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Novel Angular Furo and Thieno-quinolinones: Synthesis and Preliminary Photobiological Studies

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Abstract—A number of new furo and thienoquinolinones carrying an electron-withdrawing function or unsubstituted at the position 3 were synthesized in order to obtain new potential photochemotherapeutic agents with increased antiproliferative activity and decreased toxic side effects. Our interest in studying the SAR of these derivatives also prompted us to investigate the influence of *N*-methylation on biological activity, by preparing *N*-methyl derivatives. The antiproliferative activity of all the newly synthesized compounds was evaluated and compared to 8-methoxypsoralen (8-MOP), the drug widely used in PUVA-therapy. The 3-unsubstituted thienoquinolinones were generally the most potent derivatives, followed by the furo-analogues. In particular, the unsubstituted thieno[2,3-*h*]quinoline-2(1*H*)one showed the highest activity in T2 bacteriophage, HeLa cells and Ehrlich cells tests. All the compounds, assayed on *Escherichia coli WP2 TM9*, showed a similar mutagenic activity, very close to that of 8-MOP. Except for 2-oxo-1,2-dihydrothieno[2,3-*h*]quinoline-3-carboxylic acid, which appeared to be very effective, all compounds generated singlet oxygen to slightly larger amounts when compared to 8-MOP. The *N*-methyl analogues only induced moderate skin erythemas on albino guinea pigs, while all other derivatives appeared to be entirely inactive. On the basis of these results, the unsubstituted thieno[2,3*h*]quinoline 2(1*H*)one seems to be the most interesting potential drug for PUVA photochemotherapy and photopheresis. © 2002 Elsevier Science Ltd. All rights reserved.

Introduction

Linear furocoumarins (psoralens) are a well-known family of natural and synthetic photosensitizing compounds which exhibit very interesting photobiological and phototherapeutical activities. Some of these are used in PUVA photochemotherapy (Psoralen plus UVA) to treat a variety of skin diseases. They are also employed in extracorporeal photochemotherapy (photopheresis) to treat cutaneous T-cell lymphomas and as selective immunosuppressive agents for the cure of various autoimmune diseases and to prevent rejection in organ transplants.[1,2] Furthermore, psoralen derivatives are also used as tools in biophysical studies on nucleic acids and are now recognized as effective antiviral agents, especially against enveloped viruses such as the herpes simplex virus or HIV-1.[3,4] The preferred compound is 8-methoxypsoralen (8-MOP) but 5-methoxypsoralen (5-MOP) and 4,5′,8-trimethylpsoralen (TMP) are also used. They show good antiproliferative effect due to their capability of photodamaging DNA, leading to monofunctional and two different kinds of bifunctional adducts, inter-strand cross-links (ISC)[5] and DNA–protein cross-links (DPC), tying together a DNA base and a protein aminoacid.[6–8]

The bifunctional damage, in particular the induction of ISC, was regarded as the main cause responsible for the furocoumarin toxicity, i.e., skin erythemas, genotoxicity with induction of point mutations in bacteria.[1,9–12] The formation of chromosomal aberrations was recently mainly attributed to DPC.[8]

Therefore, to obtain less toxic compounds, various authors planned the study of new compounds characterized by a prevalent ability of forming monofunctional damage; the main research line dealt with angelicin analogues, because the angular molecular structure of angelicin prevents the formation of ISC for geometrical reasons.[13,14] The most active compound

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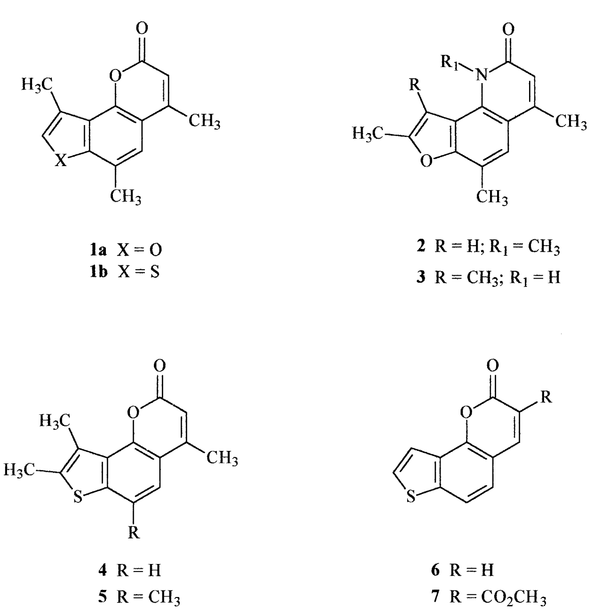
belonging to this series is 4,6,4′-trimethylangelicin **1a**, which can induce moderate amounts of ISC[15] and DPC.[6] Furthermore, various angelicin isosters, having interesting features, were prepared and studied. They are angular furoquinolinones (**2**, **3**) and thioangelicin derivatives such as **1b** and **4**–**7**[16-20] (Chart 1).



