Synthesis and Biological Evaluation of Novel Flavone-8-acetic Acid Derivatives as Reversible Inhibitors of Aminopeptidase N/CD13

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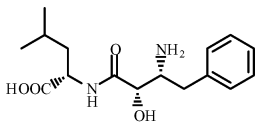
The cell surface aminopeptidase N (APN/CD13), overexpressed in tumor cells, plays a critical role in angiogenesis. However, potent, selective, and, particularly, noncytotoxic inhibitors ot this protein are lacking, and the present work was undertaken with the aim of developing a new generation of noncytotoxic inhibitors that bind to APN/CD13. In this context, we have synthesized a series of novel flavone-8-acetic acid derivatives. Among the herein described and evaluated compounds, the 2′,3-dinitroflavone-8-acetic acid (**19b**) proved to be the most efficient and exhibited an IC_{50} of 25 μM which is 2.5 times higher than that of bestatin (**1**), the natural known inhibitor of APN/CD13. However, in contrast to bestatin (**1**), the dinitroflavone **19b** did not induce any cytotoxicity to cultured human model cells. The presence of other substituents such as NO_2 or OCH_3 groups at the 3′- or 4′-position of the B phenyl group, or the existence of steric constraints (compounds **24** and **29**), did not improve selectivity and potency. The flavone **19b** affinity for APN/CD13 is not recovered with other proteases such as matrix metalloproteinase-9 (MMP-9), angiotensin converting enzyme (ACE/CD143), neutral endopeptidase (NEP/CD10), γ-glutamyl transpeptidase (CD224), or the serine proteases dipeptidyl peptidase IV (DPPIV/CD26) or cathepsin G.

Introduction

Cell surface peptidases (ectopeptidases) constitute a family of transmembrane enzymes present in a wide variety of tissues and cell types.[1,2] They have been implicated in major biological processes such as metabolism regulation, cell proliferation, survival, and invasiveness.[1,2] Aminopeptidase N (APN/CD13) (EC 3.4.11.2; CD13) is a widespread ectopeptidase which preferentially releases neutral amino acids from the N-terminal end of peptides.[3,4] The function of APN/CD13 depends on its location. In the intestinal brush border, the enzyme is involved in the terminal degradation of small peptides.[4,5] In synaptic membranes, APN/CD13 inactivates neuropeptides (endorphins and enkephalins).[4,5] Other biologically active peptides are angiotensins and chemotactic peptides.[4,5] For example, inhibition of APN/CD13 enhances neutrophil chemotaxis in response to formyl methionyl leucine phenylalanine (f-MLP) and substance P by preserving the integrity of these inflammatory peptides.[1] APN/CD13 could play a role in cell surface antigen processing trimming peptides bound to HLA DR molecules.[6]

The HELAH motif of the Zn^{2+}-binding active site of APN/CD13 reveals its metallopeptidase nature.[5] Thiol groups are efficient zinc chelators for APN, and natural inhibitors such as actinonin and bestatin (Ubenimex, **1**) have been discovered and isolated.[2] Several studies have indicated that interaction of APN/CD13 with **1** blocks cell proliferation in various cell types including leukocytes, epithelial and melanoma cells.[1,2] In addition, **1** has been shown to induce apoptosis of T and myeloid cell lines.[1,2] On the other hand, tumor cells which overexpress APN/CD13 (such as melanoma cells, acute lymphocytic leukemic cells, and urological cancer cells) are highly motile and capable of migration through extracellular matrix, and APN inhibition by **1** leads to a loss of motility.[2,4,7−9] Likewise, CD13 antibodies inhibit cell growth[1,2,4,10] and cell motility.[11] Inhibition of APN/CD13 could prevent the secretion of type IV epithelial collagenase (MMP-9) implicated in collagen degradation.[9]



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Angiogenesis is a cascade of processes emanating from microvascular endothelial cells and plays a central role in tumor growth and metastasis.[12] Recently, APN/CD13 has received a great deal of detailed attention with regard to angiogenesis.[13,14] First, APN/CD13 is expressed exclusively within the endothelial vasculature of mouse and human tumors but not in normal vasculature.[13] Second, APN antagonists (CD13 antibodies or bestatin) significantly block induced-retinal neovascularization in mice and in in vitro chorioallantoic membrane angiogenesis.[13−15] Although bestatin has shown antitumor therapeutic effects in several clinical trials,[16,17] limitations to its use in vivo include a nonspecific toxicity.[18]

A survey of the literature indicates that flavonoids could play a prominent role in cancer prevention by

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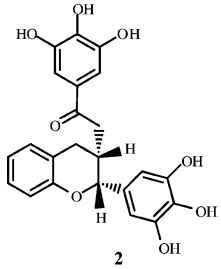
interfering with cell proliferation, survival, cell signaling, and regulating the immune system.[19−21] The antiangiogenic effects of some flavonoids have already been described.[22−26] In this context, the polyphenol epigallocatechin-3-gallate (EGCG, **2**) is a direct inhibitor of leukocyte elastase and matrix metalloproteinases (MMP) 2 and 9, enzymes often overexpressed in cancer and degrading the extracellular matrix.[27,28] Furthermore, **2** inhibits elastase at concentrations of 50, 150, and 2500 times lower than effective on MMP-2 and -9, thrombin and cathepsin G, respectively.[28] The inhibitory effects on proteases of nonphenolic constituents have also been detailed.[29,30] In the family of flavones, nobiletin (**3**) inhibits the enzymatic activity of MMP-9[30] whereas, at 0.3×10^{-3} M, baicalein (**4**) inhibits the ectopeptidases APN/CD13 and neutral endopeptidase (NEP/CD10; EC 3.4.24.11) by 57% and 36%, respectively.[29] At the same concentration, two flavanol derivatives, quercetin (**5**) and isorhamnetin (**6**), respectively, inhibit the activities of NEP/CD10 (73%) and APN/CD13 (49%).[29]



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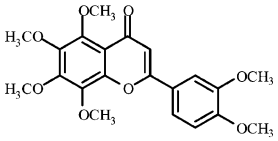

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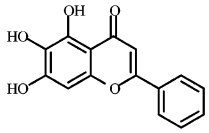

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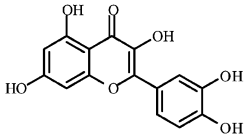

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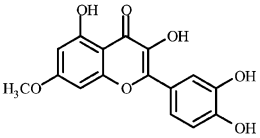

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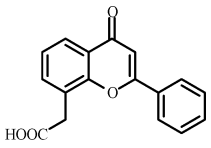
Additional studies have also indicated that some flavonoids present antiangiogenic activities by suppressing in vitro endothelial cell growth and the formation of new blood vessels in the chick embryo chorioallantoic membrane.[13] In this same context, flavone-8-acetic acid (FAA, **7**), a synthetic flavonoid known to induce significant growth delay and regression of numerous solid tumors subcutaneously implanted in mice,[31−35] has been shown to be an efficient antiangiogenic agent.[36] However, the promising activity observed in murine models was not transposed in humans as reflected by the disappointing results obtained in the numerous clinical trials carried out with FAA (**7**).[37]



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In an attempt to discover novel noncytotoxic inhibitors of APN/CD13, we became interested in exploring a flavonoid track leading to the investigation of a series of novel flavone-8-acetic acid derivatives. We report herein the synthesis and biological data for compounds belonging to this family which exhibit selective inhibition of APN/CD13 on a myeloid cell line from human origin.

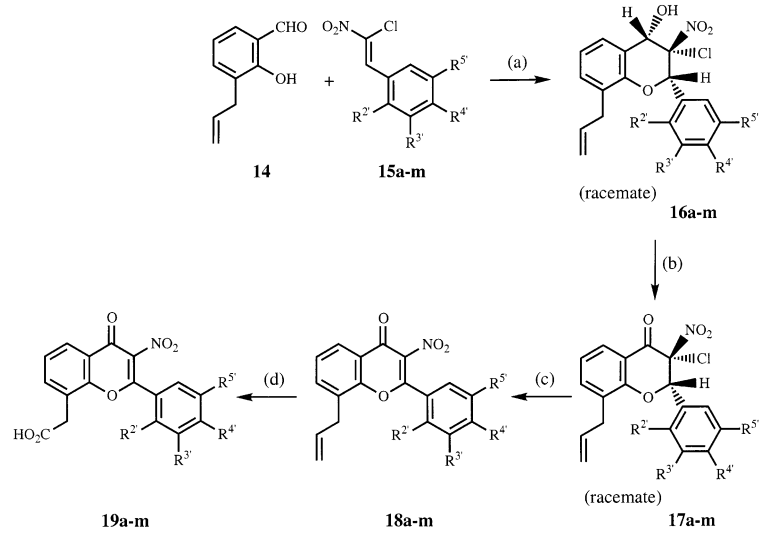
Chemistry

With regard to the chemistry, the already known 3-nitroflavone (**10**) and 2′,3-dinitroflavone (**11**),[38] as well as the new 8-substituted-3-nitroflavones **12**, **13**, and **19a**−**m** described herein, were prepared by adapting a methodology developed in our laboratory.[39] Our approach required readily available substituted aromatic aldehydes as starting materials and proved, in our context, to be more versatile and more convenient than the previously reported classical routes to flavones. The general procedure for the preparation of the desired flavone-8-acetic acid derivatives is depicted in Scheme 1.

The starting 2-hydroxy-3-allylbenzaldehyde (**14**) was easily synthesized on a large scale from salicylaldehyde via a Claisen rearrangement of the intermediate 2-allyloxybenzaldehyde.[40] The subsequent concerted Michael condensation of **14** with the appropriate *Z*-(2-chloro-2-nitroethenyl)benzenes **15a**−**m** (prepared by reacting the conveniently substituted aldehydes with bromonitromethane in the presence of an excess of dimethylammonium chloride and a small amount of potassium fluoride in refluxing xylene[41]) gave the 8-allyl-3-chloro-3,4-dihydro-4-hydroxy-3-nitro-2-phenyl-2*H*-1-benzopyrans **16a**−**m** in the exclusive 2*S*,3*S*,4*S* relative configuration. These intermediates were further oxidized in dry dichloromethane using pyridinium chlorochromate (PCC) in an ultrasonically assisted process to provide the 8-allyl-3-chloro-2,3-dihydro-3-nitro-2-phenyl-4*H*-1-benzopyran-4-ones **17a**−**m** with the 2*S*,3*R* relative configuration (it must be pointed out that these oxidation reactions never go to completion under mechanical stirring, even if a very large excess of PCC is used for a prolonged period of time). The obtained oxo derivatives **17a**−**m**, on treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in anhydrous tetrahydrofuran, were then easily converted, at room temperature, into the 8-allyl-3-nitro-2-phenyl-4*H*-1-benzopyran-4-ones **18a**−**m** by the anti elimination of a molecule of hydrochloric acid. Subsequent oxidative cleavage of the allylic double bond of the nitroflavone derivatives **18a**−**m** was next achieved by adapting the procedure reported by Sharpless and co-workers, using the ruthenium(III) chloride/sodium periodate system in the acetonitrile/water/carbon tetrachloride ternary mixture as the solvent,[42] to afford the desired 3-nitro-4-oxo-2-phenyl-4*H*-1-benzopyran-8-acetic acids **19a**−**m** in moderate to good yields.

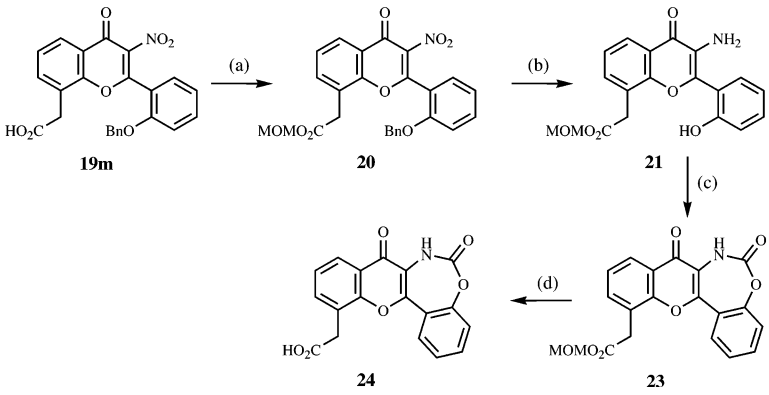
In addition, several conformationally constrained tetracyclic analogues (**24**, **28**, and **29**) of the acids **19** were also prepared. Thus, as outlined in Scheme 2, the cyclic carbamate **24** was obtained starting from the acid **19m** via its methoxymethyl ester **20**. Concomitant catalytic reduction of both nitro and benzyl groups of **20** by hydrogen in the presence of palladium-on-charcoal in ethyl acetate at room temperature gave the ami-

Scheme 1[a]



[a] Reagents, conditions, and remarks: (a) Et_3N, anhydrous THF, 20 °C, 24 h; (b) PCC, anhydrous CH_2Cl_2,)))), 19 h; (c) DBU, anhydrous THF, 3 h; (d) $RuCl_3$, $NaIO_4$, CCl_4, MeCN, H_2O. Under certain conditions previously reported in the particular case of **18a**, the related aldehyde **12** can be obtained.[39] The corresponding methyl ester **13** has also been prepared starting from the acid **19a** using a standard method.[39]

Scheme 2[a]



[a] Reagents and conditions: (a) MOMCl, Et_3N, MeCN, 20 °C; (b) H_2, Pd/C 10%, EtOAc, 20 °C, 16 h; (c) triphosgene, Et_3N, anhydrous CH_2Cl_2, 20 °C, 5 h; (d) $MgBr_2$, anhydrous CH_2Cl_2, 20 °C, 16 h.

nophenol **21**. Reaction of this intermediate with triphosgene in dry dichloromethane in the presence of triethylamine provided the tetracyclic ester **23**, whose subsequent hydrolysis under mild conditions using magnesium bromide in the same solvent at room temperature[43] yielded the desired (6,8-dioxo-6,7-dihydro-8*H*-5,13-dioxa-7-azabenzo[3,4]cyclohepta[1,2-*b*]naphthalen-12-yl)acetic acid (**24**).

