Bioorganic & Medicinal Chemistry 19 (2011) 2326–2341

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

# Pyrrolo[3,4-*h*]quinolinones a new class of photochemotherapeutic agents

Paola Barraja [a], Patrizia Diana [a], Alessandra Montalbano [a], Anna Carbone [a], Giampietro Viola [b], Giuseppe Basso [b], Alessia Salvador [c], Daniela Vedaldi [c], Francesco Dall'Acqua [c], Girolamo Cirrincione [a,*]

[a] Dipartimento di Scienze e Tecnologie Molecolari e Biomolecolari, Sezione di Chimica Farmaceutica e Biologica, Università degli Studi di Palermo, Via Archirafi 32, Palermo 90123, Italy
[b] Dipartimento di Pediatria, Laboratorio di Oncoematologia, Università degli Studi di Padova, Via Giustiniani 2, Padova 35128, Italy
[c] Dipartimento di Scienze Farmaceutiche Università degli Studi di Padova, Via Marzolo 5, Padova 35131, Italy

ARTICLE INFO

*Article history:*
Received 29 September 2010
Revised 9 February 2011
Accepted 13 February 2011
Available online 18 February 2011

*Keywords:*
Pyrrolo[3,4-*h*]quinolinones
Angelicin heteroanalogues
Photochemotherapy
Phototoxicity

ABSTRACT

Pyrrolo[3,4-*h*]quinolin-2-ones were synthesized as nitrogen isosters of the angular furocoumarin angelicin, with the aim of obtaining new photochemotherapeutic agents with increased antiproliferative activity and lower undesired toxic effects. A versatile synthetic pathway was approached to allow the isolation of derivatives of the new ring system with a good substitution pattern on the pyrrole moiety. Photobiological screenings of the new compounds revealed a potent phototoxic effect and a great UVA dose dependence, reaching $IC_{50}$ values at submicromolar level. The induced cellular photocytotoxicity was related to apoptosis with the involvement of mitochondria and lysosomes, alteration of cell cycle profile and membrane lipid peroxidation.

© 2011 Elsevier Ltd. All rights reserved.

## 1. Introduction

Furocumarins are a family of naturally occurring photosensitizing drugs that find application in the treatment of several skin diseases, such as psoriasis, vitiligo and cutaneous T-cell lymphoma when used in conjuction with long-wave (320–400 nm) ultraviolet light (UV-A).[1,2] The effectiveness of this treatment, called PUVA, is connected with the specific damage which these compounds can induce to DNA. The photoexcited furocumarins are able to react with biomolecules especially with pyrimidine bases of DNA. In fact, the tricyclic planar structure allows the initial intercalation between nucleic acid base pairs followed by light activation and formation of mono- and bis-adducts (inter-strand cross-links, ISC), which join the two complementary strands of the macromolecule; these latter are considered the main cause of the photoinduced cell killing. Nowadays, human skin diseases such as psoriasis, and vitiligo are currently treated with PUVA therapy and thanks to the development of the extracorporeal photochemotherapy (ECP), also called photopheresis, T-cell lymphoma (CTCL) is also efficiently cured. In this process, peripheral blood is exposed, in an extracorporeal system, to photoactivated 8-methoxypsoralen (8-MOP) mainly for the treatment of disorders caused by aberrant T-lymphocytes.[3] Photopheresis was approved by FDA for the cure of T-cell lymphoma, but it is also effective against various tumors, autoimmune diseases and in the prevention of rejection in organ transplantation.[4,5]

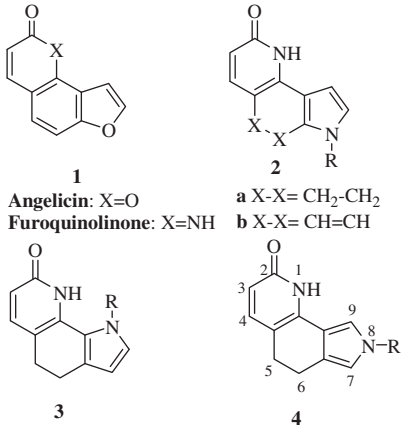



**Chart 1.** Structures of angelicin and furoquinolinone **1**, pyrrolo[2,3-*h*]quinolin-2-ones **2**, pyrrolo[3,2-*h*]quinolin-2-ones **3** and pyrrolo[3,4-*h*]quinolin-2-ones **4**.

Derivatives of angelicin **1** (Chart 1, X = O) have been synthesized with the purpose to reduce long term side effects such as genotoxicity and risk of skin cancer caused by ISC. In fact, this angular isomer of psoralen possesses the ability of causing only monofunctional damage to DNA because of its geometry.

Over the latest two decades, several heteroanalogues of angelicin have been proposed with the aim to dissociate undesirable side effects from the therapeutic ones.[6] A new family of compounds, namely the furoquinolinones **1** (Chart 1, X = NH) nitrogen isosters of angelicin, has been extensively studied.

* Corresponding author. Tel.: +39 091 6161606; fax: +39 091 23860854.
*E-mail address:* gcirrinc@unipa.it (G. Cirrincione).

0968-0896/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2011.02.023

In particular, the 1,4,6,8- and 4,6,8,9- tetramethyl derivatives (FQ) an (HFQ), respectively showed strong antiproliferative activity against tumor cell lines not only upon UVA irradiation, which was considerable higher than the most used drug in clinical photomedicine, 8-MOP, but also in the dark.[7] However, despite their inability of inducing ISC, they showed skin covalent DNA-protein cross-link (DPC).[8] Up to date several FQ analogues bearing other substituents than methyl groups and in different positions on the furoquinolinone skeleton have been synthesized to better understand the mechanism of action of this class of compounds even under no irradiation.[8,9]

Quinolines represent a class of largely investigated heterocycles for their antitumor activity.[10] Among these, pyrrolo[3,2-*f*]quinolin-9-ones and their geometric [2,3-*h*] isomers showed good antiproliferative effect.[11] Pyrroloquinolinones with photochemotherapeutic activity were poorly explored. As part of our search for pyrrolo fused heterocycles, we have recently reported the synthesis and biological activity of ring systems containing the quinolinone moiety, with the aim to investigate their antiproliferative effect either in the dark or under UVA irradiation. In particular, pyrrolo [2,3-*h*]quinolines **2**[12] and the pyrrolo[3,2-*h*]quinolines **3**[13] were studied as nitrogen isosters of angelicin (Chart 1).

Both classes of compounds revealed remarkable phototoxicity against human tumor cell lines and a great dose UV-A dependence reaching $IC_{50}$ values at submicromolar level (0.4–16.4 μM and 0.5–5.5 μM, respectively). For several derivatives, such a biological activity was higher than angelicin itself, used as reference drug. Studies on the mechanism of action for both classes of derivatives, revealed a different behavior than the lead compound angelicin as DNA is not the main target with a possible involvement of mitochondria in the photoinduced apoptosis. Undoubtedly, the most important feature of the pyrrolo[3,2-*h*]quinolines is that they exert their phototoxicity without any DNA damage (no DNA strand breaks and no DNA oxidative damages) which is the main origin of the long term side effects of the PUVA therapy. On the basis of these results, we planned the synthesis of a new series of pyrroloquinolinones, the pyrrolo[3,4-*h*]quinolin-2-ones **4**, in which the pyridine moiety is annelated to the isoindole ring with the purpose to further explore the effect of the new condensation of the pyrrole nucleus to the quinoline moiety. Such a ring system can be also regarded as a pyridine moiety fused to the isoindole system. We focused our attention on the synthesis of dihydro derivatives, since in the two previous series of pyrroloquinolinones non-aromatic compounds of type **2a** and **3** showed remarkable photoantiproliferative activity whereas the aromatic pyrrolo[2,3-*h*]quinolin-2-ones of type **2b** were inactive. The phototoxicity of the title compounds was studied and a relationship between structure and photobiological properties was hypothesized. To confirm whether also this class of pyrrolo quinolinones has a mode of action different from that of angelicin, experiments were performed to understand the mode of cell death and the cellular targets involved.

## 2. Results and discussion

### 2.1. Chemistry

We have already reported the synthesis of tetrahydroisoindole-4-ones **5a–i** as versatile precursors suitable for the annelation of heterocycles to the 4,5 isoindole positions.[14]

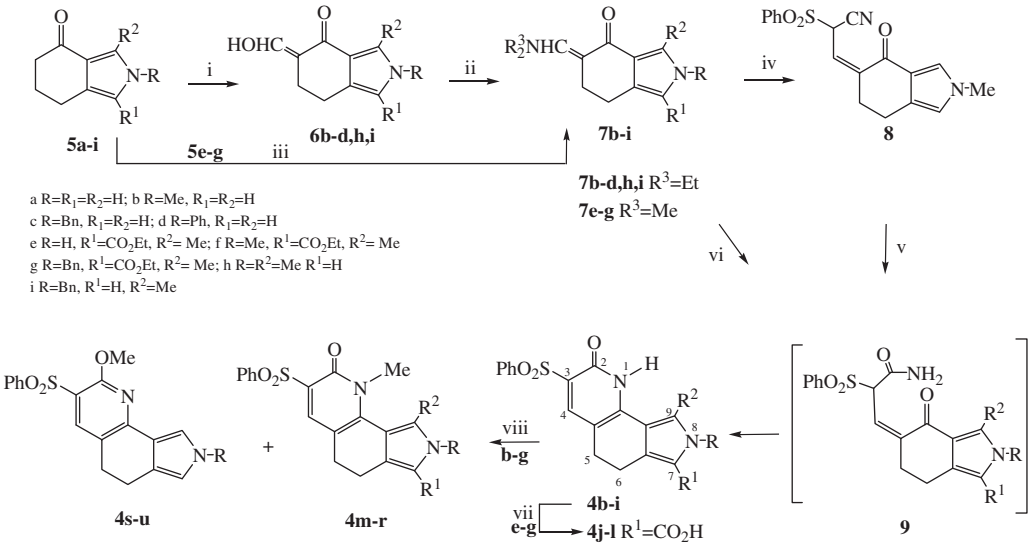
Starting from derivatives 1,3-unsubstituted pyrroles **5a–d** or from the 3-methyl ketones **5h,i** the 5-formyl derivatives **6b–d,h,i** were prepared using ethyl formate as formylating agent in the presence of *t*-BuOK, with the sole exception of **5a** which was recovered unchanged from the corresponding reaction mixture (Scheme 1). Transformation of **6b–d,h,i** into the corresponding enamino ketones **7b–d,h,i** was achieved by reaction with stoichiometric amount of diethylamine. Instead, the presence of an ethoxycarbonyl functionality at position 1 of the pyrrole ring allowed the direct introduction of the enamino functionality even starting from the NH derivative **5e**. In fact, despite the numerous publications[15] on such a reaction, **5e–g** were the only ketones capable to react with TBDMAM, producing the corresponding enaminones **7e–g**. Annelation of the pyridine ring on the isoindole moiety was accomplished using enaminones as key intermediates and phenylsulfonylacetonitrile as 1,3 dinucleophile. Such reactions were carried out in ethanol and, when reaction of the *N*-methyl derivative **7b** was conducted at room temperature for 24 h, the intermediate **8** (62%) was isolated as the main product from the reaction mixture, indicating the high electrophilicity of the α-enamino carbon atom compared with the annular carbonyl. Prolonged heating of the mixture (24–72 h) was necessary to accomplish the desired cyclization to give compounds **4b–i** (Table 1, 55–72%). The tricyclic derivative **4b** (40%) was also obtained by heating under reflux (72 h) the intermediate **8** with stoichiometric amount of diethylamine. In general, it seems likely that, by prolonging refluxing time, the hydrolysis of the cyano group to carboxamide **9** causes the reaction to proceed towards the direct closure to the 2-pyridone ring. In order to obtain pyrroloquinolinones with different solubility properties, the three ethoxycarbonyl derivatives **4e–g** (Table 1) were subjected to hydrolysis in basic media to give the corresponding carboxylic acid **4j–l** (Table 1) in very good yields (80–90%). Moreover N-methylation of the pyridone moiety of derivatives **4b–g** was achieved with stoichiometric amount of iodomethane in DMF in the presence of sodium hydride as the base leading to the 1-methyl pyrrol oquinolinones **4m–r** (34–68%, Table 1). As expected, from the reaction of **4e** along with the desired 1,9-dimethyl pyrroloquinolinone **4p** (34%), also the 8,9-dimethyl pyrroloquinolinone **4f** (12%) and the 1,8,9-trimethyl pyrroloquinolinone **4q** (15%) were isolated from the mixture. For the unsubstituted derivatives **4b–d**, together with the 1-N-methylated pyrroloquinolinones **4m–o** (55–68%), the O-methylated species **4s,t** (18–20%) were also isolated as minor products. Instead **4u** was isolated only in traces (3%).

### 2.2. Biology

#### 2.2.1. Evaluation of some physico-chemical properties

The absorption and emission spectra of pyrrolo[3,4-*h*]quinolin-2-ones were collected in phosphate buffer 10 mM pH 7.2. All compounds absorbed between the range of 250–420 nm and presented bands in UV-A region. Absorption maxima wavelengths (Table 2) exhibited a remarkable bathocromic shift compared to angelicin due to the conjugation with the substituents in 3 and 8 positions.

All compounds demonstrated higher molar extinction coefficients than angelicin thanks to the presence of a phenylsulfonyl group in position 3 and of the substituent in 8. This is probably due to the greater electronic density, to the increased extension of the molecule conjugation but also to the increased structural rigidity for the steric hindrance of the phenylsulfonyl group. The partition coefficients of the test compounds were calculated using the computational method as described by Ghose and Crippen.[17] The values obtained with this technique are close to the experimental measured ones.[18] The majority (11 out of 19) of pyrrolo[3,4-*h*]quinolin-2-ones were highly hydrophobic, reaching *c* log *P* values of +6.44 in the case of **4r**. The substituent in position 7 seems to play an important role in determining the *c* log *P* value. Thus, derivatives bearing the ethoxycarbonyl functionality are more hydrophobic; instead, unsubstituted or carboxylic acid derivatives generally resulted more hydrophilic.




**Scheme 1.** Synthesis of compounds **4b–u**. Reagents and conditions: (i) *t*-BuOK, HCOOEt, benzene, rt, 52–92%; (ii) $HNEt_2$, benzene, rt, 24 h, 50–95%; (iii) TBDMAM, toluene, reflux, 57–87%; (iv) $PhSO_2CH_2CN$, ethanol, rt, 24 h, 62%; (v) $HNEt_2$, ethanol, reflux, 72 h, 40%; (vi) $PhSO_2CH_2CN$, ethanol, reflux, 24–72 h, 55–72%; (vii) KOH, EtOH, reflux, 24 h, 80–90%; (viii) NaH, DMF, 0 °C to rt, 1 h then iodomethane at 0 °C to rt 2 h, 8–68%.

**Table 1**
Pyrrolo[3,4-*h*]quinolin-2-ones **4b–u**

| Compound | R | $R^1$ | $R^2$ | Substrate | Melting point (°C) | Yield (%) |
|---|---|---|---|---|---|---|
| **4b** | Me | H | H | **7b** | 345–346 | 65 |
| **4c** | Bn | H | H | **7c** | 293–294 | 55 |
| **4d** | Ph | H | H | **7d** | 217–220 | 60 |
| **4e** | H | $CO_2Et$ | Me | **7e** | 324–325 | 70 |
| **4f** | Me | $CO_2Et$ | Me | **7f** | 239–240 | 64 |
| **4g** | Bn | $CO_2Et$ | Me | **7g** | 194–195 | 65 |
| **4h** | Me | H | Me | **7h** | 244–245 | 68 |
| **4i** | Bn | H | Me | **7i** | 252–254 | 72 |
| **4j** | H | $CO_2H$ | Me | **4e** | 243–244 | 80 |
| **4k** | Me | $CO_2H$ | Me | **4f** | 230–231 | 85 |
| **4l** | Bn | $CO_2H$ | Me | **4g** | 185–186 | 90 |
| **4m** | Me | H | H | **4b** | 230–231 | 55 |
| **4n** | Bn | H | H | **4c** | 164–165 | 62 |
| **4o** | Ph | H | H | **4d** | 257–258 | 68 |
| **4p** | H | $CO_2Et$ | Me | **4e** | 248–249 | 34 |
| **4q** | Me | $CO_2Et$ | Me | **4f** | 165–166 | 65 |
| **4r** | Bn | $CO_2Et$ | Me | **4g** | 138–139 | 68 |
| **4s** | Me | H | H | **4b** | 202–203 | 18 |
| **4t** | Bn | H | H | **4c** | 186–187 | 20 |
| **4u** | Ph | H | H | **4d** | 257–258 | 3 |

**Table 2**
Physico-chemical properties of pyrrolo[3,4-*h*]quinolin-2-ones

| | $\lambda_{MAX}$abs[a] | $\varepsilon$[a] ($M^{-1}$ $cm^{-1}$) | $\lambda_{MAX}$abs[b] | $\lambda_{MAX}$fluo[b] | $\phi_F$[c] | *c* log *P*[d] |
|---|---|---|---|---|---|---|
| **4b** | 409 | 18849.5 | 396 | 438 | 0.018 | +2.01 |
| **4c** | 410 | 34338.6 | 408 | 462 | 0.059 | +3.88 |
| **4d** | 414 | 27901.0 | 394 | 437 | 0.197 | +3.88 |
| **4e** | 405 | 23928.0 | 393 | 431 | 0.790 | +3.98 |
| **4f** | 353 | 18641.2 | 395 | 429 | 0.870 | +4.58 |
| **4g** | 401 | 17332.8 | 380 | 429 | 0.734 | +5.84 |
| **4h** | 413 | 24463.2 | 399 | 448 | 0.023 | +2.47 |
| **4i** | 410 | 26883.3 | 401 | 486 | 0.064 | +4.34 |
| **4j** | 406 | 26281.3 | 396 | 442 | 0.006 | −0.28 |
| **4k** | 404 | 11084.5 | 399 | 460 | 0.004 | +0.51 |
| **4l** | 405 | 19301.0 | 399 | 456 | 0.004 | +2.00 |
| **4m** | 408 | 38804.6 | 395 | 435 | 0.040 | +2.36 |
| **4n** | 409 | 36753.2 | 396 | 430 | 0.175 | +3.87 |
| **4o** | 413 | 42799.2 | 401 | 441 | 0.662 | +4.23 |
| **4p** | 352 | 29983.3 | 357 | 429 | 0.387 | +4.17 |
| **4q** | 350 | 33957.8 | 364 | 422 | 0.361 | +4.93 |
| **4r** | 370 | 37263.2 | 396 | 428 | 0.615 | +6.44 |
| **4s** | 355 | 29148.4 | 351 | 460 | 0.014 | +2.01 |
| **4t** | 354 | 15740.5 | 353 | 454 | 0.058 | +3.52 |
| Ang | 299 | 9350.0 | 302 | 460 | 0.016 | +2.00 |

[a] In DMSO.
[b] In phosphate buffer 10 mM pH 7.2.
[c] Relative fluorescence quantum yield determined in 0.1 N $H_2SO_4$ as described in Ref. 16.
[d] Calculated as reported in Ref. 17.

