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Chemo-enzymatic synthesis and cell-growth inhibition activity of resveratrol analogues

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Abstract

The stilbenoid resveratrol (**1**) was subjected to regioselective acetylation catalysed by *Candida antarctica* lipase (CAL) to obtain 4′-acetylresveratrol (**2**). CAL biocatalysed regioselective alcoholysis of 3,5,4′-triacetylresveratrol (**3**), 3,5,4′-tributanoylresveratrol (**6**), and 3, 4, 5′-trioctanoylresveratrol (**9**) afforded derivatives **4**, **5**, **7**, **8**, **10**, and **11**. Further resveratrol analogues (**12–18**) were obtained through methylation and hydrogenation reactions, whereas the 3,4,4′-trimethoxystilbene (**19**) was obtained by complete synthesis. Resveratrol and its lipophylic analogues were subjected to cell-growth inhibition bioassays towards DU-145 human prostate cancer cells. Compounds **2**–**19** showed cell-growth inhibition activity comparable to or higher than resveratrol ($GI_{50} = 24.09$ μM), displaying low or very low toxicity against non-tumorigenic human fibroblast cells. Comparison of the trimethoxy stilbenes **12** ($GI_{50} = 2.92$ μM) and **19** ($GI_{50} = 25.39$ μM) indicates that the position of the substituents is important for the activity. The marked activity of methyl ethers **12**, **13**, and **18** in comparison with that of the corresponding esters suggests that the different chemical reactivity, rather than steric factors, strongly influences the activity.

Keywords: Resveratrol; *Candida antarctica* lipase; Synthesis; Cell-growth inhibition activity; DU-145 cells; Prostate cancer

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1. Introduction

Resveratrol (*trans*-3,5,4′-trihydroxystilbene, **1**), found in grapes as well as in other plants, is a natural phytoalexin biosynthesized in response to pathogenic attack or other stress conditions [1]. In recent times, it has been intensively studied for a variety of promising biological properties [2,3], among them antioxidant activity [4], inhibition of cyclooxygenase [5], inhibition of platelet aggregation [6], antiestrogenic activity [7]. Most of the popularity of resveratrol is probably due to its relatively high concentration in red wines and its possible role in the prevention of heart diseases, as evidenced by the so-called 'French paradox' [8]. More generally, to resveratrol have been ascribed chemopreventive activities against human degenerative diseases such as atherosclerosis [9] and cancer [10]. In particular, resveratrol showed cancer chemopreventive activity in assays representing anti-initiation, anti-promotion and antiprogression activity, inhibiting the development of preneoplastic lesions and tumorigenesis [10]. Further evidence showed that **1** is able to inhibit cell growth and to induce apoptosis (programmed cell death) in various human cancer cell lines [11–15]. In particular, a number of studies have been reported on the chemopreventive activity of **1** against prostate cancer [16]. More specifically, **1** showed differential effects on growth, cell cycle arrest, and induction of apoptosis in human prostate cancer cell lines [17], and resulted acutely toxic against DU-145 prostate cancer cells [18]. Thus, resveratrol may also play a role in cancer therapy and may be considered as a lead compound for preparation of analogues with enhanced bioactivity. A number of synthetic analogues of resveratrol have already been synthesized and submitted to bioassays. However, the structural requirements for the anti-tumour properties of resveratrol have not been completely established, and some conflicting results are reported in the literature [18–23]. Minor attention has been devoted to lipophylic analogues, and in particular to esters bearing acyl chains of different lengths, notwithstanding that the study of this kind of derivatives may be important in view of their enhanced affinity with lipophylic membrane constituents, and consequently of the probable increase of their cell uptake.

On this basis, we decided to carry out a study of lipophylic resveratrol derivatives, as part of our ongoing interest in bioactive natural products and their analogues. We have recently reported a chemo-enzymatic methodology for the preparation of regioselectively acylated resveratrol analogues in the presence of *Candida antarctica* lipase (CAL) [24]. This method allows preparation of resveratrol esters with different substitution patterns, a goal not trivial using traditional organic reagents in the presence of functional groups with very similar reactivity, such as the phenol groups of resveratrol. We planned also the preparation of methylated derivatives of **1**, in view of the recently reported data on the anti-tumour properties of these lipophylic analogues [19]. The prepared compounds were tested for cell-growth inhibition activity towards the above cited human prostate cancer DU-145 cell line, a model that some of us have recently used in a study on resveratrol-induced apoptosis of human prostate cancer [25]. Thus, we report here the results on the DU-145 cell-growth inhibition of a number of resveratrol analogues. To verify the possible cytotoxicity on normal cells, we performed also parallel experiments using primary lines of non-tumorigenic human fibroblasts.

2. Experimental

2.1. Materials

All reagents were of commercial quality and were used as received (Merck and Sigma-Aldrich); solvents were distilled and dried using standard techniques.

Resveratrol (**1**) was purchased from Sigma; the CAL (Chirazyme L-2, c. –f., C2, Lyo) was a gift from Roche.

The ^{1}H NMR spectra were recorded on a Varian Unity Inova spectrometer at 500 MHz and performed at constant temperature (27 °C). Electron impact (EI) and fast atom bombardment (FAB) mass spectra were recorded on a Kratos MS 50 instrument using 3-nitrobenzylalcohol (NBA) as matrix.

