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Double-Headed Molecules Based on Linear or Angular Psoralen and α-Methylene-γ-Butyrolactone: A Search for Non-Phototoxic Potential Antiproliferative Compounds

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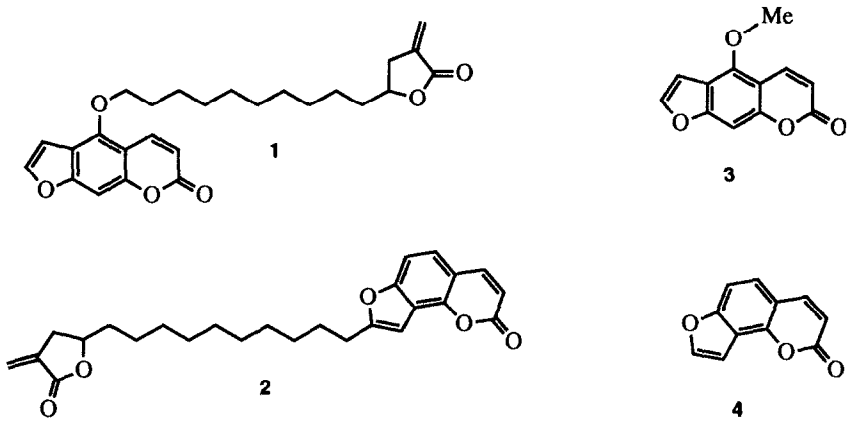
Key words: Phototoxicity; Psoralen; Angelicin; α-methylene-γ-butyrolactone.

Abstract: Two double-headed molecules have been synthesised by linking a photoreactive linear **1** or angular **2** psoralen via a straight ten-carbons chain to an electrophilic α-methylene-γ-butyrolactone. Their phototoxicity was then evaluated by the mouse ear-swelling test and compared with that of the reference compounds 5-methoxypsoralen and angelicin. Compounds **1** and **2** were found to be non-phototoxic at concentrations 120 fold higher than the minimal phototoxic dose of 5-MOP.

The use of extracts of *Ammi majus* L. or the seeds of *Psoralea corylifolia*, in conjunction with exposure to sunlight, as treatment for vitiligo (a condition resulting in hypopigmentation of the skin) can be traced back to the Egyptians in 1500 BC. In the 50s, the isolation of 8-methoxypsoralen led to the clinical study of the treatment of vitiligo, then to the development of the first synthetic furocoumarins. In the 70s, combined therapy using both psoralen and irradiation by UVA[1] (the region of the solar spectrum involved in the reaction of furocoumarins) was started, this being the beginning of photochemotherapy known by the acronym of PUVA. PUVA proved to be a good inhibitor of epidermal cell proliferation and is widely used in the treatment of psoriasis. Unfortunately most linear furocoumarins or psoralens are phototoxic[2] for the skin, resulting, after contact with these molecules and UVA irradiation, in erythema and eventually edema. The carcinogenic and phototoxic secondary effects of the psoralens have led to a search for new antiproliferative, but non-phototoxic, derivatives. During UVA irradiation, psoralens in the presence of DNA form photoadducts with the pyrimidine bases of the DNA[3]; these are C4 cycloaddition products between the 3, 4 or 4', 5' bond of psoralen and the double bond of the pyrimidine. Depending on the structure of the psoralen, mono- or di-adducts can be formed, resulting in the cross-linking of two strands of DNA[4].

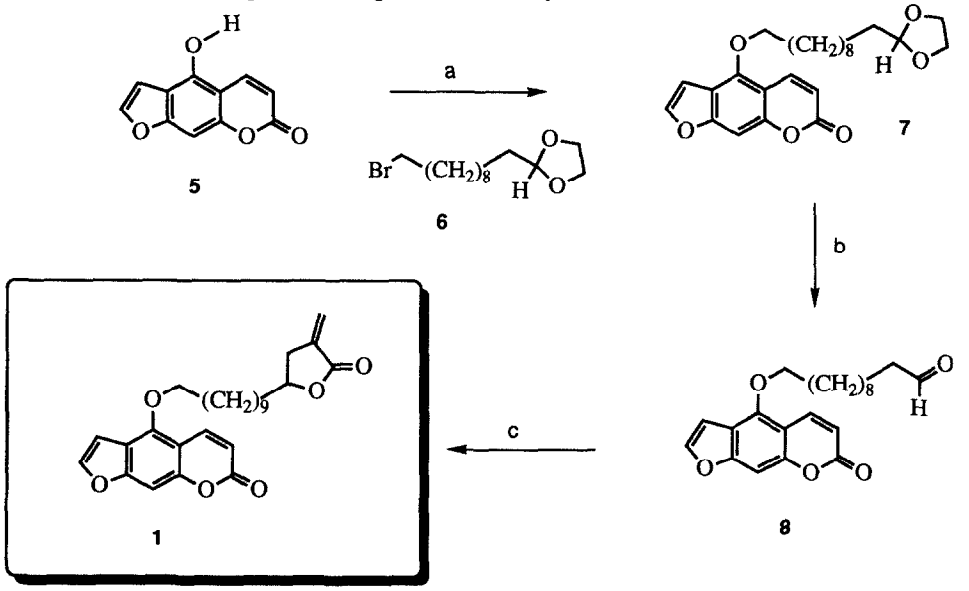
α-Methylene-γ-butyrolactones are very good electrophiles which are known to react rapidly with nucleophilic residues of proteins (amino or thiol groups), forming covalent bonds[5]. The presence of this electrophilic group, together with a furocoumarin, can lead to the formation of di-adducts, in this case, between DNA and proteins.



