Available online at www.sciencedirect.com
SCIENCE DIRECT®
Il Farmaco 58 (2003) 1083–1097
IL FARMACO
www.elsevier.com/locate/farmac

Pyran derivatives
Part XXI. Antiproliferative and cytotoxic properties of novel *N*-substituted 4-aminocoumarins, their benzo-fused derivatives, and some related 2-aminochromones

Mario Di Braccio [a], Giancarlo Grossi [a], Giorgio Roma [a,*], Cristina Marzano [b], Franca Baccichetti [b], Morena Simonato [b], Franco Bordin [b]

[a] *Dipartimento di Scienze Farmaceutiche dell'Università, Viale Benedetto XV 3, 16132 Genova, Italy*
[b] *Dipartimento di Scienze Farmaceutiche dell'Università, Centro di Studio sulla Chimica del Farmaco e dei Prodotti Biologicamente Attivi del CNR, Via F. Marzolo 5, 35123 Padova, Italy*

Received 4 March 2003; accepted 25 May 2003

Abstract

The *N*-substituted tricyclic 2-aminochromone derivatives **1a**, **2a**, and **2b** were obtained by treating the corresponding (methylthio) or (methylsulfinyl) derivatives **10**, **11**, or **12**, respectively, with an excess of the proper amines. Compound **2c** was synthesized through the reaction of 2-naphthol with the ethyl *N*,*N*-diphenylmalonamate/$POCl_3$ reagent **14**. The *N*-substituted 4-aminocoumarin bicyclic and tricyclic derivatives **5**–**8** were prepared by treating the corresponding chloro derivatives with the excess suitable amines. Compounds **1**, **2**, **5**–**8** were tested in vitro for their antiproliferative activity (DNA synthesis inhibition in Ehrlich cells) and cytotoxicity (MTT test in HeLa cells). The inhibitory properties of three selected compounds (**5c**, **5e**, **7c**) on protein and RNA syntheses in Ehrlich cells were also evaluated. Among the 27 compounds tested, 10 4-aminocoumarin derivatives **5**–**8** and two 2-aminochromone derivatives (**1a** and **2a**) showed an appreciable antiproliferative activity (IC_{50} range: 1.74–13.8 μM), whereas only four compounds **5**–**8** exhibited a comparable cytotoxic activity (IC_{50} range: 4.95–12.9 μM).

Keywords: 4-Aminocoumarins; 2-Aminochromones; Antiproliferative activity; Cytotoxicity

1. Introduction

The antiproliferative properties of photoactivated linear and angular furocoumarins (psoralens and angelicins) [1] and of photoactivated khellin [2], a natural linear furochromone, are well-known. On the other hand, the smooth muscle cell antichemotactic and antiproliferative properties of 2-morpholinochromone [3] and 2-morpholino-8-[(3-pyridinyl)methoxy]chromone [4], as well as the specific inhibitory activity of 2-morpholino-8-phenylchromone against phosphatidylinositol 3-kinase [5], have been described in the literature.

Considering the above results and pursuing our studies on *N*-substituted 2-aminochromones **1** and their benzo-fused derivatives **2**–**4**, whose first examples were previously described by us [6–11], we recently synthesized and tested for their antiproliferative and cytotoxic activities a number of compounds **1**–**4** (Fig. 1). Actually, such compounds often showed significant antiproliferative activity and, in general, low cytotoxic properties, independently of the presence of UV-A light [12].

In order to extend this investigation to the coumarin structural field, we have planned to synthesize and evaluate for their dark antiproliferative and cytotoxic activities proper series of bicyclic and tricyclic 4-aminocoumarin derivatives **5**–**8** (Fig. 1), isomers of chromone derivatives **1**–**4**.

* Corresponding author.
E-mail address: roma@unige.it (G. Roma).

0014-827X/03/$ - see front matter
doi:10.1016/S0014-827X(03)00160-5

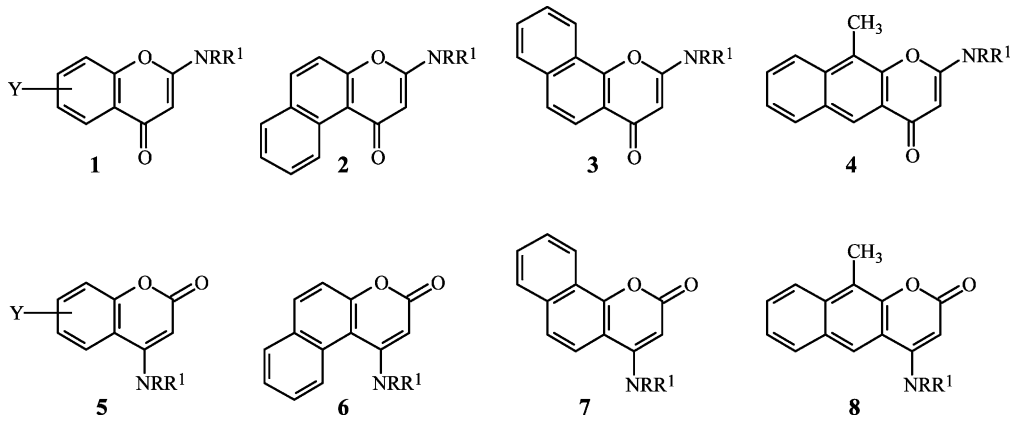


Fig. 1. Structures of the *N*-substituted 2-aminochromones **1** and 4-aminocoumarins **5**, and of their benzo-fused derivatives **2**–**4** and **6**–**8**, respectively.

The chemical and biological results of this study are reported in the present paper.

2. Chemistry

The cyclocondensation of the properly substituted 2′-hydroxyacetophenone **9** [13] with carbon disulfide in the presence of potassium *tert*-butoxide (dry toluene, room temperature) afforded the raw 4-hydroxy-6,7-methylenedioxy-2*H*-1-benzopyran-2-thione which was in turn reacted with methyl iodide (anhydrous K_2CO_3, dry acetone at reflux) to give the corresponding 2-(methylthio)chromone **10**. By treating compound **10** with excess aniline (ethylene glycol, 160 °C, H^+) the desired 6,7-methylenedioxy-2-(phenylamino)chromone (**1a**) was obtained.

The treatment of 3-(methylthio)-1*H*-naphtho[2,1-*b*]pyran-1-one (**11**) [14] with an excess of *p*-chloroaniline (1-pentanol, 160 °C, H^+) afforded the corresponding 3-[(4-chlorophenyl)amino] derivative **2a**, whereas the 3-[(2-pyridinyl)amino]-1*H*-naphtho[2,1-*b*]pyran-1-one (**2b**) was prepared by treating with excess pyridin-2-amine the corresponding 3-(methylsulfinyl) derivative **12** (Dowtherm A, 200 °C, H^+), that was obtained from the reaction [15] of **11** with *m*-chloroperbenzoic acid (1,2-dichloroethane, 0 °C). On the other hand, due to the too low nucleophilicity of diphenylamine, compound **2c** was prepared (low yield) through a different procedure, i.e. by treating 2-naphthol with the Vilsmeier-type reagent ethyl *N*,*N*-diphenylmalonamate [16] /$POCl_3$ **14** (chlorobenzene, 130 °C), in accordance with the cyclocondensation method previously applied by us for the one-pot preparation of a number of *N*-substituted 2-aminochromones [6,7] and their tricyclic analogues [8–11] (Scheme 1).

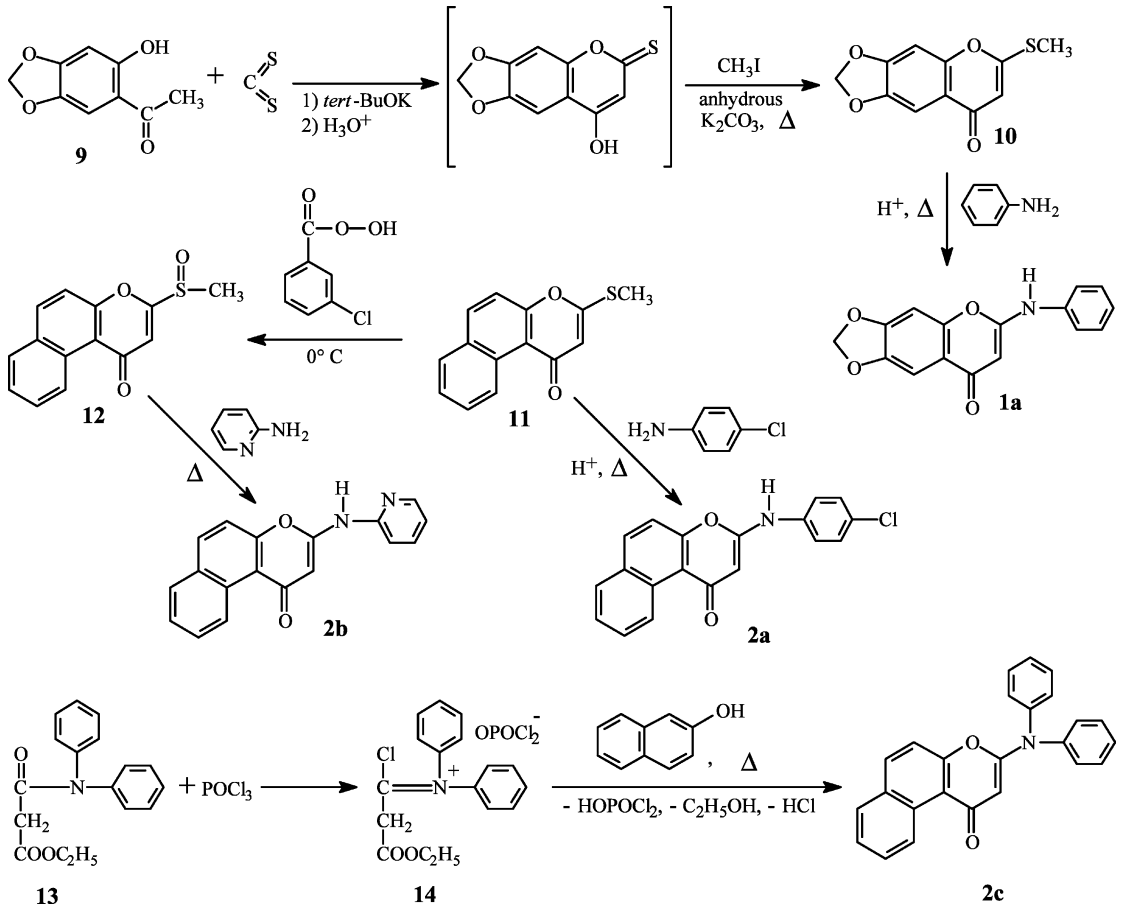
High yields of the *N*-substituted 4-aminocoumarin bicyclic and tricyclic derivatives **5a**–**e**, **6a**–**i**,**k**, **7a**–**d**, and **8a**–**c** were generally afforded by the treatment of the corresponding chloroderivatives **16a** [17], **16b** [18], **16c** [19], **18**, **20**, and **21** [11], respectively, with a large excess of the proper amines (ethanol at reflux, or ethylene glycol at 160 °C). The chloroderivatives **18** and **20**, previously described by us [14], were now obtained in notably higher yields through the reaction of the corresponding 4-hydroxycoumarin tricylic derivatives **17** [20] and **19** [20], in the presence of equimolar triethylamine, with excess phosphorus oxychloride (130 °C). Under the same novel conditions, the 4-chlorocoumarins **16a**,**b** were effectively obtained from the 4-hydroxycoumarins **15a**,**b** [21], respectively (Scheme 2). Compounds **6b**–**d** were previously prepared directly from the corresponding hydroxyderivative **17**, under severe conditions [22].

