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4-Hydroxymethyl- and 4-Methoxymethylfuro[2,3-*h*]quinolin-2(1*H*)-ones: Synthesis and Biological Properties

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Abstract—4-Hydroxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (HOFQ) was prepared by a new profitable way, which allowed to synthesize also 4-methoxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (MOFQ), and 4-hydroxymethyl-6,8-dimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (HOHFQ). Some biological activities of the three compounds were studied in comparison with 8-MOP. In the dark, they inhibited topoisomerase II, leading to a moderate antiproliferative activity in mammalian cells. The antiproliferative activity was also tested upon UVA irradiation in mammalian cells: all compounds showed higher activity than 8-MOP, without mutagenicity and skin phototoxicity, with the best results for HOFQ. Photobinding to DNA was investigated, demonstrating a different sequence specificity for these furoquinolinones in comparison with furocoumarins. For all these features, HOFQ and the other analogues appeared very promising photochemotherapeutic agents, whose mechanism of action will be further investigated.
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Introduction

Over the last decades, great efforts were made towards the search of antiproliferative compounds which cause damage to DNA, bearing in mind that drugs binding DNA or DNA-topoisomerase complex are widely clinically used for cancer chemotherapy. Working in the field of furocoumarins, well known photochemoterapeutic agents, such as 8-methoxypsoralen (8-MOP, Fig. 1), used from many years for treatment of several skin diseases,[1] we recently synthesized and studied some interesting furoquinolinones as furocoumarins bioisosters, like 1,4,6,8-tetramethylfuro[2,3-*h*]quinolin-2(1*H*)-one (FQ) and its *N*-demethylated analogue HFQ (Fig. 1), which appeared antiproliferative derivatives more effective and less toxic than the parent compounds.[2–4]

Substituting at 4 position the hydrophobic methyl group with the hydrophilic hydroxymethyl one, a new furoquinolinone was prepared, 4-hydroxymethyl-6,8-dimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (HOFQ, Fig. 2) which showed novel and interesting properties:[5] it demonstrated high antiproliferative activity on mammalian cells, due to its ability to induce large amount of DNA-protein cross-links (DPC), without forming interstrands cross-links (ISC), considered by many authors mainly responsible for furocoumarin genotoxicity.

Since by preliminary studies HOFQ appeared a very promising drug for some of its properties, such as high antiproliferative activity, very poor genotoxicity and lack of skin phototoxicity, we try to further investigate its synthetic availability, as well as some aspects of its biological activity. In this way, we planned a new synthetic pathway, in order to reduce reaction steps improving the final yield in comparison to the already reported synthetic route.[5] This new synthesis also allowed us to obtain structural analogues, in order to verify if very slight structural changes may modify the biological behaviour.

We are now reporting the new synthesis and the biological properties of three furoquinolinone derivatives, namely 4-hydroxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (HOFQ), 4-methoxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (MOFQ), and 4-hydroxymethyl-6,8-dimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (HOHFQ) (Fig. 2).

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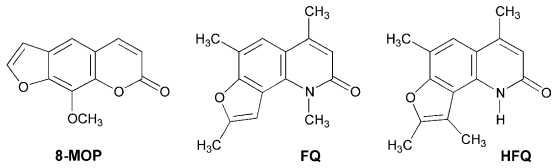


Figure 1. Structure of 8-methoxypsoralen (8-MOP), 1,4,6,8-tetramethyl-furo[2,3-*h*]quinolin-2(1*H*)-one (FQ) and 4,6,8,9-tetramethylfuro [2,3-*h*]quinolin-2(1*H*)-one (HFQ).

Figure 2. Structure of 4-hydroxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (HOFQ), 4-methoxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (MOFQ), and 4-hydroxymethyl-6,8-dimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (HOHFQ).

As a reference compound for biological assays, we used 8-MOP (Fig. 1), as it is the well-known and most used furocoumarin for photoactivation and photochemotherapeutic tests.

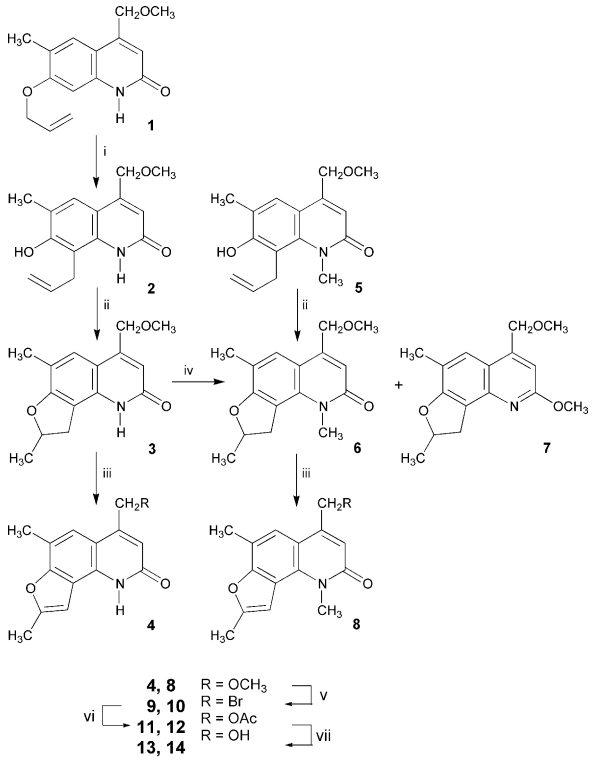
Results and Discussion

Chemistry

The title compounds were synthesized as outlined in Scheme 1. Starting product was the 7-allyloxy-4-methoxy-methyl-6-methylquinolin-2(1*H*)-one[5] (**1**), which was submitted to Claisen rearrangement yielding 8-allyl-7-hydroxy-4-methoxymethyl-6-methylquinolin-2-one (**2**). Compound **2** was cyclized by concentrated sulfuric acid treatment, yielding 8,9-dihydro-6,8-dimethyl-4-methoxy-methylfuro[2,3-*h*]quinolin-2(1*H*)-one **3**, which was dehydrogenated by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to 6,8-dimethyl-4-methoxymethylfuro[2,3-*h*]quinolin-2(1*H*)-one (**4**).

