

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

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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 cirsiolol) 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

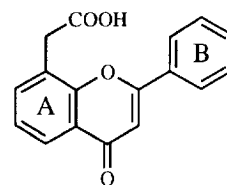
Introduction

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 amino-flavones 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].

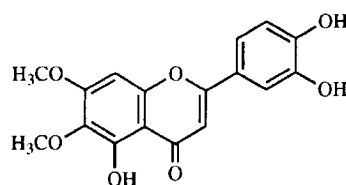
In this context, we became interested in synthesizing and evaluating the cytotoxicity of a series of 3-aminoflavones with a special interest for the 3-amino derivative of the 3'-amino-4'-methoxyflavone because the 3-unsubstituted compound proved to be one of the more cytotoxic compounds in the large 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 cirsiolol **2**, a polysubstituted flavone known to exhibit pronounced antiproliferative effects [22, 23].



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



Cirsiolol (**2**)

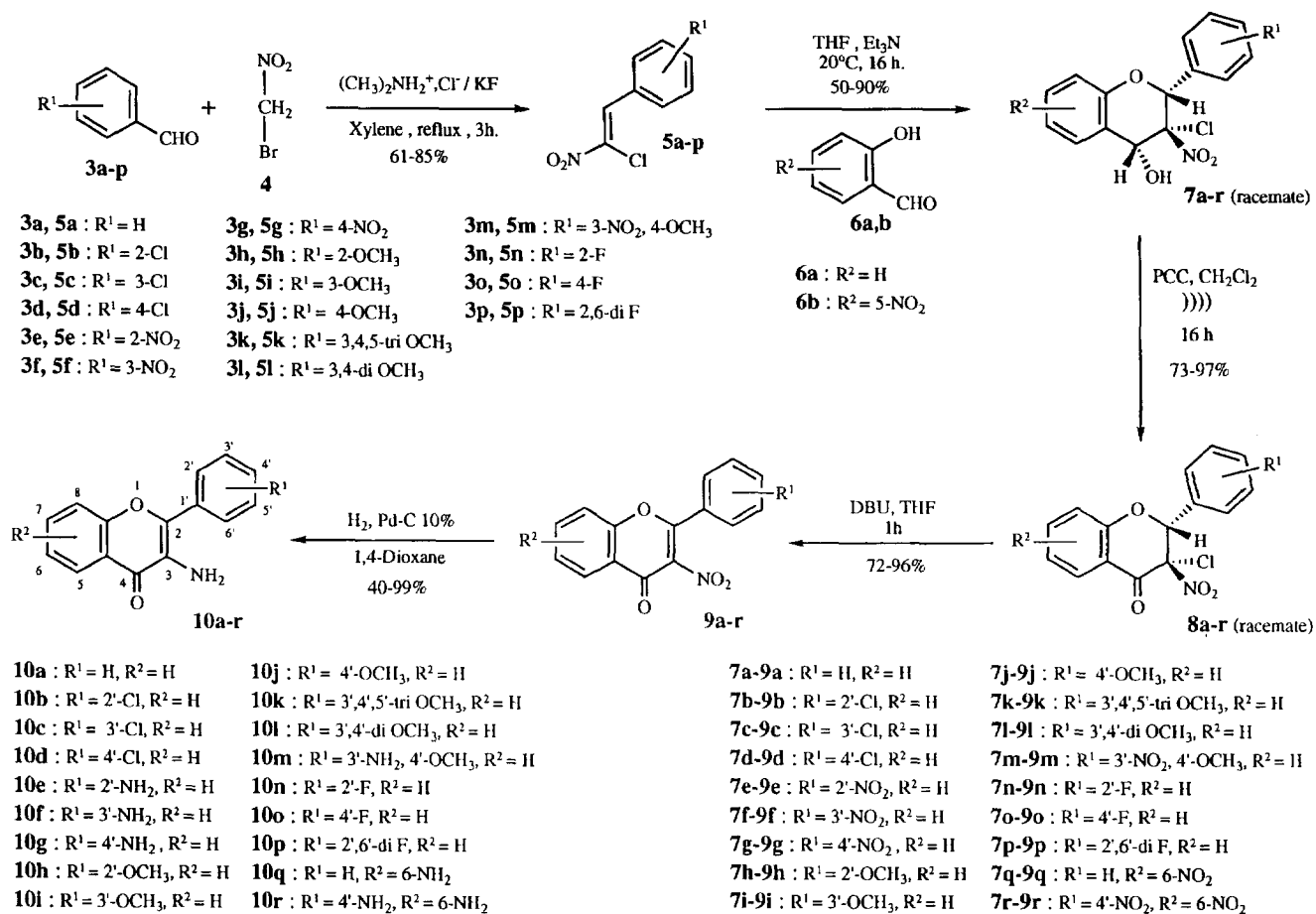
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Chemistry

