ELSEVIER

Il Farmaco 53 (1998) 602–610

IL FARMACO

Synthesis of angelicin heteroanalogues: preliminary photobiological and pharmacological studies

Luisa Mosti[a,*], Eleonora Lo Presti[a], Giulia Menozzi[a], Christine Marzano[b], Francarosa Baccichetti[b], Giuseppe Falcone[c], Walter Filippelli[c], Brunella Piucci[c]

[a]*Dipartimento di Scienze Farmaceutiche, Università degli Studi, Viale Benedetto XV 3, 16132 Genoa, Italy*
[b]*Dipartimento di Scienze Farmaceutiche, Università degli Studi, Via Marzolo 5, 35123 Padua, Italy*
[c]*Istituto di Farmacologia e Tossicologia, Facoltà di Medicina e Chirurgia, II Università degli Studi, Via S. Andrea delle Dame 8, 80138 Naples, Italy*

Received 30 July 1998; accepted 20 October 1998

Abstract

A series of angelicin heteroanalogues, in which the furan was replaced by thiophene or a 1-substituted pyrazole moiety, was synthesised in order to obtain potential therapeutic agents with antiproliferative and/or other biological activities. In general, the antiproliferative activity of the new thioangelicin, tested in different biological substrates, appeared to be higher than that of the angelicin, the natural parent compound, but lower than that of 8-MOP, the furocoumarin ordinarily used in PUVA therapy and photopheresis. Thioangelicin **6** induced strong inhibition of T2 bacteriophage infectivity and was able to significantly repress the DNA synthesis in Ehrlich ascites cells and the clonal growth in HeLa cells. The pyrazolocoumarins did not show any noticeable effect upon UVA irradiation in all the biological systems considered. All the new angelicin heteroanalogues appeared to be free of the known phototoxicity of furocoumarins on the skin. The pyrazolocoumarins have also been tested as anti-inflammatory, analgesic, antipyretic, local anaesthetic, anti-arrhythmic and platelet anti-aggregating agents by standard procedures. In this class of derivatives, **10a** showed good anti-inflammatory and antipyretic properties, while **9a** and **11a** showed significant local anaesthetic activity.

Keywords: Angelicin heteroanalogues; NSAI analogues; Photosensitizers

1. Introduction

Psoralens, a well known group of linear furocoumarins, are natural and synthetic compounds with interesting photosensitizing activity. Some are commonly used in PUVA photochemotherapy (psoralen plus UVA) of a variety of skin diseases characterized by hyperproliferative conditions [1]. Recently, psoralens have been used in extracorporeal photochemotherapy to treat cutaneous T-cell lymphomas and other autoimmune diseases [2]. Furthermore, psoralen derivatives are also used as tools for biophysical studies on nucleic acids [3] and are now recognized as effective virucidal agents, especially against enveloped viruses such as the herpes simplex virus or human immunodeficiency virus type 1 (HIV-1) [4].

However, 8-methoxypsoralen (8-MOP), the compound generally used in therapy, leads to short-term side effects (erythema) and to long-term risks such as genotoxicity; these effects were associated with the induction of covalent adducts in DNA, mostly to inter-strand cross-links [5,6].

Thus, several monofunctional derivatives have been prepared and studied, in particular angelicin analogues (iso-psoralens), in which the angular structure prevents, for geometrical reasons, the formation of inter-strand cross-links [7–10]. Some of these compounds have an evident antiproliferative activity with a genotoxicity lower than that shown by psoralens [7].

Recently, one interesting field of research, aimed at obtaining new derivatives having better features than 8-MOP, was the insertion of a heteroatom into the furocoumarin nucleus thus obtaining furocoumarin isosters, for example azapsoralens [11–13] and some sulfur and selenium derivatives [14]. These heteroanalogues exhibit a strongly increased DNA photoinactivation property and appear to be very promising for photochemotherapy [15–17].

Other interesting angelicin isosters were also studied, in particular some furoquinolinones, in which a nitrogen atom replaces the oxygen at the pyrone ring [18]; they showed a strong antiproliferative effect both in the dark and under light

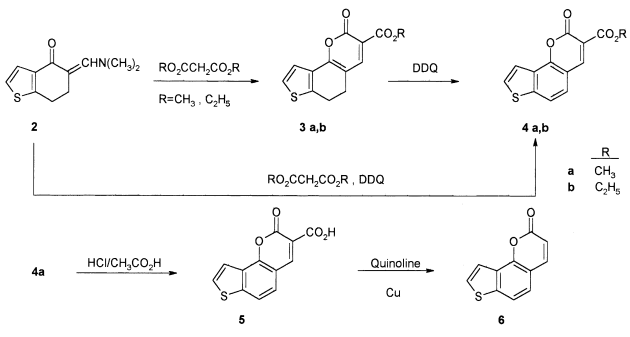
* Corresponding author. Tel: + 39-10-3538350; fax: + 39-10-3538358; e-mail: mosti@csita.unige.it

0014-827X/98/$ - see front matter
PII S0014-827X(98)00076-7

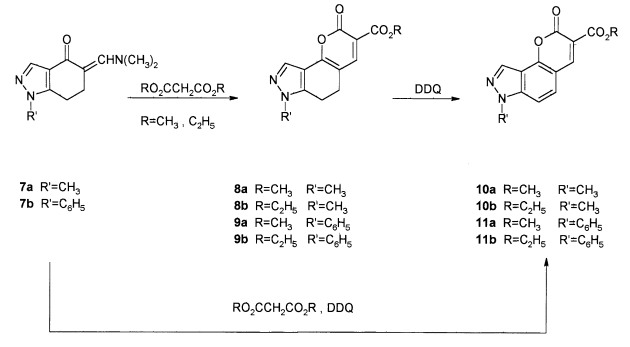
activation [19,20]. In addition, some 4-amino-3-chloroangelicin derivatives carrying a sulfur atom substituting the oxygen on the furan ring [21] and, more recently, some methyl derivatives of thienocoumarins have been also synthesised [22]. The synthesis of a new sulfur analogue of angelicin characterized by the presence of the thiopyrone in place of the pyrone moiety has been carried out and the evaluation of its photochemical and photobiological activity is in progress [23].

