Eur J Med Chem (1997) 32, 71–82

Short communication

Synthesis and in vitro cytotoxicity of a series of 3-aminoflavones

D Dauzonne[1]*, B Folléas[1], L Martinez[1], GG Chabot[2]

[1]*Laboratoire de chimie associé au CNRS (UMR 176), Institut Curie, Section de recherche, 26, rue d'Ulm, 75231 Paris cedex 05;*
[2]*Laboratoire de pharmacologie clinique associé au CNRS (URA 147), Institut Gustave-Roussy, 94805 Villejuif cedex, France*

(Received 29 March 1996; accepted 16 July 1996)

Summary — To further understand the molecular requirements for the antiproliferative activity of flavonoids, a series of 3-aminoflavones and some of their derivatives were prepared and evaluated using L1210 murine leukemia. Our novel five-step synthetic approach required easily available substituted aromatic aldehydes as starting materials and proved more convenient and more general than previously reported methods. Our results in the 3-aminoflavones series indicated that the 4'-methoxy group is important for cytotoxic activity. Moreover, for the flavone-8-acetic analogs, a marked increase in potency was observed with the addition of a 3-amino or a 3-nitro group. Methoxy groups on the 6 and 7 positions of flavonoids (as in cirsiliol) also appear to be important for antiproliferative activity. These results are essential for the further understanding of the critical molecular requirements that lead to antiproliferative properties in the flavonoid series.

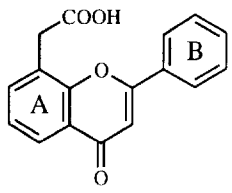
3-aminoflavone / 3-nitroflavone / flavone-8-acetic acid analog / L1210 / antiproliferative activity

Introduction

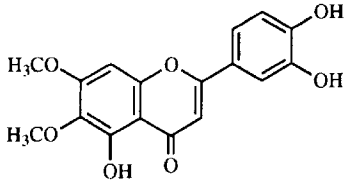
During the last decades, flavonoids have been extensively studied for their medicinal applications [1–3]. Among their various biological properties, antitumor activities and antiproliferative effects have aroused considerable attention [4–9]. In this regard, several flavonoids bearing amino groups on the A or B ring have been reported to be potential antineoplastic agents [10–15]. It is now well established that such a potency is mainly due to the ability of these aminoflavones to be competitive inhibitors of certain protein-tyrosine kinases with respect to ATP [13–15]. However, very little is known about the biological evaluation of flavonoids bearing an amino group in the 3 position since, to our knowledge, only one report deals with the 3-aminoflavone itself and its ability to inhibit cyclic AMP phosphodiesterase [16].

In this context, we became interested in synthesizing and evaluating the cytotoxicity of a series of 3-aminoflavones with a special interest for the 3-amino derivative of the 3'-amino-4'-methoxyflavone because the 3-unsubstituted compound proved to be one of the more cytotoxic compounds in the large series of flavones recently studied by Cunningham and coworkers [14]. In addition, we also synthesized some [(dialkylamino)alkyl]amino derivatives (since the favorable influence on antitumoral activity of the presence of such a side chain was demonstrated in several series of molecules) and we prepared the 3-amino derivative of flavone-8-acetic acid **1**, which is a very potent anticancer agent in murine models [17–21].

The antiproliferative activity of the newly synthesized compounds was assessed using the L1210 murine leukemia cell line and compared to the reference compounds flavone-8-acetic acid **1** and cirsiliol **2**, a polysubstituted flavone known to exhibit pronounced antiproliferative effects [22, 23].



Flavone-8-acetic acid (**1**)



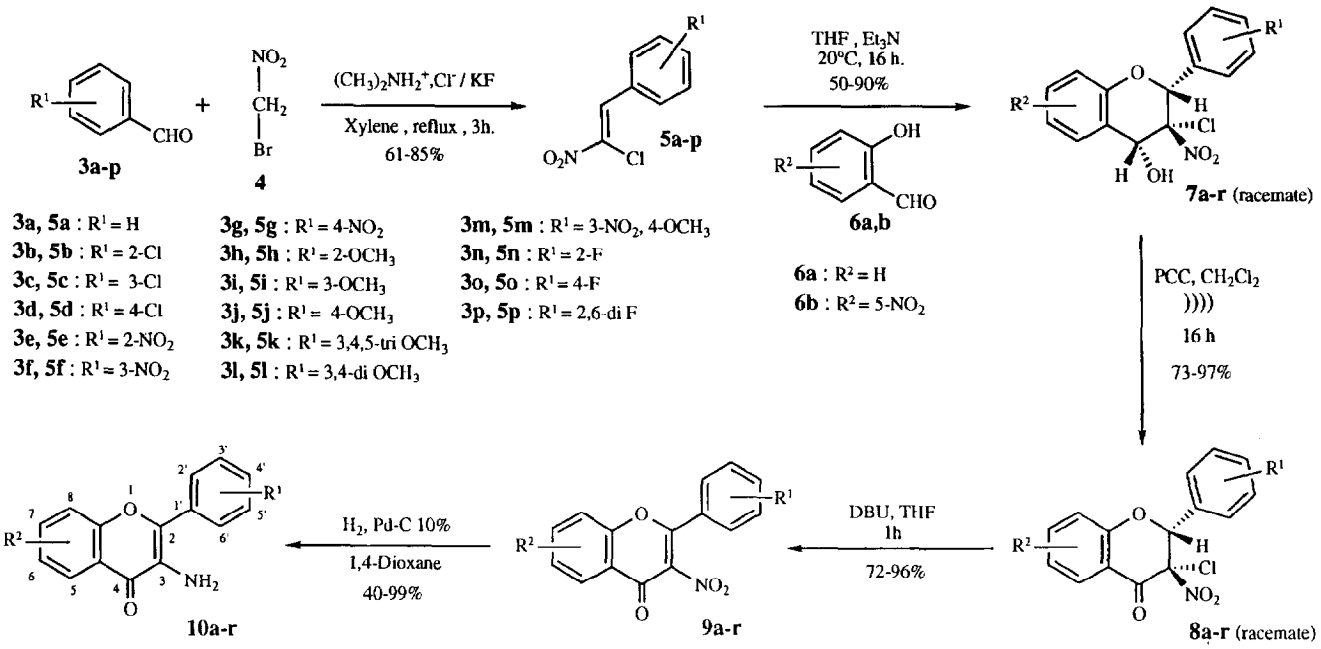
Cirsiliol (**2**)

*Correspondence and reprints.

Chemistry

With regard to chemistry, our approach requires readily available substituted aromatic aldehydes as starting materials and proved to be more versatile and more convenient than the previously reported procedures starting from not easily accessible 3-substituted flavanones often obtained from complex chalcones [24–32].

The general method for the synthesis of the desired 3-aminoflavones **10a–r** is outlined in scheme 1. Some of the required intermediate 3-nitro-2-phenyl-4*H*-1-benzopyrans (**9a–k**) and of their respective precursors (the 3-chloro-2,3-dihydro-3-nitro-2-phenyl-4*H*-1-benzopyran-4-ones **8a–k** and the 3-chloro-3,4-dihydro-4-hydroxy-3-nitro-2-phenyl-2*H*-1-benzopyrans **7a–k**) have been described in our previous communications [33, 34]. The novel 3-nitroflavones **9l–r** were especially prepared for the present work by adapting the same procedure starting from the appropriate benzaldehydes **3l–p** via a condensation with bromonitromethane **4**. The obtained (2-chloro-2-nitroethenyl)-benzenes **5l–p** were further reacted with salicylaldehydes **6a** or **6b** to provide the substituted dihydrobenzopyrans **7l–r**. Subsequent oxidation of these compounds by pyridinium chlorochromate (PCC) gave the oxo derivatives **8l–r** which were converted into the desired 3-nitroflavones **9l–r** upon basic treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The new 3-aminoflavones **10a–r** were afterwards easily obtained in good yields by catalytically reducing the corresponding nitro derivatives in the presence of palladium on activated carbon (10%) in dioxane. With regard to the preparation of the halogeno compounds **10b–d**, it is essential to add concentrated aqueous hydrochloric acid to the reaction



3a, 5a : R^1 = H
3b, 5b : R^1 = 2-Cl
3c, 5c : R^1 = 3-Cl
3d, 5d : R^1 = 4-Cl
3e, 5e : R^1 = 2-NO_2
3f, 5f : R^1 = 3-NO_2
3g, 5g : R^1 = 4-NO_2
3h, 5h : R^1 = 2-OCH_3
3i, 5i : R^1 = 3-OCH_3
3j, 5j : R^1 = 4-OCH_3
3k, 5k : R^1 = 3,4,5-tri OCH_3
3l, 5l : R^1 = 3,4-di OCH_3
3m, 5m : R^1 = 3-NO_2, 4-OCH_3
3n, 5n : R^1 = 2-F
3o, 5o : R^1 = 4-F
3p, 5p : R^1 = 2,6-di F

6a : R^2 = H
6b : R^2 = 5-NO_2

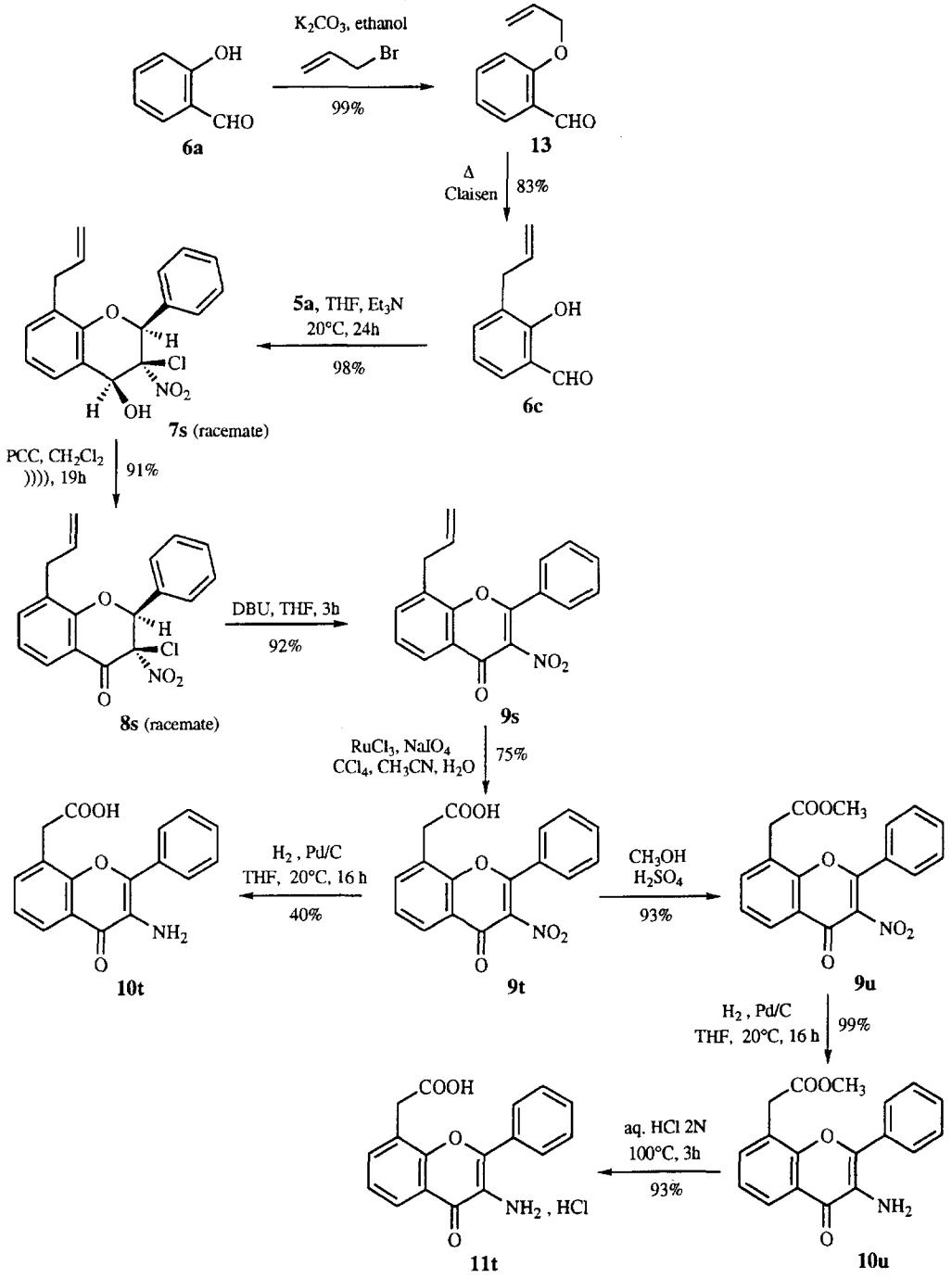
10a : R^1 = H, R^2 = H
10b : R^1 = 2'-Cl, R^2 = H
10c : R^1 = 3'-Cl, R^2 = H
10d : R^1 = 4'-Cl, R^2 = H
10e : R^1 = 2'-NH_2, R^2 = H
10f : R^1 = 3'-NH_2, R^2 = H
10g : R^1 = 4'-NH_2, R^2 = H
10h : R^1 = 2'-OCH_3, R^2 = H
10i : R^1 = 3'-OCH_3, R^2 = H
10j : R^1 = 4'-OCH_3, R^2 = H
10k : R^1 = 3',4',5'-tri OCH_3, R^2 = H
10l : R^1 = 3',4'-di OCH_3, R^2 = H
10m : R^1 = 3'-NH_2, 4'-OCH_3, R^2 = H
10n : R^1 = 2'-F, R^2 = H
10o : R^1 = 4'-F, R^2 = H
10p : R^1 = 2',6'-di F, R^2 = H
10q : R^1 = H, R^2 = 6-NH_2
10r : R^1 = 4'-NH_2, R^2 = 6-NH_2

