Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 2809–2811

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Pyrrolo[2,3-*h*]quinolinones: Synthesis and Photochemotherapic Activity

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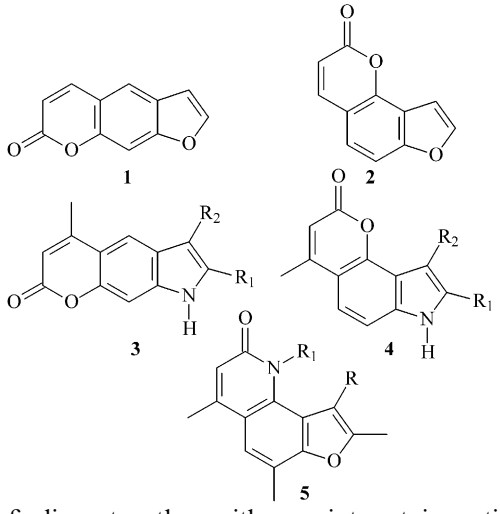
Received 24 December 2002; revised 21 February 2003; accepted 27 March 2003

Abstract—A series of derivatives of the new ring system pyrrolo[2,3-*h*]quinoline-2-one was synthesized and evaluated as photoreagents toward cultured human tumor cells. Remarkable phototoxycity resulted for some derivatives, especially those bearing the phenyl group at the 7-position.

Psoralens are a family of natural occurring linear furocoumarins, whose lead compound is **1**. They find application in the treatment of several skin diseases, such as psoriasis, vitiligo and tumors such as T-cell lymphoma[1] when used in conjunction with long-wave (320–400 nm) ultraviolet light (UV-A). The effectiveness of this treatment, called PUVA, is connected with the specific damage these linear compounds can induce to DNA. In the dark, psoralen molecules can form complex with DNA via intercalation of the furocoumarin moiety between base pairs of the nucleic acid; upon irradiation with UVA light a [2+2] photocycloaddition with the pyrimidine bases, particularly thymine, can occur involving one or both DNA strands. Thus two types of lesions can take place, monoadducts and crosslinks, which are responsible of short and long term side effects respectively: first ones involve a cycloaddition between psoralen and a pyrimidine base with a 1:1 molar ratio, and second ones a cycloaddition between one psoralen molecule and two pyrimidine moieties belonging to DNA complementary strands.[2,3]

For this reason, considerable efforts have been done in developing angular furocoumarins such as angelicin **2**, which on account of their geometry cannot crosslink DNA allowing only monofunctional photobinding and therefore reducing undesirable side effects especially long term ones such as genotoxicity and risk of skin cancer.

Many approaches have been followed to obtain new furocoumarin analogues with better DNA photobinding ability and lower toxicity. One of these involves the synthesis of heteroanalogues of **1** and **2** replacing one or both of the two intracyclic oxygen atom. The substitution with the nitrogen atom at the furan ring leads respectively to the linear or angular pyrrolocoumarins[4,5] **3**, **4** whilst the same type of replacement at the pyrone ring leads to the furoquinolinones[6] **5** which showed strong antiproliferative effect both in the dark and under light activation.



These findings together with our interest in antitumor polycondensed heterocycles containing the pyrrole/indole

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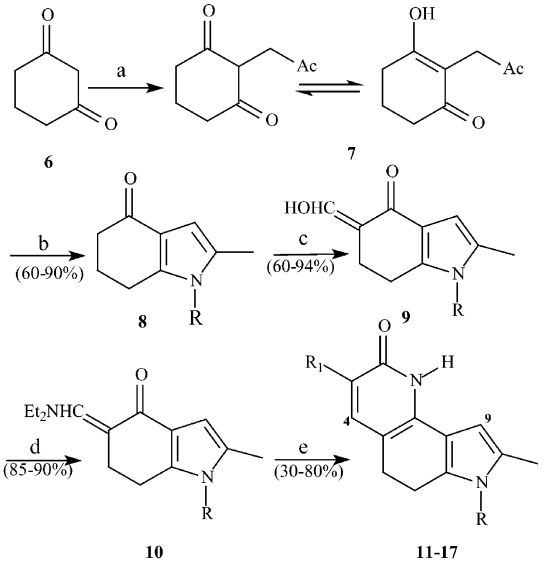
doi:10.1016/S0960-894X(03)00529-8

moiety[7a,b] prompted us to synthesize and study the photobiological activity of some new angelicin heteroanalogues in which both the oxygen atoms are replaced by nitrogens, leading to the new ring system pyrrolo[2,3-*h*]-quinoline-2-one.