These findings prompted us to synthesize and study the preliminary photobiological activity of some new angelicin heteroanalogues in which the furan is replaced by thiophene (**4a,b, 5, 6**) or 1-substituted pyrazole moiety (**10a,b** and **11a,b**) and position 3 of the α-pyrone ring retains an electron-withdrawing group, as in 3-carbethoxyangelicin [24] in order to obtain photochemotherapeutic agents with a monofunctional profile and more significant photoreactive properties.

Furthermore, taking into account the biological significance of the indazole nucleus and the results published in previous papers in which some of us reported the interesting analgesic, anti-inflammatory, antipyretic and local anaesthetic activities of a number of derivatives related to pyrazole coumarins [25], we decided to select and test a set of these compounds characterized by an indazole moiety (**10a,b**, **11a**) and of their dihydroprecursors (**8b**, **9a,b**) for the activities described. The evaluation of a potential antiarrhythmic and platelet anti-aggregating activity of the above compounds was realized to complete their biological screening.



Scheme 1.



Scheme 2.

2. Chemistry

The synthetic route for the preparation of methyl and ethyl esters of 2-oxo-2*H*-thieno[2,3-*h*]-1-benzopyran-3-carboxylic acid **4a,b** and of 1-methyl or 1-phenyl substituted 5-oxo-5*H*-pyran[2,3-*e*]indazole-6-carboxylic acid **10a,b** and **11a,b** are presented in Schemes 1 and 2.

The precursors **2** [26] and **7a,b** [27], obtained in the past in two steps from the corresponding α-hydroxymethylene derivatives with dimethylamine at room temperature, were synthesized directly, in good yield, by refluxing a solution of the suitable commercially available ketone in excess of *N,N*-dimethylformamide dimethylacetal.

In the past Maitte et al. [28] studied the reaction between malonic derivatives and some bicyclic enaminoketones in a basic medium followed by acid hydrolysis and obtained the corresponding α-pyrone derivatives only when a cyanomethylene active nucleophile was used. On the contrary, in our case, the reaction of bicyclic enaminoketones **2** and **7** with the appropriate dialkyl malonate took place without adding a base to afford, generally in good yield, the desired adducts **3a,b**, **8a,b** and **9a,b**. This reaction probably first occurs between the nucleophilic methylene of the malonic ester and the α-enamino-carbon atom, which is the most electrophilic site of the enaminone. Subsequently, the dimethylamine released in situ causes the reaction to proceed towards the direct closure of the 2-pyrone ring without isolation of intermediates.

Full aromatization of adducts **3a,b**, **8a,b** and **9a,b** was accomplished with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in refluxing anhydrous toluene (method A) [24] to afford, generally in satisfactory yield, the desired esters **4a,b**, **10a,b** and **11a,b**. Only in the case of **9a** and **9b**, even with a prolonged reaction time (up to 5 days), was the final yield poor.

Realizing the cyclization and following aromatization in one step (method B), the desired angelicin heteroanalogues **4**, **10** and **11** were also obtained but in generally smaller yield than that of method A.

These experimental data showed that the full aromatization occurred easily in the case of the adducts characterized by the benzothiophene moiety but is less satisfactory in the case of the adducts with a N-substituted indazol structure, probably due to the higher basicity of the second rather than that of the first nucleus.

Methyl ester **4a** was routinely converted into the corresponding 2-oxo-2*H*-thieno[2,3-*h*]-1-benzopyran-3-carboxylic acid **5** in a high yield by refluxing with a 50% mixture of hydrochloric and acetic acids.

Decarboxylation of acid **5** by refluxing in quinoline containing a catalytic amount of copper powder afforded thioangelicin **6**.

3. Biology

All the new angelicin heteroanalogues were investigated for the following photobiological activities: *skin phototoxicity; DNA synthesis inhibition in Ehrlich ascites tumor cells; T2 phage inactivation; HeLa cells growth inhibition*. Both angelicin and 8-MOP were used as reference compounds.

The indazole esters **10a,b**, **11a** and dihydroprecursors **8b** and **9a,b**, were submitted to a screening for: *anti-inflammatory, analgesic, antipyretic, local anaesthetic, anti-arrhythmic and platelet anti-aggregating activities*, using indomethacin, lidocaine, quinidine and acetylsalicylic acid (ASA), respectively, as reference compounds.

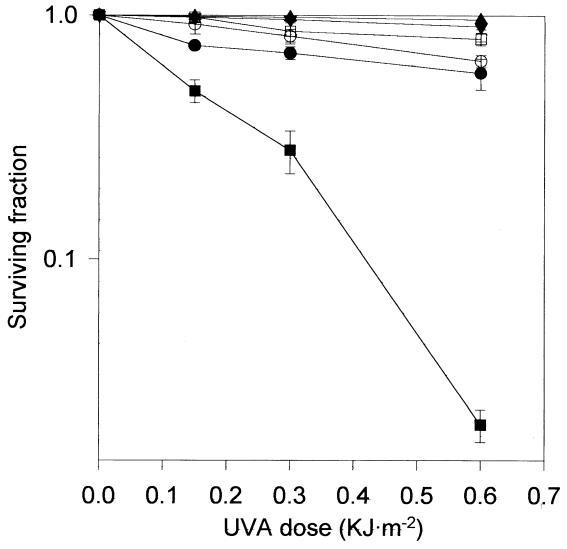


Fig. 1. T2 phage infectivity; virus particles were exposed to increasing UVA doses in the presence of thioangelicin derivatives (5 μM) and the number of the plaque forming units was determined. The symbols are: compound **4a** (▲); compound **4b** (▼); compound **5** (●); compound **6**

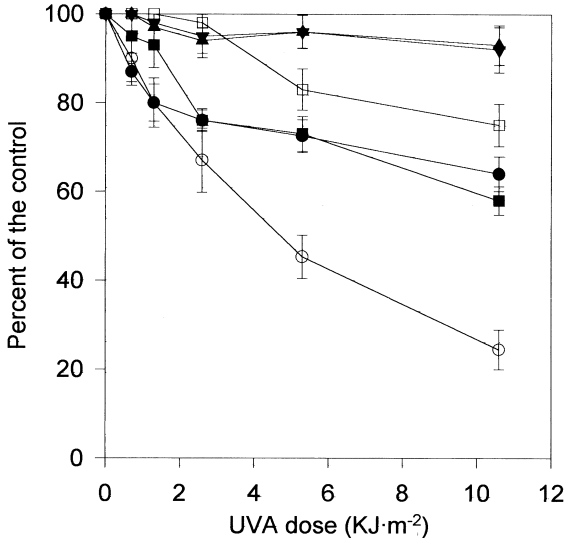


Fig. 2. Inhibition of DNA synthesis observed in Ehrlich cells. The tumor cells were incubated with tritiated thymidine and exposed to increasing UVA doses in the presence of thioangelicin derivatives (20 μM); then the acid insoluble radioactivity was determined. The symbols are: compound **4a** (▲); compound **4b** (▼); compound **5** (●); compound **6** (■); 8-MOP (○); angelicin (□). The bars represent the standard errors.

