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# Synthesis and photochemotherapeutic activity of thiopyrano[2,3-*e*]indol-2-ones

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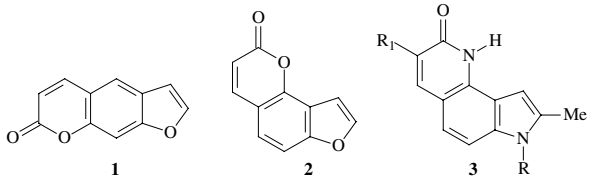
**Abstract**—A series of derivatives of the new ring system thiopyrano[2,3-*e*]indol-2-one was prepared with the aim of obtaining new photochemotherapeutic drugs. Biological screenings were performed on this new class of photoactivable drugs and a strong antiproliferative effect was observed upon irradiation with UVA light. The compound bearing a methyl substituent at the pyrrole nitrogen resulted as the most interesting showing $IC_{50}$ in the nanomolar range.


Linear furocoumarins, such as psoralen **1**, belong to an important class of photoactivable drugs which are currently used in PUVA (psoralen + UVA) therapy for the treatment of various skin diseases including psoriasis, vitiligo and tumours such as cutaneous T-cell lymphoma (CTCL).[1] This is due to their capacity to intercalate into DNA and photobind with it yielding either monoadducts or interstrand cross-links by one or two subsequent photocycloadditions.[2–4] However several short term (erythema) and long term (mutations and skin cancer) side effects are associated with this therapy. On the contrary angelicin **2**, an angular psoralen, proved to form only monoadducts with DNA. In fact its geometry avoids the simultaneous alignment of the two reactive sites.[5] We have recently reported the synthesis and biological activity of the new ring system pyrrolo[2,3-*h*]quinolin-2-one **3**, an angelicin heteroanalogue, in which nitrogen atoms replace both oxygens on the furan and the pyrone ring.[6]



**1** **2** **3**

All the tested compounds were phototoxic on HT-1080 (human fibrosarcoma) and LoVo (human intestinal adenocarcinoma) cell lines with $IC_{50}$ in the micromolar or submicromolar range (0.4–16 μM) with remarkable UVA dose-dependence. Interestingly, some compounds of this series show even higher cytotoxicity than 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP) and angelicin used as reference drugs. However, studies of linear dichroism (LD) strongly suggest that the new derivatives do not interact efficaciously with DNA, thus indicating a different mechanism in respect to that of furocoumarins.

It has been reported that the introduction of a sulfur on the pyrone moiety of psoralen improves the interaction with DNA both in the dark and under UVA light.[7] In the search for new angelicin analogues with better antiproliferative activity and lower toxicity, and considering

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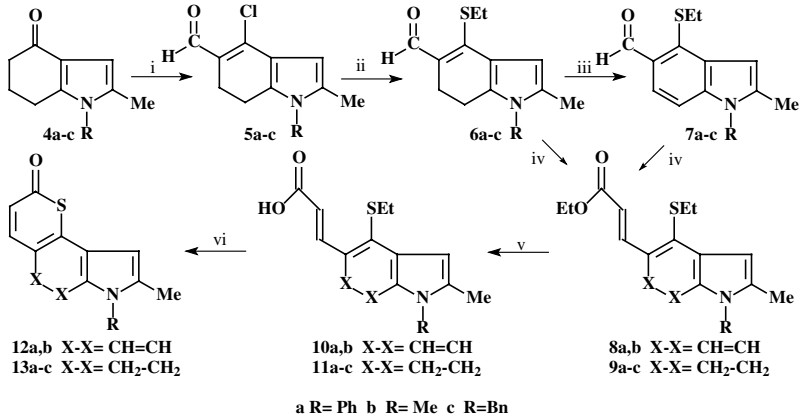


**Scheme 1.** Synthesis of compounds **12a**,**b** (X–X = CH=$CH_2$) and **13a**–**c** (X–X = $CH_2$–$CH_2$). Reagents and conditions: (i) DMF/$POCl_3$ in dichloromethane, 0 °C then reflux; (ii) HSEt/$K_2CO_3$ in DMF, rt; (iii) DDQ in benzene, reflux; (iv) $(EtO)_2P(O)CH_2CO_2Et$/*t*-BuOK in dichloromethane, rt; (v) KOH, ethanol 50%, reflux; (vi) PPA, Δ.

