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Synthesis and photocytotoxic activity of new α-methylene-γ-butyrolactone-psoralen heterodimers

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Abstract—The synthesis of a new α-methylene-γ-butyrolactone-psoralen heterodimer **2** is reported. Its photoantiproliferative activity and skin phototoxicity were compared with that of 5-methoxypsoralen (5-MOP) and another heterodimer **1**. Both derivatives show a significant phototoxicity toward malignant cell lines including melanoma cells A375 compared to their intrinsic cytotoxicity in the dark. Both compounds were found to be nonphototoxic on mice skin and therefore could be active potential drugs in photochemotherapy.

1. Introduction

The use of drugs in association with ultra-violet light for the treatment of skin diseases can be traced back to ancient Egypt, India and Greece, where plant extracts, containing psoralens, were applied on the skin in association with light to treat psoriasis and vitiligo.[1] This concept, known as photochemotherapy, represents a common basis for different therapeutic procedures, such as PUVA (psoralen+UVA)[2,3] and photodynamic therapy.[4] The mechanism of cellular damage mediated by photochemotherapy includes a variety of biochemical and molecular reactions, leading to inhibition of the hyperproliferation of skin keratinocytes or tissue destruction. Unfortunately, most photosensitizing chemicals are also phototoxic for the skin; contact with these molecules in the presence of UV irradiation results in sunburn, erythema and, eventually oedema.[4,5]

In recent years, several highly photoreactive molecules have been synthesized with the aim of developing new photochemotherapeutic drugs with less side effects.[6–8] Thus, we have reported[9] the synthesis of a new heterodimer **1** based on a furocoumarin moiety bond to a α-methylene-γ-butyrolactone by a polymethylenic chain (Fig. 1) and shown that this molecule was not phototoxic for the skin. Psoralens such as 5-methoxypsoralen (5-MOP) and 8-methoxypsoralen (8-MOP) are widely used in PUVA therapy for the treatment of a variety of epidermal proliferative diseases.[2,3] Their biological properties are generally related to their ability to bind covalently to nucleic acids.[10] On the other side, α-methylene-γ-butyrolactones are very good electrophiles and are known to react with nucleophilic residues on proteins to form covalent bonds.[11,12]

Moreover, we have shown that α-methylene-γ-butyrolactones had an interesting photoreactivity potential and

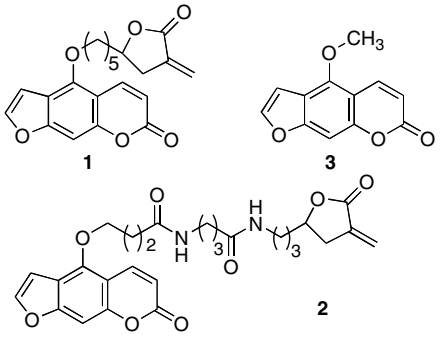

Figure 1. Structure of heterodimeric compounds **1** and **2** and of the reference compound 5-methoxypsoralen **3**.

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can form intramolecular photoadducts with psoralens[9] and intra or intermolecular photoadducts with thymine.[13,14]

The use of a psoralen to target this photoactivable/electrophilic group toward DNA could lead to the formation of diadducts of a new type, between two different sites on DNA and/or between DNA and structural proteins.

Based on these considerations, we have synthesized a new heterodimeric derivative **2** in which the psoralen is bounded to a α-methylene-γ-butyrolactone via a polyamide chain in order to increase its affinity toward DNA (Fig. 1).

In this paper we report the synthesis of **2** and the skin phototoxicity of compounds **1** and **2** in comparison with that of the 5-methoxypsoralen **3** used as reference compound. We also report the cytotoxicity properties of these molecules on three human cancer cell lines, with or without irradiation.

2. Results and discussion

2.1. Chemistry

The heterodimer **2** was synthesized according to a convergent strategy (Scheme 1). The two parts of the molecule, **4** and **5** were prepared separately before coupling and the α-methylene-γ-butyrolactone moiety was generated in the last step.

5-Hydroxypsoralen (bergaptol), prepared by dealkylation of bergamottin in a mixture of acetic and sulfuric acid[15] was converted under basic conditions into derivative **7** by reaction with the 4-bromobutyric acid derivative **6**. The deprotection was achieved by reduction with zinc dust in acidic solution and the acid derivative **4** was finally obtained in 91% yield.[16]