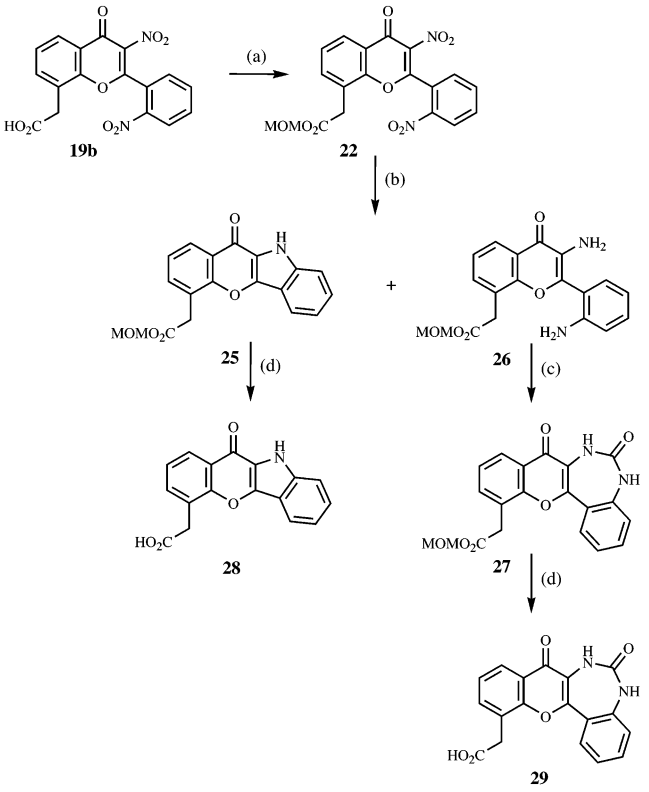
The synthesis of the cyclic urea **29** is depicted in Scheme 3 starting from **19b** via the preparation of its methoxymethyl ester **22** followed by the catalytic reduction of the two nitro groups. This latter hydrogenation afforded a mixture of the azabenzo[*b*]fluorene **25** (probably resulting from the nucleophilic attack of the in situ formed anilino nitrogen atom on the carbon 3 bearing partially reduced species of the nitro group of the flavone) and of the expected diamine **26** in a **25/26** = 55/45 ratio. Further condensation of **26** with triphosgene in anhydrous dichloromethane in the presence of triethylamine gave the tetracyclic diaza derivative **27**. Subsequent hydrolysis of the methoxymethyl esters **25** and **27** employing magnesium bromide in dry dichloromethane at room temperature yielded the corresponding (10-oxo-10,11-dihydro-5-oxa-11-azabenzo[*b*]fluoren-6-yl)-acetic acid (**28**) and (6,8-dioxo-5,6,7,8-tetrahydro-13-oxa-5,7-diazabenzo[3,4]cyclohepta[1,2-*b*]naphthalen-12-yl)-acetic acid (**29**), respectively. In this context, it is worth mentioning that the choice of the methoxymethyl group to form the intermediate esters was guided by the fact that the use of more classical alkyl esters (e.g., methyl esters) required too drastic hydrolytic conditions, incompatible with the stability of the molecules.

The hitherto unknown 2′-nitroflavone-8-acetic acid (**8**), required for comparison, was synthesized via the classical Baker−Venkataraman approach following a procedure already described to obtain related compounds (Scheme 4).[44,45] The ammonium salt **9** was prepared from the corresponding nitro derivative as previously reported.[39]

Results and Discussion

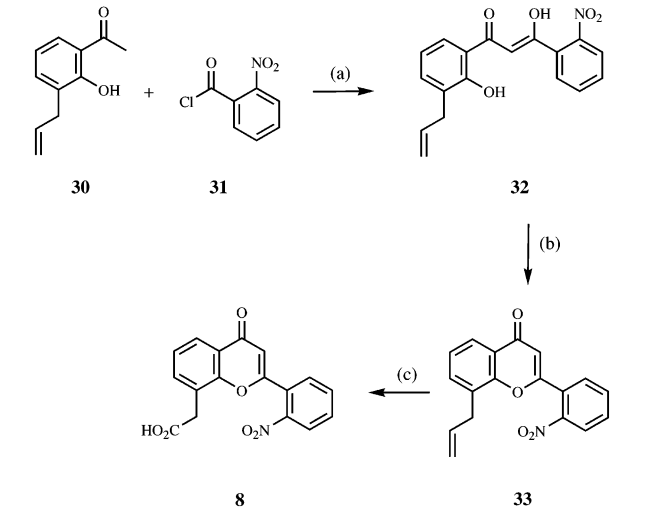
In Vitro Assessment of Cytotoxicity. The effects of the tested compounds on the U937 cell growth and cytotoxicity were evaluated upon continuous exposure at concentrations ranging from 10^{-7} to 10^{-3} M. A treatment period of 3 days was selected since the control cells were still in the exponential growth phase at this time. At the highest concentration, cell mortality ex-

Scheme 3[a]



[a] Reagents, conditions, and remarks: (a) MOMCl, Et_3N, MeCN, 20 °C; (b) H_2, Pd/C 10%, EtOAc, 20 °C, 16 h. Compounds were isolated in a **25**/**26** = 55/45 ratio; (c) triphosgene, Et_3N, anhydrous CH_2Cl_2, 20 °C, 5 h; (d) $MgBr_2$, anhydrous CH_2Cl_2, 20 °C, 16 h.

Scheme 4[a]



[a] Reagents and conditions: (a) K_2CO_3, butanone reflux, 48 h; (b) MeOH, H_2SO_4, 20 °C, 3 days, then H_2O, 0 °C; (c) $RuCl_3$, $NaIO_4$, CCl_4, MeCN, H_2O.

ceeded 40% with all the products tested (data not shown). At 10^{-4} M, some derivatives exhibited cytotoxic potency (**8**, **19m**, **20**−**23** and to a lesser degree **11**, **12**, **19h**, **19l**, **24**, **28**) (Table 1) and markedly reduced cell proliferation (over 30%). In contrast, the proliferation of U937 cells was unaffected by the non cytotoxic **7**, **9**, **10**, **13**, **18b**, **19a**−**g**, **19i**−**k**, and **29**. In this context, it is worth pointing out that bestatin (**1**) is much more toxic for U937 cells than most of the herein studied compounds. Moreover, we have assessed that the IC_{50} (concentration that caused 50% necrosis compared with the control value) for the above-mentioned derivatives were over 5×10^{-4} M (data not shown). In addition, none of the products considered in the present study induced apoptosis (data not shown).

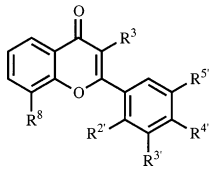
In Vitro Inhibition of APN Activity. In a first set of experiments, we assessed the ability of the tested compounds to inhibit APN activity expressed by intact U937 cells (Table 1). The potencies of these molecules varied somewhat, depending upon the nature of their substituent(s). Our results indicated that derivatives **19b**−**d**, which bear a CH_2COOH group in the 8-position and two NO_2 substituents in both 2′- and 3′-positions, inhibited efficiently APN activity, and this to the same extent as bestatin (**1**). Deletion (**19a**) or replacement (**19i**−**m**) of the NO_2 group in the 2′-position gave compounds with a lesser degree of potency against APN activity whereas the presence of an electron-donating methoxy group in the ortho (**19c**) or para position (**19d**) of the nitro substituent led to slightly lowered inhibitory effects. We have also checked that the removal of the nitro group in the 3-position (**8**), if it does not significantly affect the inhibition, provided a much more toxic compound (vide supra). A marked decreasing in potency is observed when nitro or methoxy substituents are located in 3′-and/or 4′-positions (**19e**−**h**). The other synthesized products exhibited less significant effects. In this context, it is particularly worthy of note that the flavone-8-acetic acid (**7**), the salt **9**, the 3-nitroflavone (**10**), the esters **13**, **20**−**22**, the allyl derivative **18b**, the amine **21**, the tetracyclic derivatives **23**, **24**, **28**, and **29**, and the flavone itself proved to unefficiently inhibit APN activity.

From these results, we have logically selected **19b** as the best noncytotoxic inhibitor of APN activity.

We next examined the effects of **19b** on a number of other metalloproteinases (NEP, ACE, MMP-9) and the unclassified γ-glutamyl transpeptidase (γ-GT) as well as serine proteases (DPPIV, cathepsin G). These enzymes were chosen because of their major involvement in different pathological conditions and thus represent targets for therapeutic intervention.[1,2,46,47] Proteolytic activities of APN, NEP, ACE, γ-GT, DPPIV, and cathepsin G were measured using absorbance-based assays. As expected, each protease activity was only downregulated by its specific inhibitor (Figure 1). The addition of **19b** (10^{-3} M) to the incubation medium strongly inhibited the proteolytic activity of APN, whereas the other enzyme activities were not significantly affected (Figure 1). The apparent affinity of APN present at the surface of U937 cells for Ala-pNA (i.e., $1/K_m$) exhibited a value of 10^3 M^{-1} and the apparent IC_{50} values of bestatin and **19b** were around 25 μM (data not shown). Therefore, one could consider that 1 molecule of **19b** could compete with approximately 25 molecules of the substrate Ala-pNA, thus indicating that **19b** merely exhibits a significant potent inhibitory effect toward APN.

The effect of **19b** toward the gelatinolytic activity of MMP-9 was assessed by zymography (Figure 2). By this technique, the gelatinolytic activity of MMP-9 was revealed by the presence of a 92 kDa band in native conditions (Figure 2a). Incubation of the zymograms with EDTA or the specific inhibitor of MMP-9 resulted

Table 1



compound	R^3	R^8	$R^{2'}$	$R^{3'}$	$R^{4'}$	$R^{5'}$	cytotoxicity (%)[a]	inhibition APN (%)[b]
control							<10	0
bestatin (**1**)							≥40	85 ± 3
flavone	H	H	H	H	H	H	<10	5 ± 9
FAA (**7**)	H	CH_2CO_2H	H	H	H	H	<10	30 ± 4
8	H	CH_2CO_2H	NO_2	H	H	H	≥80	83 ± 2
9	$NH_3^+Cl^-$	CH_2CO_2H	H	H	H	H	<10	33 ± 4
10	NO_2	H	H	H	H	H	<10	30 ± 9
11	NO_2	H	NO_2	H	H	H	≥40	≤5
12	NO_2	CH_2CHO	H	H	H	H	≥40	51 ± 3
13	NO_2	$CH_2CO_2CH_3$	H	H	H	H	<10	≤5
18b	NO_2	$CH_2CH{=}CH_2$	NO_2	H	H	H	<10	16 ± 2
19a	NO_2	CH_2CO_2H	H	H	H	H	<10	68 ± 6
19b	NO_2	CH_2CO_2H	NO_2	H	H	H	<10	88 ± 3
19c	NO_2	CH_2CO_2H	NO_2	OCH_3	H	H	<10	75 ± 2
19d	NO_2	CH_2CO_2H	NO_2	H	H	OCH_3	<10	80 ± 2
19e	NO_2	CH_2CO_2H	H	H	NO_2	H	<10	63 ± 1
19f	NO_2	CH_2CO_2H	H	NO_2	H	H	<10	44 ± 3
19g	NO_2	CH_2CO_2H	H	NO_2	OCH_3	H	<10	61 ± 4
19h	NO_2	CH_2CO_2H	H	H	OCH_3	H	≥40	64 ± 1
19i	NO_2	CH_2CO_2H	Cl	H	H	H	20−30	76 ± 1
19j	NO_2	CH_2CO_2H	OCH_3	H	H	H	≥20	69 ± 1
19k	NO_2	CH_2CO_2H	CO_2CH_3	H	H	H	<10	66 ± 1
19l	NO_2	CH_2CO_2H	CO_2Bn	H	H	H	≥40	77 ± 1
19m	NO_2	CH_2CO_2H	OBn	H	H	H	≥55	76 ± 1
20	NO_2	CH_2CO_2MOM[c]	OBn	H	H	H	≥95	32 ± 1
21	NH_2	CH_2CO_2MOM[c]	OH	H	H	H	≥65	19 ± 1
22	NO_2	CH_2CO_2MOM[c]	NO_2	H	H	H	≥85	48 ± 1
23							≥95	25 ± 3
24							20−30	19 ± 3
28							20−30	10 ± 2
29							<10	8 ± 4

[a] Cytotoxicity was measured after 3 days of culture of U937 for a 10^{-4} M concentration of the tested compound. [b] The reported inhibitions of APN activity (measured in the presence of intact U937 cells and Ala-pNA for 15 min at 37 °C) in the presence of the tested compounds (10^{-3} M) are the results of the mean of three to ten experiments ± SD. [c] MOM = CH_2OCH_3.

in the inhibition of the gelatinolytic activity of MMP-9 by 90% (Figure 2b and 2c). In contrast, **19b** did not alter the gelatinolytic activity of MMP-9 (Figure 2d). Similarly, the addition of bestatin (**1**), a noninhibitor of MMP-9, had no effect on enzyme activity (Figure 2e). Together, these data unambiguously demonstrated that **19b** selectively inhibits APN activity.

Specific inhibition of APN/CD13 by **19b**. Whole cell lysates from U937 cells were incubated in the absence or in the presence of MY7 antibody directed against CD13 antigen or mIgG1 (control isotype), as described in Experimental Procedures. The immunoprecipitates bound to Protein G-Sepharose were tested for APN activity. As shown in Figure 3, MY7 Ab was capable of precipitating APN activity from U937 cells. Both bestatin and **19b** efficiently inhibited APN/CD13 (Figure 3). These results confirm that **19b**, like bestatin (**1**), inhibits APN activity endowed by the ectoprotease CD13.

19b Reversibly and Competitively Inhibits APN/CD13 of U937 Cells. To investigate the reversible nature of the inhibition of APN/CD13 by **19b**, U937 cells were incubated with 10^{-3} M of **19b** or bestatin (**1**, used as a positive reversible inhibitor control) for 15 min before extensive washing and further incubation in the protease assay medium. As shown in Figure 4, APN inhibition observed with **19b** or bestatin was found to be lost upon cell washing, indicating the reversible nature of the inhibition by the dinitroflavone **19b**. We further examined the capacity of **19b** to bind to the catalytic site of APN/CD13 of intact U937 cells. For this purpose, we used two mAbs directed against CD13: WM15 Ab (which recognizes on CD13 the epitope involved in the catalytic activity) and MY7 Ab (which binds another epitope of CD13). Lineweaver−Burk plots revealed that inhibition by **19b** for WM15 Ab was competitive (Figure 5A), whereas the inhibition by **19b** for MY7 Ab fit with an uncompetitive inhibition model (Figure 5B). Together, our data indicate that **19b** is a reversible competitive inhibitor of APN/CD13.