### 2.2.2. Cellular phototoxicity

After having assessed that pyrrolo[3,4-*h*]quinolin-2ones they did not present any antiproliferative activity in the absence of irradiation, their phototoxicity was evaluated on a panel of cultured human cell lines. The phototoxicity tests were carried out against different human tumour cell lines: HL-60 (promyelocytic leukaemia), Jurkat (T-cell leukaemia), MCF-7 (breast adenocarcinoma) and LoVo (colon carcinoma). Analogous experiments were performed in an immortalized cell line of human keratinocytes, NCTC-2544. Table 3 showed the $IC_{50}$ in human cell lines after 72 h from the irradiation with two different UV-A doses (2.5 and 3.75 J/$cm^2$).

Among pyrrolo[3,4-*h*]quinolin-2-ones, compounds **4h**, **4j–m, 4o** were found to be non phototoxic at the employed concentrations, whereas compounds **4b** and **4c** demonstrated some activity only against leukaemia cell lines at micromolar level at the UVA dose of 3.75 J/$cm^2$. Derivatives **4d**, **4e** and **4p** are active at micromolar concentration (5.8–12.5 μM) against leukaemia cell lines at 3.75 J/$cm^2$, and excepted for **4d**, against LoVo tumor lines (13.6 μM and 13.2 μM, respectively). Pyrroloquinolines **4f, 4i, 4m, 4r** and **4t** show generally good activity in the micromolar range (1.3–14.4 μM) against all the cell lines used for this study with exception of **4i** for the LoVo cell line.

The best results are indeed obtained for **4g, 4q** and **4s** which showed high phototoxicity comparable and in some cases better than angelicin (**Ang**) used as the reference drug, also against solid tumors (0.2–2.5 μM) reaching the submicromolar and low micromolar range.

A relationship between activity and structure can be hypothesized thanks to the fair number of evaluated compounds, starting from the structure of the most phototoxic compounds. In the compounds bearing the carbonyl group in position 2, the importance of the presence of a substituent in position 8 and the ethoxycarbonyl group in position 7 was observed. In fact, compounds that had no

**Table 3**
Phototoxicity of pyrrolo[3,4-*h*]quinolin-2-ones in different human cell lines

| | [a]$IC_{50}$ (μM) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | [b]HL-60 | | Jurkat | | MCF-7 | | LoVo | | NCTC-2544 | |
| | [c]2.5 | 3.75 | 2.5 | 3.75 | 2.5 | 3.75 | 2.5 | 3.75 | 2.5 | 3.75 |
| **4b** | >20 | 5.6 ± 0.6 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **4c** | >20 | 14.5 ± 1.5 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **4d** | 12.2 ± 1.0 | 8.0 ± 1.0 | >20 | 7.4 ± 0.7 | >20 | >20 | >20 | >20 | >20 | >20 |
| **4e** | 13.7 ± 1.0 | 9.1 ± 0.8 | 18.0 ± 2.0 | 10.0 ± 1.0 | >20 | >20 | 17.5 ± 1.5 | 13.6 ± 0.5 | >20 | 15.0 ± 1.4 |
| **4f** | 3.6 ± 0.5 | 1.8 ± 0.2 | 2.0 ± 0.2 | 1.4 ± 0.2 | 10.0 ± 1.0 | 5.1 ± 0.6 | 7.9 ± 0.8 | 4.0 ± 0.4 | 10.1 ± 1.0 | 6.2 ± 0.6 |
| **4g** | 1.2 ± 0.2 | 0.8 ± 0.02 | 2.9 ± 0.3 | 1.9 ± 0.2 | 3.3 ± 0.4 | 1.4 ± 0.1 | 4.0 ± 0.4 | 2.3 ± 0.2 | 4.0 ± 0.5 | 2.5 ± 0.3 |
| **4h** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **4i** | 4.0 ± 0.4 | 2.5 ± 0.3 | 13.0 ± 1.5 | 3.0 ± 0.5 | >20 | 7.7 ± 0.8 | >20 | >20 | >20 | 14.4 ± 0.8 |
| **4j** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **4k** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **4l** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **4m** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **4n** | 4.4 ± 0.4 | 2.8 ± 0.3 | 3.6 ± 0.4 | 2.5 ± 0.3 | 6.6 ± 0.7 | 3.7 ± 0.4 | 3.9 ± 0.4 | 2.8 ± 0.3 | 8.2 ± 0.9 | 5.8 ± 0.6 |
| **4o** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **4p** | >20 | 5.8 ± 0.6 | >20 | 12.5 ± 1.3 | >20 | >20 | >20 | 13.2 ± 1.3 | >20 | >20 |
| **4q** | 1.2 ± 0.1 | 0.9 ± 0.02 | 1.2 ± 0.1 | 0.8 ± 0.1 | 1.7 ± 0.1 | 1.3 ± 0.1 | 0.7 ± 0.05 | 0.4 ± 0.03 | 2.4 ± 0.2 | 1.3 ± 0.2 |
| **4r** | 5.4 ± 0.6 | 2.6 ± 0.3 | 2.9 ± 0.3 | 1.3 ± 0.1 | >20 | 14.4 ± 1.5 | 12.1 ± 1.2 | 8.7 ± 0.8 | >20 | 14.3 ± 1.5 |
| **4s** | 1.6 ± 0.1 | 0.9 ± 0.1 | 1.0 ± 0.08 | 0.2 ± 0.01 | 2.5 ± 0.3 | 2.0 ± 0.1 | 1.9 ± 0.2 | 0.9 ± 0.09 | 4.9 ± 0.5 | 1.1 ± 0.1 |
| **4t** | 8.3 ± 0.8 | 7.3 ± 0.7 | 8.5 ± 0.5 | 6.3 ± 0.6 | 10.0 ± 1.2 | 7.5 ± 0.8 | 9.9 ± 0.1 | 8.7 ± 0.6 | 15.3 ± 1.4 | 9.6 ± 0.5 |
| **Ang** | 1.2 ± 0.1 | 0.9 ± 0.2 | 1.0 ± 0.2 | 0.9 ± 0.1 | 4.4 ± 0.5 | 1.5 ± 0.2 | 4.0 ± 0.4 | 1.1 ± 0.4 | 4.2 ± 0.5 | 0.9 ± 0.1 |

[a] Concentration of compound required to inhibit the cell growth by 50% after 72 h of exposure as determined by MTT assay. Values are means ± SEM of three experiences.
[b] Human cells: HL-60, promyelocytic leukaemia; Jurkat, T-cell leukaemia; MCF-7, adenocarcinoma; LoVo, colon carcinoma; NCTC-2544, immortalized keratinocytes.
[c] UV-A doses are expressed in J/cm$^2$.

substituent or a carboxylic acid functionality in position 7 (**4j, 4k** and **4l**) exhibited moderate (**4d** and **4i**) or no phototoxicity (**4h, 4j, 4k** and **4l**). Among the compounds with the ethoxycarbonyl group in position 7, the presence of a substituent of any nature (methyl or benzyl groups) in position 8 was fundamental for activity: in fact, **4e** and **4p** demonstrated a reduced phototoxicity. Despite the small number of compounds with the methoxyl group in position 2, we found a general increase in the phototoxicity. In fact, **4s** was one of the most active molecule and it is worthy to note that the correspondent compound (**4b**) with the carbonyl group in 2 was almost inactive.

### 2.2.3. Photodegradation and evaluation of photoproducts toxicity

All derivatives underwent a remarkable photodegradation, as shown by the changes in absorption spectra upon UVA irradiation in DMSO. In Figure 1, the absorption spectra of **4i**, **4q** and **4s** are depicted as representatives, recorded after exposure to increasing doses of UV-A light. Distinct isosbestic points were observed, suggesting that photoproducts are most likely formed. A similar behavior was observed for furocoumarins[19] and other tricyclic derivatives such as pyrrolo[2,3-*h*]quinolinones[12] and pyrrolo [3,2-*h*]quinolinones.[13]

Caffieri et al. demonstrated the ability of furocoumarins to generate cytotoxic products (POPs), upon photoirradiation that could play a relevant role in PUVA therapy.[20] Thus, the possible contribution of photoproducts in antiproliferative action was analysed. After having irradiated (6.5 J/cm$^2$) solutions of the most active compounds, Jurkat cells were incubated with that mixture for 72 h. The results (data not shown) revealed no cytotoxic effects, suggesting that the events that lead to cellular death are solely due to a phototoxic damage and not to a production of toxic photoproducts.

### 2.2.4. Effect of scavengers on pyrroloquinolinone-induced phototoxicity

With the purpose to evaluate the possible mechanism of action of the test compounds, we performed analogous experiments in the presence of some scavengers. These tests were performed with the most active compounds **4g**, **4q** and **4s** using MCF-7 cells. We employed the radical scavengers reduced glutathione (GSH) and 2,6-di-*tert*-butylhydroxyanisole (BHA); the hydroxyl radical scavengers dimethylthiourea (DMTU) and mannitol (MAN), and the singlet oxygen scavengers 1,4-diazabicyclo[2,2,2]octane (DABCO), sodium azide ($NaN_3$) as described before.[13] The results of these studies were presented in Figure 2. For **4g** and **4s**, the protective effect of scavengers was very slight and non-specific; whereas a major increase in cellular survival was detected with **4q**. In particular, all scavengers seemed to demonstrate a protective effect in **4q** experiments but 80% of cell viability was assessed when the irradiation was carried out in the presence of MAN and DABCO, indicating that hydroxyl radical and singlet oxygen may be involved in **4q** phototoxicity. However, from these data, an univocal mechanism of photosensitization was not distinguished.

### 2.2.5. Externalization of phosphatidylserine

The mode of cell death photoinduced by pyrrolo[3,4-*h*]quinolin-2-ones was investigated by cytofluorimetric analysis using propidium iodide (PI) and Annexin V–FITC conjugates, which stain DNA and PS residues, respectively. Thus, this cytofluorimetic measure discriminated between intact ($A^-/PI^-$), early apoptotic ($A^+/PI^-$), late apoptotic ($A^+/PI^+$) or necrotic cells ($A^-/PI^+$).[21] The biparametric analysis was conducted in Jurkat cells after 24 h from the irradiation in the presence of various concentrations of **4g** and **4q**. As control, the same tests were performed in non irradiated Jurkat or in irradiated cells.

A significant increase of early apoptotic cells was determined when the irradiation was conducted in the presence of the test compounds (Fig. 3).

A percentage of cells was positive to both probes and this was compatible with a later stage of apoptosis. A small part of treated cells (up to 5%) resulted necrotic but this amount was comparable to the irradiated control. From these data, a main apoptotic pathway can be associated to the cellular death photoinduced by pyrrolo[3,4-*h*]quinolin-2-ones.

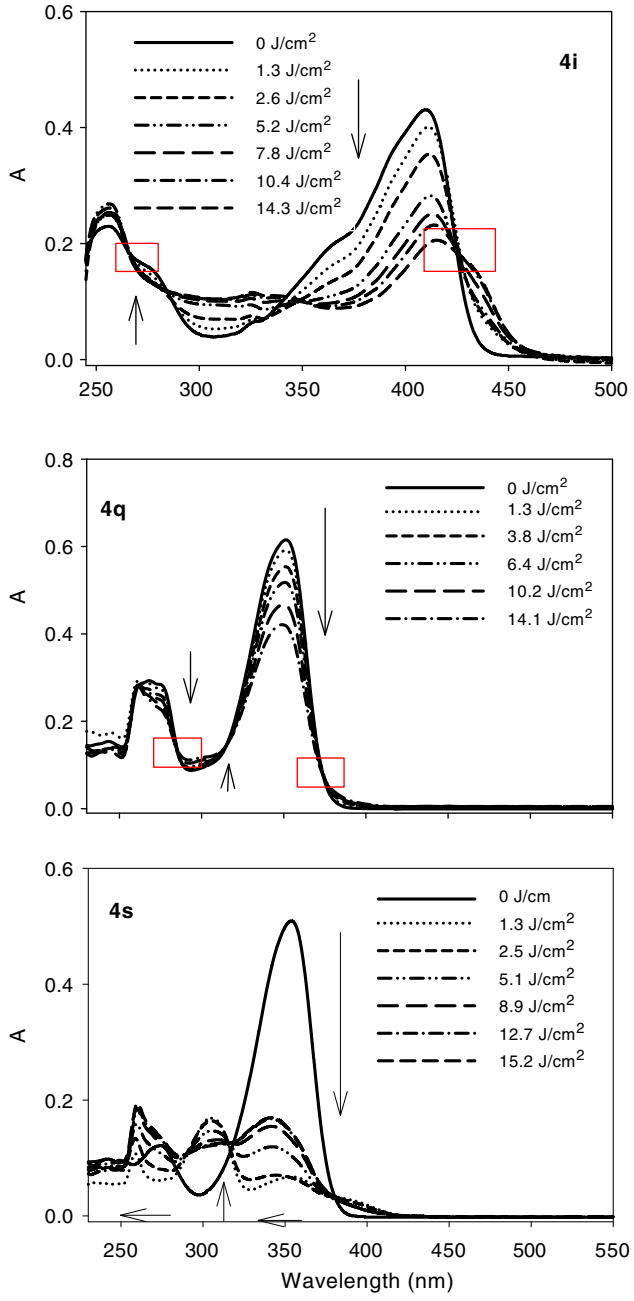



**Figure 1.** Photodegradation of pyrrolo[3,4-*h*]quinolin-2-ones. Absorption spectra of compound **4i** (upper panel), **4q** (middle panel) and **4s** (lower panel) at the concentration of 20 μM in DMSO were recorded after exposure to increasing UV-A doses. Red squares in the graphs indicate the presence of isosbetics points.

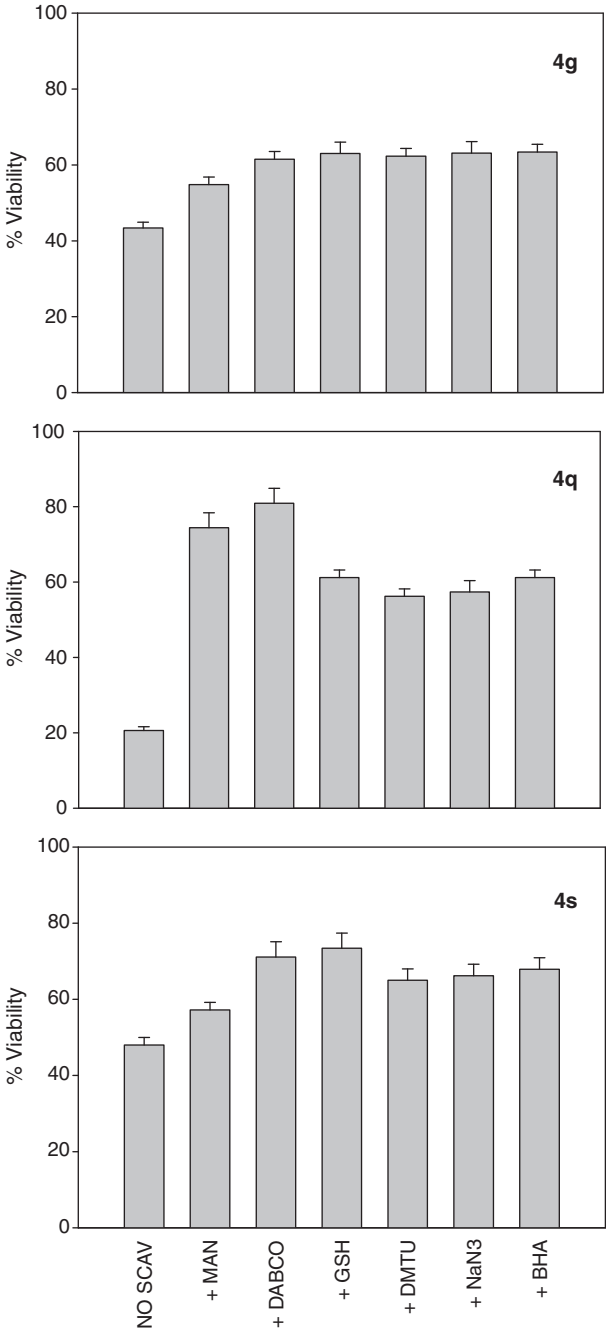



**Figure 2.** Effect of different scavengers on the phototoxicity induced by **4g**, **4q** and **4s** in MCF-7 cells. Cell viability was assayed by MTT test 72 h from the irradiation (2.5 J/cm$^2$) in the presence of the compounds and the scavengers. Data were expressed as means ± SEM for at least three independent experiments.