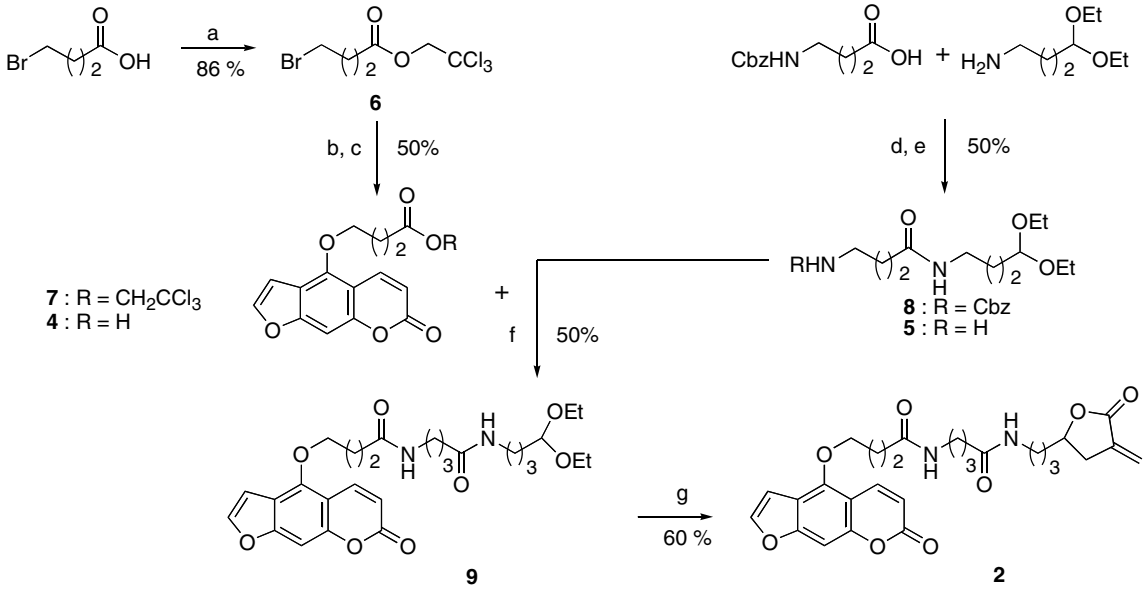
A peptidic coupling of *N*-Cbz-γ-aminobutyric acid and 4,4-diethoxybutylamine using DCC/DMAP[17,18] gave rise to compound **8** in 90% yield. The amine **5** was obtained in 99% yield from the benzyloxycarbonyl-protected precursor **8** using ammonium formate/10% palladium on carbon procedure.[19]

The two components of the target molecule were first coupled using BOP/NMM as coupling reagent.[20] This method gave rise to compound **9** in good yield (78%) but with a reaction time of three days. Using EDCI/DMAP as coupling reagent,[21] compound **9** was obtained after only 6 h with quantitative yield.

Then the α-methylene-γ-butyrolactone ring was generated in good yield directly from the protected aldehyde using 'Reformatsky type' conditions as previously described.[13] For this purpose, we have used the commercially available α-bromomethacrylic acid. The yield of the reaction could be improved (77%) by use of the methyl-α-bromomethacrylate, prepared in one step from the acid.[22]

2.2. Effect on the growth of human cancer cell lines

The antiproliferative activity of **1**, **2**, and **3** was assessed under two different experimental conditions: by incubation in the dark or after UVA irradiation. The growth inhibitory activity of these compounds against three



Scheme 1. Synthesis of **2**. Reagents and conditions: (a) 1.5 equiv CCl_3CH_2OH, 0.75 equiv *p*-TsOH, toluene, reflux, 58 h; (b) 0.9 equiv bergaptol, 1.3 equiv K_2CO_3, acetone, reflux, 18 h; (c) 19 equiv Zn, AcOH, CH_2Cl_2, rt, 19 h; (d) 1 equiv DCC, 0.9 equiv DMAP, CH_2Cl_2, rt, 18 h; (e) Pd/C, 3.5 equiv ammonium formate, MeOH, rt, 6 h; (f) 1.2 equiv EDCI, 1.1 equiv DMAP, CH_2Cl_2, rt, 6 h; (g) 1.6 equiv $SnCl_2{\cdot}H_2O$, 1.6 equiv α-bromomethacrylic acid, THF, H^+, reflux, 48 h.

Table 1. Cell growth inhibition for the compounds

Compd	Cell lines IC_{50} (μM)					
	HL-60 (promyelocytic leukemia)[a]		K1 (thyroid carcinoma)[b]		A375 (malignant melanoma)[b]	
	Dark	UVA	Dark	UVA	Dark	UVA
1	2.73	0.31	2.84	1.13	4.82	0.70
2	15.76	2.50	>15.00	6.96	27.6	3.50
3	>40.00	0.29	>39.00	29.50	>35.00	23.70

[a] Concentration inducing a 50% growth inhibition assessed by the Uptiblue test.
[b] Concentration inducing a 50% growth inhibition assessed by the SRB test.

