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# In vitro biological evaluation of novel 7-*O*-dialkylaminoalkyl cytotoxic pectolinarigenin derivatives against a panel of human cancer cell lines

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ABSTRACT

The effect of novel pectolinarigenin derivatives bearing a dialkylaminoalkyl substituent at *O*-7 on cell proliferation was evaluated in vitro in a panel of seven human cancer cell lines including renal adenocarcinoma ACHN, amelanotic melanoma C32, colorectal adenocarcinoma Caco-2, lung large cell carcinoma COR-L23, malignant melanoma A375, lung carcinoma A549 and hepatocellular carcinoma Huh-7D12 cell lines. Pectolinarigenin (**2**), obtained by hydrolysis of rutinose unit of the pectolinarin (**1**) isolated from *Linaria reflexa*, exhibited cytotoxic activity against Caco-2, A549 and A375 cell lines with $IC_{50}$ values of 5.3–8.2 μM. The most active pectolinarigenin derivative was **3** characterized by a dimethylamino-propoxy group in *O*-7 with $IC_{50}$ values of 7.2 and 7.4 μM against COR-L23 and A549 cell lines, respectively. A structure–activity relationship analysis of synthesized compounds was performed. None of the tested compounds affected the proliferation of skin fibroblasts 142BR suggesting a selective activity against tumor cells.



A significant part of drug discovery in the past few years has been focused on agents to prevent or treat cancer. This is not surprising because, in most developed countries and, to an increasing extent, cancer is among the three most common causes of death and morbidity. Cancer treatments may involve surgery, radiotherapy and chemotherapy and often a combination of two or all three is employed. Natural compounds from plants play a significant role in cancer chemotherapy but in spite of successes there is still much activity directed to finding novel anticancer agents.[1–3]

Flavonoids, a group of polyphenolic compounds derived from 2-phenylchromane, are found in considerable quantities in fruits, vegetables, seed, peel and tubers.[4,5] Besides their physiological role in plants, they have been shown to possess antiallergic, antiinflammatory, antiviral and anticarcinogenic activities.[6]

Flavonoids exert specific cytotoxic activity towards different cancer cell lines which has generated large interest in developing flavonoid-based cytostatics for anticancer therapy.[7] Different mechanisms have been linked to flavonoids mediated cytotoxicity including proteasome, topoisomerase and fatty acids synthesis, phosphatidyl-inositol 3-kinase inhibition, induction of cell cycle arrest, accumulation of p53 or enhanced expression of c-fos and c-myc genes.[8–15]

The cytotoxic activity of pectolinarigenin against human gastric adenocarcinoma MK-1, human uterus carcinoma HeLa, murine melanoma B16F10, human small lung carcinoma GLC4 and human colorectal cancer COLO 320 cell lines has been reported.[16,17] In a previous work, we determined the antiproliferative action of several flavones isolated from *Linaria reflexa* Desf. (Scrophulariaceae) and their derivatives against the large cell lung carcinoma cell line COR-L23, hepatocellular carcinoma cell line HepG-2, renal adenocarcinoma cell line ACHN, amelanotic melanoma cell line C32 and colorectal adenocarcinoma cell line Caco-2.[18] We found that pectolinarigenin and some flavonoid glycosides like pectolinarin exhibited strong cytotoxic activity on COR-L23 cell line with an $IC_{50}$ value of 5.03 and 4.07 μM, respectively.

As part of our screening program which considers the search for natural products with anticancer properties,[19–21] we further explored the potential cytotoxic effects of novel structural analogues of pectolinarigenin, able to give stable water-soluble salts.

The biological interest to develop structural analogues of antitumor agents possessing basic nitrogen atoms seemed highly desirable.[22–26] The results obtained in several classes of polyaromatic antitumour agents indicate that the introduction of an aminoalkylamino side chain permits to increase significantly the biological activity and the potency of the parent compounds.[27] The anthracenedione mitoxanthrone, anthrapyrazole losoxanthrone and pyridocarbazole retelliptine are interesting examples

