Synthesis and Biological Evaluation of a New Furo[2,3-*h*]quinolin-2(1*H*)-one

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A new furoquinolinone derivative, namely 4-hydroxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (HOFQ), was synthesized and its biological activity studied. By UVA activation, HOFQ induced strong antiproliferative effects in Ehrlich ascite cells, which lost their ability to transmit the tumor by transplantation. HOFQ exhibited poor genotoxicity and absence of skin phototoxicity. Actually, HOFQ sensitization forms DNA-protein cross-linkages but not interstrands cross-links. Therefore, HOFQ appears to be a new promising drug for PUVA photochemotherapy and photopheresis.

Introduction

Furocoumarins, currently called psoralens, are active sensitizers used in PUVA (Psoralen plus UVA) therapy to cure several skin diseases[1] and in photopheresis to treat T-cell lymphoma and various autoimmune diseases and to prevent rejection in organ transplants.[2]

PUVA therapy, performed by using linearly annulated psoralens such as 8-methoxypsoralen (8-MOP) (Figure 1), is highly effective, but some toxic effects are to be expected, such as skin erythema,[1] genotoxicity,[3,4] and carcinogenicity,[5] mostly attributed to the lesions induced in DNA by psoralen sensitization. Indeed, different kinds of DNA lesions can be recognized, i.e., covalent monoadducts (MA) with pyrimidine bases,[6] diadducts with pyrimidine bases placed on the opposite DNA strand, thus forming inter-strands cross-links (ISC),[6] and covalent DNA-protein cross-links (DPC).[7]

Since many authors considered ISC mainly responsible for furocoumarin genotoxicity, several monofunctional derivatives have been prepared and studied, such as angularly annulated furocoumarins as well as angular furoquinolinones.[8] Among them, 1,4,6,8-tetramethylfuro[2,3-*h*]quinolin-2(1*H*)-one (FQ) (Figure 1) showed a very high antiproliferative activity, without inducing ISC:[9] this was attributed to its ability to form a considerable amount of DPC, which can be considered another kind of diadduct because the molecule is a part of the covalent bridge linking together DNA and proteins.[10] Moreover, these lesions induced marked clastogenic effects in mammalian cells cultivated in vitro.[11]

We tried to modulate FQ activity reducing its toxic effects without affecting its antiproliferative activity. Considering the studies carried out on benzopsoralen derivatives in which the insertion of a hydroxymethyl group at 4 position improved the activity of such

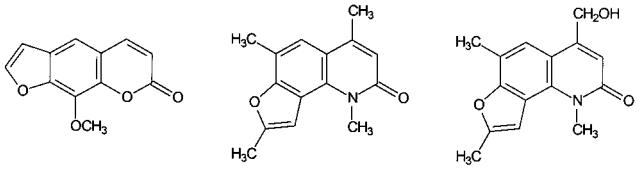

Figure 1. Structure of 8-methoxypsoralen (8-MOP), 1,4,6,8-tetramethylfuro[2,3-*h*]quinolin-2(1*H*)-one (FQ), and 4-hydroxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (HOFQ).

derivatives without increasing their toxicity,[12] we introduced a slight modification into FQ molecular structure, replacing the 4-methyl group with a hydroxymethyl one. So, we describe the synthesis and the main biological properties of a new furoquinolinone derivative, 4-hydroxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (HOFQ) (Figure 1). As a reference compound, we chose 8-MOP (Figure 1), as it is a well-known and widely used linear furocoumarin.

Chemistry

The synthetic pathway consists of building the tricyclic nucleus already carrying the 4-hydroxymethyl group protected as methyl ether and then cleaving the ether function. As shown in Scheme 1, 2,4-diaminotoluene (**1**) was reacted with methyl 4-methoxyacetoacetate to yield 7-amino-4-methoxymethyl-6-methylquinolin-2-one (**2**), which was diazotized and hydrolyzed to give 7-hydroxy-4-methoxymethyl-6-methylquinolin-2-one (**3**). Compound **3** was condensed with allyl bromide, giving the 7-*O*-allyl ether **4**, which was methylated by dimethyl sulfate to give the corresponding *N*-methyl derivative **5**. In this reaction a small amount of 2-methoxyquinoline **6** was also obtained. The 7-allyloxy-1,6-dimethyl-4-methoxymethylquinolin-2-one (**5**) was submitted to Claisen rearrangement, yielding 8-allyl derivative **7**. Compound **7** was acetylated and brominated at room temperature, affording 8-dibromopropyl derivative **9**, which was submitted to cyclization in alkaline medium,[13] to give 4-methoxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-