The structures attributed to the compounds described in this paper are supported by the results of elemental analyses and IR and ^{1}H NMR spectral data (see Section 5). In this connection, the spectral data of compounds **1**, **2**, **5**–**8** now synthesized are in accordance with those of compounds of the same structural classes previously described by us [12,14].

3. Biological results and discussion

3.1. Results

The antiproliferative and cytotoxic activities of novel compounds **1a**, **2a**–**c**, **5a**–**e**, **6a**,**e**–**i**,**k**, **7a**–**d**, **8a**–**c**, and of the previously described ones **6b**–**d** [22] and **6j** [14] were evaluated in vitro by testing such compounds for their inhibitory properties on DNA synthesis in Ehrlich ascites tumor cells and for cytotoxicity by the MTT assay, respectively (see Experimental-Biology). The 2-morpholinochromone **1b**, whose antiproliferative properties were previously reported in the literature [3], has been examined as a reference compound. The biological



Scheme 1. Synthetic routes to 2-aminochromone fused derivatives **1a**, **2a**–**c**.

data of the above compounds are summarized in Table 1.

3.1.1. Inhibition of DNA synthesis

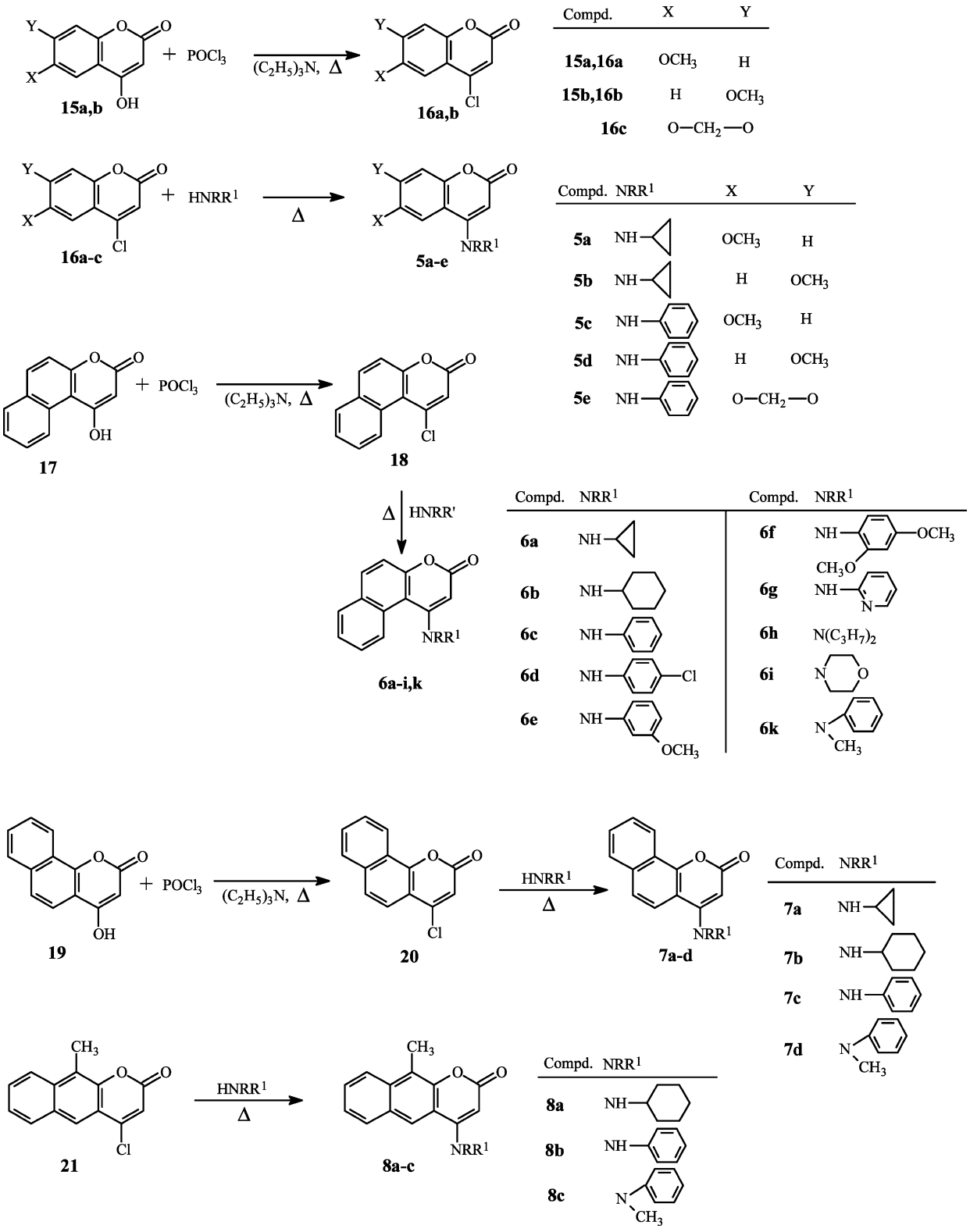
The eight most effective compounds in this test (**7c**, **5e**, **8a**, **7a**, **2a**, **6d**, **7d**, and **6c**, in order of decreasing activity) showed IC_{50} values within the range 1.74–9.03 μM. Compounds **8b**, **6g**, **1a**, **7b**, **6k**, **8c**, **5a**, **2b** and **2c** are less, but still significantly, active (IC_{50} values within the range 11.8–19.8 μM).

3.1.2. Cytotoxicity (MTT test)

The IC_{50} values of the five most active compounds (**5d**, **7c**, **5c**, **5a**, **7d**) are within the range 4.95–18.5 μM. In general, the compounds tested showed an activity lower than that shown in the preceding test: actually, only two compounds, i.e. the methoxy substituted 4-(phenylamino)coumarins **5c** and **5d**, proved to be clearly more effective as cytotoxic than as antiproliferative agents. Further, we can observe that the angular benzo-fused 4-(phenylamino)coumarin **7c** is endowed with both a high activity as a DNA synthesis inhibitor and remarkable cytotoxic properties.

3.1.3. Inhibition of RNA and protein syntheses

Considering the results obtained with the test of DNA synthesis inhibition and the MTT assay, we observed certain discrepancies: some compounds appeared to be active only in the first test, while other derivatives, having similar structures, were effective only in the second one. In order to investigate this behavior, we chose **5c**, **5e** and **7c** as compounds with very different features (i.e. active in MTT assay, or in DNA synthesis test, or in both ones, respectively), then we studied their capacity of inhibiting RNA and protein syntheses. The results are shown in Fig. 2, in which the data related to DNA synthesis inhibition are also reported, for a comparison. As we can see, among the macromolecular syntheses examined, that of RNA appears to be the less affected by the three compounds tested. Regarding the cytotoxic compound **5c**, as its concentration was rising, the inhibitory activity increased clearly more on protein than on RNA and DNA synthesis. Furthermore, it must be pointed out that, with equal concentrations of the three compounds, **5c** afforded the lowest inhibitory activity towards all the macromolecular syntheses studied (Fig. 2).

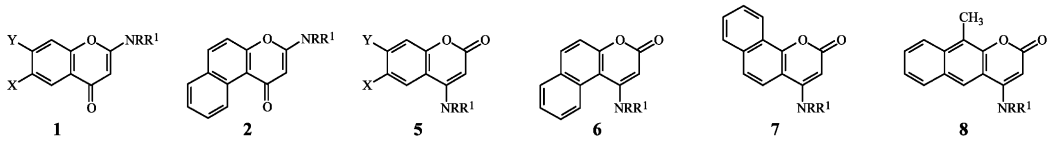


Scheme 2. Synthesis of 4-aminocoumarin bicyclic and tricyclic derivatives **5a–e**, **6a–i,k**, **7a–d** and **8a–c**.

3.2. Discussion

A series of 4-aminocoumarin and 2-aminochromone bicyclic and tricyclic derivatives was tested for their antiproliferative and cytotoxic activities. For the former test, we detected the inhibition of DNA synthesis in Ehrlich cells, an experimental tumor of the mouse, while, for the cytotoxic activity HeLa cells, a tumor cell line of human origin, were submitted to the MTT test. This test is based on the metabolic reduction of

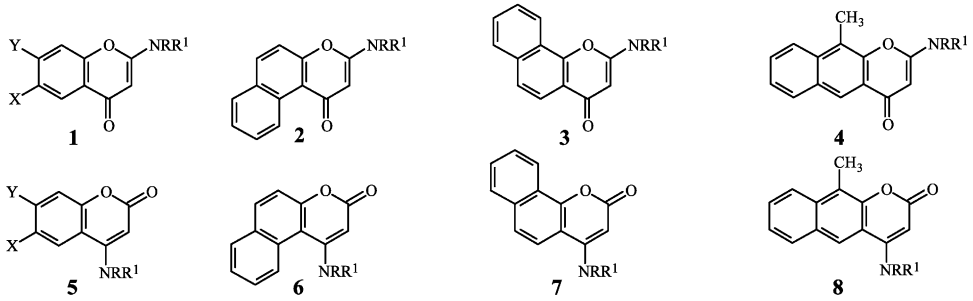
Table 1
Biological data of the *N*-substituted aminoderivatives **1a**, **2a–c**, **5a–e**, **6a–k**, **7a–d**, **8a–c**