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 bromonitroethane **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

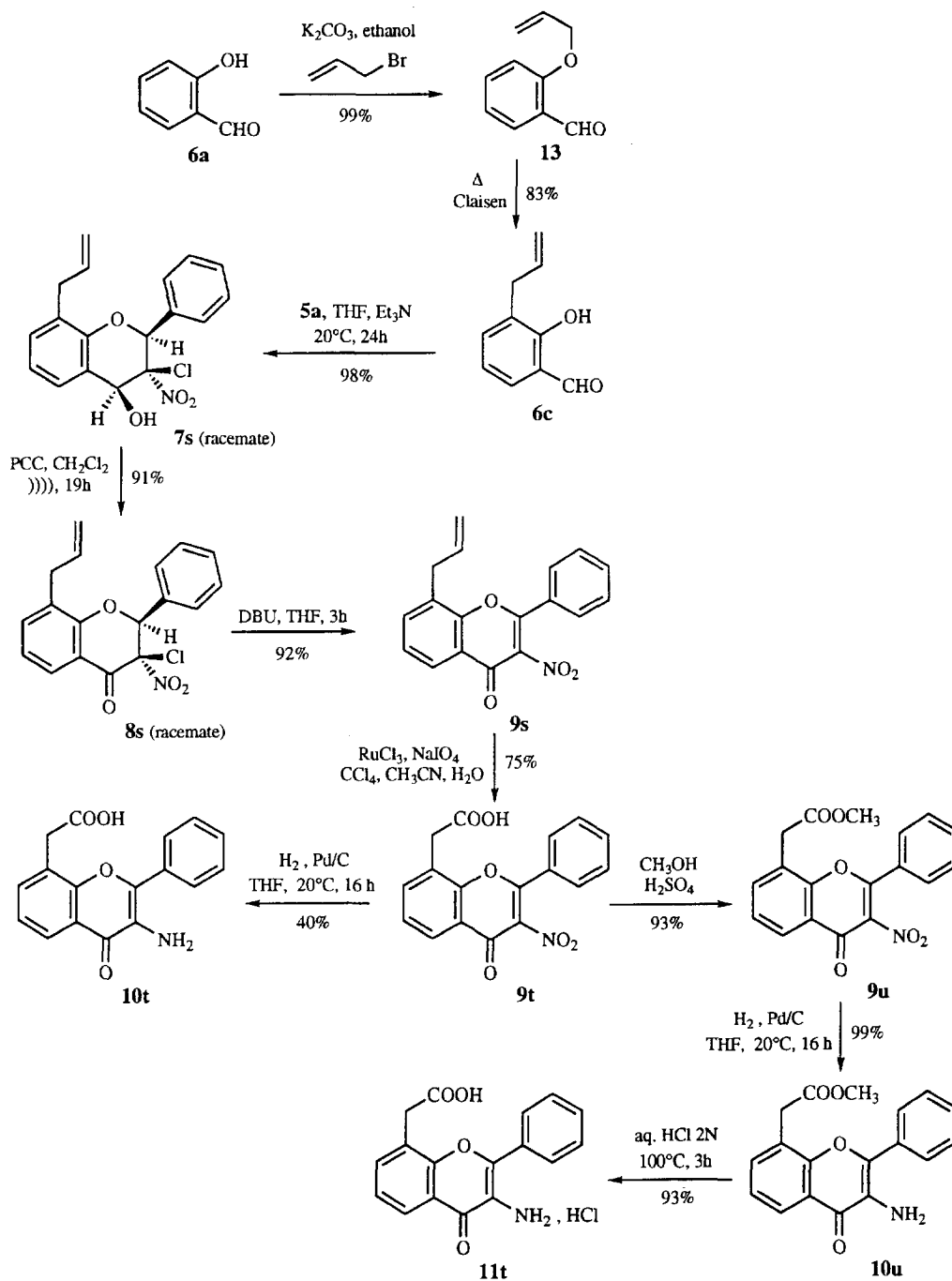


Scheme 1.