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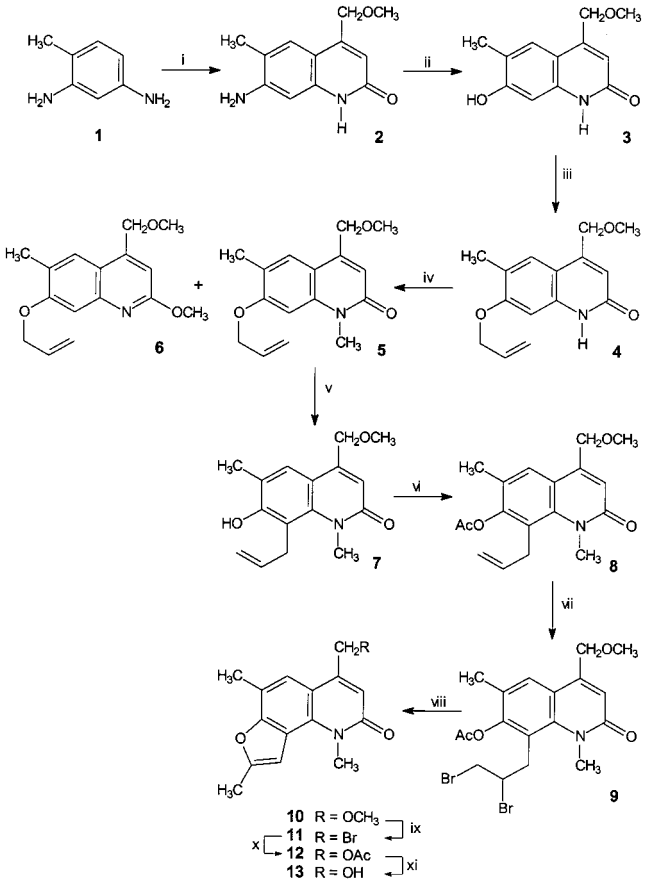
Scheme 1[a]



[a] Reagents and conditions: (i) $MeOCH_2COCH_2CO_2Et$, 150 °C; (ii) H_2SO_4, $NaNO_2$, 0 °C then H_2SO_4, 160 °C; (iii) $CH_2{=}CHCH_2Br$, DMF, K_2CO_3, RT; (iv) Me_2SO_4, acetone, K_2CO_3, reflux; (v) *N,N*-diethylaniline, reflux; (vi) Ac_2O, AcONa, reflux; (vii) Br_2, AcOH, RT; (viii) KOH, abs. EtOH, reflux; (ix) HBr, AcOH, reflux; (x) Ac_2O, AcONa, reflux; (xi) KOH, MeOH, reflux.

2(1*H*)-one (**10**). Compound **10** was reacted with hydrobromic acid, to give the 4-bromomethyl derivative **11**, then submitted to acetylation, yielding the corresponding 4-acetoxymethyl derivative **12**, and finally hydrolyzed in alkaline conditions to give the desired 4-hydroxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (**13**). Direct alkaline hydrolysis of **11** to **13** was carried out, but the reaction time was longer and the yield lower than indirect hydrolysis through **12**, owing to the formation of many degradation products.

Biological Section

Detection of DNA Damage. (a) Formation of ISC in Vitro. The induction of ISC by UVA irradiation, using PM2-linearized DNA, was tested. ISC formation was evaluated by gel-electrophoresis separation of single-stranded, not cross-linked DNA, from double-stranded, cross-linked DNA. As shown in Figure 2, HOFQ appeared to be completely incapable of inducing ISC, even at increasing UVA dose, while 8-MOP, used as reference, formed high levels of ISC, even at low UVA dose. UVA irradiation alone up to 10 kJ m^{-2} did not modify the electrophoretic migration of DNA (data not shown).

(b) Formation of DPC in Vivo. The DNA-damage-inducing ability of HOFQ was also studied detecting the formation of DPC in CHO cells by alkaline elution. As

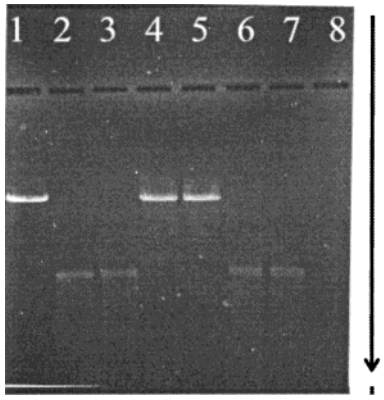


Figure 2. ISC formation in vitro in PM2-linearized DNA by sensitization with HOFQ or 8-MOP (two drug molecules per base pair) detected by electrophoresis. Lane 1: untreated double-stranded DNA. Lane 2: untreated single-stranded DNA. Lanes 3: DNA exposed to 3.3 kJ m^{-2} alone. Lanes 4, 5: DNA exposed to 1.5 and 3 kJ m^{-2} in the presence of 8-MOP, respectively. Lanes 6, 7: DNA exposed to 1.5 and 3 kJ m^{-2} in the presence of HOFQ, respectively.