human cancer cell lines, HL-60 (promyelocytic leukemia), K1 (thyroid carcinoma), A375 (malignant melanoma), is summarized in Table 1. The results are expressed in concentrations able to inhibit 50% of proliferation (IC_{50}). Different behaviors were observed for heterodimer derivatives **1**, **2** and the reference 5-MOP **3** as well as between adherent or nonadherent cell lines. On nonadherent cell lines, like HL-60, 5-MOP, **3** was found to be the most cytotoxic derivative after irradiation with UVA (Table 1). The photocytotoxicity of **1** was very similar to that of **3** (Table 1), but the heterodimer **1** was also highly cytotoxic in the dark (Fig. 2). The heterodimer **2** was found to be less photocytotoxic than **1** and **3**, but was also less cytotoxic in the dark than **1**.

On adherent cell lines, like K1 or A375, 5-MOP **3** was the less photocytotoxic derivative while heterodimers **1** and **2** showed a significant photoantiproliferative activity. It should be noticed that **1** presented also a significant cytotoxicity in the dark while **2** was less cytotoxic.

2.3. Phototoxicity study

The skin phototoxicity of compounds **1** and **2**, together with that of **3**, was tested on mice, using the mouse ear swelling test (MEST)[23] to quantify the inflammatory reaction.

As seen in Figure 3, heterodimer **2** showed no phototoxicity when tested at a concentration of 2.77 mM and

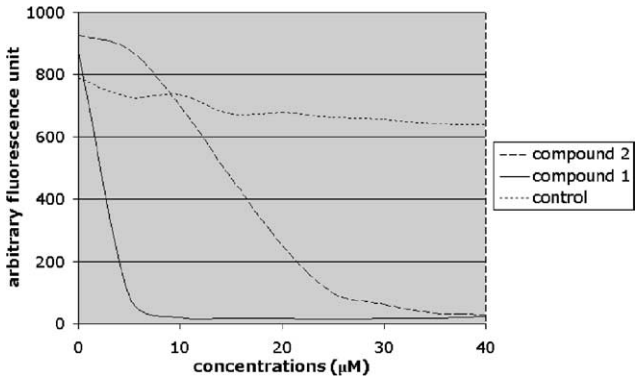


Figure 2. Cytotoxicity of compounds **1** and **2** compared to 5-MOP **3** (control) in the dark determined on HL-60 cells using the Uptiblue test.

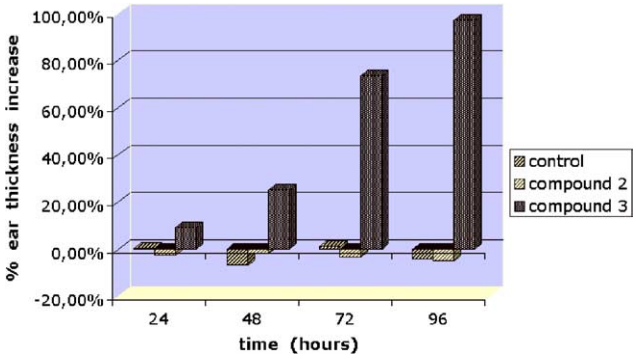


Figure 3. Phototoxicity of compounds **2** and **3** (2.77 mM) and control (vehicle) after irradiation with 6 J cm^{-2} UVA, using the MEST.

with an irradiation dose of 6 J cm^{-2}, whereas the reference compound **3** (5-MOP) induced a marked oedema. Concentration of **2** could be increased up to 27.7 mM and a UVA dose of 6 J cm^{-2} without any phototoxicity.

Following the same procedure, compound **1** was found to be nonphototoxic when irradiated at a concentration of 27.7 mM with a UVA dose of 6 J cm^{-2}.

3. Conclusion

We have synthesized a new heterodimeric derivative **2** based on a psoralen linked to an α-methylene-γ-butyrolactone by a polyamide chain in order to increase its affinity with DNA. The cytotoxicity and photocytotoxicity properties of compounds **1** and **2** were evaluated on three human cancer cell lines and compared with the one of 5-methoxypsoralen **3**. The new heterodimer **2** showed an interesting cytotoxic activity on all tumor cell lines tested with a higher cytotoxicity after irradiation and a low toxicity in the dark. Heterodimer **1** and 5-MOP **3** are cytotoxic after irradiation, but they are also highly toxic in the dark.

This toxicity could be due to the presence of the α-methylene-γ-butyrolactone group, which is a well-known Michael acceptor and can react rapidly with nucleophilic residues in proteins to form covalent bonds.[11] An inhibition of cellular enzymes activities and metabolism could be observed[24,25] leading to an antiproliferative effect.