We here report the synthesis of two heterodimeric derivatives based on a furocoumarin nucleus, linear **1** or angular **2**, bound to an α-methylene-γ-butyrolactone by a 10-carbon polymethylene chain. These compounds, when tested on mice, were found to be essentially non-phototoxic in comparison with the reference compounds **3** and **4** (5-methoxypsoralen and angelicin, respectively).

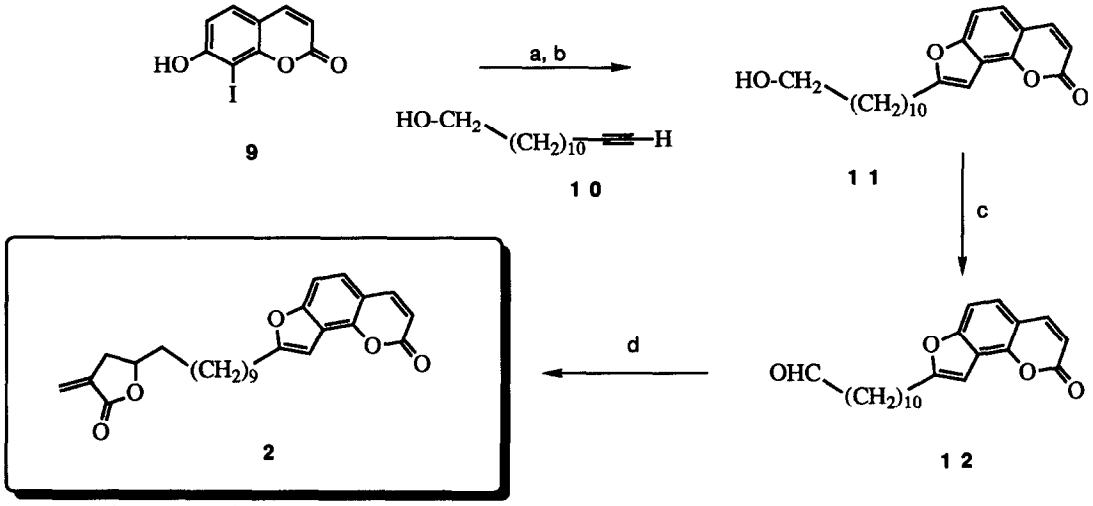
Synthesis[6].

5-hydroxypsoralen **5**, produced by dealkylation of bergamotin (AcOH, H_2SO_4) was converted, under basic conditions (K_2CO_3, DMF) to derivative **7** by reaction with the bromo derivative **6**. The acetonide group was then hydrolysed under acidic conditions (HCl 5%, THF) to give the aldehyde **8** in 71% yield. The aldehyde was finally converted to the α-methylene-γ-butyrolactone by treatment with α-bromomethacylic acid in the presence of $SnCl_2$ in THF[7], compound **1** being obtained with a yield of 64%.



Reagents and reaction conditions: a) **6**, K_2CO_3, DMF; b) HCl 5%, THF; c) $SnCl_2$, THF, α-bromomethacrylic acid

Compound **11** was prepared from iodocoumarin **9** by a Doad reaction[8] with alkyne **10**, in the presence of copper oxide in refluxing pyridine, in a yield of 90%. Deprotection of the THP group in acidic milieu (AcOH, THF, H_2O) followed by a Swern[9] oxidation (P_2O_5, DMSO, Et_3N) gave the aldehyde **12** in a yield of 77%. This was then converted to the α-methylene-γ-butyrolactone by the same reaction used previously, producing compound **2** in a yield of 40%.