1a X = O
1b X = S

2 R = H; R$_1$ = CH$_3$
3 R = CH$_3$; R$_1$ = H

4 R = H
5 R = CH$_3$

6 R = H
7 R = CO$_2$CH$_3$

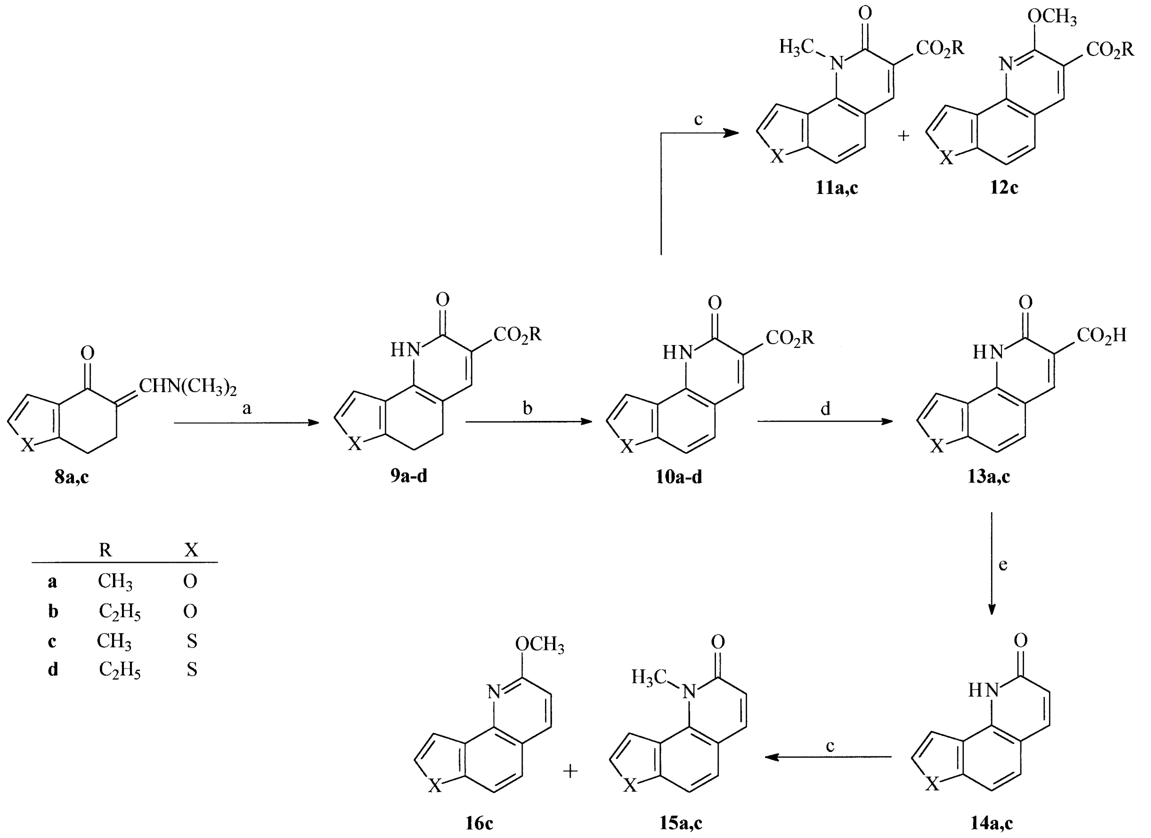
Chart 1.

So as to obtain new angelicin isosters with improved features, we studied some new furo and thienoquinolinones **10**, **13** and **14**. In all these compounds a nitrogen atom replaces the oxygen at the pyrone ring, like in the former **2** and **3**, but positions 4, 6, 8 and 9 are unsubstituted; position 3 can bear an electron-withdrawing function (**10a**–**d** and **13a**,**c**) or is unsubstituted (**14a**,**c**) such as in derivatives **6** and **7**.

We will thus compare the effect on photobiological properties of the insertion of a second heteroatom into the furocoumarin skeleton, that is, sulfur at 7 position of a furoquinolinone. This chemical modification, besides increasing molecular lipophilicity, intensifies the aromaticity of the five member ring and therefore the delocalization of the C$_{8,}$ C$_9$ double bond, which is one of the two photoreactive sites of the furocoumarin molecule. We also examined *N*-methyl derivatives **11a**,**c** and **15a**,**c** in order to evaluate the importance of the tautomerism 2-pyridone/2-hydroxypyridine on photobiological features of furo and thienoquinolinones.[7] In this paper, we are reporting the data obtained in a preliminary screening on the biological activity of the new compounds. As a reference compound, we used 8-MOP.

Chemistry

The routes used for the synthesis of the compounds assessed in this study are shown in Scheme 1. The versatile electrophilic precursors 5-[(dimethylamino)methy-



	R	X
a	CH$_3$	O
b	C$_2$H$_5$	O
c	CH$_3$	S
d	C$_2$H$_5$	S

Scheme 1. Reagents: (a) NC–CH$_2$–CO$_2$R; (b) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), toluene; (c) CH$_3$I, K$_2$CO$_3$, acetone; (d) HCl and CH$_3$COOH 50%; (e) Cu powder, quinoline.

lene]-6,7-dihydro-1-benzofuran-4(5*H*)-one **8a**[21] and 5-[(dimethylamino)methylene]-6,7-dihydro-1-benzothiophen-4(5*H*)-one **8c**[22] were synthesized directly, in good yield, by refluxing a solution of the suitable commercially available keton in excess of *N*,*N*-dimethyl-formamide dimethylacetal. Some of us recently, reported the following useful procedure to obtain derivatives characterized by the 2-pyridone nucleus by reacting enamino ketones with a 1-3 dinucleophile with *C*–C–*N* structure such as methyl cyanoacetate.[23] In order to further investigate the reactivity of this particular *C*–C–*N* dinucleophile, we carried out the synthesis of 5,6-dihydro-cycloadducts **9a–d** by reaction between **8a,c** and the appropriate ester of cyanoacetic acid. Probably this reaction occurs first between the nucleophilic methylenic group of the methyl or ethyl cyanoacetate and the α-enamino-carbon atom, which is the most electrophilic site of the enaminone. Subsequently, the dimethylamine released in situ, causes the reaction to proceed towards the direct closure of the 2-quinolinone ring without isolation of intermediates.

Full aromatization of adducts **9a–d** was accomplished with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in refluxing anhydrous toluene to afford, generally in satisfactory yield, the desired esters **10a–d**.

Compounds **10a–d** were routinely hydrolyzed to the corresponding 2-oxo-1,2-dihydro-furo[2,3-*h*]quinoline-3-carboxylic acid **13a** and 2-oxo-1,2-dihydrothieno[2,3-*h*]quinoline-3-carboxylic acid **13c**, respectively, by refluxing with a 50% mixture of hydrochloric and acetic acids.

Decarboxylation of **13a,c** which were refluxed in quinoline containing a catalytic amount of copper powder afforded 1*H*-furo[2,3-*h*]quinolin-2-one **14a** and 1*H*-thieno[2,3-*h*]qui-nolin-2-one **14c**, respectively, in good yield.

Compounds **10a,c** and **14a,c** were *N*-methylated in anhydrous DMF with a calculated excess of K_2CO_3 and iodomethane to give the expected **11a,c** and **15a,c.** Interestingly, this reaction, together with the *N*-methyl-derivatives **11c** and **15c**, also afforded the corresponding *O*-methyl isomers **12c** and **16c**, in the case of the thienoquinolinone derivatives **10c** and **14c**.

