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Anti-tumor Properties of Stilbene-based Resveratrol Analogues: Recent Results

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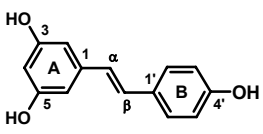
This paper is dedicated to Professor Yoshinori Asakawa for his 65th birthday.

Recent literature about stilbene-based analogues of resveratrol (**1**) has been reviewed, and a total of 94 compounds are reported (see structures **4** – **97**), selected either for their promising anti-tumor properties or as comparative terms in SAR studies. As a general outline, these recent literature data confirm the previously reported observation that minimal modification in the nature and position of the substituents on the stilbene nucleus may cause large variations in their biological activity and, more specifically, in their anti-tumor properties. Among the polyhydroxylated stilbenes, it has been established that those with either a catechol or pyrogallol moiety are far better radical scavengers than either **1** or other analogues lacking an *ortho*-dihydroxy group, and this property was shown to be related to pro-apoptotic activity. In the large majority of cases where couples of *E*- and *Z*-isomers were evaluated for either cytotoxic or pro-apoptotic activity, the *Z*-isomers were significantly more active than their *E* analogues; nevertheless, a general rule stating that stilbenoids with *Z* configuration of the double bond display a considerably higher antiproliferative activity than their *E*-isomers cannot be considered as established. A variety of methoxystilbenes has been reported recently: in many cases these analogues showed either potent antiproliferative and pro-apoptotic activity or strong inhibition of TNFα-induced activation of NF-*k*B. Globally considered, polymethoxystilbenes are a sub-group of great interest among the resveratrol analogues: these analogues appear worthy of a deeper evaluation also in connection with their potential anti-angiogenic properties. In addition, *in vivo* studies indicate that methoxystilbenes undergo different metabolic conversion and have a higher bioavailability than resveratrol. The potent activity of some amino- and halogenated stilbenes is undoubtedly worthy of attention, but the toxicity of these compounds to normal cells has rarely been evaluated. In conclusion, the synthesis and evaluation of stilbene-based resveratrol analogues proved to be a highly active field of research and has recently afforded compounds with either cytotoxic or pro-apoptotic activity in the nanomolar range. Nevertheless, the exact structural determinants to optimize the anti-tumor properties of these compounds and details of their mechanism of action remain to be clarified.

Keywords: resveratrol analogues; anti-tumor properties; antiproliferative activity; apoptotic activity; methoxystilbenes.

Among the many natural products reputed to be beneficial to health, *E*-resveratrol (*E*-3,5,4'-trihydroxystilbene, **1**) is probably the most popular, mainly due to the so-called 'French paradox', namely the inverse correlation between a high-fat diet and low mortality risk of heart disease, observed in some southern regions of France and attributed to red wine consumption [1]. This stilbenoid, first isolated from *Veratrum grandiflorum* in 1940 [2], was later obtained from the roots of the Asian medicinal plant *Polygonum cuspidatum* [3], and subsequently found in grapes, where it was studied as a phytoalexin, elicited in response to infection or injury [4].



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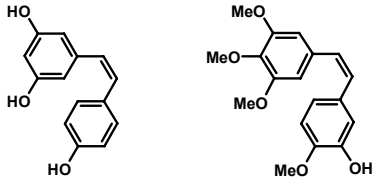
Resveratrol is also present in other edible plants such as blueberry, bilberry and peanuts [5,6]. As one of the main polyphenols present in red wine, it has been extensively evaluated for its possible role in preventing cardiovascular heart diseases (CHD) [7]. Starting from these original observations, **1** has been

the subject of an increasing number of studies, pointing essentially to its promising biological activities: thus, there is a surprising variety of beneficial properties to date attributed to resveratrol, summarised in a number of reviews [5,6,8,9] and in a recent book [10], and including antimicrobial activity, inhibition of prostaglandin biosynthesis, inhibition of platelet aggregation, anti-oestrogenic activity, neuroprotection, radio-protection, immunomodulation, and other biological activities. However, the most promising studies for possible biomedical application of resveratrol are probably those related to its cancer chemopreventive activity, originally evidenced in a widely cited article published in Science in 1997 [11]. Since then, many literature reports have shown that **1** is able to inhibit carcinogenesis and tumour cell cycle progression, as well as to interfere with intracellular signal transduction regulating cell survival and apoptosis (programmed cell death) in various human cancer cell lines [12,13]. The large amount of data available now on resveratrol indicates that it may prove useful not only in cancer chemoprevention, but also in cancer chemotherapy. In fact, evidence has been gathered showing that resveratrol is active *in vivo* as an anti-tumor agent, while at the same time being quite safe in humans [5,6]. In addition, studies highlighting its properties as an inhibitor of cell survival signal transduction, as an inhibitor of angiogenesis and as a sensitizer to stimuli inducing apoptosis [14], suggest its possible use as an adjuvant in cancer chemotherapy, for instance in reducing resistance of tumour cells to the currently used anticancer drugs.