The enzymatic reactions were incubated at 40° under shaking at 400 rpm; control reactions without enzyme were carried out under the same conditions.

The progress of the chemo-enzymatic reactions was monitored, at regular time intervals, by thin-layer chromatography (TLC). Reactions were quenched on completion by filtering off the enzyme. Solvents were removed under reduced pressure and the products were purified by flash chromatography on silica gel DIOL 40–63 m (Merck) or using the Buchi-Biotage systems FLASH 12 or FLASH 40, equipped with prepacked silica gel cartridges 12S, 12M or 40S.

Analytical TLC was performed on silica gel (Merck 60 F_{254}) plates using cerium sulphate as developing reagents.

2.2. Acylation reactions

The peracylated derivatives [3,5,4′-tri-*O*-acetylresveratrol (**3**), 3,5,4′-tri-*O*-butanoylresveratrol (**6**), and 3,5,4′-tri-*O*-octanoylresveratrol (**9**)] were prepared under standard conditions using acetic and butyric anhydride in pyridine (1:1) and octanoyl chloride in pyridine (1:1) respectively.

Mass Spectroscopy (MS)-FAB and ^{1}H NMR spectra of 3,5,4′-tri-*O*-acetylresveratrol (**3**) were in perfect agreement with those reported in the literature [26].

3,5,4′-tri-*O*-Butanoylresveratrol **(6)**: white amorphous powder; EI-MS *m*/*z*: 438 [M^+]; ^{1}H NMR ($CDCl_3$): δ 7.48 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 7.10 (d, 2H, $J = 2.0$ Hz, H-2, H-6), 7.08 (d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 7.06 (d, 1H, $J = 16.0$ Hz, H-β), 6.97 (d, 1H, $J = 16.0$ Hz, H-α), 6.81 (t, 1H, $J = 2.0$ Hz, H-4), 2.54 (t, 6H, $J = 7.5$ Hz, 3,5,4′-OCOC$\underline{H}_2$CH$_2$CH$_3$), 1.79 (sest., 6H, $J = 7.5$ Hz, 3,5,4′-OCOCH$_2$C$\underline{H}_2$CH$_3$), 1.05 (t, 9H, $J = 7.5$ Hz, 3,5,4′-OCOCH$_2$CH$_2$C$\underline{H}_3$). Anal. Calcd for $C_{26}H_{30}O_6$: C, 71.21; H, 6.90; O, 21.89. Found: C, 71.92; H, 6.83; O, 21.25.

3,5,4′-tri-*O*-Octanoylresveratrol **(9)**: white amorphous powder; FAB-MS *m*/*z* 607 [M+H]$^+$; ^{1}H NMR ($CDCl_3$): δ 7.47 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 7.10 (d, 2H, $J = 2.0$ Hz, H-2, H-6), 7.07 (d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 7.05 (d, 1H, $J = 16.0$ Hz, H-β), 6.98 (d, 1H, $J = 16.0$ Hz, H-α), 6.79 (t, 1H, $J = 2.0$ Hz, H-4), 2.55 [t, 6H, $J = 7.5$ Hz, 3,5,4′-COC$\underline{H}_2$(CH$_2$)$_5$CH$_3$], 1.75 [pent, 6H, $J = 7.5$ Hz, 3,5,4′- OCOCH$_2$C$\underline{H}_2$(CH$_2$)$_4$CH$_3$], 1.43 [pent, 6H, $J = 7.5$ Hz, 3,5,4′-OCOCH$_2$(CH$_2$)$_4$C$\underline{H}_2$CH$_3$], 1.36 [bs, 18H, 3,5,4′-OCOCH$_2$CH$_2$(C$\underline{H}_2$)$_3$CH$_2$CH$_3$], 0.90 [t, 9H,

$J = 7.5$ Hz, 3,5,4′-$OCOCH_2(CH_2)_5C\underline{H}_3$]. Anal. Calcd for $C_{38}H_{54}O_6$: C, 75.21; H, 8.97; O, 15.82. Found: C, 75.09; H, 8.83; O, 16.08.

2.3. Methylation reaction

Sample (**1**, 90 mg, 0.39 mmol) was placed into a boiling flask and dispersed with 40 mL of acetone and 60 mg of potassium carbonate; 60 μl of dimethyl sulphate was added to this suspension, then heated for 24 h under a reflux condenser. Acetone was removed from the mixture by a rotary evaporator. The resulting mixture was purified by LC (silica gel, CH_2Cl_2 in *n*-hexane from 20 to 100%), thus affording 80 mg (89% yield) of **12** and 7.5 mg (8% yield) of **13**.

MS-FAB and ^{1}H NMR data of 3,5,4′-trimethoxystilbene (**12**) and 3,4′-dimethoxystilbene (**13**) are in agreement with those reported in the literature [27,28].

2.4. Hydrogenation reactions

A solution of the each substrate (**1**, **2**, **3**, **4** or **12**, 100 mg) in anhydrous EtOH (10 mL) was stirred at room temperature under 2 atm H_2 pressure in the presence of Pd/C (10 mg); the reactions were monitored by TLC and stopped after 12 h, by filtering off the catalyst. The filtrate was evaporated in vacuo and the product (**14**, **15**, **16**, **17**, or **18**) was obtained in quantitative yield.