Biological Results and Discussion

The photobiological activity of the new furo (**9a,b**; **10a,b**; **11a**; **13a**; **14a**; **15a**) and thienoquinolinones (**9c,d**; **10c,d**; **11c**; **12c**; **13c**; **14c**; **15c**; **16c**) was assayed using four different tests. Remembering that DNA is the main target of furocoumarins and their homologues, we detected DNA synthesis and clonal growth capacity in mammalian cells (Ehrlich and HeLa cells, respectively), the infectivity of T2 bacteriophage and the formation of revertants in *E.coli WP2 TM9* bacteria. The furocoumarins inhibit DNA synthesis and clonal growth in mammalian cells,[24] reduce T2 infectivity[25] (antiproliferative effects), and induce point mutations in bacteria[26] (genotoxicity). Therefore, if a new furocoumarin homologue shows significant activity in all these tests, we can deduce that probably induces DNA damage, similarly to known furocoumarin derivatives. Moreover, remembering 3-carbethoxyangelicin can form large amounts of reactive oxygen species (ROS), we also studied the formation of singlet oxygen.[27] Finally, we assayed all the compounds for their capacity of inducing skin erythemas, another well-known furocoumarin side effect.[1]

The 3-unsubstituted furo (**14a** and **15a**) and thienoquinolinones (**14c** and **15c**) gave significant and comparable responses in all four biological tests. Figures 1 and 2 show the results obtained with these compounds. Among them, various derivatives appeared to be more active

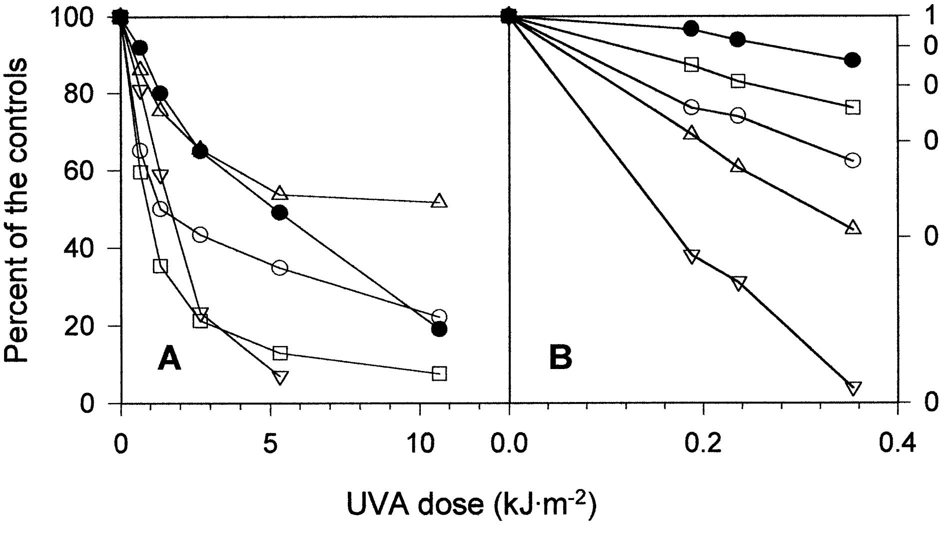


Figure 1. Antiproliferative activity in mammalian cells. Panel A: Inhibition of DNA synthesis in Ehrlich ascites cells observed after sensitization with the studied compounds (20 μM); the cells were incubated with ^{3}H-thymidine and the acid-insoluble radioactivity was determined. Panel B: Inhibition of clonal growth capacity of HeLa cells observed after sensitization with the studied compounds (5 μM). Symbols: 8-MOP: ●; **14a**: ○; **14c**: ▽; **15a**: □; **15c**: △.

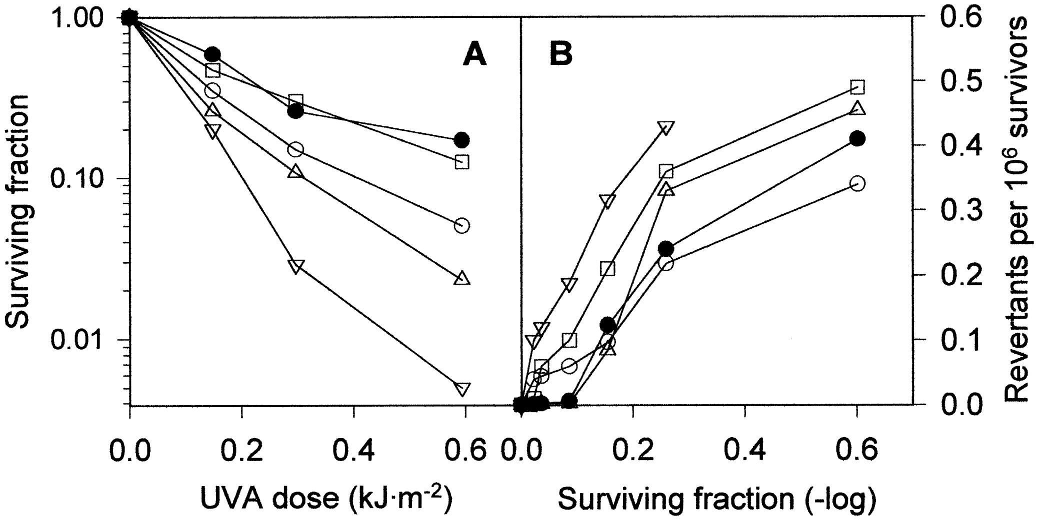


Figure 2. Effects in microorganisms. Panel A: Inactivation of T2 bacteriophage observed after sensitization with the studied compounds (2 μM). Panel B: mutagenic activity in *E. coli* TM9 observed after sensitization with the studied compounds: the revertants per 10^6 survivors are reported as a function of the survival scored in the same experiment. Symbols: 8-MOP: ●; **14a**: ○; **14c**: ▽; **15a**: □; **15c**: △.

than 8-MOP, in particular, the two thienoquinolinones **14c** and **15c**. These results are consistent with the hypothesis that they damage DNA, probably forming covalent adducts.

The data obtained by testing all the derivatives are summarized in Table 1. With a few exceptions, in general the 3-substituted analogues **10a–d**, **11a,c** and **13a** appeared to be less active in comparison with 8-MOP, often showing very different activities in the three tests: DNA synthesis, T2 inactivation and revertant induction in bacteria. This picture suggests the 3-substituted derivatives show a different mechanism of action from that of 3-unsubstituted compounds. In fact, it is known that the presence of a withdrawing group at position 3 interferes with the reactivity of the 3,4 double bound, thus abolishing one of the two reactive sites of the molecule.[27] On the other hand, such derivatives are known as being capable of generating large amounts of ROS. Considering the results on singlet oxygen formation, with a few exceptions, we can say that all the new compounds are a little more active (about 5-fold) than 8-MOP, without significant differences between 3-substituted and unsubstituted derivatives. Only **13c** forms a very large amount of singlet oxygen, even if to a less extent compared to 3-carbethoxyangelicin[27] and 3-carbethoxypsoralen, a well-known linear furocoumarin, which is intrinsically monofunctional and unable to produce carcinogenic effects in mice.[28] Therefore, these data clearly show the involvement of an oxidative damage in the mechanism of action only with **13c**.

