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Growth inhibitory activities of oxyprenylated and non-prenylated naturally occurring phenylpropanoids in cancer cell lines

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ABSTRACT

A series of 25 selected oxyprenylated natural phenylpropanoids were synthesized, and their growth inhibitory activities were evaluated in vitro together with 14 other commercially available non-alkylated compounds belonging to the same chemical series. The compounds were tested on six human cancer cell lines using MTT colorimetric assays. The data reveal that of the six chemical groups (G) studied, coumarins (G1), cinnamic and benzoic acids (G2), chalcones (G3), acetophenones (G4), anthraquinones (G5), and cinnamaldehydes and cinnamyl alcohols (G6), G2-related compounds displayed the weakest growth inhibitory activities in vitro, whereas G5-related compounds displayed the highest activities. Quantitative videomicroscopy analyses were then carried out on human U373 glioblastoma cells, which are characterized by various levels of resistance to different pro-apoptotic stimuli. These analyses revealed that compounds **20** (4,2′,4′-trihydroxychalcone), and **30** and **31** (two cinnamaldehydes) were cytostatic and able to overcome the intrinsic resistance of U373 cancer cells to pro-apoptotic stimuli.

Every year, approximately seven million people die from cancer, which makes this disease responsible for at least 12% of deaths worldwide.[1] A number of important new commercialized anticancer drugs have been obtained from natural sources,[2] including vinblastine, vincristine, vinorelbine, etoposide, teniposide, taxol®, taxotere®, topotecan and irinotecan from plants.[3] We contributed to the discovery of a *Catharantus roseus* alkaloid, vinflunine,[4] that has been recently marketed as Javlor®.[5] Trabectedin (Yondelis®) became the first marketed anticancer drug derived from a marine source in 2007.[6] In fact, as emphasized by Tan et al.[7] natural products have been the most significant sources of drugs, accounting for approximately 74% of anticancer drugs. Thus, as claimed both by Coseri[1] and Gordaliza,[2] natural products represent the most valuable potential source of novel anticancer agents. We have accumulated experience in this domain over the last two decades and have developed an original screening approach based on the combined use of the conventional MTT colorimetric assay[8–11] and computer-assisted phase-contrast microscopy, i.e., quantitative videomicroscopy.[9–11] We have used these approaches to identify anticancer drugs with potentially novel mechanisms of action. For example, these methods enabled us to identify a hemisynthetic derivative of a cardiotonic steroid (19-hydroxy-2′-oxovoruscharin) as a novel anticancer agent and enter it into Phase I clinical trials for oncology.[12,13] The parent compound was identified in the African plant *Calotropis procera* (Aiton) W.T. Aiton (Asclepiadaceae).[8] It targets the sodium-potassium pump (Na^+/K^+-ATPase) α1 subunit, which is over-expressed in renal cell carcinomas,[14] non-small-cell lung cancers (NSCLCs),[9] gliomas[10] and melanomas.[11] It also targets the 3 subunit,[9,10] which is over-expressed in colon cancers.[15] As detailed below, we adopted a similar strategy of research in this current work to investigate the potential of oxyprenylated natural compounds as anticancer agents.

Oxyprenylated natural products, such as isopentenyloxy-(C_5), geranyloxy-(C_{10}), and the farnesyloxy-(C_{15}) related compounds, represent a family of secondary metabolites that were considered for years to be merely biosynthetic intermediates of the more widespread C-prenylated derivatives. These secondary metabolites have been recognized in the last two decades as interesting and valuable biologically active phytochemicals. Approximately 300 compounds have been isolated and structurally characterized from plants, primarily from the families Rutaceae and Compositae, which are comprised of several edible vegetables and fruits. The phytochemistry and pharmacology of prenyloxyphenylpropanoids was recently reviewed.[16]

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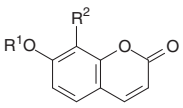
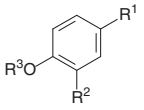
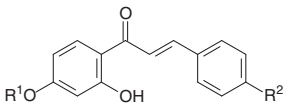


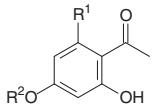
1 R^1= geranyl, R^2 = H
2 R^1= isopentenyl, R^2 = H
3 R^1= farnesyl, R^2 = H
4 R^1= isopentenyl, R^2 = OH
5 R^1= isopentenyl, R^2 = OCH_3
6 R^1= R^2 = H



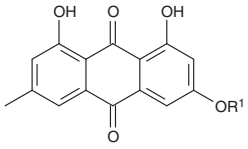
7 R^1= CH=CH-COOH, R^2 = OCH_3, R^3 = isopentenyl
8 R^1= CH=CH-COOH, R^2 = OCH_3, R^3 = geranyl
9 R^1= CH=CH-COOH, R^2 = H, R^3 = isopentenyl
10 R^1= CH=CH-COOH, R^2 = H, R^3 = geranyl
11 R^1= COOH, R^2 = H, R^3 = isopentenyl
12 R^1= COOH, R^2 = OCH_3, R^3 = isopentenyl
13 R^1= CH=CH-COOH, R^2 = OCH_3, R^3 = H
14 R^1= CH=CH-COOH, R^2 = R^3 = H
15 R^1= COOH, R^2 = R^3 = H
16 R^1= COOH, R^2 = OCH_3, R^3 = H