MS-FAB and ^{1}H NMR data of dihydroresveratrol (**14**), 3,5,4′-tri-*O*-acetyldihydroresveratrol (**15**), and 3,5,4′-trimethoxydihydrostilbene (**18**) are in agreement with those reported in the literature [27,29].

3,5-di-*O*-Acetyl-dihydroresveratrol **(16)**: white amorphous powder; EI-MS *m/z* 314 [M^+]; ^{1}H NMR ($CDCl_3$): δ 7.02 (d, 2H, $J = 8.0$ Hz, H-3′, H-5′), 6.79 (d, 2H, $J = 2.0$ Hz, H-2, H-6), 6.76 (bt, 1H, H-4), 6.74 (d, 2H, $J = 8.0$ Hz, H-2′, H-6′), 2.85 (s, 4H), 2.28 (s, 6H). Anal. Calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77; O, 25.45. Found: C, 69.02.; H, 5.93; O, 25.05.

4′-*O*-Acetyl-dihydroresveratrol **(17)**: white amorphous powder. EI-MS *m/z* 272 [M^+]; ^{1}H NMR (CD_3OD): δ 7.17 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 6.96 (d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 6.14 (bd, 2H, H-2, H-6), 6.10 (bt, 1H, H-4), 2.85 (t, 2H, $J = 7.2$ Hz, H-α), 2.73 (t, $J = 7.2$ Hz, 2H, H-β), 2.24 (s, 3H, 4′-$OCOCH_3$). Anal. Calcd for $C_{16}H_{16}O_4$: C, 70.57; H, 5.92; O, 23.5. Found: C, 70.81; H, 5.84; O, 23.35.

2.5. Enzymatic acetylation of 1

The esterification reaction was carried out in sealed flasks at 40 °C. Resveratrol (**1**, 500 mg, 2.19 mmol) was dissolved in *t*-amyl alcohol (100 mL) and vinyl acetate (2 mL, 21.6 mmol). CAL (500 mg) was added to a solution of **1**. After 90 h incubation, the reactions were quenched and the residues purified by flash chromatography on prepacked silica gel cartridge (from 1 to 4% di-MeOH/CH_2Cl_2) to give 218 mg (43 %) of **2**.

4′-*O*-Acetylresveratrol **(2)**: MS-FAB and ^{1}H NMR spectra of **2** were identical to those reported in the literature [24].

2.6. Enzymatic alcoholysis

Each substrate (**3**, **6**, or **9**, 500 mg) was dissolved in *t*-butylmethylether (50 mL) and *n*-BuOH (2 mL). CAL (500 mg) was added to this solution and the mixture was maintained under shaking (400 rpm) at 40 °C for 0.5 h (**3**) or 48 h (**6** and **9**). The reactions were quenched by filtering off the enzyme and the filtrate was evaporated in vacuo. The residue was subjected to flash chromatography to obtain the deacylated analogues.

MS-FAB and ^{1}H NMR spectra of 3,5-di-*O*-acetylresveratrol (**4**) and 3-*O*-acetylresveratrol (**5**) are in perfect agreement with those reported in the literature [24].

3,5-di-*O*-Butanoylresveratrol **(7)**: white amorphous powder, 256 mg (61% yield) purified by LC (silica gel, from CH_2Cl_2 to 3% EtOAc–CH_2Cl_2); EI-MS *m*/*z* 368 [M^+]; ^{1}H NMR ($CDCl_3$): δ 7.34 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 7.06 (d, 2H, $J = 2.0$ Hz, H-2, H-6), 6.98 (d, 1H, $J = 16.0$ Hz, H-β), 6.82 (d, 1H, $J = 16.0$ Hz, H-α), 6.78 (d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 6.76 (bt, 1H, H-4), 2.55 (t, 4H, $J = 7.5$ Hz, 3,5-OCOC$\underline{H}_2$CH$_2$CH$_3$), 1.79 (sest., 4H, $J = 7.5$ Hz, 3,5-OCOCH$_2$C$\underline{H}_2$CH$_3$), 1.05 (t, 6H, $J = 7.5$ Hz, 3,5-OCOCH$_2$CH$_2$C$\underline{H}_3$). Anal. Calcd for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57; O, 21.71. Found: C, 71.58.; H, 6.45; O, 21.97.

3-*O*-Butanoylresveratrol **(8)**: white amorphous powder, 41 mg (12% yield) purified by LC (silica gel, from CH_2Cl_2 to 5% EtOAc–CH_2Cl_2); EI-MS *m*/*z* 298 [M^+]; ^{1}H NMR (CD_3OD): δ 7.38 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 7.03 (d, 1H, $J = 16.5$ Hz, H-β), 6.87 (d, 1H, $J = 16.5$ Hz, H-α), 6.81 (bt, 1H, H-2), 6.77 (d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 6.71 (bt, 1H, H-6), 6.38 (t, 1H, $J = 2.0$ Hz, H-4), 2.55 (t, 2H, $J = 7.0$ Hz, 3-OCOC$\underline{H}_2$CH$_2$CH$_3$), 1.76 (sest., 2H, 3-OCOCH$_2$C$\underline{H}_2$CH$_3$), 1.05 (t, 3H, 3-OCOCH$_2$CH$_2$C$\underline{H}_3$). Anal. Calcd for $C_{18}H_{18}O_4$: C, 72.47; H, 6.08; O, 21.45. Found: C, 72.22; H, 5.98; O, 21.8.