our previous satisfactory results on pyrroloquinolinones, we decided to investigate the synthesis of the new ring system thiopyrano[2,3-*e*]indol-2-ones and to study the biological activity of its derivatives. The synthetic approach to such compounds outlined in Scheme 1, started from ketones **4a**–**c** prepared by known procedures, by reaction of freshly prepared 2-acetonyl-1,3-cyclohexanedione with the proper amines in acetic acid.[6] Chloroformylation was performed in dichloromethane with an excess of the Vilsmeier–Haack reagent (DMF/$POCl_3$). Strict temperature control (0 °C), during the addition of the formylating mixture, and a short refluxing time (5 min), are crucial to achieve the best yields (70–75%). The chloroformylated derivatives **5a**–**c** are unstable at room temperature and require storage at −20 °C. Nucleophilic substitution of the chlorine atom by the ethanethiolate anion yields the thioethers **6a**–**c** (94–96%).

Oxidation of the dihydro derivatives with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) effected conversion of **6a**–**c** into the stable aldehydes **7a**–**c** (80–84%). Transformation into the vinylogous esters **8a**,**b** and **9a**–**c** was achieved by reaction with the appropriate Wittig–Horner reagent either on the dihydro aldehydes (70–75%) or the aromatic ones (72–76%). Hydrolysis to the corresponding acids **10a**,**b** (86–88%) and **11a**–**c** (82–85%) and cyclization with PPA yielded thiopyrano[2,3-*e*]-indol-2-ones **12a**,**b** and **13a**–**c** (30–40%).[8] The phototoxicity of the title compounds was investigated on two cultured cell lines: HL-60 and LoVo. Table 1 shows the extent of cell survival expressed as $IC_{50}$ which is the concentration, expressed in micromolars, that induces 50% inhibition of cell growth, after irradiation at different UVA doses.[9] Control experiments with UVA light or drugs alone were carried out without significant cytotoxic effects, except that, it was not possible to use the highest UVA dose (6.5 J $cm^{-2}$) for HL-60 cells because it causes in this cell line a significant cytotoxicity (data not shown). It can be noted that the compounds exhibit different values of $IC_{50}$ depending on the substitution pattern, and a remarkable UVA dose-dependence. In particular compound **12b** showed the highest cytotoxicity, especially in the solid tumour when compared to 8-MOP, and angelicin used as reference compounds. It is interesting to note that the most active compound bears a methyl group on the pyrrole nitrogen, whereas a phenyl or a benzyl group strongly reduces the activity.

In parallel to the cytotoxic evaluation, we used flow cytometry to study cell cycle variations upon irradiation.[11] In Figure 1, the effect in the leukaemic cell line, of the most active compound **12b** is shown after 24, 48 and 72 h from the irradiation. The results indicate that treatment with **12b** in combination with UVA induces

**Table 1.** $IC_{50}$ (μM) values obtained for the test compounds at different UVA doses in two human tumour cell lines[a]

| Compounds | Cytotoxicity ($IC_{50}$, μM)[a] | | | | |
|---|---|---|---|---|---|
| | HL-60[b] | | LoVo | | |
| | 2.5[c] | 3.2 | 2.5 | 3.2 | 6.5 |
| **12a** | 6.2 ± 0.6 | 2.7 ± 0.3 | >20 | 12.3 ± 1.7 | 2.9 ± 1.1 |
| **13a** | 2.6 ± 0.3 | 1.3 ± 0.2 | 5.1 ± 1.2 | 4.2 ± 1.4 | 3.9 ± 0.4 |
| **12b** | 0.6 ± 0.06 | 0.3 ± 0.1 | 1.0 ± 0.1 | 0.8 ± 0.2 | 0.07 ± 0.02 |
| **13c** | 4.8 ± 0.9 | 2.7 ± 0.3 | >20 | 7.2 ± 1.2 | 1.8 ± 0.6 |
| **8-MOP** | 1.4 ± 0.2 | 1.2 ± 0.4 | 1.1 ± 0.4 | 0.7 ± 0.1 | 0.4 ± 0.1 |
| **Angelicin** | 1.2 ± 0.1 | 0.9 ± 0.2 | 1.6 ± 0.2 | 0.9 ± 0.1 | 0.8 ± 0.1 |