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of this modification.[28–31] The distance between the amino groups plays an important role, but the optimal number of methylene units, generally two or three, greatly depends on the structure of the parent chromophore.[28,29] Additionally, the substituents at the distal amino group are important to be considered.[32–35] In the acronycine series, the replacement of the methoxy group at C-6 by a dialkylaminoalkylamino substituent was recently shown to give an interesting entry toward stable and antitumor candidates. Indeed, 6-dialkylaminoalkylamino-3,3,12-trimethyl-3,12-dihydro-7 H-pyrano[2,3-*c*]acridine-7-ones and their benzo[*b*]pyrano[2,3-*h*]acridine-7-one homologues, exhibited increased potency when compared with the parent compounds.[36,37]

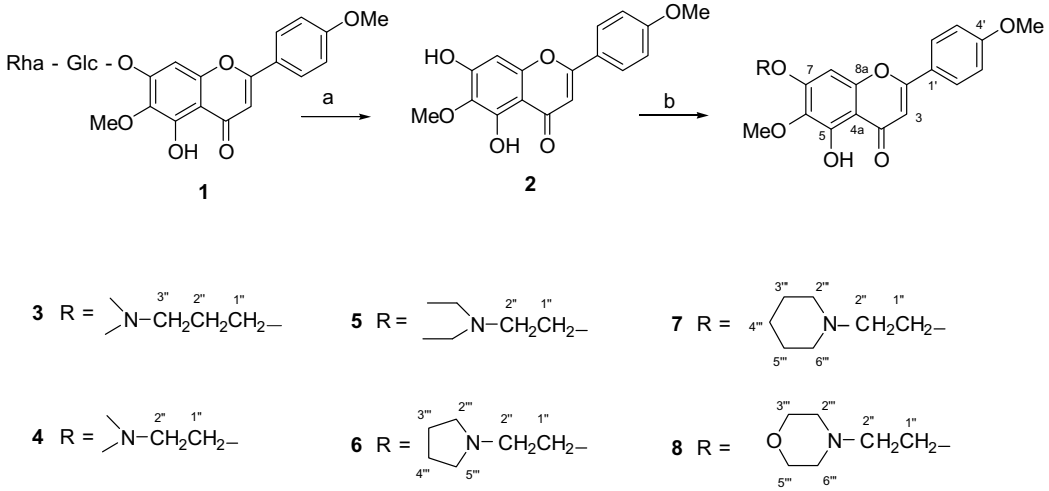
On the basis of these results and following our previous work, herein we report the synthesis of a novel series of 7-*O*-dialkylaminoalkyl pectolinarigenin derivatives and their cytotoxic potential in an in vitro cell culture system against a panel of human cancer cell lines.

The hydrolysis of the rutinose unit of pectolinarin (**1**), isolated from *L. reflexa*, was performed adding to a solution of HCl 5% (prepared using a mixture $H_2O$/MeOH 7:3) **1** (100 mg; 0.161 mmol) overnight at reflux. The reaction mixture was neutralized with an aqueous solution of NaOH and extracted with ethyl acetate. The extract was purified with column chromatography over silica gel 20–45 μm using a mixture of dichloromethane and methanol as eluent to afford pectolinarigenin (**2**) (42 mg, 0.134 mmol; 83%).

The synthesis of dialkylaminoalkyl pectolinarigenin derivatives was carried out according to the procedure described in Scheme 1.[38] The reaction of pectolinarigenin (**2**) with different dialkylaminoalkylchloride hydrochlorides produced the desired compounds **3**–**8** in good yield (63–82%). The structures of the compounds were determined on the basis of the spectral data (UV, IR, MS, $^1$H NMR, $^{13}$C NMR and 2D NMR).

The sulphorhodamine B (SRB) assay was used for measurement of cell proliferation.[39,40] Seven cancer cell lines, large cell carcinoma COR-L23 (ECACC No. 92031919), colorectal adenocarcinoma Caco-2 (ATCC No. HTB-37), amelanotic melanoma C32 (ATCC No. CRL-1585), hepatocellular carcinoma HepG-2 (ECACC No. 85011430), renal cell adenocarcinoma ACHN (ATCC No. CRL-1611), malignant melanoma A375 (ECACC No. 88113005), lung carcinoma A549 (ECACC No. 86012804), hepatocellular carcinoma Huh-7D12 (ECACC No. 01042712) and one normal cell line such as skin fibroblasts 142BR (ECACC No. 90011806) were used in our experiments. The COR-L23, C32 and ACHN cells were cultured in RPMI 1640medium while MRC-5, 142BR, Caco-2, A549, Huh-7D12, Caco-2, A375 and HepG-2 cells were cultured in DMEM. Both media were supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin. The cytotoxic assay was performed following a published protocol.[41,42]

All tested compounds **1**–**8** were able to inhibit the in vitro proliferation of seven human tumour cell lines in a concentration-dependent manner although marked differences in the degree of




**Scheme 1.** Synthesis of pectolinarigenin derivatives **3**–**8**. Reagents: (a) HCl 5% in $H_2O$/MeOH (7:3); (b) $K_2CO_3$, $CH_3OH$, argon.