In an analogous manner, the *N*-methyl analogue was prepared starting from 8-allyl-1,6-dimethyl-7-hydroxy-4-methoxy-methylquinolin-2-one[5] (**5**), as shown in Scheme 1. Compound **5** was cyclized to 8,9-dihydro-4-methoxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (**6**) by concentrated sulfuric acid treatment. Compound **6** was also obtained by methylation of **3** with dimethyl sulfate, along with the isomeric 8,9-dihydro-6,8-dimethyl-2-methoxy-4-methoxymethylfuro[2,3-*h*]-quinoline (**7**) in a comparable yield: in fact, methylation occurred at the same extent both at N position and O position. Compound **6** was dehydrogenated by DDQ to 4-methoxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (**8**). It must be pointed out that direct methylation of compound **4** gave only methoxyfuroquinoline derivative and no trace of methylfuroquinolinone **8**, as already reported.[2]

The intermediates **4** and **8** were then converted to the corresponding 4-hydroxymethyl derivatives. As already



Scheme 1. Reagents and conditions: (i) *N*,*N*-diethylaniline, CsF, reflux, 72%; (ii) concd H_2SO_4, RT, 55% (**3**) and 74% (**6**); (iii) DDQ, toluene, reflux, 53% (**4**) and 48% (**8**); (iv) Me_2SO_4, acetone, K_2CO_3, reflux, 44% (**6**) and 44% (**7**); (v) HBr, AcOH, reflux, 59% (**9**) and 55% (**10**); (vi) Ac_2O, AcONa, reflux, 98% (**11**) and 86% (**12**); (vii) KOH, MeOH, reflux, 42% (**13**) and 76% (**14**).

described for compound **4**,[5] compound **8** was reacted with hydrobromic acid, to give the 4-bromomethyl derivatives **9**, then submitted to acetylation, yielding the corresponding 4-acetoxymethyl derivatives **11**, and finally hydrolized in alkaline conditions to give the desired 4-hydroxymethyl-6,8-dimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (**13**). The same procedure was repeated for compound **4**. Direct alkaline hydrolysis of **9** and **10** to **13** and **14** was carried out, but the reaction time was longer and the yield lower than indirect hydrolysis through **11** and **12**, owing to the formation of many degradation products.

Using this new synthetic pathway the final yield for HOFQ resulted much higher than that previously reported.

Biology

Biological activity by dark incubation.

Antiproliferative activity. HOFQ, HOHFQ and MOFQ were first examined for their cytotoxic properties without light exposure. The antiproliferative activity at increasing drug concentrations was tested in mammalian cells, studying the effect on DNA synthesis in Ehrlich cells, that is a short term effect, and the clonal growth capacity of HeLa cells, that is a long term effect. The results were summarized in Table 1. *N*-Methylated compounds appeared to be the most potent derivatives, while HOHFQ showed a significantly lower activity.

Table 1. Biological activity by dark incubation

Compd	DNA synthesis Ehrilch cells[a]	Clonal growth HeLa cells[a]	Topoisomerase II inhibition[b]
MOFQ	13.26±0.43	11.89±0.01	+
HOHFQ	35.90±0.22	30.78±2.40	+
HOFQ	12.73±1.77	9.02±0.10	+
8-MOP	—	—	–

[a]IC_{50} (μM)±SD: drug concentration which induces 50% inihibition; values were calculated by probit analysis ($P < 0.05$, χ^2 test). SD = standard deviation.
[b]Relaxation assay. Symbols: +, 100% inhibition of purified topoisomerase II at concentration ≥1 μM; –, 0% inhibition at all tested concentration (1–100 μM).

8-MOP, evaluated under the same experimental conditions, was completely ineffective upon dark incubation.

Previous studies, carried out on the influence of a hydroxymethyl group at 4 position of the furocoumarin nucleus, suggested that this structural element is essential for antiproliferative activity by dark incubation.[6] The current results indicated that the substitution of the 4-hydroxymethyl group with a 4-methoxymethyl one did not influence this activity, as HOFQ and MOFQ showed comparable IC_{50} values, while *N*-methylation of the furoquinolinone moiety is crucial, as HOHFQ was considerably less active.

Inhibition of topoisomerase II activity. The antiproliferative activity after incubation in the dark exhibited by some methylfuroquinolinones was previously related to topoisomerase II inhibition.[7] The inhibition ability of the test compounds was investigated using the relaxation test of supercoiled DNA of PM2 phage in the presence of purified topoisomerase II. The data obtained at increasing drug concentrations (from 1 to 100 μM) were summarized in Table 1. DNA relaxation was not observed with all furoquinolinones, demonstrating the complete inhibition of topoisomerase II by all compounds, even when tested at the lower concentration (1 μM). On the contrary, with 8-MOP supercoiled DNA form completely disappeared, indicating no influence on topoisomerase II activity.

