarin 7

A methanol solution (10 ml) of potassium hydroxide (2.6 g, 46.3 mmol) in 10 ml of methanol was added to a solution of 7-(4-methoxymethyl-8-methylcoumarinyl)-mercapto-*N,N*-dimethylcarbamate **5** (4.5 g, 14.6 mmol) in methanol (100 ml) and the mixture was refluxed in the dark until the starting material disappeared (3 h, TLC). The mixture was cooled and acidified with diluted hydrochloric acid. After elimination of methanol, the aqueous solution was extracted with ethyl acetate; from the dried (Na_2SO_4) organic phase, the solvent was eliminated under reduced pressure. The residue was crystallized from methanol, yielding 2.8 g (81%) of 7-mercapto-4-methoxymethyl-8-methylcoumarin **7**, m.p. 130°C; ^{1}H NMR: δ (ppm) 7.23 (d, J=8.4 Hz, 1H, H-5 or H-6), 7.16 (d, J=8.4 Hz, 1H, H-6 or H-5), 6.47 (t, J=1.3 Hz, 1H, H-3), 4.58 (d, J=1.3 Hz, 2H, 4-CH_2–), 3.59

(s, 1H, –SH), 3.50 (s, 3H, –OMe), 2.43 (s, 3H, Me-8). *Anal.* ($C_{12}H_{12}O_3S$) C, H, S.

*3.1.6. 7-Mercapto-4-methoxymethyl-6-methylcoumarin **8***

This compound was prepared from 7-(4-methoxymethyl-6-methylcoumarinyl)mercapto-*N,N*-dimethylcarbamate **6** in an analogous manner to **7**. The resulting crude mixture was purified by column chromatography. Pure compound **8** was isolated from the pooled fractions containing a single spot, m.p. 193°C (75%); ^{1}H NMR: δ (ppm) 7.27 (br s, 1H, H-5), 7.23 (s, 1H, H-8), 6.44 (t, J = 1.4 Hz, 1H, H-3), 4.57 (d, J = 1.4 Hz, 2H, 4-CH_2–), 3.62 (s, 1H, –SH), 3.50 (s, 3H, –OMe), 2.34 (s, 3H, Me-6). *Anal.* ($C_{12}H_{12}O_3S$) C, H, S.

*3.1.7. 7-Acetonylmercapto-4-methoxymethyl-8-methylcoumarin **9***

Chloroacetone (1.1 g, 11.9 mmol) and anhydrous potassium carbonate (40.0 g) were added to a solution of 7-mercapto-4-methoxymethyl-8-methylcoumarin **7** (2.4 g, 10.1 mmol) in acetone (150 ml) and the mixture was refluxed until the fluorescent starting material disappeared (30 min, TLC). After cooling, the potassium carbonate was filtered off and washed with fresh acetone. The solvent was evaporated from the pooled filtrate and washed, yielding a solid which was crystallized from ethyl acetate to give 2.0 g (68%) 7-acetonylmercapto-4-methoxymethyl-8-methylcoumarin **9**, m.p. 114°C; ^{1}H NMR: δ (ppm) 7.33 (d, J = 8.5 Hz, 1H, H-5), 7.10 (d, J = 8.5 Hz, 1H, H-6), 6.48 (t, J = 1.4 Hz, 1H, H-3), 4.57 (d, J = 1.4 Hz, 2H, 4-CH_2–), 3.76 (s, 2H, H-1′), 3.49 (s, 3H, –OMe), 2.49 (s, 3H, Me-8), 2.31 (s, 3H, –Ac). *Anal.* ($C_{15}H_{17}O_4S$) C, H, S.

*3.1.8. 7-Acetonylmercapto-4-methoxymethyl-6-methylcoumarin **10***

This compound was prepared from 7-mercapto-4-methoxymethyl-6-methylcoumarin **8** in an analogous manner to **9**, m.p. 155°C (methanol, 79%); ^{1}H NMR: δ (ppm) 7.26 (d, J = 0.9 Hz, 1H, H-5), 7.01 (t, J = 1.4 Hz, 1H, H-8), 6.43 (t, J = 1.4 Hz, 1H, H-3), 4.56 (d, J = 1.4 Hz, 2H, 4-CH_2–), 3.83 (s, 2H, H-1′), 3.49 (s, 3H, –OMe), 2.37 (d, J = 0.9 Hz, 3H, Me-6), 2.33 (s, 3H, –Ac). *Anal.* ($C_{15}H_{17}O_4S$) C, H, S.

