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# Pyrano[2,3-*e*]isoindol-2-ones, new angelicin heteroanalogues

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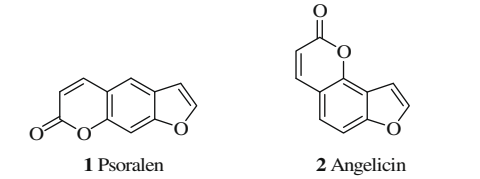
ABSTRACT

A convenient synthesis of the pyrano[2,3-*e*]isoindol-2-one ring system, an heteroanalogue of angelicin, is reported. Our synthetic approach consists of the annelation of the pyran ring on the isoindole moiety using 5-dialkylamino- or 5-hydroxymethylene intermediates as building blocks. The photoantiproliferative activity of the new derivatives was studied. Some of them bearing the benzyl group at the 8 position were active with $IC_{50}$ in the micromolar range. Cell cytotoxicity involves apoptosis, alteration of cell cycle profile and membrane photodamage.

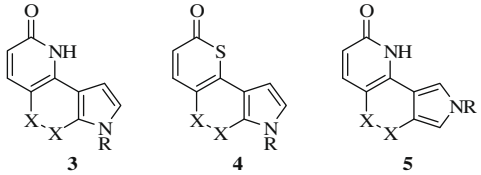


Furocoumarins constitute a group of natural or synthetic compounds of great pharmacological interest. One of the most important applications of these compounds is in the field of PUVA photochemotherapy (psoralen plus UVA) for the treatment of a variety of skin diseases characterized by hyperproliferative conditions such as psoriasis, vitiligo and tumors, such as T-cell lymphoma, when used in conjunction with long-wave (320–400 nm) ultraviolet light (UVA). Psoralen **1** is the parent compound of a relatively large number of linearly fused furocoumarins. The biological activity of these linear compounds is related to their ability to link DNA via intercalation in the dark between the base pairs of the nucleic acid followed by a [2 + 2] photocycloaddition with the pyrimidine bases, particularly thymine, when irradiated with UV-A light. The so formed monoadduct undergoes further [2 + 2] photocycloaddition thus generating inter-strand cross-links.[1] Undesirable side effects of this kind of photoreactions are mutagenicity and carcinogenicity. It explains why considerable efforts have been made to develop monofunctional furocoumarins such as angelicin **2**, parent compound of a much smaller and less available class of furocoumarins, which on account of their geometry cannot crosslink with DNA, resulting less dangerous than the linear compounds.

In consideration of above, we studied the synthesis of heteroanalogues of angelicin in which a nitrogen or a sulfur atom replaces one or both oxygen atoms of the pyran and furan rings. These heteroanalogues exhibit a strongly increased DNA photoactivation property, thus appearing very promising for photochemotherapy.[2]



**1** Psoralen **2** Angelicin



**3** **4** **5**

X-X= $CH_2$-$CH_2$ or CH=CH

With the aim of preparing and studying new photoreactive agents with increased antiproliferative activity and decreased severe toxic side effects, we have recently reported the synthesis of the new ring systems pyrrolo[2,3-*h*]quinolin-2-one **3** and thiopyrano[2,3-*e*]indole **4**, isosters of angelicin, whose derivatives showed generally high phototoxicity in comparison with 8-MOP ($GI_{50}$ 0.4–16.4 μM and 0.07–3.9 μM, respectively).[3]

On continuing our studies on photochemotherapeutic drugs, here we report the synthesis of pyrano[2,3-*e*]isoindol-2-one, an heteroanalogue of angelicin, in which the isoindole ring replaces the benzofuran one. We chose to synthesize this ring system in

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order to verify how the condensation of the pyrrole ring on the benzopyran moiety can affect the photobiological activity, considering that derivatives of the pyrrolo[3,4-*h*]quinoline system **5**, compared to their positional isomers of the series **3**, showed an increased phototoxicity (0.1–14.5 μM).[4] The active compounds of the series **5** were the dihydro derivatives, which did not intercalated into DNA but after irradiation they induced photodamage to the macromolecule. Moreover, upon irradiation, they caused remarkable oxidation of the cellular membrane lipids.[4]

Our synthetic approach to pyrano-isoindoles consisted in the annelation of the pyran ring on the isoindole moiety using dialkylaminomethylene isoindoles **13–15** as building blocks.