### 2.2.6. Cell cycle analysis

To investigate the effects of test compounds upon UVA irradiation on the cell cycle, Jurkat cells were treated with the most active compounds at different concentrations and at the light dose of 2.5 J cm$^{-2}$. After 24 h from the irradiation, the cells were fixed and labelled with propidium iodide and the different phases of the cell cycle were analyzed by flow cytometry. From the data in Table 4, an arrest of the cell cycle in S phase was observed along with a reduction of G1 cells. These data suggest that in response to phototoxic stress induced by the drugs, the progress of the cell cycle can be arrested at certain checkpoints that serve to maintain genomic integrity.

Nevertheless, the most important modification was the concentration-dependent onset of a subG1 peak, which represented apoptotic cells. The subG1 was a consequence of the ordered degradation of DNA which occurred during apoptosis. This phenomenon is considered a later event than PS externalization, thus these data were well in agreement with the ones of the previous experiment.

### 2.2.7. Intracellular localization of pyrrolo[3,4-*h*]quinolin-2-ones

It has recently been demonstrated the importance of the cellular localization for the cytotoxic action of photosensitizers.[12,22,23] The strength of a photosensitiser is determined by the nature of

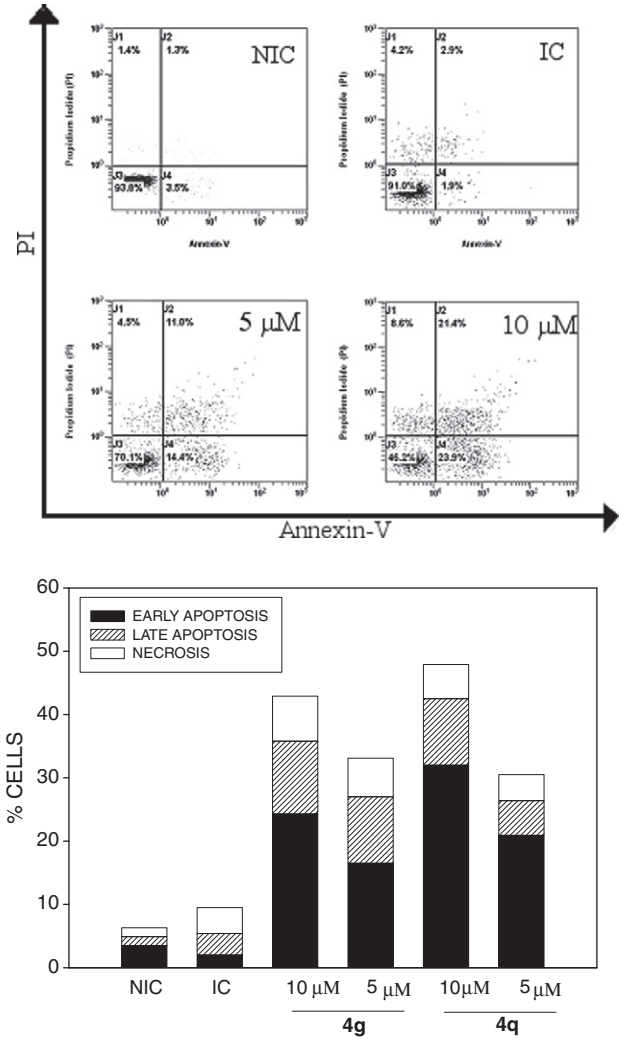



**Figure 3.** Upper panel. Representative biparametric histograms obtained 24 h after the irradiation (3.75 J $cm^{-2}$) of Jurkat cells in the presence of 4q at the indicated concentrations. The cell were labelled with Annexin-V-FITC and Propidium iodide (PI) as described in Section 4 and analyzed by flow cytometry. Lower panel. Percentage of early apoptotic cells ($A^+/PI^-$), late apoptotic cells ($A^+/PI^+$) and necrotic cells ($A^-/PI^+$) after 24 h from the irradiation (2.5 J/$cm^2$) in the presence of **4g** and **4q** at the indicated concentrations (μM). NIC = non irradiated control; IC = irradiated control. Data were expressed as mean ± SEM of three independent experiments.

**Table 4**
Percentage of Jurkat cells in each cell cycle phase 24 h after irradiation

| | G1[c] | S[c] | G2/M[c] | SubG1[d] |
|---|---|---|---|---|
| NIC[a] | 63.0 | 27.6 | 9.4 | 0.1 |
| IC[b] | 64.4 | 25.3 | 10.3 | 0.5 |
| ***4g*** | | | | |
| 5 μM | 53.8 | 41.8 | 4.4 | 3.8 |
| 10 μM | 44.8 | 54.4 | 0.8 | 12.5 |
| ***4q*** | | | | |
| 5 μM | 48.9 | 46.9 | 4.2 | 10.5 |
| 10 μM | 39.1 | 55.6 | 5.3 | 43 |
| ***4s*** | | | | |
| 5 μM | 49.6 | 45.4 | 5.0 | 4.0 |
| 10 μM | 40.1 | 50.4 | 9.5 | 15.9 |

[a] Not irradiated control.
[b] Irradiated control.
[c] The percentage of each phase of the cell cycle was calculated on living cells.
[d] Percentage of the cell population with hypodiploid DNA content peak (apoptotic cells).

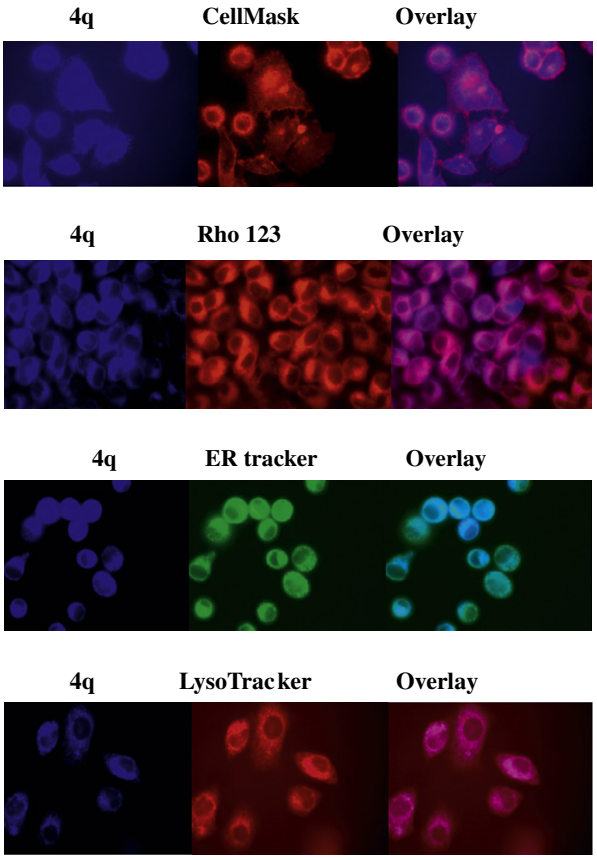



**Figure 4.** Intracellular localization of pyrrolo[3,4-*h*]quinolin-2-ones in NCTC-2544 cells. Fluorescence microphotographs showing the intracellular localization of **4q** in NCTC-2544 cells in the presence of Cellmask Orange, Rho 123, ER Tracker Green and Lysotracker RED. The cells were treated as described in Experimental Section and cellular fluorescence images were acquired with a video-confocal microscope, using a Nir Apo 60X/1.0W water immersion objective. Overlay images of **4q** localization are generated by transferring the blue color of the compound, onto the corresponding fluorescence image of the probe.

the excited states or by its ability to form reactive species whereas, in a phototoxicological context, other factors such as subcellular localization, are essential parameters. Thus, it is critically important to evaluate the distribution of photosensitisers inside cells. In order to investigate the intracellular localization of pyrroloquinolinones, we used different fluorescent probes: Rhodamine 123 (Rho 123), a lipophilic cation commonly used for the assessment of the mitochondrial potential, Lyso tracker RED, a fluorescent dye that specifically stains lysosomes,[24] cell mask orange, a plasma membrane staining and ER-tracker, which is an highly selective marker for the endoplasmic reticulum in living cells. These markers fluoresce in the visible region (about 550–630 nm) whereas the test compounds emit in the blue region. Both fluorescences can be easily separated using suitable bandpass optical filters. After an incubation in NCTC-2544 cells, compound **4q**, chosen as representative, was found to incorporate and associate with subcellular structures. It can be observed from Figure 4 that **4q** diffuses largely in the cytoplasm and co-localizes with Rho 123, Lyso tracker and ER tracker, indicating that this compound is broadly distributed in the cytoplasm and in many subcellular organelles without a particular specificity.

### 2.2.8. Mitochondrial and lysosomial integrity assay

Mitochondrial dysfunction was examined to better investigate cell death mode through the assessment of the mitochondrial membrane potential ($\Delta\psi_{mt}$). The determination of $\Delta\psi_{mt}$ was

carried out through a flow cytometric analysis using the probe JC-1. This cytofluorimetric analysis was performed after 6, 12 and 24 h from the irradiation of Jurkat cells in the presence of **4g** and **4q**. With normal cells (high $\Delta\psi_{mt}$), JC-1 displays a orange fluorescence (590 nm). This is caused by spontaneous and local formation of aggregates that are associated with a large shift in the emission. In contrast, when the mitochondrial membrane is depolarized (low $\Delta\psi_{mt}$), JC-1 forms monomers which emit at 530 nm.[25]

As depicted in Figure 5 (upper panel), a rapid mitochondrial involvement in inducing apoptosis was assessed: in fact, after 6 h from irradiation, the percentage of treated cells with low mitochondrial potential significantly increased compared to controls at both concentration used. The disruption of $\Delta\psi_{mt}$ is associated with the appearance of Annexin-V positivity in the treated cells when they are in an early apoptotic stage. In fact, the dissipation of $\Delta\psi_{mt}$ is characteristic of apoptosis and has been observed with many photosensitisers including furocoumarins, fluoroquinolones, phenothiazines, and antimalarial drugs.[26a–d]

In order to investigate the integrity of lysosomes after irradiation with the test compounds, a flow cytometric analysis was performed using the fluorescent dye acridine orange (AO).[27] As depicted in Figure 5 (lower panel), a concentration and time-dependent increase of the number of damaged lysosomes is observed after irradiation in the presence of **4g** and **4q**), indicating that lysosomes, together with mitochondria, were deeply involved in cell death.

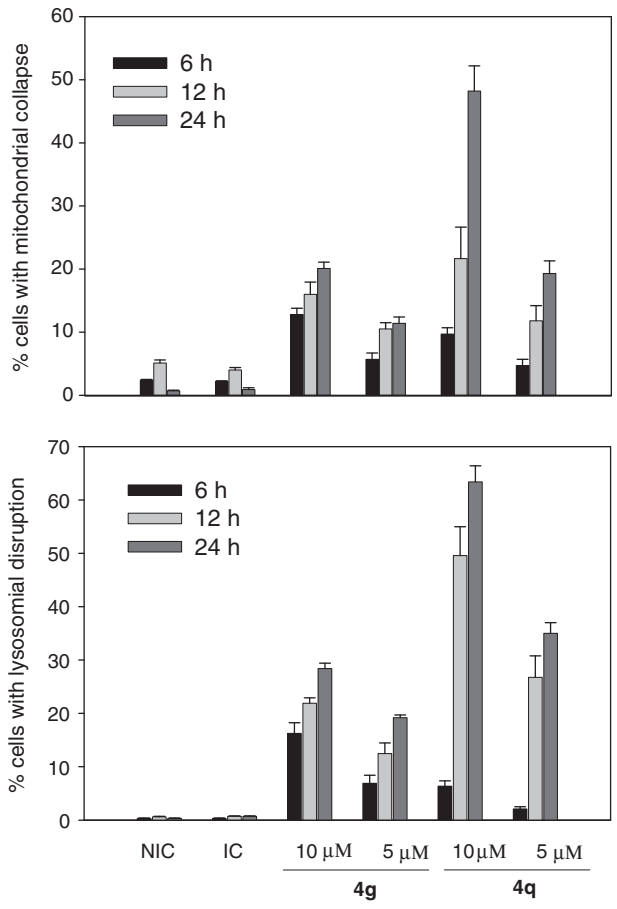



**Figure 5.** Upper panel. Percentage of Jurkat with collapsed ΔψM (fluorescence of the JC-1 monomeric form) after 6, 12 and 24 h from the irradiation (2.5 J/cm$^2$) with **4g** and **4q** at the indicated concentrations (μM). Data were expressed as mean ± -SEM of three independent experiments. Lower Panel. Percentage of Jurkat cells with damaged lysosomes after 6, 12 and 24 h from irradiation (2.5 J/cm$^2$) in the presence of **4g** and **4q** at the indicated concentrations. NIC = non irradiated control; IC = irradiated control. Data are represented as mean S.E.M. of three experiments.

### 2.2.9. DNA interaction

As DNA is considered one of the most important target for psoralen, the affinity of pyrrolo[3,4-*h*]quinolin-2-ones for this macromolecule was checked. Linear dichroism is an efficient tool to evaluate the binding mode between an organic compound and nucleic acids.[28] Linear dichroism (LD) measurements were performed on solutions with salmon testes DNA (st-DNA) and the title compounds at various molar ratios. The sign and the extend of LD signal is related to the orientation of the compound with respect to DNA helix axis.

Inspection of the spectra of DNA in the presence of the most active derivatives (**4g**, **4q** and **4s)** shows (Fig. 6) that there is an increase in the signal intensity for the DNA band (230–300 nm) indicating that the DNA becomes more oriented within the hydrodynamic field because of stiffening of the helix upon interaction of these compounds. However, even for the highest concentrations of test compounds, the spectra do not show any LD bands in the chromophore absorption region (300–450 nm), suggesting a poor interaction with this macromolecule.

Moreover, the interaction of pyrrolo[3,4-*h*]quinolin-2-ones with DNA was also monitored by fluorimetric titrations, where possible changes in emission spectra of aqueous solution of compounds were searched after the addition of st-DNA. Results are presented in Figure 7.

Fluorescence titrations carried out with compounds **4g, 4q** and **4s** showed a poor fluorescence quenching even when DNA concentration was 10 times higher than the one of the compounds. Altogether these data indicate that the compounds are not intercalated into DNA or are loosely bound to it, in contrast with natural furocoumarins which bound DNA by intercalation.[1]

### 2.2.10. DNA photodamage

DNA photodamage experiments were carried out to determine whether the new derivatives were able to photosensitize DNA strand break activity even if they did not demonstrate a high affinity for this biomolecule. pBR322 plasmid DNA was used as a model system to evaluate DNA breaking activity: frank strand breaks, the oxidative damage of purine and/or pyrimidine bases. The irradiation of supercoiled plasmid pBR322 was carried out in the presence of the test compounds at the molar ratio [DNA]/[Drug] = 1/3. After the irradiation, pBR322 aliquots were also incubated with two base excision repair enzymes formamido pyrimidin glycosilase (Fpg) and Endonuclease III (Endo III), respectively to evaluate, in addition to the formation of frank strand breaks, also oxidative damage to DNA bases.[29] The results (see Fig. 8 upper panel) pointed out that the new compounds did not provoke any formation of open circular or linear bands, indicating they did not sensitize frank strand breaks. On the contrary, the treatment with the two base excision enzymes revealed that they were able to photooxidize DNA bases in a great amount.

All compounds were able to photoinduce base oxidation but **4q** resulted the most active especially after treatment of irradiated DNA with Endo III, suggesting pyrimidinic bases as its preferential targets. To better understand the mechanism of base photooxidation, the irradiation of pBR322 was carried out in the presence of nitrogen flux: a dramatic reduction of base oxidative damages was observed (Fig. 9), indicating that the process of photoinduced DNA damage is strongly oxygen dependent. Thus, the formation of reactive oxygen species was essential for DNA photodamage by pyrrolo[3,4-*h*]quinolin-2-ones.