Scheme 1 outlines the synthetic pathway followed for the synthesis of the desired new ring system. According to the Stetter and Lauterbach procedure,[8] the reaction of 1,3-dihydroresorcin **6** with chloroacetone and potassium hydroxide in methanol was stirred at room temperature for 48 h to afford the 1,3-acetonylcyclohexandione **7** as a mixture of the two tautomers which was used without any further purification. Condensation of **7** with the suitable amine in acetic acid at 60 °C for 2 h yielded the 1-substituted 1,5,6,7-tetrahydro-2-methyl-indole-4-ones **8** (60–80%). These latter by reaction with ethyl formate and potassium *t*-butoxide in benzene, under nitrogen atmosphere gave 1-substituted 5-hydroxy-methylene-1,5,6,7-tetrahydro-2-methyl-indole-4-ones **9** in good yield (60–94%).

The enaminoketones **10**, key intermediate for the synthesis of the new ring system pyrrolo[2,3-*h*]quinolinone, were prepared in very high yield (85-90%) from compounds **9** and diethylamine in benzene at room temperature for 24 h. Ring closure was accomplished by refluxing in ethanol for 24 h the versatile precursors obtained with cyanomethylene compounds such as phenylsulphonylacetonitrile, benzoylacetonitrile and malonitrile.[9] Recrystallization from ethanol gave compounds **11**–**17** in moderate to very good yield (30–80%).

The phototoxycity of title compounds, was investigated on a cultured cell line of human fibrosarcoma (HT-1080). Table 1 shows the extent of cell survival expressed as IC_{50} which is the concentration, expressed in μM, which induce 50% of inhibition of cell growth, after irradiation at different UVA doses.[10] Control experiments with drugs in the presence/absence of UVA light or drugs alone were carried out without significant cytotoxic effects (data not shown).



11 R= Ph, R'=SO_2Ph; **12** R=Ph, R_1=COPh, **13** R=Ph, R_1=CN; **14** R=Me, R_1=SO_2Ph
15 R=Me, R_1=COPh; **16** R=CH_2Ph, R_1=SO_2Ph, **17** R=CH_2Ph, R_1=COPh

Scheme 1. Synthesis of compounds **11**–**17:** (a) $AcCH_2Cl$, KOH, methanol, rt; (b) RNH_2, AcOH, 60 °C; (c) HCOOEt, t-BuOK, benzene, rt; (d) $HNEt_2$, benzene, rt; (e) NC-CH_2R_1, ethanol, reflux.

It can be noted that the compounds exhibit different values of IC_{50} depending on the substitution pattern, and a remarkable dose UVA-dependence. In particular, compounds **11** and **12** show the highest cytotoxicity also in comparison to 8-MOP, 5-MOP and angelicin used as references compounds. The most active compounds require a phenyl group linked to the indole moiety, whereas a benzyl group in which the phenyl group is not directly linked to the indole moiety strongly reduces the activity. On the other hand, the substitution with a methyl group causes the disappearance of the phototoxic activity. In order to understand the nature of the binding between the drugs and the biological substrate, linear dichroism (LD) measurements on DNA–drug complexes were performed at various molar ratios. LD is a technique that was demonstrated to be able to characterize complexes between ligands and nucleic acid.[11,12]

In a typical LD experiment, the long axis of the DNA double helix is aligned along the flow lines, thus the DNA bases, which are oriented roughly perpendicular to the helix axis, give a negative LD signal. The same negative LD signal is recorded for drug molecules which are intercalated in the DNA structure and are almost perpendicular to the helix axis. Instead, drug molecules which are bound in the minor groove of DNA, such as netropsin or dystamicin, generally exhibit angles of 40–50° between the transition moment of the oriented chromophore and the helix axis, and give a positive LD signal.[11,12]

The absorbance spectra, and the LD spectra of two derivatives (**11** and **12**), recorded at different [Drug]/[DNA] ratios are shown in Figure 1. Inspection of the LD spectra of the derivatives in the presence of salmon testes-DNA reveals that despite a strong absorption in the 300–500 nm region, the two compounds give no LD bands in this region. Furthermore fluorescence titrations

Table 1. Values of IC_{50} (μM) obtained for the test compounds at different UVA doses in HT-1080 human fibrosarcoma[a]

Compd	Cytotoxicity (IC_{50}, μM)		
	2.6 (J cm^{-2})[b]	3.2 (J cm^{-2})	6.5 (J cm^{-2})
11	3.6±0.5	2.3±0.8	0.40±0.04
12	6.6±1.6	4.6±0.9	1.7±0.2
13	>20	13.1±1.5	11.1±1.4
14	>20	>20	16.4±1.4
15	>20	>20	>20
16	>20	15.1±0.2	1.8±0.1
17	14.7±0.9	9.0±1.1	1.9±0.02
8-MOP	7.8±0.7	2.1±0.3	1.5±0.2
5-MOP	8.5±0.6	1.8±0.4	0.9±0.3
Angelicin	15.7±1.9	2.6±0.2	2.5±0.3

[a]Values are means±SEM of three experiments.
[b]UVA dose expressed in J cm^{-2} as measured at 365 nm by a Cole Parmer Radiometer.