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-amino-8-chloro-6-phenyl-2,3-dihydro-4H-chromene (**10b**) 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 cirsiolol **2** (table I).

The 3-nitro synthesis precursors tested (**7j**, **8j**, **9t**) exhibited potent antiproliferative properties (ie, $\leq 10 \mu\text{M}$), 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'-OCH₃) and **10m** (3'-NH₂, 4'-OCH₃) had the lowest IC₅₀ 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'-NH₂ with a 4'-OCH₃ 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**, IC₅₀ = 187 μM), its 3-amino derivative **10t** had a 4.5-fold increase in potency (IC₅₀ = 41 μM) whereas its 3-nitro derivative **9t** had a 20-fold increase in antiproliferative potency (IC₅₀ = 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 cirsiolol **2**, led to a marked increase in antiproliferative potency.

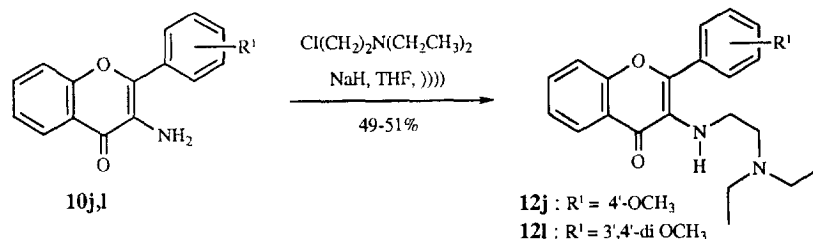
In conclusion, these results indicate that, in the 3-aminoflavone series, the 4'-methoxy group is important for activity. Moreover, in the flavone-8-acetic analogs, a marked increase in potency is observed with the addition of a 3-amino group or a 3-nitro group. Methoxy groups on the 6- and 7-positions 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 K f ler 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 ¹H-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 $\pm 0.3\%$ 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.



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 cirsiolol **2**.

<i>Compound</i>	<i>R</i> ¹	<i>R</i> ²	<i>IC</i> ₅₀ (μ <i>M</i>)
7j	4'-OCH ₃	H	4
8j	4'-OCH ₃	H	9
9j	4'-OCH ₃	H	>17
9t	H	8-CH ₂ COOH	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 ₂ COOH	41
12j	4'-OCH ₃	H	30
12l	3',4'-OCH ₃	H	58
8-FAA 1			187
Cirsiliol 2			3

IC₅₀ 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 cirsiol **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). ¹H-NMR (90 MHz; CDCl₃) 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 C₈H₇NO₄ (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. ¹H-NMR (300 MHz; CDCl₃) 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 D₂O). IR (CDCl₃) ν 1650 cm⁻¹; anal C₁₀H₁₀O₂ (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 × 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); ¹H-NMR (90 MHz; CDCl₃) 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 (CDCl₃) ν 1596, 1535, 1308 cm⁻¹; MS (EI) *m/z* 243, 245 (M⁺); anal C₁₀H₁₀ClNO₄ (C, H, N).

Z-(2-Chloro-2-nitroethenyl)-4-methoxy-3-nitrobenzene **5m**. Yield 60%; mp 126–128 °C (benzene/cyclohexane); ¹H-NMR (90 MHz; CDCl₃) 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 (CDCl₃) ν 1625, 1540, 1330 cm⁻¹; MS (EI) *m/z* 258, 260 (M⁺); anal C₉H₇ClN₂O₅ (C, H, N).

Z-(2-Chloro-2-nitroethenyl)-2-fluorobenzene **5n**. Yield 74%; mp 75–76 °C (hexane); ¹H-NMR (90 MHz; CDCl₃) δ 7.07–7.73 (m, 3H), 8.10 (dt, 1H, *J* = 7.5 Hz and 1.8 Hz), 8.60 (s, 1H); IR (CDCl₃) ν 1626, 1544, 1318 cm⁻¹; MS (EI) *m/z* 201, 203 (M⁺); anal C₈H₅ClFNO₂ (C, H, N).