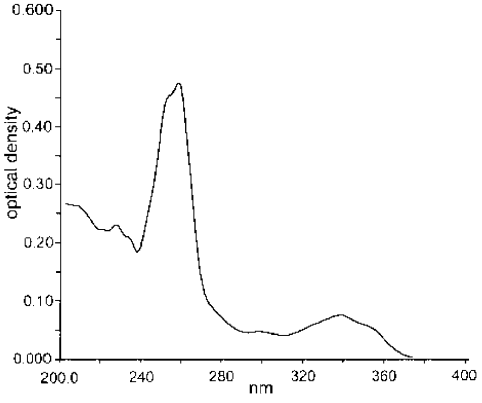


Figure 3. UV spectrum of HOFQ (1.123×10^{-5} M in absolute ethanol).

Biological activity by UVA irradiation

Spectroscopic features. The UV spectrum of HOFQ is shown in Figure 3; HOHFQ and MOFQ exhibited very similar spectral behaviours, differing only for the extinction coefficients (at 338 nm, $\varepsilon_{HOFQ} = 6680$; $\varepsilon_{HOHFQ} = 6480$; $\varepsilon_{MOFQ} = 3870$).

Antiproliferative activity. The antiproliferative activity of furoquinolinones was tested in mammalian cells also upon UVA irradiation, studying the effect on DNA synthesis in Ehrlich cells, and the clonal growth capacity of HeLa cells. As summarized in Table 2, all derivatives showed a strong antiproliferative effect, higher than 8-MOP; in particular, the HOFQ activity appeared to be from four to six-fold higher than that of 8-MOP. Even in this case, the *N*-methylation seemed to exert a considerable influence on antiproliferative activity, while substitution of the 4-hydroxymethyl group with a 4-methoxymethyl one changed not so much the potency of the compounds.

Mutagenic activity. The mutagenic ability was detected in *Escherichia coli TM9*, a bacterial strain defective in DNA repair and very sensitive to mutagens. As shown in Table 2, all furoquinolinones proved to be one order of magnitude less effective than 8-MOP, classified as a mutagen for the high numbers of revertants induced.[8] This different feature must be related to the inability of furoquinolinones to induce ISC.[5]

Skin phototoxicity. Skin phototoxic properties were evaluated on guinea pig (Table 2); all derivatives, differently from 8-MOP, appeared to be completely unable to form erythemas.

Photobinding to DNA. Since furocoumarins and analogues photoreacted with DNA forming furan-side cycloadducts with thymine, which exhibited a violet fluorescence, calf thymus DNA solutions were exposed to increasing UVA doses in the presence of HOFQ, chosen for its high activity. These solutions showed fluorescence spectra with a maximum at 384 nm ($\lambda_{ex} = 339$ nm), very different from that of HOFQ, which showed a maximum at 321 nm. As summarized in Figure 4, the fluorescence intensity increased as a function

Table 2. Biological activity by UVA irradiation

Compd	DNA synthesis Ehrlich cells[a]	Clonal growth HeLa cells[a]	Mutagenesis *E. Coli* TM9[b]	Skin phototoxicity[c]
MOFQ	1.75±0.03	0.022±0.004	0.22±0.03	– – –
HOHFQ	5.49±0.20	0.074±0.020	0.25±0.01	– – –
HOFQ	0.65±0.02	0.020±0.006	0.41±0.05	– – –
8-MOP	36.6±0.20	0.790±0.020	2.96±0.12	+ + +

[a]ID_{50}±SD: UVA dose which reduces to 50% DNA synthesis or clonal growth when delivered in the presence of 2 μM drug concentration; values were calculated by probit analysis ($P < 0.05$, χ^2 test). SD = standard deviation.
[b]Revertants per 10^6 survivors scored at 0.37 surviving fraction.
[c]Phototoxicity on guinea-pig skin at 0.5 mM cm^{-1} drug concentration and 20 kJ m^{-2} UVA dose. Symbols: – – – no erythema, + + + strong erythema.

of UVA dose, quickly until 1 kJ m^{-2} and slowing down at higher doses. This result was consistent with the formation of furan-side monoadducts, similarly to the other known furocoumarins.

It was known that 8-MOP exhibited a photobinding preference for AT-rich DNA sequences, forming cycloadducts with tymine.[9] In order to investigate the affinity of the test compounds towards pBR322 DNA sequences, restriction endonuclease analyses were carried out. Plasmid DNA was photosensitized with furoquinolinones, digested with seven restriction enzymes (*Bam*HI, *Ava*I, *Hind*III, *Ssp*I, *Ecl*XI, *Nae*I), and then processed with gel-electrophoresis.[10] The results obtained by gel densitometric analyses were shown in Table 3, as per cent inhibition of restriction enzyme activities. The inhibitory effects of drugs plus UVA irradiation on endonuclease activities were caused by recognition inhibition and were dose-dependent. It can be noted that the photoreaction between all furoquinolinone derivatives and DNA involves specific DNA sites which are not implicated in the photoreaction with 8-MOP. The restriction enzymes with a thymidine residue at the cleaving site, like *Ava*I, *Hind*III and *Ssp*I, were inhibited both by furoquinolinones and 8-MOP. This behaviour, already reported using other restriction enzymes,[11] was expected for 8-MOP, as it is well known that the photocycloaddition of psoralens to DNA involves thymine residues. This inhibitory effects observed with furoquinolinones were also demonstrated by the recent isolation of photocycloadducts between HOFQ and thymine (unpublished results).