7a-9a : R^1 = H, R^2 = H
7b-9b : R^1 = 2'-Cl, R^2 = H
7c-9c : R^1 = 3'-Cl, R^2 = H
7d-9d : R^1 = 4'-Cl, R^2 = H
7e-9e : R^1 = 2'-NO_2, R^2 = H
7f-9f : R^1 = 3'-NO_2, R^2 = H
7g-9g : R^1 = 4'-NO_2, R^2 = H
7h-9h : R^1 = 2'-OCH_3, R^2 = H
7i-9i : R^1 = 3'-OCH_3, R^2 = H
7j-9j : R^1 = 4'-OCH_3, R^2 = H
7k-9k : R^1 = 3',4',5'-tri OCH_3, R^2 = H
7l-9l : R^1 = 3',4'-di OCH_3, R^2 = H
7m-9m : R^1 = 3'-NO_2, 4'-OCH_3, R^2 = H
7n-9n : R^1 = 2'-F, R^2 = H
7o-9o : R^1 = 4'-F, R^2 = H
7p-9p : R^1 = 2',6'-di F, R^2 = H
7q-9q : R^1 = H, R^2 = 6-NO_2
7r-9r : R^1 = 4'-NO_2, R^2 = 6-NO_2

Scheme 1.

medium before starting the hydrogenation. In this way, the catalyzed replacement of the halogen by a hydrogen atom on the aromatic ring was avoided and good yields of the desired aminochloroflavones **10b–d** were obtained.

The slightly different procedure which has been developed to provide access to 3-aminoflavone-8-acetic acid **10t** is summarized in scheme 2 and has been the subject of a preliminary communication [35]. However, it has recently been possible to obtain this amino acid as its hydrochloride **11t** with a significantly improved overall yield by performing the reduction step on the methyl ester **9u** instead of the 3-nitroflavone-8-acetic acid **9t** itself. In this instance,



Scheme 2.

the aminoester **10u** was hydrolyzed in aqueous hydrochloric medium to provide **11t** almost quantitatively.

The dialkylamino derivatives **12j** and **12l** were obtained, as depicted in scheme 3, by carrying out ultrasound-assisted reactions of the corresponding aminoflavones **10j** or **10l** with 2-diethylaminoethyl chloride in the presence of sodium hydride.

Biological results and discussion

The antiproliferative activity of some of the compounds synthesized above were assessed using the murine L1210 leukemia cells and compared with the reference compounds flavone-8-acetic acid **1** and cirsiliol **2** (table I).

The 3-nitro synthesis precursors tested (**7j**, **8j**, **9t**) exhibited potent antiproliferative properties (ie, ≤ 10 μM), whereas compound **9j** was less active.

Among the 3-amino derivatives (**10a–r** and **10t**), the most potent compounds were those bearing substituent(s) in the 3'- and/or the 4'-position (table I). Compounds **10j** (4'-OCH_3) and **10m** (3'-NH_2, 4'-OCH_3) had the lowest IC_{50} of this series. The presence of more than one methoxy substituent on the B phenyl ring appeared to decrease activity (eg, **10k**, **10l** compared to **10j**), whereas the addition of a 3'-NH_2 with a 4'-OCH_3 doubled the activity (compare **10m** to **10j**).

The substitution of the 3-amino by a dialkylamino group (**12j**, **12l**) led to a similar activity compared to the corresponding 3-amino compounds (**10j**, **10l**).

Compared to flavone-8-acetic acid (**1**, $IC_{50} = 187$ μM), its 3-amino derivative **10t** had a 4.5-fold increase in potency ($IC_{50} = 41$ μM) whereas its 3-nitro derivative **9t** had a 20-fold increase in antiproliferative potency ($IC_{50} = 9$ μM) (table I).

Substitution on the A ring at the 6-position with an amino group did not appear to improve potency (eg, **10q**, **10r**), although substitution with methoxy or hydroxy groups at the 5-, 6- and 7-positions, as in cirsiliol **2**, led to a marked increase in antiproliferative potency.

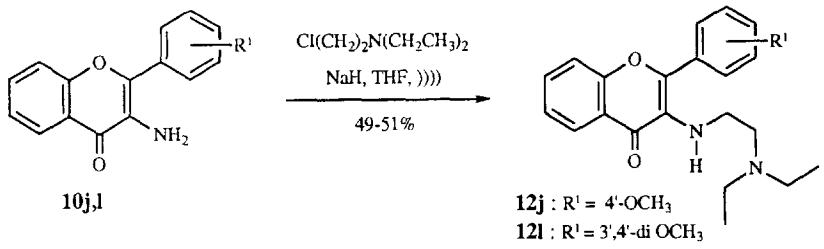
In conclusion, these results indicate that, in the 3-aminoflavone series, the 4'-methoxy group is important for activity. Moreover, in the flavone-8-acetic analogs, a marked increase in potency is observed with the addition of a 3-amino group or a 3-nitro group. Methoxy groups on the 6- and 7-positions of flavonoids also appear to play a non-negligible part in the antiproliferative potency. These results help to further understand the critical molecular requirements that lead to antiproliferative properties in the flavonoid series.

Taking into account the above observations, the synthesis and the biological evaluation of novel substituted 3-aminoflavone-8-acetic acids are currently in progress.

Experimental protocols

Chemistry

Melting points were measured on a Köfler hot stage apparatus and are uncorrected. Mass spectra were obtained with a Nermag-Ribermag R10-10C spectrometer using either electron impact method (EI) at 70 eV or applying a desorption chemical ionization technique (CI) using ammonia as the reagent gas. Infrared spectra were registered with a Perkin-Elmer 1710 spectrophotometer either as chloroform solutions or KBr discs. The ^{1}H-NMR spectra (90, 250 or 300 MHz) were recorded on a Varian EM390, a Bruker AC 250 or a Bruker AC 300 spectrometer. Chemical shifts are expressed as parts per million downfield from tetramethylsilane. Splitting patterns have been designated as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), ddd (doublet of doublets of doublets), dt (doublet of triplets), m (multiplet), br (broad signal), sh (shoulder). Coupling constants (*J* values) are listed in hertz (Hz). Microanalyses were carried out by the Service Central d'Analyse du CNRS, Vernaison, France, and analytical values are within ± 0.3% of the calculated compositions. Reactions were monitored by analytical thin-layer chromatography performed on Merck 60F254 precoated plates and products were visualized by UV light. Silica gel Merck (230-400 Mesh ASTM) was used for column chromatography. A Bransonic 220 apparatus (50 kHz) was used as the ultrasonic generator. Anhydrous tetrahydrofuran was obtained by distillation from benzophenone/sodium. Water-free dichloromethane was prepared by distillation from diphosphorus pentoxide. Triethylamine was purified by distillation from calcium hydride



10j,l

12j : R^1 = 4'-OCH_3
12l : R^1 = 3',4'-di OCH_3

Scheme 3.

Table I. Antiproliferative activity (L1210 leukemia) of 3-aminoflavones, some derivatives and some synthetic precursors, compared with reference compounds flavone-8-acetic acid **1** and cirsiliol **2**.

Compound	R^1	R^2	$IC_{50}(\mu M)$
7j	4'-OCH_3	H	4
8j	4'-OCH_3	H	9
9j	4'-OCH_3	H	>17
9t	H	8-CH_2COOH	9
10a	H	H	63
10b	2'-Cl	H	99
10c	3'-Cl	H	46
10d	4'-Cl	H	61
10e	2'-NH_2	H	163
10f	3'-NH_2	H	87
10g	4'-NH_2	H	40
10h	2'-OCH_3	H	94
10i	3'-OCH_3	H	60
10j	4'-OCH_3	H	22
10k	3',4',5'-OCH_3	H	>31
10l	3',4'-OCH_3	H	74
10m	3'-NH_2, 4'-OCH_3	H	10
10n	2'F	H	67
10o	4'F	H	>20
10p	2',6'-F	H	77
10q	H	6-NH_2	107
10r	4'-NH_2	6-NH_2	49
10t	H	8-CH_2COOH	41
12j	4'-OCH_3	H	30
12l	3',4'-OCH_3	H	58
8-FAA **1**			187
Cirsiliol **2**			3

IC_{50} is the concentration that inhibits cell growth by 50% of control cells (mean of duplicate experiments).

prior to use. Bromonitromethane (**4**, bp 149–150 °C/749 mmHg) was prepared on a multimolar scale following a previously described method [36]. The 2-chloro-*N,N*-diethylethylamine was liberated from its hydrochloride according to an already published procedure [37]. Flavone-8-acetic acid **1** and cirsiliol **2** were kindly provided by P Briet (Lipha, Lyon, France). Most of the starting substituted benzaldehydes **3** and **6** were purchased from Acros Organics or from Aldrich Chemical Co and were used without further purification. Compounds **3m** and **6c** are not commercially available and have been prepared according to previously reported methods.

*4-Methoxy-3-nitrobenzaldehyde **3m***
This product was prepared by nitration of the 4-methoxybenzaldehyde (85 g, 76 mL, 0.624 mol) following the procedure described by Einhorn and Grabfield [38]. The obtained crude product was further recrystallized (heptane/isopropanol) to give pure **3m** (91.6 g, 81%), mp 85–86 °C (lit [38] 83.5 °C). ^{1}H-NMR (90 MHz; $CDCl_3$) δ 4.10 (s, 3H), 7.20 (d, 1H, *J* = 9.0 Hz), 8.10 (dd, 1H, *J* = 9.0 Hz and 2.2 Hz), 8.37 (d, 1H, *J* = 2.2 Hz), 9.96 (s, 1H); anal $C_8H_7NO_4$ (C, H, N).