**Table 1**
Cytotoxic profile and log*P* values of pectolinarin (**1**), pectolinarigenin (**2**) and pectolinarigenin derivatives **3**-**8** against selected human cancer cell lines

| Compound | log*P* [a] | $IC_{50}$ (μM)[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 142 BR | Huh-7D12 | Caco-2 | COR-L23 | ACHN | C32 | A549 | A375 |
| **1** | −0.7 ± 0.1 | >100 | 50.8 ± 2.8 | 6.2 ± 2.2° | 5.0 ± 2.3° | 17.2 ± 3.1° | 7.2 ± 3.2° | 38.8 ± 1.2 | 32.9 ± 2.9 |
| **2** | 2.0 ± 0.3 | >100 | >100 | 5.3 ± 1.8 | 4.1 ± 2.4° | 15.2 ± 1.8° | 7.0 ± 4.1° | 5.6 ± 0.9 | 8.2 ± 1.3 |
| **3** | 2.4 ± 0.2 | >100 | >100 | >100 | 7.2 ± 2.2 | 10.8 ± 1.9 | 8.9 ± 2.5 | 7.4 ± 1.4 | 7.7 ± 1.1 |
| **4** | 2.3 ± 0.1 | >100 | >100 | >100 | 9.2 ± 1.7 | 10.5 ± 2.6 | >100 | 13.2 ± 1.0 | >100 |
| **5** | 3.0 ± 0.2 | >100 | >100 | >100 | 41.9 ± 2.1 | 54.1 ± 1.3 | >100 | 59.9 ± 1.8 | >100 |
| **6** | 2.6 ± 0.2 | >100 | >100 | >100 | >100 | 12.7 ± 2.7 | 54.7 ± 2.9 | >100 | 38.6 ± 1.3 |
| **7** | 3.0 ± 0.4 | >100 | >100 | 78.6 ± 1.8 | 34.1 ± 1.7 | 92.6 ± 2.5 | 63.6 ± 1.3 | 44.9 ± 1.9 | 52.3 ± 1.5 |
| **8** | 1.9 ± 0.1 | >100 | >100 | >100 | 39.5 ± 2.5 | 55.7 ± 3.2 | 47.6 ± 2.1 | 85.1 ± 3.4 | 58.8 ± 2.2 |

Cell lines: human malignant melanoma A375, human amelanotic melanoma C32, human Caucasian lung carcinoma A549, human hepatocellular carcinoma Huh-7D12, human Caucasian lung large cell carcinoma COR-L23, human Caucasian colon adenocarcinoma Caco-2, human renal cell adenocarcinoma ACHN, human skin fibroblast 142BR. Vinblastine sulfate salt was used as positive control for 142BR, Huh-7D12, Caco-2, COR-L23, A375 and C32 cell lines, while taxol was used for ACHN cell line.

[a] Values are means of three experiments ($n$ = 3). °Previously published.[16]

cytotoxicity have been observed (Table 1). The flavone pectolinarigenin (**2**) demonstrated to be cytotoxic not only against COR-L23, ACHN and C32 cell lines as previously published, but also against Caco-2, A549 and A375 cell lines with $IC_{50}$ values ranging from 5.3 μM for Caco-2 to 8.2 μM for A375 cell lines. Interestingly, the cytotoxic activity of the precursor pectolinarin (**1**) against A549 and A375 cell lines becomes very remarkable when the hydrolysis of the rutinose unit was performed. On the contrary, the rutinose hydrolysis did not appear to improve potency against C32 cell line ($IC_{50}$ values of 7.2 and 7.0 μM for **1** and **2**, respectively).