3,5-di-*O*-Octanoylresveratrol **(10)**: white amorphous powder, 166 mg (42% yield) purified by LC (silica gel, EtOAc-*n*-hexane from 10 to 25%); FAB-MS *m*/*z* 481 [M+H]$^+$; ^{1}H NMR ($CDCl_3$): δ 7.36 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 7.06 (d, 2H, $J = 2.0$ Hz, H-2, H-6), 6.80 (d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 7.01 (d, 1H, $J = 16.0$ Hz, H-β), 6.85 (d, 1H, $J = 16.0$ Hz, H-α), 6.76 (t, 1H, $J = 2.0$ Hz, H-4), 2.55 [t, 4H, $J = 7.5$ Hz, 3,5-COC$\underline{H}_2$(CH$_2$)$_5$CH$_3$], 1.75 [pent, 4H, $J = 7.5$ Hz, 3,5-OCOCH$_2$C$\underline{H}_2$(CH$_2$)$_4$CH$_3$], 1.41 [pent., 4H, $J = 7.5$ Hz, 3,5-OCOCH$_2$(CH$_2$)$_4$C$\underline{H}_2$CH$_3$], 1.36 [bs, 12H, 3,5-OCOCH$_2$CH$_2$(C$\underline{H}_2$)$_3$CH$_2$CH$_3$], 0.90 [t, 6H, $J = 7.5$ Hz, 3,5-OCOCH$_2$(CH$_2$)$_5$C$\underline{H}_3$]. Anal. Calcd for $C_{30}H_{40}O_5$: C, 74.97; H, 8.39; O, 16.64. Found: C, 75.01; H, 8.27; O, 16.72.

3-*O*-Octanoylresveratrol **(11)**: white amorphous powder, 32 mg (11% yield) purified by LC (silica gel, EtOAc-*n*-hexane from 10% to 30%); FAB-MS *m*/*z* 355 [M+H]$^+$; ^{1}H NMR ($CDCl_3$): δ 7.38 (d, 2H, $J = 8.5$ Hz, H-2′, H-6′), 7.06 (s, 1H, H-2), 6.79 (s, 1H, H-6), 6.81(d, 2H, $J = 8.5$ Hz, H-3′, H-5′), 7.01 (d, 1H, $J = 16.0$ Hz, H-β), 6.85 (d, 1H, $J = 16.0$ Hz, H-α), 6.48 (t, 1H, $J = 2.0$ Hz, H-4), 2.54 [t, 2H, $J = 7.5$ Hz, 3-COC$\underline{H}_2$(CH$_2$)$_5$CH$_3$], 1.74 [pent, 2H, $J = 7.5$ Hz, 3-OCOCH$_2$C$\underline{H}_2$(CH$_2$)$_4$CH$_3$], 1.41 [pent., 2H, $J = 7.5$ Hz, 3-OCOCH$_2$(CH$_2$)$_4$C$\underline{H}_2$CH$_3$], 1.36 [bs, 6H, 3-OCOCH$_2$CH$_2$(C$\underline{H}_2$)$_3$CH$_2$CH$_3$], 0.90 [t, 3H, $J = 7.5$ Hz, 3-OCOCH$_2$(CH$_2$)$_5$C$\underline{H}_3$]. Anal. Calcd for $C_{22}H_{26}O_4$.: C, 74.55 H, 7.39; O, 18.06. Found: C, 75.01; H, 7.27; O, 17.72.

2.7. Synthesis of 3,4,4′-trimethoxystilbene (**19**)

4-Methoxybenzylchloride (1.1 mL, 8.13 mmol) was heated with excess of triethyl phosphite (1.85 mL, 10.6 mmol) to 130 °C to give a 4-methoxy-diethylbenzylphosphonate (1.82 g, 7.1 mmol, yield 87%), which was cooled to 0 °C, and dry DMF (10 mL) and 0.41 g (7.6 mmol) of sodium methoxide were added. To this solution, 1.18 g (7.1 mmol) of 3,4-dimethoxybenzaldeide was added to stand at room temperature for 1 h. The mixture was heated to 100 °C, allowed to stand at this temperature for 1 h. After cooling, the reaction mixture was stirred overnight and subsequently quenched with water and extracted with diethylether. The combined organic layers were washed with water and dried over Na_2SO_4, affording 1.57 g of the product **19** (yield 83%). EI-MS *m/z* 270 [M^+]; ^{1}H NMR ($CDCl_3$): δ 7.44 (d, 2H, $J = 8.0$ Hz, H-3′, H-5′), 7.05 (d, 1H, $J = 2.0$ Hz, H-2), 7.02 (dd, 1H, $J = 8.0$, $J = 2.0$ Hz, H-6), 6.92 (bs, 2H, H-α e β), 6.89 (d, 2H, $J = 8.0$ Hz, H-2′, H-6′), 6.86 (d, 1H, $J = 8.0$ Hz, H-5, 3.94 (s, 3H, CH_3), 3.90 (s, 3H, CH_3), 3.83 (s, 3H, CH_3). Anal. Calcd for $C_{17}H_{18}O_3$: C, 75.53; H, 6.71; O, 17.76. Found: C, 75.37; H, 6.52; O, 18.11.