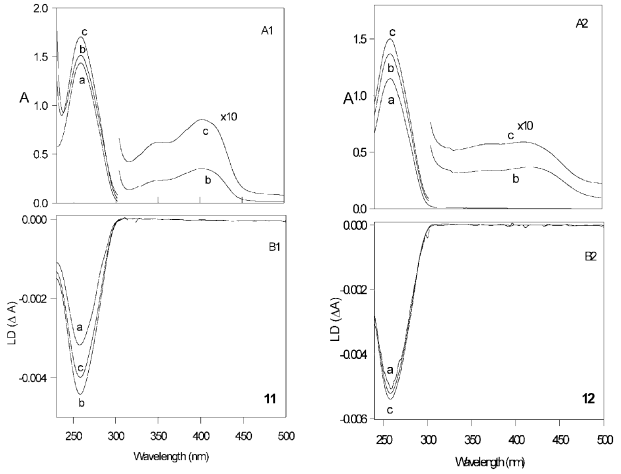


Figure 1. Absorbance (A), and linear dichroism (LD) spectra of mixtures of salmon testes DNA and compounds **11** (A1, B1) and **12** (A2, B2) at different [Drug]/[DNA] ratio (a = 0.00, b = 0.02, c = 0.04).

carried out with the same derivatives (data not shown) showed a poor fluorescence quenching confirming that the title compounds are loosely bound to DNA. This fact strongly suggests that the new derivatives do not interact efficaciously with the macromolecule. Considering these results, the new pyrroloquinolinones, appear as novel and potentially useful agents in photochemotherapy in so far as they show a remarkable photoantiproliferative activity which does not appear related to the classical mechanism (i.e., formation of mono- and bifunctional adducts to DNA) exerted by psoralens.

On the basis of the biological evaluation, experiments aimed at defining the targets at cellular level and the mechanism of phototoxycity are in progress.

Acknowledgements

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

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9. All final products were characterized by IR, ^{1}H and ^{13}C NMR and elemental analysis. A representative example, compound **11**: 80% mp > 300 °C; IR 2921 (NH), 1635 (CO) cm^{-1}; ^{1}H NMR (DMSO-d_6): 2.02 (3H, s, CH_3), 2.56 (2H, t, J = 7.6 Hz, CH_2), 2.80 (2H, t, J = 7.6 Hz, CH_2), 6.71 (1H, s, H-9), 7.38 (2H, d, J = 6.8, Ar), 7.50–7.69 (6H, m, Ar), 7.96 (2H, d, J = 6.8 Hz, Ar), 8.10 (1H, s, H-4), 12.24 (1H, s, NH); ^{13}C NMR (DMSO-d_6): 12.5 (q), 20.8 (t), 25.4 (t), 103.3 (d), 107.0 (s), 111.8 (s), 118.1 (s), 127.2 (2×d), 127.5 (2×d), 128.4 (3×d), 129.4 (2×d), 130.8 (s), 132.5 (d), 136.2 (s), 137.7 (s), 141.5(s), 142.0 (d), 148.6 (s), 158.2 (s). Anal. calcd for $C_{24}H_{20}N_2SO_3$: C, 69.21; H, 4.84; N, 6.73. Found: C, 69.11; H, 4.69; N, 6.53.
10. Antitumor assay. Exponentially growing HT-1080 human fibrosarcoma cells were resuspended at a density of 5×10^4 cells/mL in a complete medium (DMEM containing 10% fetal bovine serum, 100 UI/mL penicillin G and 100 μg/mL streptomycin) and seeded in a 96 well culture plates which were allowed to adhere for 18 h to culture plates before addition of the drugs. After the medium was removed, 100 μL of the drug solution, dissolved in DMSO and diluted with Hank's balanced salt solution (HBSS pH = 7.2), was added to each well. The plate was then incubated for 30 min in an atmosphere of 5% CO_2 at 37 °C, the control plate was placed in the dark and then irradiated with two HPW 125 Philips lamp, principally emitting at 365 nm. After irradiation, the solution was replaced by the medium and the plates were incubated for 72 h. Cell viability was assayed by the MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5diphenyl tetrazolium bromide)] method.[13] Cell growth at each drug concentration was expressed as percentage of untreated controls and the concentration resulting in 50% (IC_{50}) growth inhibition was determined by linear regression analysis.
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