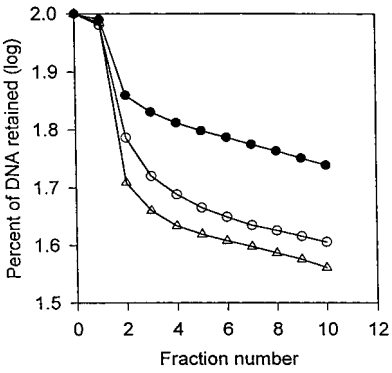


Figure 3. DPC formation in CHO cells detected by alkaline elution. Cells were exposed to UVA light (0.3 kJ m^{-2}) in the presence of HOFQ or 8-MOP (both at 2 μM concentration). Symbols: Δ, controls only submitted to γ rays (30 Gy); ○, HOFQ + 30 Gy; ●, 8-MOP + 30 Gy.

reported in Figure 3, 8-MOP generated an elution profile just above that of untreated control cells: this means that 8-MOP, tested under the same experimental conditions of HOFQ, formed small numbers of DPC. On the contrary, cells sensitized by HOFQ showed a high DNA retention, due to the formation of a considerable amount of DPC.

Mutagenesis in *Escherichia coli WP2*. The HOFQ mutagenic activity was detected in two *E. coli WP2* strains, *TM6* and *TM9* (Figure 4). In *TM6* strain, proficient in DNA repair, HOFQ and 8-MOP gave rise to two superimposed curves; in *TM9* strain, defective in DNA excision repair, HOFQ appeared to be no mutagenic, while 8-MOP induced large numbers of revertants.

Antiproliferative Activity in Mammalian Cells in Vitro. The antiproliferative effect of HOFQ was tested in mammalian cells in vitro by studying the inhibition of DNA synthesis in Ehrlich cells and of clonal growth in CHO cells. Table 1 summarizes the data obtained in various experiments showing the ID_{50} values, which represent the treatments producing a 50% inhibition. HOFQ exhibited an antiproliferative activity 40−60-fold higher than 8-MOP in both tests. To obtain further information on the mechanism of the HOFQ

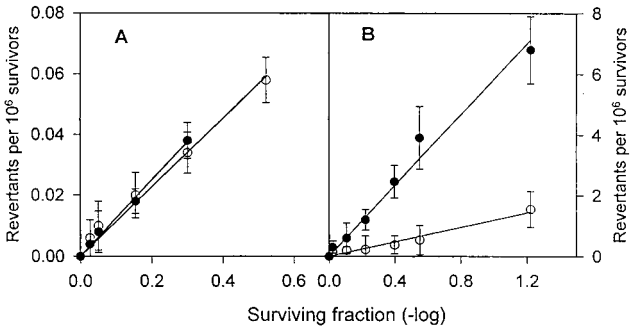


Figure 4. Mutagenesis in *E. coli WP2*. Bacteria were exposed to UVA light in the presence of HOFQ or 8-MOP (2 μM), and surviving fraction and revertants per million survivors were determined. The revertants were plotted against the surviving fraction. Panel A: *TM6* strain, proficient in DNA repair. Panel B: *TM9* strain, defective in DNA excision repair. Symbols: ○, HOFQ; ●, 8-MOP.

Table 1. Antiproliferative Effect in Mammalian Cells upon UVA Irradiation[a]

compound	DNA synthesis	clonal growth
HOFQ	0.65 ± 0.02	0.02 ± 0.006
8-MOP	36.6 ± 0.3	0.84 ± 0.04

[a] The data are expressed as ID_{50}, which indicates the treatment producing a 50% inhibition, calculated by probit analysis, that is, the UVA light dose, expressed as kJ m^{-2}, delivered in the presence of a 2 μM drug concentration.

