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Stilbene-based anticancer agents: Resveratrol analogues active toward HL60 leukemic cells with a non-specific phase mechanism

Daniele Simoni,[a,*] Marinella Roberti,[b] Francesco Paolo Invidiata,[c] Enrico Aiello,[c] Stefania Aiello,[c] Paolo Marchetti,[a] Riccardo Baruchello,[a] Marco Eleopra,[a] Antonietta Di Cristina,[d] Stefania Grimaudo,[d] Nicola Gebbia,[e] Lucia Crosta,[f] Francesco Dieli[g] and Manlio Tolomeo[d]

[a]*Dipartimento di Scienze Farmaceutiche, Via Fossato di Mortara 17-19, Università di Ferrara, 44100 Ferrara, Italy*
[b]*Dipartimento di Scienze Farmaceutiche, Università di Bologna, Italy*
[c]*Dipartimento Farmacochimico Tossicologico e Biologico, Università di Palermo, Palermo, Italy*
[d]*Divisione di Ematologia e Servizio AIDS, Policlinico, Università di Palermo, Palermo, Italy*
[e]*Dipartimento di Oncologia, Unità Operativa Oncologia Medica, Policlinico, Universitá di Palermo, Palermo, Italy*
[f]*Consorzio di Ricerca sul Rischio Biologico in Agricoltura (Co.Ri.Bi.A), Palermo, Italy*
[g]*Dipartimento di Biopatologia e Metodologie Biomediche, Università di Palermo, Palermo, Italy*

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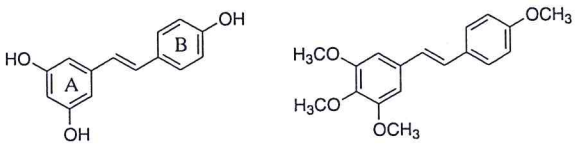
Abstract—Several stilbenes, related to known resveratrol, have been synthesized and tested for their anticancer effect on HL60 leukemia cell line, taking particular care of the cell cycle analysis. The most potent compound was the known (*Z*)-3,4′,5-trimethoxystilbene (**6b**) which was active as apoptotic agent at 0.24 μM. Differently from other stilbenes (including resveratrol) that induced a prevalent recruitment of cells in S phase of cell cycle, we found a peculiar behavior of **6b** that caused a decrease of cells in all phases of cell cycle (G_0–G_1, S, and G_2–M) and a proportional increase of apoptotic cells. The potent pro-apoptotic activity shown by compound **6b** and its effects on cell cycle make this compound of great interest for further investigations.

Resveratrol (**1**), a phytoalexin present in grapes and other food products,[1,2] has recently been suggested as a potential cancer chemopreventive agent based on its striking inhibitory effects on cellular events associated with cancer initiation, promotion, and progression.[3] This triphenolic stilbene has also been shown to induce apoptosis (programmed cell death) in different cell lines.[4–6] Since reduced apoptosis has been implicated in the development and progression of malignant tumors[7,8] and in the occurrence of chemoresistant phenotypes,[9–12] resveratrol-induced apoptosis might therefore contribute to its antitumor activity (Fig. 1).

The complex variety of biological activities shown by resveratrol and other natural polyphenolic derivatives is a feature shared with several results observed from other research fields of medicinal chemistry. The simplicity of resveratrol, associated with its interesting anticancer activity, offers promises for the rational design of new chemotherapeutic agents, and efforts have recently been devoted regarding a detail study on the structure–activity relationship (SAR) of this type of substituted stilbene derivatives.[13–16]



Resveratrol (**1**) **2**

Figure 1. Resveratrol and a known methoxylate analogue with potent cancer growth inhibitory activity.