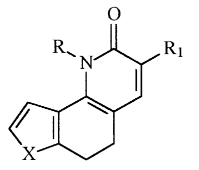
The 5,6-dihydro derivatives **9** and the methoxyl derivatives **12c** and **16c** resulted totally inactive; this behavior is probably due to different factors. Behaving differently from furocoumarins, the resonance between the pyronic and the five member ring is interrupted in the 5,6-dihydro compounds **9**, while in **12c** and **16c** derivatives the high resonance delocalizes the 3,4 and 7,8 double bonds, that is the two photoreactive sites, thus reducing their reactivity.

Compounds **14a**, **15a**, **14c** and **15c** appeared to be very effective; in particular, the latter resulted as the best antiproliferative agents, equivalent (**14c**) or one order of magnitude more active (**15c**) than 8-MOP in inhibiting DNA synthesis, one (**15c**) or two (**14c**) orders of magnitude more active than the reference compound in the inhibition of clonal growth of HeLa cells and 2 orders of magnitude more effective in reducing the surviving fraction of T2 bacteriophage. Moreover, all the new furo and thienoquinolinones induced a number of revertants comparable to 8-MOP in *E. coli WP2 TM9*. A result which is clearly consistent to the hypothesis of their capacity in damaging DNA. Surprisingly, all the derivatives appear to be entirely ineffective in inducing skin phototoxicity. Only **15a** and **15c** induce moderate erythemas, less intensive than those formed by 8-MOP. The absence of skin phototoxicity of compound **13c** in spite of its high capacity of generating singlet oxygen, is consistent with that already observed with 3-carbethoxyangelicin[27] and 3-carbethoxy-psoralen.[28]

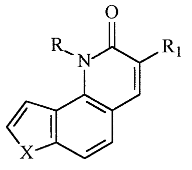
Considering all these preliminary photobiological results, we can draw the following conclusions. The 3-unsubstituted derivatives showed higher antiproliferative activity in comparison with the 3-substituted ones. In particular, the thieno derivatives generally resulted more effective than the furo analogues, a result which is consistent with the hypothesis of a increased affinity toward DNA, probably related to a higher lipophilicity of the molecule. Moreover, the increase in the aromaticity of the five-membered ring does not affect the reactivity of C_8,C_9 double bond, and the ability of photoinducing covalent adducts in the biological target.[20] As expected, the introduction of an electronwithdrawing function on the position 3 of the pyrone ring, decreases the photobiological activity of the molecule.

The *N*-methyl substitution does not significantly influence the antiproliferative activity, but increases skin phototoxicity of the molecule.

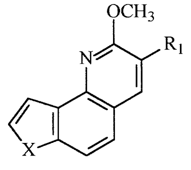
Table 1. Main photobiological properties of furo and thienoquinolinones **9a–d, 10a–d, 11a,c, 12c, 13a,c, 14a,c, 15a,c,16c**



9a-d



10a-d, 11a,c, 13a,c, 14a,c, 15a,c



12c, 16c

Compound	X	R	R_1	ID_{50} DNA[a] ± SD	ID_{50} clonal growth[b] ± SD	ID_{37} T2 inactivation[c] ± SD	Revertants at 0.5 survival[d]	Singlet oxygen production[e]	Skin photo toxicity[f]
9a	O	H	CO_2CH_3	49.70 ± 3.81	2.38 ± 0.07	48.73 ± 3.1	n.d.	13.0 ± 0.8	—
9b	O	H	$CO_2C_2H_5$	38.10 ± 0.82	1.42 ± 0.04	42.04 ± 2.8	n.d.	12.8 ± 0.4	—
9c	S	H	CO_2CH_3	> 250	> 3	12.1 ± 1.3	n.d	5.0 ± 0.2	—
9d	S	H	$CO_2C_2H_5$	> 250	> 3	14.12 ± 1.1	n.d.	7.0 ± 0.3	—
10a	O	H	CO_2CH_3	1.34 ± 0.12	0.18 ± 0.08	13.21 ± 0.9	0.25 ± 0.012	8.8 ± 0.5	—
10b	O	H	$CO_2C_2H_5$	11.80 ± 0.93	0.45 ± 0.20	20.28 ± 3.1	0.10 ± 0.02	7.6 ± 0.4	—
10c	S	H	CO_2CH_3	18.06 ± 1.39	0.11 ± 0.01	0.145 ± 0.05	0.125 ± 0.01	3.2 ± 0.2	—
10d	S	H	$CO_2C_2H_5$	27.73 ± 2.21	0.30 ± 0.02	1.61 ± 0.15	0.47 ± 0.03	9.8 ± 0.7	—
11a	O	CH_3	CO_2CH_3	7.51 ± 0.30	1.65 ± 0.20	0.416 ± 0.04	0.36 ± 0.015	10.6 ± 0.6	+ –
11c	S	CH_3	CO_2CH_3	> 250	1.60 ± 0.30	0.476 ± 0.01	0.175 ± 0.01	10.2 ± 0.9	—
12c	S	—	CO_2CH_3	> 250	> 3	n.d.	n.d.	11.0 ± 0.5	—
13a	O	H	CO_2H	17.62 ± 0.91	1.72 ± 0.53	0.416 ± 0.07	n.d.	9.3 ± 0.7	—
13c	S	H	CO_2H	13.08 ± 0.85	0.23 ± 0.03	0.118 ± 0.03	0.25 ± 0.03	36.8 ± 1.1	—
14a	O	H	H	2.17 ± 0.44	0.23 ± 0.05	6.06 ± 0.8	0.22 ± 0.014	8.6 ± 0.8	—
14c	S	H	H	2.09 ± 0.46	0.06 ± 0.01	0.069 ± 0.004	0.26 ± 0.02	10.5 ± 0.6	—
15a	O	CH_3	H	0.70 ± 0.19	0.360 ± 0.003	0.233 ± 0.025	0.25 ± 0.023	11.4 ± 0.5	+ + –
15c	S	CH_3	H	0.89 ± 0.02	0.15 ± 0.05	0.089 ± 0.003	0.31 ± 0.04	9.5 ± 0.6	+ + –
16c	S	—	H	> 250	> 3	n.d	n.d	10.5 ± 0.4	+ + –
8-MOP				3.7 ± 0.2	0.79 ± 0.02	1.55 ± 0.2	0.24 ± 0.011	2.0 ± 0.2	+ + +

n.d., not detectable.

[a] The UVA dose which reduces to 50% DNA synthesis in Ehrlich cells when delivered in the presence a 20 μM drug concentration.