Table 2. Protection against the Ehrlich Viable Cells Transplant[a]

challenge			
no. of viable cells ($\times 10^{-6}$)	days after the transplant	% mortality	mean survival time (days)
0.3	8	70	21.3
0.3	15	35	25
0.3	21	0	>60
1	21	30	10.6
4	21	100	15.8

[a] Before the challenge, mice (groups of 10 animals) were injected ip with 5×10^6 Ehrlich cells exposed to UVA light (2.7 kJ m^{-2}) in the presence of HOFQ (2 μM).

activity by UVA irradiation, its ability to inhibit RNA and protein syntheses was also checked in the experimental conditions used for DNA. HOFQ sensitization inhibits RNA synthesis, with an ID_{50} value (0.36 ± 0.02) very close to that obtained for DNA synthesis. On the contrary, protein synthesis remained completely unaffected (ID_{50} > 10).

Experiments in Vivo. (a) Ehrlich Tumor Transplantation. The effect of HOFQ sensitization was further studied in Ehrlich cells in vivo, at first testing the tumor transmitting capacity into recipient mice. The injection of untreated viable cells yielded a 100% mortality, but when they were exposed to UVA light (1.33 kJ m^{-2}) in the presence of 2 μM HOFQ, the mortality was abolished.

Then we studied the effect of the injection of such inactivated cells on mice; therefore, groups of mice were injected with the photoinactivated cells and then, at different times, they were submitted to a transplantation of different amounts of viable tumor cells. As shown in Table 2, the protection became total when the challenge was performed 21 days after the injection of photoinactivated cells. If 10^6 viable cells were injected, a 30% mortality was observed, which reached 100% when the challenge was accomplished with 4×10^6 tumor cells.

(b) Skin Phototoxicity. Skin phototoxicity was studied on albino guinea pig skin; HOFQ appeared to be completely unable of inducing skin erythemas even when tested at high concentration (30 μM cm^{-2}) and with large UVA doses (10 kJ m^{-2}). On the contrary, 8-MOP induced skin phototoxicity even in mild experimental conditions (4 μM cm^{-2}, 5 kJ m^{-2}).

Conclusions

The new furoquinolinone derivative HOFQ was tested in various biological systems. Upon UVA activation, HOFQ, like FQ,[9,10] induced large amounts of DPC, as assayed in vivo in CHO cells; moreover, HOFQ appeared to be incapable of forming ISC, as detected in vitro in PM2-linearized DNA. Unfortunately, at present a radioactive sample of HOFQ is not available; therefore we can only suppose it shows a high capacity of photobinding to DNA, like FQ.[8] On the other hand, the induction of a severe DNA damage was also demonstrated when studying macromolecular synthesis in Ehrlich cells: both DNA and RNA syntheses were strongly affected by HOFQ sensitization, while protein synthesis remained unchanged; these results are similar to those previously obtained with FQ [unpublished results].

HOFQ retained the very high antiproliferative activity in mammalian cells typical of FQ, as detected studying both DNA synthesis and clonal growth capacity in Ehrlich and CHO cells, respectively.

The HOFQ activity was further studied in Ehrlich cells in vivo; when these cells are injected intraperitoneously into recipient mice, a 100% mortality is observed within 1 or 3 weeks, according to cell amount. However, if Ehrlich cells were submitted to HOFQ photosensitization, this property was completely canceled and all mice were able to survive a long period. This means that all tumor cells lost their reproductive capacity. Moreover, we observed that animals treated with such photoinactivated cells developed a resistance against a successive transplantation of untreated viable tumor cells; this protection was only observed if tumor challenge was performed at least three weeks after the injection of inactivated cells; at shorter times, the protection decreased, with a corresponding increase in mortality in treated mice. This suggested that the injection of the inactivated cells stimulated a host immune response against tumor cells. These results can be explained supposing that DNA damage induced by HOFQ neutralizes cell reproductive ability and introduces modifications which increase cell antigenic character, so stimulating a host immune response against tumor challenge. This immune response mechanism is the same postulated as that of photopheresis.[2] On the basis of these observations, the results achieved photoinactivating Ehrlich cells in the presence of HOFQ prompt the consideration of this furoquinolinone derivative as a potential useful drug for photopheresis therapy.

HOFQ genotoxicity was investigated on two WP2 *Escherichia coli* strains, *TM6* and *TM9*, the former proficient in DNA repair, the latter defective, *uvrA*$^-$ being an essential gene of DNA excision repair.[14] In the defective strain *TM9*, HOFQ induced much lower amounts of revertants than 8-MOP and seems to be a weaker mutagen.

A similar picture has been obtained studying skin phototoxicity, a well-known side effect of 8-MOP; in fact, while FQ showed a phototoxicity comparable to that of 8-MOP,[9] HOFQ appeared to be incapable of inducing erythemas on guinea pig skin.