2.8. Cell cultures

Androgen-non-responsive DU-145 human prostate cancer cells were cultured in Earle minimal essential medium (EMEM), containing 10% fetal calf serum, 1 mM L-glutamine, antibiotics (50 IU/mL penicillin and 50 μg/mL streptomycin), and 1% non-essential amino acids (Invitrogen Life Technologies, Inc.).

Primary lines of non-tumorigenic, non-immortalized, human adult fibroblasts were grown in Dulbecco's modified eagle's medium containing 10% fetal calf serum, 100 U/mL penicillin, 100 g/mL streptomycin, and 25 g/mL fungizone (Invitrogen Life Technologies, Inc.).

The cells were incubated at 37 °C in humidified 5% CO_2/95% atmosphere and transferred to subcultures every 3 days following treatment with trypsin–EDTA.

2.9. Cell-growth inhibition assay (MTT test)

The MTT assay, used to evaluate cell viability, measures the cellular capacity to reduce 3-(4,5-dimethylthiazol-2-yl)diphenyltetrazolium bromide (MTT) to blue formazan products by various mitochondrial dehydrogenase enzymes. The method described by Mosmann and Chung [30] was employed. Briefly, DU-145 and human adult fibroblast cells (1×10^4) were set up in flat-bottomed 200 L microplates, incubated at 37 °C in a humidified 5% CO_2/95% air mixture and, 24 h later, treated with the resveratrol compounds at concentrations of 50, 25, 12.5, and 6.25 μM for 72 h, except for the compound **12**, which was tested also at concentrations of 3.12 and 1.56 μM for 72 h, before cell harvesting. Four hours before the end of the culture, 20 L of 0.5% MTT in phosphate buffer saline was added to each microwell. After incubation with the reagent, the supernatant was removed and replaced with 100 μL of dimethyl sulfoxide. The optical density of each sample was measured with

a microplate spectrophotometer reader (Titertek Multiskan, Flow Laboratories) at λ 570 nm.

2.10. Statistical analysis

Each experiment was repeated at least three times in quadruplicate and the mean $\pm$ SEM for each value was calculated. Statistical analysis of results was performed using Student's t test and one-way ANOVA by the statistical software package SYSTAT, version 9 (Systat Inc., Evanston, IL, USA). A difference was considered significant at $P < 0.01$.

3. Results and discussion

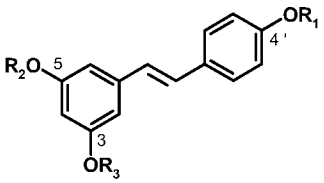
We report here the preparation of 17 resveratrol derivatives (**2**–**18**) obtained through acylation, methylation, and hydrogenation of **1**, as well as the complete synthesis of the 3,4,4′-trimethoxystilbene (**19**). These compounds were assayed for cell-growth inhibition activity towards DU-145 human prostate cancer cells and non-tumorigenic human fibroblasts. For the latter, the values of percent viability of treated samples (at the maximum concentration, 50 M) vs. untreated controls were from 80 to 100% except for the compound **19** (74%), indicating that resveratrol analogues are not cytotoxic against normal cells. Thus, we report here only the GI_{50} values on DU-145 cells (see Table 1). We planned to investigate the effect on the cell-growth inhibition activity of a regioselective substitution of the –OH groups in **1** with more lipophylic groups, like –OMe or –OCOR, which should enhance the affinity with biological membranes and consequently increase the cell uptake. In addition, we prepared also the dihydroderivatives of **1** and its acetylated (**2–4**) and permethylated (**12**) analogues in order to evaluate the possible role of the central double bond. To this purpose, we have employed both classical chemical reactions and enzymatic methods, according to our previous work [24]. The 4′-acetylresveratrol (**2**) was more conveniently obtained in high yield through direct CAL-catalysed esterification with vinyl acetate employing *t*-amyl alcohol as solvent (see Scheme 1A): in these conditions a high selectivity of CAL was observed, so that after 90 h only the 4′-acetyl derivative was obtained. The high selectivity of the direct esterification was counterbalanced by a low reactivity, so that the 4′-acylated derivatives with longer acyl chain could not be obtained with satisfactory yields. The majority of regioselectively acylated resveratrol analogues were obtained through alcoholysis of a peracylated resveratrol derivative dissolved in organic solvent and in the presence of CAL [24].

The peracylated analogues, namely 3,5,4′-triacetylresveratrol (**3**), 3,5,4′-tributanoylresveratrol (**6**), and trioctanoylresveratrol (**9**), were prepared from **1** according to standard acylation procedures (see Section 2). The biocatalysed deacylation reactions were carried out in *t*-butylmethylether, using an eccess of *n*-butanol as nucleofile (see Scheme 1B). Due to the preferential discrimination of the 4′ position by CAL, the 3,5-diacylderivative is normally obtained as the main product. This then becomes a substrate in a further deacylation step, thus giving the 3-acyl derivative.