[b] The UVA dose which reduces to 50% the clonal growth capacity of HeLa cells when delivered in the presence a 5 μM drug concentration.

[c] The UVA dose which reduces to 0.37 of the surviving fraction of T2 bacteriophage when delivered in the presence a 2 μM drug concentration.

[d] Revertants per 10^6 survivors scored in *E. coli TM9* at survival fraction 0.5.

[e] Percent of RNO bleaching scored exposing an aqueous drug solution (20 μM) to UVA light (10 kJ m^{-2}).

[f] Phototoxicity on guinea pig skin: 0.5 mM per cm^2 and 20 kJ m^{-2}.

Besides the lack of activity observed for methoxyderivatives **12c** and **16c** underlines the importance of the presence of the carbonyl function at 2-position for the interaction with DNA.[7]

According to these results, we can conclude that thienoquinolinone **14c** appears to be the most interesting compound among the newly synthesized and seem potentially attractive as photochemotherapeutic drug.

Experimental

Chemistry

All reagents were commercially obtained and used without further purification. Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Microanalyses for C, H, N, S (±0.3% of the theoretical value) were performed on an Elemental Analyzer EA 1110. UV spectra were measured in 95% ethanol on a Perkin-Elmer Lambda 3 spectrophotometer. IR spectra were taken on a Perkin-Elmer 398 instrument and are expressed in cm^{-1}. Nuclear magnetic resonance (NMR) data for ^1H NMR and ^{13}C NMR were recorded on a Varian Gemini (200 and 50.30 MHz, respectively) spectrometers. The chemical shifts are reported in δ (ppm) downfield from tetramethylsilane (TMS). Coupling constants (*J*) are expressed in Hertz (Hz). The assignments of ^{13}C signals were assisted by DEPT spectral data.

General procedure for the preparation of esters 9

A mixture of 5-[(dimethylamino)methylene]-6,7-dihydro-1-benzofuran-4(5*H*)-one **8a** or 5-[(dimethylamino)-methylene]-6,7-dihydro-1-benzothiophen-4(5*H*)-one **8c** (10 mmol) in the appropriate cyanoacetate (175 mmol) was stirred at room temperature for 24 h. The reaction mixture was cooled and filtered under reduced pressure. The crude solid residue was recrystallized from 95% ethanol.

Methyl 2-oxo-1,2,5,6-tetrahydrofuro[2,3-*h*]quinoline-3-carboxylate (9a). Yield: 1.49 g (61%), mp 294–296 °C; IR (KBr) 1724, 1624 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.94 (s, 4H, CH_2-5 + CH_2-6), 3.75 (s, 3H, CH_3O), 7.16(d, 1H, $J = 2.0$, H-9), 7.72 (d, 1H, $J = 2.0$, H-8), 8.04 (s, 1H, H-4), 12.24 (brs, 1H, NH, exchanges with D_2O). Calcd for $C_{13}H_{11}NO_4$: C 63.67%; H 4.52%; N 5.71%; found C 63.64%; H 4.52%; N 5.69%.

Ethyl 2-oxo-1,2,5,6-tetrahydrofuro [2,3-*h*]quinoline-3-carboxylate (9b). Yield: 1.81 g (70%), mp 260–262 °C; IR (KBr) 1675, 1650 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.28 (t, 3H, *J* = 7.0, $CH_2C\underline{H_3}$), 2.94 (s, 4H, CH_2-5 + CH_2-6), 4.22 (q, 2H, *J* = 7.0, $\underline{CH_2}CH_3$), 7.16 (d, 1H, *J* = 2.0, H-9), 7.72 (d, 1H, J = 2.0, H-8), 8.01 (s, 1H, H-4), 12.18 (brs, 1H, NH, exchanges with D_2O). Calcd for $C_{14}H_{13}NO_4$: C 64.86%; H 5.05%; N 5.40%; found C 64.89%; H 5.05%; N 5.40%.

Methyl 2-oxo-1,2,5,6-tetrahydrothieno[2,3-*h*]quinoline-3-carboxylate (9c). Yield: 1.49 g (57%), mp 259–261 °C; IR ($CHCl_3$) 1724, 1675, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.02 (s, 4H, CH_2-5 + CH_2-6), 3.99 (s, 3H, CH_3O), 7.26(d, 1H, *J* = 2.0, H-9), 8.04 (d, 1H, *J* = 2.0, H-8), 8.15 (s, 1H, H-4), 12.80 (brs, 1H, NH, exchanges with D_2O). Calcd for $C_{13}H_{11}NO_3S$: C 59.75%; H 4.24%; N 5.36%; found C 59.76%; H 4.23%; N 5.35%.

Ethyl 2-oxo-1,2,5,6-tetrahydrothieno[2,3-*h*]quinoline-3-carboxylate (9d). Yield: 2.09 g (76%), mp 242–243 °C; IR ($CHCl_3$) 1722, 1670, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.43 (t, 3H, *J* = 7.2, $CH_2C\underline{H_3}$), 3.02 (s, 4H, CH_2-5 + CH_2-6), 4.46 (q, 2H, *J* = 7.2, $C\underline{H_2}CH_3$), 7.23 (d, 1H, *J* = 2.0, H-9), 7.99 (d, 1H, *J* = 2.0, H-8), 8.12 (s, 1H, H-4), 12.40 (brs, 1H, NH, exchanges with D_2O). Calcd for $C_{14}H_{13}NO_3S$: C 61.07%; H 4.80%; N 5.09%; found C 60.95%; H 4.71%; N 5.03%.

General procedure for the preparation of esters 10

A warm solution of DDQ (2.27 g, 10 mmol) in anydrous toluene (50 mL) was added dropwise to a warm and stirred solution of **9** (10 mmol) in the same solvent (100–120 mL). After the addition was completed, the reaction mixture was refluxed for 48 h and then filtered. The filtrate was washed twice with 1 M NaOH and once with water, dried ($MgSO_4$) and evaporated under reduced pressure. The solid residue was recrystallized from the suitable solvent.

Methyl 2-oxo-1,2-dihydrofuro[2,3-*h*]quinoline-3-carboxylate (10a)

Yield: 1.94 g (80%), mp 286–288 °C from methanol; IR (KBr) 1692, 1625 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.83 (s, 3H, CH_3O), 7.52 (dd, 1H, *J′* = 8.8, *J″* = 0.9, H-6), 7.63 (dd, 1H, *J′* = 2.2, *J″* = 0.9, H-9), 7.78 (d, 1H, *J* = 8.8, H-5), 8.10 (d, 1H, *J* = 2.2, H-8), 8.70 (s, 1H, H-4), 12.53 (brs, 1H, NH, exchanges with D_2O); UV nm (log ε) 222 (4.37), 227 (4.38), 234 (4.36), 255 (4.60), 312 (4.02), 357 (3.86). Calcd for $C_{13}H_9NO_4$: C 64.20%; H 3.73%; N 5.76%; found C 64.11%; H 3.73%; N 5.79%.