Conclusions

We have herein reported on the capacity of some flavone-8-acetic acid derivatives to inhibit the enzymatic activity of the ectopeptidase APN/CD13. We have synthesized and tested a set of variously substituted compounds, and the best results were obtained with the compound **19b** bearing two NO_2 groups in both 2′- and 3-positions. This selected 2′,3-dinitroflavone-8-acetic acid (**19b**) proves to be noncytotoxic for the human model U937 cell line and exhibits an exclusive inhibitory APN/CD13 activity by reversibly binding to the catalytic site of the enzyme. Furthermore, the comparison of the apparent affinity of APN/CD13 of U937 cells for its

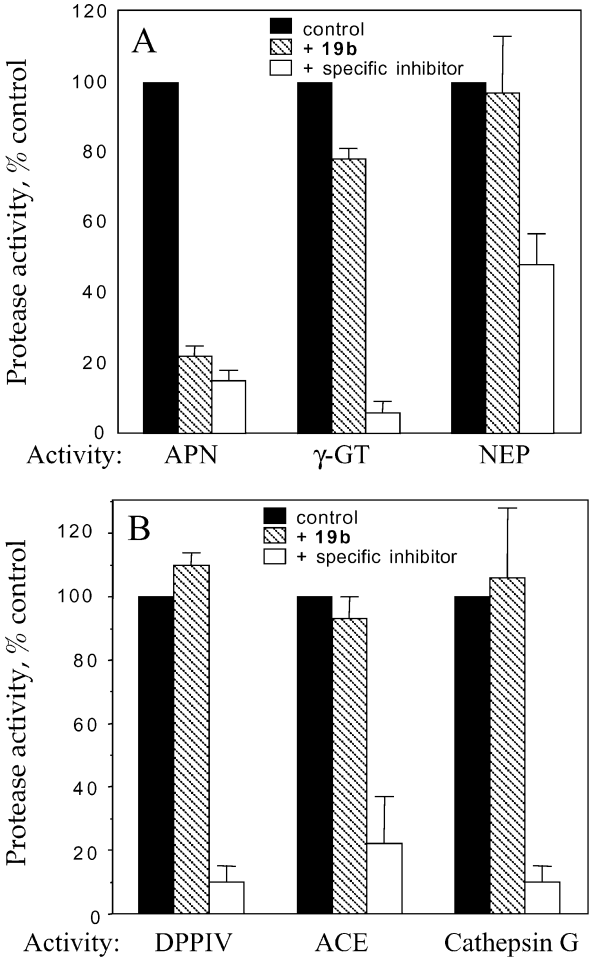


Figure 1. Effect of **19b** on various protease activities. (A) Intact U937 cells exhibit APN, γ-GT, and NEP activities at their surface. Relative enzyme activity was expressed as the percentage of the control activity without inhibitor: bestatin (APN inhibitor), acivicin (γ-GT inhibitor), phosphoramidon (NEP inhibitor), and **19b**. (B) Purified soluble proteases DPPIV, ACE, and cathepsin G were tested for their enzymatic activities in the absence or presence of their respective inhibitors (DFP for DPPIV and cathepsin G) (captopril for ACE) or **19b**. Values are expressed as percentages of controls and represent the mean ± SD (n = 3). All inhibitors were tested at 10^{-3} M.

synthetic substrate Ala-pNA (10^3 M^{-1}) with the apparent IC_{50} value of **19b** (around 25 μM) indicates that the inhibitory effect of **19b** toward APN is strong.

Apart from APN/CD13, we have examined the effect of **19b** on other metalloproteinase activities including MMP-9, NEP, and ACE, as well as on serine activities (DPPIV and cathepsin G) and on γ-GT, all proteases previously suggested to play critical roles in tumoral processes.[1,2,46,47] Our results indicate that **19b** exclusively inhibits the proteolytic activity of APN/CD13. The results obtained in the U937 cell line were also observed in other myeloid cell lines (HL-60, THP-1, Mono Mac6), T (Jurkat), B (Eskol, Ramos, RPMI 8266) and in normal (T, B, monocytes) and leukemic (LLC, LMC) leukocytes (data not shown). A growing accumulation of evidence shows the crucial importance of APN/CD13 in tumor metastasis and angiogenesis, both in vitro and in vivo.[2,4,7−9,11,13−15] When angiogenesis is blocked, tumor growth is often suppressed, and tumor cell proliferation is balanced by apoptosis. Modulation of growth arrest/

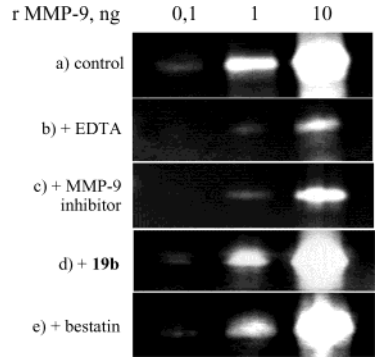


Figure 2. Absence of effect of **19b** on MMP-9 gelatinolytic activity. The gelatinolytic activity of recombinant 92 kDa pro-MMP-9 was analyzed using zymography performed with increasing amounts of protein loaded (0.1, 1, and 10 ng). Gelatinolytic activities are detected as clear bands in the gel. (a) in the absence, and in the presence of (b) EDTA, (c) the specific inhibitor of MMP-9 (2*R*)-2-[(4-biphenylsulfonyl)amino]3-phenylpropionic acid, (d) **19b**, (e) bestatin (**1**). All inhibitors were tested at 10^{-3} M.

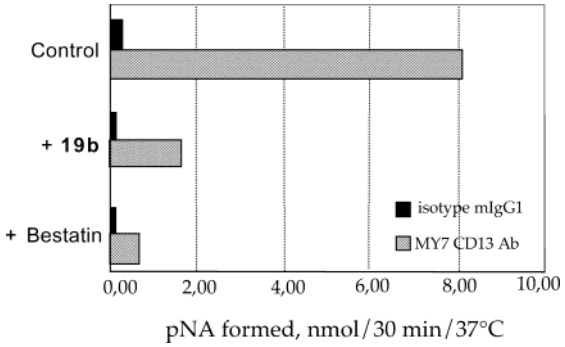


Figure 3. Immunoprecipitation of APN activity with MY-7 Ab directed against CD13, and inhibition by bestatin or **19b**. Whole cell lysates from U937 cells were immunoprecipitated with mIgG1 (control isotype) or MY7/CD13 Ab. Material bound to Protein G-Sepharose was tested for APN activity using Ala-pNA (1 mg/mL) in the absence or presence of bestatin or **19b** (10^{-3} M).

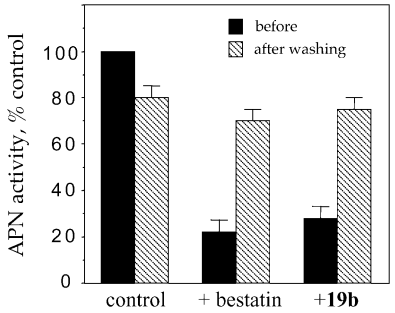


Figure 4. Reversibility of APN/CD13 inhibition by **19b**. Intact U937 cells were preincubated for 15 min in the absence (control) or presence of 10^{-3} M bestatin (**1**) or **19b**, washed twice in protease buffer, and then assayed for APN activity. Relative enzyme activity was expressed as the percentage of the control activity expressed by U937 cells which have not been washed and without inhibitor (mean ± SD, n = 3).

apoptosis is associated with signaling through protein phosphorylation.[48−50] There is evidence that tyrosine phosphorylation steps are involved in APN/CD13 signaling.[51−54] Intracellular calcium flux and MAP kinase phosphorylation are implicated in the activation of monocytes and T cells through APN/CD13.[52−54] Flavone-8-acetic derivatives, and particularly **19b**, may

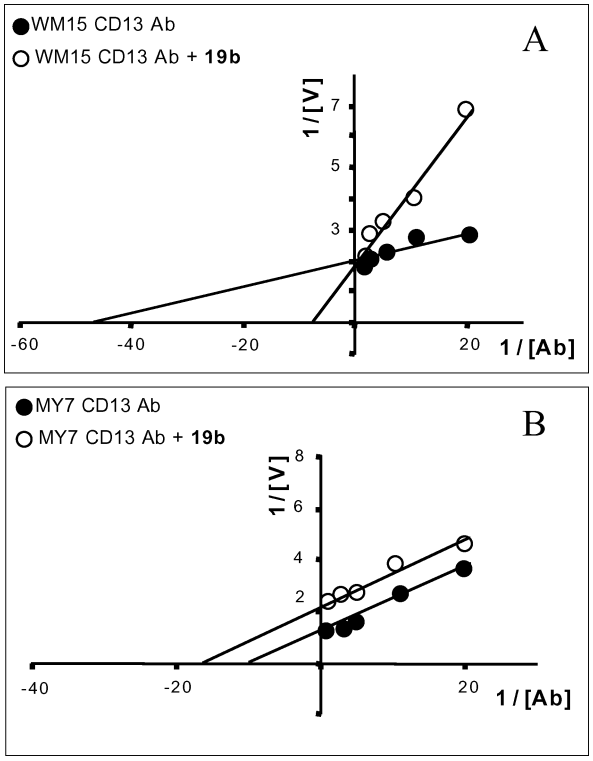


Figure 5. Lineweaver−Burk plots for **19b** and mAbs against CD13. Representative plots of 1/bound CD13 Ab to U937 cells vs 1/[CD13 Ab] in the presence or absence of **19b** (10^{-3} M) are shown. (A) WM15 Ab which recognizes on CD13 the epitope involved in the catalytic activity, and (B) MY7 Ab which binds another epitope of CD13. Staining of U937 cells with Abs in the absence or presence of **19b** was performed at 4 °C.

therefore represent valuable tools for investigating mechanisms involved in these processes.

In conclusion, this novel series of noncytotoxic inhibitors of APN bears the potential of producing selective APN-mediated antiangiogenic inhibitors. We hope to report some of these promising data soon.

Experimental Section

Chemistry. Starting materials and solvents were purchased from Acros, Aldrich or Avocado. Reference bestatin (**1**) and flavone were obtained from Sigma Chemical Co. A pure sample of flavone-8-acetic acid (**7**) was kindly provided by Merck (France). Melting points were measured on a Köfler hot stage apparatus and are uncorrected. Mass spectra were obtained with a Nermag-Ribermag R10−10C spectrometer applying a desorption chemical ionization (CI) technique using ammonia as the reagent gas. Infrared spectra were obtained with a Perkin-Elmer 1710 spectrophotometer for chloroform solutions or KBr disks. The ^{1}H NMR (300 MHz) spectra were recorded on 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), dd (doublet of doublet), ddd (doublet of doublet of doublet), dt (double triplet), m (multiplet), and br (broad signal). Coupling constants (*J* values) are listed in hertz (Hz). Reactions were monitored by analytical thin-layer chromatography, and products were visualized by exposure to UV light. Merck silica gel (230−400 Mesh ASTM) was used for column chromatography. Acetone, methanol, and dichloromethane employed as eluents for column chromatography were distilled on a rotary evaporator prior to use. Anhydrous benzene was obtained by distillation from calcium hydride. Dry THF was prepared by distillation from benzophenone/sodium. All yields reported are unoptimized. Elemental analysis for most of the new substances were performed by CNRS Laboratories (Vernaison, France) and, unless noted otherwise, the results obtained are within 0.4% of the theoretical values. However, it must be pointed out that the microanalyses have not been carried out for compounds **16b**, **16d**−**f**, **16i**, **16j**, and **16m** which have been otained as oily liquid after chromatography and have been directly used in the subsequent step. Compounds **9**−**13**, **15a**, **15b**, **15e**, **15f**, **15g**−**j**, **16a**, **17a**, **18a**, and **19a** were already prepared for a previous study. Synthetic and analytical details for these derivatives have been published elsewhere.[39] The remaining novel 3-nitroflavones **19b**−**m** used in the present work and their precursors **16b**−**m**, **17b**−**m**, and **18b**−**m** were synthesized by adapting the methodology developed in our laboratory starting from 2-hydroxy-3-allylbenzaldehyde (**14**) and appropriately substituted *Z*-2-chloro-2-nitroethenylbenzenes (**15a**−**m**).[39]

General Procedure for the Synthesis of the *Z*-(2-Chloro-2-nitroethenyl)benzenes 15a−m. The relevant benzaldehyde (0.3 mol, all of which are commercially available except 2-formylbenzoic acid methyl ester,[55] 2-formylbenzoic benzyl ester,[56] and 2-benzyloxybenzaldehyde,[57] which were prepared according to previously reported procedures), xylene (750 mL), dimethylammonium chloride (220.3 g, 2.7mol), bromonitromethane (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 fitted with a Dean−Stark apparatus (capacity about 20 mL). The mixture was vigorously refluxed with stirring for 3 h and then allowed to cool to room temperature. Removal of the volatile materials under reduced pressure left a residue which was taken up with H_2O (300 mL) and CH_2Cl_2 (800 mL). The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic extracts were dried ($MgSO_4$), filtered and then evaporated in vacuo to afford a crude product which was chromatographed on a silica gel column (950 g, eluent CH_2Cl_2). Evaporation of the solvent, followed by recrystallization, gave pure (2-chloro-2-nitroethenyl)benzenes **15a**−**m** in the exclusive *Z* configuration. Compounds **15a**, **15b**, and **15e**−**j** have been described in a previous communication.[39] Yields, recrystallization solvents, physical constants, and spectral data for the hitherto unknown derivatives **15c**, **15d**, and **15k**−**m** are reported below:

***Z*-(2-Chloro-2-nitroethenyl)-3-methoxy-2-nitrobenzene (15c).** Yield 65%; mp 127−128°C recrystallized from a benzene/heptane mixture as pale yellow crystals; ^{1}H NMR ($CDCl_3$ δ: 3.96 (s, 3H), 7.19 (dd, 1H, 8.2, 0.7), 7.48 (dd, 1H, 8.2, 0.7), 7.58 (t, 1H, 8.2), 8.20 (s, 1H); IR ν = 1553, 1344 cm^{-1}; MS *m*/*z*: 259, 261 $(M + H)^+$, 276, 278 $(M + NH_4)^+$. Anal. ($C_9H_7ClN_2O_5$). C, H, N.

***Z*-(2-Chloro-2-nitroethenyl)-5-methoxy-2-nitrobenzene (15d).** Yield 60%; mp 94−95 °C recrystallized from a benzene/heptane mixture as pale yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.94 (s, 3H), 7.07 (m, 2H), 8.30 (d, 1H, J = 9.7), 8.69 (s, 1H); IR ν = 1554, 1349 cm^{-1}; MS *m*/*z*: 259, 261 $(M + H)^+$, 276, 278 $(M + NH_4)^+$. Anal. ($C_9H_7ClN_2O_5$). C, H, N.

***Z*-2-(2-Chloro-2-nitroethenyl)benzoic Acid Methyl Ester (15k).** Yield 61%; mp 98−99 °C recrystallized from a benzene/heptane mixture as pale yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.94 (s, 1H), 7.35 (dt, 1H, J = 7.8, 1.3), 7.65 (m, 2H), 8.13 (d, 1H, J = 7.8), 8.95 (s, 1H); IR ν = 1718. 1542. 1342 cm^{-1}; MS *m*/*z*: 242, 244 $(M + H)^+$, 259, 261 $(M + NH_4)^+$. Anal. ($C_{10}H_8ClNO_4$). C, H, N.

***Z*-2-(2-Chloro-2-nitroethenyl)benzoic Acid Benzyl Ester (15l).** Yield 73%; amber-colored oily liquid; ^{1}H NMR ($CDCl_3$) δ: 5.36 (s, 2H), 7.41 (m, 5H), 7.54 (dt, 1H, J = 10.6, 1.1), 7.65 (m, 2H), 8.17 (dt, 1H, J = 10.6. 1.1), 8.93 (s, 1H); IR ν = 1714. 1546. 1324. 1265 cm^{-1}; MS *m*/*z*: 318. 320 $(M + H)^+$, 335, 337 $(M + NH_4)^+$. Anal. ($C_{16}H_{12}ClNO_4$). C, H, N.

***Z*-2-(2-Chloro-2-nitroethenyl)benzyloxybenzene (15m).** Yield 80%; amber-colored oily liquid; ^{1}H NMR ($CDCl_3$) δ: 5.18 (s, 2H), 7.15 (m, 2H), 7.4 (m, 6H), 8.15 (dd, 1H, J = 7.6, 0.5), 8.88 (s, 1H), IR ν = 1556, 1350 cm^{-1}; MS *m*/*z*: 290, 292 $(M + H)^+$, 307, 309 $(M + NH_4)^+$. Anal. ($C_{15}H_{12}ClNO_3$). C, H, N.