The phototoxicity of these derivatives was evaluated on mouse skin, as the ability of furocoumarins such as 5-methoxypsoralen **3** to induce severe erythemas on the skin is a well-known and serious side effect in PUVA therapy. Heterodimeric derivatives appeared to be unable to cause skin phototoxicity, even when tested in harsh experimental conditions (MEST experiment).

The precise cellular target affected by these compounds is so far not known. Further work is currently under investigation to fully elucidate the mechanism of action of these compounds.

4. Experimental

4.1. Chemistry

^{1}H and ^{13}C NMR spectra were recorded on Bruker AC200, Avance 300, or AM400-MHz spectrometers in $CDCl_3$ unless otherwise specified. Chemical shifts are reported in ppm (δ) with respect to TMS, and $CHCl_3$ was used as internal standard ($\delta = 7.27$ ppm). Multiplicities are indicated by s (singlet), d (doublet), t (triplet), m (multiplet). Infrared spectra were obtained on a Perkin–Elmer FT-IR 1600 spectrometer; peaks are reported in reciprocal centimeters. UV spectra were obtained using a Hewlett Packard HP 8452 diode-array spectrophotometer. Melting points were determined in capillary tubes on a Buchi Tottoli 510 apparatus and are uncorrected. Elemental analyses were performed on a Perkin–Elmer 240B and were within ±0.4% of calculated values in all cases. Dried solvents were freshly distilled before use. Tetrahydrofuran and ethyl ether were distilled from sodium benzophenone. Methylene chloride was dried over P_2O_5 before distillation. All air- or moisture-sensitive reactions were conducted in flame-dried glassware under an atmosphere of dry argon. Chromatographic purifications were conducted on silica gel columns (Merck 60, 0.040–0.063 mm) or alumina gel (Merck 90, activity II–III, 0.063–0.200 mm) according to the flash chromatography technique. Analytical TLC was performed on pre-coated silica gel plates (Merck 60 F254).

4.1.1. 2′,2′,2′-Trichloroethyl-4-bromobutyrate (6). In a two-necked round bottom flask equipped with a Dean–Stark apparatus 2,2,2-trichloroethanol (4.30 mL, 45.00 mmol, 1.50 equiv) and *p*-TsOH (4.00 g, 23.00 mmol, 0.75 equiv) were added to a solution of 4-bromobutyric acid (5.00 g, 30.00 mmol) in toluene (150 mL). After stirring under reflux for 48 h, toluene was removed under vacuum. The residue dissolved in Et_2O (150 mL) was washed with saturated aqueous $NaHCO_3$ (30 mL), water (2×30 mL), brine (2×30 mL), dried over $MgSO_4$ and concentrated. Purification by column chromatography on silica (hexane/EtOAc, 95/5) afforded a colorless oil (7.7 g, 86%). ^{1}H NMR (200 MHz, $CDCl_3$): 4.75 (s, 2H, $H_{1'}$), 3.49 (t, 2H, H_4, $J = 6.4$ Hz), 2.67 (t, 2H, H_2, $J = 7.1$ Hz), 2.23 (m, 2H, H_3). ^{13}C NMR (50 MHz, $CDCl_3$): 170.9 (C_1), 94.9 ($C_{2'}$), 74.0 ($C_{1'}$), 32.2 (C_4), 28.0 (C_2), 27.5 (C_3). IR (film), ν (cm^{-1}) 1754 (C=O ester), 720 (C–Cl). Anal. Calcd for $C_6H_8BrCl_3O_2$ (298.4): C, 24.15; H, 2.70. Found: C, 24.34; H, 2.68.

4.1.2. 5-(Trichloroethyloxybutyrate)psoralen (7). K_2CO_3 (1.70 g, 12.30 mmol) was added to a solution of bergaptol (2.00 g, 10 mmol) in dry acetone (110 mL). After stirring under reflux for 1.5 h, a solution of ester **6** (3.26 g, 11.40 mmol) in acetone (20 mL) was added and stirring continued under reflux for 21 h. The mixture was cooled down to rt and acetone was removed under vacuum. The residue was taken-up in EtOAc (100 mL) and washed with water (3×50 mL) and 10% aqueous HCl (3×50 mL). The organic layer was dried over $MgSO_4$ and concentrated under vacuum to afford **7**, which precipitated from hot EtOAc/Et_2O as a white powder (3.5 g, 80%). Mp 110–112 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 8.16 (d, 1H, H_4, $J = 9$ Hz), 7.63 (d, 1H, $H_{5'}$, $J = 3$ Hz), 7.3 (s, 1H, H_8), 6.99 (d, 1H, $H_{4'}$, $J = 3$ Hz), 6.33 (d, 1H, H_3, $J = 9$ Hz), 4.81 (s, 2H, CH_2CCl_3), 4.57 (t, 2H, $H_{4''}$, $J = 6$ Hz), 2.83 (t, 2H, $H_{2''}$, $J = 6$ Hz), 2.28 (m, 2H, $H_{3''}$). ^{13}C NMR (75 MHz, $CDCl_3$): 170.2 ($C_{1''}$), 161.1 (C_2), 158.3 (C_9), 152.7 (C_7), 148.5 (C_5), 145.1 ($C_{5'}$), 139.1 (C_4), 113.5 (C_{10}), 112.9 (C_3), 106.9 (C_6), 104.9 ($C_{4'}$), 94.3 (C_8 and CCl_3), 74.1 (CH_2CCl_3), 71.5 ($C_{4''}$), 30.4 ($C_{2''}$), 25.2 ($C_{3''}$). IR ($CHCl_3$), ν (cm^{-1}) 1726 (C=O coumarin and ester), 1216 (C–O). UV (EtOH), λ (nm): 222 ($\varepsilon = 23,576$), 250 ($\varepsilon = 18,150$), 260 ($\varepsilon = 16,266$), 268 ($\varepsilon = 16,052$), 310 ($\varepsilon = 14,419$). Anal. Calcd for $C_{17}H_{13}Cl_3O_6$ (419.64): C, 48.86; H, 3.12. Found: C, 48.92; H, 3.30.