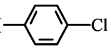


Compound [a]	NRR^1	X	Y	Inhibition of DNA synthesis IC_{50} (μM)±SD [b]	Inhibition of cell growth IC_{50} (μM)±SD [c]
1a	NH–C6H4	O–CH_2–O		13.5±0.64	21.7±3.04
2a	NH–C6H4–Cl	-	-	4.24±0.29	33.1±4.91
2b	NH–C5H4N	-	-	17.6±2.1	75.3±6.23
2c	$N(C_6H_5)_2$	-	-	19.8±1.97	31.1±3.71
5a	NH–C3H5	OCH_3	H	17.6±1.9	12.9±1.77
5b	NH–C3H5	H	OCH_3	27.7±2.2	73.6±3.58
5c	NH–C6H4	OCH_3	H	82.5±4.1	9.23±1.8
5d	NH–C6H4	H	OCH_3	30.7±1.7	4.95±2.16
5e	NH–C6H4	O–CH_2–O		2.64±0.16	77.4±5.06
6a	NH–C3H5	-	-	40.6±2.15	66.3±4.4
6b	NH–C6H10	-	-	58.5±4.34	96.2±4.04
6c	NH–C6H4	-	-	9.03±1.8	34.6±1.03
6d	NH–C6H4–Cl	-	-	6.9±1.8	27.3±1.92
6e	NH–C6H4–OCH_3	-	-	29.3±2.35	49.3±2.51
6g	NH–C5H4N	-	-	11.8±2.6	51.3±4.03
6h	$N(C_3H_7)_2$	-	-	64.6±8.54	50.5±12.4
6i	N(CH2CH2)2O	-	-	45.6±1.99	95.9±8.94
6j	N(CH2CH2)2NH	-	-	n.d. [d]	43.8±1.75
6k	$N(CH_3)$–C6H5	-	-	15.3±0.9	55.3±13.58
7a	NH–C3H5	-	-	4.05±1.36	36.1±0.81
7b	NH–C6H10	-	-	13.8±2.3	43.7±12.3
7c	NH–C6H4	-	-	1.74±0.18	8.71±1.2
7d	$N(CH_3)$–C6H5	-	-	8.37±2.0	18.5±3.72
8a	NH–C6H10	-	-	3.38±1.8	20.8±5.81
8b	NH–C6H4	-	-	11.8±1.73	61.8±7.63
8c	$N(CH_3)$–C6H5	-	-	16.6±1.78	31.8±5.32
1b [e]	N(CH2CH2)2O	H	H	19.2±17.29	52.0±2.14

[a]Compounds **6b–d** (Ref. [22]) and **6j** (Ref. [14]) were previously reported in the literature. Both IC_{50} values of compound **6f** were not detectable.[b]Drug concentration which induces a 50% inhibition of DNA synthesis in Ehrlich ascites tumor cells.[c]Drug concentration which induces a 50% inhibition of cell growth, detected by MTT assay.[d]n.d.: values not detectable.[e]Previously described in the literature (Ref. [3]). Tested as a reference compound.

Table 2
Biological data of the *N*-substituted 2-aminochromone fused derivatives **1a**, **2d**, **3a**, **4a** and 4-aminocoumarin fused derivatives **5e**, **6c**,**d**, **7c** and **8a**.



Compound [a]	NRR^1	X	Y	Inhibition of DNA synthesis IC_{50} (μM)±SD [b]	Inhibition of cell growth IC_{50} (μM)±SD [c]
1a	NH– 	O–CH_2–O		13.5±0.64	21.7±3.04
5e	NH– 	O–CH_2–O		2.64±0.16	77.4±5.06
2d	NH– 	-	-	1.98±0.70	39.1±5.49
6c	NH– 	-	-	9.03±1.8	34.6±1.03
6d	NH– 	-	-	6.90±1.8	27.3±1.92
3a	NH– 	-	-	8.21±0.66	39.7±10.08
7c	NH– 	-	-	1.74±0.18	8.71±1.2
4a	NH– 	-	-	1.74±0.20	9.92±1.50
8a	NH– 	-	-	3.38±1.8	20.8±5.81
1b [d]		H	H	19.2±17.29	52.0±2.14

[a]The biological data of compounds **1a**, **5e**, **6c**, **6d**, **7c**, **8a** and **1b** are reported in Table 1, whereas the ones of compounds **2d**, **3a** and **4a** were previously described by us (Ref. [12]).[b]Drug concentration which induces a 50% inhibition of DNA synthesis in Ehrlich ascites tumor cells.[c]Drug concentration which induces a 50% inhibition of cell growth, detected by MTT assay (compounds **1a**, **5e**, **6c, 6d**, **7c**, **8a, 1b**) or by clonogenic test (**2d**, **3a**, **4a**).[d]Previously described in the literature (Ref. [3]). Tested as a reference compound.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan [23]; it is considered useful for evaluating cytotoxicity, because only metabolically active cells can reduce MTT [24]. Therefore, the data afforded by this test, now presented, can be correctly compared with those previously obtained by us with the clonogenic assay [12].

Considering first the coumarin derivatives **5a**–**e**, **6a**–**k**, **7a**–**d** and **8a**–**c**, we can point out that, on the basis of the satisfactory activity previously [12] and now (**1a**, **2a**) shown by the (cyclopropylamino) and/or (phenylamino) substituted chromone derivatives **1**–**4** in the test of DNA synthesis inhibition, the (cycloalkylamino) and (phenylamino) groups, their derivatives and analogues were mainly used as amino substituents of compounds **5**–**8**. Actually, with respect to such test, the (phenylamino) (**5e**, **7c**), [(4-chlorophenyl)amino] (**6d**) and (cyclohexylamino) (**8a**) substituted compounds are the most active in the corresponding structural classes, being **7c** the most active (IC_{50} 1.74±0.18 μM) of all the compounds **1**, **2**, **5**–**8** described in the present paper (see Table 1). Significantly active (IC_{50} range: 4.05–16.6 μM) proved to be also the [(2-pyridinyl)amino], (cyclopropylamino) and (cyclohexylamino) substituted compounds **6g**, **7a** and **7b**, respectively, as well as the (phenylamino) substituted compounds **6c**, **8b**, and the (*N*-methyl-*N*-phenylamino) substituted **6k**, **7d** and **8c**.

In this connection, it must be noted that, as we previously observed in the case of chromone derivatives **1**–**4** [12], also the antiproliferative activity of the coumarin derivatives **5**–**8** now studied depend, not only on the type of amino substituent, but also on the structure of the cyclic system: see, for instance, the IC_{50} values of the (cycloalkylamino) substituted compounds

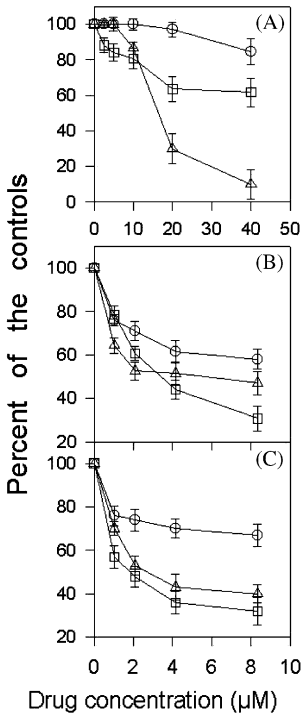


Fig. 2. Inhibition of macromolecular synthesis in Ehrlich ascites cells. Cells were incubated in the presence of increasing drug concentrations and then the syntheses of DNA, RNA and proteins were determined as described in Section 5. The tested compounds are: **5c** (panel A); **5e** (panel B); **7c** (panel C). Symbols: □, DNA; ○, RNA; △, proteins.

6a,**b**, **7a**,**b** and **8a** in the corresponding test of DNA synthesis inhibition (Table 1).

It is interesting to point out that the replacement of the (phenylamino) substituent of compounds **6c**, **7c** and **8b** with a (*N*-methyl-*N*-phenylamino) group afforded new derivatives (**6k**, **7d** and **8c**, respectively) all less active, in this test, than the corresponding parent compounds.

As regards the activity exhibited in the MTT assay by the bicyclic and tricyclic coumarin derivatives **5**–**8** (see Table 1), we before pointed out that, in general, it is lower or considerably lower than that afforded by the same compounds in the test of DNA synthesis inhibition. A similar behavior was shown both by compounds **1a**, **2a**–**c** (in the same test) and by a number of bicyclic and tricyclic 2-aminochromone derivatives **1**–**4**, previously studied by us [12], in the test of inhibition of clonal growth in HeLa cells.

Nevertheless, also in the MTT assay the (phenylamino) substituent proved to be particularly effective for the activity of the aminocoumarin derivatives studied, depending on the structure of the cyclic system. In fact, the (phenylamino) derivatives **5d**, **7c**, and **5c** were the most active of all the compounds **5**–**8** tested in this study (IC_{50} values: 4.95, 8.71, and 9.23 μM, respectively). In addition, it can be remarked that, among the compounds now studied, only 4-(phenylamino)-2*H*-naphtho[1,2-*b*]pyran-2-one (**7c**) showed both a high inhibitory activity on DNA synthesis in Ehrlich cells and notable cytotoxic properties, being it on the whole the most active compound assayed.

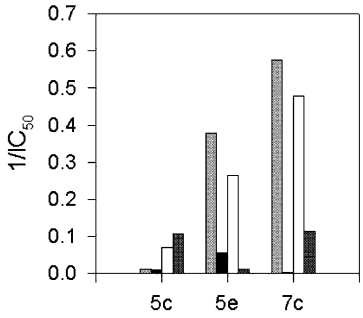


Fig. 3. Comparison between the data obtained in the tests on DNA, RNA, protein syntheses and in MTT test for compounds **5c**, **5e** and **7c**. The results are reported as the inverse of the IC_{50} (μM) values. The symbols are as follows: DNA synthesis, ▨; RNA synthesis, ■; Protein synthesis, □; MTT test, ▩.

It seems also interesting to stress the behavior of the alkoxy derivatives **5c**, **5d**, and **5e** in the above tests. Both the 6-methoxy- and 7-methoxy-4-(phenylamino)coumarins **5c** and **5d** afforded rather insignificant activities as DNA synthesis inhibitors, whereas the 6,7-methylenedioxy analogue **5e** proved to be very active. On the contrary, in the MTT assay, the methoxy derivatives **5c** and **5d** were two of the three most effective compounds tested, but the tricyclic 6,7-dialkoxy derivative **5e** appeared to be negligibly active.

We report in Table 2, for comparison, the IC_{50} values now and previously [12] afforded in the test of DNA synthesis inhibition both by the most active chromone or coumarin derivative of each structural class (**1**–**8**) and by the antiproliferative agent 2-morpholinochromone (**1b**) [3], examined as a reference compound. In this connection, also the data of cytotoxic activity are reported in Table 2.

Finally, in order to obtain information on the mechanism of action of these coumarin derivatives, we further studied three of them (**5c**, **5e** and **7c**), chosen for their different behavior in the two tests.

Fig. 3 shows a quantitative comparison between the inhibition of macromolecular synthesis and cytotoxicity. To get a better understanding, the results are reported as the inverse of the IC_{50} values. We must remember that macromolecular synthesis was detected in short term experiments (1.5 h), while the treatment for the MTT test was always carried out during 24 h. Therefore, the data on macromolecular synthesis give information on what happens just after a short treatment; on the contrary, the cytotoxicity test tells us the ultimate consequences of a long term treatment. If the effect found in the short term test is correlated with the long term consequences, we should find a quantitative correspondence between the effectiveness shown by the

compound in both tests. Our data did not allow to find such a correlation. This behavior suggests that the cytotoxicity mechanism of such compounds may not be mediated by macromolecular synthesis inhibition. On the other hand, this conclusion is also supported by the results obtained with other derivatives which proved to be ineffective (**2a**, **5d**, **6c**, **6d**, **7a**) or moderately active (**7d** and **8a**) on protein synthesis (data not shown), but exhibited an evident, even though variable, cytotoxicity (IC_{50} range: 4.95–36.1 μM). Considering these results, we can conclude that at present we have no data capable of clarifying the mechanism of action of these compounds and that further detailed studies are required, focused on the most significant compounds and based on the use of suitable cell lines. However, taking into account that 2-morpholino-8-phenylchromone, a structural analogue of herein described 1-benzopyran derivatives, was reported [5] to possess both smooth muscle cell antiproliferative properties and specific inhibitory activity against phosphatidylinositol 3-kinase, an enzyme implicated in growth factor signal transduction, we can suppose that the antiproliferative activity of compounds **1**–**8** is as well related to the inhibition of the same enzyme. We are planning to verify this working hypothesis.