Tetrahydroisoindole-4-ones **7, 9** and **10**, as suitable substrates for our purpose, were prepared by two synthetic routes with a good substitution pattern on the pyrrole moiety.[5,6] In particular, the reaction of 2-ciclohexen-1-one **6**, a good Michael acceptor, with TOSMIC afforded the unsubstituted isoindole derivative **7a** (85%), whereas the Knorr-type reductive condensation of 2-acetylcyclohexane-1,3-dione **8** with freshly prepared diethyl hydroxyiminomalonate led to 1,3-disubstituted isoindole **9a** (65%). The latter was subjected to basic hydrolysis followed by decarboxylation, with dilute hydrochloric acid in ethanol leading to 3-methyl substituted isoindole **10a** (70%). The tetrahydroisoindoles **7a**, **9a** and **10a** were functionalized on the nitrogen atom in THF or DMF using NaH as the base and an alkylating agent such as MeI, BnCl, *p*-MeBnCl or *p*-OMeBnCl (60–98%). In the attempt to obtain the N-phenyl substituted derivatives, tetrahydroisoindoles **7a**, **9a** and **10a** were subjected to the Ulman cross-coupling reaction with CuBr, iodobenzene and $K_2CO_3$ in 1-methyl-2-pyrrolidinone (NMP). However, it was impossible to obtain the corresponding *N*-phenyl derivatives from **9a** and **10a** whereas **7a** furnished **7d** in 65% yield.

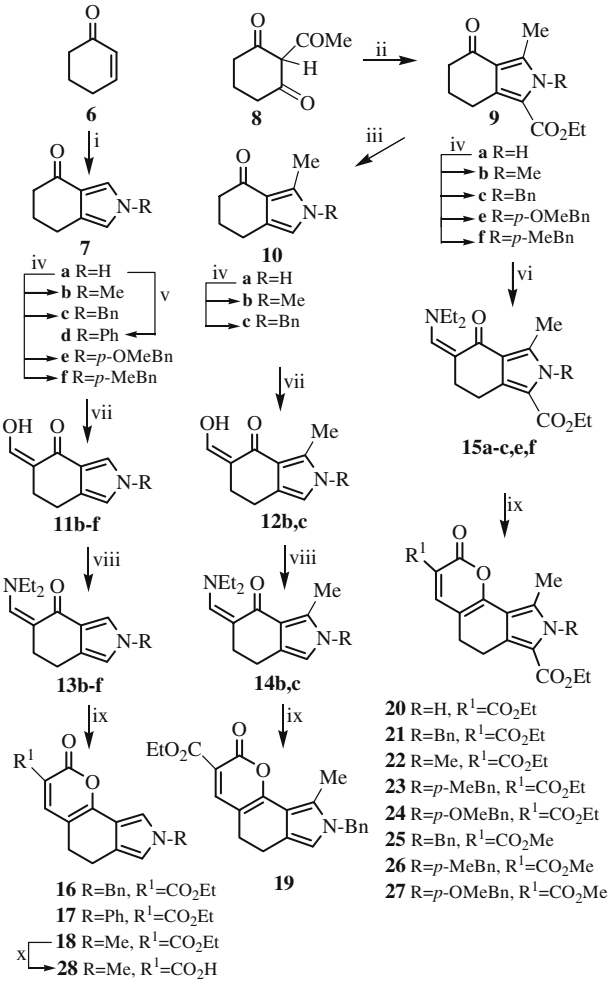
To achieve the direct introduction of the enamino functionality which allows to obtain derivatives of type **13–15**, the isoindoles **7a–f**, **9a–c,e,f** and **10a–c** were reacted with an excess of *tert*-butoxybis(dimethylamino)methane (TBDMAM). However, this reaction succeeded only with derivatives **9a–c,e,f** bearing the ethoxycarbonyl group in the 1 position which were converted into 15a–**c**,**e**,**f**.

In order to get the enaminoketons **13** and **14**, isoindoles **7b–f** and **10b,c** were subjected to a two steps sequence starting with a formylation in benzene at room temperature with ethyl formate as the formylating agent, leading to the hydroxymethylene derivatives **11b–f** and **12b,c** and subsequent reaction with diethylamine at room temperature in benzene to produce enaminoketones **13b–f** and **14b,c**.