4.1.3. 5-(Oxybutyric-acid)psoralen (4). A solution of AcOH/H_2O (9/1, 4.00 mL) was added to ester **7** (1.00 g, 2.38 mmol), in CH_2Cl_2 (5 mL). The resulting mixture was cooled down to 0 °C and powder zinc (2.94 g, 45.00 mmol, 19 equiv) was added. After 40 min at 0 °C the reaction was allowed to warmup to rt and stirred for an additional 26 h. The zinc was filtered and the organic layer was washed with water (3×50 mL) and extracted with saturated aqueous Na_2CO_3 (3×30 mL). The aqueous layer was acidified with 1 M HCl and then extracted with EtOAc (5×30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum to afford acid **4** as a white solid (623 mg, 91%). Mp 164–166 °C. ^{1}H NMR (200 MHz, acetone-d_6): 8.11 (d, 1H, H_4, $J = 9.8$ Hz), 7.55 (d, 1H, $H_{5'}$, $J = 2.4$ Hz), 7.20 (s, 1H, H_8), 6.90 (d, 1H, $H_{4'}$, $J = 2.4$ Hz), 6.26 (d, 1H, H_3, $J = 9.8$ Hz), 4.47 (t, 2H, $H_{4''}$, $J = 6.1$ Hz), 2.62 (t, 2H, $H_{2''}$, $J = 7.1$ Hz), 2.19 (m, 2H, $H_{3''}$). ^{13}C NMR (50 MHz, acetone-d_6): 174.1 ($C_{1''}$), 160.0 (C_2), 157.5 (C_9), 152.0 (C_7), 148.6 (C_5), 145.9 ($C_{5'}$), 139.5 (C_4), 112.8 (C_{10}), 112.3 (C_3), 106.0 (C_6), 105.5 ($C_{4'}$), 93.2 (C_8), 66.8 ($C_{4''}$), 30.1 ($C_{2''}$), 24.9 ($C_{3''}$). IR ($CHCl_3$), ν (cm^{-1}) 1736 (C=O coumarin), 1704 (C=O acid), 1214 (C–O). UV (EtOH), λ (nm): 222 ($\varepsilon = 24,851$), 250 ($\varepsilon = 18,326$), 260 ($\varepsilon = 17,008$), 268 ($\varepsilon = 17,338$), 310 ($\varepsilon = 15,034$). Anal. Calcd for $C_{15}H_{12}O_6$ (228.25): C, 62.50; H, 4.20. Found: C, 62.38; H, 4.28.