4. Results and discussion

4.1. Photobiology

The indazole esters **10**, **11** and related dihydroprecursors **8** and **9** were entirely ineffective in inhibiting T2 phage infectivity, DNA synthesis in Ehrlich cells and clonal growth capacity of in vitro HeLa cells (data not shown).

Fig. 1 shows the results regarding the ability of thioangelicin derivatives of inactivating T2 phage. Unsubstituted thioangelicin **6** (5 μM at the range 0.0–0.7 kJ × m^{-2} UVA) induced a strong reduction of the surviving fraction at every UVA dose, while all the other derivatives were essentially ineffective. Under the same experimental conditions, 8-MOP and angelicin also appeared to be poorly effective in repressing T2 phage activity.

Fig. 2 shows the results obtained from studying the activity of thioangelicin derivatives in inhibiting the DNA synthesis in Ehrlich cells (20 μM at the range 0–12 kJ × m^{-2} UVA). Unsubstituted thioangelin **6** shows the highest activity, although carboxylic acid **5** is only a little less effective; both compounds have an antiproliferative effect higher than that of angelicin but much lower than that of 8-MOP. The two esters **4a** and **4b** appeared to be poorly effective.

Fig. 3 shows the data obtained from studying the activity of thioangelicin derivatives (5 μM at the range 0.00–0.25 kJ × m^{-2} UVA) on the clonal growth capacity of HeLa cells cultivated in vitro. Once again, unsubstituted thioangelicin **6** was the most effective compound; its activity is higher than that of 8-MOP at a small UVA dose (0.1 kJ × m^{-2}) but at larger doses the reference compound becomes more efficient. Carboxylic acid **5**, like angelicin, induced only a moderate reduction in the colony-forming ability, while the two esters **4a** and **4b** appeared to be totally ineffective.

Finally, skin phototoxicity of the new angelicin heteroanalogues was evaluated on albino guinea-pig skin. As reported in Table 1, the compounds, which also appeared more effective in the other biological tests, are not able to induce a detectable erythema even when tested at 30 μM × cm^{-2} and with 5 kJ × m^{-2} UVA. Under these experimental conditions, angelicin was also ineffective. 8-MOP induced a barely visible erythema up to 4 μM × cm^{-2} of applied drug at the UVA dose of 5 kJ × m^{-2}, while at a drug concentration of 30 μM and with the same UVA dose it induced a very strong erythema with edema.

Summarizing the main points regarding photobiological studies of the new angelicin heteroanalogues, we can say that only the substitution of the furan with the thiophene ring increases the photoreactivity and consequently the

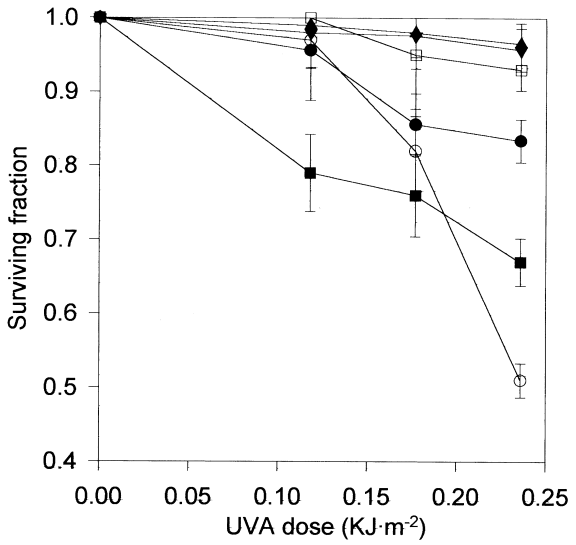


Fig. 3. Clonal growth capacity of HeLa cells cultivated in vitro; the cells were exposed to increasing UVA doses in the presence of thioangelicin derivatives (5 μM) and then their colony forming ability was determined. The symbols are: compound **4a** (▲); compound **4b** (▼); compound **5** (●); compound **6** (■); 8-MOP (○); angelicin (□). The bars represent the standard errors.

phototherapeutic activity, mainly when the pyrone moiety is unsubstituted.

In fact thioangelicin **6** is the best compound of the new angelicin isosteres.

In a simple system such as T2 phage, compound **6** quickly inactivated the virus infectivity under UVA irradiation and was the most effective compound in the in vitro HeLa cell test, resulting in a very good capacity to reduce the colony-forming ability.

The antiproliferative effect of thioangelicin **6** on the DNA synthesis in the Ehrlich cells was lower than that of 8-MOP but higher than that of angelicin, the natural furocoumarin characterized by the same structure. This is probably due to a better sensitizing activity on the macromolecule which forms the structure of the virus particle rather than on that of mammalian cell.

Table 1
Phototoxicity on guinea-pig skin

Comp.	μM × cm^{-2}	UVA dose (kJ m^{-2})	Erythema intensity[a]
None	0	10	− − − −
4a	30	5	− − − −
4b	30	5	− − − −
5	30	5	− − − −
6	30	5	− − − −
Angelicin	30	5	− − − −
8-MOP	4	5	+ − − −
8-MOP	30	5	+ + + +

[a] The symbols are: − − − −, no erythema; + − − −, barely detectable erythema; + + + +, strong erythema with edema.

Unlike 8-MOP, the new angelicin heteroanalogues were entirely ineffective in inducing erythema on guinea-pig skin, thus resembling angelicin (Table 1).

4.2. Pharmacology

The indazole esters **10a**,**b**, **11a** and their dihydroprecursors **8b**, **9a,b** gave negligible or too weak activity in the preliminary test for analgesic and platelet anti-aggregating activities (data not shown).