Z-(2-Chloro-2-nitroethenyl)-4-fluorobenzene **5o**. Yield 77%; mp 76–77 °C (hexane); ¹H-NMR (90 MHz; CDCl₃) δ 7.17 (t, 2H, *J* = 8.5 Hz), 7.77–8.02 (m, 2H), 8.37 (s, 1H); IR (CDCl₃) ν 1625, 1537, 1318 cm⁻¹; MS (EI) *m/z* 201, 203 (M⁺); anal C₈H₅ClFNO₂ (C, H, N).

Z-(2-Chloro-2-nitroethenyl)-2,6-difluorobenzene **5p**. Yield 64%; mp 49–50 °C (pentane); ¹H-NMR (90 MHz; CDCl₃) δ 7.17 (br t, 2H, *J* = 8.1 Hz), 7.30–7.67 (m, 1H), 8.23 (s, 1H); IR (CDCl₃) ν 1637, 1555, 1323 cm⁻¹; MS (EI) *m/z* 219, 221 (M⁺); anal C₈H₄ClF₂NO₂ (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-2H-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 × 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 *2R*, *3R*, *4R*. 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); ¹H-NMR (300 MHz; CDCl₃) δ 2.85 (d, 1H, *J* = 12.1 Hz, exchangeable with D₂O), 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 (CDCl₃), ν 3558, 1570, 1520, 1346 cm⁻¹; MS (EI) *m/z* 365, 367 (M⁺); anal C₁₇H₁₆ClNO₆ (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); ¹H-NMR (250 MHz; CDCl₃) δ 2.52 (d, 1H exchangeable with D₂O, *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 (CDCl₃), ν 3560, 1571, 1535, 1360, 1346 cm⁻¹; MS (EI) *m/z* 380, 382 (M⁺); anal C₁₆H₁₃ClN₂O₇ (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); ¹H-NMR (300 MHz; CDCl₃) δ 2.52 (d, 1H exchangeable with D₂O, *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 (CDCl₃), ν 3552, 1570, 1342 cm⁻¹; MS (EI) *m/z* 323, 325 (M⁺); anal C₁₅H₁₁ClFNO₄ (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); ¹H-NMR (300 MHz; CDCl₃) δ 2.49 (d, 1H exchangeable with D₂O, *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 (CDCl₃), ν 3553, 1575, 1353 cm⁻¹; MS (EI) *m/z* 323, 325 (M⁺); anal C₁₅H₁₁ClFNO₄ (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); ¹H-NMR (250 MHz; CDCl₃) δ 2.54 (d, 1H exchangeable with D₂O, *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 (CDCl₃), ν 3562, 1580, 1362 cm⁻¹; MS (EI) *m/z* 341, 343 (M⁺); anal C₁₅H₁₀ClF₂NO₄ (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); ¹H-NMR (250 MHz; CDCl₃) δ 2.85 (d, 1H, *J* = 8.2 Hz, exchangeable with D₂O), 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 (CDCl₃), ν 3557, 1581, 1530, 1348 cm⁻¹; MS (EI) *m/z* 350, 352 (M⁺); anal C₁₅H₁₁ClN₂O₆ (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); ¹H-NMR (250 MHz; CDCl₃) δ 2.86 (d, 1H exchangeable with D₂O, *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 (CDCl₃), ν 3560, 1580, 1532, 1352, 1340 cm⁻¹; MS (EI) *m/z* 395, 397 (M⁺); anal C₁₅H₁₀ClN₃O₈ (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). ¹H-NMR (250 MHz; CDCl₃) δ 2.45 (d, 1H exchangeable with D₂O, *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 (CDCl₃), ν 3557, 1571, 1348 cm⁻¹; MS (CI) *m/z* 363, 365 (M + NH₄⁺); anal C₁₈H₁₆ClNO₄ (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-4H-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-2H-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). ¹H-NMR (250 MHz; CDCl₃) 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 (CDCl₃), ν 1713, 1581, 1520, 1335 cm⁻¹; MS (EI) *m/z* 363, 365 (M⁺); anal C₁₇H₁₄ClNO₆ (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); ¹H-NMR (300 MHz; CDCl₃) 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 (CDCl₃), ν 1722, 1580, 1539, 1361, 1348 cm⁻¹; MS (EI) *m/z* 378, 380 (M⁺); anal C₁₆H₁₁ClN₂O₇ (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); ¹H-NMR (300 MHz; CDCl₃) 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 (CDCl₃), ν 1710, 1579, 1342 cm⁻¹; MS (EI) *m/z* 321, 323 (M⁺); anal C₁₅H₉ClFNO₄ (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); ¹H-NMR (250 MHz; CDCl₃) 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 (CDCl₃), ν 1719, 1579, 1344 cm⁻¹; MS (EI) m/z 321, 323 (M⁺); anal C₁₅H₉ClFNO₄ (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); ¹H-NMR (250 MHz; CDCl₃) 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 (CDCl₃), ν 1719, 1582, 1339 cm⁻¹; MS (EI) m/z 339, 341 (M⁺); anal C₁₅H₈ClF₂NO₄ (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); ¹H-NMR (300 MHz; CDCl₃) 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 (CDCl₃), ν 1730, 1581, 1540, 1362, 1350 cm⁻¹; MS (EI) m/z 348, 350 (M⁺); anal C₁₅H₈ClN₂O₆ (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); ¹H-NMR (300 MHz; CDCl₃) 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 (CDCl₃), ν 1729, 1582, 1538, 1357, 1349 cm⁻¹; MS (EI) m/z 393, 395 (M⁺); anal C₁₅H₈ClN₃O₈ (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); ¹H-NMR (250 MHz; CDCl₃) 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 (CDCl₃), ν 1713, 1580, 1341 cm⁻¹; MS (CI) m/z 361, 363 (M + NH₄⁺); anal C₁₈H₁₄ClNO₄ (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-4H-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); ¹H-NMR (250 MHz; CDCl₃) 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 (CDCl₃), ν 1661, 1517, 1330 cm⁻¹; MS (EI) m/z 327 (M⁺); anal C₁₇H₁₃NO₆ (C, H, N).