4. Conclusions

With respect to compounds **1**–**8** studied by us in the present (mainly coumarin derivatives) and in a recent paper on this topic [12] (only chromone derivatives) and considering the structural and biological data reported in Tables 1 and 2, the following structure–activity relationships (SAR) conclusions can be drawn about their antiproliferative (DNA synthesis inhibition) and cytotoxic properties (MTT test).

- In general, compounds **1**–**8** showed clearly more significant antiproliferative than cytotoxic activity.
- On the whole, the activities of compounds depended both on the type of amino substituent and on the structure of the heterocyclic nucleus, in the chromone as well as in the coumarin structural field. For instance, considering the test of DNA synthesis inhibition, phenylamino and cycloalkylamino were in general the most effective amino substituents in both fields, whereas the tricyclic nuclei of compounds **4** [12] (linear) and **7** (angular) proved to be the most suitable in the chromone and coumarin fields, respectively.
- The (cyclopropylamino) substituted chromone derivative **4a** [12] and the now synthesized (phenylamino) substituted coumarin derivative **7c** showed the highest antiproliferative activity ($IC_{50} = 1.74$ μM) and, on the whole, also appear to be the two most active compounds tested, due to their notable cytotoxic properties. Actually, the cytotoxic activity of **4a** was the highest one in the chromone derivative field [12].
- The (phenylamino) substituted chromone derivative **2d** ($IC_{50} = 1.98 \pm 0.70$ μM) [12] and the now described coumarin derivative **5e** ($IC_{50} = 2.64 \pm 0.16$ μM) can however be considered the most interesting ones as antiproliferative agents, being nearly equiactive to **4a** and **7c**, but showing almost negligible cytotoxic activity.
- Nine of the 23 4-aminocoumarin bicyclic and tricyclic derivatives **5**–**8** studied in the present paper are provided with significant antiproliferative activity (IC_{50} range: 1.74–11.8 μM), whereas only four of them showed an about similar cytotoxic activity (IC_{50} range: 4.95–12.9 μM). On the whole, this behavior is nearly equivalent to that afforded by the 2-aminochromone bicyclic and tricyclic derivatives **1**–**4** previously [12] and now studied by us.

5. Experimental

5.1. Chemistry

Melting points were determined using a Fisher–Johns apparatus (Electrothermal when above 300 °C) and are uncorrected. IR spectra were recorded on a Perkin–Elmer 398 spectrophotometer (abbreviations relative to IR bands: br = broad, s = strong, w = weak, sh = shoulder). ^{1}H NMR spectra were recorded on a Varian Gemini 200 (200 MHz) spectrometer, and chemical shifts (δ) are reported in ppm using $(CH_3)_4Si$ as an internal reference ($\delta = 0$). Spin multiplicities are given as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet). Analyses of all compounds described were performed by the Laboratorio di Microanalisi del Dipartimento di Scienze Farmaceutiche, University of Genoa. Thin-layer chromatograms were run on Merck silica gel 60 F_{254} precoated plastic sheets (layer thickness 0.2 mm). Column chromatography was performed using Carlo Erba silica gel (0.05–0.20 mm) or Carlo Erba neutral aluminium oxide (Brockmann activity I).

5.1.1. 6-(Methylthio)-8H-1,3-dioxolo[4,5-g][1]benzopyran-8-one (**10**)

To a suspension of potassium *tert*-butoxide (3.37 g, 30.0 mmol) in 50 ml of dry toluene, stirred under nitrogen, a solution of 2-acetilsesamol **9** [13] (1.80 g, 10.0 mmol) and carbon disulphide (0.84 g, 11.0 mmol) in dry toluene (50 ml) was slowly added. The resulting orange–yellow viscous mixture was stirred under nitrogen at room temperature overnight. Cold water (500 ml) was then added to this slurry and the resulting mixture was transferred to a separatory funnel, then extracted with

ethyl ether. The organic layer was discarded and the orange aqueous solution was collected, then acidified with 6 N aq. HCl so that a yellowish oil separated out. This emulsion was allowed to stir at room temperature for 1 h (under a fume hood to remove hydrogen sulphide), then subjected to exhaustive extraction with the mixture ethyl ether–tetrahydrofuran (1:1). The solvents were then removed from the dried combined extracts to give a brownish thick oil containing the desired 8-hydroxy-6*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-6-thione together with some starting compound **9** and impurities. Dry acetone (100 ml), anhyd. K_2CO_3 (1.0 g), and iodomethane (1.5 ml) were added to this raw oil, stirring then the mixture at reflux for 1 h. After cooling and removing the solvent in vacuo, the residue was partitioned between water and dichloromethane, the organic layer was collected and the aqueous one was extracted twice with dichloromethane. The oily residue obtained from the combined extracts after removal of solvents was chromatographed on a silica gel column eluting first with dichloromethane to recover compound **9** and remove some impurities, then with the mixture dichloromethane–ethyl acetate (1:1). This latter eluate was finally evaporated to dryness in vacuo to afford the pure solid compound **10** (0.42 g, 18%); whitish crystals, m.p. 174–175 °C (isopropyl ether with charcoal). ^{1}H NMR ($CDCl_3$): δ 2.52 (s, 3H, SCH_3), 6.09 (s, 2H, OCH_2O), 6.15 (s, 1H, H-7), 6.82 (s, 1H, H-4), 7.48 (s, 1H, H-9). IR ($CHCl_3$): 1634 s (CO), 1616, 1557, 1505 w cm^{-1}. *Anal.* Calc. for $C_{11}H_8O_4S$: C, 55.93; H, 3.41; S, 13.57. Found: C, 55.76; H, 3.65; S, 13.68%.

5.1.2. 6-(Phenylamino)-8H-1,3-dioxolo[4,5-g][1]benzopyran-8-one (**1a**)

A mixture of compound **10** (0.35 g, 1.5 mmol), aniline (1.40 g, 15.0 mmol), monohydrate *p*-toluenesulfonic acid (0.15 g) and ethylene glycol (10 ml) was stirred at 160 °C for 4 h. The mixture was then poured onto ice–water and exhaustively extracted with chloroform. The combined organic phases were dried (anhyd. sodium sulfate) and the solvent removed in vacuo to give a dark oil which was chromatographed on a silica gel column eluting with dichloromethane to remove excess aniline and impurities, then with dichloromethane–methanol (9:1) to recover compound **1a**. The eluate collected, after removal of solvents, afforded pure compound **1a** (0.19 g, 45%); pale-brown crystals, m.p. 282–283 °C (ethanol). ^{1}H NMR (DMSO-d_6): δ 5.46 (s, 1H, H-7), 6.19 (s, 2H, OCH_2O), 7.10–7.46 (m, 7H, H-4,9+phenyl H's), 9.90 (s, 1H, NH; disappeared with D_2O). IR (KBr): 3145 br (NH), 1640 (CO), 1615, 1600, 1588 w, 1570 s, 1523 s,br cm^{-1}. *Anal.* Calc. for $C_{16}H_{11}NO_4$: C, 68.33; H, 3.94; N, 4.98. Found: C, 68.06; H, 4.00; N, 5.00%.

5.1.3. 3-[(4-Chlorophenyl)amino]-1H-naphtho[2,1-b]pyran-1-one (**2a**)

A mixture of 3-(methylthio)-1*H*-naphtho[2,1-*b*]pyran-1-one (**11**) [14] (0.73 g, 3.0 mmol), 4-chloroaniline (3.83 g, 30.0 mmol), monohydrate *p*-toluenesulfonic acid (0.20 g) and 1-pentanol (20 ml) was stirred at 160 °C for 16 h. After cooling and standing, pure compound **2a** separated out as a whitish crystalline solid which was recovered by filtration (0.49 g, 51%); m.p. 291–291.5 °C (pyridine). ^{1}H NMR (DMSO-d_6): δ 5.72 (s, 1H, H-2), 7.42 and 7.50 [AB q, $J = 9$ Hz, 4H, (4-chlorophenyl) H's], 7.58–7.78 (m, 3H, H-5,8,9), 8.07 (m, 1H, H-7), 8.27 (d, $J_{6,5} = 9$ Hz, 1H, H-6), 10.00–10.12 (m, 2H, H-10+NH; 1H after treatment with D_2O). IR (KBr): 3160 br (NH), 1657 (CO), 1630, 1605, 1586 s, 1572, 1561, 1534 s,br cm^{-1}. *Anal.* Calc. for $C_{19}H_{12}ClNO_2$: C, 70.92; H, 3.76; N, 4.35; Cl, 11.02. Found: C, 70.72; H, 3.71; N, 4.49; Cl, 11.38%.

5.1.4. 3-(Methylsulfinyl)-1H-naphtho[2,1-b]pyran-1-one (**12**)

A mixture of the (methylthio) derivative **11** [14] (1.21 g, 5.0 mmol), 3-chloroperbenzoic acid (0.90 g, 5.2 mmol) and 1,2-dichloroethane (50 ml) was stirred at 0 °C for 1 h. The resulting suspension was partitioned between 10% aq. Na_2CO_3 (200 ml) and dichloromethane (200 ml). The organic layer was collected and the aqueous phase was extracted twice with dichloromethane. The combined organic phases (dried over anhyd. sodium sulfate), after evaporation to dryness in vacuo, afforded a white solid which was taken up in a little isopropyl ether and filtered to give pure compound **12** (1.23 g, 95%); white crystals, m.p. 206–206.5 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 3.02 (s, 3H, $SOCH_3$), 7.07 (s, 1H, H-2), 7.50 (d, $J_{5,6} = 9$ Hz, 1H, H-5), 7.66 and 7.78 (2m, 1H+1H, H-8,9), 7.92 (m, 1H, H-7), 8.13 (d, $J_{6,5} = 9$ Hz, 1H, H-6), 9.94 (m, 1H, H-10). IR ($CHCl_3$): 1643 s (CO), 1619 sh, 1594, 1572 sh, 1514, 1074 s (SO) cm^{-1}. *Anal.* Calc. for $C_{14}H_{10}O_3S$: C, 65.10; H, 3.90; S, 12.41. Found: C, 64.96; H, 3.84; S, 12.78%.

