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

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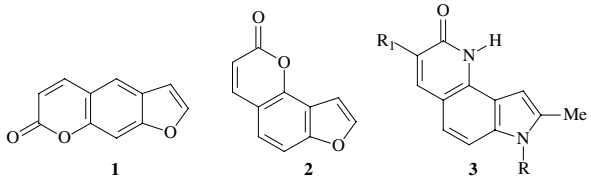
Abstract—A series of derivatives of the new ring system thiopyrano[2,3-*e*]indol-2-one was prepared with the aim of obtaining new photochemotherapeutic drugs. Biological screenings were performed on this new class of photoactivable drugs and a strong antiproliferative effect was observed upon irradiation 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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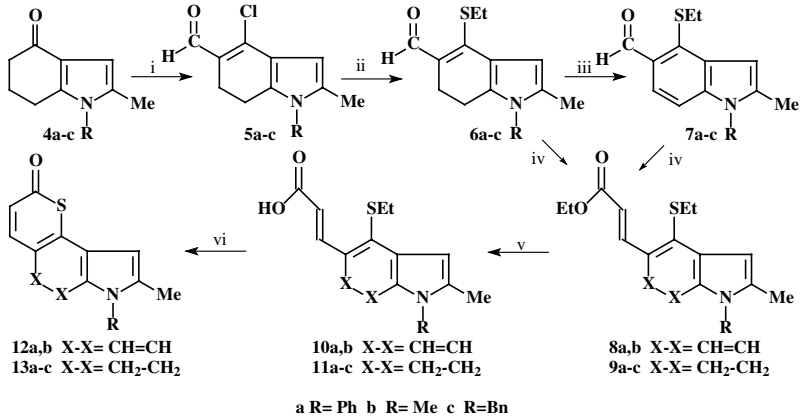

Scheme 1. Synthesis of compounds **12a**,**b** (X–X = CH=CH_2) and **13a**–**c** (X–X = CH_2–CH_2). Reagents and conditions: (i) DMF/$POCl_3$ in dichloromethane, 0 °C then reflux; (ii) HSEt/K_2CO_3 in DMF, rt; (iii) DDQ in benzene, reflux; (iv) $(EtO)_2P(O)CH_2CO_2Et$/*t*-BuOK in dichloromethane, rt;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