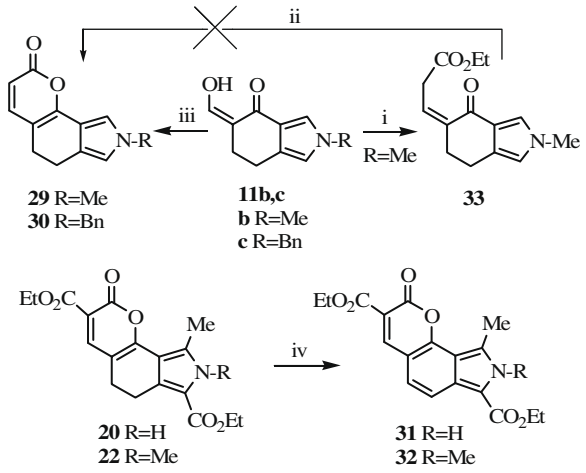
To afford the final tricyclic heteroanalogues of angelicin, enaminoketones **13b–f** were reacted with diethyl malonate, used as solvent, keeping the temperature at 80–100 °C. The reactions generally gave quite complex mixtures and was only possible to isolate derivatives **16–18** in moderate yields (30–42%); from **14b**,**c** only **19** (57%) was isolated, whereas all enaminoketones **15a–c,e,f** provided the corresponding tricyclic systems **20–24** (30–46%). Compounds **15c,e,f** were also reacted with dimethyl malonate to give the corresponding pyrano-isoindoles **25–27** (40–42%) (Scheme 1).

Considering the remarkable activity of angelicin, we also focused our attention on the synthesis of 3-H substituted derivatives of the ring system that could have been obtained simply by hydrolysis and decarboxylation of the ester derivatives. However, several attempts for the hydrolysis of the carboxyester functionality on the pyran ring of **16–18** and **19** failed. Only in the case of the reaction of **18** in ethanol/potassium hydroxide very poor yield (8%) of the corresponding acid **28** was obtained making difficult any further reaction.

Therefore, we considered an alternative route that was successfully used in the past for the synthesis of angelicin, and its derivatives, such as oroselone and oroselol, reacting 5-hydroxymethylene




**Scheme 1.** Synthesis of compounds **16–28**. Reagents and conditions: (i) TOSMIC, *t*-BuOK–THF; (ii) HON=C($CO_2Et$)$_2$, Zn–acetic acid; (iii) KOH-EtOH then 6 M HCl–EtOH; (iv) NaH, MeI or BnCl, or *p*-MeBnCl, or *p*-OMeBnCl/DMF or THF; (v) CuBr, PhI, $K_2CO_3$/NMP; (vi) TBDMAM–toluene; (vii) HCOOEt, *t*-BuOK–benzene; (viii) $HNEt_2$/benzene; (ix) $CH_2(CO_2Et)_2$ or $CH_2(CO_2Me)_2$, piperidine, 80–100 °C; (x) KOH–EtOH, reflux.




**Scheme 2.** Synthesis of compounds **29–33**. Reagents and conditions: (i) $Ph_3PCH_2CO_2Et$/xylene; (ii) xylene or DMA or TEG; (iii) $Ph_3PCH_2CO_2Et$/TEG or DMA; (iv) DDQ/benzene.