To further understand the mechanism of action, irradiation of pBR322 was carried out in the presence of **4q** and some scavengers. We used MAN, a hydroxyl radical scavenger, DABCO, a singlet oxygen scavenger, and GSH, a free radical scavenger (Fig. 10). A little reduction in the formation of OC form was observed when irradiation was conducted with GSH and MAN. Whereas, in the presence

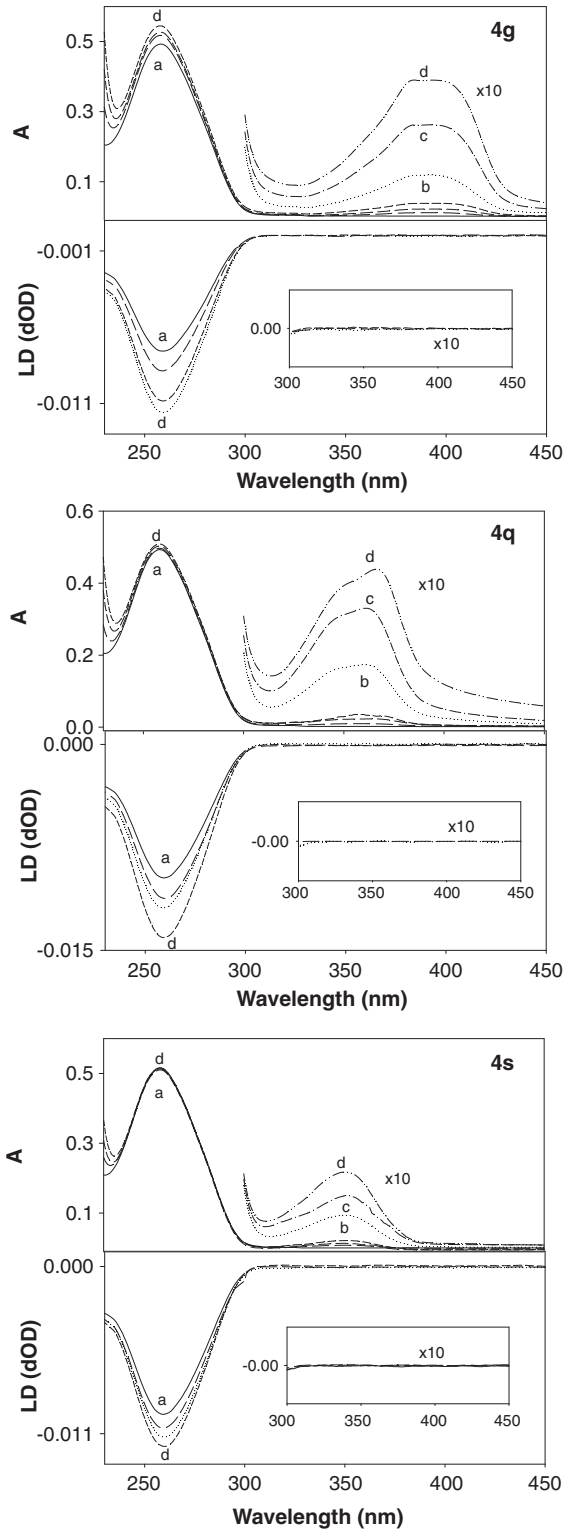



**Figure 6.** Absorbance (A) and linear dichroism (LD) spectra of mixtures of st-DNA and **4g** and **4q** (upper panels) and **4s** (lower panel) at different [drug]/[DNA] ratios (*a* = 0; *b* = 0.01; *c* = 0.02; *d* = 0.03) in phosphate buffer 10 mM, pH 7.2.

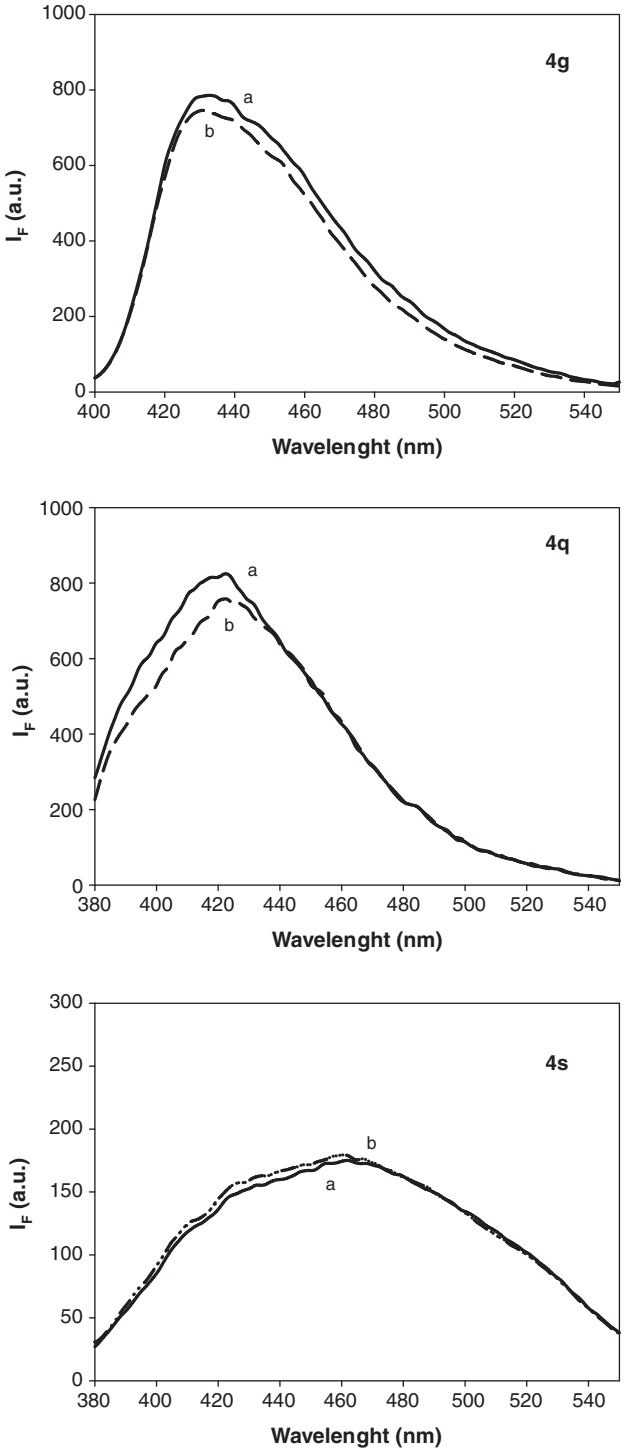



**Figure 7.** Fluorimetric titrations of stDNA (*a* = 0 μM; *b* = 100 μM) to compounds **4g**, **4q**, and **4s** at a ligand concentration of 10 μM.

of DABCO, the oxidations to DNA photoinduced by **4q** were not detected, suggesting an involvement of singlet oxygen in photosensitization reaction. As DABCO is not very specific toward singlet oxygen, these data were confirmed by irradiation in presence of $D_2O$ buffer. Interestingly, when the aqueous buffer was replaced by $D_2O$, a dramatic increase of the formation of ssb (single strand breaks) was observed. DNA damage was significantly enhanced in $D_2O$ indicating the involvement of $^1O_2$ since the lifetime of $^1O_2$ is enhanced by a factor of ca. 14 in $D_2O$.

### 2.2.11. Lipid peroxidation

As most pyrrolo[3,4-*h*]quinolin-2-ones are hydrophobic (see *c* log *P* in Table 2), they may be expected to be localized mainly in plasmatic and/or in subcellular membranes, making these structures particularly sensitive to photodamage. Lipid peroxidation was analyzed by measuring the level of malonyldialdehyde

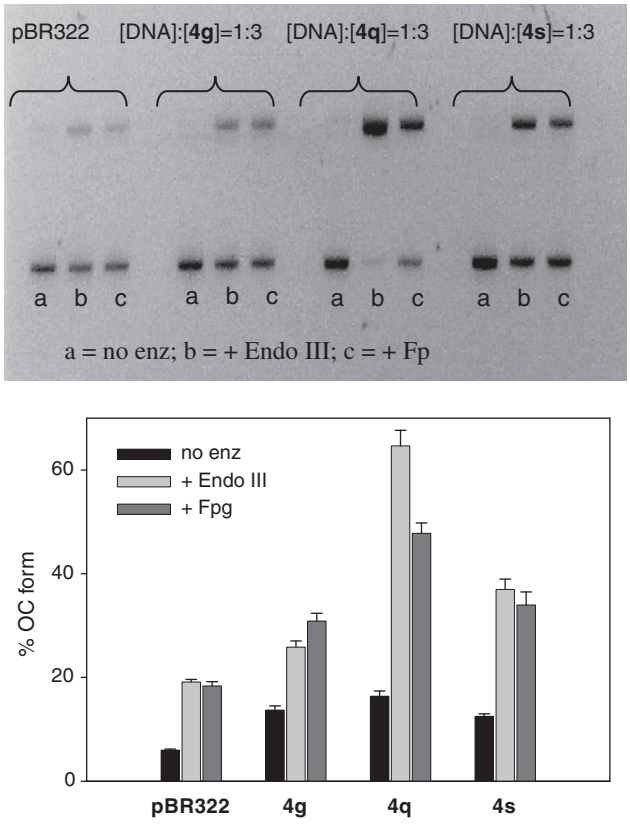



**Figure 8.** DNA base modifications photoinduced by pyrrolo[3,4-*h*]quinolin-2-ones. Upper panel. Supercoiled circular pBR322 was irradiated (3.75 J/cm$^2$) in the presence of [DNA]/[Drug]=1/3 and then treated with base excision enzyme: *a* = no enzyme; *b* = +Endo III; *c* = +Fpg. Lower panel. Percentage of II form after irradiation (3.75 J/cm$^2$) in the presence of **4g**, **4q** and **4s** at the ratio [DNA]/[Drug] = 1/3.

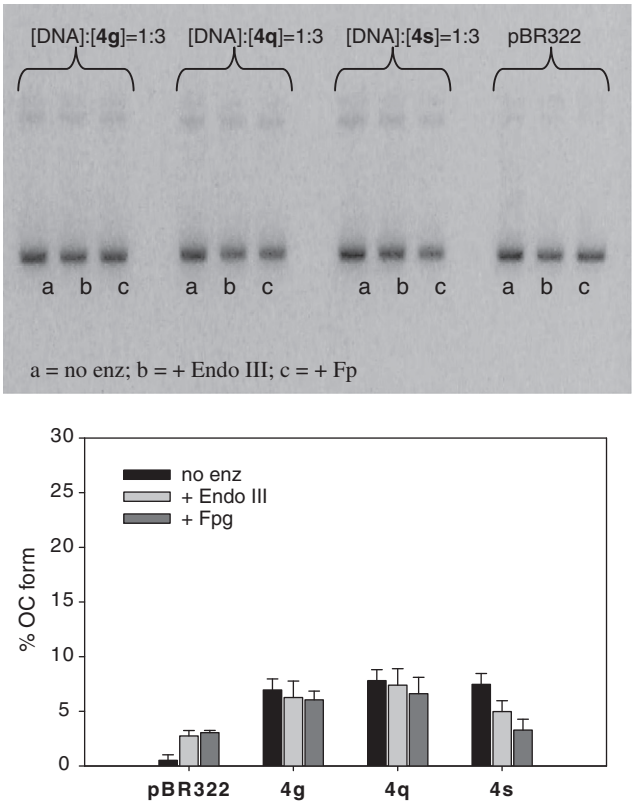



**Figure 9.** DNA base modifications photoinduced by pyrrolo[3,4-*h*]quinolin-2-ones in the presence of nitrogen flux. Upper panel. Supercoiled circular pBR322 was irradiated (3.75 J/cm$^2$) in the presence of [DNA]/[Drug] = 1/3 and then treated with base excision enzyme: *a* = no enzyme; *b* = +Endo III; *c* = +Fpg. Lower panel. Percentage of OC form after irradiation (3.75 J/cm$^2$) in the presence of **4g**, **4q** and **4s** at the ratio [DNA]/[Drug] = 1/3.

(MDA) bound to thiobarbituric acid (TBA) in treated and untreated Jurkat cells.[30]

Figure 11 shows the results obtained for compounds **4g**, **4q** and **4s** as a function of light doses. Thiobarbituric acid reactive substances (TBARS) were significantly produced in a concentration-dependent manner when the cells were exposed to the compounds and UV-A. Thus, the induced oxidative damage to membrane lipids is well correlated with the extent of cell death suggesting that an extensive lipid peroxidation could play a major role in the photokilling mechanism of the test compounds.

## 3. Conclusions

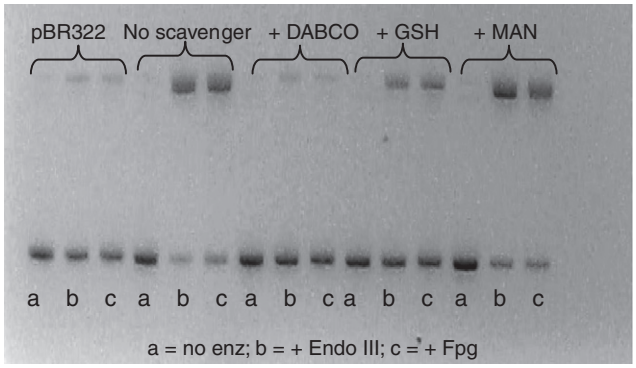
Pyrrolo[3,4-*h*]quinolin-2-ones were synthesized as heteroanalogues of angular furocoumarins, in which oxygen atoms are replaced by nitrogens. The data reported herein indicate that pyrrolo[3,4-*h*]quinolin-2-ones represent a new interesting class of potential useful compounds in photochemotherapy with probable minor side effects than angelicins.

After assessing the lack of cytotoxicity by the title compounds in the ground state, we found that several pyrrolo[3,4-*h*]quinolin-2-ones (**4d, 4e, 4f, 4i, 4m, 4p, 4r** and **4t**) exhibited UVA dose dependent phototoxicity at low micromolar concentrations against many tumor cell lines. Three compounds **4g, 4q,** and **4s** resulted the most active reaching submicromolar $IC_{50}$ values in all cell lines. Starting from these three molecules, a correlation between structure and activity was evidenced. Compounds carrying carbonyl in position 2, ethoxycarbonyl group in position 7 and a substituent in position 8, exhibited phototoxicity. Moreover, molecules carrying a methoxyl group in position 2 demonstrated some activity. Apoptosis represents the main mode of cell death photoinduced by these derivatives as demonstrated by the appearance of a subG1 peak, an index of apoptotic DNA degradation and confirmed through Annexin-V-FITC/PI test.

As a consequence of their high hydrophobic character, the title compounds easily entered the cell and diffused in the cytosol accumulating into subcellular organelles. After irradiation, apart the extensive lipid peroxidation exerted by the new derivatives, the photodamage of mitochondria and lysosomes was also shown suggesting the involvement of these organelles in the photoinduced cell death.

Interactions with DNA have been investigated as this macromolecule is supposed to be the main cause of the antiproliferative activity of furocoumarins. Pyrrolo[3,4-*h*]quinolin-2-ones did not demonstrate a significative affinity towards DNA as they did not share the planar nucleus of angelicin and psoralens: in fact, they are not fully unsaturated derivatives. Of note, the lack of affinity for the macromolecule is a characteristic shared by other isomers of this series of compounds.[13b] Moreover, through a series of plasmidic DNA photocleavage experiments, the formation of frank strand break was not assessed, although a large number of DNA bases oxidation was observed because of the production of singlet oxygen. In this case, a photoreaction of II type could explain DNA base oxidations: thus, DNA photoreaction of these new derivatives would be different from that of psoralens, which are known to mainly produce DNA-photoadducts (photoreaction of III type).

The high antiproliferative activity of the most active compounds was similar to that of angelicin while the lack of



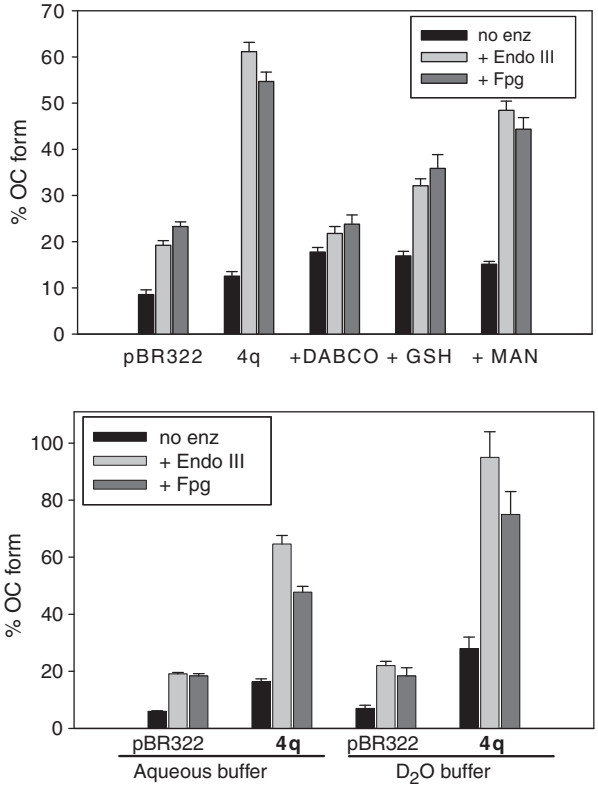




**Figure 10.** Upper panel. DNA base modifications photoinduced by **4q** in the presence of some scavengers: DABCO, GSH and MAN. Supercoiled circular pBR322 was irradiated (3.75 J/cm$^2$) in the presence of [DNA]/[**4q**] = 1/3 and then treated with base excision enzyme: *a* = no enzyme; *b* = +Endo III; *c* = +Fpg. Middle panel. Percentage of II form after irradiation (3.75 J/cm$^2$) in the presence of **4q** at the ratio [DNA]/[Drug] = 1/3 and scavengers. Lower panel. Percentage of II form after irradiation (3.75 J/cm$^2$) in the presence of **4q** at the ratio [DNA]/[Drug] = 1/3 in aqueous buffered solution and deuterated buffer.

affinity of the title compounds for DNA may be of great relevance in modulating the long term toxic effect such as skin cancer or mutagenesis exhibited by psoralen.