Compounds **8b**, **9a** and **10a** showed at a dose of 50 mg/kg p.o. an appreciable anti-inflammatory activity in comparison to that of the indomethacin (5 mg/kg p.o.) used as reference compound (Table 2). **10a** showed an ED_{50} at the third and the fourth hour of 17.73 (14.61–21.52) and 17.01 (1.61–17.94) mg/kg, respectively. **10a** also exhibited a good antipyretic effect in the yeast-induced pyrexia test (Table 3).

On the other hand, a satisfactory local anaesthetic activity was present in nearly all pyrazolocoumarins studied (with the exception of **8b** and **10b**), the most active being **9a** and **11a**, which showed a 0.082% (0.066–0.095) and 0.079% (0.066–0.095) ED_{50} after 5 and 30 min, respectively, expressed as % effective concentration (Table 4). Anti-arrhytmic activity of moderate level was shown by **9b** (Table 5).

In conclusion, from the preliminary pharmacological data, we can confirm that the indazole nucleus could be a good carrier of anti-inflammatory and antipyretic activities even if it does not bear a classic acid functionalization. The presence of the ester function together with a basic centre such as the pyrazole nucleus may justify the significant local anaesthetic effect.

5. Experimental

5.1. Photobiological methods

5.1.1. T2 phage inactivation

T2 phage was grown in *E. coli B48* cultures prepared in brain–heart infusion (Difco Laboratories, Detroit, Michigan, USA). Irradiation was performed by diluting the phage suspension up to 10^9 virus particles per ml with Hank's solution containing the tested compound (final concentration 20 μM). Phage titles were determined using the two-layer method and *E. coli B48* as host bacteria, according to Adams [29].

5.1.2. DNA synthesis inhibition in Ehrlich ascites tumor cells

The capacity of inhibiting DNA synthesis in Ehrlich ascites tumor cells was evaluated as already described [13]. Results were calculated as percentages of radioactivity incorporated into the DNA with respect to untreated control cells.

Table 2
Anti-inflammatory activity by carrageenan-induced rat paw edema test[a]

Comp.	Dose (mg/kg 4p.o.)	Edema volume (ml ± SE[b]) at the following times (h) after treatment (in parentheses per cent increase of volume relative to 0 value)				
		0	1	2	3	4
Control	–	1.6 ± 0.1	2.1 ± 0.1 (+ 31)	2.3 ± 0.1 (+ 44)	2.4 ± 0.1 (+ 50)	2.6 ± 0.1 (+ 63)
Indomethacin	5	1.4 ± 0.1	1.5 ± 0.1 (+ 7)	1.5 ± 0.1 (+ 7)	1.5 ± 0.1 (+ 7)	1.5 ± 0.1 (+ 7)
8b	50	1.8 ± 0.1	2.0 ± 0.1 (+ 11)	2.0 ± 0.1 (+ 11)	2.0 ± 0.1 (+ 11)	2.0 ± 0.1 (+ 11)
9a	50	1.8 ± 0.1	1.9 ± 0.1 (+ 6)	1.9 ± 0.1 (+ 6)	2.0 ± 0.1 (+ 11)	2.0 ± 0.1 (+ 11)
9b	50	1.7 ± 0.1	1.9 ± 0.1 (+ 12)	2.0 ± 0.1 (+ 18)	2.2 ± 0.1 (+ 29)	2.3 ± 0.1 (+ 35)
10a	12.5	2.2 ± 0.1	2.3 ± 0.1 (+ 5)	2.5 ± 0.1 (+ 13)	2.6 ± 0.1 (+ 18)	2.7 ± 0.1 (+ 23)
	25	1.9 ± 0.1	2.0 ± 0.1 (+ 5)	2.1 ± 0.1 (+ 10)	2.1 ± 0.1 (+ 10)	2.2 ± 0.1 (+ 16)
	50	2.3 ± 0.1	2.4 ± 0.1 (+ 4)	2.4 ± 0.1 (+ 4)	2.4 ± 0.1 (+ 4)	2.4 ± 0.1 (+ 4)
10b	50	2.2 ± 0.1	2.4 ± 0.1 (+ 9)	2.4 ± 0.1 (+ 9)	2.5 ± 0.1 (+ 14)	2.5 ± 0.1 (+ 14)
11a	50	1.3 ± 0.1	1.5 ± 0.1 (+ 15)	1.6 ± 0.1 (+ 23)	1.7 ± 0.1 (+ 31)	1.9 ± 0.1 (+ 46)

[a] Each compound was tested on a group of five albino rats (200–250 g). Compounds were given by gastric probe 30 min before carrageenan (0.1 ml of 1% solution).
[b] SE was always smaller than ± 0.10 ml and so rounded up to this value.

5.1.3. Clonal growth of HeLa cells

HeLa cells ((1.5–2) × 10^5), grown in Nutrient Mixture F-12 Ham medium (Sigma Chemical Co, St Louis, MO, USA.), containing 5% foetal calf serum, were seeded in Petri dishes in growth medium (4 ml). After 24 h, the medium was replaced with a fresh one containing the compound to be studied. The cells were then incubated for 15 min at 37°C in the dark and then exposed to UVA light. Aliquots of 200 cells were seeded in the same medium, incubated for 7 days and then the colonies were stained and counted, discarding colonies with less than 50 cells. The efficiency of the clonal growth, that is the ratio between the number of the formed colonies and the number of the cells seeded, was then calculated. The plating efficiency was about 90%.

5.1.4. Skin phototoxicity

All new heteroangelicins were evaluated following a standard procedure already described [30]. Compounds were applied on the depilated skin of albino guinea-pigs (outbred Dunkin–Hartley strain) as 0.1% methanol solutions up to 5×10^{-2} mg cm^{-2} and the area was exposed to UVA light. The animals were observed for 48 h.

5.1.5. Ultraviolet light source

All irradiations were performed by Philips HPW 125 W lamps provided with built-in Philips filters. The emission spectrum was in the range 320–400 nm, with a maximum at 365 nm (over 90% of the total). Irradiation intensity was determined on a UVX radiometer (Ultraviolet Products Co., Cambridge, UK) at 5.5×10^{-3} kJ s^{-1} m^{-2}.

5.2. Pharmacological methods

For the most active compounds, the ED_{50} values were determined by administration of three dosages (12.5, 25 and 50 mg p.o.).