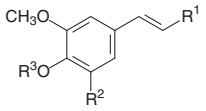
17 R^1= isopentenyl, R^2 = H
18 R^1= isopentenyl, R^2 = OH
19 R^1= R^2 = H
20 R^1= H, R^2 = OH



21 R^1= H, R^2 = isopentenyl
22 R^1= H, R^2 = geranyl
23 R^1= OH, R^2 = geranyl
24 R^1= OH, R^2 = farnesyl
25 R^1= R^2 = H
26 R^1 = OH, R^2 = H

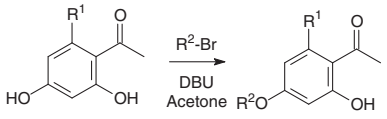


27 R^1 = geranyl
28 R^1 = isopentenyl
29 R^1 = H



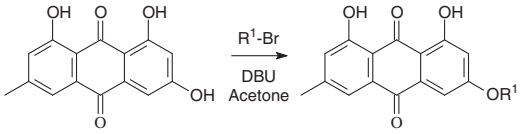
30 R^1= CHO, R^2 = H, R^3 = H
31 R^1= CHO, R^2 = OCH_3, R^3 = H
32 R^1= CHO, R^2 = OCH_3, R^3 = isopentenyl
33 R^1= CHO, R^2 = OCH_3, R^3 = geranyl
34 R^1= CHO, R^2 = H, R^3 = geranyl
35 R^1= CH_2OH, R^2 = OCH_3, R^3 = isopentenyl
36 R^1= CH_2OH, R^2 = OCH_3, R^3 = geranyl
37 R^1= CH_2OH, R^2 = H, R^3 = geranyl
38 R^1= CH_2OH, R^2 = OCH_3, R^3 = H
39 R^1= CH_2OH, R^2 = R^3 = H

Figure 1. Illustration of the chemical structures studied. The 39 compounds that we studied belong to six chemical groups: coumarins (compounds **1–6**; Table 1), cinnamic and benzoic acids (compounds **7–16**; Table 1), chalcones (compounds **17–20**; Table 1), acetophenones (compounds **21–26**; Table 1), anthraquinones (compounds **27–29**; Table 1) and cinnamaldehydes and cinnamyl alcohols (compounds **30–39**; Table 1).



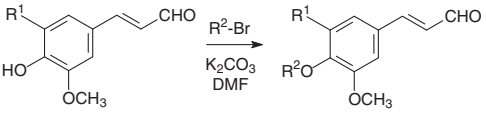
R^1 = H, OH
R^2 = geranyl, farnesyl

Scheme 1.



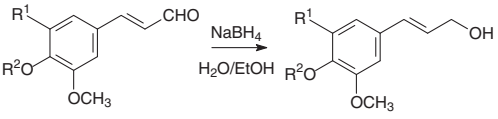
R^1 = isopentenyl, geranyl

Scheme 2.



R^1 = H, OCH_3
R^2 = isopentenyl, geranyl

Scheme 3.



R^1 = H, OCH_3
R^2 = H, isopentenyl, geranyl

Scheme 4.

In the current work, we characterized the in vitro growth inhibitory activity of 25 selected oxyprenylated phenylpropanoids compared to 14 non-prenylated compounds. We adopted the same pharmacological approach as the one described above for the novel cardiotonic steroid that we identified as a potential anticancer agent.[8–11] The in vitro IC_{50} growth inhibitory concentrations were determined for each compound in six human cancer cell lines. Then, we used computer-assisted phase-contrast microscopy, that is, quantitative videomicroscopy,[9–11] to determine whether the growth inhibitory activity of a given compound was due to cytotoxic or cytostatic effects.

Table 1
Physicochemical characteristics of compounds **1–39**

	P[a]	S[a]
1	99	100
2	99	94
3	99	43
4	98	–
5	98	–
6	99	–
7	99	–
8	99	–
9	99	–
10	99	–
11	99	–
12	99	–
13	99	–
14	99	–
15	99	–
16	99	–
17	98	100
18	98	–
19	98	–
20	99	–
21	99	–
22	99	–
23	98	–
24	98	–
25	99	–
26	99	–
27	98	–
28	98	–
29	99	95
30	99	98
31	99	81
32	99	88
33	99	85
34	99	–
35	99	–
36	99	–
37	99	–
38	99	–
39	99	–

[a] P means purity expressed as %, which was assessed after selective precipitation and/or crystallization. The stability (S), expressed as % of the products was measured by HPLC analyses following incubation in a physiological solution at 37 °C over 3 days. Results are expressed as the % of the incubated compound recovered.