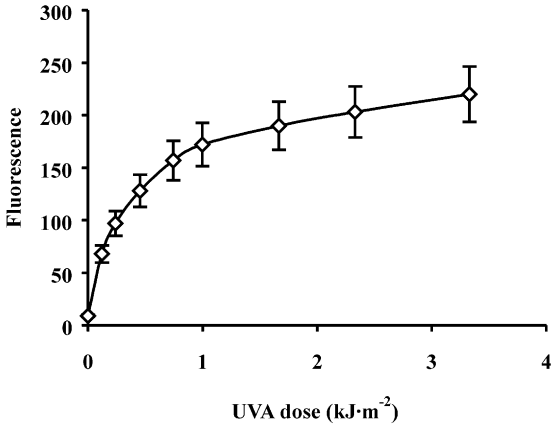


Figure 4. Photobinding of HOFQ to calf thymus DNA in vitro; the irradiated DNA was precipitated, hydrolyzed and submitted to fluorescence determinations ($\lambda_{ex} = 339$ nm; $\lambda_{em} = 384$ nm).

On the other hand, restriction enzymes with 5′-TpA sequence at the recognition site but not at the cleaving site (*Bam*HI) and enzymes that cut DNA sequences lacking thymine or adenine residues (*Ecl*XI, *Nae*I) were strongly inhibited by MOFQ, HOHFQ, and HOFQ, but not by 8-MOP even tested using higher UVA doses. This suggested that 5′-GpA might be a specific DNA damage site for furoquinolinones after UVA irradiation.

Conclusion

HOFQ was prepared by a new synthetic strategy, which allowed to synthesize even the *N*-demethyl analogue (HOHFQ). This alternative route proved to be more straightforward as compared with the synthesis previously reported.[5] HOFQ, HOHFQ and MOFQ, an intermediate of this new synthetic route, were tested for their biological activity.

The three furoquinolinones appeared to be quite active by dark incubation, with a mechanism of action related to topoisomerase II inhibition. For this *dark effect* the isosteric substitution of the oxygen atom of furocoumarins with a nitrogen one in furoquinolinones was essential, as already known, but also the *N*-methylation at 1 position played a crucial role, since HOFQ was the most potent of the three tested compounds. All compounds were characterized by an extremely high photosensitizing activity, indicating that the furoquinolinone moiety was an essential requisite for increasing photoreactivity in comparison with furocoumarins. HOFQ showed the higher activity, again demonstrating that N-methylation afforded more active compounds. Moreover, all tested compounds proved to be nor mutagenic neither phototoxic, both useful properties for a future therapeutic use.

So we can conclude that, among the three tested compounds, HOFQ presents the best structural requirements

Table 3. Inhibition of restriction enzyme activity

Compd	Dose UVA (kJ m^{-2})	% Inhibition of restriction enzyme activity					
		*Bam*HI	*Ava*I	*Hind*III	*Ssp*I	*Ecl*XI	*Nae*I
		G/GATCC	C/TCGGG	A/AGCTT	AA/TATT	C/GGCCG	GCC/GGC
MOFQ	1	26.7	29.5	49.3	60.7	46.2	51.2
	3	32.3	34.6	52.1	69.4	54.3	55.6
HOHFQ	1	19.5	21.7	39.8	55.7	41.6	38.6
	3	20.5	33.4	55.4	59.0	45.2	45.3
HOFQ	1	37.2	27.6	56.1	62.6	52.8	45.5
	3	46.1	44.7	68.9	64.9	53.1	49.3
8-MOP	3	n.d.	15.5	49.4	54.9	n.d.	n.d.
	5	n.d.	35.7	62.9	56.5	n.d.	n.d.

Photosensitized DNA samples digested with restriction enzymes were analysed by gel electrophoresis. Densitometry of the cut versus the uncut DNA fractions was performed to estimate the enzymatic activity.

for the higher activity, so representing a potential chemotherapeutic agent.

The preliminary data obtained with fluorimetric titrations suggest HOFQ damages DNA by UVA irradiation forming 2 + 2-cycloadducts with pyrimidine bases, very probably with thymine. However, the most interesting feature of the present studies on HOFQ was its different sequence specificity toward DNA in comparison with 8-MOP: in fact, it damaged DNA also in a different site than 8-MOP. Since HOFQ induced large amount of DPC without forming ISC,[5] contrary to 8-MOP, probably the different damage site was related to DPC formation. Further experiments are now in progress on the mechanism of action at molecular level in order to understand the damage induced in DNA and its relationship with the biological consequences.