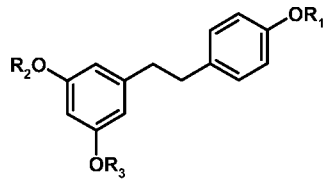
Table 1
DU-145 growth inhibition[a] of resveratrol (**1**) and analogues (**2**–**19**)

GI_{50} (μM)[b]				
1	**2**	**3**	**4**	**5**
24.09 ± 0.4	32.18 ± 0.7	23.34 ± 0.5	25.21 ± 0.9	25.42 ± 0.4
6	**7**	**8**	**9**	**10**
23.87 ± 0.5	19.07 ± 0.9	21.63 ± 1.1	25.07 ± 0.6	30.82 ± 0.7
11	**12**	**13**	**14**	**15**
25.20 ± 0.8	2.92 ± 0.9	12.24 ± 1.4	22.85 ± 0.6	29.76 ± 0.8
16	**17**	**18**	**19**	
21.91 ± 1.3	23.66 ± 0.6	12.37 ± 0.5	25.39 ± 0.5	

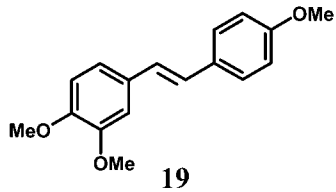
[a] Human prostate tumour cell line.
[b] GI_{50} calculated after 72 h of continuous exposure relative to untreated controls. Values are the mean (±SD) of three experiments.



	R_1	R_2	R_3
1	H	H	H
2	$COCH_3$	H	H
3	$COCH_3$	$COCH_3$	$COCH_3$
4	H	$COCH_3$	$COCH_3$
5	H	$COCH_3$	H
6	COC_3H_7	COC_3H_7	COC_3H_7
7	H	COC_3H_7	COC_3H_7
8	H	COC_3H_7	H
9	COC_7H_{15}	COC_7H_{15}	COC_7H_{15}
10	H	COC_7H_{15}	COC_7H_{15}
11	H	COC_7H_{15}	H
12	CH_3	CH_3	CH_3
13	CH_3	CH_3	H

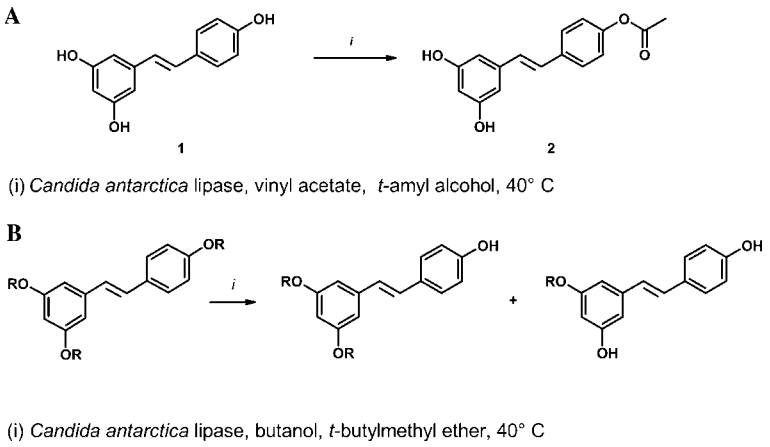


	R_1	R_2	R_3
14	H	H	H
15	$COCH_3$	$COCH_3$	$COCH_3$
16	H	$COCH_3$	$COCH_3$
17	$COCH_3$	H	H
18	CH_3	CH_3	CH_3



19

Further progression of the reaction would have afforded **1**, so we stopped the reaction when a convenient amount of the diacylated and monoacylated derivatives had been obtained. It is worth noting here that the little difference in the length of the acyl chain from acetyl to butanoyl causes a dramatic decrease in the reaction rate: the 3,5-diacetylresveratrol is obtained with good yield in 30 min, while a comparable amount of the 3,5-dibutanoyl can be recovered only after 30 h. Also, the diacetate **3** is more reactive than the dibutanoate **6**: the latter, after 48 h, was still present in the reaction mixture to an extent of 60%, affording also 12% of the monobutanoate **8** and only trace amounts of resveratrol. In contrast, **3** is rapidly converted into **5** and the reaction had to be stopped after 30–40 min to avoid a massive conversion to resveratrol. A standard duration of 48 h was used for the conversion of trioctano-



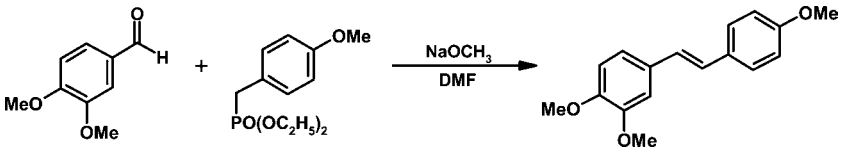
Scheme 1.

ate **9**, in order to have a satisfactory yield of both the main product **10** and the monoacylated analogue **11**.