One of the challenges faced by researchers when setting up new strategies to combat cancer is that several types of cancer, including glioblastoma (GBM),[17] non-small-cell lung cancer (NSCLC),[18,19] esophageal cancer,[20,21] pancreatic cancer,[22] melanoma[11,23] and metastatic cancers,[24] display significant levels of resistance to pro-apoptotic stimuli. This resistance indicates that cytotoxic pro-apoptotic agents are inefficient at curing cancers associated with intrinsic resistance to pro-apoptotic stimuli.[25,26] Compounds that induce non-apoptotic cell death or that display sustained cytostatic effects are, therefore, needed to combat 'apoptosis-resistant' cancer cells. The goal of this current work was to investigate whether certain oxyprenylated phenylpropanoids could induce sustained cytostatic effects in cancer cells that have significant levels of resistance to pro-apoptotic stimuli. We used the human U373 GBM cell line, which indeed exhibits various levels of resistance to pro-apoptotic stimuli.[10,27–29]

The chemical structures of the compounds that we studied are illustrated in Fig. 1.

The main natural sources of auraptene (**1**), 7-isopentenyloxycoumarin (**2**), umbelliprenin (**3**), 8-hydroxy-7-isopentenyloxy-coumarin (**4**), lacinartin (**5**), boropinic acid (**7**), 4′-geranyloxyferulic acid (**8**), isopentenyloxy-*p*-coumaric acid (**9**), geranyloxy-*p*-coumaric acid (**10**), valencic acid (**11**), isopentenyloxy vanillic acid (**12**), cordoin (**17**), 4′-hydroxycordoin (**18**), 2-hydroxy-4-isopentenyloxyacetophenone (**21**), and 2-hydroxy-4-geranyloxyacetophenone (**22**) have been described previously.[16]

The compounds 2′,6′-dihydroxy-4′-geranyloxyacetophenone (**23**) and 2′,6′-dihydroxy-4′-farnesyloxyacetophenone (**24**) have been previously extracted from the fruits of *Evodia merrilli* Kanehira & Sasaki,[30] from the aerial parts of *Boronia ramosa* Benth.,[31] from the fruits and aerial parts of *Melicope obscura* (Coode) T.G. Hartley, *M. obtusifolia* sp. *obtusifolia* var. *arborea* (Coode) T.G. Hartley[32] and *M. semecarpifolia* (Merr.) T.G. Hartley,[33] all belonging to the Rutaceae family. The anthranoids 3′-geranyloxyemodin (**27**) and madagascin (**28**) have been isolated from several species belonging to the *Vismia* and *Psorospermum* genera (Fam. Clusiaceae),[34,35] whereas (*E*)-3-(4-(3-methylbut-2-enyloxy)-3,5-dimethoxyphenyl)acrylaldehyde (**32**), nelumal A (**33**), and nelumol A (**36**) have been isolated from *Ligularia nelumbifolia* Hand. Mazz. (Fam. Asteraceae).[36] Boropinol C (**35**) has been obtained from the aerial parts of *Boronia pinnata* Sm. (Fam. Rutaceae).[37] (2*E*)-3-(4-((*E*)3,7-Dimethylocta-2,6-dienyloxy)-3-methoxyphenyl)acrylaldehyde (**34**) and (2*E*)-3-(4-((E)-3,7-dimethylocta-2,6-dienyloxy)-3-methoxyphenyl)prop-2-en-1ol (**37**) have been extracted from the bark of *Fagara rhetza* (Roxb.) DC (Fam. Rutaceae).[38]

To investigate the effects of O-prenylation of the phenylpropanoid core on the growth inhibition of cancer cells, we also investigated the effects of the parent compounds with free hydroxyl groups.

The syntheses of auraptene (**1**), 7-isopentenyloxycoumarin (**2**), umbelliprenin (**3**), 8-hydroxy-7-isopentenyloxycoumarin (**4**), lacinartin (**5**), boropinic acid (**7**), 4′-geranyloxyferulic acid (**8**), *p*-isopentenyloxycoumaric acid (**9**), *p*-geranyloxycoumaric acid (**10**), valencic acid (**11**), isopentenyloxy vanillic acid (**12**), cordoin (**17**), 4-hydroxycordoin (**18**), 2′,4′-dihydroxychalcone (**19**), 4,2′,4′-trihydroxychalcone (**20**), 2-hydroxy-4-isopentenyloxyacetophenone (**21**), and 2-hydroxy-4-geranyloxyacetophenone (**22**) were accomplished according to the procedures described previously.[39–42] The compounds 2′,6′-dihydroxy-4′-geranyloxyacetophenone (**23**) and 2′,6′-dihydroxy-4′-farnesyloxyacetophenone (**24**) were prenylated selectively at the 4′ position with geranyl and farnesyl bromide, respectively, in the presence of DBU as the base in acetone at room temperature for 2 h. The yields of the desired adducts were 55% and 62%, respectively (Scheme 1).