Experimental

Chemistry

Melting points were determined on a Gallenkamp MFB-595-010M melting point apparatus and are uncorrected. Analytical TLC was performed on precoated 60 F_{254} silica gel plates (0.25 mm; Merck) developing with a $CHCl_3$/MeOH mixture (9:1). Preparative column chromatography was performed using silica gel 60 (0.063–0.100 mm; Merck), eluting with $CHCl_3$. Fluorimetric determinations were carried out on a spectrophotofluorimeter Kontron Instruments model SFM. ^{1}H NMR spectra were recorded on a Bruker AMX300 spectrometer with TMS as internal standard. Elemental analyses were obtained on all intermediates and final compounds and are within ±0.4% of theoretical values. Starting 7-allyloxy-4-methoxymethyl-6-methylquinolin-2-one (**1**) and 8-allyl-7-hydroxy-4-methoxymethyl-6-methylquinolin-2-one (**5**) were prepared according to literature methods.[5]

8-Allyl-7-hydroxy-4-methoxymethyl-6-methylquinolin-2-one (2). A solution of **1**[5] (2.10 g, 8.1 mmol) in *N*,*N*-diethylaniline (12 mL) was refluxed until **1** disappeared (4 h, TLC). After cooling, the precipitate was collected, washed with cyclohexane and crystallized from MeOH to give **2** (1.51 g, 72%): mp 174 °C; ^{1}H NMR (DMSO-d_6) δ 10.34 (broad s, 1H, –NH or –OH), 9.01 (broad s, 1H, –NH or –OH), 7.33 (br s, 1H, 5-H), 6.31 (s, 1H, 3-H), 5.96.77 (m, 1H, 2′-H), 4.98.86 (m, 2H, 3′-H), 4.63 (s, 2H, 4-$\underline{CH_2}$OMe), 3.70 (d, 2H, J = 5.0 Hz, 1′-H), 3.40 (s, 3H, 4-$\underline{CH_2}$O$\underline{Me}$), 2.25 (br s, 3H, 6-Me); anal. calcd for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40; found: C, 69.57; H, 6.55; N, 5.39.

8,9-Dihydro-6,8-dimethyl-4-methoxymethylfuro[2,3-*h*]quinolin-2(1*H*)-one (3). Compound **2** (1.51 g, 5.8 mmol) was dissolved in concd H_2SO_4 (60 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, washed with water and crystallized from MeOH to give **3** (0.83 g, 55%): mp 263 °C; ^{1}H NMR ($CDCl_3$) δ 11.49 (broad s, 1H, –NH), 7.28 (q, 1H, J = 0.8 Hz, 5-H), 6.56 (t, 1H, J = 1.1 Hz, 3-H), 5.13 (qdd, 1H, J_q = 6.3 Hz, J_d = 9.3, 7.5 Hz, 8-H), 4.65 (d, 2H, J = 1.1 Hz, 4-$\underline{CH_2}$OMe), 3.64 (dd, 1H, J = 15.8, 9.3 Hz, 9-H), 3.48 (s, 3H, 4-CH_2O$\underline{Me}$), 3.06 (dd, 1H, J = 15.8, 7.5 Hz, 9-H), 2.26 (d, 3H, J = 0.8 Hz, 6-Me), 1.55 (d, 3H, J = 6.3 Hz, 8-Me); anal. calcd for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40; found: C, 69.43; H, 6.57; N, 5.42.

6,8-Dimethyl-4-methoxymethylfuro[2,3-*h*]quinolin-2(1*H*)-one (4). A mixture of **3** (0.83 g, 3.2 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.93 g, 4.1 mmol) in anhydrous toluene (35 mL) was refluxed until starting product disappeared (40 h, TLC). After cooling, the solid was filtered off and the solvent evaporated under reduced pressure. The residue was purified by column chromatography and crystallized from MeOH to give **4** (0.44 g, 53%): mp > 300 °C; ^{1}H NMR ($CDCl_3$) δ 12.18 (broad s, 1H,-NH), 7.28 (q, 1H, J = 1.0 Hz, 5-H), 7.17 (q, 1H, J = 1.1 Hz, 9-H), 6.79 (t, 1H, J = 1.2 Hz, 3-H), 4.76 (d, 2H, J = 1.2 Hz, 4-$\underline{CH_2}$OMe), 3.53 (s, 3H, 4-CH_2O$\underline{Me}$), 2.56 (d, 3H, J = 1.1 Hz, 8-Me), 2.55 (d, 3H, J = 1.0 Hz, 6-Me); anal. calcd for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.88; N, 5.44; found: C, 69.98; H, 5.80; N, 5.39.

8,9-Dihydro-4-methoxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (6). From 5. Compound **5**[5] (2.48 g, 9.1 mmol) was dissolved in concd H_2SO_4 (40 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, washed with water and crystallized from AcOEt/*n*-hexane to give **6** (1.84 g, 74%): mp 153 °C; ^{1}H NMR ($CDCl_3$) δ 7.32 (br s, 1H, 5-H), 6.57 (s, 1H, 3-H), 4.96 (qdd, 1H, J_q = 6.2 Hz, J_d = 8.8, 8.0 Hz, 8-H), 4.58 (s, 2H, 4-$\underline{CH_2}$OMe), 3.93 (dd, 1H, J = 15.0, 8.8 Hz, 9-H), 3.84 (s, 3H, –NMe), 3.43 (s, 3H, 4-CH_2O$\underline{Me}$), 3.38 (dd, 1H, J = 15.0, 8.0 Hz, 9-H), 2.24 (br s, 3H, 6-Me), 1.51 (d, 3H, J = 6.2 Hz, 8-Me); anal. calcd for $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.12; found: C, 70.18; H, 7.00; N, 5.19.