*2-Hydroxy-3-(prop-2-enyl)benzaldehyde **6c***
This compound was synthesized on a large scale according to the method reported by Claisen and Eisleb [39] starting from the salicylaldehyde **6a** (244 g, 2 mol) and allyl bromide (242 g, 2 mol) via the formation of the 2-allyloxybenzaldehyde **13**. Distillation of the crude product at bp 118–122 °C/16 mmHg (lit [39] 245.5–246 °C/755 mmHg) provided pure **6c** (269 g, 83% overall) as a pale yellow oil. ^{1}H-NMR (300 MHz; $CDCl_3$) δ 3.40 (d, 2H, *J* = 6.0 Hz), 5.13 (m, 2H), 5.80–6.32 (m, 1H), 6.82–7.31 (m, 2H), 7.50 (d, 1H, *J* = 7.5 Hz), 9.86 (s, 1H), 11.35 (s, 1H exchangeable with D_2O). IR ($CDCl_3$) ν 1650 cm^{-1}; anal $C_{10}H_{10}O_2$ (C, H).

*Preparation of Z-(2-chloro-2-nitroethenyl)benzenes **5a–p**. General procedure*
Large quantities of starting β-chloro-β-nitrostyrenes **5a–p** were prepared by scaling up the syntheses previously described for **5a–k** [31]. The appropriate benzaldehyde **3a–p** (0.3 mol, all of which are commercially available except **3m** which has been prepared as described above), xylene (750 mL), dimethylammonium chloride (220.3 g, 2.7 mol), bromonitromethane (**4**, 78.4 g, 39.12 mL, 0.56 mol) and potassium fluoride (2.61 g, 45 mmol) were placed in a 2 L conical flask equipped with a Dean–Stark water trap (capacity about 20 mL). The mixture was vigorously refluxed with stirring for 3 h, then allowed to cool to room temperature. Removal of the volatile materials under reduced pressure left a residue which was taken up with water (300 mL) and dichloromethane (800 mL). The organic layer was separated and the aqueous phase was extracted with dichloromethane (3 x 200 mL). The combined organic extracts were dried (magnesium sulfate), filtered, then evaporated in vacuo to afford a crude product which was chromatographed on a silica-gel column (950 g, 200–400 mesh, eluent dichloromethane). Evaporation of the solvent followed by recrystallization gave pure (2-chloro-2-nitroethenyl)benzenes **5a–p** exclusively in the *Z* form. Compounds **5a–k** have been described in a previous communication [33]. Yields, recrystallization solvents, physical constants and spectral data for hitherto unknown derivatives **5l–p** are reported below.

*Z-(2-Chloro-2-nitroethenyl)-3,4-dimethoxybenzene **5l**.* Yield 80%; mp 113–114 °C (heptane); ^{1}H-NMR (90 MHz; $CDCl_3$) δ 3.95 (s, 3H), 3.97 (s, 3H), 6.97 (d, 1H, *J* = 9.0 Hz), 7.40–7.57 (m, 2H), 8.37 (s, 1H); IR ($CDCl_3$) ν 1596, 1535, 1308 cm^{-1}; MS (EI) *m/z* 243, 245 (M$^+$); anal $C_{10}H_{10}ClNO_4$ (C, H, N).

*Z-(2-Chloro-2-nitroethenyl)-4-methoxy-3-nitrobenzene **5m**.* Yield 60%; mp 126–128 °C (benzene/cyclohexane); ^{1}H-NMR (90 MHz; $CDCl_3$) δ 4.07 (s, 3H), 7.23 (d, 1H, *J* = 9.0 Hz), 8.05 (dd, 1H, *J* = 9.0 Hz and 3.0 Hz), 8.35 (s, 1H), 8.73 (d, 1H, *J* = 3.0 Hz); IR ($CDCl_3$), ν 1625, 1540, 1330 cm^{-1}; MS (EI) *m/z* 258, 260 (M$^+$); anal $C_9H_7ClN_2O_5$ (C, H, N).

*Z-(2-Chloro-2-nitroethenyl)-2-fluorobenzene **5n**.* Yield 74%; mp 75–76 °C (hexane); ^{1}H-NMR (90 MHz; $CDCl_3$) δ 7.07–7.73 (m, 3H), 8.10 (dt, 1H, *J* = 7.5 Hz and 1.8 Hz), 8.60 (s, 1H); IR ($CDCl_3$), ν 1626, 1544, 1318 cm^{-1}; MS (EI) *m/z* 201, 203 (M$^+$); anal $C_8H_5ClFNO_2$ (C, H, N).

*Z-(2-Chloro-2-nitroethenyl)-4-fluorobenzene **5o**.* Yield 77%; mp 76–77 °C (hexane); ^{1}H-NMR (90 MHz; $CDCl_3$) δ 7.17 (t, 2H, *J* = 8.5 Hz), 7.77–8.02 (m, 2H), 8.37 (s, 1H); IR ($CDCl_3$), ν 1625, 1537, 1318 cm^{-1}; MS (EI) *m/z* 201, 203 (M$^+$); anal $C_8H_5ClFNO_2$ (C, H, N).

*Z-(2-Chloro-2-nitroethenyl)-2,6-difluorobenzene **5p**.* Yield 64%; mp 49–50 °C (pentane); ^{1}H-NMR (90 MHz; $CDCl_3$) δ 7.17 (br t, 2H, *J* = 8.1 Hz), 7.30–7.67 (m, 1H), 8.23 (s, 1H); IR ($CDCl_3$), ν 1637, 1555, 1323 cm^{-1}; MS (EI) *m/z* 219, 221 (M$^+$); anal $C_8H_4ClF_2NO_2$ (C, H, N).

*Preparation of 2-aryl-3-chloro-3,4-dihydro-4-hydroxy-3-nitro-2H-1-benzopyrans **7a–s**. General procedure*
Multigram quantities of 2-aryl-3-chloro-3,4-dihydro-4-hydroxy-3-nitro-2*H*-1-benzopyrans **7a–s** were prepared by scaling up the syntheses previously described for **7a–k** [33]. In a dry 1 L flask fitted with a drying tube, the appropriate starting *Z*-(2-chloro-2-nitroethenyl)benzene **5a–p** (0.1 mol) and salicylaldehyde **6a–c** (0.56 mol) were dissolved in a minimal volume of anhydrous tetrahydrofuran (about 100 mL in most cases, 300 mL when **6b** was involved in the reaction). Freshly distilled triethylamine (5.45 g, 7.5 mL, 0.054 mol) was quickly added via a syringe and the mixture was stirred at room temperature for 24 h (48 h in the cases of **7p**, **7q** and **7r**). Acetic acid (about 1 mL) was then added and the volatile materials were rotary-evaporated in vacuo (0.6 mmHg) at 45–50 °C (excessive heating and moisture must be avoided). The crude residue was taken up with a tepid solution (50 °C) of Girard's reagent T (prepared by dissolving the commercial reagent (94 g, 0.56 mol) in a mixture ethanol/acetic acid/water 8:1:1 (700 mL)). This mixture was efficiently stirred for 2 h at room temperature, then diluted with dichloromethane (600 mL) and water (500 mL). When an insoluble material remained, it was filtered on a sintered funnel and meticulously rinsed with dichloromethane. The organic layer was separated and the aqueous phase was extracted with dichloromethane (3 x 300 mL). The combined organic extracts were dried (magnesium sulfate), filtered then concentrated under reduced pressure to leave an aldehyde-free material. Chromatography of the crude product on silica gel (600 g, eluent dichloromethane) provided the desired derivatives **7a–s** which, after evaporation of the solvent, were recrystallized in appropriate conditions. These compounds are exclusively obtained in the relative configuration 2*R*, 3*R*, 4*R*. The benzopyrans **7a–k** have been described in a former communication [33]. Yields, recrystallization solvents, physical constants and spectral data for novel derivatives **7l–s** are reported below.

The excess of unreacted salicylaldehydes **6a–c** were almost quantitatively recovered from the hydrazone derivatives (formed with Girard's reagent) after acidic hydrolysis and subsequent extraction.

3-Chloro-3,4-dihydro-2-(3,4-dimethoxyphenyl)-4-hydroxy-3-nitro-2H-1-benzopyran ***7l***. Yield 65%; mp 174–175 °C (benzene/heptane); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 2.85 (d, 1H, *J* = 12.1 Hz, exchangeable with D_2O), 3.90 (s, 3H), 3.91 (s, 3H), 5.73 (s, 1H), 6.03 (d, 1H, *J* = 12.1 Hz), 6.87 (d, 1H, *J* = 8.3 Hz), 6.99 (dd, 1H, *J* = 8.3 Hz and 1.9 Hz), 7.02–7.08 (m, 1H), 7.14 (ddd, 1H, *J* = 8.0 Hz, 7.1 Hz and 1.2 Hz), 7.31–7.40 (m, 2H), 7.59 (dd, 1H, *J* = 8.0 Hz and 1.2 Hz); IR ($CDCl_3$), ν 3558, 1570, 1520, 1346 cm^{-1}; MS (EI) *m/z* 365, 367 (M^+); anal $C_{17}H_{16}ClNO_6$ (C, H, N).

3-Chloro-3,4-dihydro-4-hydroxy-2-(4-methoxy-3-nitrophenyl)-3-nitro-2H-1-benzopyran ***7m***. Yield 76%; mp 159–160 °C (benzene/heptane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 2.52 (d, 1H exchangeable with D_2O, *J* = 12.1 Hz), 4.00 (s, 3H), 5.81 (s, 1H), 6.01 (d, 1H, *J* = 12.1 Hz), 6.95–7.55 (m, 6H), 8.10 (d, 1H, *J* = 2.2 Hz); IR ($CDCl_3$), ν 3560, 1571, 1535, 1360, 1346 cm^{-1}; MS (EI) *m/z* 380, 382 (M^+); anal $C_{16}H_{13}ClN_2O_7$ (C, H, N).

3-Chloro-3,4-dihydro-2-(2-fluorophenyl)-4-hydroxy-3-nitro-2H-1-benzopyran ***7n***. Yield 79%; mp 113–115 °C (benzene/hexane); ^{1}H-NMR (300 MHz; $CDCl_3$), δ 2.52 (d, 1H exchangeable with D_2O, *J* = 11.8 Hz), 6.15 (d, 1H, *J* = 11.8 Hz), 6.18 (s, 1H,), 6.99 (br d, 1H, *J* = 8.3 Hz), 7.09 (ddd, 1H, *J* = 9.1 Hz, 8.4 Hz and 1.0 Hz), 7.16 (dt, 1H, *J* = 7.6 Hz and 1.0 Hz), 7.28 (br t, 1H, *J* = 7.6 Hz), 7.35 (ddd, 1H, *J* = 8.2 Hz, 7.3 Hz and 0.7 Hz), 7.40–7.50 (m, 1H), 7.61 (dd, 1H, *J* = 7.7 Hz and 0.9 Hz), 7.88 (dt, 1H, *J* = 7.9 Hz and 1.5 Hz); IR ($CDCl_3$), ν 3552, 1570, 1342 cm^{-1}; MS (EI) *m/z* 323, 325 (M^+); anal $C_{15}H_{11}ClFNO_4$ (C, H, N).

3-Chloro-3,4-dihydro-2-(4-fluorophenyl)-4-hydroxy-3-nitro-2H-1-benzopyran ***7o***. Yield 74%; mp 111–113 °C (benzene/hexane); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 2.49 (d, 1H exchangeable with D_2O, *J* = 12.2 Hz), 5.79 (s, 1H), 6.04 (d, 1H, *J* = 12.2 Hz), 7.02 (d, 1H, *J* = 8.3 Hz), 7.08–7.20 (m, 3H), 7.35 (br t, 1H, *J* = 7.6 Hz), 7.43–7.54 (m, 2H), 7.59 (d, 1H, *J* = 7.8 Hz); IR ($CDCl_3$), ν 3553, 1575, 1353 cm^{-1}; MS (EI) *m/z* 323, 325 (M^+); anal $C_{15}H_{11}ClFNO_4$ (C, H, N).