[a] Values are means ± SEM of three independent experiments.
[b] HL-60, human promyelocytic leukaemia; LoVo intestinal human adenocarcinoma.
[c] UVA dose expressed in joule per centimetre square as measured at 365 nm by a Cole Parmer radiometer.

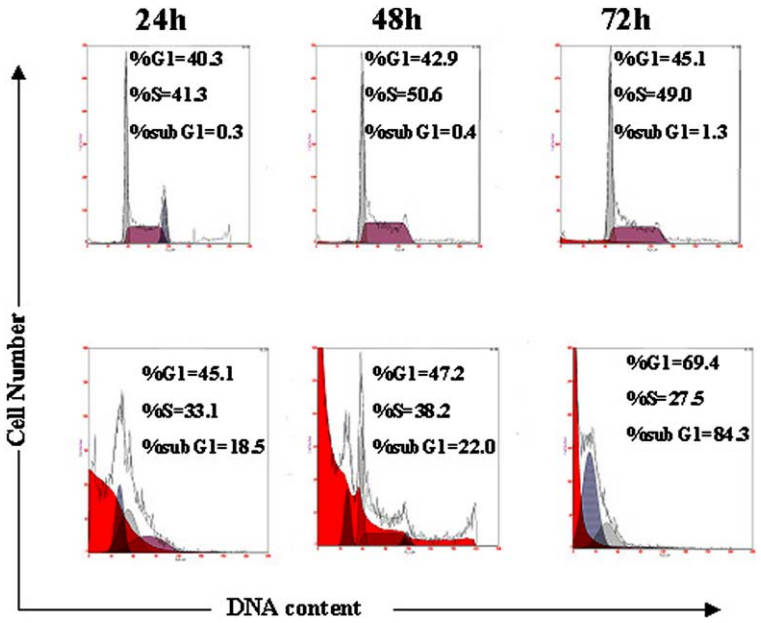


**Figure 1.** DNA flow cytometric analysis. HL-60 cells were irradiated with compound **12b** at concentration of 5 μM and a UVA dose of 2.5 J $cm^{-2}$. The cells were harvested for designated times as shown in the figure and described in Ref. 11. The data shown here are from a representative experiment repeated two times with similar results. Upper panels: irradiated controls; lower panels **12b** treated cells.

a remarkable reduction of the S phase at 24 h from irradiation. This is followed by massive induction of apoptosis, as observed by the appearance of a sub G1 peak (apoptotic peak) that refers to cells with DNA content lower than G1. In fact, apoptosis induces the activation of endogenous nucleases which are responsible for nucleic acid degradation.[12]

Considering that DNA is the major target of psoralen, we have investigated the binding between the drugs and the macromolecule. UV–vis and fluorimetric titration with DNA have shown neither variation of the absorption spectra nor the presence of isosbestic points indicative of the formation of a complex between the test compounds and the DNA. Furthermore, a poor fluorescence quenching of the emission of the free drug in the presence of DNA, suggests that the title compounds are loosely bound to DNA. These data are also confirmed by LD measurements on DNA–drug complexes.[13,14] The obtained results (data not shown) indicate a complete absence of any signal in the chromophore absorption region of the thiopyrano derivatives. This fact strongly suggests that the new derivatives do not significantly interact with the macromolecule as already observed for other series of angelicin heteroanalogues.[6]

According to these preliminary results, we can conclude that the new thiopyrano derivatives seem to be interesting as potential drugs for PUVA photochemotherapy.

However, further studies are necessary to identify the biological targets involved in their mechanism of action as well as the downstream events involved in the activation of the apoptosis. Identification of these specific targets may have significant implications for the therapeutic application of the new derivatives.