The 3,5,4′-trimethoxystilbene (**12**) and the related 3,4′-dimethoxy-5-hydroxystilbene (**13**) were obtained as the main product and as a minor product, respectively, following standard methylation of **1** (see Section 2). Some of our preliminary results from bioassays as well as recent literature data prompted us to investigate the effect of a different substitution pattern of the stilbenoids nucleus bearing three methoxy group. Thus, we carried out a complete synthesis of *trans*-3,4,4′-trimethoxystilbene (**19**), according to Scheme 2. We employed a classical method [31] for the synthesis of stilbenoids, through a Wittig-like reaction of 4-methoxy-diethylbenzylphosphonate with 3,4-dimethoxybenzaldehyde to obtain the stilbenoid **19**.

The dihydroderivatives, namely dihydroresveratrol (**14**), 3,5,4′-triacetyldihydroresveratrol (**15**), 3,5-diacetyldihydroresveratrol (**16**), 4′-acetyldihydroresveratrol (**17**), and 3,5,4′-trimethoxydihydrostilbene (**18**), were obtained by catalytic hydrogenation of the corresponding unsaturated compounds (see Section 2).

The results of the cell-growth inhibition assays on a total of 19 compounds, including resveratrol (**1**), are reported as GI_{50} (μM) and summarised in Table 1. It is apparent at a glance that none of the analogues shows absent or negligible activity with respect to **1**. Conversely, nine compounds show an activity higher than that of resveratrol. In particular, the most active analogue is the 3,5,4′-trimethoxystilbene



Scheme 2.

12, showing $GI_{50} = 2.92$ μM. Also, the 3,4′-dimethoxystilbene **13** ($GI_{50} = 12.24$ μM) and the 3,4,4′-dihydrotrimethoxystilbene **18** ($GI_{50} = 12.37$ μM) are significantly more active than resveratrol, while all the other compounds show cell-growth inhibition activity comparable to that of resveratrol, and in some cases (3,5,4′-triacetylresveratrol **3**, 3,5,4′-tributanoylresveratrol **6**, 3,5-dibutanoylresveratrol **7**, 3-butanoylresveratrol **8**, dihydroresveratrol **14**, 3,5-dihydrodiacetylresveratrol, **16**), slightly higher. Two hydrogenated derivatives are more active than the parent compound, namely **14** and **16**, but small differences are observed. Conversely, the dihydroderivative **18** is less active than the parent compound **12**, although it maintains an activity higher than resveratrol. These results suggest that the central double bond is not essential for the activity but may have a role, for instance, in blocking the conformational motion of the trimethoxy structure **12** in the highly active configuration 19. Of course, this point requires further evidence to be established. Interestingly, the 3,4,4′-trimethoxystilbene **19** ($GI_{50} = 25.39$ μM) results in dramatically less activity than the permethylated resveratrol **12**, nothwithstanding the minimal structural difference between these compounds.

4. Conclusions

The present study confirmed the usefulness of biocatalysed reactions carried out in organic solvents for the regioselective modification of bioactive polyphenols. This methodology — employing CAL — allowed us to obtain resveratrol analogues with a different substitution pattern, that is, 4′-acetylresveratrol (**2**) as well as 3-acyl, 3,5-diacyl, and triacyl derivatives with C-2, C-4, and C-8 acyl chain (**3–11**). Conventional chemical conversions and synthesis afforded also compounds **12–19**, and cell-growth inhibition activity towards DU-145 prostate cancer cells was evaluated for this panel of 19 compounds. The first conclusion we can draw is that lipophylic analogues of resveratrol maintain cell-growth inhibition activity, which is enhanced in particular in methyl ethers with 3,5,4′ substitution pattern. A second important observation is that the activity is maintained or increased even in the absence of free hydroxyl groups. In fact, we report here nine derivatives showing activity comparable or higher than resveratrol and lacking free hydroxyl groups. This is worth noting in connection with previous literature data reporting that chemically available hydroxyl groups are required for DU-145 cell cycle arrest and decreased cell viability [18], and at least the 4′-OH group is required for inhibition of cell proliferation [23]. Comparison of the two trimethoxy stilbenes **12** and **19** also indicates that the position of the substituents is important for cell-growth inhibition activity, at least towards DU-145 cells. The marked activity of methyl ethers **12**, **13**, and **18** in comparison with that of the corresponding esters suggests that the different chemical reactivity, rather then steric factors, strongly influences the activity. Resveratrol and its esters might be ‘prodrugs’ of the metabolite active in vivo, that is, a methoxystilbene formed through biological methylation. In this hypothesis, lipophylic analogues would be better absorbed into the cell, but esters would require hydrolysis by cellular esterases to be converted into the active O-methylated form. Of course, further studies are needed

for better elucidation of the structural determinants and the mechanism of action of these cell-growth inhibition stilbenoids. However, the results reported here confirm the promising biological properties of resveratrol analogues and may offer a further impulse to the development of stilbenoids-based anti-tumour drugs with low or absent toxicity against non-tumorigenic cells.