As reported previously,[40] the use of a sterically hindered base like DBU results in the selective alkylation of the more accessible OH moiety at the 4′ position. A very similar methodology, except that the reaction time was prolonged up to 24 h, was employed for the synthesis of 3′-geranyloxyemodin (**27**) and madagascin (**28**), which were obtained with 54% and 59% yields, respectively (Scheme 2).

Cinnamaldehydes (**30** and **31**) were prenylated using either isopentenyl bromide or geranyl bromide in DMF at 80 °C in the presence of K_2CO_3 as the base, resulting in (*E*)-3-(4-(3-methylbut-2-enyloxy)-3,5-dimethoxyphenyl)acrylaldehyde (**32**), nelumal A (**33**), and (2*E*)-3-(4-((*E*)3,7-dimethylocta-2,6-dienyloxy)-3-methoxyphenyl)acrylaldehyde (**34**) with yields of 89%, 65%, and 60%, respectively (Scheme 3).

It is important to emphasize that the use of a 'classic' solvent, such as acetone, instead of a polar aprotic solvent, such as DMF, to perform this type of phenolic etherification failed to produce the desired prenyloxy adduct. All of the synthesized oxyprenylated cinnamaldehydes were also reduced to the corresponding cinnamyl alcohol by treatment with $NaBH_4$ in a hydroalcoholic solution at room temperature for 30 min (Scheme 4). Boropinol C (**35**), nelumol A (**36**), and (2*E*)-3-(4-((*E*)-3,7-dimethylocta-2,6-dienyloxy)-3-methoxyphenyl)prop-2-en-1ol (**37**) were obtained with 64 %, 56 %, and 67 % yields, respectively. A similar procedure was employed for the synthesis of unprenylated 4-[(1*E*)-3-hydroxyprop-1-enyl]-2,6-dimethoxyphenol (**38**) and 4-[(1*E*)-3-hydroxy-

Table 2
In vitro growth inhibitory activity induced by treatment with the 39 compounds.

	MTT colorimetric assay (IC_{50}) (μM)[a]						Mean ± SEM
	CL1	CL2	CL3	CL4	CL5	CL6	
1	82	58	82	65	87	66	71±4
2	>100	79	>100	>100	>100	82	>100
3	72	30	71	75	66	50	61±4
4	>100	>100	>100	>100	>100	92	>91
5	70	>100	95	46	>100	40	>40
6	>100	33	>100	>100	>100	97	>33
7	>100	>100	>100	>100	>100	>100	>100
8	>100	>100	>100	>100	>100	>100	>100
9	>100	>100	>100	>100	>100	>100	>100
10	>100	>100	>100	>100	>100	>100	>100
11	>100	>100	>100	>100	>100	>100	>100
12	>100	>100	>100	>100	>100	>100	>100
13	>100	68	>100	>100	>100	95	>68
14	>100	66	>100	>100	>100	82	>66
15	>100	71	>100	>100	>100	94	>71
16	>100	61	>100	>100	>100	91	>61
17	53	39	54	71	81	58	54±6
18	87	58	66	>100	>100	57	>57
19	>100	49	>100	>100	75	81	>49
20	68	48	85	99	75	57	72 ±7
21	>100	>100	>100	>100	>100	>100	>100
22	48	37	51	27	44	38	40±3
23	29	34	34	48	40	35	36±3
24	33	18	26	38	33	32	29±3
25	>100	45	>100	>100	>100	97	>45
26	>100	42	100	>100	>100	90	>42
27	18	>100	32	30	40	14	23±4
28	5	10	20	6	7	8	10 ± 2
29	31	29	28	35	35	27	31 ± 1
30	28	29	59	35	33	23	35+5
31	56	39	>100	63	66	33	>33
32	30	29	68	28	32	31	37 ± 6
33	8	27	71	39	26	23	34 ± 9
34	7	34	5	24	28	12	16 ± 5
35	>100	>100	>100	>100	>100	>100	>100
36	42	39	76	40	72	38	47 ± 7
37	25	23	29	21	31	27	25 ± 1
38	>100	80	>100	>100	>100	>100	>80
39	97	60	85	84	>100	64	>100

[a] IC_{50} growth inhibitory concentrations were determined in vitro by MTT colorimetric assays. The cell lines (**CL**) included the human U373 (**CL1**; ECACC code 89081403) glioblastoma cell line, the OE21 (**CL2**; ECACC code 96062201) esophageal carcinoma cell line, the A549 (**CL3**; DSMZ code ACC107) NSCLC cell line, the PC-3 (**CL4**; DSMZ code ACC465) prostate cancer cell line, the SKMEL-28 (**CL5**; ATCC code HTB-72) melanoma cell line and the LoVo (**CL6**; DSMZ code ACC350) colon cancer cell line

prop-1-enyl]-2-methoxyphenol (**39**) (Scheme 4)**.** Finally, all products were purified by crystallization without the need for chromatographic separation, and yields were always calculated on the isolated product.