Therefore, we can conclude that HOFQ represents a new furoquinolinone derivative markedly more efficient in promoting antiproliferative effects than 8-MOP and very close to FQ.[9,11] Pointing out some preliminary interesting properties of HOFQ, including scarce genotoxicity and lack of skin phototoxicity, extensive studies will be made in the future on the relationship between the damage induced into DNA and the observed biological effects.

Experimental Section

Chemistry. TLC, mp, ^{1}H NMR spectra, and CHN were obtained as noted.[9] See Supporting Information for synthesis of **2** and **3**, mp, NMR, and microanalysis data.

7-Allyloxy-4-methoxymethyl-6-methylquinolin-2-one (4). A mixture of **3** (7.4 g, 34.0 mmol), allyl bromide (6.1 g, 50.4 mmol), and K_2CO_3 (10.0 g) in DMF (150 mL) was stirred at 20 °C for 40 h. The mixture was diluted with water (350 mL) and the solid collected, washed with water, and crystallized from MeOH to give **4** (2.1 g, 24%).

7-Allyloxy-1,6-dimethyl-4-methoxymethylquinolin-2-one (5) and 7-Allyloxy-6-methyl-2-methoxy-4-methoxymethylquinoline (6). A mixture of **4** (5.0 g, 19.3 mmol), dimethyl sulfate (2.8 g, 22.5 mmol), and K_2CO_3 (10.0 g) in acetone (250 mL) was refluxed for 15 h. After cooling, the solid was filtered off and the filtrate evaporated under reduced pressure. The residue was purified by column chromatography to give **6** (0.44 g, 8%), followed by **5** (3.5 g, 66%).

8-Allyl-1,6-dimethyl-7-hydroxy-4-methoxymethylquinolin-2-one (7). A solution of **5** (3.5 g, 12.8 mmol) in *N*,*N*-diethylaniline (50 mL) was refluxed for 7 h. After cooling, the precipitate was collected, washed with cyclohexane, and crystallized from AcOEt to give **7** (2.5 g, 75%).

7-Acetoxy-8-allyl-1,6-dimethyl-4-methoxymethylquinolin-2-one (8). A mixture of **7** (5.4 g, 19.7 mmol) and anhydrous AcONa (1.0 g) in acetic anhydride (30 mL) was refluxed for 1 h. The mixture was cautiously diluted with water (600 mL), and the collected precipitate was crystallized from AcOEt/*n*-hexane to give **8** (4.2 g, 68%).

7-Acetoxy-8-(2′,3′-dibromopropyl)-1,6-dimethyl-4-methoxymethylquinolin-2-one (9). A solution of Br_2 (1.4 g, 8.9 mmol) in acetic acid (10 mL) was added dropwise to a solution of **8** (2.8 g, 8.9 mmol) in acetic acid (60 mL) at 20 °C, and after the addition was completed the solution was stirred for 30 min. The solvent was evaporated under reduce pressure, and the residue was crystallized from MeOH to give **9** (3.9 g, 92%).

4-Methoxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (10). A solution of KOH (2.5 g) in absolute EtOH (50 mL) was added to a solution of **9** (3.9 g, 8.2 mmol) in absolute EtOH (100 mL), and the mixture was refluxed for 45 min. The cooled mixture was acidified with 2 N HCl and diluted with water (500 mL). The solid was collected, washed with water, and crystallized from AcOEt to give **10** (1.8 g, 83%).

4-Bromomethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (11). A mixture of **10** (1.5 g, 5.5 mmol), HBr 48% (5 mL), and acetic acid (50 mL) was refluxed for 10 h. The mixture was poured into water (250 mL), and the collected precipitate was crystallized from EtOAc to give **11** (0.97 g, 55%).

4-Acetoxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (12). Compound **12** (0.74 g, 86%) was prepared from **11** (0.95 g, 2.9 mmol) as described for compound **8**.

4-Hydroxymethyl-1,6,8-trimethylfuro[2,3-*h*]quinolin-2(1*H*)-one (13). To a solution of **12** (0.70 g, 2.3 mmol) in MeOH (20 mL) was added a 5% methanolic KOH solution (20 mL), and the mixture was refluxed for 1 h. The solution was diluted with water (100 mL) and acidified with 2 N HCl. The collected precipitate was crystallized from MeOH to give **13** (0.45 g, 76%).

Biological Assays. Detection of DNA damage, mutagenesis, antiproliferative activity in vitro and in vivo, and skin phototoxicity have been recently described.[10−12] For further details, see Supporting Information.

Supporting Information Available: Detailed procedures for the syntheses of compounds **2** and **3**; mp, NMR, and microanalysis data for all compounds; and biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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