From 3. A mixture of **3** (0.54 g, 2.1 mmol), dimethyl sulfate (0.32 g, 2.5 mmol) and anhydrous K_2CO_3 (5.0 g) in acetone (100 mL) was refluxed until **3** disappeared (12 h, TLC: AcOEt). After cooling, the solid was filtered off and washed with fresh acetone. The solvent was evaporated under reduced pressure from the combined filtrate and washings. The residue was purified by column chromatography to give **7** (0.25 g, 44%), followed by product **6** (0.25 g, 44%).

Compound **6**: mp and ^{1}H NMR as reported above.

Compound **7**: mp 85 °C (MeOH); ^{1}H NMR ($CDCl_3$) δ 7.46 (br s, 1H, 5-H), 6.76 (s, 1H, 3-H), 5.14 (qdd, 1H, J_q = 6.3 Hz, J_d = 9.2, 7.7 Hz, 8-H), 4.78 (s, 2H, 4-$\underline{CH_2}$OMe), 4.03 (s, 3H, 2-OMe), 3.69 (dd, 1H, J = 15.8, 9.2 Hz, 9-H), 3.46 (s, 3H, 4-CH_2O$\underline{Me}$), 3.15 (dd, 1H, J = 15.8, 7.7 Hz, 9-H), 2.36 (br s, 3H, 6-Me), 1.55 (d, 3H, J = 6.3 Hz, 8-Me); anal. calcd for $C_{16}H_{19}NO_3$: C, 70.31; H, 7.01; N, 5.12; found: C, 70.27; H, 6.98; N, 5.17.

4-Methoxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (8). A mixture of **7** (1.01 g, 3.7 mmol) and

2,3 - dichloro - 5,6 - dicyano - 1,4 - benzoquinone (1.00 g, 4.4 mmol) in anhydrous toluene (150 mL) was refluxed until starting compound disappeared (10 h, TLC). After cooling, the solid was filtered off and the solvent evaporated under reduced pressure. The residue was purified by column chromatography (eluting with AcOEt) and crystallized from AcOEt to give **8** (0.48 g, 48%): mp 171 °C; ^{1}H NMR ($CDCl_3$) δ 7.35 (q, 1H, J = 0.9 Hz, 5-H), 6.92 (q, 1H, J = 1.1 Hz, 9-H), 6.77 (t, 1H, J = 1.0 Hz, 3-H), 4.70 (d, 2H, J = 1.0 Hz, 4-CH_2OMe), 4.03 (s, 3H, –NMe), 3.47 (s, 3H, 4-CH_2O**Me**), 2.54 (d, 3H, J = 0.9 Hz, 6-Me), 2.52 (d, 3H, J = 1.1 Hz, 8-Me); anal. calcd for $C_{16}H_{17}NO_3$: C, 70.83; H, 6.31; N, 5.16; found: C, 70.79; H, 6.18; N, 5.09.

4-Bromomethylfuro[2,3-*h*]quinolin-2(1*H*)-ones (9 and 10). General procedure. A mixture of **4** or **8** (1.0 mmol), HBr 48% (1 mL) and acetic acid (10 mL) was refluxed until starting product disappeared (5–10 h, TLC). The mixture was poured into cold water (50 mL) and the precipitate was collected, washed with water and crystallized from EtOAc to give **9** or **10**.

4-Bromomethyl-6,8-dimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (9). Yield 59%; mp 207 °C; ^{1}H NMR ($CDCl_3$) δ 11.88 (br s, 1H, –NH), 7.41 (q, 1H, J = 0.9 Hz, 5-H), 7.09 (q, 1H, J = 1.0 Hz, 9-H), 6.78 (br s, 1H, 3-H), 4.65 (br s, 2H, 4-CH_2Br), 2.58 (d, 3H, J = 0.9 Hz, 6-Me), 2.57 (d, 3H, J = 1.0 Hz, 8-Me); anal. calcd for $C_{14}H_{12}BrNO_2$: C, 54.92; H, 3.95; N, 4.57; Br, 26.10; found: C, 54.89; H, 3.85; N, 4.47; Br, 26.02.

4-Bromomethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (10). Yield 55%; mp 229 °C; ^{1}H NMR ($CDCl_3$) δ 7.46 (br s, 1H, 5-H), 6.92 (q, 1H, J = 1.0 Hz, 9-H), 6.77 (br s, 1H, 3-H), 4.61 (br s, 2H, 4-CH_2Br), 4.02 (s, 3H, –NMe), 2.58 (br s, 3H, 6-Me), 2.53 (d, 3H, J = 1.0 Hz, 8-Me); anal. calcd for $C_{15}H_{14}BrNO_2$: C, 56.27; H, 4.41; N, 4.37; Br, 24.96; found: C, 56.28; H, 4.37; N, 4.37; Br, 25.00.

4-Acetoxymethylfuro[2,3-*h*]quinolin-2(1*H*)-ones (11 and 12). General procedure. A mixture of **9** or **10** (1 mmol) and anhydrous AcONa (0.5 g) in acetic anhydride (10 mL) was refluxed for 1 h. The mixture was cautiously diluted with water (10 mL) and poured into cold water (100 mL). The precipitate obtained was filtered, washed with water and crystallized from AcOEt to give **11** or **12**.

4-Acetoxymethyl-6,8-dimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (11). Yield 98%; mp 159 °C; ^{1}H NMR ($CDCl_3$) δ 7.19 (q, 1H, J = 0.8 Hz, 5-H), 7.14 (q, 1H, J = 0.8 Hz, 9-H), 6.78 (t, 1H, J = 1.1 Hz, 3-H), 5.44 (d, 2H, J = 1.1 Hz, 4-CH_2OAc), 2.56 (d, 3H, J = 0.8 Hz, 6-Me or 8-Me), 2.55 (d, 3H, J = 0.8 Hz, 6-Me or 8-Me), 2.24 (s, 3H, –OAc); anal. calcd for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91; found: C, 67.32; H, 5.35; N, 4.88.