*3.1.9. 4-Methoxymethyl-6,9-dimethyl–2H-thieno[3,2-g]-1-benzopyran-2-one **11***

An ethanol solution of potassium hydroxide (3.7 g, 65.9 mmol) was added to a solution of 7-acetonyl-4-methoxymethyl-8-methylmercaptocoumarin **9** (2.0 g, 6.8 mmol) in absolute ethanol (50 ml) and the mixture refluxed in the dark (1.5 h). After this period, TLC showed the disappearance of the starting material and the presence of a fluorescent spot. The crude mixture was acidified with diluted hydrochloric acid. After elimination of the methanol, the aqueous solution was extracted with ethyl acetate; the solvent was eliminated under reduced pressure from the dried (Na_2SO_4) organic phase. The residue was crystallized from methanol, yielding 1.7 g (91%) of 4-methoxymethyl-6,9-dimethyl-2*H*-thieno[3,2-g]-1-benzopyran-2-one **11**, m.p. 202°C; ^{1}H NMR: δ (ppm) 7.66 (s, 1H, H-5), 7.10 (q, J = 1.2 Hz, 1H, H-7), 6.56 (t, J = 1.4 Hz, 1H, H-3), 4.75 (d, J = 1.4 Hz, 2H, 4-CH_2–), 3.55 (s, 3H, –OMe), 2.65 (s, 3H, Me-9), 2.48 (d, J = 1.2 Hz, 3H, Me-6). *Anal.* ($C_{15}H_{14}O_3S$) C, H, S.

*3.1.10. 4-Methoxymethyl-6,9-dimethyl-2H-thieno[2,3-h]-1-benzopyran-2-one **12***

This compound was prepared from 7-acetonylmercapto-4-methoxymethyl-6-methylcoumarin **10** in an analogous manner to **11**, m.p. 168°C (methanol, 74%); ^{1}H NMR: δ (ppm) 7.20 (qd, J_q = 0.9 and J_d = 0.5 Hz, 1H, H-5), 7.10 (qd, J_q = 1.2 and J_d = 0.5 Hz, 1H, H-8), 6.55 (t, J = 1.4 Hz, 1H, H-3), 4.68 (d, J = 1.4 Hz, 2H, 4-CH_2–), 3.54 (s, 3H, –OMe), 2.81 (d, J = 1.2 Hz, 3H, Me-9), 2.56 (d, J = 0.9 Hz, 3H, Me-6). *Anal.* ($C_{15}H_{14}O_3S$) C, H, S.

*3.1.11. 4-Acetoxymethyl-6,9-dimethyl-2H-thieno[3,2-g]-1-benzopyran-2-one **13***

2.6 ml of a 48% solution of hydrobromic acid in acetic acid were added to a solution of 4-methoxymethyl-6,9-dimethyl-2*H*-thieno[3,2-g]-1-benzopyran-2-one **11** (0.60 g, 2.2 mmol) in glacial acetic acid (150 ml) and the mixture refluxed for 16 h. After this period, TLC showed the disappearance of the starting material and the presence of two fluorescent spots, due to bromomethyl and acetoxymethyl derivatives. The mixture was diluted with water, neutralized with $NaHCO_3$ and extracted with ethyl acetate. The dried (Na_2SO_4) organic phase was concentrated to dryness and the crude product submitted to successive reaction. The solid was then dissolved in acetic anhydride (30 ml) and the solution was refluxed for 40 min, resulting in the complete transformation of the bromomethyl derivative in the acetoxymethyl compound. 30 ml of water were carefully added to the solution and heated, up to complete hydrolysis of acetic anhydride. The reaction mixture was diluted with abundant water, neutralized and extracted with ethyl acetate. The solid, isolated from the dried (Na_2SO_4) organic phase, was crystallized from methanol, yielding 0.39 g (59%) of 4-acetoxymethyl-6,9-dimethyl-2*H*-thieno[3,2-g]-1-benzopyran-2-one **13**, m.p. 175°C; ^{1}H NMR: δ (ppm) 7.63 (s, 1H, H-5), 7.13 (q, J = 1.2 Hz, 1H, H-7), 6.55 (t, J = 1.5 Hz, 1H, H-3), 5.45 (d, J = 1.5 Hz, 2H, 4-CH_2–), 2.67 (s, 3H, Me-9), 2.48 (d, J = 1.2 Hz, 3H, Me-6), 2.25 (s, 3H, –Ac). *Anal.* ($C_{16}H_{14}O_4S$) C, H, S.