The following pharmacological activities were evaluated by standard procedures:

Table 3
Antipyretic activity by yeast-induced pyrexia test[a]

Comp.	Dose (mg/kg p.o.)	Mean values of rectal temperature (°C ± SE) at the following times (h) after treatment, 18 h from yeast treatment (in parentheses difference from 0 value)[b]				
		0	1	2	3	4
Control	–	38.7 ± 0.2	38.7 ± 0.2 (+ 0)	38.6 ± 0.3 (− 0.1)	38.6 ± 0.3 (− 0.1)	38.5 ± 0.2 (− 0.2)
Indomethacin	5	38.7 ± 0.2	38.0 ± 0.2 (− 0.7)	37.3 ± 0.2 (− 1.4)	37.2 ± 0.3 (− 1.5)	36.8 ± 0.2 (− 1.9)
8b	50	39.2 ± 0.3	38.5 ± 0.3 (− 0.7)	38.5 ± 0.3 (− 0.7)	38.4 ± 0.2 (− 0.8)	38.2 ± 0.4 (− 1.0)
9a	50	39.0 ± 0.3	38.9 ± 0.2 (− 0.1)	38.4 ± 0.1 (− 0.6)	38.3 ± 0.2 (− 0.7)	38.2 ± 0.2 (− 0.8)
9b	50	38.9 ± 0.2	38.8 ± 0.2 (− 0.1)	38.6 ± 0.1 (− 0.3)	38.4 ± 0.1 (− 0.5)	38.4 ± 0.1 (− 0.5)
10a	50	39.0 ± 0.2	38.3 ± 0.4 (− 0.7)	37.8 ± 0.5 (− 1.2)	37.6 ± 0.5 (− 1.4)	36.8 ± 0.2 (− 2.2)
10b	50	38.7 ± 0.1	38.5 ± 0.2 (− 0.2)	38.3 ± 0.2 (− 0.4)	38.0 ± 0.3 (− 0.7)	37.8 ± 0.3 (− 0.9)
11a	50	38.8 ± 0.2	38.5 ± 0.1 (− 0.3)	38.6 ± 0.2 (− 0.2)	38.7 ± 0.1 (− 0.1)	38.7 ± 0.1 (− 0.1)

[a] Each compound was tested on a group of five albino rats (200–220 g).
[b] Mean value of five determinations.

Table 4
Infiltration anesthesia by pinch-tail test[a]

Comp.	Dose[b]	Activity[c]	
		5 min	30 min
Lidocaine	0.1	80	100
8b	0.1	20	40
9a	0.1	60	60
9b	0.1	40	40
10a	0.1	40	40
10b	0.1	20	40
11a	0.025	10	10
	0.05	30	30
	0.1	60	60

[a] Ten mice (20–25 g)/group.
[b] Percent of glycofurol solution (0.2 ml).
[c] Percentage of animals showing anesthesia, 5 and 30 min after infiltration of test compounds into the tail root. Dieffenbach tweezers were applied for 10 s.

Table 5
Activity against ventricular fibrillation caused by aconitine in albino rats (anti-arrhythmic activity)[a]

Comp.	Dose (mg/kg p.o.)	Appearance time (s ± SE) of extrasystoles	Death time (s ± SE)
Control (aconitine HCl)	–[b]	187 ± 18.3	652 ± 22.3
Quinidine	25	376 ± 24.8[d]	1086 ± 32.4[d]
8b	50	192 ± 18.4	676 ± 28.2
9a	50	244 ± 20.5[c]	746 ± 34.3[c]
9b	50	344 ± 25.9[d]	856 ± 41.2[d]
10a	50	210 ± 20.4	723 ± 31.4
10b	50	254 ± 22.3[c]	718 ± 19.4[c]
11a	50	184 ± 21.8	672 ± 28.4

[a] Five animals (200–250 g)/group.
[b] 15 μg/kg i.v./min.
[d] Statistically significant value calculated in comparison with the test performed with aconitine only ($P < 0.01$).
[c] Statistically significant value calculated in comparison with the test performed with aconitine only ($P < 0.05$).

1. anti-inflammatory activity, evaluated by carrageenan-induced paw edema in rats (Table 2);
2. analgesic activity evaluated by acetic acid writhing test in mice;
3. antipyretic activity (Table 3);
4. local anaesthetic activity, evaluated as infiltration anaesthesia in rats (Table 4);
5. anti-arrhythmic activity, evaluated as protection index against ecgraphic effects from aconitine in rats (Table 5);
6. platelet anti-aggregating activity, evaluated in vitro by the inhibitory test of platelet aggregation induced by collagen.

5.3. Chemistry

Melting points were determined with a Fisher-Johns apparatus and are uncorrected. UV spectra were measured in 95% ethanol on a Perkin Elmer Lambda 3 spectrophotometer. IR spectra were registered on a Perkin Elmer 398