4-Acetoxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (12). Yield 86%; mp 171 °C; ^{1}H NMR ($CDCl_3$) δ 7.20 (q, 1H, J = 0.9 Hz, 5-H), 6.93 (q, 1H, J = 1.1 Hz, 9-H), 6.78 (t, 1H, J = 1.3 Hz, 3-H), 5.39 (t, 2H, J = 1.3 Hz, 4-CH_2OAc), 4.03 (s, 3H, –NMe), 2.54 (d, 3H, J = 1.1 Hz, 8-Me), 2.53 (d, 3H, J = 0.9 Hz, 6-Me), 2.21 (s, 3H, –OAc); anal. calcd for $C_{17}H_{17}NO_4$: C, 68.22; H, 5.72; N, 4.68; found: C, 68.20; H, 5.75; N, 4.60.

4-Hydroxymethylfuro[2,3-*h*]quinolin-2(1*H*)-ones (13 and 14). General procedure. To a methanolic solution (10 mL) of **11** or **12** (1.0 mmol) a 5% methanolic potassium hydroxide solution (10 mL) was added and the mixture was refluxed for 1 h. The solution was cooled, diluted with cold water (50 mL) and acidified with diluted HCl. The precipitate obtained was collected and crystallized from MeOH to give **13** or **14**.

4-Hydroxymethyl-6,8-dimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (13). Yield 42%; mp > 300 °C; ^{1}H NMR ($CDCl_3$) δ 11.89 (broad s, 1H, –NH), 7.32 (q, 1H, J = 0.8 Hz, 5-H), 7.22 (q, 1H, J = 1.0 Hz, 9-H), 6.53 (t, 1H, J = 1.2 Hz, 3-H), 5.50 (t, 1H, J = 5.5 Hz, 4-CH_2OH), 4.79 (dd, 2H, J = 5.5, 1.2 Hz, 4-CH_2OH), 2.50 (d, 3H, J = 1.0 Hz, 8-Me), 2.49 (d, 3H, J = 0.8 Hz, 6-Me); anal. calcd for $C_{14}H_{13}NO_3$: C, 69.12; H, 5.39; N, 5.76; found: C, 69.19; H, 5.85; N, 5.68.

4-Hydroxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (14). Yield 76%; mp 302 °C; ^{1}H NMR (DMSO-d_6) δ 7.40 (br s, 1H, 5-H), 7.24 (s, 1H, 9-H), 6.65 (s, 1H, 3-H), 5.54 (t, 1H, J = 5.5 Hz, 4-CH_2OH), 4.79 (d, 2H, J = 5.5 Hz, 4-CH_2OH), 3.90 (s, 3H, –NMe), 2.52 (br s, 3H, 6-Me or 8-Me), 2.49 (br s, 3H, 6-Me or 8-Me); anal. calcd for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.88; N, 5.44; found: C, 70.09; H, 5.85; N, 5.42.

Biology

8-MOP was obtained from Chinoin (Milan, Italy) and calf thymus DNA from Sigma-Chemie (Deisenhofen, Germany). Plasmid pBR322 DNA and the restriction enzymes *Bam*HI, *Ava*I, *Hind*III, *Ssp*I, *Pst*I, *Ecl*XI, and *Nae*I were purchased from Boehringer Mannheim GmbH, (Manneheim, Germany). The test compounds were dissolved in dimethyl sulfoxide (DMSO; 4.5 mM) and the solutions kept at −20 °C in the dark. Just before the experiment, a calculated amount of compound solution was added in the dark to phosphate-buffered saline (PBS) or to the growth medium containing cells, to a final solvent concentration never exceeding 0.5%. The same amount of DMSO was added to untreated controls.

UVA irradiation. Cell suspensions containing the tested compound were incubated at room temperature for 15 min in the dark, put into Petri dishes (5 cm in diameter, 3 mL) and exposed to UVA light. UVA exposure were performed with a Philips HPW 125 lamp, provided with a built-in Philips filter. The emission spectrum was in the range 320–400 nm, with a maximum, over 90% of the total, at 365 nm. Irradiation intensity was determined on a UV radiometer (model 97507, Cole–Parmer Instrument Co., Niles, IL) at 5.5×10^{-3} kJ s^{-1} m·

Experiments with DNA in vitro

Inhibition of topoisomerase II activity. The inhibition of topoisomerase II activity was studied using a purified enzyme from *Drosophila melanogaster* embryos (USB, Amersham Italia S.r.l., Milano, Italy).[12] PM2 DNA (0.125 μg) was incubated for 15 min at 30° C in the presence of 2 units topoisomerase II (1 unit is defined as the activity capable of relaxing 0.3 μg of supercoiled DNA) in the reaction buffer containing 10 mM Tris–HCl (pH 7.9), 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 μg mL^{-1} BSA, 1 mM ATP. Aliquots of compound solution (2 μL) in DMSO (4.5 μM) were added to reach the following final concentrations: 1, 5, 50, 100 μM. A suitable amount of reaction buffer was then added to every sample to reach the final volume of 20 μL. The reaction was blocked by adding 7 mM EDTA (3 μL) containing 0.77% SDS. Bromophenol blue (2 μL) containing 15% glycerol was added to the samples, which were then analyzed by agarose gel (0.7%) electrophoresis containing TAE (40 mM Tris-sodium acetate, pH 8.2; 1 mM EDTA) for 90 min. The gel was stained during 1 h in aqueous ethidium bromide (0.5 μg mL^{-1}). The bands were detected by exposure to an UV transilluminator TM36 (UVP Inc., San Gabriel, CA USA) and recorded with an Uvitec GAS9200 camera equipped with UVIdoc software.