**Table 1**
Phototoxicity of pyrano[2,3-*e*]isoindol-2-ones **16–32** at different UVA doses

| Compound | $IC_{50}$ (μM)[a,b] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NCTC-2544 | | | LoVo | | | K562 | | | Jurkat | | |
| | 0[c] | 2.5 | 3.75 | 0 | 2.5 | 3.75 | 0 | 2.5 | 3.75 | 0 | 2.5 | 3.75 |
| **16** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **17** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **18** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **19** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **20** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **21** | >20 | 14.6 ± 1.3 | 10.9 ± 0.9 | >20 | 10.5 ± 1.0 | 9.6 ± 0.8 | >20 | 7.5 ± 0.8 | 7.3 ± 0.6 | >20 | 8.2 ± 0.9 | 7.2 ± 0.7 |
| **22** | >20 | >20 | >20 | >20 | >20 | 11.1 ± 1.1 | >20 | 13.9 ± 1.4 | 13.1 ± 1.2 | >20 | 15.4 ± 1.7 | 15.2 ± 1.6 |
| **23** | >20 | >20 | 17.0 ± 1.7 | >20 | >20 | 15.0 ± 1.7 | >20 | 13.5 ± 1.4 | 9.3 ± 0.8 | >20 | 16.1 ± 1.8 | 12.8 ± 1.4 |
| **24** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | 14.0 ± 1.7 | >20 | >20 | 13.5 ± 1.8 |
| **25** | >20 | 14.0 ± 1.2 | 10.0 ± 0.8 | >20 | 7.7 ± 0.6 | 7.3 ± 0.6 | >20 | 6.1 ± 0.5 | 2.7 ± 0.4 | >20 | 8.4 ± 0.8 | 3.7 ± 0.4 |
| **26** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | 16.6 ± 1.7 | >20 | >20 | 17.8 ± 2.1 |
| **27** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | 12.6 ± 1.9 | >20 | >20 | 11.8 ± 1.1 |
| **28** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **29** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **30** | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 | >20 |
| **31** | >20 | >20 | >20 | >20 | >20 | 17.8 ± 1.9 | >20 | >20 | >20 | >20 | >20 | >20 |
| **32** | >20 | >20 | >20 | >20 | 16.2 ± 1.8 | 6.7 ± 0.5 | >20 | >20 | >20 | >20 | >20 | >20 |
| **8MOP** | >20 | 5.5 ± 0.6 | n.d.[d]. | >20 | 1.1 ± 0.4 | 0.7 ± 0.1 | >20 | n.d. | n.d. | >20 | 1.2 ± 0.3 | n.d. |
| **Ang** | >20 | 4.2 ± 0.5 | n.d. | >20 | 1.6 ± 0.2 | 0.9 ± 0.1 | >20 | n.d. | n.d. | >20 | 0.9 ± 0.2 | n.d. |

[a] Concentration of compound required to inhibit the cell growth by 50% after 72 h of exposure as determined by MTT assay.
[b] Values are means ± SEM of three experiences.
[c] UVA doses are expressed as J/cm$^2$.
[d] Not determined.

benzofuranones with (carbethoxymethylene) triphenylphosphorane in refluxing xylene.[7] However, under these conditions the 5-hydroxymethylene derivative **11b** reacted only yielding the uncyclized ester **33** as main product (70%). No evidence of the desired tricyclic system was observed nor when the open chain intermediate, once isolated, was heated in boiling xylene for 24 h. It was thus reasonable to consider a solvent with an higher boiling point such as *N*,*N*-dimethylaniline (DMA) or triethylene glycol (TEG) to force the ring closure. However, this reaction did not give the desired result and only the starting material from the reaction mixture was recovered. On the contrary, straight heating of 5-hydroxymethylene derivatives **11b,c** with (carbethoxymethylene) triphenylphosphorane in the already mentioned high boiling solvents pyrano-isoindole derivatives **29** and **30** were isolated in good yields (60–73%)