Ethyl 2-oxo-1,2-dihydrofuro[2,3-*h*]quinoline-3-carboxylate (10b). Yield: 2.16 g (84%), mp 286–288 °C from 95% ethanol; IR (KBr) 1688, 1658, 1625 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.33 (t, 3H, *J* = 7.0, $CH_2C\underline{H_3}$), 4.29 (q, 2H, *J* = 7.0, $C\underline{H_2}CH_3$), 7.52 (dd, 1H, *J′* = 8.7, *J″* = 0.8, H-6), 7.64 (near d, 1H, *J* = 2.2, H-9), 7.78 (d, 1H, *J* = 8.7, H-5), 8.10 (d, 1H, *J* = 2.2, H-8), 8.66 (s, 1H, H-4), 12.51 (brs, 1H, NH, exchanges with D_2O); UV nm (log ε) 222 (4.39), 228 (4.39), 234 (4.31), 256 (4.49), 312 (3.92), 357 (3.89). Calcd for $C_{14}H_{11}NO_4$: C 65.37%; H 4.31%; N 5.44%; found C 65.11%; H 4.30%; N 5.38%.

Methyl 2-oxo-1,2-dihydrothieno[2,3-*h*]quinoline-3-carboxylate (10c). Yield: 1.58 g (61%), mp 301–302 °C from methanol; IR (KBr) 1697, 1655, 1610 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.90 (s, 3H, CH_3), 7.76 (d, 1H, *J* = 8.6, H-6), 7.86 (d, 1H, *J* = 8.6, H-5), 7.91 (d, 1H, *J* = 5.8, H-9), 8.34 (d, 1H, *J* = 5.8, H-8), 8.71 (s, 1H, H-4), 12.47 (brs, 1H, NH, exchanges with D_2O); UV nm (log ε) 222 (4.23), 244 (4.53), 255 (4.49), 264 (4.51), 277 (4.28), 318 (3.97), 375 (3.99). Calcd for $C_{13}H_9NO_3S$: C 60.22%; H 3.50%; N 5.40%; found C 60.38%; H 3.65%; N 5.36%.

Ethyl 2-oxo-1,2-dihydrothieno[2,3-*h*]quinoline-3-carboxylate (10d). Yield: 1.33 g (49%), mp 306–308 °C from 95% ethanol; IR (KBr) 1695, 1655, 1610 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.34 (t, 3H, *J* = 7.1, $CH_2C\underline{H_3}$), 4.31 (q, 2H, *J* = 7.1, $C\underline{H_2}CH_3$), 7.76 (d, 1H, *J* = 8.5, H-6), 7.87 (d, 1H, *J* = 8.5, H-5), 7.92 (d, 1H, *J* = 5.6, H-9), 8.35 (d, 1H, *J* = 5.6, H-8), 8.68 (s, 1H, H-4), 12.45 (brs, 1H, NH, exchanges with D_2O); UV nm (log ε) 222 (4.22), 244 (4.51), 255 (4.46), 264 (4.48), 277 (4.26), 318 (3.96), 374 (3.99). Calcd for $C_{14}H_{11}NO_3S$: C 61.52%; H 4.06%; N 5.12%; found C 61.33%; H 4.00%; N 5.05%.

General procedure for the preparation of carboxylic acids 13

A solution of ester **10** (10 mmol) in a 50% hydrochloric and acetic acid mixture (100 mL) was refluxed for 24h. After cooling, the solid residue was filtered, washed with water and recrystallized from 95% ethanol.

2-Oxo-1,2-dihydrofuro[2,3-*h*]quinoline-3-carboxylic acid (13a). Yield: 1.58 g (69%), mp > 380 °C; IR (KBr) 3300–2500, 1710, 1658 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.66–7.70 (m, 2H, H-6 + H-9), 7.96 (d, 1H, *J* = 8.8, H-5), 8.17 (d, 1H, *J* = 2.2, H-8), 9.06 (s, 1H, H-4), 13.60 (brs, 1H, NH, exchanges with D_2O), 14.65 (near s, 1H, COOH, exchanges with D_2O); UV nm (log ε) 228 (4.32), 254 (4.51), 262 (4.53), 320 (3.82), 350 (3.64). Calcd for $C_{12}H_7NO_4$: C 62.89%; H 3.08%; N 6.11%; found C 62.45%; H 3.05%; N 6.04%.

2-Oxo-1,2-dihydrothieno[2,3-*h*]quinoline-3-carboxylic acid (13c). Yield: 2.25 g (92%), mp 347–349 °C; IR (KBr) 3300-2200, 1718, 1640 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.95 (d, 1H, *J* = 8.6, H-6), 8.01 (d, 1H, *J* = 5.6, H-9), 8.04 (d, 1H, *J* = 8.6, H-5), 8.40 (d, 1H, *J* = 5.6, H-8), 9.09 (s, 1H, H-4), 13.52 (near s, 1H, NH, exchanges with D_2O), 14.75 (near s, 1H, COOH, exchanges with D_2O); UV nm (log ε) 222 (4.27), 244 (4.40), 258 (4.49), 278 (4.13), 325 (3.91), 363 (3.85). Calcd for $C_{12}H_7NO_3S$: C 58.77%; H 2.88%; N 5.71%; found C 58.51%; H 2.95%; N 5.59%.

General procedure for the preparation of compounds 14

A solution of acid **13** (10 mmol) in quinoline (20 mL) containing copper powder (0.16 g) was refluxed for 6 h. The hot mixture was cooled and filtered, the solid residue was washed once with diethyl ether and recrystallized from 95% ethanol to give the expected product.

Furo[2,3-*h*]quinolin-2(1*H*)one (14a). Yield: 1.35 g (73%), mp 280–281 °C; IR (KBr) 1664, 1560, cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.55 (d, 1H, *J* = 9.0, H-3), 7.50 (d, 1H, *J* = 8.4, H-6), 7.70 (d, 1H, *J* = 8.4, H-5), 7.71 (d, 1H, *J* = 2.4, H-9), 8.09 (d, 1H, *J* = 9.0, H-4), 8.14 (d, 1H, *J* = 2.4, H-8), 12.30 (brs, 1H, NH, exchanges with D_2O); UV nm (log ε) 223 (4.32), 229 (4.38), 248 (4.53), 254 (4.58), 264 (4.07), 295 (3.69), 332 (3.87). Calcd for $C_{11}H_7NO_2$: C 71.35%; H 3.81%; N 7.56%; found C 71.22%; H 3.75%; N 7.47%.