2-(4-Methoxy-3-nitrophenyl)-3-nitro-4H-1-benzopyran-4-one 9m. Yield 87%; mp 221–223 °C (benzene/hexane); ¹H-NMR (250 MHz; CDCl₃) δ 4.07 (s, 3H), 7.15–7.30 (m, 2H), 7.42–7.68 (m, 2H), 7.74–7.91 (m, 2H), 8.22–8.38 (m, 1H); IR (CDCl₃), ν 1664, 1539, 1349 cm⁻¹; MS (EI) m/z 342 (M⁺); anal C₁₆H₁₀N₂O₇ (C, H, N).

2-(2-Fluorophenyl)-3-nitro-4H-1-benzopyran-4-one 9n. Yield 88%; mp 154–156 °C (benzene/heptane); ¹H-NMR (250 MHz; CDCl₃) 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 (CDCl₃), ν 1671, 1529, 1368 cm⁻¹; MS (EI) m/z 285 (M⁺); anal C₁₅H₈FNO₄ (C, H, N).

2-(4-Fluorophenyl)-3-nitro-4H-1-benzopyran-4-one 9o. Yield 94%; mp 136–138 °C (benzene/heptane); ¹H-NMR (250 MHz; CDCl₃) 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 (CDCl₃), ν 1670, 1538, 1364 cm⁻¹; MS (EI) m/z 285 (M⁺); anal C₁₅H₈FNO₄ (C, H, N).

2-(2,6-Difluorophenyl)-3-nitro-4H-1-benzopyran-4-one 9p. Yield 92%; mp 210–211 °C (benzene); ¹H-NMR (250 MHz; CDCl₃) 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 (CDCl₃), ν 1671, 1541, 1349 cm⁻¹; MS (EI) m/z 303 (M⁺); anal C₁₅H₇F₂NO₄ (C, H, N).

3,6-Dinitro-2-phenyl-4H-1-benzopyran-4-one 9q. Yield 81%; mp 203–205 °C (benzene/heptane); ¹H-NMR (300 MHz; CDCl₃) δ 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 (CDCl₃), ν 1679, 1553, 1362 cm⁻¹; MS (EI) m/z 312 (M⁺); anal C₁₅H₈N₂O₆ (C, H, N).