4.1.4. 4-Carbobenzyloxyamino-*N*-(4′,4′-diethoxybutyl)butanamide (8). DMAP (2.10 g, 17.22 mmol) was added to a solution of *N*-Cbz-γ-aminobutyric acid (5.00 g, 21.00 mmol) in dry CH_2Cl_2 (100 mL). After cooling the mixture to −10 °C, DCC (3.85 g, 18.70 mmol) in dry CH_2Cl_2 (30 mL) and aminobutanal diethylacetal (3.00 mL, 17.58 mmol) were added. The reaction mixture was stirred at −10 °C for 20 min and then allowed to warmup to rt for further 18 h. Formed urea was filtered off and the reaction washed with brine (2 × 100 mL). The organic layer was dried over $MgSO_4$ and concentrated under vacuum. Purification by column chromatography on silica (hexane/EtOAc, 10/90) afforded **8** as a white solid (6.0 g, 90%). Mp 60–62 °C. ^{1}H NMR (200 MHz, $CDCl_3$): 7.31–7.24 (m, 5H, –Ph), 7.10 (br s, 1H, NH), 6.20 (br s, 1H, NH), 5.03 (s, 2H, CH_2Ph), 4.42 (t, 1H, $H_{4''}$, $J = 5.0$ Hz), 3.73 (m, 2H, $H_{5'}$), 3.42 (m, 2H, $H_{5'}$), 3.17 (m, 4H, H_4 and $H_{1'}$), 2.14 (t, 2H, H_2, $J = 6.9$ Hz), 1.83 (m, 2H), 1.50 (m, 4H), 1.18 (t, 6H, $H_{6'}$, $J = 4.8$ Hz). ^{13}C NMR (50 MHz, $CDCl_3$): 172.0 (*CO*Cbz), 160.0 (C_1), 136.6 (C_{arom}), 128.4 (C_{arom}), 128.0 (C_{arom}), 102.6 ($C_{4'}$), 66.6 (CH_2Ph), 61.5 ($C_{5'}$), 40.4, 39.3, 33.9, 31.1, 26.1, 24.6, 15.4 ($C_{6'}$). IR ($CHCl_3$), ν (cm^{-1}) 1711 (C=O amide), 1668 (C=O carbamate), 1220 (C–O). Anal. Calcd for $C_{20}H_{32}N_2O_5$ (380.23): C, 63.13; H, 8.48; N, 7.36. Found: C, 63.25; H, 8.51; N, 7.28.

4.1.5. 4-Amino-*N*-(4′,4′-diethoxybutyl)butanamide (5). Pd/C (10%, 0.44 g) and ammonium formate (0.46 g, 7.22 mmol) were added to a solution of **8** (0.80 g, 2.11 mmol) in methanol (20 mL). After stirring at rt until the starting material has disappeared, the catalyst was removed by filtration on Celite and washed with MeOH (5 × 10mL). The residue was concentrated under vacuum to afford a colorless oil (518 mg, 99%), which we used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): 6.09 (br s, 1H, NH), 4.47 (t, 1H, $H_{4'}$, $J = 5.2$ Hz), 3.63 (m, 2H, $H_{5'}$), 3.47 (m, 2H, $H_{5'}$), 3.25 (m, 2H, $H_{1'}$), 2.75 (t, 2H, H_4, $J = 6.7$ Hz), 2.24 (t, 2H, H_2, $J = 7,0$ Hz), 1.81 (br s, 2H, NH_2), 1.78 (m, 2H, $H_{3'}$), 1.60 (m, 4H, H_3 and $H_{2'}$), 1.20 (t, 6H, $H_{6'}$, $J = 7.0$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 172.7 (C_1), 102.7 ($C_{4'}$), 61.4 ($C_{5'}$), 40.9, 39.2, 33.9, 31.1, 28.2, 24.6, 15.3 ($C_{6'}$). IR ($CHCl_3$), ν (cm^{-1}) 3320 (–NH_2), 1658 (C=O amide), 1560 (NH_2).

4.1.6. *N*-[3-(4,4-Diethoxybutylcarbamoyl)-propyl]-4-(7-oxo-7*H*-furo[3,2-*g*]chromen-4-yloxy)-butyramide (9). DMAP (8 mg, 0.06 mmol) and EDCI (164 mg, 1.03 mmol) were added to a solution of acid **4** (268 mg, 0.93 mmol) in dry CH_2Cl_2 (15 mL) at −10 °C. After stirring for 20 min at −10 °C, amine **5** (270 mg, 1.10 mmol) in dry CH_2Cl_2 (10 mL) was added. The reaction was stirred at 10 °C for 1 h 30 and 20 h at room. The mixture was concentrated under vacuum and purified by column chromatography on alumina (CH_2Cl_2/MeOH, 98/2) to afford **9** as a white solid (480 mg, 99%). Mp 116–118 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 8.13 (d, 1H, H_4, $J = 9.5$), 7.56 (d, 1H, $H_{5'}$, $J = 2.3$ Hz), 7.06 (s, 1H, H_8), 6.96 (d, 1H, $H_{4'}$, $J = 2.3$ Hz), 6.76 (br s, 1H, NH), 6.27 (br s, 1H, NH), 6.23 (d, 1H, H_3, $J = 9.5$ Hz), 4.48 (m, 3H, $H_{1''}$ and $H_{12''}$), 3.62 (m, 2H, $H_{13''}$), 3.46 (m, 2H, $H_{13''}$), 3.26 (m, 4H, $H_{5''}$ and $H_{9''}$), 2.45 (t, 2H, $H_{3''}$, $J = 7.3$ Hz), 2.22 (m, 4H, $H_{2''}$ and $H_{7''}$), 1.81 (m, 2H, $H_{11''}$), 1.61 (m, 4H, $H_{10''}$ and $H_{6''}$), 1.18 (t, 6H, $H_{14''}$, $J = 7.1$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 172.8 ($C_{4''}$ or $C_{8''}$), 172.2 ($C_{4''}$ or $C_{8''}$), 161.3 (C_2), 158.2 (C_9), 152.5 (C_7), 148.7 (C_5), 144.9 ($C_{5'}$), 139.4 (C_4), 1123.1 (C_{10}), 112.4 (C_3), 106.5 (C_6), 105.1 ($C_{4'}$), 102.6 ($C_{12''}$), 93.8 (C_8), 71.9 ($C_{1''}$), 61.5 ($C_{13''}$), 39.4, 34.1, 32.6, 31.1, 25.9, 25.1, 24.5, 15.3 ($C_{14''}$). IR ($CHCl_3$), ν (cm^{-1}) 1732 (C=O coumarin), 1672 (C=O amide), 1209 (C–O). UV (EtOH), λ (nm): 208 ($\varepsilon = 16,496$), 222 ($\varepsilon = 17,121$), 250 ($\varepsilon = 11,796$), 260 ($\varepsilon = 10,704$), 268 ($\varepsilon = 10,785$), 310 ($\varepsilon = 8884$). Anal. Calcd for $C_{27}H_{36}N_2O_8$ (516.25): C, 62.78; H, 7.02; N, 5.42. Found: C, 62.76; H, 7.28; N, 5.35.