Thieno[2,3-*h*]quinoline-2(1*H*)one (14c). Yield: 1.76 g (88%), mp 300–302 °C; IR (KBr) 1665, 1595 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.56 (d, 1H, *J* = 9.5, H-3), 7.62 (d, 1H, *J* = 8.5, H-6), 7.81 (d, 1H, *J* = 8.5, H-5), 7.87 (d, 1H, *J* = 5.6, H-9), 8.05 (d, 1H, *J* = 9.5, H-4), 8.30 (d, 1H, *J* = 5.6, H-8), 12.18 (brs, 1H, NH, exchanges with D_2O); UV nm (log ε) 202 (4.35), 219 (4.25), 243 (4.54), 258 (4.53), 344 (4.02), 358 (3.92). Calcd for $C_{11}H_7NOS$: C 65.65%; H 3.51%; N 6.96%; found C 65.92%; H 3.46%; N 7.00%.

General procedure for the preparation of compounds 11, 12, 15, 16

To a warm mixture of **10** or **14** (5 mmol) and anhydrous K_2CO_3 (6.8 g, 50 mmol) in acetone (500 mL) methyliodide (2.13 g, 15 mmol) was added. The reaction mixture was heated and stirred for 24 h at 50 °C, then K_2CO_3 was filtered off and washed with fresh acetone. The solvent was removed under reduced pressure and the solid residue was crystallized from a suitable solvent.

Methyl 1-methyl-2-oxo-1,2-dihydrofuro[2,3-*h*]quinoline-3-carboxylate (11a). Yield: 0.69 g (54%), mp 160–161 °C; IR ($CHCl_3$) 1738, 1709, 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.97 (s, 3H, O–CH_3), 4.10 (s, 3H, N-CH_3), 7.32 (dd, 1H, *J′* = 2.4, *J″* = 0.9, H-9), 7.45 (dd, 1H, *J′* = 8.6, *J″* = 0.9, H-6), 7.56 (d, 1H, *J* = 8.6, H-5), 7.76 (d, 1H, *J* = 2.4, H-8), 8.58 (d, 1H, H-4); UV nm (log ε) 228 (4.25), 234 (4.22), 256 (4.59), 263 (4.60), 312 (3.88), 359 (3.71). Calcd for $C_{14}H_{11}NO_4$: C 65.37%; H 4.31%; N 5.44%; found C 65.42%; H 4.11%; N 5.52%.

Methyl 1-methyl-2-oxo-1,2-dihydrothieno[2,3-*h*]quinoline-3-carboxylate (11c). Yield: 0.15 g (11%), mp 190–192 °C from ethyl acetate; IR ($CHCl_3$) 1740, 1705, 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.97 (s, 3H, O–CH_3), 4.17 (s, 3H, N–CH_3), 7.54 (d, 1H, *J* = 8.4, H-6), 7.59 (d, 1H, *J* = 5.8, H-9), 7.76 (dd, 1H, *J′* = 8.4, *J″* = 0.7, H-5), 8.10 (dd, 1H, *J′* = 5.8′, *J″* = 0.7, H-8), 8.59 (s, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 36.40 (NCH_3), 53.06 (OCH_3), 116.32 (C_q), 118.50 (CH_{ar}), 120.16 (C_q), 125.11, 126.18, 126.69 (3 CH_{ar}), 127.49, 136.96 (C_q), 145.70 (CH_{ar}), 147.30 (C_q), 160.37, (C=O), 166.15 (C_q); UV nm (log ε) 218 (4.22), 243 (4.46), 258 (4.65), 290 (3.80), 308 (3.73), 343 (3.68). Calcd for $C_{14}H_{11}NO_3S$: C 61.52%; H 4.05%; N 5.12%; found C 61.72%; H 4.13%; N 5.06%.

Methyl 2-methyoxythieno[2,3-*h*]quinoline-3-carboxylate (12c). Yield: 0.61 g (45%), mp 114–115 °C from diethyl ether; IR ($CHCl_3$) 1720, 1610 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.98 (s, 3H, $COOCH_3$), 4.24 (s, 3H, OCH_3), 7.57 (d, 1H, *J* = 5.4, H-9), 7.65 (d, 1H, *J* = 8.7, H-6), 7.83 (d, 1H, *J* = 8.7, H-5), 8.19 (d, 1H, *J* = 5.4, H-8), 8.73 (d, 1H, H-4); ^{13}C NMR ($CDCl_3$) δ 52.87, 54.63 (2 OCH_3), 114.35 (C_q), 119.99 (CH_{ar}), 120.87 (C_q), 123.79, 124.72, 126.39 (3 CH_{ar}), 136.69 (C_q), 143.29 (CH_{ar}), 144.87, 160.32, (2 C_q), 165.91 (C_q + C=O); UV nm (log ε) 218 (4.22), 243 (4.46), 258 (4.65), 290 (3.80), 308 (3.73), 343 (3.68). Calcd for $C_{14}H_{11}NO_3S$: C 61.52%; H 4.05%; N 5.12%; found C 61.62%; H 4.15%; N 5.09%.

1-Methylfuro[2,3-*h*]quinolin-2(1*H*)one (15a). Yield: 0.69 g (70%), mp 198–199 °C from 95% ethanol; IR ($CHCl_3$) 1645, 1588 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.01 (s, 3H, NCH_3), 6.65 (d, 1H, *J* = 9.4, H-3), 7.24 (dd, 1H, *J′* = 2.4, *J″;* = 0.6, H-9), 7.35 (dd, 1H, *J′* = 8.6, *J″* = 0.6, H-6), 7.41 (d, 1H, *J* = 8.6, H-5), 7.69 (d, 1H, *J* = 2.4, H-8), 7.70 (d, 1H, *J* = 9.4, H-4); ^{13}C NMR ($CDCl_3$) δ 33.35 (NCH_3), 107.97, 108.30 (2 CH_{ar}), 115.77, 116.51 (2C_q), 119.31, 126.32 (2CH_{ar}), 136.24 (C_q), 140.51, 144.55 (2 CH_{ar}), 157.35 (C_q), 163.23 (C=O); UV nm (log ε) 204 (4.07), 224 (4.02), 229 (4.07), 248 (4.37), 255 (4.43), 295 (3.44), 333 (3.59). Calcd for $C_{12}H_9NO_2$: C 72.35%; H 4.55%; N 7.03%; found C 72.15%; H 4.65%; N 7.12%.