3-Chloro-2-(2,6-difluorophenyl)-3,4-dihydro-4-hydroxy-3-nitro-2H-1-benzopyran ***7p***. Yield 50%; mp 123–125 °C (benzene/hexane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 2.54 (d, 1H exchangeable with D_2O, *J* = 11.8 Hz), 6.07 (d, 1H, *J* = 11.8 Hz), 6.23 (s, 1H), 6.88–7.03 (m, 3H), 7.14 (br t, 1H, *J* = 7.5 Hz), 7.30–7.45 (m, 2H), 7.58 (d, 1H, *J* = 7.7 Hz); IR ($CDCl_3$), ν 3562, 1580, 1362 cm^{-1}; MS (EI) *m/z* 341, 343 (M^+); anal $C_{15}H_{10}ClF_2NO_4$ (C, H, N).

3-Chloro-3,4-dihydro-3,6-dinitro-4-hydroxy-2-phenyl-2H-1-benzopyran ***7q***. Yield 70%; mp 193–194 °C (benzene/heptane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 2.85 (d, 1H, *J* = 8.2 Hz, exchangeable with D_2O), 5.94 (s, 1H), 6.09 (d, 1H, *J* = 8.2 Hz), 7.15 (d, 1H, *J* = 8.8 Hz), 7.44 (m, 5H), 8.25 (dd, 1H, *J* = 8.8 Hz and 2.7 Hz), 8.55 (dd, 1H, *J* = 2.7 Hz, and 0.9 Hz); IR ($CDCl_3$), ν 3557, 1581, 1530, 1348 cm^{-1}; MS (EI) *m/z* 350, 352 (M^+); anal $C_{15}H_{11}ClN_2O_6$ (C, H, N).

3-Chloro-3,4-dihydro-3,6-dinitro-4-hydroxy-2-(4-nitrophenyl)-2H-1-benzopyran ***7r***. Yield 81%; mp 233–235 °C (benzene/heptane); ^{1}H-NMR (250 MHz; $CDCl_3$), δ 2.86 (d, 1H exchangeable with D_2O, *J* = 10.4 Hz), 6.07 (s, 1H), 6.11 (d, 1H, *J* = 10.4 Hz), 7.18 (d, 1H, *J* = 8.9 Hz), 7.66 (br d, 2H, *J* = 8.8 Hz), 8.26 (br dd, 1H, *J* = 8.9 Hz and 2.7 Hz), 8.30 (br d, 2H, *J* = 8.8 Hz), 8.56 (dd, 1H, *J* = 2.7 Hz and 1.0 Hz); IR ($CDCl_3$), ν 3560, 1580, 1532, 1352, 1340 cm^{-1}; MS (EI) *m/z* 395, 397 (M^+); anal $C_{15}H_{10}ClN_3O_8$ (C, H, N).

3-Chloro-3,4-dihydro-4-hydroxy-3-nitro-2-phenyl-8-(prop-2-enyl)-2H-1-benzopyran ***7s***. Yield 98% (viscous oil). ^{1}H-NMR (250 MHz; $CDCl_3$) δ 2.45 (d, 1H exchangeable with D_2O, *J* = 12.2 Hz), 3.31–3.53 (m, 2H), 4.97–5.11 (m, 2H,), 5.81 (s, 1H), 5.89–6.08 (m, 1H), 6.05 (d, 1H, *J* = 12.2 Hz), 7.08 (t, 1H, *J* = 7.6 Hz), 7.23 (br d, 1H, *J* = 6.8 Hz), 7.36–7.51 (m, 6H); IR ($CDCl_3$), ν 3557, 1571, 1348 cm^{-1}; MS (CI) *m/z* 363, 365 ($M + NH_4^+$); anal $C_{18}H_{16}ClNO_4$ (C, H, N).

Preparation of 2-aryl-3-chloro-2,3-dihydro-3-nitro-4H-1-benzopyran-4-ones ***8a–s***. *General procedure*

Multigram quantities of 2-aryl-3-chloro-2,3-dihydro-3-nitro-4*H*-1-benzopyran-4-ones **8a–s** were prepared by scaling up the syntheses previously described for **8a–k** [34]. In a dry 1 L round-bottomed flask equipped with a condenser surmounted by a drying tube, the appropriate 2-aryl-3-chloro-3,4-dihydro-4-hydroxy-3-nitro-2*H*-1-benzopyrans **7a–s** (65 mmol) was dissolved in anhydrous dichloromethane (350–500 mL). Pyridinium chlorochromate (28.1 g, 0.13 mol) was then added in one portion and the flask was dipped into an ultrasound bath for 24 h. The temperature of the bath increased progressively from room temperature to 55 °C. The reaction mixture was then suction-filtered through a short pad of Celite, and the solid was exhaustively rinsed with several portions of dichloromethane. Evaporation of the solvent under reduced pressure left a residue which was directly chromatographed on a silica-gel column (700 g, eluent dichloromethane). Removal of the solvent in vacuo, followed by recrystallization in suitable solvents, afforded the desired derivatives **8a–s** with the exclusive relative configuration 2*R*,3*S*. The benzopyran-4-ones **8a–k** have been described in a previous report [34]. Yields, recrystallization solvents, physical constants and spectral data for novel derivatives **8l–s** are reported below.

3-Chloro-2,3-dihydro-2-(3,4-dimethoxyphenyl)-3-nitro-4H-1-benzopyran-4-one ***8l***. Yield 99%; mp 132–134 °C (benzene/heptane); (allotropic change at 115–120 °C). ^{1}H-NMR (250 MHz; $CDCl_3$) δ 3.90 (s, 3H), 3.91 (s, 3H), 6.21 (s, 1H), 6.88 (d, 1H, *J* = 8.2 Hz), 6.97–7.07 (m, 2H), 7.18–7.28 (m, 2H), 7.69 (ddd, 1H, *J* = 8.9 Hz, 7.4 Hz and 1.7 Hz), 8.06 (dd, 1H, *J* = 7.9 Hz and 1.7 Hz); IR ($CDCl_3$), ν 1713, 1581, 1520, 1335 cm^{-1}; MS (EI) *m/z* 363, 365 (M^+); anal $C_{17}H_{14}ClNO_6$ (C, H, N).

3-Chloro-2,3-dihydro-2-(4-methoxy-3-nitrophenyl)-3-nitro-4H-1-benzopyran-4-one ***8m***. Yield 99%; mp 159–160 °C (benzene/heptane); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 4.01 (s, 3H), 6.31 (s, 1H), 7.14 (d, 1H, *J* = 8.8 Hz), 7.21 (d, 1H, *J* = 8,4 Hz), 7.29 (dd, 1H, *J* = 7.5 Hz and 8.1 Hz), 7.55 (dd, 1H, *J* = 8.7 Hz and 2.2 Hz), 7.73 (ddd, 1H, *J* = 8.4 Hz, 7.2 Hz and 1.5 Hz), 8.06 (dd, 1H, *J* = 8.5 Hz and 1.5 Hz), 8.15 (d, 1H, *J* = 2.2 Hz); IR ($CDCl_3$), ν 1722, 1580, 1539, 1361, 1348 cm^{-1}; MS (EI) *m/z* 378, 380 (M^+); anal $C_{16}H_{11}ClN_2O_7$ (C, H, N).

3-Chloro-2,3-dihydro-2-(2-fluorophenyl)-3-nitro-4H-1-benzopyran-4-one ***8n***. Yield 99%; mp 112–114 °C (heptane); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 6.63 (s, 1H), 6.99 (br d, 1H, *J* = 8.3 Hz), 7.11 (ddd, 1H, *J* = 8.4 Hz, 7.5 Hz and 1.2 Hz), 7.18 (dd, 1H, *J* = 8.4 Hz and 0.7 Hz), 7.28 (dt, 1H, *J* = 7.9 Hz and 1.0 Hz), 7.31 (dd, 1H, *J* = 7.7 Hz and 1.2 Hz), 7.43–7.52 (m, 1H), 7.70 (ddd, 1H, *J* = 8.6 Hz, 7.3 Hz and 1.8 Hz), 7.88 (ddd, 1H, *J* = 8.3 Hz, 7.5 Hz and 1.7 Hz), 8.09 (dd, 1H, *J* = 8.0 Hz and 1.7 Hz); IR ($CDCl_3$), ν 1710, 1579, 1342 cm^{-1}; MS (EI) *m/z* 321, 323 (M^+); anal $C_{15}H_9ClFNO_4$ (C, H, N).

3-Chloro-2,3-dihydro-2-(4-fluorophenyl)-3-nitro-4H-1-benzopyran-4-one ***8o***. Yield 98%; mp 109–111 °C (hexane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 6.28 (s, 1H), 7.09–7.21 (m, 3H),

7.26 (dt, 1H, J = 7.6 Hz and 0.9 Hz), 7.45–7.54 (m, 2H), 7.70 (ddd, 1H, J = 8.6 Hz, 7.3 Hz and 1.7 Hz), 8.07 (dd, 1H, J = 7.9 Hz and 1.7 Hz); IR ($CDCl_3$), ν 1719, 1579, 1344 cm^{-1}; MS (EI) *m/z* 321, 323 (M^+); anal $C_{15}H_9ClFNO_4$ (C, H, N).

3-Chloro-2-(2,6-difluorophenyl)-2,3-dihydro-3-nitro-4H-1-benzopyran-4-one ***8p***. Yield 55%; mp 135–137 °C (benzene/hexane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 6.71 (s, 1H), 7.00 (ddd, 2H, J = 10.3 Hz, 8.6 Hz and 1.7 Hz), 7.19 (dd, 1H, J = 8.4 Hz and 0.8 Hz), 7.26 (ddd, 1H, J = 8.1 Hz, 7.3 Hz and 1.0 Hz), 7.38–7.54 (m, 1H), 7.69 (ddd, 1H, J = 8.6 Hz, 7.3 Hz and 1.7 Hz), 8.09 (dd, 1H, J = 7.9 Hz and 1.7 Hz); IR ($CDCl_3$), ν 1719, 1582, 1339 cm^{-1}; MS (EI) *m/z* 339, 341 (M^+); anal $C_{15}H_8ClF_2NO_4$ (C, H, N).

3-Chloro-2,3-dihydro-3,6-dinitro-2-phenyl-4H-1-benzopyran-4-one ***8q***. Yield 91%; mp 181–183 °C (benzene/heptane); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 6.40 (s, 1H), 7.38 (d, 1H, J = 9.2 Hz), 7.42–7.56 (m, 5H), 8.54 (dd, 1H, J = 9.2 Hz and 2.7 Hz), 8.96 (d, 1H, J = 2.7 Hz); IR ($CDCl_3$), ν 1730, 1581, 1540, 1362, 1350 cm^{-1}; MS (EI) *m/z* 348, 350 (M^+); anal $C_{15}H_9ClN_2O_6$ (C, H, N).

3-Chloro-2,3-dihydro-3,6-dinitro-2-(4-nitrophenyl)-4H-1-benzopyran-4-one ***8r***. Yield 61%; mp 158–160 °C (benzene/hexane); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 6.53 (s, 1H), 7.42 (d, 1H, J = 9.2 Hz), 7.71 (br d, 2H, J = 8.8 Hz), 8.34 (br d, 2H, J = 8.8 Hz), 8.58 (dd, 1H, J = 9.2 Hz and 2.7 Hz), 8.97 (d, 1H, J = 2.7 Hz); IR ($CDCl_3$), ν 1729, 1582, 1538, 1357, 1349 cm^{-1}; MS (EI) *m/z* 393, 395 (M^+); anal $C_{15}H_8ClN_3O_8$ (C, H, N).