General Procedure for the Preparation of the 8-Allyl-3-chloro-3,4-dihydro-4-hydroxy-3-nitro-2-phenyl-2*H*-1-benzopyrans 16am. In a dry 1-L flask fitted with a drying

tube, the appropriate starting *Z*-(2-chloro-2-nitroethenyl)-benzene **15a**−**m** (80 mmol) and 3-allylslicylaldehyde (**14**,[40] 73 g, 0.45 mol) were dissolved in a minimal volume of anhydrous THF (80 mL). Dry and freshly distilled triethylamine (4.36 g, 6.06 mL, 43 mmol) was quickly added via a syringe, and the mixture was stirred at room temperature for 24−48 h under inert atmosphere (monitoring by TLC). 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 lukewarm solution (50 °C) of Girard's reagent T [prepared by dissolving the commercial reagent (75 g, 0.45 mol) in a 8/1/1 mixture of CH_3CH_2OH, CH_3COOH and H_2O (560 mL)]. The reaction medium was efficiently stirred for 2 h at room temperature and then diluted with CH_2Cl_2 (500 mL) and water (400 mL). When an insoluble material remained, it was filtered off on a sintered funnel and thoroughly rinsed with CH_2Cl_2. The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 250 mL). The combined organic extracts were dried ($MgSO_4$), filtered then concentrated under reduced pressure to leave an aldehyde-free material. Chromatography of the crude product on silica gel (500 g, eluent CH_2Cl_2) afforded the desired derivatives **16a**−**m** which, after evaporation of the solvent, were recrystallized in appropriate conditions. These benzopyrans were exclusively obtained as a unique diastereomer with the relative configuration 2*R*,3*R*,4*R*. Compound **16a** has been described in a former communication.[39] Yields, recrystallization solvents, physical constants, and spectral data for the novel derivatives **16b**−**m** are reported below:

8-Allyl-3-chloro-3,4-dihydro-4-hydroxy-3-nitro-2-(2-nitrophenyl)-2*H*-1-benzopyran (16b). Yield 63%; viscous yellow oil, ^{1}H NMR ($CDCl_3$) δ 2.64 (d, 1H, J = 10.7, exchangeable with D_2O), 3.34−3.40 (m, 2H), 5.00 (dd, 1H, J = 17.0, 1.7), 5.07 (dd, 1H, J = 10.1, 1.7), 5.85−5.95 (m, 1H), 6.05 (d, 1H, J = 10.7), 6.72 (s, 1H), 7.12 (br t, 1H, J = 8.0), 7.22 (br d, 1H, J = 7.5), 7.50 (br d, 1H, J = 7.5), 7.64 (dt, 1H, J = 8.3.1.2), 7.76 (dt, 1H, J = 8.3. 1.2), 7.98 (dd, 1H, J = 8.3, 1.2), 8.16 (dd, 1H, J = 8.4, 1.3), IR ν = 3560−3080, 1570, 1540, 1400, 1350 cm^{-1}; MS *m*/*z*: 408, 410 $(M + NH_4)^+$.

8-Allyl-3-chloro-3,4-dihydro-4-hydroxy-3-nitro-2-(3-methoxy-2-nitrophenyl)-2*H*-1-benzopyran (16c). Yield 61%; viscous amber-colored oil, ^{1}H NMR ($CDCl_3$) δ: 2.60 (d, 1H, J = 11.0, exchangeable with D_2O), 3.42−3.55 (m, 2H), 3.90 (s, 3H), 4.90 (dd, 1H, J = 17.1, 1.3), 5.15 (dd, 1H, J = 10.1, 1.3), 5.60 (s, 1H), 5.83−5.98 (m, 2H), 7.10 (t, 1H, J = 7.6), 7.13−7.32 (m, 2H), 7.40 (d, 1H, J = 7.3), 7.49−7.56 (m, 2H); IR ν = 3480, 1580, 1523, 1340 cm^{-1}; MS *m*/*z*: 421, 423 $(M + H)^+$, 438, 440 $(M + NH_4)^+$.

8-Allyl-3-chloro-3,4-dihydro-4-hydroxy-3-nitro-2-(5-methoxy-2-nitrophenyl)-2*H*-1-benzopyran (16d). Yield 77%; viscous amber-colored oil, ^{1}H NMR (DMSO-d_6) δ: 2.41 (d, 1H, J = 10.9, exchangeable with D_2O), 3.20−3.42 (m, 2H), 3.90 (s, 3H), 4.93 (dd, 1H, J = 16.9, 1.4), 5.05 (dd, 1H, J = 10.3, 1.4), 5.70 (s, 1H), 5.82−6.01 (m, 2H), 7.00 (t, 1H, J = 7.6), 7.18−7.29 (m, 2H), 7.30 (d, 1H, J = 8.7), 7.40 (d, 1H, J = 7.6), 8.10 (d, 1H, J = 8.7), IR ν = 3480, 1582, 1526, 1341 cm^{-1}; MS *m*/*z*: 421, 423 $(M + H)^+$, 438, 440 $(M + NH_4)^+$.

8-Allyl-3-chloro-3,4-dihydro-4-hydroxy-3-nitro-2-(4-nitrophenyl)-2*H*-1-benzopyran (16e). Yield 90%; viscous yellow oil; ^{1}H NMR ($CDCl_3$) δ: 2.70 (d, 1H, J = 11.0, exchangeable with D_2O), 3.29−3.42 (m, 2H), 4.90 (dd, 1H, J = 17.1, 1.6), 5.10 (dd, 1H, J = 10.8, 1.6), 5.30 (s, 1H), 5.84−5.96 (m, 1H), 6.00 (d, 1H, J = 11.0), 7.04−7.13 (m, 2H), 7.20 (d, 1H, J = 7.4), 7.60 (d, 1H, J = 8.8), 8.30 (d, 2H, J = 8.8), IR ν = 3490, 1578, 1530, 1345 cm^{-1}; MS *m*/*z*: 391, 393 $(M + H)^+$, 408, 410 $(M + NH_4)^+$.

8-Allyl-3-chloro-3,4-dihydro-4-hydroxy-3-nitro-2-(3-nitrophenyl)-2*H*-1-benzopyran (16f). Yield 95%; viscous yellow oil; ^{1}H NMR ($CDCl_3$) δ: 2.67 (d, 1H, J = 11.9, exchangeable with D_2O), 3.30−3.49 (m, 2H), 5.02 (dd, 1H, J = 17.1, 1.3), 5.12 (dd, 1H, J = 10.1, 1.3), 5.90 (s, 1H), 5.91−6.00 (m, 1H), 6.03 (br d, 1H, J = 11.9), 7.08−7.75 (m, 5H), 8.31 (m, 1H), 8.45 (d, 1H J = 1.8). IR ν = 3450, 1570, 1540, 1400, 1350 cm^{-1}; MS *m*/*z*: 391, 393 $(M + H)^+$, 408, 410 $(M + NH_4)^+$.

8-Allyl-3-chloro-3,4-dihydro-4-hydroxy-2-(4-methoxy-3-nitrophenyl)-3-nitro-2*H*-1-benzopyran (16g). Yield 66%; mp 134−135 °C, recrystallized from a benzene/heptane mixture as pale yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 2.50 (d, 1H, J = 11.8, exchangeable with D_2O), 3.30−3.47 (m, 2H), 3.99 (s, 3H), 5.01 (dd, 1H, J = 16.8, 1.3), 5.09 (dd, 1H, J = 9.9, 1.3), 4.97−5.10 (m, 2H), 5.82 (s, 1H), 5.82−6.03 (m, 1H), 6.00 (d, 1H J = 11.8), 7.08 (m, 2H), 7.24 (m, 1H), 7.45 (d, 1H, J = 6.6), 7.54 (dd, 1H, J = 8.7, 2.2), 8.06 (d, 1H, J = 2.2), IR ν = 3450. 1572. 1544. 1403. 1355 cm^{-1}; MS *m*/*z*: 421, 423 $(M + H)^+$, 438, 440 $(M + NH_4)^+$. Anal. ($C_{19}H_{17}ClN_2O_7$). C, H, N.

8-Allyl-3-chloro-3,4-dihydro-4-hydroxy-2-(4-methoxyphenyl)-3-nitro-2*H*-1-benzopyran (16h). Yield 76%; mp 128−129 °C, recrystallized from a benzene/heptane mixture as colorless crystals; ^{1}H NMR ($CDCl_3$) δ: 2.47 (d, 1H, J = 11.9, exchangeable with D_2O), 3.29−3.51 (m, 2H), 3.82 (s, 3H), 5.02 (dd, 1H, J = 17.0, 1.7), 5.09 (dd, 1H, J = 9.4, 1.7), 5.75 (s, 1H), 5.89−6.01 (m, 1H), 6.03 (d, 1H, J = 11.9), 6.91 (br d, 2H = 8.8), 7.07 (br t, 1H J = 7.7), 7.22 (m, 1H), 7.39 (br d, 2H, J = 8.8), 7.43 (d, 1H, J = 7.7). IR ν = 3450. 1575. 1542. 1406. 1350 cm^{-1}; MS *m*/*z*: 376. 378 $(M + H)^+$, 393. 395 $(M + NH_4)^+$. Anal. ($C_{19}H_{18}ClNO_5$). C, H, N.

8-Allyl-3-chloro-3,4-dihydro-4-hydroxy-2-(2-chlorophenyl)-3-nitro-2*H*-1-benzopyran (16i). Yield 68%; viscous amber-colored oil; ^{1}H NMR ($CDCl_3$) δ: 2.80 (d, 1H, J = 11.2, exchangeable with D_2O), 3.28−3.51 (m, 2H), 5.02 (dd, 1H, J = 17.1, 1.3), 5.16 (dd, 1H, J = 10.8, 1.3), 5.85−6.12 (m, 2H), 6.42 (s, 1H), 6.82 (t, 1H, J = 7.6), 6.94 (d, 1H, J = 7.6), 7.04−7.21 (m, 2H), 8.01 (d, 1H, J = 7.8); IR ν = 3480, 1570, 1523 cm^{-1}; MS *m*/*z*: 380, 382, 384 $(M + H)^+$, 397, 399, 401 $(M + NH_4)^+$. Anal. ($C_{18}H_{15}Cl_2NO_4$) C, H, N.

8-Allyl-3-chloro-3,4-dihydro-4-hydroxy-2-(2-methoxyphenyl)-3-nitro-2*H*-1-benzopyran (16j). Yield 42%; viscous amber-colored oil; ^{1}H NMR ($CDCl_3$) δ: 2.53 (d, 1H, J = 11.6, exchangeable with D_2O), 3.28−3.49 (m, 2H), 3.72 (s, 3H), 4.93 (dd, 1H, J = 17.2, 1.6), 5.24 (dd, 1H, J = 9.5, 1.6), 5.30 (s, 1H), 5.82−6.04 (m, 1H), 6.32 (d, 1H, J = 11.6), 6.92 (dd, 1H, J = 8.3, 2.0), 7.00−7.13 (m, 2H), 7.20 (d, 1H, J = 7.4), 7.32−7.51 (m, 2H), 7.83 (dd, 1H, J = 8.3. 2.0), IR ν= 3480, 1570, 1544 cm^{-1}; MS *m*/*z*: 376, 378 $(M + H)^+$, 393, 395 $(M + NH_4)^+$.

2-[8-Allyl-3-chloro-3,4-dihydro-4-hydroxy-3-nitro-2*H*-1-benzopyran-2-yl]benzoic Acid Methyl Ester (16k). Yield 51%; mp 155−156 °C, recrystallized from a benzene/heptane mixture as white crystals; ^{1}H NMR ($CDCl_3$) δ: 2.62 (d, 2H, J = 11.1, exchangeable with D_2O), 3.25−3.51 (m, 2H), 3.92 (s, 3H), 5.01 (dd, 1H, J = 17.4, 1.2), 5.13 (dd, 1H, J = 11.6, 1.2), 5.83−6.05 (m, 1H), 6.12 (d, 1H, J = 11.1), 7.02−7.20 (m, 2H), 7.26 (d, 1H, J = 7.4), 7.42−7.60 (m, 2H), 7.64 (dt, 1H, J = 7.8, 0.8), 7.86 (dd, 1H, J = 7.8, 0.8), 8.16 (dd, 1H, J = 7.8, 0.8), IR ν = 3480, 1718, 1570, 1458, 1271 cm^{-1}; MS *m*/*z*: 404, 406 $(M + H)^+$, 421, 423 $(M + NH_4)^+$. Anal. ($C_{20}H_{18}ClNO_6$) C, H, N.

2-[8-Allyl-3-chloro-3,4-dihydro-4-hydroxy-3-nitro-2*H*-1-benzopyran-2-yl]benzoic Acid Benzyl Ester (16l). Yield 73%; mp 122−123 °C, recrystallized from a benzene/heptane mixture as white crystals; ^{1}H NMR ($CDCl_3$) δ: 2.77 (d, 2.H, J = 11.0, exchangeable with D_2O), 3.28−3.54 (m, 2H), 5.02 (dd, 1H, J = 18.2, 1.8), 5.13 (dd, 1H, J = 10.8, 1.8), 5.28 (d, 1H, J = 12.3), 5.40 (d, 1H, J = 12.3), 5.85−6.00 (m, 1H), 6.01 (d, 1H, J = 11.0), 7.02−7.13 (m, 3H), 7.28 (dd, 1H, J = 7.1, 1.1), 7.40−7.59 (m, 6H), 7.64 (dt, 1H, J = 7.1, 1.1), 7.92 (dd, 1H, J = 7.8, 1.3), 8.10 (dd, 1H, J = 7.1, 1.1); IR ν = 3480, 1569, 1458, 1262 cm^{-1}; MS *m*/*z*: 480, 482 $(M + H)^+$, 497, 499 $(M + NH_4)^+$. Anal. ($C_{26}H_{22}ClNO_6$) H, N; C, calcd, 65.07; found, 64.46.

8-Allyl-3-chloro-3,4-dihydro-4-hydroxy-2-(2-benzyloxyphenyl)-3-nitro-2*H*-1-benzopyran (16m). Yield 83% from chromatography as a viscous amber-colored oil. ^{1}H NMR ($CDCl_3$) δ: 2.70 (d, 2.H, J = 10.9, exchangeable with D_2O), 3.42 (d, 1H, J = 6.4), 4.90−5.22 (m, 5H), 5.88−6.12 (m, 1H), 6.31 (s, 1H), 6.82 (br d, 1H, J = 7.8), 6.88 (dd, 1H, J = 7.0, 1.0), 6.94 (dd, 1H, J = 7.8, 1.1), 7.03−7.20 (m, 3H), 7.29−750 (m, 5H), 7.82 (dd, 1H, J = 6.8, 1.4), IR ν = 3460, 1558, 1461, 1273

cm^{-1}; MS *m/z*: 452, 454 (M + H)$^+$, 469, 471(M + NH$_4$)$^+$. Anal. ($C_{25}H_{22}ClNO_5$) C, H, N.