*3.1.12. 4-Acetoxymethyl-6,9-dimethyl-2H-thieno[2,3-h]-1-benzopyran-2-one **14***

This compound was prepared from 4-methoxymethyl-6,9-dimethyl-2*H*-thieno[2,3-h]-1-benzopyran-2-one **12** in an analogous manner to **13**, m.p. 175°C (methanol, 64%); ^{1}H NMR: δ (ppm) 7.16 (qd, J_q = 0.9 and J_d = 0.5 Hz, 1H, H-5), 7.12 (qd, J_q = 1.2 and J_d = 0.5 Hz, 1H, H-8), 6.52 (t, J = 1.5 Hz, 1H, H-3), 5.37 (d, J = 1.5 Hz, 2H, 4-CH_2–), 2.81 (d, J = 1.2 Hz, 3H, Me-9), 2.57 (d, J = 0.9 Hz, 3H, Me-6), 2.24 (s, 3H, –Ac). *Anal.* ($C_{16}H_{14}O_4S$) C, H, S.

3.2. Photobiology

3.2.1. Materials

Calf thymus DNA was purchased from Sigma (St. Louis, MO, USA). Its hypochromicity, determined according to Marmur and Doty [24], was over 35%.

3.2.2. Spectroscopic measurements

UV absorption spectra were recorded at 25°C using a Perkin-Elmer model Lambda 5 spectrometer.

3.2.3. Irradiation procedure

Irradiations were performed by means of two Philips HPW 125 lamps, equipped with a Philips filter, emitting over 90% at 365 nm. Irradiation intensity, determined on a Cole-Parmer radiometer (model 97503-00, Cole-Parmer, Illinois, USA), was 8.48 mW cm^{-2}.

3.2.4. Cross-linking evaluation

Interstrand cross-link experiments were carried out according to Blais et al. [25]. DNA solutions were irradiated at 365 nm for various times, in the presence of the thienofurocoumarins ([DNA]/[drug] ratio = 75). After irradiation, the DNA was denatured thermally (100°C for 10 min) and rapidly cooled to room temperature. The renaturation capacity of DNA, due to cross-link formation, was evaluated, and absorbance at 260 nm was recorded.

3.2.5. Inhibition growth assay

Human cervix adenocarcinoma cells (HeLa) were grown in Nutrient Mixture F-12 [HAM] medium (Sigma, N6760), 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.

1×10^5 cells 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, D2902) without phenol red, and ethanol solutions of the test compound at various concentrations were added.

The cells were kept in the dark for 1 h and then 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.

A trypan blue assay was performed to determine cell viability. Cytotoxicity data are expressed as IC_{50} values, i.e. the concentration of test compound inducing 50% reduction in cell number with respect to control cultures.

3.2.6. Phototoxicity evaluation

Skin phototoxicity was tested on depilated albino guinea pigs (Dunkin-Hartley), as previously reported [26]. An ethanol solution of each new compound was applied on the skin at the concentrations indicated (see Tables 1–3). 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 48 h after irradiation.

4. Results and discussion

4.1. Antiproliferative activity

The antiproliferative activity of new compounds **11–14** and of 8-MOP, taken as the reference drug, was evaluated on HeLa (human cervix adenocarcinoma) cells. Experiments were carried out as described in Section 3. Results are reported in Table 1.

The test compounds show strong antiproliferative activity, ten times greater than that of 8-MOP, with the exception of angular derivative **12**, which shows activity twice that of 8-MOP. Conversely, they do not have any effect in the dark.

Many authors report that antiproliferative activity is usually well correlated with covalent photocombination to DNA [4,27,28]. In this context, the presence in the angular derivative of the methoxymethyl group in position 4 probably has an unfavourable influence on C-4 cycloaddition to the pyrimidine moieties.

Studies are in progress, using labelled samples, to correlate quantitatively the biological effects of the examined compounds to their covalent bonds in the macromolecule.

4.2. Skin phototoxicity

The ability of the new compounds to induce erythema was tested as described in Section 3. The results obtained, reported in Table 2, are compared with those of 8-MOP, taken as reference drug.

Interestingly, compounds **11**, **12** and **14** are not phototoxic until applied at concentrations of 0.16–0.18 μmol cm^{-2}, whereas 8-MOP is highly toxic at a lower concentration (0.05 μmol cm^{-2}). Instead, compound **13**, at the above-mentioned concentrations of 0.16–0.18 μmol cm^{-2}, causes only barely detectable erythema.

It is known that, in most cases, angular derivatives do not exert skin phototoxicity. This property has been related to their monofunctional behaviour towards DNA, due to their

Table 1
Growth cell inhibition by irradiation at 365 nm in the presence of the tested compounds and 8-MOP as reference drug

Compound	IC_{50} (μM) [a]
11	0.8 ± 0.2
12	4.6 ± 0.4
13	0.85 ± 0.2
14	0.8 ± 0.2
8-MOP	10 ± 3

[a] Concentration (μM) of tested agent inducing 50% reduction of viability compared with control cultures.