Among the pectolinarigenin derivatives, compound **3** revealed to be the most active of the synthesized flavonoids tested with $IC_{50}$ values ranging from 7.2 to 10.8 μM against all tumor cell lines, except against Caco-2 and Huh-7D12 ($IC_{50}$ > 100 μM). Compound **4** showed selective cytotoxic activity against the two lung carcinoma cell lines such as COR-L23 and A549 and the renal adenocarcinoma cell line ACHN ($IC_{50}$ values of 9.2, 13.2 and 10.5 μM, respectively). The substitution of the dimethylamino-ethoxy group (in **4**) with the diethylamino-ethoxy group (in **5**) decreased the cytotoxic activity. Except for A549 and COR-L23 cell lines, compound **6**, characterized by a pyrrolidin-1-yl-ethoxy group in 7-position, was more active than compound **7** that present a piperidin-1-yl-ethoxy group. In particular, compound **6** showed an $IC_{50}$ value of 12.7 μM against ACHN cell line unlike compound **7** that showed an $IC_{50}$ value of 92.6 μM.

None of the tested compounds affected the proliferation of skin fibroblasts at the maximum concentration tested (100 μM) suggesting a selective activity against tumor cells.

Moreover, log*P* values for pectolinarin (**1**), pectolinarigenin (**2**) and pectolinarigenin derivatives (**3**–**8**) were evaluated. Data were reported in Table 1. It has been reported that for good absorption after oral administration a $\log P \geqslant 2$ is required.[43] Compounds **3**–**7** showed log*P* values ranging from 2.3 to 3.0. These findings, taken together with the ability to give stable water-soluble salts, suggest that pectolinarigenin derivatives might be candidate cytotoxic drugs with good absorption after oral administration.

Perusal of the literature revealed a promising agreement between our results and the reported activities of a number of flavonoids. Indeed, an impressive body of information exists on the antitumor action of plant flavonoids. Many works have concentrated on the direct and indirect actions of flavonoids on tumor cells and have found a variety of anticancer effects such as cell growth and kinase activity inhibition, apoptosis induction, suppression of the secretion of matrix metallo-proteinases and tumor invasive behaviour.[8,10,44] In recent years, many studies revealed that flavones, a class of flavonoids, exhibited a potent cytotoxic activity.[16,45–47]

In summary, a series of novel 7-*O*-dialkylaminoalkyl cytotoxic pectolinarigenin derivatives was prepared. Our results supported published data about flavones as interesting cytotoxic secondary metabolites. Certainly, the presence of methoxy groups in 6- and 4′-positions of pectolinarigenin (**2**) appear to play a non-negligible part in the antiproliferative potency of tested compounds. In our previous work we reported the cytotoxic activity profile of some natural pectolinarigenin glycosides, pectolinarin, linarin, isolinarin A, isolinarin B and pectolinarigenin-7-*O*-β-glucoside. In the newly synthesized series of amino-alkyloxy derivatives, the highest potency is obtained when a dimethylamino-propoxy group or a dimethylamino-ethoxy group is present in position 7.

These results contribute to further understanding the critical molecular requirements that lead to antiproliferative properties in the flavones series. Further in vivo studies are warranted to confirm the biological activity of the newly synthesized flavones and to investigate the molecular mechanisms responsible for the antiproliferative activity of the most active compounds with a potential pharmaceutical use.

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38. *General procedure for synthesis of pectolinarigenin derivatives.* To a solution of pectolinarigenin (**2**) (1 equiv) in DMF anhydrous was added a quantity of $K_2CO_3$ (5 equiv) and the different amino-alkylchlorides hydrochlorides (2.5 equiv) [2-diethylaminoethyl chloride; 3-dimethylaminopropyl chloride; 2-dimethylaminoethyl chloride; *N*-(2-chloethyl)piperidine; 4-(2-chloroethyl) morpholine; 1-(2-chloroethyl)pyrrolidine)]. The mixture was stirred at temperature ranged from 80 to 120 °C under argon for 24–48 h. The mixture was evaporated in vacuo and the residue was purified by column flash chromatography using the mixture dichloromethane/methanol/$(CH_3CH_2)_3$ N as eluent to afford the desired pectolinarigenin derivatives.
Reactions were monitored by thin-layer chromatography (TLC) using silica gel plates (VWR, Italy, Si gel $60F_{254}$), zones were detected visually under ultraviolet irradiation (254 and 365 nm) and read after spraying with sulfuric acid 50% (v/v) followed by heating. Flash column chromatography was performed with silica gel 230–400 mesh (VWR, Milan, Italy). NMR spectra were recorded with a Bruker AC 300 using $CDCl_3$ as solvent. $^1H$ NMR and $^{13}C$ NMR signals were unambiguously attributed by 2D NMR experiments. Mass spectra (MS) were recorded with a ZQ2000 Waters mass spectrometer (ESI). Infrared spectra have been performed with FT-IR Jasco 4200 spectrometer. The

UV spectra were recorded on Jasco V-530 spectrometer using 1 cm quartz cells. All solvents were dried according to standard procedures. All reagents were used as purchased without further treatment unless otherwise stated.