3-Chloro-2,3-dihydro-3-nitro-2-phenyl-8-(prop-2-enyl)-4H-1-benzopyran-4-one ***8s***. Yield 91%; mp 70–72 °C (hexane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 3.39–3.58 (m, 2H), 5.02–5.14 (m, 2H), 5.87–6.05 (m, 1H), 6.29 (s, 1H), 7.18 (t, 1H, J = 7.7 Hz), 7.39–7.52 (m, 5H), 7.56 (dd, 1H, J = 7.3 Hz and 1.0 Hz), 7.94 (dd, 1H, J = 8.0 Hz and 1.7 Hz); IR ($CDCl_3$), ν 1713, 1580, 1341 cm^{-1}; MS (CI) *m/z* 361, 363 ($M + NH_4^+$); anal $C_{18}H_{14}ClNO_4$ (C, H, N).

Preparation of 2-aryl-3-nitro-4H-1-benzopyran-4-ones ***9a–s***. *General procedure*

Multigram amounts of 2-aryl-3-nitro-4*H*-1-benzopyran-4-ones **9a–s** were synthesized by scaling up the preparations previously described for **9a–k** [34]. The related benzopyran-4-one **8a–s** (60 mmol) was placed, under inert atmosphere, in a dry 500 mL two-necked round-bottomed flask equipped with a septum inlet. The compound was dissolved in anhydrous tetrahydrofuran (300 mL), then 1,8-diazabicyclo[5.4.0]undec-7-ene (10.05 g, 9.87 mL, 66 mmol) was added in one portion via a syringe. The reaction was slightly exothermic and its progress was monitored by thin-layer chromatography (eluent dichloromethane). When the starting material had completely disappeared (reaction times comprised in the interval 1–3 h), aqueous hydrochloric acid (0.5 N, 175 mL) then dichloromethane (600 mL) were added. The organic layer was separated, and the aqueous phase was extracted with dichloromethane (3 x 250 mL). The combined organic extracts were dried (magnesium sulfate), filtered, and then evaporated under reduced pressure to leave a residue which was chromatographed on a silica-gel column (600 g, eluent dichloromethane). Removal of the volatile materials gave pure compounds **9a–s** which were further recrystallized in an appropriate solvent. The 3-nitroflavones **9a–k** have been described in a previous communication [34]. Yields, physical constants, recrystallization solvents, and spectral data for so far unknown derivatives **9l–s** are reported below.

2-(3,4-Dimethoxyphenyl)-3-nitro-4H-1-benzopyran-4-one ***9l***. Yield 92%; mp 191–193 °C (benzene/cyclohexane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 3.93 (s, 3H), 3.96 (s, 3H), 6.98 (d, 1H, J = 8.5 Hz), 7.22 (d, 1H, J = 2.2 Hz), 7.39 (dd, 1H, J = 8.5 Hz and 2.2 Hz), 7.51 (br t, 1H, J = 7.4 Hz), 7.60 (d, 1H, J = 8.4 Hz), 7.79 (ddd, 1H, J = 8.5 Hz, 7.4 Hz and 1.6 Hz), 8.30 (dd, 1H, J = 7.9 Hz and 1.6 Hz); IR ($CDCl_3$), ν 1661, 1517, 1330 cm^{-1}; MS (EI) *m/z* 327 (M^+); anal $C_{17}H_{13}NO_6$ (C, H, N).

2-(4-Methoxy-3-nitrophenyl)-3-nitro-4H-1-benzopyran-4-one ***9m***. Yield 87%; mp 221–223 °C (benzene/hexane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 4.07 (s, 3H), 7.15–7.30 (m, 2H), 7.42–7.68 (m, 2H), 7.74–7.91 (m, 2H), 8.22–838 (m, 1H,); IR ($CDCl_3$), ν 1664, 1539, 1349 cm^{-1}; MS (EI) *m/z* 342 (M^+); anal $C_{16}H_{10}N_2O_7$ (C, H, N).

2-(2-Fluorophenyl)-3-nitro-4H-1-benzopyran-4-one ***9n***. Yield 88%; mp 154–156 °C (benzene/heptane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 7.21–7.37 (m, 2H), 7.50–7.67 (m, 4H), 7.81 (ddd, 1H, J = 8.5 Hz, 7.1 Hz and 1.5 Hz), 7.81 (dd, 1H, J = 7.9 Hz and 1.6 Hz); IR ($CDCl_3$), ν 1671, 1529, 1368 cm^{-1}; MS (EI) *m/z* 285 (M^+); anal $C_{15}H_8FNO_4$ (C, H, N).

2-(4-Fluorophenyl)-3-nitro-4H-1-benzopyran-4-one ***9o***. Yield 94%; mp 136–138 °C (benzene/heptane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 7.19–7.30 (m, 2H), 7.54 (ddd, 1H, J = 8.1 Hz, 7.0 Hz and 1.1 Hz), 7.60 (dd, 1H, J = 7.9 Hz and 0.8 Hz), 7.72–7.84 (m, 3H), 8.32 (dd, 1H, J = 7.9 Hz and 1.8 Hz); IR ($CDCl_3$), ν 1670, 1538, 1364 cm^{-1}; MS (EI) *m/z* 285 (M^+); anal $C_{15}H_8FNO_4$ (C, H, N).

2-(2,6-Difluorophenyl)-3-nitro-4H-1-benzopyran-4-one ***9p***. Yield 92%; mp 210–211 °C (benzene); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 7.11 (br t, 2H, J = 8.0 Hz), 7.53–7.65 (m, 3H), 7.84 (ddd, 1H, J = 9.1 Hz, 6.3 Hz and 1.8 Hz), 8.37 (dd, 1H, J = 8.2 Hz and 1.8 Hz); IR ($CDCl_3$), ν 1671, 1541, 1349 cm^{-1}; MS (EI) *m/z* 303 (M^+); anal $C_{15}H_7F_2NO_4$ (C, H, N).

3,6-Dinitro-2-phenyl-4H-1-benzopyran-4-one ***9q***. Yield 81%; mp 203–205 °C (benzene/heptane); ^{1}H-NMR (300 MHz; $CDCl_3$), 7.55–7.62 (m, 2H), 7.65–7.73 (m, 1H), 7.75–7.83 (m, 3H), 8.65 (dd, 1H, J = 9.1 Hz and 2.8 Hz), 9.19 (d, 1H, J = 2.8 Hz); IR ($CDCl_3$), ν 1679, 1553, 1362 cm^{-1}; MS (EI) *m/z* 312 (M^+); anal $C_{15}H_8N_2O_6$ (C, H, N).

3,6-Dinitro-2-(4-nitrophenyl)-4H-1-benzopyran-4-one ***9r***. Yield 66%; mp 228–230 °C (benzene/hexane); ^{1}H-NMR (250 MHz; $CDCl_3$/DMSO-d_6 1:1) δ 7.73 (d, 1H, J = 9.2 Hz), 7.84 (br d, 2H, J = 8.8 Hz), 8.29 (br d, 2H, J = 8.8 Hz), 8.55 (dd, 1H, J = 9.0 Hz and 2.8 Hz), 8.97 (d, 1H, J = 2.8 Hz); IR ($CDCl_3$), ν 1720, 1538, 1351 cm^{-1}; MS (EI) *m/z* 357 (M^+); anal $C_{15}H_7N_3O_8$ (C, H, N).

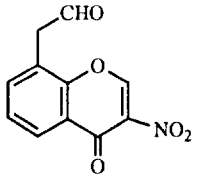
3-Nitro-2-phenyl-8-(prop-2-enyl)-4H-1-benzopyran-4-one ***9s***. Yield 92%; mp 156–157 °C (benzene/hexane); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 3.72 (d, 2H, J = 6.3 Hz), 5.08–5.21 (m, 2H), 5.96–6.09 (m, 1H), 7.48 (t, 1H, J = 7.6 Hz), 7.52–7.68 (m, 4H), 7.72–7.80 (m, 2H), 8.21 (dd, 1H, J = 8.0 Hz and 1.5 Hz); IR ($CDCl_3$), ν 1663, 1538, 1378 cm^{-1}; MS (CI) *m/z* 325 ($M + NH_4^+$), 308 ($M + H$)$^+$; anal $C_{18}H_{13}NO_4$ (C, H, N).

Preparation of 3-nitro-4-oxo-2-phenyl-4H-1-benzopyran-8-acetic acid ***9t***

The benzopyran-4-one **9s** (0.43 g, 1.4 mmol) was placed in a 50 mL round-bottomed flask, then dissolved in a mixture of carbon tetrachloride (2.8 mL), acetonitrile (2.8 mL) and water

(4.2 mL). This biphasic solution was efficently stirred at room temperature using a magnetic bar. Sodium periodate (2.48 g, 10.7 mmol) and ruthenium(III) chloride hydrate (17 mg) were successively added. Stirring was continued for 2 h before dichloromethane (14 mL) and then water (14 mL) were added. The reaction mixture was filtered through a sintered funnel (porosity 3) and the insoluble material was rinsed with dichloromethane. The organic phase was decanted off and the aqueous layer was extracted with a mixture diethyl ether/tetrahydrofuran 1:1 (4 x 10 mL). The combined organic extracts were dried (magnesium sulfate), then evaporated under reduced pressure to leave a residue which was flash-chromatographed on silica gel (30 g, eluent dichloromethane/methanol 9:1). Evaporation of the solvents gave the satisfactorily pure acid **9t** (342 mg, 75%). mp 222–224 °C (toluene); ^{1}H-NMR (300 MHz; DMSO-d_6) δ 3.97 (s, 2H), 7.58–7.80 (m, 6H), 7.91 (d, 1H, J = 7.0 Hz), 8.11 (dd, 1H, J = 8.0 Hz and 1.0 Hz), the signal of the acidic proton is indiscernible; IR (KBr), ν sh 3200–2800, 1713, 1658, 1532, 1380 cm^{-1}; MS (CI) *m/z* 326 $(M + H)^+$; anal $C_{17}H_{11}NO_6$ (C, H, N).

This reaction produced also a small amount of the 3-nitro-4-oxo-2-phenyl-4*H*-1-benzopyran-8-acetaldehyde as by-product:



A few milligrams of this compound were isolated from the above chromatography and fully characterized. In this context, it must be pointed out that, working on the same scale, larger amounts of this aldehyde (160 mg, 37%) have been prepared as well as the acid **9t** (182 mg, 40%) by using smaller quantities of sodium periodate (1.24 g, 5.8 mmol) and ruthenium(III) chloride hydrate (8.5 mg): mp 168–170 °C (benzene/heptane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 4.08 (br s, 2H), 7.48–7.69 (m, 7H,), 8.29 (dd, 1H, J = 7.8 Hz and 1.7 Hz), 9.88 (t, 1H, J = 1.1 Hz); IR ($CDCl_3$), ν 1729, 1667, 1628, 1539, 1376 cm^{-1}; MS (CI) *m/z* 310 $(M + H)^+$; anal $C_{17}H_{11}NO_5$ (C, H, N).

It is noteworthy that the sodium periodate/ruthenium(III) chloride procedure described herein gave much better results than the oxidation by permanganate in the presence of a phase-transfer catalyst [40] which, in our hands, never gave more than 35% yield of the desired acid **9t** starting from **9s**.