## 4. Experimental

### 4.1. Chemistry

All melting points were taken on a Buchi-Tottoli capillary apparatus and are uncorrected; IR spectra were determined with a Jasco FT/IR 5300 spectrophotometer; $^1$H and $^{13}$C NMR spectra were measured in DMSO-$d_6$ solutions, unless otherwise specified (TMS as internal reference), at 200 and 50.3 MHz, respectively, using a

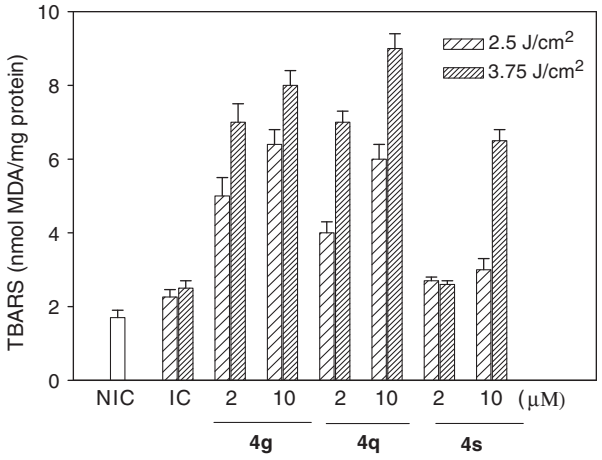



**Figure 11.** Lipid peroxidation in Jurkat cells after 24 h from the irradiation (2.5 and 3.75 J/cm$^2$) in the presence of **4g**, **4q** and **4s** at the indicated concentrations (μM). NIC = non irradiated control; IC = irradiated control. Data are represented as mean SEM of three experiments.

Bruker Avance II series 200 MHz spectrometer. Column chromatography was performed with Merck silica gel 230–400 Mesh ASTM or with a SEPACORE BÜCHI chromatography apparatus. Elemental analyses (C, H, N) were within ±0.4% of the theoretical values.

We have already reported the synthesis of ketones **5a–i**.[14]

#### 4.1.1. General procedure for the preparation of 5-(hydroxymethylene)-2,5,6,7-tetrahydro-4*H*-isoindol-4-one (6b–d,h,i)

To a suspension of *t*-BuOK (3.36 g, 30 mmol) in dry benzene (30 mL), a solution of **5b–d,h,i** (12 mmol) in dry benzene (40 mL) was added dropwise at 0 °C. After 2 h stirring at room temperature the reaction was cooled at 0 °C and a solution of ethyl formate (1.45 mL, 18 mmol) in benzene (20 mL) was added and the mixture was kept stirring at room temperature for 24 h, then the solvent was removed under vacuum. The residue was dissolved in water and the solution was washed with diethyl ether. The aqueous solution was then acidified with 6 M HCl and extracted with dichloromethane. The organic extracts were dried ($Na_2SO_4$) and evaporated under reduced pressure. The residue was purified by chromatography using dichloromethane/ethyl acetate (95:5) as eluent.

**4.1.1.1. 5-(Hydroxymethylene)-2-methyl-2,5,6,7-tetrahydro-4*H*-isoindol-4-one (6b).** Brown oil; yield 60%; IR 3394 (OH), 1653 (CO) cm$^{-1}$; $^1$H NMR: $\delta$ 2.42–2.53 (4H, m, 2 × $CH_2$), 3.64 (1H, s, $CH_3$), 6.56 (1H, s, H-1); 7.31 (1H, s, H-3), 7.61 (1H, s, CH), 10.37 (1H, br s, OH); $^{13}$C NMR: $\delta$ 20.6 (t), 24.8 (t), 36.1 (q), 109.3 (s), 118.7 (d), 123.9 (d), 126.1 (s), 164.9 (d), 189.5 (s), 201.6 (CO). Anal. Calcd for $C_{10}H_{11}NO_2$: C, 67.78; H, 6.26; N, 7.90. Found: C, 68.08; H, 6.41; N, 7.63.

**4.1.1.2. 2-Benzyl-5-(hydroxymethylene)-2,5,6,7-tetrahydro-4*H*-isoindol-4-one (6c).** Brown solid; yield 86%; mp 82–83 °C; IR 2933 (OH), 1633 (CO) cm$^{-1}$; $^1$H NMR ($CDCl_3$): $\delta$ 2.47 (2H, t, *J* = 6.0 Hz, $CH_2$), 2.63 (2H, t, *J* = 6.0 Hz, $CH_2$), 5.00 (2H, s, $CH_2$), 6.41 (1H, s, H-1), 7.14–7.34 (6H, m, Ar and H-3), 7.48 (1H, s, CH), 14.38 (1H, br s, OH); $^{13}$C NMR ($CDCl_3$): $\delta$ 21.0 (t), 25.7 (t), 53.9 (t), 109.2 (s), 117.3 (d), 122.5 (d), 125.7 (s), 127.4 (2 × d), 128.1 (d), 128.8 (2 × d), 136.3 (s), 165.4 (d), 186.7 (s), 200.5 (CO). Anal. Calcd for $C_{16}H_{15}NO_2$: C, 75.87; H, 5.97; N, 5.53. Found: C, 75.50; H, 5.73; N, 5.77.

**4.1.1.3. 5-(Hydroxymethylene)-2-phenyl-2,5,6,7-tetrahydro-4*H*-isoindol-4-one (6d).** Pale yellow oil; yield 85%; IR 2933 (OH), 1635 (CO) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 2.50 (2H, t, $J$ = 5.9 Hz, $CH_2$), 2.68 (2H, t, $J$ = 5.9 Hz, $CH_2$), 6.80 (1H, s, H-1), 7.15–7.44 (6H, m, Ar and H-3), 7.58 (1H, s, CH), 14.42 (1H, br s, OH); $^{13}C$ NMR ($CDCl_3$): $\delta$ 20.8 (t), 25.4 (t), 109.2 (s), 115.8 (d), 120.0 (d), 120.5 (2 × d), 126.3 (s), 126.7 (d), 129.5 (2 × d), 139.5 (s), 166.8 (d), 185.8 (s), 200.5 (CO). Anal. Calcd for $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48; N, 5.85. Found: C, 75.59; H, 5.64; N, 5.56.

**4.1.1.4. 5-(Hydroxymethylene)-2,3-dimethyl-2,5,6,7-tetrahydro-4*H*-isoindol-4-one (6h).** Pale yellow oil; yield 52%; IR 2933 (OH), 1635 (CO) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 2.42 (2H, t, $J$ = 6.6 Hz, $CH_2$), 2.51 (3H, s, $CH_3$), 2.58 (2H, t, $J$ = 6.6 Hz, $CH_2$), 3.49 (3H, s, $CH_3$), 6.27 (1H, s, H-1), 7.39 (1H, s, CH), 14.60 (1H, br s, OH); $^{13}C$ NMR ($CDCl_3$): $\delta$ 10.9 (q), 21.1 (t), 26.1 (t), 33.1 (q), 109.6 (s), 116.5 (d), 123.8 (s), 163.8 (d), 175.8 (s), 188.1 (s), 201.0 (CO). Anal. Calcd for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.32. Found: C, 69.32; H, 6.57; N, 6.99.

**4.1.1.5. 2-Benzyl-5-(hydroxymethylene)-3-methyl-2,5,6,7-tetrahydro-4*H*-isoindol-4-one (6i).** Yellow oil; yield 58%; mp 64–65 °C; IR 2933 (OH), 1635 (CO) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 2.43–2.51 (5H, m, $CH_2$ and $CH_3$), 2.62 (2H, t, $J$ = 6.1 Hz, $CH_2$), 5.00 (2H, s, $CH_2$), 6.34 (1H, s, H-1), 7.04 (2H, d, $J$ = 6.8, H-2′ and H-6′), 7.26–7.39 (3H, m, H-3′, H-4′ and H-5′), 7.44 (1H, s, CH), 14.63 (1H, br s, OH); $^{13}C$ NMR ($CDCl_3$): $\delta$ 11.2 (q), 21.3 (t), 26.2 (t), 50.1 (t), 109.8 (s), 116.3 (d), 117.5 (s), 124.3 (s), 126.6 (2 × d), 127.8 (d), 128.9 (2 × d), 134.8 (s), 136.7 (s), 164.3 (d), 188.4 (CO). Anal. Calcd for $C_{17}H_{17}NO_2$: C, 76.38; H, 6.41; N, 5.24. Found: C, 76.09; H, 6.88; N, 4.89.

**4.1.2. General procedure for the preparation of 5-[(diethylamino)methylene]-2,5,6,7-tetrahydro-4*H*-isoindol-4-one (7b–d,h,i)**

To a cooled solution of diethylamine (2.6 mL, 25 mmol) in dry benzene (30 mL), a solution of **6b–d,h,i** (25 mmol) in dry benzene (30 mL) was added. After 24 h stirring at room temperature the solvent was removed and the residue was washed with diethyl ether and filtered off.

**4.1.2.1. 5-[(Diethylamino)methylene]-2-methyl-2,5,6,7-tetrahydro-4*H*-isoindol-4-one (7b).** Brown oil; yield 85%; IR 1635 (CO) $cm^{-1}$; $^1H$ NMR: $\delta$ 1.14 (6H, t, $J$ = 7.3 Hz, 2 × $CH_3$), 2.53 (2H, t, $J$ = 6.4 Hz, $CH_2$), 2.69 (2H, t, $J$ = 6.4 Hz, $CH_2$), 3.28 (4H, q, $J$ = 7.3 Hz, 2 × $CH_2$), 3.59 (3H, s, $CH_3$), 6.45 (1H, s, H-1), 7.06 (1H, s, H-3), 7.33 (1H, s, CH); $^{13}C$ NMR: $\delta$ 14.6 (2 × q), 21.2 (t), 25.1 (t), 35.9 (q), 47.2 (2 × t), 102.8 (s), 117.1 (d), 122.1 (d), 122.8 (s), 124.2 (s), 144.8 (d), 182.7 (CO). Anal. Calcd for $C_{14}H_{20}N_2O$: C, 72.38; H, 8.68; N, 12.06. Found: C, 72.15; H, 8.81; N, 12.40.

**4.1.2.2. 2-Benzyl-5-[(diethylamino)methylene]-2,5,6,7-tetrahydro-4*H*-isoindol-4-one (7c).** Pale brown solid; yield 95% mp 80–81 °C; IR 1637 (CO) $cm^{-1}$; $^1H$ NMR: $\delta$ 1.21 (6H, t, $J$ = 7.1 Hz, 2 × $CH_3$), 2.64 (2H, t, $J$ = 7.1 Hz, $CH_2$), 2.80 (2H, t, $J$ = 7.1 Hz, $CH_2$), 3.32 (4H, q, $J$ = 7.1 Hz, 2 × $CH_2$), 5.00 (2H, s, $CH_2$), 6.34 (1H, s, H-1), 7.14–7.35 (6H, m, Ar and H-3), 7.59 (1H, s, CH); $^{13}C$ NMR: $\delta$ 14.6 (2 × q), 21.5 (t), 25.4 (t), 47.2 (2 × t), 53.6 (t), 103.6 (s), 115.8 (d), 122.0 (d), 123.4 (s), 125.5 (s), 127.3 (2 × d), 127.7 (d), 128.6 (2 × d), 137.0 (s), 146.2 (d), 184.7 (CO). Anal. Calcd for $C_{20}H_{24}N_2O$: C, 77.89; H, 7.84; N, 9.08. Found: C, 77.51; H, 8.04; N, 9.33.

**4.1.2.3. 5-[(Diethylamino)methylene]-2-phenyl-2,5,6,7-tetrahydro-4*H*-isoindol-4-one (7d).** Pale brown solid; yield 85%; mp 114–115 °C; IR 1637 (CO) $cm^{-1}$; $^1H$ NMR: $\delta$ 1.23 (6H, t, $J$ = 6.8 Hz, 2 × $CH_3$), 2.57–2.80 (4H, m, 2 × $CH_2$), 3.39 (4H, q, $J$ = 6.8 Hz, 2 × $CH_2$), 7.22 (1H, s, H-1), 7.34–7.76 (7H, m, Ar, H-3 and CH); $^{13}C$ NMR: $\delta$ 14.6 (2 × q), 21.1 (t), 24.7 (t), 47.3 (2 × t), 102.6 (s), 112.0 (s), 114.4 (d), 118.6 (d), 119.5 (2 × d), 125.0 (s), 125.8 (d), 129.6 (2 × d), 139.5 (s), 145.6 (d), 182.6 (CO). Anal. Calcd for $C_{19}H_{22}N_2O$: C, 77.52; H, 7.53; N, 9.52. Found: C, 77.84; H, 7.75; N, 9.25.

**4.1.2.4. 5-[(Diethylamino)methylene]-2,3-dimethyl-2,5,6,7-tetrahydro-4*H*-isoindol-4-one (7h).** Brown oil; yield 50%; IR 1633 (CO) $cm^{-1}$; $^1H$ NMR: $\delta$ 1.13 (6H, t, $J$ = 6.9 Hz, 2 × $CH_3$), 2.41 (3H, s, $CH_3$), 2.45–2.65 (4H, m, 2 × $CH_2$), 3.26 (4H, q, $J$ = 6.9 Hz, 2 × $CH_2$), 3.44 (3H, s, $CH_3$), 6.36 (1H, s, H-1), 7.29 (1H, s, CH); $^{13}C$ NMR: $\delta$ 10.4 (q), 14.6 (2 × q), 21.3 (t), 25.1 (t), 32.7 (q), 47.1 (2 × t), 103.6 (s), 115.2 (d), 118.1 (s), 122.4 (s), 131.4 (s), 144.4 (d), 183.9 (CO). Anal. Calcd for $C_{15}H_{22}N_2O$: C, 73.13; H, 9.00; N, 11.37. Found: C, 72.80; H, 9.32; N, 11.61.

**4.1.2.5. 2-Benzyl-5-[(diethylamino)methylene]-3-methyl-2,5,6,7-tetrahydro-4*H*-isoindol-4-one (7i).** Brown oil; yield 60%; IR 1633 (CO) $cm^{-1}$; $^1H$ NMR: $\delta$ 1.14 (6H, t, $J$ = 7.1 Hz, 2 × $CH_3$), 2.37 (3H, s, $CH_3$), 2.52 (2H, t, $J$ = 5.2 Hz, $CH_2$), 2.68 (2H, t, $J$ = 5.2 Hz, $CH_2$), 3.27 (4H, q, $J$ = 7.1 Hz, 2 × $CH_2$), 5.05 (2H, s, $CH_2$), 6.50 (1H, s, H-1), 7.09 (2H, d, $J$ = 7.1 Hz, H-2′ and H-6′), 7.22–7.37 (4H, m, H-3′, H-4′, H-5′ and CH); $^{13}C$ NMR: $\delta$ 10.6 (q), 14.6 (2 × q), 21.3 (t), 25.1 (t), 47.2 (2 × t), 48.9 (t), 103.7 (s), 115.1 (d), 118.6 (s), 122.9 (s), 126.7 (2 × d), 127.2 (d), 128.6 (2 × d), 131.2 (s), 138.1 (s), 144.6 (d), 184.0 (CO). Anal. Calcd for $C_{21}H_{26}N_2O$: C, 78.22; H, 8.13; N, 8.69. Found: C, 77.99; H, 7.82; N, 8.93.

**4.1.3. General procedure for the preparation of ethyl 5-[(dimethylamino)methylene]-3-methyl-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (7e–g)**

To a solution of **5e–g** (4.5 mmol) in dry toluene (10 mL), *tert*-butoxybis(dimethylamino) methane (2.79 mL, 13.5 mmol) was added. The reaction was heated under reflux for a proper time. Upon cooling at room temperature, a solid separated from the reaction mixture, which was collected and dried.

**4.1.3.1 Ethyl 5-[(dimethylamino)methylene]-3-methyl-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (7e).** This compound was obtained after 5 h. Pale green solid; yield 87%; mp 193–194 °C; IR 3278 (NH), 1668 (CO), 1637 (CO) $cm^{-1}$; $^1H$ NMR: $\delta$ 1.28 (3H, t, $J$ = 7.2 Hz, $CH_3$), 2.43 (3H, s, $CH_3$), 2.81–2.92 (4H, m, 2 × $CH_2$), 3.03 (6H, s, 2 × $CH_3$), 4.22 (2H, q, $J$ = 7.2 Hz, $CH_2$), 7. 27 (1H, s, CH), 11.75 (1H, br s, NH); $^{13}C$ NMR: 12.6 (q), 14.4 (q), 22.1 (t), 24.3 (t), 43.1 (2 × q), 59.3 (t), 103.4 (s), 114.2 (s), 119.8 (s), 132.8 (s), 136.4 (s), 147.2 (d), 160.7 (CO), 183.6 (CO). Anal. Calcd for $C_{15}H_{20}N_2O_3$: C, 65.20; H, 7.30; N, 10.14. Found: C, 65.44; H, 6.99; N, 10.36.

**4.1.3.2. Ethyl 5-[(dimethylamino)methylene]-2,3-dimethyl-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (7f).** This compound was obtained after 3 h. Brown solid; yield 72%; mp 78–79 °C; IR 1682 (CO), 1637 (CO) $cm^{-1}$; $^1H$ NMR: $\delta$ 1.28 (3H, t, $J$ = 7.1 Hz, $CH_3$), 2.52 (3H, s, $CH_3$), 2.74–2.85 (4H, m, 2 × $CH_2$), 3.03 (6H, s, 2 × $CH_3$), 3.73 (3H, s, $CH_3$), 4.21 (2H, q, $J$ = 7.1 Hz, $CH_2$), 7.29 (1H, s, CH); $^{13}C$ NMR: $\delta$ 10.9 (q), 14.3 (q), 22.8 (t), 24.1 (t), 32.0 (q), 43.1 (2 × q), 59.3 (t), 103.2 (s), 115.8 (s), 118.7 (s), 133.6 (s), 138.8 (s), 147.4 (d), 161.1 (CO), 183.1 (CO). Anal. Calcd for $C_{16}H_{22}N_2O_3$: C, 66.18; H, 7.64; N, 9.65. Found: C, 65.82; H, 7.33; N, 9.98.