3,6-Dinitro-2-(4-nitrophenyl)-4H-1-benzopyran-4-one 9r. Yield 66%; mp 228–230 °C (benzene/hexane); ¹H-NMR (250 MHz; CDCl₃/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 (CDCl₃), ν 1720, 1538, 1351 cm⁻¹; MS (EI) m/z 357 (M⁺); anal C₁₅H₇N₃O₈ (C, H, N).

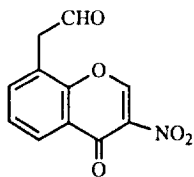
3-Nitro-2-phenyl-8-(prop-2-enyl)-4H-1-benzopyran-4-one 9s. Yield 92%; mp 156–157 °C (benzene/hexane); ¹H-NMR (300 MHz; CDCl₃) δ 3.72 (d, 2H, $J = 6.3$ Hz), 5.08–5.21 (m, 2H), 5.96–6.09 (m, 1H), 7.48 (t, 1H, $J = 7.6$ Hz), 7.52–7.68 (m, 4H), 7.72–7.80 (m, 2H), 8.21 (dd, 1H, $J = 8.0$ Hz and 1.5 Hz); IR (CDCl₃), ν 1663, 1538, 1378 cm⁻¹; MS (CI) m/z 325 (M + NH₄⁺), 308 (M + H)⁺; anal C₁₈H₁₃NO₄ (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 efficiently 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); ¹H-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 C₁₇H₁₁NO₆ (C, H, N).

This reaction produced also a small amount of the 3-nitro-4-oxo-2-phenyl-4H-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); ¹H-NMR (250 MHz; CDCl₃) 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 (CDCl₃), ν 1729, 1667, 1628, 1539, 1376 cm⁻¹; MS (CI) *m/z* 310 (M + H)⁺; anal C₁₇H₁₁NO₅ (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 dichloro-

methane) to afford pure **9u** which was combined with the previously filtered product. Yield 93%; mp 148–150 °C (benzene/heptane); ¹H-NMR (250 MHz; CDCl₃) 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 (CDCl₃), ν 1741, 1666, 1538, 1377 cm⁻¹; MS (CI) *m/z* 340 (M + H)⁺; anal C₁₈H₁₃NO₆ (C, H, N).

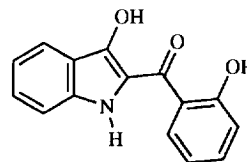
Preparation of 3-amino-2-aryl-4H-1-benzopyran-4-ones **10a**, **10e–r**, **10t** and **10u**

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); ¹H-NMR (300 MHz; CDCl₃) δ 4.04 (br s, 2H, exchangeable with D₂O), 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 (CDCl₃), ν 3450, 3360, 1626, 1585, 1561, 1480, 1471, 1419 cm⁻¹; MS (EI) *m/z* 237 (M⁺); anal C₁₅H₁₁NO₂ (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); ¹H-NMR (300 MHz; DMSO-*d*₆) δ 5.00–7.00 (sh, 3H, exchangeable with D₂O), 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 D₂O); IR (KBr), ν 1636, 1557, 1495, 1448 cm⁻¹; MS (EI) *m/z* 252 (M⁺); anal C₁₅H₁₂N₂O₂ (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.

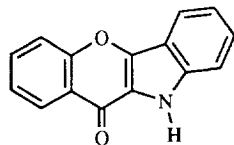
Side-product 1: 3-hydroxy-2-(2-hydroxybenzoyl)indole.



Yield 25%; mp 142–144 °C with sublimation (benzene/heptane); ¹H-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 D₂O), 10.77 (s, 1H, exchangeable with D₂O); 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 C₁₅H₁₁NO₃ (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); ¹H-NMR (300 MHz; DMSO-*d*₆) δ 7.28 (ddd, 1H, *J* = 8.0 Hz, 6.8 Hz and 1.3 Hz), 7.49–7.60 (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 D₂O); IR (KBr), ν 1641, 1607, 1537, 1510, 1459 cm⁻¹; MS (EI) m/z 235 (M⁺); anal C₁₅H₉NO₂ (C, H, N).