General Procedure for the Preparation of the 8-Allyl-3-chloro-2,3-dihydro-3-nitro-2-phenyl-4*H*-1-benzopyran-4-ones 17a−m. In a previously dried 500-mL round-bottomed flask equipped with a condenser surmounted by a drying tube, the appropriate 3-chloro-3,4-dihydro-4-hydroxy-3-nitro-2-phenyl-2*H*-1-benzopyrans **16a−m** (45 mmol) was dissolved in anhydrous CH_2Cl_2 (200−350 mL). Pyridinium chlorochromate (19.5 g, 90 mmol) was then added in one portion, and the flask was immersed 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 CH_2Cl_2. Evaporation of the solvent under reduced pressure left a residue which was directly chromatographed on a silica gel column (500 g, eluent CH_2Cl_2). Removal of the solvent in vacuo, followed by recrystallization in suitable solvents, afforded the desired derivatives **17a−m** with the exclusive relative configuration 2*R*,3*S*. The benzopyran-4-one **17a** has been described in a previous report.[39] Yields, recrystallization solvents, physical constants, and spectral data for the new compounds **17b−m** are reported below:

8-Allyl-3-chloro-2,3-dihydro-3-nitro-2-(2-nitrophenyl)-4*H*-1-benzopyran-4-one (17b). Yield 80%; mp 119−120 °C, recrystallized from a benzene/heptane mixture as pale yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.43 (br d, 2H, J = 5.6), 5.05 (dd, 1H, J = 17.1, 1.4), 5.12 (dd, 1H, J = 10.3, 1.4), 5.86−5.99 (m, 1H), 7.19−7.25 (m, 2H), 7.57 (br d, 1H J = 7.8), 7.59−7.80 (m, 3H), 7.96 (dd, 1H, J = 7.8, 1.3), 8.11 (br d, 1H, J = 7.8), IR ν = 1714, 1538, 1356 cm^{-1}; MS *m/z*: 389. 391 (M + H)$^+$, 406. 408 (M + NH$_4$)$^+$. Anal. ($C_{18}H_{13}ClN_2O_6$) C, H, N.

8-Allyl-3-chloro-2,3-dihydro-3-nitro-2-(3-methoxy-2-nitrophenyl)-4*H*-1-benzopyran-4-one (17c). Yield 83%; mp 153−154 °C, recrystallized from a benzene/heptane mixture as yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.42 (d, 2H, J = 6.5), 3.94 (s, 3H), 5.02 (dd, 1H, J = 17.0, 1.6), 5.16 (dd, 1H, J = 10.1, 1.6), 5.80−6.01 (m, 1H), 6.52 (s, 1H), 7.09−7.30 (m, 2H), 7.44 (d, 1H, J = 8.2), 7.56 (t, 1H, J = 8.2), 7.63 (dd, 1H, J = 7.9. 1.6), 7.94 (dd, 1H, J = 7.9. 1.6), IR ν = 1715, 1584, 1529, 1352 cm^{-1}; MS *m/z*: 419, 421 (M + H)$^+$, 436, 438 (M + NH$_4$)$^+$. Anal. ($C_{19}H_{15}ClN_2O_7$) C, H, N.

8-Allyl-3-chloro-2,3-dihydro-3-nitro-2-(5-methoxy-2-nitrophenyl)-4*H*-1-benzopyran-4-one (17d).Yield 62%; mp 161−162 °C recrystallized from a benzene/heptane mixture as pale yellow crystals; ^{1}H NMR (CD_3OD) δ: 3.42 (d, 2H, J = 6.5), 3.92 (s, 3H), 4.92 (dd, 1H, J = 18.2, 1.6), 5.16 (dd, 1H, J = 10.0, 1.6), 5.87−6.06 (m, 1H), 7.24−7.38 (m, 2H), 7.39 (s, 1H), 7.54 (d, 1H, J = 2.7), 7.76 (dd, 1H, J = 7.9, 1.6), 7.93 (dd, 1H, J = 7.9, 1.6), 8.15 (d, 1H, J = 9.1); IR ν= 1715, 1584, 1529, 1352 cm^{-1}; MS *m/z*: 419, 421 (M + H)$^+$, 436, 438 (M + NH$_4$)$^+$. Anal. ($C_{19}H_{15}ClN_2O_7$) C, H, N.

8-Allyl-3-chloro-2,3-dihydro-3-nitro-2-(4-nitrophenyl)-4*H*-1-benzopyran-4-one (17e). Yield 50%; mp 112−113 °C recrystallized from a benzene/heptane mixture as pale beige crystals; ^{1}H NMR ($CDCl_3$) δ: 3.55 (d, 2H, J = 6.5), 5.02 (dd, 1H, J = 17.0, 1.5), 5.17 (dd, 1H, J = 10.1, 1.5), 5.80−6.06 (m, 1H), 6.42 (s, 1H), 7.24 (t, 1H, J = 7.8), 7.66 (d, 1H, J = 7.8), 7.73 (d, 2H, J = 8.3), 7.91 (d, 1H, J = 7.8), 8.30 (d, 2H, J = 7.8); IR ν = 1714, 1583, 1530, 1354 cm^{-1}; MS *m/z*: 389, 391 (M + H)$^+$, 406, 408 (M + NH$_4$)$^+$. Anal. ($C_{18}H_{13}ClN_2O_6$): C, H, N.

8-Allyl-3-chloro-2,3-dihydro-3-nitro-2-(3-nitrophenyl)-4*H*-1-benzopyran-4-one (17f). Yield 68%; mp 110−112 °C recrystallized from a benzene/heptane mixture as pale yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.49 (d, 2H, J = 5.6), 5.09 (dd, 1H, J = 17.0, 1.2), 5.16 (dd, 1H, J = 10.1, 1.2), 5.85−6.02 (m, 1H), 6.43 (s, 1H), 7.24 (t, 1H, J = 7.6), 7.60−7.74 (m, 3H), 7.97 (dd, 1H, J = 7.9, 1.5), 8.36 (br d, J = 8.1), 8.51 (s, 1H), IR ν = 1712, 1578, 1351 cm^{-1}; MS *m/z*: 389, 391 (M + H)$^+$, 406, 408 (M + NH$_4$)$^+$. Anal. ($C_{18}H_{13}ClN_2O_6$): C, H, N.

8-Allyl-3-chloro-2,3-dihydro-3-nitro-2-(4-methoxy-3-nitrophenyl)-4*H*-1-benzopyran-4-one (17g). Yield 62%; mp 122−124°C recrystallized from a benzene/hexane mixture as pale yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.46 (d, 2H, J = 6.4), 4.00 (s, 3H), 5.06 (dd, 1H, J = 17.5, 1.4), 5.13 (dd, 1H, J = 10.4, 1.4), 5.87−6.06 5.81−6.01(m, 1H), 6.29 (s, 1H), 7.12−7.25 (m, 2H,), 7.53−7.59 (m, 2H), 7.92 (dd, 1H, J = 8.9, 2.0), 8.10 (d, 1H, J = 2.0), IR ν = 1710, 1579, 1359 cm^{-1}; MS *m/z*: 419, 421 (M + H)$^+$, 436, 438 (M + NH$_4$)$^+$. Anal. ($C_{19}H_{15}ClN_2O_7$): C, H, N.

8-Allyl-3-chloro-2,3-dihydro-3-nitro-2-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one (17h). Yield 98% as a pale yellow oil from chromatography; ^{1}H NMR ($CDCl_3$) δ: 3.35−3.55 (m, 2H), 3.84 (s, 3H), 5.06 (dd, 1H, J = 17.1, 1.5), 5.11 (dd, 1H, J = 10.7, 1.5), 5.88−6.02 (m, 1H), 6.23 (s, 1H), 6.96 (br d, 2H, J = 8.8), 7.20 (t, 1H J = 7.7), 7.43 (br d, 2H, J = 8.8), 7.55 (dd, 1H, J = 7.3, 1.5), 7.94 (dd, 1H, J = 8.0, 1.6), IR ν = 1711, 1570, 1342 cm^{-1} ; MS *m/z*: 374, 376 (M + H)$^+$, 391, 393 (M + NH$_4$)$^+$. Anal. ($C_{19}H_{16}ClNO_5$): C, H, N.

8-Allyl-3-chloro-2,3-dihydro-3-nitro-2-(2-chlorophenyl)-4*H*-1-benzopyran-4-one (17i). Yield 89%; mp 91−92 °C recrystallized from a benzene/heptane mixture as yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.55 (d, 2H, J = 6.5), 4.92 (dd, 1H, J = 17.3, 1.4), 5.16 (dd, 1H, J = 10.6, 1.4), 5.79−6.00 (m, 1H), 6.73 (s, 1H), 7.24 (t, 1H, J = 7.8), 7.42−7.48 (m, 3H), 7.65 (d, 1H, J = 8), 7.89−8.04 (m, 2H); IR ν = 1713, 1581, 1287 cm^{-1}; MS *m/z*: 378, 380, 382 (M + H)$^+$, 395, 397, 399 (M + NH$_4$)$^+$. Anal. ($C_{18}H_{13}Cl_2NO_4$) C, H, N.

8-Allyl-3-chloro-2,3-dihydro-3-nitro-2-(2-methoxyphenyl)-4*H*-1-benzopyran-4-one (17j). Yield 93%; mp 113−114 °C recrystallized from a benzene/heptane mixture as yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.43 (d, 2H, J = 6.5), 3.74 (s, 3H), 5.02 (dd, 1H, J = 17.4, 1.4), 5.17 (dd, 1H, J = 10.5, 1.4), 5.80−6.02 (m, 1H), 6.73 (s, 1H), 6.94 (d, 1H, J = 8), 7.03−7.29 (m, 2H), 7.40 (t, 1H, J = 7.9), 7.52 (d, 1H, J = 8), 7.83 (dd, 1H, J = 7.9. 1.6), 8.03 (dd, 1H, J = 7.9. 1.6), IR ν = 1711, 1583, 1290 cm^{-1}; MS *m/z*: 374. 376 (M + H)$^+$, 391. 393 (M + NH$_4$)$^+$. Anal. ($C_{19}H_{16}ClNO_5$) C, H, N.

2-[8-Allyl-3-chloro-2,3-dihydro-3-nitro-4-oxo-4*H*-1-benzopyran-2-yl]benzoic Acid Methyl Ester (17k). Yield 97%; mp 143−144 °C recrystallized from a benzene/heptane mixture as white crystals; ^{1}H NMR ($CDCl_3$) δ: 3.52 (d, 2H, J = 6.5), 3.81 (s, 3H), 4.92 (dd, 1H, J = 16.8, 1.3), 5.16 (dd, 1H, J = 11.4, 1.3), 5.78−5.97 (m, 1H), 7.22 (s, 1H), 7.47 (t, 1H, J = 7.1), 7.49−7.68 (m, 2H), 7.68−7.76 (m, 2H), 8.12−8.30 (m, 2H), IR ν = 1718, 1579, 1476, 1289, 1272 cm^{-1}; MS *m/z*: 402. 404 (M + H)$^+$, 419. 421 (M + NH$_4$)$^+$. Anal. ($C_{20}H_{16}ClNO_6$) H, N; C, calcd, 59.78; found, 59.28.

2-[8-Allyl-3-chloro-2,3-dihydro-3-nitro-4-oxo-4*H*-1-benzopyran-2-yl]benzoic Acid Benzyl Ester (17l). Yield 78%; mp 95−97 °C recrystallized from a benzene/hexane mixture as pale yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.44 (d, 2H, J = 6.6), 5.03 (dd, 1H, J = 18.3, 1.4), 5.16 (dd, 1H, J = 10.1, 1.4), 5.34 (s, 2H), 5.84−6.03 (m, 1H), 7.22 (t, 1H, J = 8), 7.28−7.40 (m, 3H), 7.39−7.49 (m, 2H), 7.51−7.59 (m, 2H), 7.60 (t, 1H, J = 8.1), 7.83 (dd, 1H, J = 8.1, 1.5), 7.96 (dd, 1H, J = 8.1, 1.5), 8.13 (d, 1H, J = 8.1); IR ν = 1714, 1578, 1476, 1289, 1266 cm^{-1}; MS *m/z*: 478, 480 (M + H)$^+$, 495, 497 (M + NH$_4$)$^+$. Anal. ($C_{26}H_{20}ClNO_6$) C, H, N.

8-Allyl-3-chloro-2,3-dihydro-3-nitro-2-(2-benzyloxyphenyl)-4*H*-1-benzopyran-4-one (17m). Yield 61%; mp 161−162 °C recrystallized from a benzene/heptane mixture as pale yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.25−3.52 (m, 2H), 5.01 (dd, 1H, J = 17.9, 1.5), 5.02 (s, 2H), 5.16 (dd, 1H, J = 12.3, 1.5), 5.82−6.03 (m, 1H), 6.75 (s, 1H), 6.95 (br d 1H, J = 8.2), 7.08−7.18 (m, 2H), 7.29−7.42 (m, 6H), 7.51 (dd, 1H, J = 9.2, 1.3), 7.85 (dd, 1H, J = 7.3, 1.8), 7.93 (dd, 1H, J = 9.2, 1.3), IR ν = 1714, 1581, 1282 cm^{-1}; MS *m/z*: 450, 452 (M + H)$^+$, 467, 469 (M + NH$_4$)$^+$. Anal. ($C_{25}H_{20}ClNO_5$) C, H, N.

General Procedure for the Preparation of the 8-Allyl-3-nitro-2-phenyl-4*H*-1-benzopyran-4-ones 18a−m. The related benzopyran-4-one **17a−m** (30 mmol) was placed, under argon atmosphere, in a 500-mL two-necked round-bottomed flask fitted with a septum inlet. The compound was dissolved in anhydrous THF (150 mL), and then 1,8-diazabicyclo[5.4.0]-undec-7-ene (5.03 g, 4.94 mL, 33 mmol) was added in one

portion via a syringe. The reaction was slightly exothermic, and its progress was monitored by thin-layer chromatography (eluent CH_2Cl_2). When the starting material had completely disappeared (reaction times: 1−3 h), aqueous HCl (0.5 N, 80 mL) and then CH_2Cl_2 (250 mL) were added. The organic layer was separated, and the aqueous phase was extracted with CH_2Cl_2 (3 × 125 mL). The combined organic extracts were dried ($MgSO_4$), filtered and then evaporated under reduced pressure to leave a residue which was chromatographed on a silica gel column (320 g, eluent: CH_2Cl_2). Removal of the volatile materials gave pure compounds **18a**−**m** which were further recrystallized in an appropriate solvent. The 8-allyl-3-nitroflavone (**18a)** has already been described in a previous communication.[39] Yields, physical constants, recrystallization solvents, and spectral data for the novel derivatives **18b**−**m** are reported below:

8-Allyl-3-nitro-2-(2-nitrophenyl)-4*H*-1-benzopyran-4-one (18b). Yield 63%; mp 162−163 °C recrystallized from a benzene/heptane mixture as small yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.50 (d, 2H, J = 6.5), 4.98 (dd, 1H, J = 17.0, 1.5), 5.08 (dd, 1H, J = 10.1, 1.5), 5.79−5.95 (m, 1H), 7.49 (t, 1H, J = 7.8), 7.60−7.72 (m, 2H), 7.81−7.91 (m, 2H), 8.24 (dd, 1H, J = 7.8, 1.4), 8.39 (dd, 1H, J = 6.1, 3.5); IR ν = 1672, 1589, 1536, 1348 cm^{-1}; MS m/z: 353 (M + H)$^+$, 370 (M + NH_4)$^+$. Anal. ($C_{18}H_{12}N_2O_6$) C, H, N.