Table 1 details the levels of purity obtained for each of the 39 compounds under study for which the IC_{50} growth inhibitory concentrations have been determined in vitro by means of the MTT colorimetric assay in six human cancer cell lines. Table 1 also provides the stability levels in MEM cell culture medium for 72 h for those compounds for which sufficient amounts were still available after having carried out the pharmacological analyses described below. The data thus show that most compounds analyzed, accepted **3**, were stable in MEM cell culture medium when maintained for 72 h at 37 °C. The fact that compound **3** revealed itself as not being stable in cell culture medium emphasizes the need to characterize the stability of a given compound before entering pharmacochemical modulations to improve its anticancer activity.

Table 2 shows the growth inhibitory activity data of the synthesized compounds. Of the six chemical groups that were studied, cinnamic and benzoic acids displayed weak or no (IC_{50} >100 μM) inhibitory activity, whereas anthraquinones were the most active compounds (Table 1).

We did not notice any differences between the effects of the 0active compounds (IC_{50} <100 μM) on the growth inhibition of a cancer cell line with low levels of resistance to pro-apoptotic stimuli (the PC3 prostate cancer cell line[43]) and cell lines with certain levels of resistance (the SKMEL-28 cell line,[11] the U373 glioblastoma cell line,[10,27–29] and the OE21 esophageal cancer cell line[21]) (Table 2). We then submitted the human U373 GBM cell line to quantitative videomicroscopy analyses in order to determine as whether the compounds that were studied induced cytotoxic or cytostatic effects. While each compound has been analyzed at its MTT assay-related IC_{50} growth inhibitory concentration (Table 3), Figure 2 shows that this concentration appeared more efficient under quantitative videomicroscopy than MTT assay analyses, may be because distinct conditions of cell cultures. Three compounds assayed at their respective MTT assay-related IC_{50} growth inhibitory concentration (Table 3) also reduced by about 50% the growth of the U373 GBM cell line. These compounds are **20** (4,2′,4′-trihydroxychalcone), and **30** and **31** (two cinnamaldehydes).

Morphological analyses as illustrated in Figure 2 revealed that the growth inhibitory effects induced by these three compounds were cytostatic, not cytotoxic. The remaining compounds displayed mixed pattern of cytostatic versus cytotoxic effects (Fig. 3).

Prenylation of phenylpropanoids and alkaloids is a common metabolic reaction in nature, most frequently occurring in bacteria, fungi, and plants.[44] Very frequently, the addition of an isoprenoid chain renders the molecule pharmacologically more effective than

Table 3
Quantitative videomicroscopy data[a]

	% of Growth inhibition as compared to control	% Cell death after 72 h of culture
1	90	58 ± 14
2	–	–
3	90	40 ± 9
4	–	–
5	80	37 ± 10
6	–	–
7	–	–
8	–	–
9	–	–
10	––	–
11	–	–
12	–	–
13	–	–
14	–	–
15	–	–
16	–	–
17	95	88 ± 12
18	70	17 ± 5
19	-	–
20	60	7 ± 3
21	–	–
22	80	17 ± 7
23	80	15 ± 2
24	70	42 ± 28
25	–	–
26	–	–
27	90	100
28	80	95 ± 4
29	90	99 ± 1
30	50	13 ± 5
31	50	2 ± 1
32	90	100
33	80	100
34	80	26 ± 3
35	–	–
36	80	17 ± 3
37	80	41 ± 3
38	–	–
39	80	26 ± 15

[a] '–' Indicates not determined.

the parent non-prenylated compound. One of the clearest examples of this phenomenon was recently reported by Kretzschmar

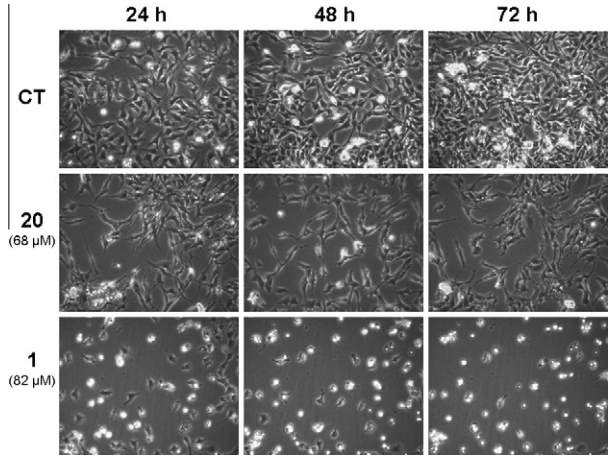


Figure 2. Morphological illustrations (quantitative videomicroscopy analyses) of changes induced by compounds **1** and **20** on the growth of the human U373 glioblastoma cell line. Cytostatic effects are defined as growth delay without observing numerous cell death as illustrated for compound **20**. In contrast, cytotoxic effects induce numerous cell deaths as illustrated for compound **1**. The % of cell deaths induced by each compound under study are detailed in Table 3.