Table 6
Physical and spectral data of compounds **3a,b**, **8a,b** and **9a,b**

Comp.	R	R′	Yield (%)	M.p. (°C)[a]	Formula	IR (cm^{-1}) ($CHCl_3$)	^1H NMR (δ, ppm) ($CDCl_3$)
3a	CH_3		48	190–192	$C_{13}H_{10}O_4S$	1752, 1698	3.03 (m, 4H, CH_2-5 + CH_2-6), 3.96 (s, 3H, CH_3O), 7.24 (d, $J = 5$, 1H, H-9), 7.41 (d, $J = 5$, 1H, H-8), 8.26 (s, 1H, H-4)
3b	C_2H_5		40	130–132	$C_{14}H_{12}O_4S$	1755, 1690	1.36 (t, $J = 7$, 3H, CH_3), 3.02 (m, 4H, CH_2-5 + CH_2-6), 4.38 (q, $J = 7$, 2H, CH_2), 7.25 (d, $J = 5$, 1H, H-9), 7.42 (d, $J = 5$, 1H, H-8), 8.22 (s, 1H, H-4)
8a	CH_3	CH_3	43	250–252 (dec)	$C_{13}H_{12}N_2O_4$	1745, 1695	2.93 (s, 4H, CH_2-8 + CH_2-9), 3.84 (s, 3H, CH_3N), 3.88 (s, 3H, CH_3O), 7.81 (s, 1H, H-7), 8.19 (s, 1H, H-3)
8b	C_2H_5	CH_3	54	194–195 (dec)	$C_{14}H_{14}N_2O_4$	1758, 1690	1.37 (t, $J = 7$, 3H, CH_3–CH_2), 2.98 (s, 4H, CH_2-8 + CH_2-9), 3.87 (s, 3H, CH_3N), 4.37 (q, $J = 7$, 2H, CH_3–CH_2), 7.84 (s, 1H, H-7), 8.20 (s, 1H, H-3)
9a	CH_3	C_6H_5	56	238–239 (dec)	$C_{18}H_{14}N_2O_4$	1753,1700	3.05 (m, 4H, CH_2-8 + CH_2-9), 3.89 (s, 3H, CH_3O), 7.56 (s, 5H, C_6H_5), 8.07 (s, 1H, H-7), 8.27 (s, 1H, H-3)
9b	C_2H_5	C_6H_5	60	178–179 (dec)	$C_{19}H_{16}N_2O_4$	1757, 1692	1.37 (t, $J = 7$, 3H, CH_3–CH_2), 3.06 (m, 4H, CH_2-8 + CH_2-9), 4.37 (q, $J = 7$, 2H, CH_3–CH_2), 7.56 (s, 5H, C_6H_5), 8.06 (s, 1H, H-7), 8.27 (s, 1H, H-3)

[a] From ethyl acetate.

Table 7
Physical and spectral data of compounds **4a,b**, **5**, **6**, **10a,b** and **11a,b**

Comp.	R	R′	Yield (%)	M.p. (°C)	Formula	UV, λ_{max} (nm) (log ϵ)	IR (cm^{-1})	^1H NMR (δ, ppm)
4a	CH_3		65 (A), 65 (B)	184–185[a]	$C_{13}H_8O_4S$	235 (4.30), 257 (4.26), 275 (4.30), 324 (4.17), 366 (4.02)	1760, 1735[d]	4.01 (s, 3H, CH_3O), 7.30–7.80 (m, 4H, H-5 + H-6 + H-8 + H-9), 8.74 (s, 1H, H-4)[f]
4b	C_2H_5		80 (A), 44 (B)	130–132[a] (dec)	$C_{14}H_{10}O_4S$	236 (4.31), 257 (4.27), 276 (4.32), 324 (4.18), 366 (4.03)	1760, 1735[d]	1.43 (t, $J = 7$, 3H, CH_3), 4.47 (q, $J = 7$, 2H, CH_2), 7.30–8.00 (m, 4H, H-5 + H-6 + H-8 + H-9), 8.69 (s, 1H, H-4)[f]
5			93	253–255[b] (dec)	$C_{12}H_6O_4S$	237 (4.21), 258 (4.20), 276 (4.14), 320 (4.00), 363 (3.86)	3100–2500, 1745, 1686[e]	7.40–8.20 (m, 4H, H-5 + H-6 + H-8 + H-9), 8.94 (s, 1H, H-4), 13.20 (br s, 1H, CO_2H; disappears with D_2O)[g]
6			70	149–150[c] (dec)	$C_{11}H_6O_2S$	236 (4.26), 258 (4.38), 268 (4.23), 308 (3.95), 342 (3.88)	1723, 1600[d]	6.47 (d, $J = 10$, 1H, H-4), 7.34 (d, $J = 2$, 1H, H-9), 7.40–8.00 (m, 3H, H-5 + H-6 + H-8), 7.87 (d, $J = 10$, 1H, H-3)[f]
10a	CH_3	CH_3	40 (A), 28 (B)	245–246[b] (dec)	$C_{13}H_{10}N_2O_4$	227 (4.29), 269 (4.26), 278 (4.35), 323 (3.75), 368 (4.13)	1762, 1730[d]	3.99 (s, 3H, CH_3O), 4.17 (s, 3H, CH_3N), 7.20–7.80 (m, 2H, H-8 + H-9), 8.44 (s, 1H, H-7), 8.78 (s, 1H, H-3)[f]
10b	C_2H_5	CH_3	42 (A), 13 (B)	174–175[a] (dec)	$C_{14}H_{12}N_2O_4$	226 (4.37), 268 (4.35), 277 (4.44), 323 (3.85), 367 (4.22)	1760, 1735[d]	1.42 (t, $J = 7$, 3H, CH_3–CH_2), 4.17 (s, 3H, CH_3N), 4.46 (q, $J = 7$, 2H, CH_3–CH_2), 7.20–7.70 (m, 2H, H-8 + H-9), 8.39 (s, 1H, H-7), 8.72 (s, 1H, H-3)[f]
11a	CH_3	C_6H_5	22 (A), 13 (B)	227–228[a] (dec)	$C_{18}H_{12}N_2O_4$	242 (4.14), 277 (4.42), 368 (4.18)	1763, 1738[d]	4.00 (s, 3H, CH_3), 7.40–7.90 (m, 7H, C_6H_5 + H-8 + H-9), 8.62 (s, 1H, H-7), 8.76 (s, 1H, H-3)[f]
11b	C_2H_5	C_6H_5	32 (A), 8 (B)	160–161[a]	$C_{19}H_{14}N_2O_4$	240 (4.22), 277 (4.42), 368 (4.18)	1765, 1738[d]	1.43 (t, $J = 7$, 3H, CH_3–CH_2), 4.54 (q, $J = 7$, 2H, CH_3–CH_2), 7.45–7.85 (m, 7H, C_6H_5 + H-8 + H-9), 8.59 (s, 1H, H-7), 8.71 (s, 1H, H-3)[f]

[a] From ethyl acetate.
[b] From ethanol 95°C.
[c] From diethyl ether.
[d] $CHCl_3$.
[e] KBr.
[f] $CDCl_3$.
[g] DMSO-d_6. (A) Method A; (B) method B.

spectrophotometer. ^1H NMR spectra were recorded on a Hitachi Perkin Elmer R-600 (60 MHz) or a Varian Gemini 200 (200 MHz) spectrometer; chemical shifts are reported as δ (ppm) relative to TMS as internal standard; J in Hz. Elemental analyses were performed at the Microanalytical Laboratory of the Dipartimento di Scienze Farmaceutiche, University of Genova, using a Carlo Erba Elemental Analyzer Model 1106. Analytical results for C, H and N were within $\pm$ 0.3% of the theoretical values.