Table 2
Skin phototoxicity in guinea pigs after exposure to the tested compounds and 20 kJ m^{-2} of UVA [a]

Compound	Dose (μmol cm^{-2})	Erythema intensity
11	0.16	− − −
12	0.16	− − −
13	0.18	+ − −
14	0.18	− − −
8-MOP	0.05	+ + + (with oedema)

[a] Symbols: + + +, strong erythema; + − −, mild erythema; − − −, no erythema.

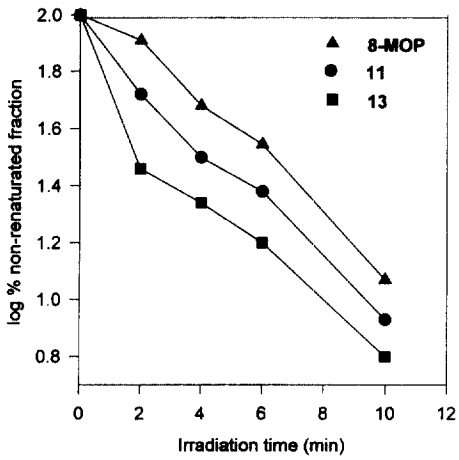

Fig. 1. Cross-linking of the tested compounds to calf thymus DNA, in comparison with 8-MOP, as a function of irradiation time.

angular structure. Results obtained with compounds **12** and **14** fit the literature data [29]. On the contrary, linear psoralens, able to form interstrand cross-links in DNA, mainly induce skin phototoxicity: in this regard, the new linear thienocoumarins show peculiar behaviour. In fact, as shown in Fig. 1, their cross-linking ability is clearly higher than that of the reference drug 8-MOP even though, as noted above, they are practically devoid of skin phototoxicity.

In order to correlate this interesting property with a definite structural pattern, the photobiological behaviour of derivatives **11** and **13** was compared with that of 4,8,4′-trimethylthienopsoralen (TMP-S) [30] and 4,8,4′-trimethylpsoralen (TMP-O). Under our experimental conditions, these two reference compounds showed antiproliferative activity which was very similar to that of **11** and **13** ($IC_{50} = 0.4 \pm 0.1$ μM) and cross-linking capacity slightly better than that of **13**. Phototoxicity data are reported in Table 3: TMP-O, even at a concentration of 0.009 μmol cm^{-2}, was able to exert a strong skin-photosensitizing effect.

The results obtained with TMP-S reveal that just substitution of the oxygen atom with sulfur in the furane ring of the tricyclic moiety weakens the cutaneous response, inducing an erythema intensity similar to that of TMP-O at a concentration almost five times higher.

Table 3
Skin phototoxicity in guinea pigs after exposure to the tested compounds and 20 kJ m^{-2} of UVA [a]

Compound	Dose (μmol cm^{-2})	Erythema intensity
11	0.16	− − −
13	0.18	+ − −
TMP-S	0.04	+ + +
TMP-O	0.009	+ + + (with oedema)

[a] Symbols: + + +, strong erythema; + − −, mild erythema; − − −, no erythema.

If the above chemical modification is accompanied by a methoxymethyl or acetoxymethyl side-chain in position 4 (pyrone ring) in place of a methyl group, as in compounds **11** and **13**, this undesired side-effect disappears.

5. Conclusions

The antiproliferative activity of the new thienocoumarins, tested on human cell line, shows that the compounds are able to exert a strong effect under UVA irradiation. In some cases, their reactivity is one order of magnitude greater than that of the reference drug 8-MOP.

Experiments to determine the potency of these compounds in inducing skin phototoxicity on guinea pig, revealed the almost total lack of this side-effect, even though they were applied at concentrations higher than that of 8-MOP.

It is known that derivatives behave as bifunctional agents, generally showing skin phototoxicity. In this context, the behaviour of the new linear thienocoumarins **11** and **13** is noteworthy because, notwithstanding their higher capacity to form interstrand cross-links in DNA in comparison with 8-MOP, they are practically non-phototoxic on skin. In order to understand the chemical structural pattern responsible for these effects, the photobiological behaviour of compounds **11** and **13** was compared with that of TMP-O and TMP-S. All examined compounds are able to cross-link DNA to similar values. These results show that the phototoxicity of TMP-S is significantly lower than that of TMP-O. Thus, just substitution of the oxygen belonging to the furane ring with a sulfur atom decreases this side-effect. Furthermore, if the 4-methyl group is replaced by a methoxymethyl **11** or acetoxymethyl **13** side-chain, this effect is practically abolished.

The lack of skin phototoxicity combined with better antiproliferative activity — in comparison with that of 8-MOP — indicates that the new thieno derivatives deserve further evaluation to assess fully their potential as photochemotherapeutic agents.

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