Preparation of 3-nitro-4-oxo-2-phenyl-4H-1-benzopyran-8-acetic acid methyl ester ***9u***

A solution of the above acid **9t** (858 mg, 2.64 mmol) in methanol (12 mL) was placed in a 25 mL round-bottomed flask fitted with a condenser. A few drops of concentrated sulfuric acid were added and the reaction mixture was smoothly refluxed for 1 h using an oil bath. After cooling at room temperature, a white precipitate was filtered off and was carefully washed with methanol and then with pentane to leave a first crop of analytically pure **9u**. The filtrate was evaporated in vacuo to leave a residue which was taken up with dichloromethane (25 mL). The obtained solution was treated with 1 M aqueous sodium hydrogenocarbonate (10 mL), then washed with water (3 x 10 mL). The organic layer was dried with magnesium sulfate before the solvent was evaporated under reduced pressure to give a second crop of the desired ester. This material was chromatographed on silica gel (20 g, eluent dichloromethane) to afford pure **9u** which was combined with the previously filtered product. Yield 93%; mp 148–150 °C (benzene/heptane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 3.72 (s, 3H), 3.95 (s, 2H), 7.46–7.69 (m, 4H), 7.70–7.77 (m, 3H), 8.27 (dd, 1H, J = 8.0 Hz and 1.5 Hz), 9.88; IR ($CDCl_3$), ν 1741, 1666, 1538, 1377 cm^{-1}; MS (CI) *m/z* 340 $(M + H)^+$; anal $C_{18}H_{13}NO_6$ (C, H, N).

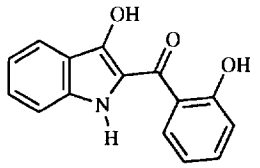
Preparation of 3-amino-2-aryl-4H-1-benzopyran-4-ones ***10a, 10e–r, 10t and 10u***

A solution of the appropriate 3-nitrobenzopyran **9a**, **9e–r**, **9t** or **9u** (5 mmol) in dioxan (100 mL for most of the reported cases) or tetrahydrofuran (100 mL in the cases of **9t**, and **9u**) was placed in a 250 mL round-bottomed flask before palladium on carbon (10%; 0.8 g) was added. The mixture was vigorously stirred under hydrogen at atmospheric pressure for about 16 h. When the starting material had completely disappeared as judged by thin-layer chromatography (eluent dichloromethane/methanol 96:4), the reaction mixture was suction-filtered through a short pad of Celite. The solid was thoroughly rinsed with tetrahydrofuran and, after evaporation of the filtrate to dryness, the crude material was chromatographed over a silica gel column (100 g, eluent dichloromethane/methanol 96:4). Removal of the solvent in vacuo provided pure amino derivatives **10a, 10e–r**, **10t** or **10u** which were then recrystallized.

3-Amino-2-phenyl-4H-1-benzopyran-4-one ***10a***. Yield 98%; mp 137–138 °C (benzene/hexane) (lit [26]: 137–138 °C); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 4.04 (br s, 2H, exchangeable with D_2O), 7.37 (br t, 1H, J = 7.8 Hz), 7.43–7.60 (m, 4H), 7.65 (br t, 1H, J = 7.7 Hz), 7.94 (d, 2H, J = 7.8 Hz), 8.29 (br d, 1H, J = 8.0 Hz); IR ($CDCl_3$), ν 3450, 3360, 1626, 1585, 1561, 1480, 1471, 1419 cm^{-1}; MS (EI) *m/z* 237 (M^+); anal $C_{15}H_{11}NO_2$ (C, H, N).

2-(2-Aminophenyl)-3-amino-4H-1-benzopyran-4-one ***10e.*** Yield 40% (after chromatography on silica gel (300 g, eluent dichloromethane, then dichloromethane/methanol 96:4)), mp > 260 °C (benzene/ethanol); ^{1}H-NMR (300 MHz; DMSO-d_6) δ 5.00–7.00 (sh, 3H, exchangeable with D_2O), 7.00–7.12 (m, 2H), 7.22 (ddd, 1H, J = 7.9 Hz, 6.8 Hz and 1.0 Hz), 7.41 (dt, 1H, J = 7.7 Hz and 1.4 Hz), 7.46 (dd, 1H, J = 7.4 Hz and 1.4 Hz), 7.53 (ddd, 1H, J = 8.0 Hz, 6.7 Hz and 1.3 Hz), 7.62 (br d, 1H, J = 8.2 Hz), 8.14 (dd, 1H, J = 8.1 Hz and 1.0 Hz), 10.35 (br s, 1H, exchangeable with D_2O); IR (KBr), ν 1636, 1557, 1495, 1448 cm^{-1}; MS (EI) *m/z* 252 (M^+); anal $C_{15}H_{12}N_2O_2$ (C, H, N). In this particular case, two side-products were isolated and fully characterized. These compounds, which are less polar than **10e**, result from a rearrangement or from an intramolecular cyclization and are described below.

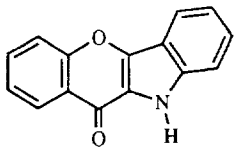
Side-product 1: 3-hydroxy-2-(2-hydroxybenzoyl)indole.



Yield 25%; mp 142–144 °C with sublimation (benzene/heptane); ^{1}H-NMR (300 MHz; DMSO-d_6) δ 6.89–7.01 (m, 3H), 7.24–7.35 (m, 2H), 7.40 (ddd, 1H, J = 8.5 Hz, 7.1 Hz and 1.5 Hz), 7.64 (dd, 1H, J = 7.6 Hz and 1.6 Hz), 7.77 (d, 1H, J =

8.1 Hz), 10.35 (br s, 2H, exchangeable with D_2O), 10.77 (s, 1H, exchangeable with D_2O); IR (KBr), ν 1620, 1582, 1530, 1488 cm^{-1}; MS (CI) *m/z* 254 $(M + H)^+$. In addition, the structure of this compound has been unambiguously confirmed by an X-ray study; anal $C_{15}H_{11}NO_3$ (C, H, N).

Side-product 2: [1]benzopyrano[3,2-b]indol-11(10H)-one.



Yield 35%; mp > 260 °C with sublimation (benzene/ethanol) (lit [41]: 288 °C (dec) with partial sublimation beforehand); ^{1}H-NMR (300 MHz; DMSO-d_6), δ 7.28 (ddd, 1H, *J* = 8.0 Hz, 6.8 Hz and 1.3 Hz), 749–7.60 (m, 3H), 7.84–7.90 (m, 2H), 8.03 (br d, 1H, *J* = 8.1 Hz), 8.34 (br d, 1H, *J* = 7.4 Hz), 12.10 (s, 1H, exchangeable with D_2O); IR (KBr), ν 1641, 1607, 1537, 1510, 1459 cm^{-1}; MS (EI) *m/z* 235 (M^+); anal $C_{15}H_9NO_2$ (C, H, N).

2-(3-Aminophenyl)-3-amino-4H-1-benzopyran-4-one ***10f***. Yield 98%; mp 213–215 °C (benzene/cyclohexane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 3.90 (br s, 2H, exchangeable with D_2O), 4.05 (br s, 2H, exchangeable with D_2O), 6.79 (dt, 1H, *J* = 7.0 Hz and 2.1 Hz), 7.21–7.40 (m, 4H), 7.49 (br d, 1H, *J* = 8.2 Hz), 7.64 (ddd, 1H, *J* = 8.6 Hz, 7.1 Hz and 1.6 Hz), 8.28 (dd, 1H, *J* = 8.1 Hz and 1.5 Hz); IR ($CDCl_3$), ν sh 3500–3300, 1622, 1559, 1517, 1480, 1472, 1418 cm^{-1}; MS (EI) *m/z* 252 (M^+); anal $C_{15}H_{12}N_2O_2$ (C, H, N).

2-(4-Aminophenyl)-3-amino-4H-1-benzopyran-4-one ***10g***. Yield 96%; mp 162–165 °C with allotropic change at 155–160 °C (benzene/cyclohexane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 3.96 (br s, 2H, exchangeable with D_2O), 4.02 (br s, 2H, exchangeable with D_2O), 6.81 (br d, 2H, AA'BB' system, *J* = 8.7 Hz), 7.35 (ddd, 1H, *J* = 8.0 Hz, 7.0 Hz and 1.0 Hz), 7.47 (br d, 1H, *J* = 8.5 Hz), 7.61 (ddd, 1H, *J* = 8.5 Hz, 7.0 Hz and 1.5 Hz), 7.78 (br d, 2H, AA'BB' system, *J* = 8.7 Hz), 8.26 (dd, 1H, *J* = 8.0 Hz and 1.5 Hz); IR ($CDCl_3$), ν sh 3500–3300, 1620, 1558, 1518, 1479, 1471, 1413 cm^{-1}; MS (EI) *m/z* 252 (M^+); anal $C_{15}H_{12}N_2O_2$ (C, H, N).

3-Amino-2-(2-methoxyphenyl)-4H-1-benzopyran-4-one ***10h***. Yield 76% (in this case, an uncharacterized by-product was formed), mp 144–145 °C (benzene/heptane); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 3.93 (s, 3H), 3.99 (br s, 2H, exchangeable with D_2O), 7.09 (d, 1H, *J* = 8.4 Hz), 7.15 (br t, 1H, *J* = 7.3 Hz), 7.37 (br t, 1H, *J* = 7.8 Hz), 7.43–7.54 (m, 2H), 7.58–7.66 (m, 2H), 8.30 (dd, 1H, *J* = 8.1 Hz and 1.4 Hz); IR ($CDCl_3$), ν 3430, 3360, 1626, 1583, 1563, 1471, 1418 cm^{-1}; MS (EI) *m/z* 267 (M^+); anal $C_{16}H_{13}NO_3$ (C, H, N).

3-Amino-2-(3-methoxyphenyl)-4H-1-benzopyran-4-one ***10i***. Yield 99%; mp 135–136 °C (benzene/cyclohexane) (lit [26]: 132 °C); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 3.90 (s, 3H), 4.07 (br s, 2H, exchangeable with D_2O), 7.02 (ddd, 1H, *J* = 7.9 Hz, 2.7 Hz and 1.2 Hz), 7.37 (br t, 1H, *J* = 7.2 Hz), 7.43–7.53 (m, 4H), 7.64 (ddd, 1H, *J* = 8.7 Hz, 7.6 Hz and 1.4 Hz), 8.28 (dd, 1H, *J* = 8.1 Hz and 1.4 Hz); IR ($CDCl_3$), ν 3445, 3360, 1626, 1583, 1561, 1481, 1471, 1416 cm^{-1}; MS (EI) *m/z* 267 (M^+); anal $C_{16}H_{13}NO_3$ (C, H, N).

3-Amino-2-(4-methoxyphenyl)-4H-1-benzopyran-4-one ***10j***. Yield 98%; mp 152–154 °C with allotropic change at 125–128 °C (benzene/hexane) (lit [26]: 152–153 °C); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 3.88 (s, 3H), 4.07 (br s, 2H, exchangeable with D_2O), 7.06 (br d, 2H, AA'BB' system, *J* = 8.7 Hz), 7.36 (br t, 1H, *J* = 7.8 Hz), 7.48 (d, 1H, *J* = 8.2 Hz), 7.63 (ddd, 1H, *J* = 8.5 Hz, 7.1 Hz and 1.6 Hz), 7.91 (br d, 2H, AA'BB' system, *J* = 8.7 Hz), 8.24 (dd, 1H, *J* = 8.0 Hz and 1.5 Hz); IR ($CDCl_3$), ν 3440, 3355, 1622, 1561, 1513, 1471 cm^{-1}; MS (EI) *m/z* 267 (M^+); anal $C_{16}H_{13}NO_3$ (C, H, N).