8-Allyl-3-nitro-(3-methoxy-2-nitrophenyl)-4*H*-1-benzopyran-4-one (18c). Yield 66%; mp 181−182 °C recrystallized from a benzene/heptane mixture as pale beige needles; ^{1}H NMR ($CDCl_3$) δ: 3.53 (d, 2H, J = 6.5), 4.00 (s, 3H), 5.01 (dd, 1H, J = 17.0, 1.2), 5.16 (dd, 1H, J = 10.1, 1.2), 5.79−6.04 (m, 1H), 7.22 (dd, 1H, J = 8.6, 0.9), 7.38 (dd, 1H, J = 8.6, 0.9), 7.42 (t, 1H, J = 7.9), 7.56 (t, 1H, J = 8.6), 7.67 (dd, 1H, J = 7.9, 1.5), 8.25 (dd, 1H, J = 7.9, 1.5); IR ν = 1671, 1582, 1381 cm^{-1}; MS m/z: 383 (M + H)$^+$, 400 (M + NH_4)$^+$. Anal. ($C_{19}H_{14}N_2O_7$) C, H, N.

8-Allyl-3-nitro-2-(5-methoxy-2-nitrophenyl)-4*H*-1-benzopyran-4-one (18d). Yield 70%; mp 172−173 °C recrystallized from a benzene/heptane mixture as pale beige needles; ^{1}H NMR (CD_3OD) δ: 2.69 (d, 2H, J = 6.5), 3.11 (s, 3H), 4.12 (dd, 1H, J = 17.8, 1.5), 4.17 (dd, 1H, J = 10.1, 1.5), 4.95−5.13 (m, 1H), 6.62 (dd, 1H, J = 9.2, 2.8), 6.69−6.87 (m, 2H), 7.01 (dd, 1H, J = 8.0, 1.5), 7.29 (dd, 1H, J = 8.0, 1.5), 7.62 (d, 1H, J = 9.2); IR ν = 1676, 1584, 1380 cm^{-1}; MS m/z: 383 (M + H)$^+$, 400 (M + NH_4)$^+$. Anal. ($C_{19}H_{14}N_2O_7$) C, H, N.

8-Allyl-3-nitro-2-(4-nitrophenyl)-4*H*-1-benzopyran-4-one (18e). Yield 79%; mp 159−160 °C recrystallized from a benzene/heptane mixture as yellow needles; ^{1}H NMR ($CDCl_3$) δ: 3.60 (d, 2H, J = 6.5), 5.01 (d, 1H, J = 17.0), 5.17 (d, 1H, J = 10.2), 5.00−5.28 (m, 1H), 6.64 (t, 1H, J = 7.1), 6.85 (d, 1H, J = 7.1), 6.96−7.19 (m, 2H), 7.23 (d, 1H, J = 7.1), 7.43−7.67 (m, 2H); IR ν = 1676, 1580, 1379 cm^{-1}; MS m/z: 353 (M + H)$^+$, 370 (M + NH_4)$^+$. Anal. ($C_{18}H_{12}N_2O_6$) C, H, N.

8-Allyl-3-nitro-2-(3-nitrophenyl)-4*H*-1-benzopyran-4-one (18f). Yield 80%; mp 140−142 °C recrystallized from a benzene/heptane mixture as pale yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.71 (d, 2H, J = 6.3), 5.13 (dd, 1H, J = 17.1, 1.5), 5.24 (dd, 1H, J = 10.2, 1.5), 5.90−6.11 (m, 1H); 7.51 (t, 1H, J = 7.7), 7.72 (dd, 1H, J = 7.3, 1.9), 7.79 (t, 1H, J = 8.1), 8.02 (dd, 1H, J = 7.0, 1.0), 8.20 (dd, 1H, J = 8.0, 1.6), 8.49 (dd, 1H, J = 8.4, 1.0), 8.66 (t, 1H, J = 1.9); IR ν = 1670, 1550, 1381 cm^{-1}; MS m/z: 353(M + H)$^+$, 370 (M + NH_4)$^+$. Anal. ($C_{18}H_{12}N_2O_6$) C, H, N.

8-Allyl-3-nitro-2-(4-methoxy-3-nitrophenyl)-4*H*-1-benzopyran-4-one (18g). Yield 73%; mp 181−183 °C recrystallized from a benzene/heptane mixture as pale yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.69 (d, 2H, J = 6.3), 4.08 (s, 3H), 5.13 (dd, 1H, J = 17.4, 1.3), 5.21 (dd, 1H, J = 12.8, 1.3), 5.95−6.02 (m, 1H), 7.24 (d, 1H, J = 8.9), 7.47 (t, 1H, J = 7.7), 7.68 (dd, 1H, J = 8.6, 1.2), 7.85 (dd, 1H, J = 8.8, 2.3), 8.20 (dd, 1H, J = 8.1, 1.4), 8.35 (d, 1H, J = 2.3), IR ν = 1672, 1545, 1380 cm^{-1}; MS m/z: 383 (M + H)$^+$, 400 (M + NH_4)$^+$. Anal. ($C_{19}H_{14}N_2O_7$) C, H, N.

8-Allyl-3-nitro-2-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one (18h). Yield 88%; mp 136−137 °C recrystallized from a benzene/heptane mixture as yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.70 (d, 2H, J = 6.3), 3.90 (s, 3H), 5.12 (dd, 1H, J = 17.0, 1.4), 5.19 (dd, 1H, J = 10.1, 1.4), 5.95−6.11 (m, 1H), 7.03 (br d, 2H, J = 9.0), 7.43 (t, 1H, J = 7.6), 7.62 (dd, 1H, J = 7.3, 1.3), 7.72 (br d, 2H, J = 9.0), 8.18 (dd, 1H, J = 8.0, 1.6); IR ν = 1662, 1541, 1376 cm^{-1}; MS m/z: 338 (M + H)$^+$, 355 (M + NH_4)$^+$. Anal. ($C_{19}H_{15}NO_5$) C, H, N.

8-Allyl-3-nitro-2-(2-chlorophenyl)-4*H*-1-benzopyran-4-one (18i). Yield 58%; mp 103−104 °C recrystallized from a benzene/heptane mixture as yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.60 (d, 2H, J = 6.2), 5.01 (dd, 1H, J = 17.1, 1.4), 5.16 (dd, 1H, J = 10.1, 1.4), 5.85−6.04 (m, 1H), 7.32−7.60 (m, 5H), 7.57 (d, 1H, J = 8.0), 8.22 (d, 1H, J = 7.6); IR ν = 1671, 1582, 1370 cm^{-1}; MS m/z: 342, 344 (M + H)$^+$, 359, 361 (M + NH_4)$^+$. Anal. ($C_{18}H_{12}ClNO_4$) C, H, N.

8-Allyl-3-nitro-2-(2-methoxyphenyl)-4*H*-1-benzopyran-4-one (18j). Yield 87%; mp 141−143 °C recrystallized from a benzene/heptane mixture as yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.60 (d, 2H J = 6.4), 3.82 (s, 3H), 5.04 (dd, 1H, J = 18.5, 1.2), 5.20 (dd, 1H, J = 10.1, 1.2), 5.88−6.08 (m, 1H), 7.01 (d, 1H, J = 7.8), 7.14 (t, 1H, J = 7.8), 7.43 (t, 1H, J = 7.8), 7.51−7.69 (m, 3H), 8.21 (dd, 1H, J = 7.9, 1.4); IR ν = 1676, 1558, 1377 cm^{-1}; MS m/z: 338 (M + H)$^+$, 355 (M + NH_4)$^+$. Anal. ($C_{19}H_{15}NO_5$) C, H, N.

2-[8-Allyl-3-nitro-4-oxo-4*H*-1-benzopyran-2-yl]benzoic Acid Methyl Ester (18k). Yield 70%; mp 119−121 °C recrystallized from a benzene/heptane mixture as white crystals; ^{1}H NMR ($CDCl_3$) δ: 3.34−3.54 (m, 2H), 3.91 (s, 3H), 5.04 (dd, 1H, J = 17.1, 1.5), 5.20 (dd, 1H, J = 10.1, 1.5), 5.82−6.03 (m, 1H), 7.18 (t, 1H, J = 8.4), 7.46−7.57 (m, 3H), 7.65 (dt, 1H, J = 7.9, 1.5), 7.83 (dd, 1H, J = 7.9, 1.5), 7.93 (dd, 1H, J = 8.4, 1.6), 8.07 (dd, 1H, J = 8.4, 1.6); IR ν = 1719, 1668, 1537, 1381, 1284 cm^{-1}; MS m/z: 366 (M + H)$^+$, 383 (M + NH_4)$^+$. Anal. ($C_{20}H_{15}NO_6$) C, H, N.

2-[8-Allyl-3-nitro-4-oxo-4*H*-1-benzopyran-2-yl]benzoic Acid Benzyl Ester (18l). Yield 60%; mp 103−104 °C recrystallized from a benzene/heptane mixture as white crystals; ^{1}H NMR ($CDCl_3$) δ: 3.39 (d, 2H, J = 6.5), 5.01 (br d, 1H, J = 17.1), 5.06 (br d, 1H, J = 10.1), 5.16 (s, 2H), 5.71−5.92 (m, 1H), 7.00−7.20 (m, 5H), 7.40 (t, 1H, J = 8.7), 7.45−7.59 (m, 2H), 7.60−7.76 (m, 2H), 8.04 (d, 1H, J = 7.9), 8.26 (dd, 1H, J = 8.7, 3.2); IR ν = 1718, 1669, 1537, 1381, 1284 cm^{-1}; MS m/z: 442 (M + H)$^+$, 459 (M + NH_4)$^+$. Anal. ($C_{26}H_{19}NO_6$) C, H, N.

8-Allyl-3-nitro-2-(2-benzyloxyphenyl)-4*H*-1-benzopyran-4-one (18m). Yield 87%; mp 147−148 °C recrystallized from a benzene/heptane mixture as pale yellow crystals; ^{1}H NMR (DMSO-d_6) δ: 3.58 (d, 2H, J = 7.6), 5.04 (dd, 1H, J = 16.9, 1.5), 5.06 (dd, 1H, J = 10.2, 1.5), 5.20 (s, 2H), 5.90−6.04 (m, 1H), 7.19 (dt, 1H, J = 7.8, 0.4), 7.25−7.37 (m, 6H), 7,57 (br t, 1H, J = 7.8), 7.62 (dt, 1H, J = 7.8, 1.7), 7.7 (dd, 1H, J = 7.8, 1.7), 7,78 (dd, 1H, J = 7.8, 1.4), 8,06 (dd, 1H, J = 7.8, 1.4), IR ν = 1676, 1558, 1377 cm^{-1}; MS m/z: 414 (M + H)$^+$, 431 (M + NH_4)$^+$. Anal. ($C_{25}H_{19}NO_5$) C, H, N.

General Procedure for the Preparation of the 3-Nitro-4-oxo-2-phenyl-4*H*-1-benzopyran-8-acetic Acid 19a−m. The relevant benzopyran-4-one **18a**−**m** (2 mmol) was placed in a 50 mL round-bottomed flask and then dissolved in a mixture of CCl_4 (4 mL), CH_3CN (4 mL), and H_2O (6 mL). The biphasic solution was vigorously stirred at room temperature. Sodium periodate (3.54 g, 15.3 mmoL) and ruthenium(III) chloride hydrate (24.3 mg) were successively added. Stirring was continued for 2 h before CH_2Cl_2 (20 mL) and H_2O (20 mL) were added. The reaction mixture was filtered through a sintered funnel, and the insoluble material was rinsed with CH_2Cl_2. The organic phase was decanted, and the aqueous layer was extracted with a Et_2O/THF 1/1 mixture (4 × 15 mL). The combined organic extracts were dried ($MgSO_4$) and then evaporated under reduced pressure to leave a residue which was flash-chromatographed on silica gel (45 g, eluent CH_2Cl_2/CH_3OH 9/1). Evaporation of the solvents gave the satisfactorily pure acids **19a**−**m**. It is worth mentioning that, in most cases, these reactions also produced variable amounts (10−25%) of the corresponding 3-nitro-4-oxo-2-phenyl-4*H*-1-benzopyran-8-

acetaldehyde as byproduct. 3-Nitroflavone-8-acetic acid (**19a)** has been described in an earlier publication.[39] Yields, physical constants, recrystallization solvents, and spectral data for the hitherto unknown 3-nitro-4-oxo-2-phenyl-4*H*-1-benzopyran-8-acetic acid **19b**−**m** are reported below:

3-Nitro-2-(2-nitrophenyl)-4-oxo-4*H*-1-benzopyran-8-acetic Acid (19b). Yield 85%; mp 230−233 °C recrystallized from a benzene/heptane mixture as a white powder; ^{1}H NMR (DMSO-d_6) δ: 3,80 (s, 2H), 7.65 (br t, 1H, J = 7.5), 7.92 (br d, 1H, J = 7.5), 7.97−8.09 (m, 3H), 8.18 (br d, 1H, J = 7.5), 8,48 (br d, 1H, J = 7.6), 12.49 (br s, 1H, exchangeable with D_2O); IR ν = 3010, 1713, 1662, 1530, 1347 cm^{-1}; MS m/z: 371 (M + H)$^+$. Anal. ($C_{17}H_{10}N_2O_8$) C, H, N.

3-Nitro-2-(3-methoxy-2-nitrophenyl)-4-oxo-4*H*-1-benzopyran-8-acetic Acid (19c). Yield 52%; mp 213−214 °C recrystallized from a benzene/heptane mixture as pale yellow crystals; ^{1}H NMR (DMSO-d_6) δ: 3.83 (s, 2H), 4.02 (s, 3H), 7.58 (t, 1H, J = 8.1), 7.69 (d, 1H, J = 8.5), 7.81 (t, 1H, J = 8.5), 7.95 (dd, 1H, J = 8.1, 1.5), 8.11 (dd, 1H, J = 8.1, 1.5), 12.73 (br s, 1H, exchangeable with D_2O); IR ν = 3028, 1718, 1669, 1582, 1381 cm^{-1}; MS m/z: 401 (M + H)$^+$, 418 (M + NH$_4$)$^+$. Anal. ($C_{18}H_{12}N_2O_9$) C, H, N.