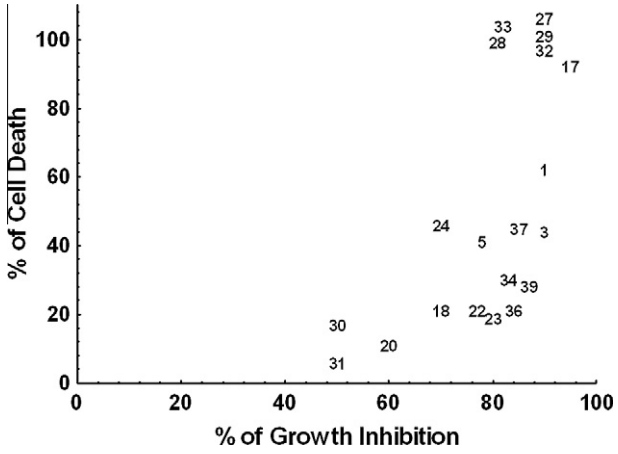


Figure 3. Illustration of the % of cell deaths induced by each compound under study (quantitative videomicroscopy analyses) in relation to the growth inhibitory effects induced by these compounds. Cell cultures have been monitored in triplicate for each experimental condition for 72 h with one image digitized each four minutes. Thus, 1,080 images have been recorded for each experimental condition. Growth inhibitory effects have been evaluated by counting the number of cells in the 1080th image and dividing this number by the number of cells present in the 1st image. The results have been normalized to control conditions arbitrarily normalized at 100% (Table 3).

and coworkers,[45] who observed that prenylated genistein and naringenin, among the most common flavonoids extracted from plants, exert stronger estrogenic activities than their respective parent compounds. In this context, selected prenyloxyphenylpropanoids (e.g., coumarins and cinnamic acids) were also shown to exert beneficial chemotherapeutic effects in vivo on chemically induced colon adenocarcinoma.[46]

In this study, we evaluated the ability of several naturally occurring oxyprenylated phenylpropanoids to inhibit growth in vitro. Table 1 reveals that in terms of structure–activity relationships, only one group of compounds among the chemical classes tested exhibited virtually no activity. This ineffective group of compounds was comprised of carboxylic acids, including prenyloxycinnamic and prenyloxybenzoic compounds. These compounds displayed IC_{50} values >100 μM. These IC_{50} values are higher than those recorded for their non-prenylated counterparts (**13–16**), for which marginal activity was revealed (Table 2). For the other oxyprenylated phytochemicals, prenylation of the hydroxyl group enhanced the observed anticancer effects in vitro (e.g., anthraquinones, acetophenones, and cinnamic aldehydes) (Table 2). Exceptions included coumarins, for which umbelliferone (**6**) exhibited a level of activity comparable to compounds **1–5**, and cinnamic alcohols, for which compound **35** had a markedly lower effect than the unprenylated parent molecules **38** and **39** (Table 2). The effect of the length of the O-side chain was strongly dependent on the class of oxyprenylated secondary metabolites. Among coumarins (compounds **1–5**), no significant differences were observed with respect to in vitro growth inhibitory activity (Table 2), although a farnesyloxy- or an isopentenyloxy- moiety appeared to enhance this activity. In the case of acetophenones, the presence of a C_5 side chain was not a necessary structural requirement for anticancer effects, at least in vitro.

Increasing the length of the side chain from C_{10} (geranyl) to C_{15} (farnesyl) resulted in a significant improvement of the observed growth inhibitory activity (Table 2). In contrast, among the anthraquinones, madagascin (**28**), which has an isopentenyloxy chain, was significantly more effective than 3′-geranyloxyemodin (**27**) (Table 2). Finally, for cinnamic aldehydes and alcohols, the influence of side chain length on the observed activity was similar. For these secondary metabolites, the most significant

enhancement of the observed effects occurred when the length of the O-chain increased from isopentenyl to geranyl (compounds **32–37**) (Table 2). When evaluating the growth inhibitory activity of substituents other than the prenylated ones, each class of natural products can be considered separately. Thus, for coumarins, the effects were slightly higher for compound **5,** which possesses a methoxy group in position 8, than for compounds **1–4,** which are deprived of this moiety (Table 2). When we compared chalcones **17** and **18,** the recorded growth inhibitory activity was improved for the compounds that do not have an hydroxy group in the 4′ position (Table 2). The presence of two hydroxy substituents in positions 2′ and 6′ (compounds **23** and **24**), rather than only one hydroxy substituent (compound **22**), increased the anticancer effects of prenyloxyacetophenones. For cinnamic aldehydes and alcohols, the structural features leading to higher growth inhibitory effects were similar (Table 2). Compounds having only one methoxy group in the 3′ position performed more effectively than those having two in positions 3′ and 5′ (Table 2).

In conclusion, oxyprenylated natural phenylpropanoids display either cytostatic or cytotoxic growth inhibitory effects in vitro. Three compounds, that is, **20** (4,2′,4′-trihydroxychalcone), and **30** and **31** (two cinnamaldehydes), displayed cytostatic effects in human U373 glioblastoma cells that usually resist to pro-apoptotic cytotoxic insults.