*5.3.1. General procedure for the preparation of N,N-dimethylaminomethyleneketones (**2**) [25] and **7a,b** [26]*

This procedure was realized directly by refluxing of the suitable ketone (20 mmol) in *N*,*N*-dimethylformamide dimethylacetal (3.57 g, 30 mmol) for different times (**2**, 6 h, 51%; **7a**, 24 h, 40%; **7b**, 24 h, 55%).

*5.3.2. General procedure for the preparation of esters of 5,6-dihydro-2-oxo-2H-thieno[2,3-h]-1-benzopyran-3-carboxylic acid **3a,b** and of 1-methyl or 1-phenyl substituted 8,9-dihydro-5-oxo-5H-pyran[2,3-e]indazol-6-carboxylic acid **8a,b**, **9a,b***

A mixture of 5-dimethylaminomethylene-6,7-dihydrobenzo[*b*]thiophen-4(5*H*)-one **2** or 5-dimethylaminomethylene-1,5,6,7-tetrahydro-(1-methyl) (1-phenyl)-4*H*-indazol-4-ones **7a,b** (10 mmol) in the appropriate dialkylmalonate (175 mmol) was refluxed for 6 h (only 1 h in the case of **7a** in dimethylmalonate). Then the malonic ester excess was evaporated under reduced pressure, diethyl ether was added to the resulting residue, cooled, and filtered to give a raw solid which was recrystallized from ethyl acetate. Yields, melting points, IR and ^1H NMR spectral data are reported in Table 6.

*5.3.3. General procedure for the preparation of esters of 2-oxo-2H-thieno[2,3-h]-1-benzopyran-3-carboxylic acid **4a,b** and of 1-methyl or 1-phenyl substituted 5-oxo-5H-pyran[2,3-e]indazol-6-carboxylic acid **10a,b**, **11a,b***

Method A. A warm solution of DDQ (2.27 g, 10 mmol) in anhydrous toluene (50 ml) was added dropwise to a warm and stirred solution of dihydroprecursors **3a,b**, **8a,b** and **9a,b** (10 mmol) in the same solvent (100–120 ml). After the addition was completed, the reaction mixture was refluxed (24 h for **3a,b** and **8a,b**; up to 5 days for **9a**,**b**) and then filtered. The filtrate was washed twice with 1 M sodium hydroxide and once with water, dried ($MgSO_4$) and evaporated under reduced pressure. The solid residue was recrystallized from a suitable solvent. Yields, melting points, UV, IR and ^{1}H NMR spectral data are reported in Table 7.

Method B. A warm solution of DDQ (2.27 g, 10 mmol) in anhydrous toluene (50 ml) was added dropwise to a warm and stirred solution of **2** or **7a,b** (10 mmol) and the appropriate dialkylmalonate (175 mmol) in the same solvent (100 ml). After the addition was completed, the reaction mixture was refluxed for 24 h and then filtered. The filtrate was washed twice with 1 M sodium hydroxide and once with water, dried ($MgSO_4$) and evaporated under reduced pressure. The solid raw residue was recrystallized from a suitable solvent as in Method A.

*5.3.4. 2-Oxo-2H-thieno[2,3-h]-1-benzopyran-3-carboxylic acid **5***

A solution of ester **4a** (2.60 g, 10 mmol) in a 50% hydrochloric and acetic acid mixture (100 ml) was refluxed for 24 h. After cooling, the solid residue was filtered, washed with water and recrystallized from 95% ethanol. Yields, melting points, UV, IR and ^{1}H NMR spectral data are reported in Table 7.

*5.3.5. 2H-Thieno[2,3-h]-1-benzopyran-2-one **6***

A solution of acid **5** (1.23 g, 10 mmol) in quinoline (20 ml) containing copper powder (0.16 g) was refluxed for 3 h. The hot mixture was filtered, the liquid was cooled and chloroform (20–30 ml) was added. The solution was extracted twice with 6 M hydrochloric acid, washed once with water, dried ($MgSO_4$) and evaporated under reduced pressure. The solid residue was recrystallized from diethyl ether. Yields, melting points, UV, IR and ^{1}H NMR spectral data are reported in Table 7.

Acknowledgements

Financial support from C.N.R., from Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST) and from Università di Genova is gratefully acknowledged.