3-Amino-2-(3,4,5-trimethoxyphenyl)-4H-1-benzopyran-4-one ***10k***. Yield 93%; mp 170–172 °C (benzene/cyclohexane); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 3.93 (s, 3H), 3.95 (s, 6H), 4.03 (br s, 2H, exchangeable with D_2O), 7.17 (s, 2H), 7.38 (dd, 1H, *J* = 8.1 Hz, and 7.0 Hz), 7.51 (d, 1H, *J* = 8.4 Hz), 7.65 (ddd, 1H, *J* = 8.4 Hz, 7.0 Hz and 1.4 Hz), 8.28 (dd, 1H, *J* = 8.1 Hz and 1.4 Hz); IR ($CDCl_3$), ν 3440, 3355, 1625, 1584, 1561, 1505, 1479, 1466, 1424 cm^{-1}; MS (EI) *m/z* 327 (M^+); anal $C_{18}H_{17}NO_5$ (C, H, N).

3-Amino-2-(3,4-dimethoxyphenyl)-4H-1-benzopyran-4-one ***10l***. Yield 94%; mp 126–127 °C (benzene/cyclohexane) (lit [26]: 125–126 °C); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 3.93 (s, 3H), 3.95 (s, 3H), 4.10 (br s, 2H, exchangeable with D_2O), 7.02 (d, 1H, *J* = 8.5 Hz), 7.37 (br t, 1H, *J* = 7.9 Hz), 7.45–7.56 (m, 3H), 7.63 (ddd, 1H, *J* = 8.6 Hz, 7.3 Hz and 1.4 Hz), 8.27 (dd, 1H, *J* = 8.0 Hz and 1.4 Hz); IR ($CDCl_3$), ν 3440, 3350, 1614, 1560, 1516, 1471, 1421 cm^{-1}; MS (EI) *m/z* 297 (M^+); anal $C_{17}H_{15}NO_4$ (C, H, N).

3-Amino-2-(3-amino-4-methoxyphenyl)-4H-1-benzopyran-4-one ***10m***. Yield 89%; mp 141–142 °C (benzene/heptane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 3.80 (br s, 4H, exchangeable with D_2O), 3.93 (s, 3H), 6.91 (d, 1H, *J* = 8.3 Hz), 7.22–7.38 (m, 3H), 7.47 (d, 1H, *J* = 8.4 Hz), 7.60 (ddd, 1H, *J* = 8.3 Hz, 7.1 Hz and 1.5 Hz), 8.25 (dd, 1H, *J* = 8.0 Hz and 1.2 Hz); IR ($CDCl_3$), ν sh 3500–3300, 1618, 1558, 1516, 1471, 1421 cm^{-1}; MS (EI) *m/z* 282 (M^+); anal $C_{16}H_{14}N_2O_3$ (C, H, N).

3-Amino-2-(2-fluorophenyl)-4H-1-benzopyran-4-one ***10n***. Yield 99%; mp 128–130 °C (cyclohexane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 3.49 (br s, 2H, exchangeable with D_2O), 7.22–7.57 (m, 5H), 7.64 (ddd, 1H, *J* = 8.5 Hz, 7.1 Hz and 1.6 Hz), 7.71 (dd, 1H, *J* = 7.4 Hz and 1.6 Hz), 8.28 (dd, 1H, *J* = 8.0 Hz and 1.6 Hz); IR ($CDCl_3$), ν 3455, 3365, 1631, 1563, 1480, 1471, 1420 cm^{-1}; MS (EI) *m/z* 255 (M^+); anal $C_{15}H_{10}FNO_2$ (C, H, N).

3-Amino-2-(4-fluorophenyl)-4H-1-benzopyran-4-one ***10o***. Yield 92%; mp 163–164 °C (heptane) (lit [29]: 162–164 °C); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 3.99 (br s, 2H, exchangeable with D_2O), 7.18–7.29 (m, 2H), 7.38 (ddd, 1H, *J* = 8.1 Hz, 7.0 Hz and 1.2 Hz), 7.49 (br d, 1H, *J* = 8.3 Hz), 7.65 (ddd, 1H, *J* = 8.6 Hz, 7.0 Hz and 1.7 Hz), 7.89–7.99 (m, 2H), 8.27 (dd, 1H, *J* = 8.1 Hz and 1.6 Hz); IR ($CDCl_3$), ν 3438, 3340, 1626, 1562, 1480, 1471, 1424 cm^{-1}; MS (EI) *m/z* 255 (M^+); anal $C_{15}H_{10}FNO_2$ (C, H, N).

3-Amino-2-(2,6-difluorophenyl)-4H-1-benzopyran-4-one ***10p***. Yield 98%; mp 141–143 °C (benzene/cyclohexane); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 3.19 (br s, 2H, exchangeable with D_2O), 7.11 (br t, 2H, *J* = 8.6 Hz), 7.39 (t, 1H, *J* = 7.7 Hz), 7.45 (d, 1H, *J* = 8.4 Hz), 7.52 (ddd, 1H, *J* = 8.5 Hz, 7.2 Hz and 1.8 Hz), 7.66 (ddd, 1H, *J* = 8.2 Hz, 7.1 Hz and 1.1 Hz), 8.30 (dd, 1H, *J* = 8.1 Hz and 1.1 Hz); IR ($CDCl_3$), ν 3465, 3370, 1624, 1585, 1564, 1479, 1420 cm^{-1}; MS (EI) *m/z* 273 (M^+); anal $C_{15}H_9F_2NO_2$ (C, H, N).

3,6-Diamino-2-phenyl-4H-1-benzopyran-4-one ***10q***. Yield 90%; mp 162–164 °C (benzene/heptane); ^{1}H-NMR (250 MHz; DMSO-d_6) δ 4.55 (br s, 2H, exchangeable with D_2O), 5.41 (br s, 2H, exchangeable with D_2O), 7.06 (dd, 1H, J = 8.9 Hz, and 2.7 Hz), 7.15 (d, 1H, J = 2.7 Hz), 7.39 (d, 1H, J = 8.9 Hz), 7.45–7.62 (m, 3H), 7.95 (br d, 2H, J = 7.9 Hz); IR (KBr), ν 1614, 1590, 1568, 1494, 1446, 1411 cm^{-1}; MS (EI) *m/z* 252 (M^+); anal $C_{15}H_{12}N_2O_2$ (C, H, N).

2-(4-Aminophenyl)-3,6-diamino-4H-1-benzopyran-4-one ***10r***. Yield 94%; mp 262 °C (benzene/acetonitrile); ^{1}H-NMR (300 MHz; DMSO-d_6) δ 4.28 (br s, 2H, exchangeable with D_2O), 5.34 (br s, 2H, exchangeable with D_2O), 5.69 (br s, 2H, exchangeable with D_2O), 6.71 (br d, 2H, J = 8.5 Hz), 7.01 (dd, 1H, J = 8.8 Hz and 2.7 Hz), 7.12 (d, 1H, J = 2.7 Hz), 7.35 (d, 1H, J = 8.8 Hz), 7.69 (br d, 2H, J = 8.5 Hz); IR (KBr), ν 1611, 1560, 1515, 1489, 1438, 1407 cm^{-1}; MS (EI) *m/z* 267 (M^+); anal $C_{15}H_{13}N_3O_2$ (C, H, N).

3-Amino-4-oxo-2-phenyl-4H-1-benzopyran-8-acetic acid ***10t***. In this case, the crude product was eluted with a mixture dichloromethane/methanol 9:1. The obtained compound, after it had been adsorbed on a small amount of silica gel, was then further purified by extraction with a Soxhlet apparatus using pure dichloromethane as solvent. Yield 40%; mp 190–192 °C. ^{1}H-NMR (300 MHz; DMSO-d_6) δ 3.92 (s, 2H), 4.79 (br s, 2H, exchangeable with D_2O), 7.39 (dd, 1H, J = 7.9 Hz and 7.3 Hz), 7.47–7.62 (m, 3H), 7.69 (dd, 1H, J = 7.3 Hz and 1.1 Hz), 8.00 (br d, 2H, J = 7.5 Hz), 8.30 (dd, 1H, J = 7.9 Hz and 1.1 Hz), the signal of the acidic proton is indiscernible; IR (KBr), ν sh 3300–2900, 1712, 1637, 1562, 1489, 1448, 1417 cm^{-1}; MS (CI) *m/z* 296 (M + H)$^+$; anal $C_{17}H_{13}NO_4$ (C, H, N).

3-Amino-4-oxo-2-phenyl-4H-1-benzopyran-8-acetic acid methyl ester ***10u***. Yield 99%; mp 155–156 °C (benzene/heptane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 3.70 (s, 3H), 3.94 (s, 2H), 4.08 (br s, 2H, exchangeable with D_2O), 7.35 (dd, 1H, J = 8.0 and 7.4 Hz), 7.45–7.61 (m, 4H), 7.88–7.96 (m, 2H), 8.24 (dd, 1H, J = 8.0 Hz and 1.6 Hz); IR ($CDCl_3$), ν 3450, 3360, 1737, 1627, 1566, 1489, 1449, 1420 cm^{-1}; MS (EI) *m/z* 310 (M + H)$^+$; anal $C_{18}H_{15}NO_4$ (C, H, N).

Preparation of 3-amino-2-aryl-4H-1-benzopyran-4-ones ***10b–d***
These chloro derivatives were prepared on the same scale according to the modified procedure above. In these cases, concentrated aqueous hydrochloric acid (12 N, 0.5 mL) was added to the reaction mixture before starting the hydrogenation process. Under these circumstances, at the end of the reaction, it was necessary to make the medium alkaline (before filtration through Celite) by adding aqueous sodium hydroxide (12 N, 5 mL). After several washings of the insoluble material with tetrahydrofuran, the filtrate was evaporated to dryness. The residue was taken up with water (40 mL) and dichloromethane (120 mL). The aqueous layer was extracted with dichloromethane (3 x 50 mL). The combined extracts were dried (magnesium sulfate), then evaporated in vacuo to give a material which was chromatographed and recrystallized (see above).

3-Amino-2-(2-chlorophenyl)-4H-1-benzopyran-4-one ***10b***. Yield 90%; mp 121–122 °C (heptane); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 3.50 (br s, 2H, exchangeable with D_2O), 7.30–7.45 (m, 4H), 7.49–7.62 (m, 3H), 8.24 (dd, 1H, J = 8.0 Hz and 1.3 Hz); IR ($CDCl_3$) ν 3450, 3370, 1632, 1584, 1563, 1472, 1424 cm^{-1}; MS (EI) *m/z* 271, 273 (M^+); anal $C_{15}H_{10}ClNO_2$ (C, H, N).

3-Amino-2-(3-chlorophenyl)-4H-1-benzopyran-4-one ***10c***. Yield 85%; mp 131–133 °C (benzene/cyclohexane); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 3.70 (br s, 2H, exchangeable with D_2O), 7.29–7.49 (m, 4H), 7.61 (ddd, 1H, J = 8.4 Hz, 7.1 Hz and 1.4 Hz), 7.80 (dd, 1H, J = 7.3 Hz and 1.3 Hz), 7.88 (br s, 1H), 8.21 (dd, 1H, J = 8.0 Hz and 1.3 Hz); IR ($CDCl_3$), ν 3445, 3360, 1631, 1585, 1562, 1473, 1419 cm^{-1}; MS (EI) *m/z* 271, 273 (M^+); anal $C_{15}H_{10}ClNO_2$ (C, H, N).