5.1.5. 3-[(2-Pyridinyl)amino]-1H-naphtho[2,1-b]pyran-1-one (**2b**)

A mixture of the 3-(methylsulfinyl) derivative **12** (0.77 g, 3.0 mmol), 2-aminopyridine (2.82 g, 30.0 mmol) and Dowtherm A (7 ml) was stirred at 200 °C for 1.5 h. The mixture was then cooled and partitioned between 2 N aq. HCl (200 ml) and ethyl ether (100 ml). After separating some insoluble tars by filtration, the aqueous phase was collected, whereas the organic one was extracted twice with 2 N aq. HCl, then discarded. The combined acidic phases were made alkaline with 30% aq. NH_3 and the resulting mixture was exhaustively extracted with chloroform. After drying (anhyd. sodium sulfate) and evaporating in vacuo the combined extracts, the resulting oil was chromatographed on a silica gel

column eluting with the mixture dichloromethane–ethyl acetate (1:1). The eluate collected, evaporated to dryness in vacuo, gave a solid residue which was treated with a little ethyl ether to yield the pure compound **2b** (0.11 g, 13%); whitish crystals, m.p. 263–263.5 °C (ethanol). ^{1}H NMR ($CDCl_3$): δ 6.72 (s, 1H, H-2), 7.05 (m, 1H, pyridinyl H-5′), 7.26 (m, 1H, pyridinyl H-3′), 7.46 (d, $J_{5,6} = 9$ Hz, 1H, H-5), 7.56–7.94 (m, 5H, H-7,8,9+ pyridinyl H-4′+NH; 4H after treatment with D_2O), 8.05 (d, $J_{6,5} = 9$ Hz, 1H, H-6), 8.40 (m, 1H, pyridinyl H-6′), 10.12 (m, 1H, H-10). IR (KBr): 3160 br (NH), 1638 (CO), 1618, 1600, 1587 s, 1568, 1520 s,br cm^{-1}. *Anal.* Calc. for $C_{18}H_{12}N_2O_2$: C, 74.99; H, 4.20; N, 9.72. Found: C, 74.68; H, 4.18; N, 9.80%.

*5.1.6. 3-(Diphenylamino)-1H-naphtho[2,1-b]pyran-1-one (**2c**)*

Phosphorus oxychloride (5.75 g, 37.5 mmol) was added dropwise with stirring to an ice-cooled solution of ethyl *N*,*N*-diphenylmalonamate [16] (7.79 g, 27.5 mmol) in chlorobenzene (10 ml). The resulting solution was stirred at room temperature for 1 h to obtain reagent **14**, then a suspension of 2-naphthol (3.60 g, 25.0 mmol) in chlorobenzene (50 ml) was added and the mixture was heated at 130 °C for 7 h, while stirring. After cooling, a solution of trihydrate sodium acetate (34 g) in water (150 ml) was added and the resulting mixture was vigorously stirred at 60 °C for 1 h. After cooling, the organic layer was collected and the aqueous one was exhaustively extracted with chloroform. The combined organic phases were washed twice with 1 N aq. NaOH, then with water until neutral, and dried (anhyd. sodium sulfate). The solvents were then removed in vacuo to give a dark thick oil which was chromatographed on a silica gel column eluting with chloroform–petroleum ether (2:1). The eluate collected, after removal of solvents, afforded a thick oil which was treated with a little ethyl acetate and allowed to stand until the nearly pure solid compound **2c** separated out (0.77 g, 8.5%); whitish crystals, m.p. 247–248 °C (ethyl acetate with charcoal). ^{1}H NMR ($CDCl_3$): δ 5.75 (s, 1H, H-2), 7.10–8.04 (m, 15H, H-5,6,7,8,9+phenyl H's), 10.11 (m, 1H, H-10). IR ($CHCl_3$): 1631 s (CO), 1614, 1594, 1562 s, 1514 w, 1493 cm^{-1}. *Anal.* Calc. for $C_{25}H_{17}NO_2$: C, 82.63; H, 4.72; N, 3.85. Found: C, 82.36; H, 4.56; N, 3.95%.

*5.1.7. General procedure for the synthesis of the 4-chlorocoumarin derivatives **16a,b, 18, 20***

A mixture of the proper 4-hydroxycoumarin derivative **15a**,**b**, **17** or **19** (10.0 mmol), triethylamine (1.01 g, 10.0 mmol) and phosphorus oxychloride (10 ml) was stirred at 130 °C for 30 min (compounds **18**, **20**) or 3 h (compounds **16a**,**b**). The mixture was then poured onto ice-water and stirred, then exhaustively extracted with dichloromethane. The combined extracts, after drying (anhyd. sodium sulfate) and removal of solvents, afforded a dark oily or nearly solid residue which was chromatographed on a silica gel column eluting with the mixture dichloromethane–petroleum ether (4:1). The eluate collected, after removal of solvents, afforded the desired 4-chlorocoumarin derivative as a solid which was taken up in a little petroleum ether, separated by filtration, then recrystallized from the proper solvent.

*5.1.7.1. 4-Chloro-6-methoxy-2H-1-benzopyran-2-one (**16a**).* Starting from 1.92 g of **15a** [21], the pure compound **16a** (1.16 g, 55%) was obtained; white crystals, m.p. 170–171 °C (ethyl acetate–isopropyl ether) (lit. [17] m.p. 159–161 °C). ^{1}H NMR ($CDCl_3$): δ 3.89 (s, 3H, OCH_3), 6.62 (s, 1H, H-3), 7.14–7.35 (m, 3H, H-5,7,8). IR (KBr): 1720 s,br (CO), 1603 w, 1570 s, 1550 sh cm^{-1}. *Anal.* Calc. for $C_{10}H_7ClO_3$: C, 57.03; H, 3.35; Cl, 16.83. Found: C, 57.20; H, 3.27; Cl, 16.95%.

*5.1.7.2. 4-Chloro-7-methoxy-2H-1-benzopyran-2-one (**16b**).* Starting from 1.92 g of **15b** [21], the pure compound **16b** (1.18 g, 56%) was obtained; white crystals, m.p. 139–140 °C (isopropyl ether) (lit. [18] m.p. 139–140 °C). ^{1}H NMR ($CDCl_3$): δ 3.90 (s, 3H, OCH_3), 6.44 (s, 1H, H-3), 6.84 (d, $J_{8,6} = 2$ Hz, 1H, H-8), 6.92 (dd, $J_{6,5} = 9$ Hz, $J_{6,8} = 2$ Hz, 1H, H-6), 7.76 (d, $J_{5,6} = 9$ Hz, 1H, H-5). IR (KBr): 1730 s, br (CO), 1620, 1604, 1551, 1503 w, br cm^{-1}. *Anal.* Calc. for $C_{10}H_7ClO_3$: C, 57.03; H, 3.35; Cl, 16.83. Found: C, 56.88; H, 3.31; Cl, 16.66%.

*5.1.7.3. 1-Chloro-3H-naphtho[2,1-b]pyran-3-one (**18**).* The reaction carried out with 2.12 g of **17** [20] yielded **18** (1.98 g, 86%); white crystalline solid, m.p. 159–160 °C (ethanol), identified by comparison (m.p., TLC, IR) with an authentic sample [14].

*5.1.7.4. 4-Chloro-2H-naphtho[1,2-b]pyran-2-one (**20**).* The reaction carried out with 2.12 g of **19** [20] afforded **20** (1.84 g, 80%); white crystalline solid, m.p. 164–165 °C (ethanol), identified by comparison (m.p., TLC, IR) with an authentic sample [14].

*5.1.8. General procedures for the preparation of the 4-aminocoumarin derivatives **5a–e, 6a–i,k, 7a–d, 8a–c** from the corresponding 4-chlorocoumarin derivatives **16a–c, 18, 20, 21***

A) Reaction with aliphatic amines (compounds **5a**,**b**, **6a**,**b**,**h**,**i**, **7a**,**b**, **8a**): a mixture of 2.0 mmol of the proper chloroderivative **16a**,**b** (0.42 g), **18** (0.46 g), **20** (0.46 g), or **21** (0.49 g), 20.0 mmol of the suitable amine [cyclopropylamine (1.14 g), cyclohexylamine (1.98 g), dipropylamine (2.02 g), or morpholine (1.74 g)] and 30 ml of ethanol was refluxed for 0.5–2 h, with stirring. After concentrating and cooling the

final reaction mixture, the desired aminoderivative separated out as a crystalline solid only in the case of compounds **6b**,**i**. In all other cases, ethanol was removed in vacuo and the residue was partitioned between dichloromethane and 5% aq. $NaHCO_3$. The organic phase was then dried (anhyd. sodium sulfate) and the solvent removed to give a nearly solid residue from which, after addition of a suitable solvent (compounds **5a**,**b**, **6a**, **8a**) or chromatography on a silica gel column eluting with dichloromethane–petroleum ether–ethyl acetate (6:3:1) (compounds **6h**, **7a**,**b**), the pure aminoderivative was finally obtained.

B) Reaction with aromatic amines (compounds **5c**–**e**, **6c**–**g**,**k**, **7c**,**d**, **8b**,**c**): a mixture of 2.0 mmol of the proper chloroderivative **16a**,**b** (0.42 g), **16c** (0.45 g), **18** (0.46 g), **20** (0.46 g), or **21** (0.49 g), 20.0 mmol of the suitable amine [aniline (1.86 g), 4-chloroaniline (2.55 g), 3-methoxyaniline (2.46 g), 2,4-dimethoxyaniline (3.06 g), 2-aminopyridine (1.88 g), or *N*-methylaniline (2.14 g)] and 10 ml of ethylene glycol (glycerol in the case of **6g**) was stirred at 160 °C for 1 h. Only in the case of compounds **6d**, **7c**, **8b** a suspension was finally obtained which was cooled, diluted with a little ethanol and filtered to yield the pure aminoderivative. The solution otherwise obtained was poured into water (200 ml) and stirred, affording a suspension (compounds **5c**, **6c**,**e**,**g**) or an emulsion (**5d**,**e**, **6f**,**k**, **7d**, **8c**). In the first case, the rough aminoderivative was recovered by filtration as an amorphous solid, washed with water then ethyl ether, and further purified by crystallization from the proper solvent (compounds **5c**, **6c**,**e**) or by column chromatography [silica gel, dichloromethane–ethyl acetate (1:1)] (**6g**). In the second case, the resulting emulsion was thoroughly extracted with dichloromethane. After removal of solvent from the dried combined extracts, the resulting residue yielded the desired aminoderivative by simple addition of a little ethyl ether (compounds **5d**,**e**) or through column chromatography [silica gel, dichloromethane–ethyl acetate (1:1)] (compounds **6f**,**k**, **7d**, **8c**).