*Compound 3:* a yellow solid (yield 63%). mp 99–100 °C. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 12.74 (1H, s, $C_5$–OH), 7.83 (2H, d, $J$ = 9.0 Hz, H-2′, H-6′), 7.01 (2H, d, $J$ = 9.0 Hz, H-3′, H-5′) 6.58 (1H, s, H-3), 6.57 (1H, s, H-8), 4.15 (2H, t, $J$ = 7.5 Hz, H-1″), 3.90 (3H, s, $C_6$–$OCH_3$), 3.89 (3H, s, $C_4$′-$OCH_3$), 2.51 (2H, t, $J$ = 7.5 Hz, H-3″), 2.27 (6H, s, (N-$(CH_3)_2$), 2.09 (2H, m, H-2″); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 182.7 (C-4), 163.9 (C-2), 162.5 (C-4′), 158.3 (C-7), 153.2 (C-5), 153.1 (C-8a), 132.7 (C-6), 128.1 (C-2′, C-6′), 123.6 (C-1′), 114.4 (C-3′, C-5′), 106.0 (C-4a), 104.1 (C-3), 91.4 (C-8), 67.4 (C-1″), 60.9 ($C_6$–$OCH_3$), 56.1 (C-3″), 55.5 ($C_4$′-$OCH_3$), 45.5 ($N$-$(CH_3)_2$), 27.1 (C-2″); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3433, 2923, 2853, 1606, 1586, 1495, 1463, 1419, 1363, 1298, 1243, 1178, 1118, 1032, 833; ESIMS $m/z$: 400 $[MH]^+$, 422 $[MNa]^+$; Anal. calcd ($C_{22}H_{25}NO_6$) C = 66.15%, H = 6.31%. Found C = 66.22%, H = 6.37%.

*Compound 4:* a yellow solid (yield 65%). mp 140–141 °C. $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 7.95 (2H, d, $J$ = 8.3 Hz, H-2′, H-6′), 7.08 (2H, d, $J$ = 8.3 Hz, H-3′, H-5′), 6.82 (1H, s, H-3), 6.68 (1H, s, H-8), 4.29 (2H, t, $J$ = 5.2 Hz, H-1″), 3.90 (3H, s, $C_6$–$OCH_3$), 3.87 (3H, s, $C_4$′–$OCH_3$), 2.94 (2H, t, $J$ = 5.2 Hz, H-2″), 2.46 (6H, s, ($N$-$(CH_3)_2$); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 182.8 (C-4), 164.8 (C-2), 163.1 (C-4′), 158.1 (C-7), 153.3 (C-5), 152.5 (C-8a), 132.5 (C-6), 128.0 (C-2′, C-6′), 123.0 (C-1′), 114.2 (C-3′, C-5′), 105.5 (C-4a), 102.9 (C-3), 91.7 (C-8), 67.1 (C-1″), 59.7 ($C_6$-$OCH_3$), 57.3 (C-2″), 54.7 ($C_4$′–$OCH_3$), 44.6 ($N$-$(CH_3)_2$), IR (KBr) $\nu_{max}$ cm$^{-1}$: 3433, 2956, 2931, 2360, 1601, 1496, 1464, 1426, 1367, 1254, 1186, 1128, 1116, 1024, 910, 835, 574; ESIMS $m/z$: 386 $[MH]^+$; Anal. calcd ($C_{21}H_{23}NO_6$) C = 65.44%, H = 6.02%. Found C = 65.05%, H = 5.90%.