Reagents and reaction conditions: a) **10**, CuO, pyridine, ; b) AcOH, THF, H_2O; c) P_2O_5, DMSO, CH_2Cl_2; Et_3N; d) $SnCl_2$, THF, α-bromomethacrylic acid.

Study of the phototoxicity of compounds 1 and 2[10].

The phototoxicity of compounds **1** and **2**, together with that of reference agents **3** and **4**, was tested in the mouse, using ear-swelling[11] to quantify the inflammatory reaction. As seen in figure 1, compounds **1**, at a concentration of 1.4 mM and with an irradiation dose of 6 J/cm^2, showed no phototoxicity, whereas compound **3** (5-MOP) induces a marked erythema. Compound **1** showed no phototoxicity at a concentration of 27.7 mM at a UVA dose of 12 J/cm^2.

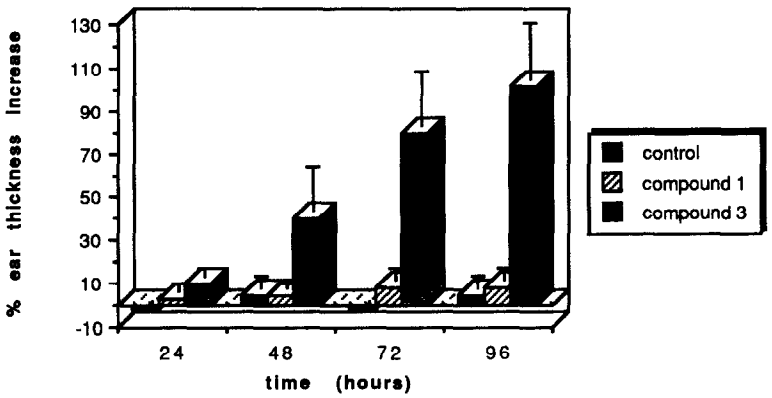


Figure 1: Phototoxicity of compounds **1** and **2** at 1.4 mM and vehicule after irradiation with 6 J/cm^2 UVA.

Compound **2** was found to be non-phototoxic at a concentration of 13.9 mM (its limit of solubility) and a UVA dose of 10 J/cm^2.

The two derivatives we have synthesised show no phototoxicity at the maximal concentration tested (their limits of solubility) and at usual irradiation doses. Their antiproliferative properties and mechanism of action are currently under investigation.

Acknowledgments.