**4.1.3.3. Ethyl 2-benzyl-5-[(dimethylamino)methylene]-3-methyl-4-oxo-4,5,6,7-tetrahydro-2*H*-isoindole-1-carboxylate (7g).** This compound was obtained after 6 h. Green solid; yield 57%; mp 133–134 °C; IR 1685 (CO), 1639 (CO) $cm^{-1}$; $^1H$ NMR: $\delta$

1.21 (3H, t, $J$ = 6.9 Hz, $CH_3$), 2.46 (3H, s, $CH_3$), 2.75–2.87 (4H, m, 2 × $CH_2$), 3.04 (6H, s, 2 × $CH_3$), 4.14 (2H, q, $J$ = 6.9 Hz, $CH_2$), 5.61 (2H, s, $CH_2$), 6.92 (2H, d, $J$ = 6.9 Hz, H-2′ and H-6′), 7.18–7.32 (4H, m, H-3′, H-4′, H-5′ and CH), $^{13}$C NMR: $\delta$ 10.8 (q), 14.1 (q), 22.9 (t), 24.0 (t), 43.1 (2 × q), 47.2 (t), 59.4 (t), 103.2 (s), 115.5 (s), 119.3 (s), 125.7 (2 × d), 126.9 (d), 128.5 (2 × d), 134.2 (s), 138.0 (s), 138.9 (s), 147.6 (d), 160.9 (CO), 183.1 (CO). Anal. Calcd for $C_{22}H_{26}N_2O_3$: C, 72.11; H, 7.15; N, 7.64. Found: C, 72.42; H, 6.92; N, 7.53.

### 4.1.4. General procedure for the preparation of 8-substituted-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinolin-2-ones (4b–i)

To a suspension of **7b–i** (4 mmol) in anhydrous ethanol (30 mL), phenylsulfonylacetonitrile (1.09 g, 6 mmol) in anhydrous ethanol (50 mL) was added dropwise under nitrogen atmosphere, and the reaction mixture was heated under reflux for 24–72 h. Upon cooling, a precipitate formed which was filtered and purified by recrystallization or by column chromatography (Sepacore Büchi) using DCM/AcOEt 9:1 as eluent.

**4.1.4.1. 8-Methyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinolin-2-one (4b).** This product was obtained from the reaction of **7b** after 72 h (yield 65%), or by heating for the same time the intermediate **8** with stoichiometric amount of diethylamine (yield 40%). Yellow solid; mp: 345–346 °C; IR: $\nu$ 3106 (NH), 1633 (CO) cm$^{-1}$; $^1$H NMR: $\delta$ 2.59–2.66 (2H, t, $J$ = 6.0 Hz, $CH_2$), 2.66 (2H, t, $J$ = 6.0 Hz, $CH_2$), 3.62 (3H, s, $CH_3$), 6.63 (1H, s, H-7), 7.49–7.64 (4H, m, H-3′, H-4′, H-5′, H-9), 7.96 (2H, d, $J$ = 7.8 Hz, H-2′, H-6′), 8.09 (1H, s, H-4), 12.23 (1H, s, NH); $^{13}$C NMR: $\delta$ 19.5 (t), 26.4 (t), 36.4 (q), 109.2 (s), 112.7 (s), 119.3 (d), 120.4 (s), 121.5 (s), 123.7 (d), 127.7 (2 × d), 128.6 (2 × d), 132.9 (d), 141.1 (s), 143.7 (d), 146.4 (s), 157.4 (CO). Anal. Calcd for $C_{18}H_{16}N_2O_3S$: C, 63.51; H, 4.74; N, 8.23. Found: C, 63.84; H, 4.60; N, 8.00. When the same reaction from **7b** was conducted at room temperature for 24 h the intermediate *3-(2-methyl-4-oxo-2,4,6,7-tetrahydro-5H-isoindol-5-ylidene)-2-(phenylsulfonyl) propanenitrile* **(8)** was isolated. Yellow solid; yield 62%; mp: 221–222 °C; IR: $\nu$ 2198 (CN), 1695 (CO) cm$^{-1}$; $^1$H NMR: $\delta$ 2.59 (4H, m, 2 × $CH_2$), 3.68 (3H, s, $CH_3$), 5.24 (1H, d, $J$ = 10.2 Hz, CH), 5.76 (1H, s, H-1), 5.88 (1H, d, $J$ = 10.2 Hz, CH), 6.63 (1H, s, H-3), 7.58–7.81 (5H, m, Ar-H-H); $^{13}$C NMR: $\delta$ 22.7 (t), 27.6 (t), 36.8 (q), 116.6 (s), 122.6 (d), 124.7 (s), 127.8 (d), 128.9 (2 × d), 129.0 (2 × d), 129.4 (d), 133.8 (d), 134.3 (d), 137.4 (s), 140.4 (s), 141.1 (s), 163.9 (CO). Anal. Calcd for $C_{18}H_{16}N_2O_3S$: C, 63.51; H, 4.74; N, 8.23. Found: C, 63.18; H, 4.86; N, 8.44.

**4.1.4.2. 8-Benzyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinolin-2-one (4c).** This product was obtained from the reaction of **7c** after 48 h. Yellow solid; yield 55%; mp: 293–294 °C; IR: $\nu$ 2933 (NH), 1641 (CO), cm$^{-1}$; $^1$H NMR: $\delta$ 2.59–2.69 (4H, m, 2 × $CH_2$), 5.10 (2H, s, $CH_2$), 6.75 (1H, s, H-7), 7.22–7.36 (5H, m, Ar-H), 7.52–7.63 (4H, m, H-3′, H-4′, H-5′, H-9), 7.96 (2H, d, $J$ = 7.8 Hz, H-2′, H-6′), 8.11 (1H, s, H-4), 12.29 (1H, s, NH); $^{13}$C NMR: $\delta$ 19.4 (t), 26.35 (t), 52.8 (t), 109.3 (s), 113.0 (s), 118.5 (d), 120.6 (s), 121.1 (d), 121.5 (s), 127.4 (d), 127.5 (2 × d), 127.7 (2 × d), 128.6 (2 × d), 128.7 (2 × d), 132.8 (d), 137.6 (s), 141.1 (s), 143.7 (d), 146.3 (s), 157.3 (CO). Anal. Calcd for $C_{24}H_{20}N_2O_3S$: C, 69.21; H, 4.84; N, 6.73. Found: C, 69.04; H, 4.60; N, 6.80.

**4.1.4.3. 8-Phenyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinolin-2-one (4d).** This product was obtained from the reaction of **7d** after 48 h. Yellow solid; yield 60%; mp: 217–220 °C; IR: $\nu$ 2933 (NH), 1633 (CO) cm$^{-1}$; $^1$H NMR: $\delta$ 2.68–2.81 (4H, m, 2 × $CH_2$), 7.31 (1H, s, H-7), 7.50–7.66 (6H, m, H-3′, H-4′, H-5′, H-3″, H-4″, H-5″), 7.96 (2H, d, $J$ = 7.8 Hz, H-2′ and H-6′), 8.00 (2H, d, $J$ = 7.8 Hz, H-2″ and H-6″), 8.12 (1H, s, H-9), 8.18 (1H, s, H-4), 12.34 (1H, s, NH); $^{13}$C NMR: $\delta$ 19.4 (t), 26.2 (t), 110.1 (s), 115.1 (s), 116.5 (d), 118.4 (d), 119.5 (2 × d), 121.9 (s), 123.2 (s), 126.3 (d), 127.8 (2 × d), 128.7 (2 × d), 129.9 (2 × d), 132.9 (d), 139.1 (s), 140.9 (s), 144.0 (d), 145.4 (s), 157.2 (CO). Anal. Calcd for $C_{23}H_{18}N_2O_3S$: C, 68.64; H, 4.51; N, 6.96. Found: C, 69.00; H, 4.32; N, 6.68.

**4.1.4.4. Ethyl 9-methyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinoline-2-one-7-carboxylate (4e).** This product was obtained from the reaction of **7e** after 24 h. Yellow solid; yield 70%; mp: 324–325 °C; IR: $\nu$ 3299 (broad, 2 × NH), 1702 (CO), 1643 (CO) cm$^{-1}$; $^1$H NMR: $\delta$ 1.29 (3H, t, $J$ = 7.0 Hz, $CH_3$), 2.51 (3H, s, $CH_3$), 2.72 (2H, t, $J$ = 6.4 Hz, $CH_2$), 2.88 (2H, t, $J$ = 6.4 Hz, $CH_2$), 4.24 (2H, q, $J$ = 7.0 Hz, $CH_2$), 7.54–7.66 (3H, m, H-3′, H-4′, H-5′), 7.97 (2H, d, $J$ = 7.2 Hz, H-2′ and H-6′), 8.14 (1H, s, H-4), 11.23 (1H, s, NH), 11.97 (1H, s, NH); $^{13}$C NMR: $\delta$ 12.5 (q), 14.3 (q), 20.3 (t), 26.4 (t), 59.6 (t), 113.4 (s), 116.0 (s), 119.5 (s), 127.8 (2 × d), 128.2 (s), 128.7 (2 × d), 130.2 (s), 133.0 (d), 134.2 (s), 140.9 (s), 141.7 (s), 143.2 (d), 157.4 (CO), 160.4 (CO). Anal. Calcd for $C_{21}H_{20}N_2O_5S$: C, 61.15; H, 4.89; N, 6.79. Found: C, 60.54; H, 5.10; N, 6.58.

**4.1.4.5. Ethyl 8,9-dimethyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinoline-2-one-7-carboxylate (4f).** This product was obtained from the reaction of **7f** after 24 h reflux. Yellow solid; yield 64%; mp: 239–240 °C; IR: $\nu$ 2981 (NH), 1699 (CO), 1646 (CO) cm$^{-1}$; $^1$H NMR: $\delta$ 1.30 (3H, t, $J$ = 6.7 Hz, $CH_3$), 2.53 (3H, s, $CH_3$), 2.73 (2H, t, $J$ = 7.1 Hz, $CH_2$), 2.88 (2H, t, $J$ = 7.1 Hz, $CH_2$), 3.77 (3H, s, $CH_3$), 4.23 (2H, q, $J$ = 6.7 Hz, $CH_2$), 7.54–7.69 (3H, m, H-3′, H-4′, H-5′), 7.94 (2H, d, $J$ = 7.8 Hz, H-2′ and H-6′), 8.11 (1H, s, H-4), 11.97 (1H, s, NH); $^{13}$C NMR: $\delta$ 11.7 (q), 14.1 (q), 21.1 (t), 26.5 (t), 32.4 (q), 59.5 (t), 113.5 (s), 117.4 (s), 118.6 (s), 127.7 (2 × d), 127.8 (s), 128.6 (2 × d), 130.9 (s), 132.9 (d), 136.40 (s), 140.4 (s), 140.9 (s), 142.6 (d), 157.7 (CO), 160.7 (CO). Anal. Calcd for $C_{22}H_{22}N_2O_5S$: C, 61.96; H, 5.20; N, 6.57. Found: C, 62.08; H, 5.36; N, 6.30. This product was also isolated (12%) from the methylation of **4e**.

**4.1.4.6. Ethyl 8-benzyl-9-methyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinoline-2-one-7-carboxylate (4g).** This product was obtained from the reaction of **7g** after 24 h reflux. Yellow solid; yield 65%; mp: 194–195 °C; IR: $\nu$ 2900 (NH), 1695 (CO), 1643 (CO) cm$^{-1}$; $^1$H NMR: $\delta$ 1.22 (3H, t, $J$ = 7.1 Hz, $CH_3$), 2.50 (3H, s, $CH_3$), 2.81 (2H, t, $J$ = 7.1 Hz, $CH_2$), 2.95 (2H, t, $J$ = 7.1 Hz, $CH_2$), 4.16 (2H, q, $J$ = 7.1 Hz, $CH_2$), 5.64 (2H, s, $CH_2$), 6.92–6.95 (2H, m, H-2″ and H-6″), 7.21–7.34 (3H, m, H-3″, H-4″, H-5″), 7.54–7.67 (3H, m, H-3′, H-4′, H-5′), 7.95–7.98 (2H, m, H-2′ and H-6′), 8.16 (1H, s, H-4), 11.53 (1H, s, NH); $^{13}$C NMR: $\delta$ 11.6 (q), 14.1 (q), 21.2 (t), 26.5 (t), 47.5 (t), 59.7 (t), 117.2 (s), 125.6 (2 × d), 125.7 (s), 126.9 (d), 127.8 (2 × d), 128.5 (2 × d), 128.6 (s), 128.8 (2 × d), 131.7 (s), 133.1 (d), 136.7 (s), 137.8 (s), 140.8 (d), 140.9 (s), 141.0 (s), 144.8 (s), 157.7 (CO), 160.6 (CO). Anal. Calcd for $C_{28}H_{26}N_2O_5S$: C, 66.92; H, 5.21; N, 5.57. Found: C, 67.18; H, 5.02; N, 5.26.

**4.1.4.7. 8,9-Dimethyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinolin-2-one (4h).** This product was obtained from the reaction of **7h** after 24 h reflux. Brown solid; yield 68%; mp: 244–245 °C; IR: $\nu$ 2915 (NH), 1635 (CO) cm$^{-1}$; $^1$H NMR: $\delta$ 2.33 (3H, s, $CH_3$), 2.47 (2H, t, $J$ = 6.0 Hz, $CH_2$), 3.32 (2H, t, $J$ = 6.0 Hz, $CH_2$), 3.42 (3H, s, $CH_3$), 6.51 (1H, s, H-7), 7.46–7.53 (3H, m, H-3′, H-4′, H-5′), 7.90 (2H, d, $J$ = 7.0 Hz, H-2′ and H-6′), 8.02 (1H, s, H-4), 11.21 (1H, s, NH); $^{13}$C NMR: $\delta$ 10.9 (q), 19.5 (t), 26.9 (t), 32.8 (q), 110.8 (s), 111.9 (s), 117.3 (d), 118.1 (s), 120.0 (s), 127.3 (2 × d), 128.2 (2 × d), 129.2 (s), 132.4 (d), 140.8 (s),

141.7 (d), 148.8 (s), 157.0 (CO). Anal. Calcd for $C_{19}H_{18}N_2O_3S$: C, 64.39; H, 5.12; N, 7.90. Found: C, 64.14; H, 5.00; N, 8.18.

**4.1.4.8. 8-Benzyl-9-methyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinolin-2-one (4i).** This product was obtained from the reaction of **7i** after 24 h reflux. Yellow solid; yield 72%; mp: 252–254 °C; IR: *v* 2938 (NH), 1641 (CO) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 2.55 (3H, s, $CH_3$), 2.58–2.62 (4H, m, 2 × $CH_2$), 5.06 (2H, s, $CH_2$), 6.49 (1H, s, H-7), 7.06–7.42 (8H, m, Ar-H), 7.89 (2H, d, *J* = 7.3 Hz, H-2′ and H-6′), 8.16 (1H, s, H-4), 12.21 (1H, s, NH); $^{13}C$ NMR ($CDCl_3$): $\delta$ 12.4 (q), 20.9 (t), 28.3 (t), 51.0 (t), 112.1 (s), 112.7 (s), 117.7 (d), 121.3 (s), 122.5 (s), 127.2 (2 × d), 128.4 (d), 128.6 (2 × d), 128.8 (2 × d), 129.5 (2 × d), 130.1 (s), 133.0 (d), 137.0 (s), 141.3 (s), 144.4 (d), 148.0 (s), 159.3 (CO). Anal. Calcd for $C_{25}H_{22}N_2O_3S$: C, 69.75; H, 5.15; N, 6.51. Found: C, 69.54; H, 5.30; N, 6.78.

**4.1.5. General procedure for the preparation of the 9-methyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinoline-2-one-7-carboxylic acids (4j–l)**

To a suspension of **4e–g** (1 mmol) in ethanol (5 mL), KOH (0.34 g, 6 mmol) dissolved in a small amount of water (2 mL) was added. The mixture was heated under reflux for 24 h, then poured onto crushed ice and extracted with diethyl ether. The aqueous layer was acidified with 6 M hydrochloric acid and the precipitate formed was filtered off and washed with small amount of ethanol to afford the desired carboxylic acid derivatives.

**4.1.5.1. 9-Methyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinoline-2-one-7-carboxylic acid (4j).** This product was obtained from **4e**. White solid; yield 80%; mp: 243–244 °C; IR: *v* 3401 (OH), 3299 (broad, 2 × NH), 1672 (CO), 1655 (CO) $cm^{-1}$; $^1H$ NMR: $\delta$ 2.51 (3H, s, $CH_3$), 2.71 (2H, t, *J* = 6.2 Hz, $CH_2$), 2.89 (2H, t, *J* = 6.2 Hz, $CH_2$), 7.53–7.65 (3H, m, H-3′, H-4′, H-5′), 7.97 (2H, d, *J* = 7.5 Hz, H-2′ and H-6′), 8.14 (1H, s, H-4), 11.90 (1H, s, NH), 12.02 (2H, br s, NH and OH); $^{13}C$ NMR: $\delta$ 13.0 (q), 20.7 (t), 26.9 (t), 113.4 (s), 113.7 (s), 117.4 (s), 119.9 (s), 128.2 (2 × d), 129.2 (2 × d), 130.5 (s), 133.6 (d), 134.3 (s), 141.1 (s), 142.6 (d), 149.2 (s), 157.9 (CO), 162.4 (CO). Anal. Calcd for $C_{19}H_{16}N_2O_5S$: C, 59.37; H, 4.20; N, 7.29. Found: C, 59.57; H, 4.10; N, 7.38.