4.1.7. *N*-{3-[3-(3-Methylene-5-oxo-tetrahydrofuran-2-yl)-propylcarbamoyl]-propyl}-4-(7-oxo-7*H*-furo[3,2-*g*]chromen-4-yloxy)-butyramide (2). To a solution of compound **9** (205 mg, 0.40 mmol) in THF (4 mL) was added 8 mL of an acidic solution (37.5 mL $CH_3O(CH_2)_2OH$, 20 mL H_2O, 10 mL CH_3COOH, 0.5 mL HCl_{concd}), α-bromomethacrylic acid (118 mg, 0.2 mmol, 1.8 equiv) and $SnCl_2{\cdot}2H_2O$ (129 mg, 0.68 mmol, 1.7 equiv). The mixture was heated under reflux for 21 h, and the solvents removed under reduced pressure. The residue was taken up in EtOAc (50 mL) and water (20 mL). pH was adjusted with saturated aqueous $NaHCO_3$ until 8 and the aqueous layer was extracted with EtOAc (5 × 20 mL). Combined organic layers were dried on $MgSO_4$, filtered and concentrated under vacuum to afford the compound **2**, which crystallized from EtOAc/Et_2O as a white solid (124 mg, 61%). Mp 84-86 °C. ^{1}H NMR (400 MHz, DMSO-d_6): 8.21 (d, 1H, H_4, $J = 9.8$ Hz), 8.02 (d, 1H, $H_{5'}$, $J = 2.4$ Hz), 7.88 (br t, 1H, –NH–, $J = 5.4$ Hz), 7.79 (br t, 1H, –NH–, $J = 5.5$ Hz), 7.34 (s, 1H, H_8), 7.28 (d, 1H, $H_{4'}$, $J = 2.4$ Hz), 6.29 (d, 1H, H_3, $J = 9.8$ Hz), 5.98 (dd, 1H, $H_{15''}$, $J_1 = J_2 = 2.7$ Hz), 5.65 (dd, 1H, $H_{15''}$, $J_1 = J_2 = 2.3$ Hz), 4.55 (m, 1H, $H_{12''}$), 4.49 (t, 3H, $H_{1''}$, $J = 6.2$ Hz), 3.05 (m, 5H, $H_{5''}$, $H_{9''}$, and $H_{13''}$), 2.56 (m, 1H, $H_{13''}$), 2.33 (t, 2H, $H_{3''}$, $J = 7.3$ Hz), 2.06 (m, 4H, $H_{2''}$ and $H_{7''}$), 1.61 (m, 2H, $H_{6''}$), 1.58 (m, 2H, $H_{11''}$), 1.46 (m, 2H, $H_{10''}$). ^{13}C NMR (100 MHz, DMSO-d_6): 171.5 ($C_{4''}$), 171.3 ($C_{8''}$), 169.9 ($C_{16''}$), 160.1 (C_2), 157.6 (C_9), 152.1 (C_7), 148.6 (C_5), 145.9 ($C_{5'}$), 139.5 (C_4), 135.2 ($C_{14''}$), 121.3 ($C_{15''}$), 110.8 (C_{10}), 112.3 (C_3), 106.0 (C_6), 105.5 ($C_{4'}$), 93.2 (C_8), 76.9 ($C_{12''}$), 72.1 ($C_{1''}$), 38.6 ($C_{5''}$ or $C_{9''}$), 38.0 ($C_{5''}$ or $C_{9''}$), 33.3 ($C_{7''}$ or $C_{11''}$), 32.8 ($C_{7''}$ or $C_{11''}$), 32.7 ($C_{13''}$), 31.7 ($C_{3''}$), 25.5 ($C_{2''}$ or $C_{6''}$), 25.4 ($C_{2''}$ or $C_{6''}$), 24.6 ($C_{10''}$). IR ($CHCl_3$), ν (cm^{-1}) 3480 (NH), 1756 (C=O lactone), 1731 (C=O coumarin), 1662 (C=O amide). UV (EtOH), λ (nm): 210 ($\varepsilon = 28,893$), 250 ($\varepsilon = 15,399$), 268 ($\varepsilon = 14,064$), 310 ($\varepsilon = 12,100$). Anal. Calcd for $C_{27}H_{30}N_2O_8$ (510.20): C, 63.52; H, 5.92; N, 5.49. Found: C, 63.96; H, 6.28; N, 5.21.