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References and notes

1. Coseri, S. *Mini Rev. Med. Chem.* **2009**, *9*, 560–571.
2. Gordaliza, M. *Clin. Transl. Oncol.* **2007**, *9*, 767–776.
3. Wang, H. K. *Drugs* **1998**, *1*, 92–102.
4. Jacquesy, J.C.; Fahy, J.; Berrier, C.; Bigg, D.; Jouannetaud, M.P.; Zunino, F.; Kruczynski, A.; Kiss, R. WO95/03312.
5. Bennouna, J.; Delord, J. P.; Campone, M.; Nguyen, L. *Clin. Cancer Res.* **2008**, *14*, 1625–1632.
6. Molinski, T. F.; Dalisay, D. S.; Lievens, S. L.; Saludes, J. P. *Nat. Rev. Drug Discov.* **2009**, *8*, 69–85.
7. Tan, G.; Gyllenhaal, C.; Soejarto, D. D. *Curr. Drug Targets* **2006**, *7*, 265–277.
8. Van Quaquebeke, E.; Simon, G.; Andre, A.; Dewelle, J.; Yazidi, M. E.; Bruyneel, F.; Tuti, J.; Nacoulma, O.; Guissou, P.; Decaestecker, C.; Braekman, J. C.; Kiss, R.; Darro, F. *J. Med. Chem.* **2005**, *48*, 849–856.
9. Mijatovic, T.; Roland, I.; Van Quaquebeke, E.; Nilsson, B.; Mathieu, A.; Van Vynckt, F.; Darro, F.; Blanco, G.; Facchini, V.; Kiss, R. *J. Pathol.* **2007**, *212*, 170–179.
10. Lefranc, F.; Mijatovic, T.; Kondo, Y.; Sauvage, S.; Roland, I.; Krstic, D.; Vasic, V.; Gailly, P.; Kondo, G.; Blanco, G.; Kiss, R. *Neurosurgery* **2008**, *62*, 211–222.
11. Mathieu, V.; Pirker, C.; Martin de Lassalle, E.; Vernier, M.; Mijatovic, T.; De Neve, N.; Gaussin, J. F.; Dehoux, M.; Lefranc, F.; Berger, W.; Kiss, R. *J. Cell Mol. Med.* **2009**, *13*, 3960–3972.
12. Mijatovic, T.; Lefranc, F.; Van Quaquebeke, E.; Van Vynckt, F.; Darro, F.; Kiss, R. *Drug Dev. Res.* **2007**, *68*, 1–10.
13. Mijatovic, T.; Van Quaquebeke, E.; Delest, B.; Debeir, O.; Darro, F.; Kiss, R. *BBA Rev. Cancer* **2007**, *1776*, 32–57.
14. Seligson, D. B.; Rajasekaran, S. A.; Yu, H.; Liu, X.; Eeva, M.; Tze, S.; Ball, W., Jr.; Horvath, S.; de Kernion, J. B.; Rajasekaran, A. K. *J. Urol.* **2008**, *179*, 338–345.
15. Sakai, H.; Suzuki, T.; Maeda, M.; Takahashi, Y.; Horikawa, N.; Minamimura, T.; Tsukada, K.; Takeguchi, N. *FEBS Lett.* **2004**, *563*, 151–154.
16. Epifano, F.; Genovese, S.; Menghini, L.; Curini, M. *Phytochemistry* **2007**, *68*, 939–953.
17. Lefranc, F.; Brotchi, J.; Kiss, R. *J. Clin. Oncol.* **2005**, *23*, 2411–2422.
18. Mathieu, A.; Remmelink, M.; D'Haene, N.; Penant, S.; Gaussin, J. F.; Van Ginckel, R.; Darro, F.; Kiss, R.; Salmon, I. *Cancer* **2004**, *101*, 1908–1918.
19. Han, S.; Roman, J. *Curr. Cancer Drug Targets* **2010**, *10*, 566–574.
20. D'Amico, T. A.; Harpole, D. H., Jr. *Chest Surg. Clin. N. Am.* **2000**, *10*, 451–469.
21. Bruyère, C.; Lonez, C.; Duray, A.; Cludts, S.; Ruysschaert, J. M.; Saussez, S.; Yeaton, P.; Kiss, R.; Mijatovic, T. *Cancer* **2010**. doi:10.1002/cncr.25687.
22. Wong, H. H.; Lemoine, N. R. *Nat. Rev. Gastroenterol. Hepatol.* **2009**, *8*, 412–422.
23. Soengas, M. S.; Lowe, S. W. *Oncogene* **2003**, *22*, 3138–3151.
24. Simpson, C. D.; Anyiwe, K.; Schimmer, A. D. *Cancer Lett.* **2008**, *272*, 177–185.
25. Savage, P.; Stebbing, J.; Bower, M.; Crook, T. *Nat. Clin. Pract. Oncol.* **2009**, *6*, 43–52.
26. Wilson, T. R.; Johnston, P. G.; Longley, D. B. *Curr. Cancer Drug Targets* **2009**, *9*, 307–319.