**4.1.5.2. 8,9-Dimethyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinoline-2-one-7-carboxylic acid (4k).** This product was obtained from **4f**. White solid; yield 85%; mp: 230–231 °C; IR: *v* 3197 (OH), 2956 (NH), 1648 (CO), 1646 (CO) $cm^{-1}$; $^1H$ NMR: $\delta$ 2.52 (3H, s, $CH_3$), 2.71 (2H, t, *J* = 7.0 Hz, $CH_2$), 2.91 (2H, t, *J* = 7.0 Hz, $CH_2$), 3.78 (3H, s, $CH_3$), 7.54–7.71 (3H, m, H-3′, H-4′, H-5′), 7.96 (2H, d, *J* = 7.0 Hz, H-2′, H-6′), 8.11 (1H, s, H-4), 12.03 (2H, br s, NH and OH); $^{13}C$ NMR: $\delta$ 11.8 (q), 21.1 (t), 26.6 (t), 32.4 (q), 113.1 (s), 115.7 (s), 118.1 (s), 118.7 (s), 127.8 (2 × d), 128.7 (2 × d), 130.1 (d), 133.1 (s), 136.1 (s), 139.9 (s), 140.9 (d), 150.0 (s), 157.6 (CO), 162.3 (CO). Anal. Calcd for $C_{20}H_{18}N_2O_5S$: C, 60.29; H, 4.55; N, 7.03. Found: C, 60.58; H, 4.32; N, 7.20.

**4.1.5.3. 8-Methyl-9-benzyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinoline-2-one-7-carboxylic acid (4l).**

This product was obtained from of **4g**. White solid; yield 90%; mp: 185–186 °C; IR: *v* 3457 (OH), 2969 (NH), 1656 (CO), 1654 (CO) $cm^{-1}$; $^1H$ NMR: $\delta$ 2.48 (3H, s, $CH_3$), 2.78 (2H, t, *J* = 6.4 Hz, $CH_2$), 2.99 (2H, t, *J* = 6.4 Hz, $CH_2$), 5.69 (2H, s, $CH_2$), 6.96 (2H, d, *J* = 6.5 Hz, H-2″ and H-6″), 7.21–7.34 (3H, m, H-3″, H-4″, H-5″), 7.58–7.66 (3H, m, H-3′, H-4′, H-5′), 7.96 (2H, d, *J* = 6.9 Hz, H-2′, H-6′), 8.16 (1H, s, H-4), 12.03 (2H, br s, NH and OH); $^{13}C$ NMR: $\delta$ 11.7 (q), 21.3 (t), 26.6 (t), 47.3 (t), 113.9 (s), 116.4 (s), 117.8 (s), 118.8 (s), 125.8 (2 × d), 126.9 (d), 127.8 (2 × d), 128.5 (2 × d), 128.8 (2 × d), 131.6 (s), 133.1 (d), 136.2 (s), 138.0 (d), 140.7 (s), 140.9 (s), 150.6 (s), 157.7 (CO), 162.2 (CO). Anal. Calcd for $C_{26}H_{22}N_2O_5S$: C, 65.81; H, 4.67; N, 5.90. Found: C, 65.55; H, 4.42; N, 6.02.

**4.1.6. General procedure for the preparation of 8-substituted 1-methyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinolin-2-ones (4m–r) and 8-substituted 2-methoxy-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinolines (4s–u)**

To a solution of the proper 8-substituted 3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinolin-2-one **4b–i** (15 mmol) dissolved in anhydrous DMF (20 mL), NaH (0.38 g, 16 mmoles) was added at 0 °C and the reaction mixture was stirred for 1 h at rt, then iodomethane (1 mL, 16 mmol) was added at 0 °C. The reaction mixture was stirred for 2 h, then poured onto crushed ice and the precipitate was filtered off. Column chromatography of the residue, using DCM as eluent, gave the expected product.

**4.1.6.1. 1,8-Dimethyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinolin-2-one (4m).** This product was obtained from **4b**. Yellow solid; yield 55%; mp: 230–231 °C; IR: *v* 1643 (CO) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 2.65–2.74 (4H, m, 2 × $CH_2$), 3.71 (3H, s, $CH_3$), 3.73 (3H, s, $CH_3$), 6.51 (1H, s, H-9), 7.04 (1H, s, H-3), 7.46–7.51 (3H, m, H-3′, H-4′, H-5′), 8.10 (2H, d, *J* = 6.9 Hz, H-2′, H-6′), 8.19 (1H, s, H-4); $^{13}C$ NMR ($CDCl_3$): $\delta$ 20.2 (t), 29.3 (t), 33.7 (q), 36.7 (q), 112.1 (s), 114.2 (s), 118.3 (d), 120.9 (s), 123.3 (d), 124.2 (s), 128.4 (2 × d), 128.6 (2 × d), 132.7 (d), 140.7 (s), 141.8 (d), 148.4 (s), 157.9 (CO). Anal. Calcd for $C_{19}H_{18}N_2O_3S$: C, 64.39; H, 5.12; N, 7.90. Found: C, 64.12; H, 5.40; N, 7.68. From the same reaction mixture *2-methoxy-8-methyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-5H-pyrrolo[3,4-h]quinoline* **(4s)** was isolated as white solid; yield 18%; mp: 202–203 °C; IR: *v* 1572 (C═N) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 2.73 (2H, t, *J* = 7.5 Hz, $CH_2$), 2.90 (2H, t, *J* = 7.5 Hz, $CH_2$), 3.66 (3H, s, $CH_3$), 3.91 (3H, s, $CH_3$), 6.40 (1H, s, H-7), 7.13 (1H, s, H-9), 7.45–7.51 (3H, m, H-3′, H-4′, H-5′), 8.00 (2H, d, *J* = 6.8 Hz, H-2′, H-6′), 8.10 (1H, s, H-4); $^{13}C$ NMR ($CDCl_3$): $\delta$ 20.0 (t), 28.5 (t), 36.4 (q), 53.5 (q), 118.1 (d), 120.0 (d), 120.6 (s), 122.1 (s), 122.4 (s), 128.2 (2 × d), 128.4 (2 × d), 132.7 (d), 138.0 (d), 141.4 (s), 144.3 (s), 154.8 (s), 158.7 (s). Anal. Calcd for $C_{19}H_{18}N_2O_3S$: C, 64.39; H, 5.12; N, 7.90. Found: C, 64.56; H, 4.98; N, 8.08.

**4.1.6.2. 8-Benzyl-1-methyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinolin-2-one (4n).** This product was obtained from **4c**. Yellow solid; yield 62%; mp: 164–165 °C; IR: *v* 1643 (CO) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 2.67 (2H, t, *J* = 6.5 Hz, $CH_2$), 2.71 (2H, t, *J* = 6.5 Hz, $CH_2$), 3.70 (3H, s, $CH_3$), 5.09 (2H, s, $CH_2$), 6.56 (1H, s, H-7), 7.15–7.54 (9H, m, Ar-H and H-3), 8.12 (2H, d, *J* = 6.4 Hz, H-2′, H-6′), 8.20 (1H, s, H-4); $^{13}C$ NMR ($CDCl_3$): $\delta$ 20.3 (t), 29.2 (t), 33.7 (q), 53.9 (t), 112.2 (s), 114.5 (s), 117.7 (d), 121.1 (s), 122.7 (d), 124.3 (s), 127.1 (2 × d), 128.2 (d), 128.4 (2 × d), 128.6 (2 × d), 128.9 (2 × d), 132.7 (d), 136.4 (s), 140.6 (s), 141.9 (d), 148.3 (s), 157.9 (CO). Anal. Calcd for $C_{25}H_{22}N_2O_3S$: C, 69.75; H, 5.15; N, 6.51. Found: C, 70.02; H, 4.86; N, 6.84. From the same reaction mixture *8-benzyl-2-methoxy-3-(phenylsulfonyl)-1,56,8-tetrahydro-2H-pyrrolo[3,4-h]quinoline* **(4t)** was isolated as white solid; yield 20%; mp: 186–187 °C; IR: *v* 1572 (C═N) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): $\delta$ 2.74 (2H, t, *J* = 6.9 Hz, $CH_2$), 2.92 (2H, t, *J* = 6.9 Hz, $CH_2$), 3.90 (3H, s, $CH_3$), 5.05 (2H, s, $CH_2$), 6.46 (1H, s, H-7), 7.15–7.55 (9H, m, Ar-H and H-9), 7.98 (2H, d, *J* = 6.9 Hz, H-2′, H-6′), 8.12 (1H, s, H-4); $^{13}C$ NMR ($CDCl_3$): $\delta$ 20.1 (t), 28.5 (t), 53.5 (q), 53.7 (t), 117.6 (d), 117.9 (s), 119.5 (d), 120.9 (s), 122.2 (s), 122.6 (s), 127.1 (2 × d), 127.9 (d), 128.2 (2 × d), 128.4 (2 × d), 128.8 (2 × d), 132.7 (d), 137.3 (s), 138.2 (d), 141.4 (s), 148.6 (s), 158.7 (s). Anal. Calcd for $C_{25}H_{22}N_2O_3S$: C, 69.75; H, 5.15; N, 6.51. Found: C, 69.48; H, 5.40; N, 6.42.

**4.1.6.3. 1-Methyl-8-phenyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinolin-2-one (4o).** This product was obtained from the reaction of **4d**. Yellow solid; yield 68%; mp: 257–258 °C; IR: *ν* 1645 (CO) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): *δ* 2.67–2.77 (4H, m, 2 × $CH_2$), 3.80 (3H, s, $CH_3$), 6.97 (1H, s, H-7), 7.33–7.52 (9H, m, Ar-H and H-9), 8.12 (2H, d, *J* = 6.5 Hz, H-2′, H-6′), 8.24 (1H, s, H-4); $^{13}C$ NMR ($CDCl_3$): *δ* 20.2 (t), 29.1 (t), 33.9 (q), 112.7 (s), 116.0 (s), 116.3 (d), 120.4 (d), 120.9 (2 × d), 122.1 (s), 125.4 (s), 127.0 (d), 128.4 (2 × d), 128.6 (2 × d), 129.8 (2 × d), 132.8 (d), 139.4 (s), 140.5 (s), 142.0 (d), 147.7 (s), 157.8 (CO). Anal. Calcd for $C_{24}H_{20}N_2O_3S$: C, 69.21; H, 4.84; N, 6.73. Found: C, 69.02; H, 5.12; N, 6.54. From the same reaction mixture *8-phenyl-2-methoxy-3-(phenylsulfonyl)-6,8-dihydro-5H-pyrrolo[3,4-h]quinoline* **(4u)** was isolated as white solid; yield 8%; mp 284–286 °C; IR: *ν* 1572 (C=N) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): *δ* 2.80–3.01 (4H, m, 2 × $CH_2$), 4.14 (3H, s, $CH_3$), 6.95 (1H, s, H-7), 7.28–7.57 (9H, m, Ar-H, H-9), 8.06 (2H, d, *J* = 6.0 Hz, H-2′, H-6′), 8.12 (1H, s, H-4); $^{13}C$ NMR: *δ* 20.0 (t), 30.1 (t), 63.7 (q), 115.7 (d), 116.5 (s), 118.4 (s), 119.1 (d), 120.7 (2 × d), 123.2 (s), 126.6 (d), 128.0 (2 × d), 128.9 (2 × d), 129.7 (2 × d), 132.3 (s), 132.8 (d), 133.4 (d), 135.3 (s), 136.9 (s), 139.9 (s), 141.4 (s). Anal. Calcd for $C_{24}H_{20}N_2O_3S$: C, 69.21; H, 4.84; N, 6.73. Found: C, 69.42; H, 4.68; N, 6.86.

**4.1.6.4. Ethyl 1,9-dimethyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinoline-2-one-7-carboxylate (4p).** This product was obtained from the reaction of **4e**. Yellow solid; yield 34%; mp: 248–249 °C; IR: *ν* 3284 (NH), 1660 (CO), 1655 (CO) $cm^{-1}$; $^1H$ NMR: *δ* 1.30 (3H, t, *J* = 6.8 Hz, $CH_3$), 2.56 (3H, s, $CH_3$), 2.88–2.93 (4H, m, 2 × $CH_2$), 3.85 (3H, s, $CH_3$), 4.24 (2H, q, *J* = 6.8 Hz, $CH_2$), 7.57–7.73 (3H, m, H-3′, H-4′, H-5′), 7.93 (2H, d, *J* = 6.8 Hz, H-2′, H-6′), 8.11 (1H, s, H-4), 11.90 (1H, s, NH); $^{13}C$ NMR: *δ* 13.0 (q), 14.4 (q), 20.2 (t), 27.0 (t), 53.8 (q), 59.5 (t), 115.6 (s), 117.1 (s), 122.2 (s), 127.7 (2 × d), 129.1 (2 × d), 129.9 (s), 133.4 (d), 134.7 (s), 137.6 (d), 140.9 (s), 147.2 (s), 154.9 (s), 157.9 (CO), 160.5 (CO). Anal. Calcd for $C_{22}H_{22}N_2O_5S$: C, 61.96; H, 5.20; N, 6.57. Found: C, 61.82; H, 5.08; N, 6.84.

**4.1.6.5. Ethyl 1,8,9-trimethyl-3-(phenylsulfonyl)-2,5,6,8-tetrahydro-1*H*-pyrrolo[3,4-*h*]quinoline-2-one-7-carboxylate (4q).** This product was obtained from the reaction of **4f**. Yellow solid; yield 65%; mp: 165–166 °C; IR: *ν* 1684 (CO), 1682 (CO) $cm^{-1}$; $^1H$ NMR: *δ* 1.30 (3H, t, *J* = 7.2 Hz, $CH_3$), 2.65 (3H, s, $CH_3$), 2.88 (2H, t, *J* = 6.5 Hz, $CH_2$), 2.95 (2H, t, *J* = 6.5 Hz, $CH_2$), 3.78 (3H, s, $CH_3$), 3.86 (3H, s, $CH_3$), 4.23 (2H, q, *J* = 7.2 Hz, $CH_2$), 7.58–7.71 (3H, m, H-3′, H-4′, H-5′), 7.95 (2H, d, *J* = 6.8 Hz, H-2′, H-6′), 8.13 (1H, s, H-4); $^{13}C$ NMR: *δ* 11.3 (q), 14.2 (q), 20.9 (t), 26.8 (t), 32.3 (q), 53.8 (q), 59.5 (t), 116.0 (s), 117.1 (s), 117.3 (s), 122.6 (s), 127.8 (2 × d), 129.1 (2 × d), 130.8 (s), 133.4 (d), 137.0 (s), 137.6 (d), 140.8 (s), 154.5 (s), 157.7 (CO), 160.9 (CO). Anal. Calcd for $C_{23}H_{24}N_2O_5S$: C, 62.71; H, 5.49; N, 6.36. Found: C, 62.90; H, 5.18; N, 6.63. This product was also isolated (15%) from the methylation of **4e**.

**4.1.6.6. Ethyl-1,9-dimethyl-8-benzyl-3-(phenylsulfonyl)-1,5,6,8-tetrahydro-2*H*-pyrrolo[3,4-*h*]quinoline-2-one-7-carboxylate (4r).** This product was obtained from the reaction of **4g**. Yellow solid; yield 68%; mp: 138–139 °C; IR: *ν* 1692 (CO), 1690 (CO) $cm^{-1}$; $^1H$ NMR ($CDCl_3$): *δ* 1.30 (3H, t, *J* = 6.9 Hz, $CH_3$), 2.64 (3H, s, $CH_3$), 2.93 (2H, t, *J* = 7.0 Hz, $CH_2$), 3.11 (2H, t, *J* = 7.0 Hz, $CH_2$), 3.90 (3H, s, $CH_3$), 4.24 (2H, q, *J* = 6.9 Hz, $CH_2$), 5.62 (2H, s, $CH_2$), 6.95 (2H, d, *J* = 6.9 Hz, H-2″ and H-6″), 7.19–7.30 (3H, m, H-3″, H-4″, H-5″), 7.47–7.56 (3H, m, H-3′, H-4′, H-5′), 8.01 (2H, d, *J* = 6.6 Hz, H-2′, H-6′), 8.15 (3H, s, H-4), 12.3 (1H, s, NH); $^{13}C$ NMR ($CDCl_3$): *δ* 11.6 (q), 14.3 (q), 21.6 (t), 27.8 (t), 48.1 (t), 53.9 (q), 59.9 (t), 117.4 (s), 117.5 (s), 118.0 (s), 122.8 (s), 125.7 (2 × d), 127.0 (d), 128.3 (2 × d), 128.5 (2 × d), 128.6 (2 × d), 132.1 (s), 132.9 (d), 136.9 (s), 137.8 (d), 137.9 (s), 141.2 (s), 154.8 (s), 158.3 (CO), 161.5 (CO). Anal. Calcd for $C_{29}H_{28}N_2O_5S$: C, 67.42; H, 5.46; N, 5.42. Found: C, 67.74; H, 5.23; N, 5.60.

## 4.2. Biology

### 4.2.1. Chemicals

Plasmid pBR322 was bought from Fermentas (Burlington, Canada). Base excision repair enzymes (BER), Formamydo-pyrimidine glycosilase (Fpg) and Endonuclease III (Endo III), were a generous gift from Dr. S. Boiteux (CEA, Fontenay aux Roses, France). If not otherwise indicated, all the chemicals were purchased from Sigma-Aldrich (Milano, Italy).