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References

[1] P. Sarig, Y. Zutkhi, A. Monjauze, N. Lisker, R. Ben-Arie, Physio. Mol. Plant Pathol. 50 (1997) 337–347.
[2] J. Gusman, H. Malonne, G. Atassi, Carcinogenesis 22 (2001) 1111–1117.
[3] L. Fremont, Life Sci. 66 (2000) 633–673.
[4] L. Belguendouz, L. Fremont, A. Linard, Biochem. Pharmacol. 53 (1997) 1347–1355.
[5] M. Maccarrone, T. Lorenzon, P. Guerrieri, A. Finazzi, Agró, Eur. J. Biochem. 265 (1999) 27–34.
[6] C.R. Pace-Asciak, O. Rounova, S.E. Hahn, E.P. Diamandis, D.M. Goldberg, Clin. Chimi. Acta 246 (1996) 163–182.
[7] B.D. Gehm, J.M. McAndrews, P.-Y. Chien, L. Jameson, Proc. Natl. Acad. Sci. USA 94 (1997) 14138–14143.
[8] A.Y. Sun, A. Simonyi, G. Sun, Free Radic. Biol. Med. 32 (2002) 314–318.
[9] C.R. Pace-Asciak, S. Hahn, E.P. Diamandis, G. Soleas, D.M. Goldberg, Clin. Chim. Acta 235 (1995) 207–219.
[10] M. Jang, L. Cai, G.O. Udeani, K.V. Slowing, C.F. Thomas, C.W.W. Beecher, H.H.S. Fong, N.R. Farnsworth, A.D. Kinghorn, R.G. Mehta, R.C. Moon, J.M. Pezzuto, Science 275 (1997) 218–220.
[11] Y.-J. Surh, Y.-J. Hurh, J.-Y. Kang, E. Lee, G. Kong, S.J. Lee, Cancer Lett. 140 (1999) 1–10.
[12] Y. Schneider, V. Florence, D. Benoit, B. Lassina, F. Gossé, C. Bergmann, N. Seilers, F. Raoul, Cancer Lett. 158 (2000) 85–91.
[13] K. Joe, H. Liu, M. Suzui, M.E. Vural, D. Xiao, I.B. Weinstein, Clin. Cancer Res. 8 (2002) 893–903.
[14] P.-L. Kuo, L.-C. Chiang, C.-C. Lin, Life Sci. 72 (2002) 23–34.
[15] O.P. Mgbonyebi, J. Russo, I.H. Russo, Int. J. Oncol. 12 (1998) 865–869.
[16] H.L. Ratan, W.P. Steward, A.J. Gesher, J.K. Mellon, Urol. Oncol. 7 (2002) 223–227.
[17] T.-C. Hsieh, J.M. Wu, Exp. Cell Res. 249 (1999) 109–115.
[18] G.Z. Morris, R.L. Williams, M.S. Elliot, S.J. Beebe, Prostate 52 (2002) 319–329.
[19] G.R. Pettit, M.P. Grealish, M.K. Jung, E. Hamel, R.K. Pettit, J.-C. Chapuis, J.M. Schidt, J. Med. Chem. 45 (2002) 2534–2542.
[20] M. Roberti, D. Pizzirani, D. Simoni, R. Rondanin, R. Baruchello, C. Bonora, F. Buscemi, S. Grimaudo, M. Tolomeo, J. Med. Chem. 46 (2002) 3546–3554.
[21] Y. Schneider, P. Chabert, J. Stutzamann, D. Coelho, A. Fougerousse, F. Gossé, J.-F. Launay, R. Brouillard, F. Raul, Int. J. Cancer 107 (2003) 189–196.
[22] F. Raul, Y. Schneider, R. Brouillard, A. Fougerousse, P. Chabert, E. Gonzalez, PCT Int. Appl. (2003) 38 (CODEN: PIXXD2 WO 2003031381).

[23] L.A. Stivala, M. Savio, F. Carafoli, P. Perucca, L. Bianchi, G. Maga, L. Forti, U.M. Pagnoni, A. Albini, E. Prosperi, V. Vannini, J. Biol. Chem. 276 (2001) 22586–22594.
[24] G. Nicolosi, C. Spatafora, C. Tringali, J. Mol. Catal., B: Enzym. 16 (2002) 223–229.
[25] V. Cardile, C. Scifo, A. Russo, M. Falsaperla, G. Morgia, M. Motta, M. Renis, E. Imbriani, G. Silvestre, Anticancer Res. 23 (2003) 4921–4926.
[26] G.S. Jayatilake, B.J. Baker, J.B. McClintock, J. Nat. Prod. 58 (1995) 1958–1960.
[27] E. Mannila, A. Talvitie, E. Kolehmainen, Phytochemistry 33 (1993) 813–816.
[28] M.J.T.G. Gonzales, M.M.M. Pinto, A. Kijjoa, C. Anantachoke, W. Herz, Phytochemistry 32 (1993) 433–438.
[29] El-Feraly, S. Farouk, J. Nat. Prod. 47 (1984) 89–92.
[30] B.T. Mossman, A. Churg, Am. J. Respir. Crit. Care Med. 157 (1998) 1666–1680.
[31] F.W. Bachelor, A.A. Loman, L.R. Snowdon, Can. J. of Chem. 48 (1970) 1554–1557.