27. Lefranc, F.; James, S.; Camby, I.; Gaussin, J. F.; Darro, F.; Brotchi, J.; Gabius, H. J.; Kiss, R. *J. Neurosurg.* **2005**, *102*, 706–714.
28. Mégalizzi, V.; Mathieu, V.; Mijatovic, T.; Gailly, P.; Debeir, O.; De Neve, N.; Van Damme, M.; Bontempi, G.; Haibe-Kains, B.; Decaestecker, C.; Kondo, Y.; Kiss, R.; Lefranc, F. *Neoplasia* **2007**, *9*, 358–369.
29. Ingrassia, L.; Lefranc, F.; Dewelle, J.; Pottier, L.; Mathieu, V.; Spiegl-Kreinecker, S.; Sauvage, S.; El Yazidi, M.; Dehoux, M.; Berger, W.; Van Quaquebeke, E.; Kiss, R. *J. Med. Chem.* **2009**, *52*, 1100–1114.
30. Chou, C. J.; Lin, L. C.; Chen, K. T.; Chen, C. F. *J. Nat. Prod.* **1992**, *55*, 795–799.
31. Ahsan, M.; Gray, A. I.; Waterman, P. G.; Armstrong, J. A. *J. Nat. Prod.* **1994**, *57*, 673–676.
32. Adsersen, A.; Smitt, U. W.; Simonsen, H. T.; Christensen, S. B.; Jaroszewski, J. W. *Biochem. System Ecol.* **2007**, *35*, 447–453.
33. Chen, J. J.; Cho, J. Y.; Hwang, T. S.; Chen, I. S. *J. Nat. Prod.* **2008**, *71*, 71–75.
34. Noungoue, D. T.; Chaabi, M.; Ngouela, S.; Antheaume, C.; Boyom, F. F.; Gut, J.; Rosenthal, P. J.; Lobstein, A.; Tsamo, E. *Z. Naturforsc. C. J. Biosci.* **2009**, *64*, 210–214.
35. Tsaffack, M.; Nguemeving, J. R.; Kuete, V.; Ndejouong, T.; Le Sage, B.; Mkounga, P.; Beng, P.; Hultin, V.; Gregory, P.; Tsamo, E.; Nkengfack, A. E. *Chem. Pharm. Bull.* **2009**, *57*, 1113–1118.
36. Zhao, Y.; Lu, W.; Hao, X. J.; Cai, J. C.; Yu, H.; Sevenet, T.; Gueritte, F. *Chin. Chem. Lett.* **2002**, *13*, 201–204.
37. Ito, C.; Itoigawa, M.; Otsuka, T.; Tokuda, H.; Nishino, H.; Furukawa, H. *J. Nat. Prod.* **2000**, *63*, 1344–1348.
38. Shibuya, H.; Takeda, Y.; Zhang, R.; Tong, R. X.; Kitagawa, I. *Chem. Pharm. Bull.* **1992**, *40*, 2325–2330.
39. (a) Locatelli, M.; Tammaro, F.; Menghini, L.; Carlucci, G.; Epifano, F.; Genovese, S. *Phytochemistry Lett.* **2009**, *2*, 223–226; (b) Genovese, S.; Tammaro, F.; Menghini, L.; Carlucci, G.; Epifano, F.; Locatelli, M. *Phytochem. Anal.* **2010**, *21*, 261–267; (c) Genovese, S.; Epifano, F.; Carlucci, G.; Marcotullio, M. C.; Curini, M.; Locatelli, M. *J. Pharm. Biomed. Anal.* **2010**, *53*, 212–214.
40. Genovese, S.; Epifano, F.; Curini, M.; Dudra-Jastrzebska, M.; Luszczki, J. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5419–5422.
41. Epifano, F.; Menghini, L.; Pagiotti, R.; Angelini, P.; Genovese, S.; Curini, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5523–5525.
42. Epifano, F.; Curini, M.; Genovese, S.; Blaskovich, M.; Hamilton, A.; Sebti, S. M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2639–2642.
43. Dumont, P.; Ingrassia, L.; Rouzeau, S.; Ribaucour, F.; Thomas, S.; Roland, I.; Darro, F.; Lefranc, F.; Kiss, R. *Neoplasia* **2007**, *9*, 766–776.
44. Kuzuyama, T.; Noel, J. P.; Richard, S. B. *Nature* **2005**, *435*, 983–987.
45. Kretzschmar, G.; Zierau, O.; Wober, J.; Tischer, S.; Metz, P.; Vollmer, G. *J. Steroid Biochem. Mol. Biol.* **2010**, *118*, 1–6.
46. Tanaka, T.; de Azevedo, M. B.; Durán, N.; Alderete, J. B.; Epifano, F.; Genovese, S.; Tanaka, M.; Tanaka, T.; Curini, M. *Int. J. Cancer* **2010**, *126*, 830–840.