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A new psoralen derivative with enlarged antiproliferative properties

Lisa Dalla Via [a], Jose Carlos González-Gómez [b,†], Lázaro Guillermo Pérez-Montoto [b], Lourdes Santana [b], Eugenio Uriarte [b], Sebastiano Marciani Magno [a], Ornella Gia [a,*]

[a] Department of Pharmaceutical Sciences, School of Pharmacy, University of Padova, Via Marzolo 5, 35131 Padova, Italy
[b] Department of Organic Chemistry, Universidad de Santiago de Compostela, 15782, Spain

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ABSTRACT

Following our results with benzopsoralens as potent photochemotherapeutic agents, we report the antiproliferative evaluation of nitrogenated isoster upon and without UVA irradiation. The evaluated pyridazinopsoralen showed a higher photochemotherapeutic activity with respect to the well-known drug, 8-MOP, and a significant cytotoxicity, also in the dark. This result enlarges the interest in this tetracyclic psoralen derivative skeleton in the search of new anticancer agents.

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Furocoumarins are a class of compounds of high pharmacological interest due to their capacity to link covalently to DNA upon irradiation with long-wavelength UV light (365 nm). This photoreactivity is generally greatest for linear furocoumarins (psoralens), among which 8-methoxypsoralen (8-MOP) is currently the most common reference drug for PUVA (Psoralen + UVA) therapy of various skin diseases.[1–4] Nevertheless, some serious side-effects both short-term (erythema, hyperpigmentation) and long-term (premalignant keratoses, skin cancers) have been detected.[5–7] We have previously shown that widening the tricyclic psoralen nucleus, by condensing a cyclohexane or benzene to 4′,5′-furan-side double bond, and inserting a dimethylaminopropoxy side chain in position 5 or 8 of the psoralen moiety with a view to increase the low aqueous solubility, very interesting results are produced.[8] In particular, all tetracyclic derivatives showed a significant decrease of skin phototoxicity, and the disappearance of this undesired side effect was obtained with benzopsoralens. Moreover, the photoantiproliferative activity resulted considerably high for benzopsoralen carrying the protonable side chain in position 8 which actually appeared 100 times more efficient in inhibiting HeLa growth, than 8-MOP. These results stimulated further studies; in fact, tetracyclic congeners with a cyclohexane or benzene ring condensed at 3,4-pyrone-side double bond, and a dimethylaminopropoxy side chain in position 8, were prepared and studied.[9] For the benzo derivative, the disappearance of skin phototoxicity was confirmed, but it showed a significantly lower photoantiproliferative ability on HeLa cells with respect to the furan-side analogue.[8,9] Furthermore, the comparison of the photobiological properties of a series of tetracyclic psoralens, carrying a cyclopentane, cyclohexane or benzene ring, evidenced that the intensity of skin phototoxicity strongly depends on the type of the condensed ring.[10] In particular, the planar six membered aromatic nucleus appears to be the most suitable.

According to these previous results, we designed and synthesised a series of tetracyclic psoralens carrying a fourth pyridazino ring fused at the level of the 4′,5′ photoreactive double bond which should ensure the weakening of skin phototoxicity.[11,12] Moreover, on the basis of the crucial role played in the photoantiproliferative effect, a dimethylaminopropoxy side-chain was inserted in position 8 of the new tetracyclic chromophore.

We describe here the synthesis of a new pyridazino[4,3-*h*]psoralen and the biological activity evaluation.

Compound **3** was prepared from commercially available 8-MOP as follows (Scheme 1). Pyridazinopsoralen **1** was obtained in an excellent 98% yield from 8-MOP and 3,6-dichlorotetrazine, in a Diels–Alder reaction followed by intramolecular cyclization.[13] This procedure was successfully used in a 2-g scale and the compound crystallized with high purity from the reaction mixture.[14,15]

Hydrolysis of compound **1** to the corresponding pyridazinone, followed by triflate preparation and palladium catalyzed hydrogenolysis under transfer conditions, afforded compound **2** in about 65% yield over three steps.[13] Hydrolysis of methoxy group of compound **2**, using $AlCl_3/CH_2Cl_2$ at room temperature, was followed by alkylation under Williamson conditions, with 3-

* Corresponding author. Tel.: +39 0498275710; fax: +39 0498275366.
E-mail address: ornellamaria.gia@unipd.it (O. Gia).
† Present address: Department of Organic Chemistry, Universidad de Alicante, Apdo. 99, 03080, Spain.