1-Methylthieno[2,3-*h*]quinolin-2(1*H*)one (15c). Yield: 0.16 g (15%), mp 176–177 °C from ethyl acetate; IR ($CHCl_3$) 1660, 1583 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.16 (s, 3H, NCH_3), 6.77 (d, 1H, *J* = 9.3, H-3), 7.47 (d, 1H, *J* = 8.3, H-6), 7.55 (d, 1H, *J* = 5.8, H-9), 7.73 (dd, 1H, *J′* = 8.3, *J″* = 0.9, H-5), 7.79 (d, 1H, *J* = 9.3, H-4), 8.09 (dd, 1H, *J′* = 5.8, *J″* = 0.9, H-8); ^{13}C NMR ($CDCl_3$) δ 36.08 (NCH_3), 117.99, 120.14 (2 CH_{ar}), 120.14 (C_q), 124.90, 125.67 (3 CH_{ar}), 127.86, 138.03 (2 C_q), 140.51 (CH_{ar}), 144.95 (C_q), 163.79 (C=O); UV nm (log ε) 206 (4.02), 218 (4.15), 243 (4.46), 257 (4.55), 290 (3.62), 303 (3.40), 346 (3.93), 361 (3.82). Calcd for $C_{12}H_9NOS$: C 66.95%; H 4.21%; N 6.50%; found C 67.12%; H 4.20%; N 6.51%.

2-Methoxythieno-[2,3-*h*]quinoline (16c). Yield: 0.36 g (34%), mp 84–85 °C from diethyl ether; IR ($CHCl_3$) 1650, 1610 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.19 (s, 3H, OCH_3), 6.95 (d, 1H, *J* = 8.8, H-3), 7.55 (dd, 1H, *J′* = 6.0, *J″* = 0.5, H-9), 7.61 (d, 1H, *J* = 8.6, H-6), 7.81 (dd, 1H, *J′* = 8.6, *J″* = 0.74, H-5), 8.06 (d, 1H, *J* = 8.8, H-4), 8.22 (dd, 1H, *J′* = 6.0, *J″* = 0.74, H-8); ^{13}C NMR ($CDCl_3$) δ 53.88 (OCH_3), 111.97, 119.10 (2 CH_{ar}), 121.90 (C_q), 123.58, 124.29, 125.82 (3 CH_{ar}), 137.23 (C_q), 139.34 (CH_{ar}), 141.16, 143.42, 162.83 (3 C_q); UV nm (log ε) 220 (4.23), 240 (4.62), 282 (3.34), 309 (3.18), 322 (3.73), 336 (3.86). Calcd for $C_{12}H_9NOS$: C 66.95%; H 4.21%; N 6.50%; found C 67.00%; H 4.10%; N 6.48%.

Biology

8-MOP was obtained from Chinoin (Milan, Italy). Compounds **9a–d**, **10a–d**, **11a**,**c**, **12c**, **13a**,**c**, **14a**,**c**, **15a**,**c** and **16c** were dissolved in dimethyl sulfoxide (DMSO) 5×10^{-6} M 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 phospate–buffered saline (PBS) or to the growth medium containing cells, to a final DMSO concentration which never exceeded 0.5%; a similar amount of pure DMSO was also added to the control cells. Every experiment was carried out at least three times.

UVA irradiation

Cell suspensions containing the test compound were incubated at room temperature for 15 min in the dark, put into Petri dishes (5 cm in diameter, 5 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^{-2}.

Experiments with T2 phage

The host bacteria (*E. coli* B48) were grown in cultures prepared in brain–heart infusion (Difco Laboratories, Detroit, MI, USA) at 37 °C, collected in log phase, suspended in $MgSO_4$ (2 mM) at a density of 10^9 cells mL^{-1}, and then infected with T2 phage at a multiplicity of 1. The culture was then incubated at 37 °C for 3 h. Phage titers were determined using the standard bilayer method[29] and the same *E. coli* strain. Phage suspensions were diluted to 10^{10} viral particles/mL with $MgSO_4$ (2 mL) containing the compound to be studied. Aliquots (5 mL) of these virus suspensions were poured into Petri dishes (5 cm in diameter), incubated at 37 °C for 25 min in the dark and then exposed to UVA light. After irradiation, the viral suspensions further diluted with the same medium and the numbers of plaque-forming units/mL were scored.

DNA synthesis in Ehrlich cells

DNA synthesis was assayed in Ehrlich ascites tumor cells (Lettrè strain) as already described.[24] Just after UVA irradiation (cell density: 2×10^7 cells mL^{-1} in PBS), the samples (10^6 cells in 0.5 mL PBS) were 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). The filters were dried and counted. Data are expressed as ID_{50}, that is the UVA. dose, expressed in KJ m^{-2} which, in the presence of 20 μM drug concentration, induced 50% inhibition of the macromolecular synthesis. Calculations were accomplished by means of probit analysis.

Clonal growth of HeLa cells

HeLa cells were grown in nutrient mixture F-12 Ham medium (Sigma Chemical Co, St. Louis, MO, USA), containing 10% foetal calf serum, and supplemented with antibiotics. Cell growth was accomplished at 37 °C in a 10% 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 compound to be studied. The cells were then incubated for 15 min at 37 °C in the dark and then exposed to UVA light. Aliquots of 100 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%. Data are expressed as ID_{50}, that is the UVA. dose, expressed in kJ m^{-2} which, in the presence of 5 μM drug concentration, induced 50% inhibition of the clonal growth.

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[30] and therefore by formation of C_4-cycloadducts of furocoumarins.[10] 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 phosphate-buffered saline (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 suspentions 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.

Skin phototoxicity

All new furo and thienoquinolinones were evaluated following a standard procedure already described.[31] Compounds were applied on the depilated skin of albino guinea-pigs (outbred Dunkin–Hartley strain) as 0.5% methanol solutions up to 5×10^{-2} mg cm^{-2}. The animals were kept in the dark for 15 min and then exposed to a UVA light dose of 20 kJ m^{-2}. The animals were observed for 7 days.

Singlet oxygen determination

The formation of $^1O_2$ was determined according to the method of Kraljic and El Mohsni.[32] Aqueous solutions, containing phosphate buffer (0.02 M, pH 7.3), RNO (4×10^{-5}M), L-histidine (1×10^{-3}M) and the test compound at a concentration of 20 μM were irradiated for increasing times in quartz cuvettes. The RNO bleaching was determined by reading the optical density at 440 nm.

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