### 4.2.2. Irradiation procedure

HPW 125 Philips lamps, mainly emitting at 365 nm, were used for irradiation experiments. The spectral irradiance of the source was 4.0 mW $cm^{-2}$ as measured, at the sample level, by a Cole-Parmer Instrument Company radiometer (Niles, IL), equipped with a 365-CX sensor.

### 4.2.3. Spectrophotometric measurement

All spectrophotometric measures were performed using a Perkin–Elmer Lambda 15 spectrophotometer and emission spectra were recorded with a Perkin–Elmer LS-50B fluorimeter.

### 4.2.4. Cellular phototoxicity

Human promyelocytic leukaemia cells (HL-60) and human lymphoblastoid cells (Jurkat) were grown in RPMI-1640 medium, human breast adenocarcinoma cells (MCF-7) and human kerat inocytes (NCTC-2544) were grown in DMEM medium, intestinal adenocarcinoma cells (LoVo) were grown in Ham's F12 medium. All cellular media were supplemented with 115 units/mL of penicillin G, 115 μg/ml streptomycin and 10% foetal bovine serum (Invitrogen, Milano, Italy). Individual wells of a 96-well tissue culture microtiter plate were inoculated with 100 μl of complete medium containing 8 × $10^3$ HL-60, Jurkat cells or 5 × $10^3$ MCF-7, LoVo and NCTC-2544 cells. Plates were incubated at 37 °C in a humidified 5% $CO_2$ incubator for 18 h prior the experiments. Drugs were dissolved in DMSO and then diluted with Hank's Balanced Salt Solution (HBSS, pH 7.2) for phototoxicity test. After medium removal, 100 μl of the drug solution were added to each well and incubated at 37 °C for 30 min and then irradiated (2.5 or 3.75 J/$cm^2$). After irradiation, drug solutions were replaced with medium and plates were incubated for 72 h. Cell viability was assayed by MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide)] test, as previously described.[12] Analogous experiments were performed in the presence of various additives.

### 4.2.5. Cellular localization studies

NCTC-2544 cells were allowed to attach in a sterile Petri dishes and treated with 4q at the concentration of 10 μM for 2 h, then washed with HBSS, and incubated for further 30 min in the presence of Rhodamine 123 a fluorescence probe which stains mitochondria,[26b] LysoTracker RED, as fluorescent probe to stain lysosomes, CellMask™ Orange, a plasma membrane probes and ER Tracking™ a specific stain for the endoplasmic reticulum. All of these probes were purchase d by Molecular Probes. Cellular fluorescence images were acquired with an video-confocal microscope (NIKON), using a Nir Apo 60X/1.0W water immersion objective (NIKON). Emission filter settings were used to separate the emission of the probes from that of the test compound.

### 4.2.6. Externalization of phosphatidylserine

Surface exposure of phosphatidylserine (PS) by apoptotic cells was measured by flow cytometry with a Coulter Cytomics FC500 (Becton Dickinson) by adding Annexin V–FITC to cells according

to the manufacturer's instructions (Annexin V Fluos Roche Diagnostic). Simultaneously cells were stained with PI. Excitation was set at 488 nm and the emission filters were at 525 nm.[19]

#### 4.2.7. Cell cycle analysis

For flow cytometric analysis of DNA content, $5 \times 10^5$ Jurkat cells in exponentially growth were irradiated (2.5 J/cm$^2$) in the presence of various concentrations of compounds. After 24 h of incubation, cells were fixed in ice-cold ethanol (70%), then treated with lysis buffer containing RNAseA, and finally stained with propidium iodide (PI, 10 μg/ml). Samples were analyzed on a Coulter Cytomics FC500 (Becton Dickinson) flow cytometer. For cell cycle analysis, results were examined using MultiCycle for Windows (Phoenix Flow Systems, San Diego, CA), as previously reported.[14] Data were expressed as fractions of cells in the different cycle phases.

#### 4.2.8. Mitochondrial membrane potential

The mitochondrial membrane potential was measured with the lipophilic cation 5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazolcarbocyanine (JC-1, Molecular Probes). After 6, 12 and 24 h from the irradiation (2.5 J/cm$^2$), Jurkat were collected by centrifugation and resuspended in HBSS containing the JC-1 at the concentration of 1 μg/ml. The cytofluorimetric analysis (Coulter Cytomics FC500) was performed collecting green (FL1) and orange (FL2) fluorescence in at least 10000 events for each sample.[25]

#### 4.2.9. Lysosomal stability assessment

Jurkat cells were assessed for lysosomal stability using the acridine orange (AO) uptake method.[27] After 6, 12 and 24 h from irradiation (2.5 J/cm$^2$), cells were collected by centrifugation and resuspended in RPMI-1640 containing AO at the concentration of 1 μM. The fluorescence was directly recorded with the flow cytometer (Coulter Cytomics FC500) using the 488 nm wavelength as excitation and the emission in FL3 channel.

#### 4.2.10. Linear dichroism

LD measurements were performed with a Jasco J500A circular dichroism spectropolarimeter (Jasco, Cremella Italy), converted for LD and equipped with an IBM PC and Jasco J interface. For these analysis, the sample orientation was obtained using a flow device designed by Wada and Kozawa,[31] which presents a cylindrical rotating couvette, a 0.14 cm optical path and a constant flow of 800 rpm. LD spectra were performed using different [Drug]/[DNA] ratios, dissolved in phosphate buffer (10 mM, pH 7.2).

#### 4.2.11. pBR322 DNA stand breaks

Each pBR322 DNA sample (100 ng) dissolved in phosphate buffer (10 mM, pH 7.2) was irradiated with UV-A (3.75 J/cm$^2$) in the presence of the compounds and of some additives. Some experiments were also performed under Nitrogen flux or in deuterated phosphate buffer as described previously.[32,33] After irradiation, two aliquots of sample were incubated at 37 °C with Fpg (Formamydo pyrimidin glycosilase) and Endo III (Endonuclease III), respectively as described by Epe et al.[29] Prior to the run, loading buffer (0.25% bromophenol blue, 0.25% xilene cianol, 30% glycerol in water) was added to DNA. Samples were loaded on 1% agarose gel and the run was carried out in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) at 80 V for 2 h. After staining with ethidium bromide solution, gel was washed with water and the DNA bands were detected under UV radiation with a UV transilluminator. Photographs were taken by a digital photocamera Kodak DC256 and the quantification of the bands was achieved by image analyzer software Quantity One (Bio Rad, Milano Italy). The fractions of supercoiled (Form I) and open circular DNA (Form II) were calculated as described by others.[34]

#### 4.2.12. Lipid peroxidation

500.000 Jurkat cells were irradiated (2.5 and 3.75 J/cm$^2$) in the presence of the most active compounds and then they were incubated for 24 h. Lipid peroxidation was measured using a thiobarbituric acid assay as described by Morliere et al.[30] Briefly, after cell centrifugation, 900 μl of supernatant were collected and put at −20 °C after having added 90 μl of 2,6-di-tert-butyl-*p*-cresol (BHT, 2% in absolute ethanol). Cells were washed, resuspended in 500 μl of water. Cells (400 μl) were lysed with 400 μl of SDS (1% in water). This suspension was divided into two aliquots: 50 μl of BHT were added in 500 μl; 300 μl were used for protein quantification with Peterson method.[35] A standard curve of 1,1,3,3 tetraethoxypropane was used to quantify the amount of produced malondialdehyde. Data were expressed in terms of nanomoles of TBARS normalized to the total protein content in an aliquot of the cell extract.

## Acknowledgment

This work was financially supported by Ministero dell'Istruzione dell'Università e della Ricerca.

## References and notes

1. Dall'Acqua, F.; Viola, G. D. Vedaldi. In *The CRC Handbook of Organic Photochemistry and Photobiology*; Hoorspool, W. M., Lenci, F., Eds.; CRC Press: London, 2004; pp 1–17.
2. (a) Stern, R. S. *N. Eng. J. Med.* **2007**, *357*, 682; (b) Danno, K. *J. Dermatol. Sci.* **1999**, *19*, 85; (c) Falabella, R.; Barona, M. I. *Pigment Cell Melanoma Res.* **2009**, *22*, 42.
3. Edelson, R.; Berger, C.; Gasparro, F.; Jegasothy, B.; Heald, P.; Wintroub, B.; Vonderheid, E.; Knobler, R.; Wolff, K.; Plewig, G.; McKiernan, G.; Christiansen, I.; Oster, M.; Honigsmann, H.; Wilford, H.; Koroska, E.; Rehle, T.; Stingl, G.; Laroche, L. *N. Eng. J. Med.* **1987**, *316*, 297.
4. Dalla Via, L.; Marciani Magno, S. *Curr. Med. Chem.* **2001**, *8*, 1405.
5. (a) Hivelin, M.; Siemionow, M.; Grimbert, P.; Lantieri, L. *Transpl. Immunol.* **2009**, *21*, 117; (b) Voss, C. Y.; Fry, T. J.; Coppes, M. J.; Blajchman, M. A. *Transfus. Med. Rev.* **2010**, *24*, 22.
6. (a) Mosti, L.; Lo Presti, E.; Menozzi, G.; Marzano, C.; Baccichetti, F.; Falcone, G.; Filippelli, W.; Piucci, B. *Il Farmaco* **1998**, *53*, 602; (b) Vedaldi, D.; Dolmella, A.; Moro, S.; Miolo, G.; Viola, G.; Caffieri, S.; Dall'Acqua, F. *Il Farmaco* **2004**, *59*, 125; (c) Rodighiero, P.; Pastorini, G.; Chilin, A.; Marotto, A. *J. Heterocycl. Chem.* **1998**, *35*, 847.
7. (a) Rodighiero, P.; Guiotto, A.; Chilin, A.; Bordin, F.; Baccichetti, F.; Carlassare, F.; Vedaldi, D.; Caffieri, S.; Pozzan, A.; Dall'Acqua, F. *J. Med. Chem.* **1996**, *39*, 1293; (b) Bordin, F.; Marzano, C.; Carlassare, F.; Rodighiero, P.; Guiotto, A.; Caffieri, S.; Baccichetti, F. *J. Photochem. Photobiol., B: Biol.* **1996**, *34*, 159; (c) Bettio, F.; Baccichetti, F.; Simonato, M.; Marzano, C.; Bordin, F. *Chem. Med. Chem.* **2006**, *1*, 525.
8. Marzano, C.; Baccichetti, F.; Carlassare, F.; Chilin, A.; Lora, S.; Bordin, F. *Photochem. Photobiol.* **2000**, *71*, 263.
9. (a) Chilin, A.; Dodoni, G.; Frezza, C.; Guiotto, A.; Barbieri, V.; Di Lisa, F.; Canton, M. *J. Med. Chem.* **2005**, *48*, 192; (b) Marzano, C.; Chilin, A.; Baccichetti, F.; Bettio, F.; Guiotto, A.; Miolo, G.; Bordin, F. *Eur. J. Med. Chem.* **2004**, *39*, 411; (c) Fossa, P.; Mosti, L.; Menozzi, G.; Marzano, C.; Baccichetti, F.; Bordin, F. *Bioorg. Med. Chem.* **2002**, *10*, 743; (d) Xie, L.; Qian, X.; Cui, J.; Xiao, Y.; Wang, K.; Wu, P.; Cong, L. *Bioorg. Med. Chem.* **2008**, *16*, 8713.
10. (a) Chen, I.-L.; Chang, K.-M.; Miaw, C.-L.; Liao, C.-H.; Chena, J.-J.; Wang, T.-C. *Bioorg. Med. Chem.* **2007**, *15*, 6527; (b) Cai, S. X.; Zhang, H.; Kemmitzer, W. E.; Jiang, S.; Drewe, J. A.; Storer, R. PCT Int. Appl. WO 2002092076, 2002.; (c) Soural, M.; Hlaváč, J.; Hradil, P.; Fryšová, I.; Hajdúch, M.; Bertolasi, V.; Maloň, M. *Eur. J. Med. Chem.* **2006**, *41*, 467.
11. (a) Gasparotto, V.; Castagliuolo, I.; Chiarelotto, G.; Pezzi, V.; Montanaro, D.; Brun, P.; Palù, G.; Viola, G.; Ferlin, M. G. *J. Med. Chem.* **2006**, *49*, 1910; (b) Ferlin, M. G.; Chiarelotto, G.; Gasparotto, V.; Dalla Via, L.; Pezzi, V.; Barzon, L.; Palù, G.; Castagliuolo, I. *J. Med. Chem.* **2005**, *48*, 3417.
12. Barraja, P.; Diana, P.; Montalbano, A.; Dattolo, G.; Cirrincione, G.; Viola, G.; Vedaldi, D.; Dall'Acqua, F. *Bioorg. Med. Chem.* **2006**, *14*, 8712.
13. (a) Barraja, P.; Cirrincione, G.; Dall'Acqua, F.; Salvador, A. ITAPD2009A000224.; (b) Barraja, P.; Caracausi, L.; Diana, P.; Carbone, A.; Montalbano, A.; Cirrincione, G.; Brun, P.; Palù, G.; Castagliuolo, I.; Dall'Acqua, F.; Vedaldi, D.; Salvador, A. *Bioorg. Med. Chem.* **2010**, *18*, 4830.
14. Barraja, P.; Spanò', V.; Diana, P.; Carbone, A.; Cirrincione, G.; Vedaldi, D.; Salvador, A.; Viola, G.; Dall'Acqua, F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1711.
15. Stanovnik, B.; Svete, J. *Chem. Rev.* **2004**, *104*, 2433.
16. Demas, J. N.; Crosby, G. A. *J. Phys. Chem.* **1971**, *75*, 991.
17. Ghose, A. K.; Crippen, G. M. *J. Comput. Chem.* **1986**, *7*, 565.
18. Eros, D.; Kövesdi, I.; Orfi, L.; Takács-Novák, K.; Acsády, G.; Kéri, G. *Curr. Med. Chem.* **2002**, *9*, 1819.
19. Caffieri, S. *Photochem. Photobiol. Sci.* **2002**, *1*, 149.

20. Caffieri, S.; Di Lisa, F.; Facco, M.; Semenzato, G.; Dall'Acqua, F.; Canton, M. *Blood* **2007**, *109*, 4988.
21. Vermes, I.; Haanen, C.; Steffens-Nakken, H.; Reutelingsperger, C. *J. Immunol. Methods* **1995**, *184*, 39.
22. Ouedraogo, G.; Morlière, P.; Bazin, M.; Santus, R.; Kratzer, B.; Miranda, M. A.; Castell, J. V. *Photochem. Photobiol.* **1999**, *70*, 123.
23. Ho, Y.; Wu, M.; Cheng, B.; Chen, Y.; Shin, M. *Biochim. Biophys. Acta* **2009**, *1788*, 1287.
24. Yuan, M. X.; Li, W.; Dalen, H.; Kama, R.; Sachs, L.; Brunk, U. T. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6286.
25. Salvioli, S.; Ardizzoni, A.; Franceschi, C.; Cossarizza, A. *FEBS Lett.* **1997**, *411*, 77.
26. (a) Viola, G.; Salvador, A.; Vedaldi, D.; Dall'Acqua, F.; Bianchi, N.; Zuccato, C.; Borgatti, M.; Lampronti, I.; Gambari, R. *Ann. N.Y. Acad. Sci.* **2009**, *1171*, 334; (b) Ouédraogo, G.; Morlière, P.; Santus, R.; Miranda, M. A.; Castell, J. V. *J. Photochem. Photobiol.* **2000**, *58*, 20; (c) Bastianon, C.; Zanoni, R.; Miolo, G.; Caffieri, S.; Reddi, E. *Int. J. Biochem. Cell Biol.* **2005**, *37*, 901; (d) Viola, G.; Salvador, A.; Cecconet, L.; Basso, G.; Vedaldi, D.; Dall'Acqua, F.; Aloisi, G.; Amelia, M.; Barbafina, A.; Latterini, L.; Elisei, F. *Photochem. Photobiol.* **2007**, *83*, 1415.
27. Zhao, M.; Eaton, J. W.; Brunk, U. T. *FEBS Lett.* **2000**, *485*, 104.
28. Nordén, B.; Kurucsev, T. *J. Mol. Recognit.* **1994**, *7*, 141.
29. Epe, B.; Pflaum, M.; Boiteux, S. *Mutat. Res.* **1993**, *299*, 135.
30. Morlière, P.; Moysan, A.; Santus, R.; Hüppe, G.; Mazière, J.; Dubertret, L. *Biochem. Biophys. Acta* **1991**, *1084*, 261.
31. Wada, A.; Kozawa, S. *J. Polim. Sci. A* **1964**, *2*, 853.
32. Viola, G.; Latterini, L.; Vedaldi, D.; Aloisi, G. G.; Dall'Acqua, F.; Gabellini, N.; Elisei, F.; Barbafina, A. *Chem. Res. Toxicol.* **2003**, *16*, 644.
33. Aloisi, G.; Amelia, M.; Barbafina, A.; Latterini, L.; Elisei, F.; Dall'Acqua, F.; Vedaldi, D.; Faccio, A.; Viola, G. *Photochem. Photobiol.* **2007**, *83*, 664.
34. Ciulla, T. A.; Van Camp, J. R.; Rosenfeld, E.; Kochevar, I. *Photochem. Photobiol.* **1989**, *49*, 293.
35. Peterson, G. L. *Anal. Biochem.* **1977**, *83*, 346.