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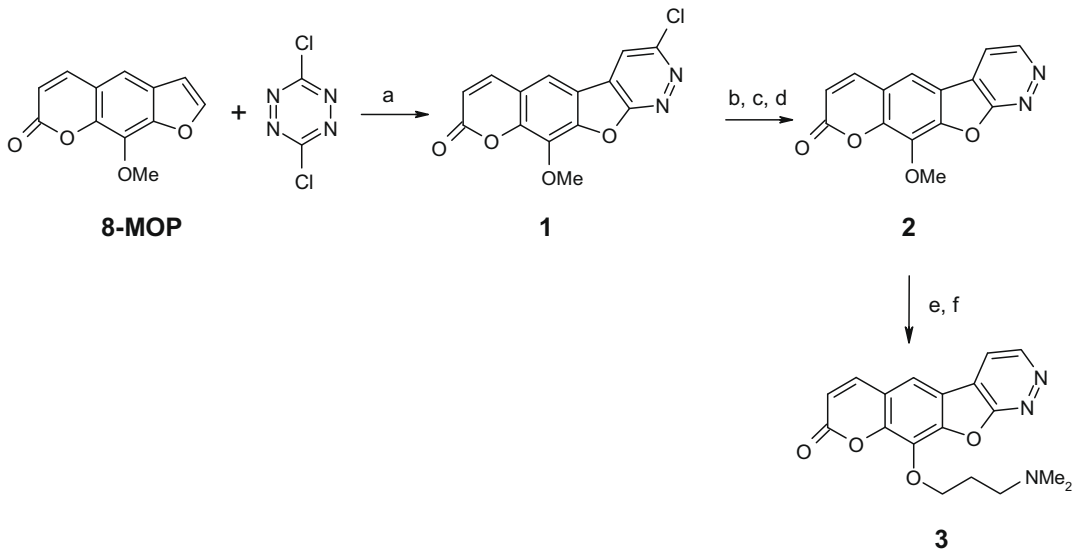

Scheme 1. Reagents and conditions: (a) CH_2Cl_2, 140 °C, then *i*-Pr_2NEt; (b) 20:1 AcOH/H_2O, reflux; (c) Tf_2O, Py, rt; (d) HCO_2H, cat. $Pd(OAc)_2$/dppf, *i*-Pr_2NEt, DMF; (e) $AlCl_3$, CH_2Cl_2, then H_2O; (f) $Cl(CH_2)_3NMe_2$, NaH, DMF.

chloro-*N*,*N*-dimethyl-propylamine hydrochloride, to afford target compound **3** in 40% yield over two steps.[16]

The antiproliferative activity of compound **3** was evaluated by means of a growth inhibition assay on HeLa, human cervix adenocarcinoma, cell line and expressed as IC_{50} values, that is, the concentration (μM) of compound able to induce 50% of cell death with respect to the control culture. Experiments were performed both in the absence and in the presence of UVA light (365 nm, 0.793 J cm^{-2}) following previously established procedures.[17] The well-known photochemotherapeutic drug 8-MOP was taken as reference compound and the results are reported in Table 1.

After exposure to UVA light, compound **3** showed a notable capacity to inhibit cell growth. Indeed, the measured IC_{50} value is significantly lower than that obtained for the reference drug. Moreover, the new pyridazinopsoralen showed a cytotoxic activity even in the dark, and this latter effect is about four times lower with respect to that exerted upon UVA irradiation. For previous tetracyclic derivatives[8,9] an antiproliferative effect in the dark was also observed; nevertheless, for the pyridazinopsoralen **3** the difference between photobiological and biological activity decreases. In this connection, it is well known that in ground state, psoralens and psoralen analogues are able to form a molecular complex with DNA through an intercalative mode of binding. Figure 1 shows

Table 1
HeLa cell growth inhibition and skin phototoxicity in guinea pigs in the presence of **3** and 8-MOP as reference drug

Compd	IC_{50} (μM)		Formation of erythema	
	Dark	UVA	Intensity[a]	μmol cm^{-2}
3	13.5 ± 1.7	2.9 ± 0.5	+–	0.046
8-MOP	>20	10 ± 3	++	0.046

[a] Symbols: ++, erythema with edema; +–, mild.