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   Kanne, D.; Straub, K.; Hearst, J.E.; Rapoport, H. *J. Amer. Chem. Soc.* **1982**, *104*, 6754-6764.
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6. All compounds were fully characterized and gave satisfactory microanalysis.
   Compound **11:** white solid, m.p. 108-109°C, ^{1}H NMR δ 9.76 (t, 1H, CHO, J = 1.9 Hz); 8.16 (d, 1H, H_4, J = 9.9 Hz); 7.58 (d, 1H, $H_{5'}$, J = 2.4 Hz); 7.14 (s, 1H, H_8); 6.96 (dd, 1H, $H_{4'}$, J = 2.4 Hz, J = 1.0 Hz); 6.28 (d,1H, H_3, J = 9.9 Hz); 4.45 (t, 2H, CH_2Opso, J = 6.4 Hz); 2.41 (dt,2H,CH_2-CHO, J = 7.3 Hz, J = 1.9 Hz); 1.80-1.29 (m, 16H, $(CH_2)_8$).
   Compound **1:** To aldehyde **11** (180 mg, 0.48 mmol) in THF (0.5 mL) were added 1 mL of an acidic solution (37.5 mL $CH_3(CH_2)_2OH$, 20 mL H_2O, 10 mL CH_3COOH, 0.5 mL HCl), α-bromo-methacrylic acid (98 mg, 0.58 mmol) and $SnCl_2$ (256 mg, 1.21 mmol). The mixture was heated to reflux for 4 h, THF was removed under reduced pressure and the residue taken up in Et_2O. The ethereal layer was washed with HCl 1% (25 mL), water (25 mL), $NaHCO_3$ (25 mL) and water (25 mL). Organic solvents were removed under vacuum and the crude lactone purified by column chromatography (hexane, 18 % AcOEt, 10 % CH_2Cl_2) to give 136 mg (64 % yield) of compound **1** as a white solid. m.p. 64°C, ^{1}H NMR δ 8.26 (dd, 1H, H_4, J = 9.8 Hz, J = 0.6 Hz); 7.86 (d, 1H, $H_{5'}$, J = 2.4 Hz); 7.25 (dd, 1H, $H_{4'}$, J = 2.4 Hz, J = 1.0 Hz); 7.17 (m, 1H, H_8); 6.26 (d, 1H, H_3, J = 9.8 Hz); 6.04 (dt, 1H, H_i ou H_g, J = 2.8 Hz, J = 0.7 Hz); 5.63 (dt, 1H, H_i ou H_g, J = 2.5 Hz, J = 0.7 Hz); 4.58 (t , 2H, CH_2Opso, J = 6.4 Hz); 4.60-4.47 (m, 1H, H_j, X part of an ABX system); 3.13 (1H, H_e ou H_f detriplet A part of an ABX system, J_{AB} = 17.2 Hz, J_{BX}= 7.6 Hz, J= 2.6 Hz); 2.62 (1H, H_e or H_f detriplet B part of an ABX system, J_{AB} = 17.2 Hz, J_{AX} = 6.1 Hz, J = 3.0 Hz); 1.98 -1.64 (m, 2H, CH_2-lactone); 1.73-1.15 (m, 16H, $(CH_2)_8$); IR ν 1755 (C=O γ-lactone), 1723 (C=O pyrone); UV (CH_3CN) 222 nm (ε = 28740), 250 nm (ε = 24100), 292 nm (ε = 18800), 352 nm (ε = 15460).
   Compound **18:** white crystals, m.p. 53°C, ^{1}H NMR δ 9.76 (t, 1H, CHO, J = 1.9 Hz); 7.78 (d, 1H, H_4, J = 9.5 Hz); 7.33 (1H, H_6, dedoublet A part of an AB system, J_{AB} = 8.4 Hz, J = 0.9 Hz); 7.28 (1H, H_5, B part of an AB system, J_{AB} = 8.4 Hz); 6.72 (m, 1H, $H_{4'}$); 6.35 (d, 1H, H_3, J = 9.5 Hz); 2.80 (dt, 2H, CH_2-furane, J = 7.5 Hz, J = 0.7 Hz); 2.41 (dt, 2H, CH_2-CHO, J = 7.3 Hz, J = 1.9 Hz); 1.80-1.29 (m, 16H, $(CH_2)_8$); IR ν 1734 cm^{-1} (C=O coumarin), 1700 cm^{-1} (C=O aldehyde).
   Compound **2:** white crystals, m.p. 110-111°C, ^{1}H NMR δ 7.78 (d, 1H, H_4, J = 9.5 Hz); 7.34 (1H, H_6, dedoublet part of an AB system, J_{AB} = 8.8 Hz, J = 0.8 Hz); 7.29 (1H, H_5, B part of an AB system J_{AB} = 8.8 Hz); 6.73 (d, 1H, $H_{4'}$, J = 0.8 Hz); 6.35 (d, 1H, H_3, J = 9.5 Hz); 6.22 (t, 1H, H_h, J = 3.0 Hz); 5.61 (t, 1H, H_i, J =); 4.53-4.44 (m, 1H, H_e, X part of an ABX system); 3.04 (1H, H_f ou H_g, detriplet part of an ABX system, J_{AB} = 17.0 Hz, J_{BX} = 7.6 Hz, J = 2.5 Hz); 2.80 (t, 2H, CH_2-furane, J = 7.2 Hz); 2.57 (1H, H_f ou H_g, detriplet part of an ABX system J_{AB} = 17.0 Hz, J_{AX} = 6.2 Hz, J = 3.1 Hz); 1.80-0.85 (m, 18H, $(CH_2)_9$); UV (CH_3CN) 204 nm (ε = 27900), 254 nm (ε = 22700), 298 nm (ε = 9800); IR ν 1763 cm^{-1} (C=O γ-lactone); 1728 cm^{-1} (C=O coumarin).
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10. ***Animals***: Female BALB/c mice, 8 weeks of age, were obtained from IFFA CREDO (Lyon, France). They were maintained in an animal care facility at constant temperature and fed with dry food and water *ad libitum*.
    Application of the compound to be tested. The compound to be tested in solution in acetone/olive oil (4:1; 50 µL) was applied on the ventral side of the right ear one hour before irradiation. Increase in ear thickness was measured with an engineering micrometer ODITEST™ (Germany) 24, 48, 72 and 96 hours after ear irradiation .
    Irradiation. A high pressure UVA lamp (Heraeus 400W®, Sunlamp® type) was used. Prior to irradiation, power was measured (mW/cm^2) using a Dixwell® UVA-364 radiometer.
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