*Compound 5:* a yellow solid (yield 80%). mp 92–93 °C. $^1$H NMR (300 MHz, $CD_3OD$) δ7.96 (2H, d, $J$ = 8.9 Hz, H-2′, H-6′), 7.09 (2H, d, $J$ = 8.9 Hz, H-3′, H-5′), 6.83 (1H, s, H-3), 6.69 (1H, s, H-8), 4.34 (2H, t, $J$ = 4.5 Hz, H-1″), 3.90 (3H, s, $C_6$–$OCH_3$), 3.87 (3H, s, $C_4$′–$OCH_3$), 3.25 (2 H, t, $J$ = 4.5 Hz, H-2″), 2.96 (4H, q, ($N$-$(CH_2–CH_3)_2$), 1.24 (6H, t, ($N$-$(CH_2–CH_3)_2$); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 182.8 (C-4), 164.8 (C-2), 163.1 (C-4′), 157.8 (C-7), 153.3 (C-5), 152.5 (C-8a), 132.4 (C-6), 128.0 (C-2′, C-6′), 123.0 (C-1′), 114.2 (C-3′, C-5′), 105.6 (C-4a), 102.9 (C-3), 91.7 (C-8), 66.4 (C-1″), 59.7 ($C_6$–$OCH_3$), 54.7 ($C_4$′–$OCH_3$), 54.5 (C-2″), 50.7 (($N$-$(CH_2–CH_3)_2$), 9.6 (($N$-$(CH_2–CH_3)_2$); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3434, 2965, 2934, 2842, 1644, 1606, 1510, 1496, 1463, 1427, 1357, 1297, 1254, 1193, 1117, 1030, 826, 605; ESIMS $m/z$: 414 $[MH]^+$; Anal. calcd ($C_{23}H_{27}NO_6$) C = 66.81%, H = 6.58%. Found C = 66.70%, H = 6.51%.

*Compound 6:* a yellow solid (yield 76%). mp 86–87 °C. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.85 (2H, d, $J$ = 8.7 Hz, H-2′, H-6′), 7.03 (2H, d, $J$ = 8.7 Hz, H-3′, H-5′), 6.59 (1H, s, H-3), 6.56 (1H, s, H-8), 4.26 (2H, t, $J$ = 6.1 Hz, H-1″), 3.92 (3H, s, $C_6$–$OCH_3$), 3.90 (3H, s, $C_4$′–$OCH_3$), 3.04 (2 H, t, $J$ = 6.1 Hz, H-2″), 2.71 (4H, m, H-2‴, H-5‴), 1.86 (4H, m, H-3‴, H-5‴); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 182.7 (C-4), 164.0 (C-2), 162.6 (C-4′), 158.0 (C-7), 153.2 (C-5), 153.1 (C-8a), 132.8 (C-6), 128.0 (C-2′, C-6′), 123.6 (C-1′), 114.5 (C-3′, C-5′), 106.2 (C-4a), 104.1 (C-3), 91.4 (C-8), 68.5 (C-1″), 60.8 ($C_6$–$OCH_3$), 55.5 ($C_4$′–$OCH_3$), 54.8 (C-2‴, C-5‴), 54.4 (C-2″), 23.6 (C-3‴, C-5‴); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3425, 3080, 2931, 1654, 1606, 1511, 1496, 1463, 1428, 1363, 1299, 1254, 1195, 1118, 1031,830, 606; ESIMS $m/z$: 412 $[MH]^+$, 434 $[MNa]^+$, 450 $[MK]^+$; Anal. calcd ($C_{23}H_{25}NO_6$) C = 67.14%, H = 6.12%. Found C = 67.12%, H = 6.08%.

*Compound 7:* a yellow solid (yield 65%). mp 148–149 °C. $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 8.03 (2H, d, $J$ = 9.0 Hz, H-2′, H-6′), 7.36 (1H, s, H-3), 7.12 (2H, d, $J$ = 9.0 Hz, H-3′, H-5′), 6.82 (1H, s, H-8), 4.60 (2H, t, H-1″), 3.95 (3H, s, $C_6$–$OCH_3$), 3.90 (3H, s, $C_4$′–$OCH_3$), 3.58 (2H, t, H-2″), 3.17 (4H, m, H-3‴, H-7‴), 2.00 (6H, m, H-4‴, H-5‴, H-6‴); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 178.9 (C-4), 163.4 (C-2), 163.1 (C-4′), 156.6 (C-7), 154.6 (C-5), 149.8 (C-8a), 140.2 (C-6), 128.0 (C-2′, C-6′), 122.6 (C-1′), 114.3 (C-3′, C-5′), 111.6 (C-4a), 105.7 (C-3), 98.6 (C-8), 67.3 (C-1″), 60.7 ($C_6$–$OCH_3$), 57.0 (C-2″), 54.7 ($C_4$′–$OCH_3$), 54.0 (C-2‴), 53.6 (C-6‴), 22.8 (C-3‴, C-4‴, C-5‴); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3435, 2934, 2656, 1625, 1605, 1584, 1509, 1463, 1363, 1305, 1263, 1187, 1132, 1033, 993, 830, 575; ESIMS $m/z$: 426 $[MH]^+$, 448 $[MNa]^+$, 464 $[MK]^+$; Anal. calcd ($C_{24}H_{27}NO_6$) C = 67.72%, H = 6.40%. Found C = 67.72%, H = 6.38%.