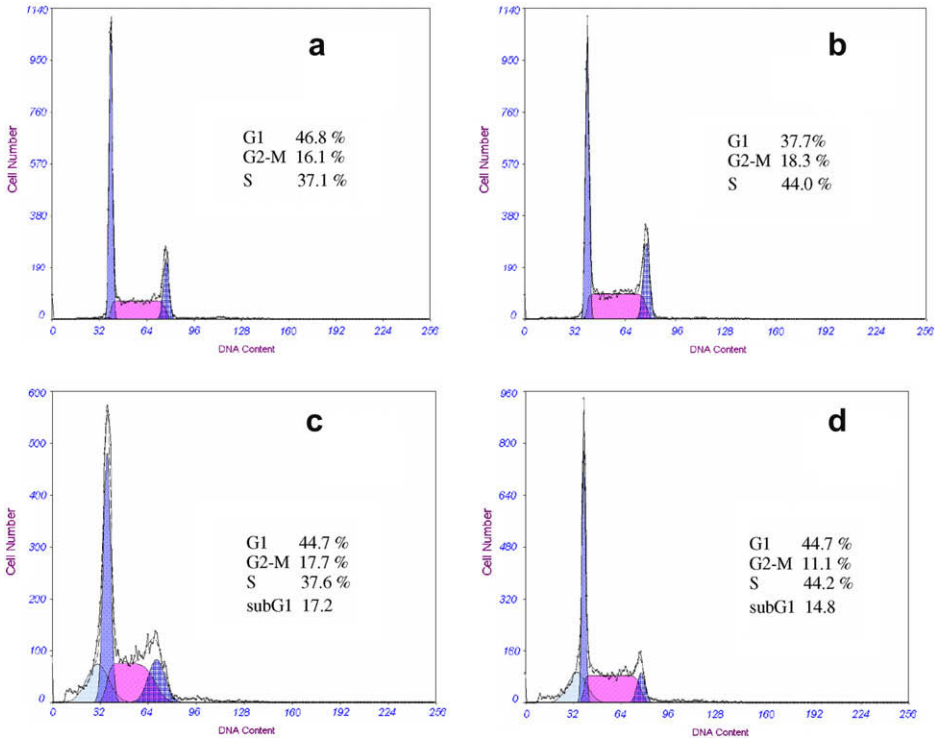



**Figure 1.** DNA flow cytometric analysis. Jurkat cells were irradiated (2.5 J/cm$^2$) with 5 μM **21** and **25** (panels c and d, respectively). Panels a and b are non irradiated and 2.5 J/cm$^2$ irradiated control, respectively. The data shown here are from a representative experiment repeated two times with similar results.

(Scheme 2). Getting flat pyrano-isoindoles would allow to verify whether such compounds, being aromatic could achieve intercalation into DNA; the dihydro derivatives were therefore subjected to oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in refluxing anhydrous benzene. From such a reaction only two aromatic derivatives **31** and **32** in moderate yields (30–35%) were isolated. The structure of all synthesized compounds was confirmed by spectroscopic data (IR, $^{1}H$ and $^{13}C$ NMR) and elemental analysis (C, H, N) reported in the Supporting information.

All test compounds absorbed in UV–Vis range (230–500 nm) and underwent photolysis after UVA irradiation.

The phototoxicity of pyrano[2,3-*e*]isoindol-2-ones was evaluated on a panel of cultured human cell lines: NCTC-2544 (immortalized keratinocytes), LoVo (intestinal adenocarcinoma), K562 (chronic myeloid leukemia) and Jurkat (T-cell leukemia). No cytotoxicity was found in the dark by MTT[8] test after 72 h from the incubation with these compounds. Table 1 shows the extent of cell survival expressed as $IC_{50}$, which is the concentration that induces 50% inhibition of cell growth, after irradiation at different UVA doses (2.5 and 3.75 J/cm$^2$).

Some of the test compounds resulted phototoxic in the micromolar range: in particular, the lowest cellular survival was assessed irradiating cells in the presence of **21** and **25**. The presence of a COOEt group in position 7 and of a benzyl one in the isoindole nitrogen seemed to be important for phototoxicity. It is worth remarking that the most active compounds – which were active against all the four cell lines – are not fully unsaturated such as angelicin but on the contrary are dihydroderivatives. Whereas the aromatic pyrano-isoindoles **31** and **32** were active against LoVo cell line only. Parallel behaviour was also observed in the pyrrolo[2,3-*h*]quinolin-2-one **3** series, in which the unsaturated derivative was devoid of phototoxicity,[3b] and in the pyrrolo[3,4-*h*]quinolin-2-one **5** series as already mentioned in this text.[4]

In order to have better insight into the mechanism of action of compounds **16–32**, we used flow cytometry to study cell cycle variations upon irradiation. In Figure 1, the effect of the most active compounds **21** and **25** was shown after 24 h from Jurkat cells irradiation (2.5 J/cm$^2$). The irradiation in the presence of the test compounds caused the appearance of a subG1 peak (apoptotic peak) in cell cycle profile and the latter consisted of apoptotic cells with DNA content lower than G1, as a consequence of endonuclease activation.

As pyrano[2,3-*e*]isoindol-2-ones were highly hydrophobic, we assessed the lipid peroxidation of Jurkat cell membranes using TBA test as described by Morlière et al.[9] and we found a clear increase in lipid peroxidation in Jurkat cells irradiated in the presence of test compounds (data reported in the Supporting information).

In conclusion, we found a convenient and versatile synthetic pathway to achieve pyrano[2,3-*e*]isoindol-2-ones in moderate to good yield. The new compounds tested as photoantiproliferative agents showed no activity in the dark, whereas some of them showed photoantiproliferative activity in the micromolar range under UVA activation. Cell cytotoxicity involves apoptosis, alteration of cell cycle profile and membrane photodamage.

## Acknowledgment

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.01.096.

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