3-Nitro-2-(5-methoxy-2-nitrophenyl)-4-oxo-4*H*-1-benzopyran-8-acetic Acid (19d). Yield 70%; mp 184−185 °C recrystallized from a benzene/heptane mixture as pale yellow crystals; ^{1}H NMR (DMSO-d_6) δ: 3.59 (s, 2H), 3.87 (s, 3H), 7.03 (d, 1H, J = 2.6), 7.16 (dd, 1H, J = 9.2, 2.6), 7.43 (t, 1H, J = 7.9), 7.58 (dd, 1H, J = 7.9, 1.3), 8.11 (dd, 1H, J = 7.9, 1.3), 8.32 (d, 1H, J = 9.2), 12.73 (br s, 1H, exchangeable with D_2O); IR ν = 3030, 1719, 1671, 1582, 1380 cm^{-1}; MS m/z: 401 (M + H)$^+$, 418 (M + NH$_4$)$^+$. Anal. ($C_{18}H_{12}N_2O_9$) C, H, N.

3-Nitro-2-(4-nitrophenyl)-4-oxo-4*H*-1-benzopyran-8-acetic Acid (19e). Yield 53%; mp 243−244 °C recrystallized from a benzene/heptane mixture as light beige crystals; ^{1}H NMR (DMSO-d_6) δ: 3.86 (s, 2H), 7.49 (t, 1H, J = 8), 7.84 (d, 1H, J = 8.0), 7.88 (d, 2H, J = 8.6), 8.12 (d, 1H, J = 8.0), 8.36 (d, 2H, J = 8.6), 12.89 ((br s, 1H, exchangeable with D_2O); IR ν= 3028, 1714, 1669, 1578, 1376 cm^{-1}; MS m/z: 371 (M + H)$^+$, 388 (M + NH$_4$)$^+$. Anal. ($C_{17}H_{10}N_2O_8$) H, N; C: calcd, 55.14; found, 54.61.

3-Nitro-2-(3-nitrophenyl)-4-oxo-4*H*-1-benzopyran-8-acetic Acid (19f). Yield 87%; mp 240−242 °C recrystallized from toluene as small beige crystals; ^{1}H NMR (DMSO-d_6) δ: 3.98 (s, 2H), 7.63 (br t, 1H, J = 7.7), 7.92 (dd, 1H, J = 7.7, 1.5), 7.94 (t, 1H, J = 8.2), 8.14 (dd, 1H, J = 8.0, 1.4), 8.18 (dd, 1H, J = 8.8, 1.0), 8.51−8.54 (m, 1H), 8.57 (dd, 1H, J = 8.0, 1.5), 12.84 (br s, 1H, exchangeable with D_2O); IR ν = 3016, 1729, 1668, 1385, 1232 cm^{-1}; MS m/z: 371 (M + H)$^+$. Anal. ($C_{17}H_{10}N_2O_8$) C, H, N.

3-Nitro-2-(4-methoxy-3-nitrophenyl)-4-oxo-4*H*-1-benzopyran-8-acetic Acid (19g). Yield 75%; mp 235−238 °C (dec) pale yellow powder after washings in chloroform; ^{1}H NMR ($CDCl_3$) δ: 3.86 (s, 2H), 4.02 (s, 3H), 7.52−7.62 (m, 2H), 7.86 (d, 1H, J = 6.9), 8.06 (br d, 2H, J = 7.4), 8,31 (br s, 1H), 12.96 (br s, 1H, exchangeable with D_2O); IR ν = 3100, 1720, 1670, 1530, 1375 cm^{-1}; MS m/z: 401 (M + H))$^+$, 418 (M + NH$_4$)$^+$. Anal. ($C_{18}H_{12}N_2O_9$) C, H, N.

3-Nitro-2-(4-methoxyphenyl)-4-oxo-4*H*-1-benzopyran-8-acetic Acid (19h). Yield 76%; mp 157−160 °C recrystallized from a toluene/acetonitrile mixture as small pale yellow crystals; ^{1}H NMR (DMSO-d_6) δ: 3.80 (s, 3H), 3.94 (s, 2H), 7.15 (br d, 2H, J = 8.9), 7.57 (t, 1H, J = 8.8), 7.71 (br d, 2H, J = 8,9), 7.85 (dd,1H, J = 7.3,1.0), 8.07 (dd,1H, J = 8.0, 1.3), 13.28 (br s, 1H, exchangeable with D_2O); IR ν = 3050, 1712, 1668, 1532, 1378 cm^{-1}; MS m/z: 356 (M + H))$^+$, 373 (M + NH$_4$)$^+$. Anal. ($C_{18}H_{13}NO_7$) C, H, N.

3-Nitro-2-(2-chlorophenyl)-4-oxo-4*H*-1-benzopyran-8-acetic Acid (19i). Yield 45%; mp 229−230 °C recrystallized from a benzene/heptane mixture as light beige crystals; ^{1}H NMR (DMSO-d_6) δ: 3.86 (s, 2H), 7.69 (m, 5H), 7.51−7.80 (d, 1H, J = 8.0), 8.23 (d, 1H, J = 7.7), 12.71 (br s, 1H, exchangeable with D_2O); IR ν= 3028, 1718, 1671, 1582 cm^{-1}; MS m/z: 360, 362 (M + H)$^+$, 377, 379 (M + NH$_4$)$^+$. Anal. ($C_{17}H_{10}ClNO_6$) C, H, N.

3-Nitro-2-(2-methoxyphenyl)-4-oxo-4*H*-1-benzopyran-8-acetic Acid (19j). Yield 68%; mp 212−213 °C recrystallized from a benzene/heptane/acetonitrile mixture as pale yellow crystals; ^{1}H NMR (DMSO-d_6) δ: 3.85 (s, 3H), 3.91 (s, 2H), 7.0.07−7.29 (m, 2H), 7.50−7.74 (m, 3H), 7.86 (dd, 1H, J = 7.9, 1.3), 8.12 (dd, 1H, J = 7.9, 1.3), 12.64 (br s, 1H, exchangeable with D_2O); IR ν = 3030, 1720, 1667, 1585 cm^{-1}; MS m/z: 356 (M + H)$^+$, 373 (M + NH$_4$)$^+$. Anal. ($C_{18}H_{13}NO_7$) C, H, N.

2-(8-Carboxymethyl-3-nitro-4-oxo-4*H*-chromen-2-yl)-benzoic Acid Methyl Ester (19k). Yield 60%; mp 200−202 °C recrystallized from a benzene/heptane mixture as pale yellow crystals; ^{1}H NMR (DMSO-d_6) δ: 3.87 (s, 2H), 4.01 (s, 3H), 7.67−7.86 (m, 2H), 7.88−8.02 (m, 3H), 8.42 (dd, 1H, J = 8.5, 1.1), 8.48 (dd, 1H, J = 8.0, 1.3), 12.64 (br s, 1H, exchangeable with D_2O); IR ν= 3029, 1718, 1671, 1537, 1381, 1284 cm^{-1}; MS m/z: 384 (M + H)$^+$, 401 (M + NH$_4$)$^+$. Anal. ($C_{19}H_{13}NO_8$) H, N; C, calcd, 59.54; found, 59.11.

2-(8-Carboxymethyl-3-nitro-4-oxo-4*H*-chromen-2-yl)-benzoic Acid Benzyl Ester (19l). Yield 84%; mp 164−166 °C recrystallized from a benzene/heptane mixture as white crystals; ^{1}H NMR (DMSO-d_6) δ: 3.87 (s, 2H), 5.31 (s, 2H), 6.92−7.26 (m, 6H), 7.42 (dt, 1H, J = 8.6, 1.9), 7.49 (dd, 1H, J = 8.6, 1.9), 7.52−7.71 (m, 2H), 8.02 (dd, 1H, J = 7.8, 1.1), 8.26 (dd, 1H, J = 8.6, 1.9), 12.76 (br s, 1H, exchangeable with D_2O); IR ν = 3028, 1719, 1669, 1536, 1380, 1284 cm^{-1}; MS m/z: 460 (M + H)$^+$, 477 (M + NH$_4$)$^+$. Anal. ($C_{25}H_{17}NO_8$) C, H, N.

3-Nitro-2-(2-benzyloxyphenyl)-4-oxo-4*H*-1-benzopyran-8-acetic Acid (19m). Yield 62%; mp 155−157 °C recrystallized from a benzene/heptane mixture as a white powder; ^{1}H NMR (DMSO-d_6) δ: 3.90 (s, 2H), 5.19 (s, 2H), 7.19 (br t, 1H, J = 7.8), 7.22−7.38 (m, 6H), 7.59 (br t, 1H, J = 7.7), 7.63 (dd, 1H, J = 8.0, 1.6), 7.68 (dd, 1H, J = 7.6, 1.6), 7.88 (dd, 1H, J = 7.7, 1.6), 8,10 (dd, 1H, J = 7.7, 1.2); 12.64 (br s, 1H, exchangeable with D_2O); IR ν = 3035, 1715, 1649, 1530, 1347 cm^{-1}; MS m/z: 432 (M + H)$^+$, 449 (M + NH$_4$)$^+$. Anal. ($C_{24}H_{17}NO_7$) C, H, N.

3-Nitro-2-(2-benzyloxyphenyl)-4-oxo-4*H*-1-benzopyran-8-acetic Acid Methoxymethyl Ester (20). A stirred solution of the above acid **19m** (0.863 g, 2 mmol) in anhydrous acetonitrile (18 mL) was placed under argon in a 50 mL round-bottomed flask. Chloromethyl methyl ether (209 mg, 196 mL, 2.6 mmol) and triethylamine (263 mg, 362 mL, 2.6 mmol) were then successively added at room temperature via a syringe. The yellow solution became cloudy and then limpid while it turned brown. The mixture (monitored by TLC) was stirred for 5 additional hours. Evaporation of the solvent under reduced pressure followed by chromatography (35 g SiO_2, eluent: CH_2Cl_2/CH_3OH 95/5) gave pure ester **20**. Yield 98%; mp 144−146 °C recrystallized from a benzene/heptane mixture as pale yellow crystals; ^{1}H NMR (DMSO-d_6) δ: 3.12 (s, 3H), 4.10 (s, 2H,), 5,12 (s, 2H), 5,21 (s, 2H), 7,18 (t, 1H, J = 7.6), 7.20−7.41 (m, 6H), 7.55−7.65 (m, 3H), 7,92 (dd, 1H, J = 7.8, 0.8), 8.13 (dd, 1H, J = 7.8, 0.8); IR ν = 1742, 1641, 1539, 1345 cm^{-1}. MS m/z: 476 (M + H)$^+$. Anal. ($C_{26}H_{21}NO_8$) C, H, N.

3-Amino-2-(2-hydroxyphenyl)-4-oxo-4*H*-1-benzopyran-8-acetic Acid Methoxymethyl Ester (21). The benzyl ether **20** (475 mg, 1 mmol) was dissolved in ethyl acetate (20 mL). Palladium on charcoal (10%, 150 mg) was then added, and the stirred mixture was maintened under hydrogen atmosphere (1 atm) for 16 h. The suspension was filtered through a short pad of Celite (thoroughly rinsing the solid with successively a mixture CH_2Cl_2/CH_3COCH_3 9/1 and then a mixture CH_2Cl_2/CH_3OH 9/1). The obtained filtrate was evaporated in vacuo to provide a crude residue which was further chromatographed (35 g SiO_2, eluent: CH_2Cl_2/CH_3OH 97/3). Yield 89%; mp 172−174 °C recrystallized from a benzene/heptane mixture as a pale beige powder; ^{1}H NMR (DMSO-d_6) δ: 3.19 (s, 3H), 4.03 (s, 2H), 5.17 (s, 2H), 6.96−7.07 (m, 2H), 7.34−7.43 (m, 2H), 7.55 (dd, 1H, J = 7.8, 1.5), 7.72 (dd, 1H, J = 7.2, 1.1), 8,06 (dd, 1H, J = 8.0, 1.3); MS m/z: 356 (M + H)$^+$. Anal. ($C_{19}H_{17}NO_6$) C, H, N.

3-Nitro-2-(2-nitrophenyl)-4-oxo-4*H*-1-benzopyran-8-acetic Acid Methoxymethyl Ester (22). This ester was prepared from **19b** on a 3 mmol scale using the procedure above-described for **20**. Yield 98%; mp 130−132 °C recrystallized from

a benzene/heptane mixture as a pale yellow powder; ^{1}H NMR (CDCl3) δ: 3.28 (s, 3H), 3.83 (s, 2H), 5.16 (s, 2H), 7.53 (t, 1H, J = 7.8), 7.66 (dd, 1H, J = 6.1, 3.5), 7.73 (dd, 1H, H7, J = 7.8, 1.6), 7.80−7.91 (m, 2H), 8.31 (dd, 1H, J = 7.8, 1.6), 8.38 (dd, 1H, J = 6.1, 2.5); IR ν = 1745, 1639, 1537, 1348 cm^{-1}; MS *m/z*: 415 (M + H)$^+$, 432 (M + NH$_4$)$^+$. Anal. ($C_{19}H_{14}N_2O_9$) C, H, N.

(6,8-Dioxo-6,7-dihydro-8*H*-5,13-dioxa-7-azabenzo[3,4]cyclohepta[1,2-*b*]naphthalen-12-yl)acetic Acid Methoxymethyl Ester (23). A solution of the aminophenol **21** (355 mg, 1 mmol) in anhydrous CH_2Cl_2 (50 mL) was placed, under inert atmosphere, in a 100 mL two-necked flask. Triphosgene (111.4 mg, 0.375 mmol) dissolved in anhydrous CH_2Cl_2 (12 mL) was added via a syringe at room temperature to the stirred mixture. The yellow solution turned orange. After 3 h, a precipitate appeared, and triethylamine was added dropwise until the mixture became limpid again. Two hours later, removal of the solvent under reduced pressure followed by chromatography (40 g SiO_2, eluent: CH_2Cl_2/CH_3OH 98/2) afforded pure tetracyclic ester **23**. Yield 65%; mp 162−165 °C recrystallized from a benzene/heptane mixture as an orange powder; ^{1}H NMR (DMSO-d_6) δ: 3.25 (s, 3H), 4.19 (s, 2H), 5.21 (s, 2H), 7.43 (d, 1H, J = 8.4), 7.53 (t, 1H, J = 7.8), 7.57 (t, 1H, J = 8.4), 7.76 (dt, 1H, J = 8.4, 1.2), 7.86 (dd, 1H, J = 7.8, 1.2), 7.95 (dd, 1H, J = 8.4, 1.2), 8.12 (dd, 1H, J = 7.8, 1.2), 9.83 (s, 1H, exchangeable with D_2O). MS *m/z*: 382 (M + H)$^+$, 399 (M + NH$_4$)$^+$. Anal. ($C_{20}H_{15}N\ O_7$) H, N; C, calcd, 62.99; found, 62.54.