*5.1.8.1. 4-(Cyclopropylamino)-6-methoxy-2H-1-benzopyran-2-one (**5a**).* Following the procedure above described, from the reaction of **16a** with cyclopropylamine (2 h), a solid residue was finally obtained which was taken up in a little isopropyl ether to give **5a** (0.29 g, 63%); white crystals, m.p. 196–197 °C (ethyl acetate–isopropyl ether). ^{1}H NMR (DMSO-d_6): δ 0.57–0.70 and 0.81–0.94 (2m, 2H+2H, cyclopropyl CH_2's), 2.57 (m, 1H, cyclopropyl CH), 3.82 (s, 3H, OCH_3), 5.45 (s, 1H, H-3), 7.19 (dd, $J_{7,8}=9$ Hz, $J_{7,5}=2$ Hz, 1H, H-7), 7.27 (d, $J_{8,7}=9$ Hz, 1H, H-8), 7.57 (d, $J_{5,7}=2$ Hz, 1H, H-5), 7.90 (s, 1H, NH; disappeared with D_2O). IR (KBr): 3310 s (NH), 1663 s, br (CO), 1606, 1563 s, br, 1545 s, br, 1492 w cm^{-1}. *Anal.* Calc. for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.54; H, 5.40; N, 6.06%.

*5.1.8.2. 4-(Cyclopropylamino)-7-methoxy-2H-1-benzopyran-2-one (**5b**).* From **16b** and cyclopropylamine, pure **5b** was obtained as described for **5a**. Yield: 0.33 g (71%); white crystals, m.p. 209–210 °C (ethanol). ^{1}H NMR (DMSO-d_6): δ 0.56–0.68 and 0.80–0.92 (2m, 2H+2H, cyclopropyl CH_2's), 2.57 (m, 1H, cyclopropyl CH), 3.85 (s, 3H, OCH_3), 5.33 (s, 1H, H-3), 6.85–6.96 (m, 2H, H-6,8), 7.81 (s, 1H, NH; disappeared with D_2O), 7.93 (d, $J_{5,6}=9$ Hz, 1H, H-5). IR (KBr): 3300 s (NH), 1680 s, br (CO), 1618 s, br, 1548, 1496 cm^{-1}. *Anal.* Calc. for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.59; H, 5.78; N, 6.16%.

*5.1.8.3. 4-(Phenylamino)-6-methoxy-2H-1-benzopyran-2-one (**5c**).* The amorphous solid obtained from **16a** and aniline, according to general procedure, was crystallized to give pure **5c** (0.35 g, 65%); whitish crystals, m.p. 269–270 °C (methanol). ^{1}H NMR (DMSO-d_6): δ 3.87 (s, 3H, OCH_3), 5.30 (s, 1H, H-3), 7.24–7.60 (m, 7H, H-7,8+phenyl H's), 7.80 (d, $J_{5,7}=2$ Hz, 1H, H-5), 9.30 (s, 1H, NH; disappeared with D_2O). IR (KBr): 3275 (NH), 1648 s, br (CO), 1610 w, 1592, 1560 s, 1530 s, 1498 w cm^{-1}. *Anal.* Calc. for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.63; H, 4.71; N, 5.23%.

*5.1.8.4. 4-(Phenylamino)-7-methoxy-2H-1-benzopyran-2-one (**5d**).* Obtained from **16b** and aniline according to general procedure. Yield: 0.34 g (64%); whitish needles, m.p. 248–249 °C (ethanol). ^{1}H NMR (DMSO-d_6): δ 3.89 (s, 3H, OCH_3), 5.19 (s, 1H, H-3), 6.95–7.08 (m, 2H, H-6,8), 7.26–7.57 (m, 5H, phenyl H's), 8.17 (d, $J_{5,6}=9$ Hz, 1H, H-5), 9.26 (s, 1H, NH; disappeared with D_2O). IR (KBr): 3285 (NH), 1665 s, br (CO), 1620, 1590, 1543, 1500 cm^{-1}. *Anal.* Calc. for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.76; H, 4.80; N, 5.25%.

*5.1.8.5. 8-(Phenylamino)-6H-1,3-dioxolo[4,5-g][1]benzopyran-6-one (**5e**).* Obtained from **16c** [19] and aniline according to general procedure. Yield: 0.36 g (64%); pale brown crystals, m.p. 310–312 °C (ethanol). ^{1}H NMR (DMSO-d_6): δ 5.20 (s, 1H, H-7), 6.20 (s, 2H, OCH_2O), 7.06 (s, 1H, H-4), 7.25–7.57 (m, 5H, phenyl H's), 7.77 (s, 1H, H-9), 9.08 (s, 1H, NH; disappeared with D_2O). IR (KBr): 3290 (NH), 1662 s, br (CO), 1630 w, 1597, 1572, 1540 s, br, 1496 w cm^{-1}. *Anal.* Calc. for $C_{16}H_{11}NO_4$: C, 68.33; H, 3.94; N, 4.98. Found: C, 68.09; H, 3.92; N, 5.04%.

*5.1.8.6. 1-(Cyclopropylamino)-3H-naphtho[2,1-b]pyran-3-one (**6a**).* The reaction (30 min) of **18** with cyclopropylamine afforded a solid residue which was taken up in a little isopropyl ether to give **6a** (0.43 g,

86%); white crystals, m.p. 173–175 °C (ethyl acetate–isopropyl ether). ^{1}H NMR ($CDCl_3$): δ 0.74–1.14 (m, 4H, cyclopropyl CH_2's), 2.60 (m, 1H, cyclopropyl CH), 5.94 (s, 1H, H-2), 6.11 (near s, 1H, NH; disappeared with D_2O), 7.24–8.14 (m, 5H, H-5,6,7,8,9), 8.42 (m, 1H, H-10). IR ($CHCl_3$): 3420 (NH), 1680 s, br (CO), 1626, 1606 w, 1587, 1551, 1503 cm^{-1}. *Anal.* Calc. for $C_{16}H_{13}NO_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.29; H, 5.34; N, 5.51%.

*5.1.8.7. 1-(Cyclohexylamino)-3H-naphtho[2,1-b]pyran-3-one (**6b**).* Obtained from **18** and cyclohexylamine according to general procedure. Yield: 0.30 g (51%); white crystals, m.p. 203.5–204 °C (ethyl acetate) (lit. [22] m.p. 190 °C). ^{1}H NMR ($CDCl_3$): δ 1.20–1.93 and 2.09–2.31 (2m, 8H+2H, cyclohexyl CH_2's), 3.50 (m, 1H, cyclohexyl CH), 5.49 (s, 1H, H-2), 5.69 (near d, 1H, NH; disappeared with D_2O), 7.36–7.71 and 7.85–7.99 (2m, 3H+2H, H-5,6,7,8,9), 8.47 (m, 1H, H-10). IR ($CHCl_3$): 3438 (NH), 1680 s, br (CO), 1628, 1609 w, 1588, 1553, 1518 cm^{-1}. *Anal.* Calc. for $C_{19}H_{19}NO_2$: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.60; H, 6.51; N, 4.85%.

*5.1.8.8. 1-(Phenylamino)-3H-naphtho[2,1-b]pyran-3-one (**6c**).* The amorphous solid obtained from **18** and aniline, according to general procedure, was crystallized to give pure **6c** (0.40 g, 70%); white crystals, m.p. 236–237 °C (ethanol) (lit. [22] m.p. 225 °C). ^{1}H NMR (DMSO-d_6): δ 5.61 (s, 1H, H-2), 7.17–7.74 (m, 8H, H-5,8,9+phenyl H's), 8.08 (m, 1H, H-7), 8.20 (d, $J_{6,5}=9$ Hz, 1H, H-6), 8.87 (m, 1H, H-10), 9.29 (s, 1H, NH; disappeared with D_2O). IR ($CHCl_3$): 3419 (NH), 1690 s, br (CO), 1629, 1610 w, 1587, 1557, 1513 cm^{-1}. *Anal.* Calc. for $C_{19}H_{13}NO_2$: C, 79.43; H, 4.56; N, 4.87. Found: C, 79.42; H, 4.57; N, 4.89%.

*5.1.8.9. 1-[(4-Chlorophenyl)amino]-3H-naphtho[2,1-b]pyran-3-one (**6d**).* Obtained from **18** and 4-chloroaniline as described in general procedure. Yield: 0.41 g (64%); white crystals, m.p. 271–272 °C (ethanol–dichloromethane) (lit. [22] m.p. 265 °C). ^{1}H NMR (DMSO-d_6): δ 5.71 (s, 1H, H-2; nearly completely disappeared with D_2O), 7.26–8.41 [m, 9H, H-5,6,7,8,9+(4-chlorophenyl) H's], 8.90 (m, 1H, H-10), 9.36 (s, 1H, NH; disappeared with D_2O). IR (KBr): 3200 br (NH), 1652 s, br (CO), 1630 sh, 1608 sh, 1581, 1548, 1510 cm^{-1}. *Anal.* Calc. for $C_{19}H_{12}ClNO_2$: C, 70.92; H, 3.76; N, 4.35; Cl, 11.02. Found: C, 70.70; H, 3.62; N, 4.32; Cl, 11.29%.

*5.1.8.10. 1-[(3-Methoxyphenyl)amino]-3H-naphtho[2,1-b]pyran-3-one (**6e**).* Obtained from **18** and 3-methoxyaniline as described for **6c**. Yield: 0.49 g (77%); whitish crystals, m.p. 210–211 °C (ethyl acetate). ^{1}H NMR (DMSO-d_6): δ 3.77 (s, 3H, OCH_3), 5.71 (s, 1H, H-2), 6.81, 6.99 and 7.31–7.76 [3m, 1H+2H+3H, H-8,9+(3-methoxyphenyl) H's], 7.52 (d, $J_{5,6}=9$ Hz, 1H, H-5), 8.08 (m, 1H, H-7), 8.20 (d, $J_{6,5}=9$ Hz, 1H, H-6), 8.87 (m, 1H, H-10), 9.26 (s, 1H, NH; disappeared with D_2O). IR ($CHCl_3$): 3410 (NH), 1710 sh and 1690 s (CO), 1630, 1605, 1588, 1556, 1513 cm^{-1}. *Anal.* Calc. for $C_{20}H_{15}NO_3$: C, 75.70; H, 4.76; N, 4.41. Found: C, 75.81; H, 4.84; N, 4.47%.

*5.1.8.11. 1-[(2,4-Dimethoxyphenyl)amino]-3H-naphtho[2,1-b]pyran-3-one (**6f**).* Obtained from **18** and 2,4-dimethoxyaniline after purification by column chromatography according to general procedure. Yield: 0.51 g (73%); whitish crystals, m.p. 247–248 °C (pyridine). ^{1}H NMR (DMSO-d_6): δ 3.84 (s, 6H, OCH_3), 4.90 (s, 1H, H-2), 6.65 [dd, $J_{5',6'}=9$ Hz, $J_{5',3'}=2$ Hz, 1H, (2,4-dimethoxyphenyl) H-5′], 6.79 [d, $J_{3',5'}=2$ Hz, 1H, (2,4-dimethoxyphenyl) H-3′], 7.22 [d, $J_{6',5'}=9$ Hz, 1H, (2,4-dimethoxyphenyl) H-6′], 7.52 (d, $J_{5,6}=9$ Hz, 1H, H-5), 7.57–7.78 (m, 2H, H-8,9), 8.12 (m, 1H, H-7), 8.21 (d, $J_{6,5}=9$ Hz, 1H, H-6), 8.75 (s, 1H, NH; disappeared with D_2O), 8.90 (m, 1H, H-10). IR (KBr): 3305 (NH), 1667 s, br (CO), 1622 sh, 1611, 1586, 1548, 1500 s cm^{-1}. *Anal.* Calc. for $C_{21}H_{17}NO_4$: C, 72.61; H, 4.93; N, 4.03. Found: C, 72.42; H, 4.90; N, 4.28%.