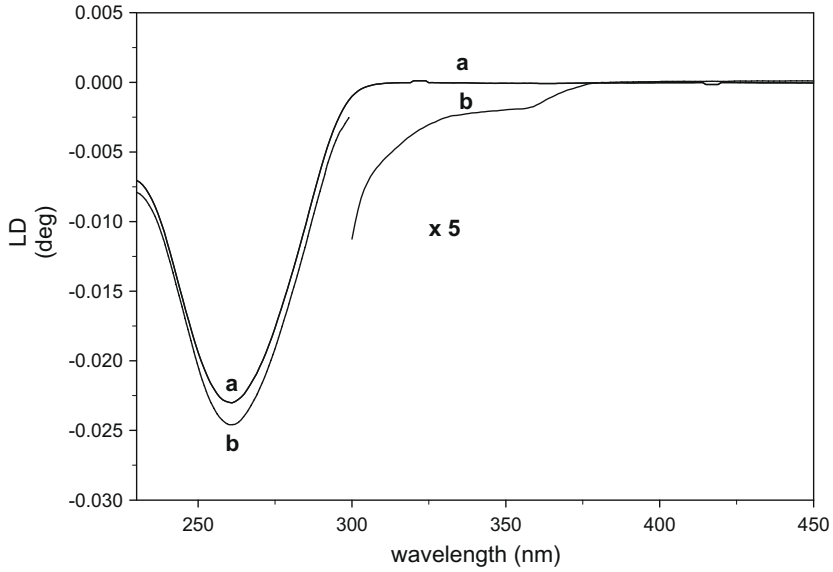

Figure 1. Linear flow dichroism spectra for compound **3** at [drug]/[DNA] ratios: *a* = 0, *b* = 0.04; [DNA] = 1.8 × 10^{-3} M.

the spectra of DNA alone (line a) and in the presence of **3** (line b) at [drug]/[DNA] = 0.04, obtained from flow dichroism experiments performed as previously reported.[17] The occurrence of a negative dichroic signal at wavelengths at which the pyridazinopsoralen chromophore absorbs (300–380 nm), also confirmed for derivative **3** the ability to intercalate between base pairs of the macromolecule and this property could account for the occurrence of the UVA-independent cytotoxicity.

Skin phototoxicity was evaluated for compound **3** on depilated skin in guinea pigs in comparison with 8-MOP (Table 1).[17] Interestingly, the pyridazinopsoralen derivative is significantly less phototoxic than the reference drug and this property acquires more significance thanks to the evidenced difference in photocytotoxicity between the two compounds.

In conclusion, the new pyridazinopsoralen derivative **3** appears to be characterized by some peculiar biological properties. Indeed, **3** is endowed with an interesting photobiological profile deriving from both a significant photoantiproliferative activity and a weak skin phototoxicity. Moreover, a significant cytotoxic effect in the dark occurs. This capacity was also exhibited by the benzo analogue,[8] nevertheless the difference in the cytotoxicity in the presence of UVA light and in the dark is less for **3**. This behavior can be attributed to an efficient DNA binding process mediated by the pyridazine ring. This latter, thanks to the tendency to protonation of its nitrogens,[18,19] with respect to benzene ring, can make the intercalative capacity between DNA base pairs more suitable for the antiproliferative activity in the dark.

A further contribution in DNA interaction ability can derive from the insertion of the dimethylaminopropoxy side-chain. Indeed, a cationic side-chain linked to a planar structure, can generate additional electrostatic interactions with the polyanionic DNA target, hence favouring the binding process.[20] Both the pyridazine ring and the protonable side-chain can account for the significant shift of the psoralen derivative **3** toward a dark antiproliferative activity and actually indicate the possibility to modulate the photo-dependent and -independent cellular effect by just modifying suitably the intercalative arrangement of the tetracyclic psoralen skeleton.

Acknowledgments

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16. 6-(*N*,*N*-Dimethylaminopropyloxy)pyridazino[4,3-*h*]psoralen (**3**) ^{1}H NMR ($CDCl_3$): 9.31 (d, *J* = 5.0, 1H), 8.04 (d, *J* = 5.0, 1H), 7.86 (s, 1H), 7.83 (d, *J* = 9.6, 1H), 6.50 (d, *J* = 9.6), 4.70 (t, *J* = 6.3, 2H), 2.67 (m, 2H), 2.32 (s, 6H), 2.12 (m, 2H). ^{13}C NMR ($CDCl_3$): 166.2, 158.9, 148.3, 147.6 (CH), 143.0 (CH), 132.7, 120.8, 119.2 (CH), 118.1, 117.3, 116.1 (CH), 115.6 (CH), 72.8 (CH_2), 55.9 (CH_2), 45.5 (CH_3), 28.2 (CH_2). HRMS (EI) found 339.1229, $C_{18}H_{17}ClN_3O_4^+$ requires 339.1219.
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