2-(3-Aminophenyl)-3-amino-4H-1-benzopyran-4-one 10f. Yield 98%; mp 213–215 °C (benzene/cyclohexane); ¹H-NMR (250 MHz; CDCl₃) δ 3.90 (br s, 2H, exchangeable with D₂O), 4.05 (br s, 2H, exchangeable with D₂O), 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 (CDCl₃), ν sh 3500–3300, 1622, 1559, 1517, 1480, 1472, 1418 cm⁻¹; MS (EI) m/z 252 (M⁺); anal C₁₅H₁₂N₂O₂ (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); ¹H-NMR (250 MHz; CDCl₃) δ 3.96 (br s, 2H, exchangeable with D₂O), 4.02 (br s, 2H, exchangeable with D₂O), 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 (CDCl₃), ν sh 3500–3300, 1620, 1558, 1518, 1479, 1471, 1413 cm⁻¹; MS (EI) m/z 252 (M⁺); anal C₁₅H₁₂N₂O₂ (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); ¹H-NMR (300 MHz; CDCl₃) δ 3.93 (s, 3H), 3.99 (br s, 2H, exchangeable with D₂O), 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 (CDCl₃), ν 3430, 3360, 1626, 1583, 1563, 1471, 1418 cm⁻¹; MS (EI) m/z 267 (M⁺); anal C₁₆H₁₃NO₃ (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); ¹H-NMR (300 MHz; CDCl₃) δ 3.90 (s, 3H), 4.07 (br s, 2H, exchangeable with D₂O), 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 (CDCl₃), ν 3445, 3360, 1626, 1583, 1561, 1481, 1471, 1416 cm⁻¹; MS (EI) m/z 267 (M⁺); anal C₁₆H₁₃NO₃ (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); ¹H-NMR (250 MHz; CDCl₃) δ 3.88 (s, 3H), 4.07 (br s, 2H, exchangeable with D₂O), 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 (CDCl₃), ν 3440, 3355, 1622, 1561, 1513, 1471 cm⁻¹; MS (EI) m/z 267 (M⁺); anal C₁₆H₁₃NO₃ (C, H, N).

3-Amino-2-(3,4,5-trimethoxyphenyl)-4H-1-benzopyran-4-one 10k. Yield 93%; mp 170–172 °C (benzene/cyclohexane); ¹H-NMR (300 MHz; CDCl₃) δ 3.93 (s, 3H), 3.95 (s, 6H), 4.03 (br s, 2H, exchangeable with D₂O), 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 (CDCl₃), ν 3440, 3355, 1625, 1584, 1561, 1505, 1479, 1466, 1424 cm⁻¹; MS (EI) m/z 327 (M⁺); anal C₁₈H₁₇NO₅ (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); ¹H-NMR (300 MHz; CDCl₃) δ 3.93 (s, 3H), 3.95 (s, 3H), 4.10 (br s, 2H, exchangeable with D₂O), 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 (CDCl₃), ν 3440, 3350, 1614, 1560, 1516, 1471, 1421 cm⁻¹; MS (EI) m/z 297 (M⁺); anal C₁₇H₁₅NO₄ (C, H, N).

3-Amino-2-(3-amino-4-methoxyphenyl)-4H-1-benzopyran-4-one 10m. Yield 89%; mp 141–142 °C (benzene/heptane); ¹H-NMR (250 MHz; CDCl₃) δ 3.80 (br s, 4H, exchangeable with D₂O), 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 (CDCl₃), ν sh 3500–3300, 1618, 1558, 1516, 1471, 1421 cm⁻¹; MS (EI) m/z 282 (M⁺); anal C₁₆H₁₄N₂O₃ (C, H, N).

3-Amino-2-(2-fluorophenyl)-4H-1-benzopyran-4-one 10n. Yield 99%; mp 128–130 °C (cyclohexane); ¹H-NMR (250 MHz; CDCl₃) δ 3.49 (br s, 2H, exchangeable with D₂O), 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 (CDCl₃), ν 3455, 3365, 1631, 1563, 1480, 1471, 1420 cm⁻¹; MS (EI) m/z 255 (M⁺); anal C₁₅H₁₀FNO₂ (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); ¹H-NMR (250 MHz; CDCl₃) δ 3.99 (br s, 2H, exchangeable with D₂O), 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 (CDCl₃), ν 3438, 3340, 1626, 1562, 1480, 1471, 1424 cm⁻¹; MS (EI) m/z 255 (M⁺); anal C₁₅H₁₀FNO₂ (C, H, N).