*Compound 8:* amorphous (yield 82%). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 7.93 (2 H, d, $J$ = 8.9 Hz, H-2′, H-6′), 7.10 (1H, s, H-3), 7.06 (2H, d, $J$ = 8.9 Hz, H-3′, H-5′), 6.59 (1H, s, H-8), 4.32 (2H, t, H-1″), 3.88 (6H, s, $C_6$–$OCH_3$, $C_4$′–$OCH_3$), 3.74 (4H, m, H-3‴, H-5‴), 2.93 (2H, m, H-2″), 2.68 (4H, m, H-2‴, H-6‴); $^{13}$C NMR (75 MHz, $CD_3OD$) $\delta$ 178.0 (C-4), 162.7 (C-2), 162.3 (C-4′), 157.5 (C-7), 154.6 (C-5), 150.9 (C-8a), 140.6 (C-6), 127.6 (C-2′, C-6′), 123.0 (C-1′), 114.2 (C-3′, C-5′), 112.1 (C-4a), 105.3 (C-3), 97.4 (C-8), 67.0 (C-1″), 66.4 (C-3‴, C-5‴), 60.5 ($C_6$–$OCH_3$), 58.0 (C-2″), 54.6 ($C_4$′–$OCH_3$), 53.8 (C-2‴, C-6‴); IR (KBr) $\nu_{max}$ cm$^{-1}$: 3433, 2954, 2853, 1636, 1603, 1512, 1455, 1426, 1351, 1302, 1260, 1182, 1116, 1025, 948, 915, 835, 607, 564; ESIMS $m/z$: 428 $[MH]^+$, 450 $[MNa]^+$, 466 $[M]^+$; Anal. calcd ($C_{23}H_{25}NO_7$) C = 64.63%, H = 5.90%. Found C = 64.58%, H = 5.88%.


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42. *SRB assay.* One hundred microliters per well of this cell suspension was seeded in 96-well microtiter plates and incubated to allow for cell attachment. After 24 h, the cells were treated with serial dilutions of pure compounds. Each compound was initially dissolved in an amount of DMSO and diluted further in medium to produce six concentrations. Hundred microliters per well of each concentration was added to the plates in six replicates. By these serial dilutions, the final mixture used for treating the cells contained not more than 0.5% of the solvent (DMSO), the same as in the solvent- control wells. The final volume in each well was 200 μL. The plates were incubated for a select exposure time of 48 h. At the end of exposure time, 100 μL of ice-cold 40% trichloroacetic acid (TCA) was added to each well, left at 4 °C for 1 h, and washed five times with distilled water. The TCA-fixed cells were stained for 30 min with 50 μL of 0.4% (w/v) SRB in 1% HOAc. The plates were washed five times with 1% HOAc and air-dried overnight. Vinblastine sulfate salt was used as positive control for 142BR, Huh-7D12, Caco-2, COR-L23, A375, and C32 cell lines, while taxol was used for ACHN cell line. On the day of reading the plates, bound dye was solubilized with 100 μL of 10 mM TRIS base (tris[hydroxymethyl] aminomethane). The absorbance of each well was read on an ELISA reader at 564 nm. Cell survival was measured as the percentage absorbance compared to the control (non-treated cells). All values are expressed as means ± standard deviation of the mean (SD). All products are purchased from Sigma, Italy. The inhibitory concentration 50% ($IC_{50}$) was calculated from a dose–response curve obtained by plotting the percentage of inhibition versus the concentrations with the use of GraphPad Prism 4 software.


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