Binding to calf thymus DNA. Samples (2 mL) of 6 mM DNA solution, treated with HOFQ (100 μg mL^{-1}), were exposed to increasing UVA doses. DNA was precipitated adding NaCl up to 2 mL followed by 2 volumes of absolute ethanol. DNA samples were washed twice with 80% ethanol and heated at 100 °C for 1 h with HCl 1 N. The solutions were neutralized by adding NaOH and 1 mL of PBS at pH 7.4, and submitted to fluorimetric analysis.

Binding to plasmid pBR322 DNA. Binding to plasmid pBR322 DNA was detected as previously described.[10] Briefly, pBR322 DNA (1.4×10^{-4} M) was incubated for 15 min at 37 °C with different doses of the test compounds in TE buffer (10 mM Tris–HCl, pH 7.5; 0.1 mM EDTA) to a total volume of 22 μL and exposed to UVA light. The prescribed incubation buffer containing the enzyme was then added to the mixture; the enzyme amount used was that necessary to cut 1 μg of DNA in 1 h at 37 °C. The digestion was stopped by adding 2 μL of loading buffer (0.25% bromophenol blue, 40% sucrose, 50 mM EDTA; pH 7.5). The samples of restriction fragments were analysed by agarose gel (1%) electrophoresis in TAE containing ethidium bromide (0.5 μg mL^{-1}) at 100 V. Bands were detected as above.

Experiments in vivo

DNA synthesis in Ehrlich cells. DNA synthesis was assayed in Ehrlich ascites tumor cells (Lettrè strain) as already described.[13] Cells (2×10^7 cells mL^{-1} in PBS) were incubated for 1 h in the dark with increasing concentrations of tested compounds or exposed to UVA light according to the selected treatment, as above described. The samples (10^6 cells in 0.5 mL PBS) were then incubated for 30 min at 37° in the presence of 40 kBq mL^{-1} of [^{3}H]thymidine (4.77 TBq mM^{-1}; Amersham International Inc., UK). The acid-insoluble fraction was precipitated by adding ice-cold 5% 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 percentage of radioactivity incorporated into the DNA with respect to untreated control cells (approximately 3–6 kBq). Filtrations were carried out with a Sample Manifold apparatus (Millipore Corp., Bedford, USA). Filters from DNA synthesis determinations were counted using a toluene based scintillator (PPO 5 g, dimethyl-POPOP 0.25 g, toluene up to 1 L of solution) and a Packard Tri-Carb 1900TR spectrometer. Counting was accomplished automatically on the basis of quenching curves obtained using [^{3}H]radioactivity standards.

Clonal growth of HeLa cells. HeLa cells were grown in nutrient mixture F-12Ham medium (Sigma Chemical Co, St. Louis, MO, USA), containing 10% fetal calf serum, and supplemented with antibiotics. Cell growth was accomplished at 37 °C in a 5% carbon dioxide atmosphere. HeLa cells ($1.5–2\times10^5$) were seeded in Petri dishes in growth medium (4 mL). After 24 h, the medium was replaced with a fresh one containing the tested compound. The cells were incubated for 3 h in the dark or exposed to the UVA light according to the selected treatment, as above described. Aliquots of 200 cells were seeded in the same medium, incubated for 7 days and then the colonies were stained and counted, discarding colonies with less than 50 cells. The efficiency of the clonal growth, that is the ratio between the number of the formed colonies and the number of the cells seeded, was then calculated. The plating efficiency was about 90%.

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 by most base pair substitution mutagens[14] and therefore by formation of C_4-cycloadducts of furocoumarins.[15] Bacteria were grown overnight in a minimal Davis–Mingioli salt glucose medium supplemented with tryptophan (20 mg L^{-1}). *E. coli* cells were washed and then suspended in PBS (pH 7.0) containing the test compound (20 μM) at a density of 10^8 cells mL^{-1}. Bacteria were irradiated with UVA. 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). To determine the surviving fraction, the irradiated cells (0.1 mL) were diluted with phosphate buffer, added to 2 mL of molten 0.6% agar, and plateled on Davis–Mingioli minimal medium supplemented with tryptophan. The plates were incubated for 24 h at 37 °C in the dark, and the colonies were then counted. The mutation frequency was expressed as mutants per 10^6 survivors, computed by dividing the number of revertants observed per plate by the number of surviving bacteria at the same treatment and subtracting from result the number of revertant colonies per million survivors observed in controls (0.03 ± 0.01).

Skin phototoxicity. Skin phototoxicity was tested applied on depilated albino guinea-pigs (outbred Dunkin–Hartley strain) as already described.[16] Compounds were applied topically as 0.5% methanolic solutions up to 5×10^{-2} mg cm· The animals were kept in the dark for 15 min and then exposed to UVA light (20 KJ m^{-2}). The animals were observed for 7 days.

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