3-Amino-2-(2,6-difluorophenyl)-4H-1-benzopyran-4-one 10p. Yield 98%; mp 141–143 °C (benzene/cyclohexane); ¹H-NMR (300 MHz; CDCl₃) δ 3.19 (br s, 2H, exchangeable with D₂O), 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 (CDCl₃), ν 3465, 3370, 1624, 1585, 1564, 1479, 1420 cm⁻¹; MS (EI) m/z 273 (M⁺); anal C₁₅H₉F₂NO₂ (C, H, N).

3,6-Diamino-2-phenyl-4H-1-benzopyran-4-one 10q. Yield 90%; mp 162–164 °C (benzene/heptane); ¹H-NMR (250 MHz; DMSO-*d*₆) δ 4.55 (br s, 2H, exchangeable with D₂O), 5.41 (br s, 2H, exchangeable with D₂O), 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 C₁₅H₁₃N₂O₂ (C, H, N).

2-(4-Aminophenyl)-3,6-diamino-4H-1-benzopyran-4-one 10r. Yield 94%; mp 262 °C (benzene/acetonitrile); ¹H-NMR (300 MHz; DMSO-*d*₆) δ 4.28 (br s, 2H, exchangeable with D₂O), 5.34 (br s, 2H, exchangeable with D₂O), 5.69 (br s, 2H, exchangeable with D₂O), 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 C₁₅H₁₃N₃O₂ (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. ¹H-NMR (300 MHz; DMSO-*d*₆) δ 3.92 (s, 2H), 4.79 (br s, 2H, exchangeable with D₂O), 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 C₁₇H₁₃NO₄ (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); ¹H-NMR (250 MHz; CDCl₃) δ 3.70 (s, 3H), 3.94 (s, 2H), 4.08 (br s, 2H, exchangeable with D₂O), 7.35 (dd, 1H, *J* = 8.0 Hz 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 (CDCl₃), ν 3450, 3360, 1737, 1627, 1566, 1489, 1449, 1420 cm⁻¹; MS (EI) *m/z* 310 (M + H)⁺; anal C₁₈H₁₅NO₄ (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); ¹H-NMR (300 MHz; CDCl₃) δ 3.50 (br s, 2H, exchangeable with D₂O), 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 (CDCl₃) ν 3450, 3370, 1632, 1584, 1563, 1472, 1424 cm⁻¹; MS (EI) *m/z* 271, 273 (M⁺); anal C₁₅H₁₀ClNO₂ (C, H, N).

3-Amino-2-(3-chlorophenyl)-4H-1-benzopyran-4-one 10c. Yield 85%; mp 131–133 °C (benzene/cyclohexane); ¹H-NMR (300 MHz; CDCl₃) δ 3.70 (br s, 2H, exchangeable with D₂O), 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 (CDCl₃), ν 3445, 3360, 1631, 1585, 1562, 1473, 1419 cm⁻¹; MS (EI) *m/z* 271, 273 (M⁺); anal C₁₅H₁₀ClNO₂ (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); ¹H-NMR (300 MHz; CDCl₃) δ 3.20 (br s, 2H, exchangeable with D₂O), 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 (CDCl₃), ν 3445, 3360, 1626, 1584, 1563, 1472, 1422 cm⁻¹; MS (EI) *m/z* 271, 273 (M⁺); anal C₁₅H₁₀ClNO₂ (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. ¹H-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 C₁₇H₁₄ClNO₄ (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); ¹H-NMR (250 MHz; CDCl₃) 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 D₂O); IR (CDCl₃), ν 1611, 1567, 1511, 1467, 1380 cm⁻¹; MS (CI) m/z 367 (M + H)⁺; anal C₂₂H₂₆N₂O₃ (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); ¹H-NMR (250 MHz; CDCl₃) 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 D₂O); IR (CDCl₃), ν 1614, 1569, 1515, 1468, 1420, 1377 cm⁻¹; MS (CI) m/z 397 (M + H)⁺; anal C₂₃H₂₈N₂O₄ (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% CO₂ 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 (IC₅₀), ie, the concentration at which the growth was 50% of control cells.

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