In previous works, we described the synthesis of a series of *cis*- and *trans*-stilbene-based resveratrols with the aim

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* Corresponding author. Tel.: +39 0532 291291; fax: +39 0532 291296; e-mail: smd@unife.it

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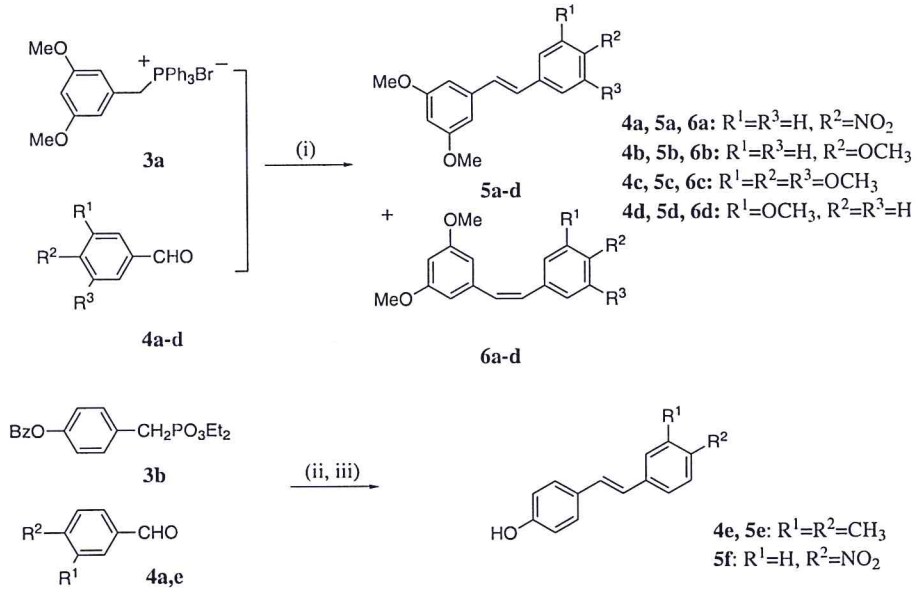
of discovering new lead compounds with clinical potential.[17,18] The best results were obtained with *cis*-3,5-dimethoxy analogues of rhapontigenin and its 3′-amino- and 3′-hydroxy derivatives, which showed apoptotic activity at nanomolar concentration.[17] We have also demonstrated that 3′-hydroxy stilbenes possess interesting antileukemic properties and they may constitute effective and powerful drugs in MDR and apoptosis-resistant hematological malignancies.[18] Of interest, intriguing results were recently described regarding the 3,4,5,4′-tetramethoxystilbene (**2**), a methoxylated analogue of resveratrol, which was found to potently inhibit the growth of cancer cell lines, but with almost no inhibitory effect on the growth of normal cells.[19] Thus, on the basis of our previous observations regarding the importance of the 3,5-dimethoxy motif in conferring pro-apoptotic activity, together with the interesting selective tumor growth inhibitory activity of **2**, we further explored other modified resveratrols and, therefore, we planned the preparation of a small library mainly based on methoxylated analogues with the aim to obtain potent and selective proapoptotic agents.

In this letter, we observe that some of the synthesized stilbenes are potent inducers of apoptosis but, for the first time, we demonstrate that the 3,4′,5-trimethoxystilbene **6b** causes a not phase-specific block of cell cycle, suggesting that its mechanism of cytotoxicity on neoplastic cells could be different from those of other stilbenes. Our data suggest that **6b** could be a compound effective in cancers with different kinetics and it should be useful alone or in combination with other anticancer agents to decrease the percentage of minimal residual disease caused by kinetic factors.

Stilbenes were prepared through classic Wittig couplings from ylide **3a** and aldehydes **4a–d** (Scheme 1) or, for *trans* compounds **5e,f**, (3,4-dimethyl- or 4-nitro-4′-hydroxystilbene) through Horner–Emmons–Wadsworth reaction with (4-benzyloxybenzyl)-phosphonic acid diethyl ester (**3b**), NaH, and 3,4-dimethyl benzaldehyde (**4e**) or 4-nitrobenzaldehyde (**4a**), respectively, in THF (40–50% yield), and subsequent debenzylation with *N*,*N*-dimethylaniline (3 equiv), $AlCl_3$ (4 equiv) in dichloromethane (60% yield). Meanwhile, procedures were attempted also with employment of resin-bound triphenyl phosphine and 3,5-dimethoxybenzyl bromide (for compounds **5a–d** and **6a–d**) or Wang-bromo resin as analogue of resin-bound 4-hydroxybenzyl bromide for compounds **5e,f**, but we did not find improvements in synthetic yields.