(6,8-Dioxo-6,7-dihydro-8*H*-5,13-dioxa-7-azabenzo[3,4]cyclohepta[1,2-*b*]naphthalen-12-yl)acetic Acid (24). Adapting a previously reported method,[43] dry magnesium bromide (552 mg, 3 mmol) was added in one portion to a stirred solution of the above methoxymethyl ester **23** (190 mg, 0.5 mmol) in anhydrous CH_2Cl_2 (25 mL). The reaction medium became cloudy and then yellow and was stirred overnight at room temperature. Removal of the solvent under reduced pressure followed by chromatography (90 g of SiO_2, eluent: CH_2Cl_2/CH_3-OH 90/10) provided pure carboxylic acid **24**. Yield 51%; mp > 260 °C yellow powder; ^{1}H NMR (DMSO-d_6) δ: 3.94 (s, 2H), 7.42 (d, 1H, J = 7.0), 7.49 (br t, 1H, J = 7.0), 7.57 (br t, 1H, J = 7.0), 7.69−7,85 (m, 2H), 7.98−8,10 (m, 2H). MS *m/z*: 338 (M + H)$^+$, 355 (M + NH$_4$)$^+$. Anal. ($C_{18}H_{11}N\ O_6$) C, H, N.

(10-Oxo-10,11-dihydro-5-oxa-11-azabenzo[*b*]fluoren-6-yl)acetic Acid Methoxymethyl Ester (25) and 3-Amino-2-(2-aminophenyl)-4-oxo-4*H*-1-benzopyran-8-acetic Acid Methoxymethyl Ester (26). The dinitro derivative **22** (1.76 g, 4.24 mmol) was dissolved in EtOAc (160 mL). Palladium on charcoal (10%, 450 mg) was then added, and the stirred mixture was maintained under hydrogen atmosphere (1 atm) for 16 h. The suspension was filtered through a short pad of Celite (thoroughly rinsing the solid with successively a mixture CH_2Cl_2/CH_3COCH_3 9/1 and then a mixture CH_2Cl_2/CH_3OH 9/1). The obtained filtrate was evaporated in vacuo to give a crude residue which was further chromatographed (200 g SiO_2, eluent: CH_2Cl_2/ CH_3COCH_3 in the successive proportions 9/1, 8/2, and, finally, 7/3) to provide **25** and then **26**.

25: Yield 49%; mp 209−211 °C recrystallized from a benzene/heptane mixture as flakey pale yellow crystals; ^{1}H NMR ($CDCl_3$) δ: 3.38 (s, 3H, CH_3), 4.17 (s, 2H), 5.30 (s, 2H), 7.29 (br t, 1H, J = 7.8), 7.46 (br t, 1H, J = 7.5), 7.54 (dt, 1H, J = 7.5, 0.9), 7,62 (dd, 1H, J = 7.8, J = 0,5), 7.69 (d, 1H, J = 7.5), 8.01 (dd, 1H, J = 7.8, 0.5), 8,47 (dd, 1H, J = 7.5, J = 0.9), 9.7 (br s, 1H, exchangeable with D_2O). MS *m/z*: 338 (M + H)$^+$, 355 (M + NH$_4$)$^+$. Anal. ($C_{19}H_{15}N\ O_5$) C, H, N.

26 : Yield 40%; mp 190−192 °C orange clear powder; ^{1}H NMR (DMSO d_6) δ: 3.34 (s, 3H), 3.79 (s, 2H), 5.19 (s, 2H), 7.07 (t, 1H, J = 7.5), 7.27 (t, 1H, J = 8.0), 7.42 (d, 1H, J = 7.5), 7.50−7.61 (m, 2H), 7.69 (d, 1H, J = 8.0), 8.13 (d, 1H, J = 8.0). MS *m/z*: 372 (M + H)$^+$, 355 (M + NH$_4$)$^+$. Anal. ($C_{19}H_{18}N_2\ O_5$) C, H, N.

(6,8-Dioxo-5,6,7,8-tetrahydro-13-oxa-5,7-diazabenzo-[3,4]cyclohepta[1,2-*b*]naphthalen- 12-yl)acetic Acid Methoxymethyl Ester (27). This tetracyclic ester was prepared from **26** (354 mg, 1 mmol) according to the procedure above-described for **23**. Yield 65%; mp 250−252 °C yellow powder; ^{1}H NMR (DMSO d_6) δ: 3.24 (s, 3H), 4.12 (s, 2H), 5.19 (s, 2H), 6,97 (t, 1H, J = 7.4), 7.37 (d, 1H, J = 7.4), 7.60−7.91 (m, 3H), 8.01 (d, 1H, J = 8.2), 8.09 (d, 1H, J = 8.2), 12.51 (s, 1H exchangeable with D_2O), 14,40 (s, 1H exchangeable with D_2O). MS *m/z*: 381 (M + H)$^+$, 398 (M + NH$_4$)$^+$. Anal. ($C_{20}H_{16}N_2O_6$) C, H, N.

(10-Oxo-10,11-dihydro-5-oxa-11-azabenzo[*b*]fluoren-6-yl)acetic Acid (28). This carboxylic acid was prepared from **25** (337 mg, 1 mmol) according to the procedure above-described for **24**. Yield 51%; mp > 260 °C pale yellow powder; ^{1}H NMR (DMSO-d_6) δ: 4.01 (s, 2H), 7.28 (ddd, 1H, J = 8.2, 7.4, 1.3), 7.41−7.62 (m, 3H), 7.74 (dd, 1H, J = 7.4, 1.3), 7.95 (dd, 1H, J = 7.7, 0.9), 8.20 (d, 1H, J = 8.2), 9.7 (br s, 1H exchangeable with D_2O). MS *m/z*: 294 (M + H)$^+$, 311 (M + NH$_4$)$^+$. Anal. ($C_{17}H_{11}NO_4$) C, H, N.

(6,8-Dioxo-5,6,7,8-tetrahydro-13-oxa-5,7-diazabenzo-[3,4]cyclohepta[1,2-*b*]naphthalen- 12-yl)acetic Acid (29). This carboxylic acid was prepared from **27** (380 mg, 1 mmol) according to the procedure above-described for **24**. Yield 57%; mp > 260 °C, yellow powder; ^{1}H NMR (DMSO-d_6) δ: 3.71 (s, 2H), 7.02 (t, 1H, J = 7.6), 7.25−7.43 (m, 3H), 7.72 (br s, 2H), 8.18 (d, 1H, J = 8.9), 12.29 (br s, 1H exchangeable with D_2O). MS *m/z*: 337 (M + H)$^+$, 354 (M + NH$_4$)$^+$. Anal. ($C_{18}H_{12}N_2O_5$) C, H, N.

1-(3-Allyl-2-hydroxyphenyl)-3-hydroxy-3-(2-nitrophenyl)propenone (32). A mixture of 3-allyl-2-hydroxyacetophenone (**30**)[45] (4.6 g, 26 mmol), commercial 2-nitrobenzoyl chloride (**31**, 3.6 g, 19 mmol), and anhydrous K_2CO_3 (10 g) in anhydrous butanone (150 mL) was heated under reflux for 48 h. The reaction mixture was allowed to cool to room temperature, and the solid was filtered off. The filtrate was then poured onto a mixture of ice and water (400 mL). The precipitate formed was collected by filtration and recrystallized to give pure **32**. Yield 68%; mp 228−229 °C recrystallized from methanol as small yellow crystals; ^{1}H NMR (DMSO-d_6) δ: 2.92−3.34 (m, 1H), 4.08 (s, 1H), 4.92 (dd, 1H, J = 16.9, 1.2), 5.01 (dd, 1H, J = 9.8, 1.2), 5.81−6.00 (m, 1H), 6.56 (t, 1H, J = 7.6), 6.98 (d, 1H, J = 8.4), 7.26−7.38 (m, 1H), 7.38−7.47 (m, 2H), 7.58 (dt, 1H, J = 8.4, 0.8), 7.87 (dd, 1H, J = 8.4, 0.8), 16.43 (s, 1H exchangeable with D_2O); IR ν = 3560−3040, 1713, 1650, 1540 cm^{-1}; MS *m/z*: 326 (M + H)$^+$, 343 (M + NH$_4$)$^+$. Anal. ($C_{18}H_{15}NO_5$) H, N; C, calcd, 66.46; found, 65.95.

8-Allyl-2-(2-nitrophenyl)-4*H*-1-benzopyran-4-one (33). The preparation of this flavone was performed on a 1.2 mmol scale starting from compound **32** by adapting a method previously described for similar cases.[44,58] Yield 97%; mp 129−131 °C recrystallized from a benzene/heptane mixture as a white powder; ^{1}H NMR ($CDCl_3$) δ: 3.49 (d, 2H, J = 6.8), 5.01 (br d, 1H, J = 16.6), 5.12 (d, 1H, J = 8.9), 5.82−6.01 (m, 1H), 6.63 (s, 1H), 7.40 (t, 1H, J = 7.8), 7.56 (d, 1H, J = 7.1), 7.63−7.86 (m, 3H), 8.08 (d, 1H, J = 7.8), 8.14 (d, 1H, J = 8); IR ν = 1670, 1582, 1381 cm^{-1}; MS *m/z*: 308 (M + H)$^+$, 325 (M + NH$_4$)$^+$. Anal. ($C_{18}H_{13}NO_4$) C, H, N.

2-(2-Nitrophenyl)-4-oxo-4*H*-1-benzopyran-8-acetic Acid (8). This carboxylic acid was prepared from the allyl derivative **33** (307 mg, 1 mmol) according to the procedure above-described for compounds **19**. Yield 48%; mp 186−188 °C recrystallized from a benzene/heptane mixture as a pale yellow powder; ^{1}H NMR (DMSO-d_6) δ: 3.87 (s, 2H), 6.63 (s, 1H), 7.40 (t, 1H, J = 7.8), 7.56 (d, 1H, J = 7.1), 7.67−7.83 (m, 3H), 8.08 (d, 1H, J = 7.8), 8.14 (d, 1H, J = 8.0); IR ν = 1718, 1670, 1582, 1381 cm^{-1}; MS *m/z*: 326 (M + H)$^+$, 343 (M + NH$_4$)$^+$. Anal. ($C_{17}H_{11}NO_6$) C, H, N.

Materials for Biological Assays. The synthetic substrates *p*-nitroanilide (pNA) derivatives, protease inhibitors, bovine cathepsin G, and human angiotensin converting enzyme (ACE) were provided by Sigma Chemical Co. (St Louis, MO). Human recombinant pro-matrix metalloproteinase-9 (pro-MMP-9) with a Mr 92 kDa was from R&D (UK). Purified human dipeptidyl peptidase IV(DPPIV)/CD26 was a gift of Dr I De Meester.[59] Goat F(ab′)$_2$ fragment anti-mouse fluorescein-conjugated Ig, monoclonal antibody (mAb) specific for CD13 (MY7, mIgG1) and the control isotype mIgG1 were obtained from Coulter

Immunotech (Coultronics, France). Monoclonal Ab specific for CD13 (WM15, mIgG1) was from Pharmingen International (Becton Dickinson Company). Protein G-Sepharose was from Pharmacia (Uppsala, Sweden).

Cell Culture Conditions. Human monoblastic U937 cells were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated FCS (Gibco, Parsley, Scotland, LPS levels < 0.1 ng/mL), 2 mM L-glutamine, 1 mM sodium pyruvate and 40 μg/mL gentamycin (Flow Laboratories, Rockville, MD) in a 5% CO_2 humidified atmosphere at 37 °C. Cells (0.5×10^5 cells/mL) were resuspended in fresh medium containing FCS in tissue culture flasks and were grown for various periods of time, in the absence or presence of different concentrations of the tested compounds. In this context, it must be pointed out that some of these derivatives (**8**, **20**−**23** and **29**) precipitate at high concentration (10^{-3} M). Cells were collected, washed twice, and counted with a Coulter Counter ZM equipped with a Coultronic 256 channelizer, and their viability was determined by trypan blue exclusion. Cell protease assays were performed as described below. Apoptosis was assessed by flow cytometry as described below by measuring the appearance of Apo 2.7 antigen (Coulter Immunotech), a mitochondrial membrane protein lately exposed on the surface of cells undergoing programmed cell death.

Protease Assays. Protease activities were assayed with a number of *p*-nitroanilide (pNA) derivatives as previously described.[60] In a typical experiment, 5×10^5 cells were incubated for 15 min at 37 °C in 0.1 mL peptidase buffer in the absence or in the presence of 0.5 mg/mL of pNA derivative. Formation of pNA was recorded at 405 nm. Aminopeptidase N (APN/CD13) and neutral endopeptidase (NEP/CD10) activities at the surface of intact U937 cells were respectively measured using Ala-pNA or Succ-Ala-Ala-Pro-Phe-pNA as substrates, and bestatin or phosphoramidon as specific inhibitors. γ-glutamyl transpeptidase (γ-GT/CD224) activity of U937 cells was assayed using γ-Glu-pNA as a substrate of γ-GT hydrolytic activity and Gly-Gly as glutamate acceptor for the transpeptidation reaction. Acivicin is an irreversible inhibitor of γ-GT. Results were expressed as nmoles of pNA formed per 10^5 cells at 37 °C. To quantify the effect of inhibitors, the remaining activity was expressed as the percentage of the control activity without inhibitor. Soluble activities of two purified serine proteases dipeptidyl peptidase IV (DPPIV/CD26) and cathepsin G were respectively measured using Gly-Pro-pNA and Succ-Ala-Ala-Phe-pNA as substrates, and diisopropyl phosphorofluoridate (DFP) as their inhibitor. Angiotensin converting enzyme (ACE/CD143) activity was assessed by measuring the cleavage of Hip-His-Leu at 490 nm and its inhibition by captopril.[61] Assays to immunoprecipitate APN activity used the monoclonal antibody (mAb) MY7/CD13. Whole cell lysates were obtained by lysing cells in protease buffer and 1% (w/v) *n*-octyl-β-D-glucoside (lysis buffer). Ten micrograms of protein in 50 μL was incubated for 18 h at 4 °C with mAb against CD13 (10 μg) or an equivalent amount of control isotype mIgG1, and the immunoprecipitates were obtained by adding Protein G-Sepharose (20 μL) for 3 h at 4 °C. After centrifugation, the immunoprecipitates bound to Protein G-Sepharose beads (washed twice in cold PBS) were tested for APN activity by incubation with Ala-pNA in the absence and in the presence of bestatin (**1**) or **19b** at the concentration 10^{-3} M.

Gelatinolytic Activity of MMP-9. Analysis of MMP-9 activity was carried out in 7.5% (w/v) SDS-polyacrylamide gels containing 0.1% gelatin (w/v) as described.[62] Various amounts of pro-MMP-9 (0.1, 1 and 10 ng) were applied to the gel in Laemmli sample buffer lacking β-mercaptoethanol. Gelatinolytic activities of pro-MMP-9 were detected as transparent bands on the background of Coomassie-blue stained gelatin. The NIH Image 1.44 β11 software was used for the analysis of the bands, after acquisition in an Appligene densitometer (Oncor)

Flow Cytometry Analysis. Intact cells were immunostained with CD13 antibodies as previously described.[63] The matched-isotype (mIgG1) and fluorescein isothiocyanate (FITC)-conjugated goat F(ab′)2 anti-mouse IgG were from Coulter Beckman (Margency, France). Analysis was performed in a FACS flow cytometer analyzer (Becton-Dickinson, Mountain View, CA). Fluorescence data were expressed in relative fluorescence intensity (%) and antigen relative density per cell.

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