*5.1.8.12. 1-[(2-Pyridinyl)amino]-3H-naphtho[2,1-b]pyran-3-one (**6g**).* The amorphous solid, obtained from **18** and 2-aminopyridine according to general procedure, was purified by column chromatography to give pure **6g** (0.18 g, 31%); whitish crystals, m.p. 276–277 °C (ethanol). ^{1}H NMR (DMSO-d_6): δ 6.49 (s, 1H, H-2), 7.28–8.09 (m, 9H, H-5,6,7,8,9+pyridinyl H's), 9.09 (m, 1H, H-10), 9.88 (broad s, 1H, NH; disappeared with D_2O). IR (KBr): 3120 br (NH), 1690 sh and 1658 s (CO), 1628, 1594 w, 1583 w, 1520, 1508 cm^{-1}. *Anal.* Calc. for $C_{18}H_{12}N_2O_2$: C, 74.99; H, 4.20; N, 9.72. Found: C, 74.66; H, 4.34; N, 9.66%.

*5.1.8.13. 1-(Dipropylamino)-3H-naphtho[2,1-b]pyran-3-one (**6h**).* The reaction (1.5 h) of **18** with dipropylamine afforded a nearly solid residue which was chromatographed as described in general procedure to give **6h** (0.23 g, 39%); white crystals, m.p. 135–136 °C (isopropyl ether–petroleum ether). ^{1}H NMR ($CDCl_3$): δ 0.84 [t, 6H, $N(CH_2CH_2\mathit{CH_3})_2$], 1.40–1.80 [m, 4H, $N(CH_2\mathit{CH_2}CH_3)_2$], 3.05–3.38 [m, 4H, $N(\mathit{CH_2}CH_2CH_3)_2$], 5.85 (s, 1H, H-2), 7.37–7.64 and 7.80–7.98 (2m, 3H+2H, H-5,6,7,8,9), 8.80 (m, 1H, H-10). IR ($CHCl_3$): 1690 s, br (CO), 1623 w, 1603 w, 1583, 1542, 1512 w cm^{-1}. *Anal.* Calc. for $C_{19}H_{21}NO_2$: C, 77.26; H, 7.17; N, 4.74. Found: C, 77.14; H, 7.18; N, 4.72%.

*5.1.8.14. 1-Morpholino-3H-naphtho[2,1-b]pyran-3-one (**6i**).* Obtained from the reaction (30 min) of **18** with

morpholine, according to general procedure. Yield: 0.47 g (84%); white needles, m.p. 206–207 °C (ethanol). ^{1}H NMR ($CDCl_3$): δ 2.76–3.66 (m, 4H, N–CH_2), 3.86–4.20 (m, 4H, O–CH_2), 5.93 (s, 1H, H-2), 7.30–8.20 (m, 5H, H-5,6,7,8,9), 9.18 (m, 1H, H-10). IR ($CHCl_3$): 1695 s, br (CO), 1620 w, 1602 w, 1584 w, 1544, 1512 w cm^{-1}. *Anal.* Calc. for $C_{17}H_{15}NO_3$: C, 72.58; H, 5.37; N, 4.98. Found: C, 72.61; H, 5.39; N, 4.98%.

*5.1.8.15. 1-(N-Methyl-N-phenylamino)-3H-naphtho[2,1-b]pyran-3-one (**6k**).* The oily residue obtained from **18** and *N*-methylaniline, according to general procedure, was purified by column chromatography to yield pure **6k** (0.22 g, 36%); pale yellow crystals, m.p. 137–138 °C (isopropyl ether). ^{1}H NMR ($CDCl_3$): δ 3.32 (s, 3H, CH_3), 6.19 (s, 1H, H-2), 6.96–7.53 (m, 8H, H-5,8,9+phenyl H's), 7.82 (m, 1H, H-7), 7.95 (d, $J_{6,5}=9$ Hz, 1H, H-6), 8.78 (m, 1H, H-10). IR ($CHCl_3$): 1703 s, br (CO), 1627 w, 1602 w, 1588, 1550, 1498 cm^{-1}. *Anal.* Calc. for $C_{20}H_{15}NO_2$: C, 79.72; H, 5.02; N, 4.65. Found: C, 79.55; H, 5.02; N, 4.63%.

*5.1.8.16. 4-(Cyclopropylamino)-2H-naphtho[1,2-b]pyran-2-one (**7a**).* The reaction (1 h) of **20** with cyclopropylamine afforded a nearly solid residue which was chromatographed to give **7a** (0.41 g, 82%); white crystals, m.p. 262–263 °C (acetone). ^{1}H NMR ($CDCl_3$): δ 0.76 and 0.95 (2m, 2H+2H, cyclopropyl CH_2's), 2.65 (m, 1H, cyclopropyl CH), 5.65 (s, 1H, NH; disappeared with D_2O), 5.86 (s, 1H, H-3), 7.39 (d, $J_{6,5}=9$ Hz, 1H, H-6), 7.52–7.73 and 7.85 (2m, 3H+1H, H-5,7,8,9), 8.58 (m, 1H, H-10). IR ($CHCl_3$): 3460 and 3310 (free and associated NH), 1688 s, br (CO), 1642 w, 1612 s, 1564 w, 1528, 1504 w cm^{-1}. *Anal.* Calc. for $C_{16}H_{13}NO_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.16; H, 5.21; N, 5.63%.

*5.1.8.17. 4-(Cyclohexylamino)-2H-naphtho[1,2-b]pyran-2-one (**7b**).* Pure **7b** was obtained from **20** and cyclohexylamine, as described for **7a**. Yield: 0.41 g (70%); white crystals, m.p. 252.5–253 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 1.20–1.93 and 2.08–2.28 (2m, 8H+2H, cyclohexyl CH_2's), 3.46 (m, 1H, cyclohexyl CH), 5.16 (d, 1H, NH; disappeared with D_2O), 5.43 (s, 1H, H-3), 7.41 (d, $J_{6,5}=9$ Hz, 1H, H-6), 7.54–7.72 and 7.85 (2m, 3H+1H, H-5,7,8,9), 8.58 (m, 1H, H-10). IR ($CHCl_3$): 3455 and 3350 br,w (free and associated NH), 1693 sh and 1678 s (CO), 1642 sh, 1610 s, 1550 sh, 1536, 1505 sh cm^{-1}. *Anal.* Calc. for $C_{19}H_{19}NO_2$: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.65; H, 6.53; N, 4.87%.

*5.1.8.18. 4-(Phenylamino)-2H-naphtho[1,2-b]pyran-2-one (**7c**).* Obtained from **20** and aniline as described in general procedure. Yield: 0.48 g (84%); white crystals, m.p. 342–344 °C (pyridine). ^{1}H NMR (DMSO-d_6): δ 5.40 (s, 1H, H-3), 7.28–7.61, 7.77 and 8.10 (3m, 5H+2H+1H, H-7,8,9+phenyl H's), 7.96 (d, $J_{6,5}=9$ Hz, 1H, H-6), 8.30 (d, $J_{5,6}=9$ Hz, 1H, H-5), 8.42 (m, 1H, H-10), 9.48 (s, 1H, NH; disappeared with D_2O). IR ($CHCl_3$): 3275 br (NH), 1657 s, br (CO), 1607, 1589 s, 1523 s, br, 1500 sh cm^{-1}. *Anal.* Calc. for $C_{19}H_{13}NO_2$: C, 79.43; H, 4.56; N, 4.87. Found: C, 79.19; H, 4.57; N, 4.98%.

*5.1.8.19. 4-(N-Methyl-N-phenylamino)-2H-naphtho[1,2-b]pyran-2-one (**7d**).* The oily residue obtained from **20** and *N*-methylaniline according to general procedure was purified by column chromatography to recover pure **7d** (0.29 g, 48%); pale yellow crystals, m.p. 205–206 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 3.46 (s, 3H, CH_3), 6.02 (s, 1H, H-3), 6.98 (d, $J_{6,5}=9$ Hz, 1H, H-6), 7.08–7.41 and 7.55–7.77 (2m, 6H+3H, H-5,7,8,9+phenyl H's), 8.60 (m, 1H, H-10). IR ($CHCl_3$): 1712 sh and 1695 s, br (CO), 1634 w, 1598, 1555, 1497 cm^{-1}. *Anal.* Calc. for $C_{20}H_{15}NO_2$: C, 79.72; H, 5.02; N, 4.65. Found: C, 79.49; H, 5.10; N, 4.74%.

*5.1.8.20. 4-(Cyclohexylamino)-10-methyl-2H-naphtho[2,3-b]pyran-2-one (**8a**).* The reaction (2 h) of **21** [11] with cyclohexylamine afforded a solid residue which was taken up in a little acetone and filtered to give **8a** (0.43 g, 70%); whitish crystals, m.p. 251–252 °C (ethanol). ^{1}H NMR ($CDCl_3$): δ 1.20–1.94 and 2.08–2.28 (2m, 8H+2H, cyclohexyl CH_2's), 2.75 (s, 3H, 10-CH_3), 3.47 (m, 1H, cyclohexyl CH), 5.26 (d, 1H, NH; disappeared with D_2O), 5.44 (s, 1H, H-3), 7.47 and 7.61 (2m, 1H+1H, H-7,8), 7.83 (s, 1H, H-5), 7.90 and 8.05 (2m, 1H+1H, H-6,9). IR ($CHCl_3$): 3340 br (NH), 1692 sh and 1678 s, br (CO), 1633, 1616, 1570, 1526 br cm^{-1}. *Anal.* Calc. for $C_{20}H_{21}NO_2$: C, 78.15; H, 6.89; N, 4.56. Found: C, 77.89; H, 6.97; N, 4.65%.

*5.1.8.21. 10-Methyl-4-(phenylamino)-2H-naphtho[2,3-b]pyran-2-one (**8b**).* Obtained from **21** and aniline as described in general procedure. Yield: 0.47 g (78%); whitish crystals, m.p. 322–324 °C (pyridine). ^{1}H NMR (DMSO-d_6): δ 2.71 (s, 3H, 10-CH_3), 5.46 (s, 1H, H-3), 7.27–7.78 (m, 7H, H-7,8+phenyl H's), 8.04 and 8.18 (2m, 1H+1H, H-6,9), 8.80 (s, 1H, H-5), 9.50 (s, 1H, NH; disappeared with D_2O). IR ($CHCl_3$): 3305 br (NH), 1662 s, br (CO), 1626, 1612, 1588, 1563, 1530 br, 1496 cm^{-1}. *Anal.* Calc. for $C_{20}H_{15}NO_2$: C, 79.72; H, 5.02; N, 4.65. Found: C, 79.49; H, 5.00; N, 4.73%.