In this work, the effects of 10 different stilbenes on the myeloblastic leukemia cell line HL60 cells (myeloblastic acute leukemia) are described. Antiproliferative (IC_{50}) and apoptosis-inducing (AC_{50}) activities are reported in Table 1. Apoptosis was detected by morphological examination and confirmed by Annexin *V* test. The most active compounds resulted in the stilbenes **5b**,[13,14,16,20] **e**,[21] **f**,[22] **6b**,[13,16,23] **c**,[24] **d**.[25] Compounds **5b,e,f** and **6d** showed a similar antiproliferative and apoptotic-inducing activity, a little less than that of compound **6c**. Compound **5b** showed an apoptotic activity about 10–12 times higher than the natural analogues resveratrol and piceatannol, and 19 times higher than that of pterostilbene.[18] The most active compound of this series, however, was **6b**, which was about 10 and 17 times more active than compounds **6c** and **5b**. From a structure–activity relationship point of view, remarkable results were obtained when the hydroxyl group of pterostilbene was changed to the methoxy derivative **5b**, having the latter about 14 times smaller IC_{50}.[18] Analogous results were also obtained with the *cis*-methoxy derivative **6b** that is about 13 times more active than the cor-



Scheme 1. Reagents and conditions: (i) **3a** (1.1 equiv), **4a–d** (1 equiv), NaH (1.2 equiv), THF, 4–16 h, room or reflux temperature, 30–50% yield, 1:1 to 1:3 *Z*/*E* isomer ratio; (ii) same conditions as (i), but only *E*-isomers were recovered; (iii) $AlCl_3$, *N*,*N*-dimethylaniline, CH_2Cl_2, rt, 60% yield.

Table 1. Antiproliferative effects (expressed as IC_{50}) and apoptosis-inducing effects (expressed as AC_{50}) of stilbene derivatives on HL60 cells

Compound	IC_{50} (μM)	AC_{50} (μM)
1[a]	5 (±2)	50 (±6)
5a	35 (±3)	68 (±5.6)
5b	2.5 (±0.6)	4 (±2.1)
5c	22 (±3.7)	42 (±6.2)
5d	37 (±2.9)	70 (±10)
5e	3.5 (±0.2)	6 (±0.7)
5f	2.5 (±0.3)	4.8 (±0.6)
6a	10 (±1.8)	28 (±2.1)
6b	0.15 (±0.01)	0.24 (±0.017)
6c	1.8 (±0.22)	2.6 (±0.3)
6d	2.8 (±0.4)	5.4 (±0.4)

[a] Ref. 18.

responding 4′-hydroxy derivative.[17] Of note, by locating the methoxy substituent at the 4′-position, as in **5b** and **6b**, we obtained compounds with significantly better activity than the corresponding 3′-methoxy derivatives **5d** and **6d**.

The effects on cell cycle of the most active *cis* (**6b,c**) and *trans* (**5b,f**) were examined by flow cytometry after staining of cells with propidium iodide. HL60 cells were exposed to each compound at the concentrations reported in Figure 2. In previous studies, we observed that most stilbene compounds cause a block of cells in a specific phase of cell cycle, acting as phase-specific drugs; in particular many *trans*-stilbenes (*trans*-resveratrol and natural resveratrol analogues such as piceatannol) induced a prevalent block in S phase, while *cis*-stilbenes (such as combretastatin analogues) in G_2–M phase,[26,27] although some *trans*-stilbenes can still cause a block in G_2–M.[16] Similar to resveratrol, compounds **5b,f** and **6c** induced a partial block of cells in S phase and an apoptotic sub-G_0–G_1 peak corresponding of about 20%, suggesting that these compounds act on HL60 cells as phase-specific cytotoxic agents. In contrast, compound **6b** caused an evident sub-G_0–G_1 peak increase but no modification in cell cycle distribution (phases G_0–G_1, S, and G_2–M) respect to the control.