3-Amino-2-(4-chlorophenyl)-4H-1-benzopyran-4-one ***10d***. Yield 97%; mp 160–162 °C (heptane) (lit [29]: 159–161 °C); ^{1}H-NMR (300 MHz; $CDCl_3$) δ 3.20 (br s, 2H, exchangeable with D_2O), 7.34 (br t, 1H, J = 7.5 Hz), 7.40–7.51 (m, 3H), 7.62 (ddd, 1H, J = 8.5 Hz, 7.2 Hz and 1.6 Hz), 7.88 (br d, 2H, J = 8.5 Hz), 8.17 (dd, 1H, J = 8.0 Hz and 1.2 Hz); IR ($CDCl_3$), ν 3445, 3360, 1626, 1584, 1563, 1472, 1422 cm^{-1}; MS (EI) *m/z* 271, 273 (M^+); anal $C_{15}H_{10}ClNO_2$ (C, H, N).

Preparation of the 3-amino-4-oxo-2-phenyl-4H-1-benzopyran-8-acetic acid hydrochloride ***11t***
The methyl ester **10u** (309 mg, 1 mmol) and 2 N aqueous hydrochloric acid (40 mL) were placed in a 100 mL round-bottomed flask equipped with a condenser. The mixture was gently refluxed with stirring for 3 h using an oil bath. The obtained clear solution was allowed to cool to room temperature, then evaporated under reduced pressure to afford a white solid which was dissolved in distilled water (25 mL). The water was removed again (this operation was repeated three times). The residue was then taken up with anhydrous acetone (20 mL) and then left overnight. Filtration of the solid, followed by drying in a vacuum oven, gave analytically pure **11t** (310 mg, 93%), mp 200–202 °C with an allotropic change at 190–195 °C. ^{1}H-NMR (300 MHz; DMSO-d_6) δ 3.94 (s, 2H), 7.43 (br t, 1H, J = 7.5 Hz), 7.51–7.65 (m, 3H), 7.73 (br d, 1H, J = 7.2 Hz), 7.98 (br dd, 2H, J = 7.9 Hz and 1.5 Hz), 8.05 (dd, 1H, J = 8.0 Hz and 1.5 Hz), the signal of the acidic and amino protons are indiscernible; IR (KBr), ν sh 3200–2800, 1697, 1661, 1608, 1560, 1482, 1447, 1398 cm^{-1}; MS (CI) *m/z* 296 (M + H)$^+$; anal $C_{17}H_{14}ClNO_4$ (C, H, N).

Preparation of 2-aryl-3-[(2-diethylamino)ethyl]amino-4H-1-benzopyran-4-ones ***12j and 12l***
A solution of the appropriate aminoflavone **10j** or **10l** (2.5 mmol) in anhydrous tetrahydrofuran (20 mL) was placed, under argon atmosphere, in a two-necked round-bottomed flask fitted with a condenser and a septum inlet. Powdered sodium hydride (180 mg, 7.5 mmol) and freshly distilled *N,N*-diethyl-2-chloroethylamine (1.02 g, 1.1 mL, 7.5 mmol) were added and the flask was immersed into an ultrasound bath for 20 h (the temperature of the bath increased progressively from room temperature to 55 °C and the progress of the reaction was monitored by thin-layer chromatography). The reaction mixture was allowed to cool to room temperature, then filtered through a short pad of Celite. The Celite was successively washed with dichloromethane and methanol. The filtrate was evaporated in vacuo to leave a crude material which was chromatographed over a silica-gel column (150 g, eluent dichloromethane/methanol 8:2). Removal of the solvents followed by recrystallization gave analytically pure **12j** or **12l**.

3-[(2-Diethylamino)ethyl]amino-2-(4-methoxyphenyl)-4H-1-benzopyran-4-one ***12j***. Yield 49%; mp 85–86 °C (benzene/hexane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 0.96 (t, 6H, J = 7.2 Hz), 2.40–2.54 (m, 6H), 2.74 (t, 2H, J = 6.5 Hz), 3.89 (s, 3H), 7.02 (br d, 2H, AA'BB' system, J = 8.8 Hz), 7.35 (ddd, 1H, J = 8.0 Hz, 7.0 Hz and 1.0 Hz), 7.47 (dd, 1H, J = 8.5 Hz

and 1.0 Hz), 7.62 (ddd, 1H, J = 8.5 Hz, 7.0 Hz and 1.6 Hz), 7.98 (br d, 2H, AA'BB' system, J = 8.8 Hz), 8.26 (dd, 1H, J = 8.0 Hz and 1.6 Hz), 11.0 (sh, 1H, exchangeable with D_2O); IR ($CDCl_3$), ν 1611, 1567, 1511, 1467, 1380 cm^{-1}; MS (CI) *m/z* 367 $(M + H)^+$; anal $C_{22}H_{26}N_2O_3$ (C, H, N).

3-[(2-Diethylamino)ethyl]amino-2-(3,4-dimethoxyphenyl)-4H-1-benzopyran-4-one ***12l***. Yield 51%; mp 131–133 °C (benzene/cyclohexane); ^{1}H-NMR (250 MHz; $CDCl_3$) δ 1.32 (t, 6H, J = 7.2 Hz), 2.98–3.15 (m, 6H), 3.38 (t, 2H, J = 6.7 Hz), 3.96 (s, 3H), 3.97 (s, 3H), 7.03 (d, 1H, J = 8.5 Hz), 7.35–7.51 (m, 3H), 7.55 (dd, 1H, J = 8.3 Hz and 2.1 Hz), 7.67 (ddd, 1H, J = 8.3 Hz, 7.0 Hz and 1.6 Hz), 8.21 (dd, 1H, J = 8.0 Hz and 1.6 Hz), 11.75 (br s, 1H, exchangeable with D_2O); IR ($CDCl_3$), ν 1614, 1569, 1515, 1468, 1420, 1377 cm^{-1}; MS (CI) *m/z* 397 $(M + H)^+$; anal $C_{23}H_{28}N_2O_4$ (C, H, N).

L1210 leukemia antiproliferative assay

Murine L1210 leukemia cells were grown in RPMI 1640 medium containing 10% fetal calf serum, penicillin (100 U/mL) and streptomycin (100 μg/mL). Exponentially growing cells were seeded at 5000 cells in 1 mL in 24-well petri dishes (2 mL wells). The tested compounds were dissolved in dimethyl sulfoxide, mixed with 1 mL of culture medium and added to the above 1 mL of cells. Final concentration of compounds were between 0 and 50 μg/mL, or up to the maximum solubility. Final concentration of dimethyl sulfoxide was ≤ 0.1%. Cells were grown in an incubator (37 °C; 5% CO_2 and 95% air; 100% humidity) for 72 h and counted using a Coulter counter. Results are the mean of duplicate experiments and are presented as the 50% inhibitory concentration (IC_{50}), ie, the concentration at which the growth was 50% of control cells.

Acknowledgment

We gratefully thank P Demerseman and C Monneret for their support throughout this work. We also express our gratitude to Geneviève Flad for recording the ^{1}H-NMR spectra, C Bois (Laboratoire de chimie des métaux de transition, UA No 419, CNRS, Université Pierre-et-Marie-Curie, Paris) for the X-ray crystallographic analysis of a by-product and MA Verjus for the L1210 leukemia assay. We are also indebted to the CNRS for financial support.

References

1 Pathak D, Pathak K, Singla AK (1991) *Fitoterapia* 62, 371-389
2 *Progress in Clinical and Biological Research, Vol 213, Plant Flavonoids in Biology and Medicine* (1986) (Cody V, Middleton E, Harborne JB, eds) Alan R Liss Inc, New York
3 *Progress in Clinical and Biological Research, Vol 280, Plant Flavonoids in Biology and Medicine II* (1988) (Cody V, Middleton E, Harborne JB, Beretz A, eds) Alan R Liss Inc, New York
4 Molnár J, Béládi I, Domonkos K, Földeak S, Boda K, Veckenstedt A (1981) *Neoplasma* 28, 11–18
5 Konoshima T, Takasaki M, Kosuka M et al (1989) *Shoyakugaku Zasshi* 43, 135–141
6 Hirano T, Oka K, Akiba M (1989) *Res Commun Chem Path Pharmacol* 64, 69–78
7 Cushman M, Nagarathnam D (1991) *J Nat Prod* 54, 1656–1660
8 Post JFM, Varma RS (1992) *Cancer Lett* 67, 207–213
9 Middleton Jr E, Kandaswami C (1993) In: *The Flavonoids, Advances in research since 1986* (Harborne JB, ed) Chapman & Hall, London, 619-652
10 Sugaya T, Akinaga S, Morimoto M, Shida Y, Gomi K, Kasai M (1990) Jpn Kokai Tokkyo Koho JP 02 138 277; *Chem Abstr* 113, 171775
11 Shida Y, Sagaya T, Gomi K, Kasai M, Morimoto M (1990) Eur Patent Appl EP 374 789; *Chem Abstr* 113, 231085
12 Akama T, Shida Y, Ikeda S et al (1993) Eur Patent Appl EP 556 720; *Chem Abstr* 120, 106766
13 Cushman M, Nagarathnam D, Burg DL, Geahlen RL (1991) *J Med Chem* 34, 798–806
14 Cunningham BDM, Threadgill MD, Groundwater PW, Dale IL, Hickman JA (1992) *Anti Cancer Drug Des* 7, 365–384
15 Cushman M, Zhu H, Geahlen RL, Kraker AJ (1994) *J Med Chem* 37, 3353–3362
16 Ferrell Jr JE, Chang Sing PDG, Loew G, King R, Mansour JM, Mansour TE (1979) *Mol Pharmacol* 16, 556–568
17 Cummings J, Smyth JF (1989) *Cancer Chemother Pharmacol* 24, 269–272
18 Anon (1989) *Drugs of the Future* 14, 180–183
19 Wurm G (1990) *Deutsch Apothek Zeit* 130, 2306–2312
20 Bibby MC (1991) *Br J Cancer* 63, 3–5
21 Bibby MC, Double JA (1993) *Anti-Cancer Drugs* 4, 3–17
22 Tsukada T, Nakshima K, Shirakawa S (1986) *Biochem Biophys Res Commun* 140, 832–836
23 Blomgren H, Kling-Andersson G (1992) *Anticancer Res* 12, 981–986
24 Bognár R, O'Brien C, Philbin EM, Ushioda S, Wheeler TSA (1960) *Chem Ind* 1186–1187
25 Kidd D (1962) *J Chem Soc* 5121–5125
26 O'Brien C, Philbin EM, Ushioda S, Wheeler TS (1963) *Tetrahedron* 19, 373–377
27 Bognár R, Rákosi M (1966) *Liebigs Ann Chem* 693, 225–232
28 Patonay T, Rákosi M, Litkei G, Bognár R (1979) *Liebigs Ann Chem* 161–173
29 Patonay T, Bognár R, Litkei G (1984) *Tetrahedron* 40, 2555–2562
30 Patonay T, Litkei G, Bognár R (1984) *Tetrahedron* 40, 3425–3430
31 De Meyer N, Haemers A, Mishra L et al (1991) *J Med Chem* 34, 736–746
32 Michalska M (1968) *Bull Acad Pol Sci, Ser Sci Chim* 16, 567–573
33 Dauzonne D, Demerseman P (1990) *Synthesis* 66–70
34 Dauzonne D, Grandjean C (1992) *Synthesis* 677–680
35 Dauzonne D, Martinez L (1995) *Tetrahedron Lett* 36, 1845–1848
36 Slagh HR (1953) US Patent 2 632 776; *Chem Abstr* 48, 1412
37 Burtner RR (1949) *J Am Chem Soc* 71, 2578
38 Einhorn A, Grabfield JP (1888) *Liebigs Ann Chem* 243, 362–378
39 Claisen L, Eisleb O (1913) *Liebigs Ann Chem* 401, 21–119
40 Lee DG, Lamb SE, Chang VS, Lorah PD, Kende AS (1990) In: *Organic Syntheses Coll Vol VII* (Freeman JP, ed), J Wiley & Sons, New York, 397
41 Görlitzer K (1974) *Arch Pharm (Weinheim, Ger)* 307, 523–539