4.2. Biology

4.2.1. Cell cultures. HL-60 cells were grown in RPMI 1640 (Sigma Chemical Co.) supplemented with 15%

heat-inactivated fetal calf serum (Seromed), 1% sodium pyruvate. K1 cells were grown in complete DMEM medium with phenol red supplemented with 8.6% heat-inactivated fetal calf serum (Seromed), and Nutrient Mixture F-12 (HAM). A375 cells were grown in DMEM with phenol red supplemented with 15% heat-inactivated fetal calf serum (Seromed). Penicillin–streptomycin (1%) was added to the three media. Cells were cultured at 37 °C in a moist atmosphere with 5% carbon dioxide.

4.2.2. Irradiation procedure. Irradiations were performed with a Tecima-Dixwell® block equipped with a mercury vapor lamp Heraus® 400 W emitting between 320 and 400 nm. Irradiation intensity was checked on Dixwell® radiometers UVA-365 and UVB-312 before each experiment.

4.2.3. Proliferation assays. Cells (4.5×10^3) were seeded into each well of a 96-well cell culture plate. After 24 h of incubation, various concentrations of the test agents in DMSO solution were administered so that the final DMSO concentration never exceeded 1%. Controls were treated with the same concentration of DMSO in medium solution.

After incubation for 5 h in the dark, the medium containing phenol red was replaced with an equal volume of PBS in the case of K1 and A375 cells and cells were irradiated with a UVA dose of 0.8 J cm^{-2}. After irradiation PBS was removed and cells were incubated in DMEM medium for 60 h. A sulforhodamine B (SRB) test was performed 64 h after irradiation to assess cell viability of K1 and A375 cells. Cells in 96-well plates were washed with PBS, fixed with 10% ice-cold trichloroacetic acid at 4 °C for 1 h, then washed with water five times and dried at room temperature. The cellular proteins in each well were stained with 100 μL of 0.4% SRB in 1% acetic acid at room temperature for 20 min and then washed with 1% acetic acid four times and dried at 37 °C for another 30 min. To dissolve the SRB bound to cellular protein, 200 μL of 10 mM Tris were added to each well and incubated at room temperature with mechanical agitation until the color became homogeneous. SRB bound to protein was measured by absorbance at a 550 nm wavelength using MRX microplate reader (Dynech, France).

HL-60 cells were kept in the dark for 5 h and then irradiated with a UVA dose of 0.8 J cm^{-2}. After irradiation, cells were incubated in the dark for further 60 h. Cell growth on HL-60 cells was determined using the Uptiblue (UB) reagent (Interchim, Montluçon, France), according to the manufacturer's instructions. After 20, 40, and 60 h of incubation at 37 °C after irradiation, 20 μL of UB solution were added to each wells and 96-wells microplates were read at 590 nm (excitation at 560 nm), using a Fluorolite 1000 microplate reader (Dynex, Issy-les-Moulineaux, France). Each experiment was repeated at least four times.

Cytotoxicity data were expressed in IC_{50} values, corresponding to the concentrations of the test agent inducing 50% reduction of the measured parameter (quantity of proteins...) compared with control cultures.

4.2.4. Skin phototoxicity. Skin phototoxicity was tested on BALB/c female mice according to the mouse ear swelling test (MEST). An acetone/olive oil solution of each compound (2.77 and 27.7 mM) was applied topically to the right ear of each mouse, the left ear was not treated and used as an internal control. The animals were kept in the dark for 1 h and then irradiated with a UVA dose of 6 J cm^{-2}. After irradiation the ear's swelling was recorded every 24 h for six days.

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