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## References and notes

1. Dall'Acqua, F.; Viola, G.; Vedaldi, D. Molecular Basis of Psoralen Photochemotherapy Chapter 142. In *CRC Handbook of Organic Photochemistry and Photobiology*; Hoorspool, W. M., Lenci, F., Eds.; CRC: Boca Raton USA, 2004; p 1.
2. Tessman, J. W.; Isaacs, S. T.; Hearst, J. E. *Annu. Rev. Biochem.* **1985**, *54*, 1151.
3. Musajo, L.; Rodighiero, G. In *Photophysiology*; Giese, A. C., Ed.; Accademic: New York, 1972; Vol. 7, p 115.
4. Dall'Acqua, F.; Viola, G. In *Photobiology for the 21st Century*; Coohil, T. P., Valenzeno, D. P., Eds.; Valdenmar Publishing company: Overland park Kansas, 2001; p 325.
5. Bordin, F.; Dall'Acqua, F.; Guiotto, A. *Pharmacol. Ther.* **1991**, *52*, 331.
6. Barraja, P.; Diana, P.; Lauria, A.; Montalbano, A.; Almerico, A. M.; Dattolo, G.; Cirrincione, G.; Viola, G.; Dall'Acqua, F. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2809, and references herein cited.
7. (a) Jacobs, A. E.; Piette, J. *J. Photochem. Photobiol. B* **1994**, *22*, 9; (b) Jacobs, A. E.; Piette, J. *J. Med. Chem.* **1995**, *38*, 869.
8. All final products were characterized by IR, $^{1}H$ and $^{13}C$ NMR and elemental analysis. A representative example, 7,8-dimethyl-6,7-dihydrothiopyrano[2,3-*e*]indol-2(5*H*)-one **13b**: 40% mp 172–173 °C; IR 1620 (CO) $cm^{-1}$; $^{1}H$ NMR (DMSO-$d_6$): 2.22 (3H, s, $CH_3$), 2.77–2.79. (4H,

m, H-5 and H-6), 3.43 (3H, s, $CH_3$), 5.99 (1H, s, H-9), 6.16 (1H, d, $J$ = 10.2 Hz, H-3), 7.22 (1H, d, $J$ = 10.2 Hz, H-4); $^{13}$C NMR (DMSO-$d_6$): 12.1 (q), 20.8 (t), 29.4 (t), 30.3 (q), 101.5 (d), 108.2 (s), 116.5 (s), 117.4 (d), 120.2 (s), 130.8 (s), 131.7 (s)144.6 (d), 184.4 (s, CO). Anal. Calcd for $C_{13}H_{13}NOS$: C, 67.50; H, 5.66; N, 6.06. Found: C, 67.80; H, 5.69; N, 6.40.

9. Exponentially growing HL-60 leukaemia cells were resuspended at a density of $5 \times 10^4$ cells/mL in a complete medium (RPMI containing 10% foetal bovine serum, 100 UI/mL penicillin G and 100 μg/mL streptomycin) and seeded in 96 well culture plates. LoVo, intestinal human adenocarcinoma cells were resuspended at a density of $5 \times 10^4$ cells/mL in a complete medium (Ham'F12 containing 10% foetal 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, by centrifugation, 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 lamps, principally emitting at 365 nm. After irradiation, the solution was replaced by the complete medium by centrifugation and the plates were incubated for 72 h. Cell viability was assayed by the MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)] method.[10] 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.
10. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
11. For cell cycle experiments $5 \times 10^5$ HL-60 cells were treated, as described for phototoxicity experiments, fixed in ethanol 70% after 24, 48 and 72 h, respectively, from the treatment and stained with propidium iodide. Samples were analyzed on flow cytometer (Epics XL-MCL, Coulter), using the Multicycle® software (Phoenix, CA).
12. Compton, M. M. *Cancer Metast. Rev.* **1992**, *11*, 105.
13. Nordén, B.; Kubista, M.; Kurucsev, T. *Q. Rev. Biophys.* **1992**, *25*, 51.
14. Nordén, B.; Kurucsev, T. *J. Mol. Recognit.* **1994**, *7*, 141.