*5.1.8.22. 10-Methyl-4-(N-methyl-N-phenylamino)-2H-naphtho[2,3-b]pyran-2-one (**8c**).* The sticky residue obtained from **21** and *N*-methylaniline, according to general procedure, was purified by column chromatography to recover pure **8c** (0.20 g, 32%); pale yellow crystals, m.p. 227–228 °C (ethyl acetate–isopropyl ether). ^{1}H NMR ($CDCl_3$): δ 2.77 (s, 3H, 10-CH_3), 3.47 (s, 3H, N–CH_3), 6.00 (s, 1H, H-3), 7.07–7.59 and 7.98 (2m, 9H+1H, H-5,6,7,8,9+phenyl H's). IR

($CHCl_3$): 1690 s, br (CO), 1624, 1604, 1582 w, 1556, 1492 cm^{-1}. *Anal.* Calc. for $C_{21}H_{17}NO_2$: C, 79.98; H, 5.43; N, 4.44. Found: C, 80.05; H, 5.41; N, 4.57%.

5.2. Biology

5.2.1. Chemicals

Test compounds were dissolved in DMSO (4.5 mmol l^{-1}) and the solutions were stored frozen in plastic tubes. Just before the experiments were performed, a calculated amount of the compound solution was added to PBS or to the growth medium containing cells to a final solvent concentration of 0.5%. [^{3}H]-thymidine (4.77 TBq $mmol^{-1}$), [^{3}H]-uridine (1.1 TBq $mmol^{-1}$) and [^{3}H]-leucine (2.37 TBq $mmol^{-1}$) were obtained from Amersham International (UK).

5.2.2. Macromolecular synthesis in Ehrlich cells

Ehrlich ascites tumor cells (Lettrè strain from Heidelberg) were routinely transferred by injecting intraperitoneally 2×10^6 cells per animal into NCL mice. For the experiments, samples of 2×10^7 cells/ml, collected on days 6–7 after transplant, suspended in Hank's solution containing the compound to be studied were incubated at 37 °C for 60 min; then 40 kBq ml^{-1} of [^{3}H]-thymidine, [^{3}H]-uridine or [^{3}H]-leucine (for DNA, RNA or protein synthesis, respectively), in a small volume of the same medium, were added and the cells were further incubated at 37 °C for 30 min. The acid insoluble fraction was then precipitated by adding 5% ice-cold trichloroacetic acid and filtered through Whatman GF/C filters. After several washings with cold 1% trichloroacetic acid, the filters were dried and counted. The results were calculated as the percentage of radioactivity incorporated into untreated control cells (about 3–6 MBq); filtrations were carried out on a Sample Manifold apparatus (Millipore, Bedford, MA, USA). The radioactivity measurements were performed by dipping the dried filters into 5 ml of a toluene based scintillator (PPO 5 g, dimethyl-POPOP 0.25 g, toluene up to 1 l of solution). Counting was accomplished by a Packard Tri-Carb 1900TR spectrometer.

5.2.3. HeLa cell cultures

HeLa cells (kindly provided by Professor F. Majone, Department of Biology, Padua University, Italy) were grown as monolayers in nutrient mixture F12 Ham medium (Sigma) supplemented with 10% foetal calf serum (Biological Industries, Kibbutz Beth Haemek, Israel) and the antibiotics penicillin (50 units ml^{-1}) and streptomycin (50 μg ml^{-1}) [12]. Trypsin (0.25% Boehringer, Mannheim) was routinely used for subculture.

5.2.4. MTT test

Cytotoxicity was evaluated by means of MTT (tetrazolium salts reduction) test [23]. Briefly, HeLa cells (5×10^4 cells ml^{-1}) were seeded in 96-well microplates in growth medium (100 μl) and then incubated at 37 °C in a 10% carbon dioxide atmosphere. After 24 h, the medium was removed and replaced with a fresh one containing the compound to be studied at the appropriate concentrations. Quadruplicate cultures were established for each treatment. After other 24 h, each well was treated with 10 μl of a 5 mg ml^{-1} MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) saline solution, and after 5 h incubation 100 μl of a SDS (sodium dodecylsulfate) solution in HCl 0.1 M were added. After an overnight incubation, the inhibition of cell growth by the various complexes was monitored using a Camberra-Packard spectrophotometer at 570 nm.

5.2.5. Calculations

The data related to macromolecular synthesis and MTT test were elaborated using probit analysis, thus obtaining the IC_{50}, i.e. the compound concentration (μmol/l) which induces a 50% inhibition of the biological function examined. All the biological experiments were carried out at least in triplicate.

Acknowledgements

The authors wish to thank R. Raggio, C. Rossi, F. Tuberoni for spectra recording, and A. Panaro for elemental analyses. Financial support from Consiglio Nazionale delle Ricerche, Rome, Italy, is gratefully acknowledged.

References

[1] F. Bordin, F. Dall'Acqua, A. Guiotto, Angelicins, angular analogs of psoralens: chemistry, photochemistry, photobiological and phototerapeutic properties, Pharm. Ther. 52 (1991) 331–363.
[2] D. Vedaldi, S. Caffieri, F. Dall'Acqua, L. Andreassi, L. Bovalini, P. Martelli, Khellin, naturally occurring furochromone, used for the photochemotherapy of skin diseases: mechanism of action, Farmaco 43 (1988) 333–346.
[3] P. Bonin, J.P. Singh, R. Gammill, L. Erickson, Inhibition of fibroblast and smooth muscle cell proliferation and migration in vitro by a novel aminochromone U-67154, J. Vasc. Res. 30 (1993) 108–115.
[4] L. Erickson, et al., In vitro and in vivo inhibition of rat vascular smooth muscle cell migration and proliferation by a 2-aminochromone U-86983, J. Pharmacol. Exp. Ther. 271 (1994) 415–421.
[5] C. Vlahos, W. Matter, K. Hui, R. Brown, A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one (LY294002), J. Biol. Chem. 269 (1994) 5241–5248.
[6] A. Ermili, M. Mazzei, G. Roma, C. Cacciatore, Ricerche chimiche e farmacologiche su derivati piranici. Nota VIII. Sintesi di 2-dialchilammino-7-metossicromoni e derivati, Farmaco Ed. Sci. 32 (1977) 375–387.

[7] A. Ermili, A. Balbi, M. Di Braccio, G. Roma, Ricerche chimiche e farmacologiche su derivati piranici. Nota IX. Sintesi di 2-dialchilamminocromoni, Farmaco Ed. Sci. 32 (1977) 713–724.
[8] A. Ermili, G. Roma, La reazione di Vilsmeier-Haack applicata alle *N*,*N*-dialchiletossicarbonilacetammidi. Nota I. Sintesi del 1-oxo-3-dietilammino-1*H*-nafto[2,1-*b*]pirano, Gazz. Chim. Ital. 101 (1971) 269–280.
[9] A. Ermili, G. Roma, A. Balbi, La reazione di Vilsmeier-Haack applicata alle *N*,*N*-dialchiletossicarbonilacetammidi. Nota II. Sintesi di 1-oxo-3-dialchilammino-1*H*-nafto[2,1-*b*]pirani, Gazz. Chim. Ital. 101 (1971) 651–660.
[10] A. Ermili, A. Balbi, G. Roma, A. Ambrosini, N. Passerini, Ricerche chimiche e farmacologiche su derivati piranici. Nota VI. 2-Dialchilammino-4-oxo-4*H*-nafto[1,2-*b*]pirani e derivati, Farmaco Ed. Sci. 31 (1976) 627–648.
[11] G. Roma, E. Vigevani, M. Mazzei, A. Ermili, A. Ambrosini, N. Passerini, Ricerche chimiche e farmacologiche su derivati piranici. Nota XI. Sintesi di 2-dialchilammino-4-oxo-10-metil-4*H*-nafto[2,3-*b*]pirani, Farmaco Ed. Sci. 33 (1978) 822–837.
[12] G. Roma, M. Di Braccio, G.C. Grossi, C. Marzano, M. Simonato, F. Bordin, Pyran derivatives XX. 2-Aminochromone benzo-fused derivatives with antiproliferative properties, Farmaco 53 (1998) 494–503.
[13] K. Fukui, M. Nakayama, Synthetic studies of sesamol derivatives. IV. Preparation and reaction of 2-hydroxy-4,5-methylenedioxyacetophenones, Bull. Chem. Soc. Japan 37 (1964) 300–302.
[14] M. Di Braccio, G. Roma, G. Leoncini, M. Poggi, Pyran derivatives XIX. (Dialkylamino)substituted 1-benzopyranones and naphthopyranones with platelet antiaggregating activity, Farmaco 50 (1995) 703–711.
[15] J.R. Bantick, J.L. Suschitzky, Synthesis of 2-aminochromones. Studies on the nucleophilic displacement of sulphinyl and sulphonyl groups in the 2-position of 5,8-dimethoxychromone, J. Heterocyclic Chem. 18 (1981) 679–684.
[16] L. Lafon, Acetamide derivatives, U.S. Patent 4,177,290, 1979; Chem. Abstr. 93 (1980), 7872q.
[17] C.M. Bonnin, P.A. Cadby, C.G. Freeman, A.D. Ward, Acetylenic acids. IV. The reactions of alkoxy-substituted phenylpropiolic acids and esters with sulfur halides, particularly thionyl chloride, Aust. J. Chem. 32 (1979) 833–847.
[18] M. Mazzei, A. Balbi, A. Ermili, E. Sottofattori, G. Roma, P. Schiantarelli, S. Cadel, Ricerche Chimiche e farmacologiche su derivati piranici. Nota XVI. Derivati del 2-(dialchilammino)-7-metossicromone ad attività antiallergica, Farmaco Ed. Sci. 40 (1985) 895–908.
[19] S.S. Soman, K.N. Trivedi, A synthesis of ayapin, Indian J. Chem. 32B (1993) 372–373.
[20] M. Darbarwar, V. Sundaramurthy, N.V. Subba Rao, Synthesis of amino-, halo-, methyl- & benz-11,12-oxygenated-coumestans, Indian J. Chem. 11 (1973) 115–117.
[21] D.R. Buckle, B.C.C. Cantello, H. Smith, B.A. Spicer, Antiallergic activity of 4-hydroxy-3-nitrocoumarins, J. Med. Chem. 18 (1975) 391–394.
[22] A. Mustafa, O.H. Hishmat, M.I. Ali, M.E. Wassef, E.M. Khalil, A.K.M. Gohar, Reaktionem mit substituierten 4-Hydroxy-cumarinen und Furocumarinen, Justus Liebigs Ann. Chem. 712 (1968) 107–115.
[23] D.T. Vistica, P. Skehan, D.A. Scudiero, A. Monks, A. Pittman, M.R. Boyd, Tetrazolium-based assays for cellular viability: a critical examination of selected parameters affecting formazan production, Cancer Res. 51 (1991) 2515–2520.
[24] J. Carmichael, W.G. De Graff, A.F. Gazdar, J.D. Minna, J.B. Mitchell, Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing, Cancer Res. 47 (1987) 936–942.