References

[1] J.A. Parrish, R.S. Stern, M.A. Pathak, T.B. Fitzpatrick, Photochemotherapy of skin disease, in: J.D. Regan, J.A. Parrish (Eds.), The Science of Photomedicine, Plenum, New York, 1982, p. 595.
[2] F.P. Gasparro, Extracorporeal photochemotherapy, Clinical aspects and the molecular basis for efficacy, R.G. Landes, Austin, TX, 1994.
[3] G.D. Cimino, H.B. Gamper, S.T. Isaacs, J.E. Hearst, Psoralens as photoreactive probes of nucleic acid structure and function: organic chemistry, photochemistry and biochemistry, Annu. Rev. Biochem. 54 (1985) 1151–1193.
[4] J. North, H. Neyndorff, J.P. Levy, Photosensitizers as virucidal agents, J. Photochem. Photobiol. B: Biol. 17 (1993) 99–108.
[5] F. Dall'Acqua, S. Marciani Magno, F. Zambon, G. Rodighiero, Kinetic analysis of the photoreaction (365 nm) between psoralen and DNA, Photochem. Photobiol. 29 (1979) 489–495.
[6] R.S. Stern, R. Lange, Members of the photochemotherapy follow-up study. Non-melanoma skin cancer occurring in patients treated with PUVA five to ten years after first treatment, J. Invest. Dermatol. 91 (1988) 120–124.
[7] A. Guiotto, P. Rodighiero, P. Manzini, G. Pastorini, F. Bordin, F. Baccichetti, F. Carlassare, D. Vedaldi, F. Dall'Acqua, M. Tamaro, G. Recchia, M. Cristofolini, 6-Methylangelicins: a new series of potential photochemoterapeutic agents for the treatment of psoriasis, J. Med. Chem. 27 (1984) 959–967 and references cited therein.
[8] D. Vedaldi, F. Dall'Acqua, F. Baccichetti, F. Carlassare, F. Bordin, P. Rodighiero, P. Manzini, A. Guiotto, Methylangelicins: structure activity studies on the role of methyl groups present in 3, 4 and 4′, 5′ photoreactive sites, Farmaco 46 (1991) 1381–1406.
[9] L. Mosti, P. Schenone, G. Menozzi, G. Romussi, F. Baccichetti, F. Carlassare, D. Vedaldi, F. Bordin, Synthesis and photobiological activity of some 4-amino-3-chloroangelicin derivatives, Eur. J. Med. Chem. 18 (1983) 113–120.
[10] L. Mosti, G. Menozzi, P. Schenone, F. Carlassare, F. Baccichetti, F. Bordin, Synthesis and photobiological activity of new N,N-disubstituted 4-amino-3-chloroangelicins, Farmaco 43 (1988) 775–792.
[11] D. Vedaldi, F. Dall'Acqua, S. Caffieri, F. Baccichetti, F. Carlassare, F. Bordin, A. Chilin, A. Guiotto, 4,4′,5′-trimethyl-8-azapsoralen, a new photoactive and non skin-phototoxic bifunctional bioisoster of psoralen, Photochem. Photobiol. 53 (1991) 143–148.
[12] B. Pani, M. Tamaro, F. Baccichetti, F. Carlassare, M. Simonato, F. Bordin, Aspects of genotoxic activity of some 8-azapsoralen derivatives, Med. Biol. Environ. 20 (1992) 117–124.
[13] F. Baccichetti, F. Bordin, M. Simonato, L. Toniolo, C. Marzano, P. Rodighiero, A. Chilin, F. Carlassare, Photobiological activity of certain new methylazapsoralens, Farmaco 47 (1992) 1529–1541.
[14] A.E. Jakobs, L.E. Christiaens, M.J. Renson, Synthesis of monosulfur and monoselenium analogues of psoralen, Tetrahedron 50 (1994) 9315–9320.
[15] A.E. Jakobs, J. Piette, Photobiological activity of sulfur and selenium analogues of psoralen, J. Photochem. Photobiol. B: Biol. 22 (1994) 9–15.
[16] D. Vedaldi, S. Caffieri, S. Frank, F. Dall'Acqua, A.E. Jakobs, J. Piette, Sulfur and selenium analogues of psoralen as novel potential photochemotherapeutic agents, Farmaco 50 (1995) 527–536.
[17] D. Vedaldi, G. Piazza, S. Moro, S. Caffieri, G. Miolo, G.G. Aloisi, F. Elisei, F. Dall'Acqua, 1-Thiopsoralen, a new photobiologically active heteropsoralen. Photophysical, photochemical and computer aided studies, Farmaco 52 (1997) 645–652.
[18] P. Rodighiero, A. Guiotto, A. Chilin, F. Bordin, F. Baccichetti, F. Carlassare, D. Vedaldi, S. Caffieri, A. Pozzan, F. Dall'Acqua, Angular furoquinolinones, psoralen analogs: novel antiproliferative agents for skin disease. Synthesis, biological activity, mechanism of action and computer-aided studies, J. Med. Chem. 39 (1996) 1293–1302.
[19] F. Bordin, C. Marzano, F. Carlassare, P. Rodighiero, A. Guiotto, S. Caffieri, F. Baccichetti, Photobiological properties of a new tetra-

methylfuroquinolinone, J. Photochem. Photobiol. B: Biol. 34 (1996) 159–168.
[20] C. Marzano, E. Severin, B. Pani, A. Guiotto, F. Bordin, DNA damage and cytotoxicity induced in mammalian cells by a tetramethylfuroquinolinone derivative, Environ. Mol. Mutagen. 29 (1997) 256–264.
[21] L. Mosti, P. Schenone, G. Menozzi, G. Romussi, F. Baccichetti, F. Carlassare, F. Bordin, Synthesis and photobiological activity of N,N-disubstituted 4-amino-3-chloro-2H-thieno[2,3-h]-1-benzopyran-2-ones 7-thioisosters of 4-amino-3-chloroangelicins, Farmaco (1984) 81–94.
[22] A. Guiotto, A. Chilin, P. Manzini, F. Dall'Acqua, F. Bordin, P. Rodighiero, Synthesis and antiproliferative activity of furocoumarin isosters, Farmaco 50 (1995) 479–488.
[23] A.E. Jakobs, L.E. Christiaens, A convenient synthesis of thiopyrano[2,3-e]benzofuran: a new sulfur analogue of angelicin, J. Org. Chem. 61 (1996) 4842–4844.
[24] M. Iester, P. Fossa, G. Menozzi, L. Mosti, F. Baccichetti, C. Marzano, M. Simonato, Synthesis and photobiological properties of 3-acylangelicins, 3-alkoxycarbonylangelicins and related derivatives, Farmaco 50 (1995) 669–678.
[25] L. Mosti, L. Sansebastiano, P. Fossa, P. Schenone, F. Mattioli, 1-Phenyl-1H-indazole derivatives with analgesic and anti-inflammatory activities, Farmaco 47 (1992) 357–365 and references cited therein.
[26] L. Mosti, P. Schenone, G. Menozzi, G. Romussi, Reaction of sulfene with heterocyclic N,N-disubstituted α-aminomethyleneketones. X. Synthesis of thieno[2,3-h]-1,2-benzoxathiin derivatives, J. Heterocycl. Chem. 19 (1982) 1057–1059.
[27] L. Mosti, G. Menozzi, P. Schenone, Reaction of dichloroketene and sulfene with N,N-disubstituted α-aminomethyleneketones. Synthesis of pyrano[2,3-e]indazole and 1,2-oxathiino[6,5-e]indazole derivatives, J. Heterocycl. Chem. 21 (1984) 361–367.
[28] M.C. Bellassoued-Fargeau, B. Graffe, M.C. Sacquet, P. Maitte, Addition de dérivés maloniques sur des β-énaminones cycliques, J. Heterocycl. Chem. 22 (1984) 713–717.
[29] M.H. Adams, Bacteriophages, Interscience, New York, 1959.
[30] F. Bordin, F. Carlassare, F. Baccichetti, A. Guiotto, P. Rodighiero, D. Vedaldi, F. Dall'Acqua, 4,5′-Dimethylangelicin: a new DNA-photobinding monofunctional agent, Photochem. Photobiol. 29 (1979) 1063–1070.