To better understand the effects of **6b** on cell cycle, we exposed HL60 cells to different concentration of **6b**

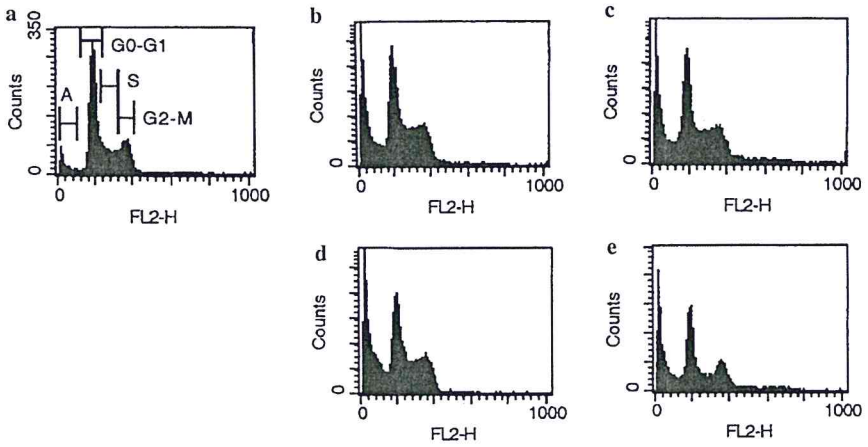


Figure 2. Flow cytometry analysis of cell cycle. HL60 cells were exposed 24 h to 3.5 μM **5b** (b), 2.5 μM **6c** (c), 4 μM **5f** (d) and 0.2 μM **6b** (e). (a) Control; A, sub-G_0–G_1 peak.

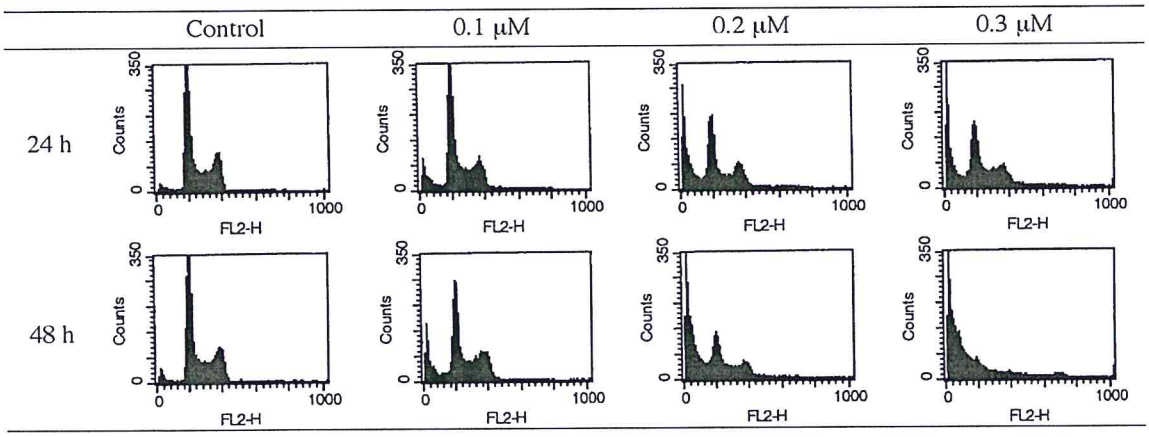


Figure 3. Flow cytometry analysis of cell cycle in HL60 cells exposed 24 and 48 h to different concentrations of compound **6b**.

for 24 and 48 h. As shown in Figure 3, **6b** caused a decrease of G_0–G_1, S, and G_2–M peaks, and a proportional increase of the apoptotic sub-G_0–G_1 peak which was correlated to the time of exposure (24 or 48 h.) and to the concentration used. These data indicate that the effect of **6b** on HL60 cells is not phase-specific and suggest that **6b** could be a compound effective in cancers with different kinetics. This is particularly interesting, because it could be useful alone or in combination with other anticancer agents to decrease the percentage of minimal residual disease caused by kinetic factors.

In conclusion, our results indicate that introduction of methoxy groups at the stilbene motif of resveratrol is important to confer cytotoxic and apoptotic activity to this class of compounds and, in some cases, the methoxy derivatives were more active than the corresponding phenols. The potent apoptosis-inducing activity and the ability of **6b** to decrease the number of neoplastic cells in all phases of cell cycle make this compound of great interest for further investigations.

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