Nevertheless, the available *in vivo* studies indicate that **1**, although absorbed to a great extent by the organism, has a poor bioavailability, and is largely metabolized to either glucuronide or sulphate [15]. In addition, it has been hypothesized that resveratrol is converted *in vivo* into compounds maintaining its anti-oxidative properties, but lacking its anti-proliferative activity [9]. Thus, a possible clinical use of **1** requires a better understanding of its pharmacokinetic properties and a careful evaluation of its effective doses *in vivo*. In this scenario, the chemical modification of this natural 'lead compound' to obtain analogues with better bioavailability and/or enhanced activity is receiving increasing attention. In addition, comparative studies of the biological properties of resveratrol analogues are undoubtedly useful in view of a better understanding of structure-activity relationships (SAR), as well as of the mechanism of action of stilbene-based bioactive compounds.

On this basis, we report here a short summary of the state-of-the-art on resveratrol analogues as a starting point for future studies. Nevertheless, there is a plethora of natural products (and synthetic analogues) based on a stilbenoid structure [16], many of them being either dimers or oligomers of the simplest stilbenoids or only remotely related to **1**. Thus, in view of the limited scope of this review, we have focused our report on the most recent results on anti-tumor activity (and related properties) of compounds strictly related to the natural lead **1**, namely those based on the *trans*-stilbene structure with the exact biogenetic skeleton C_6-C_2-C_6. As a general outline, alkylated and hydrogenated stilbenoids, oligomers, phenanthrenes and other related analogues, as well as the oestrogenic drug diethylstilbestrol (DES) and derivatives were not included. With the exception of some compounds strictly related to *Z*-resveratrol (**2**) (an isomer of **1** found in red wine [17] and other natural sources [18,19]), *cis*-stilbenoids, and in particular the potent tubulin inhibitor combretastatin A4 (**3**) and its analogues are not reviewed here. In fact, excellent reviews on combretastatins and analogues have been recently published [20-22].



2 **3**

In order to facilitate the search for a specific stilbenoid, compounds included here are grouped according to their substitution pattern, namely the number of oxygenated functions (mainly hydroxyl or methoxyl groups) on the *trans*-stilbene nucleus; a separate section (miscellaneous compounds) was devoted to stilbenes with nitrogenated or halogenated constituents; some alkylated stilbenes were also included in this section. Apart from a few exceptions, only the most recent results (2004 – 2006) have been reviewed, due to space limitations, as well as the recent publication of some interesting reviews on resveratrol analogues [23-26].

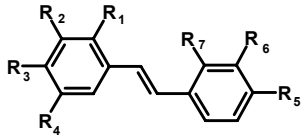
Mono- and dioxygenated analogues

Among the natural products strictly related to resveratrol, the 3,5-dihydroxystilbene, pinosylvin (**4**), found mainly in Pine wood, is known to possess antimicrobial and anti-insect properties [16,27], and was also reported as a potent antiproliferative agent in both oestrogen dependent breast cancer cell lines MCF-7 and T-47D [25]. This compound has recently been evaluated against HepG2 liver cancer cells and MDA-MB-231 (oestrogen non-dependent) breast cancer cells, displaying IC_{50} values in the range 10-12 μg/mL [28].

The higher antioxidant activity of *ortho*- and *para*-dihydroxy stilbenes in comparison with other substitution patterns has been reported previously [29]. More recently, a possible relationship of the 3,4-dihydroxy structural moiety with the anti-tumor properties has been reported [24,30]: in fact, the *ortho*-dihydroxystilbene **5**, as well as the trihydroxystilbenes **38** and **39** (see next Section), resulted not only in more effective antioxidants (linoleic acid peroxidation) than **1**, but also showed higher pro-apoptotic activity towards Jurkat cells and HL-60 leukaemia cells (EC_{50} values in the range 20 – 38 μM) with respect to **1** (EC_{50} = 85 μM). Conversely, the analogues **4**, **6**, **7** and **8**, lacking the catechol group, showed lower antioxidant and pro-apoptotic activity than that of **1**.

In the frame of SAR studies of resveratrol analogues, a parallel solution-phase synthesis afforded a library of 30 *E*-stilbene analogues with a monohydroxylated ring, and including methoxylated and fluorinated substituents on the other ring (see miscellaneous compounds) [31]; these products were evaluated against the HCT-116 (colon) and MDA-MB-468 (breast) cancer cell lines, the latter being more sensitive and showing growth inhibition by the synthetic stilbenes in the low micromolar range ($1.4 \leq GI_{50} \leq 25.7$ μM). Against the MDA-MB-468 cell line, all the analogues proved more active than **1** (GI_{50} = 41.1 μM); in particular, one of the most active compounds, **9** (GI_{50} = 2.7 μM), was significantly more active than its isomer **10** (GI_{50} = 25.7 μM).

It is known that the transcription nuclear factor *k*B (NF-*k*B), which regulates the expression of numerous genes promoting the prosurvival, anti-apoptotic state, is up-regulated in many cancer cells. A very recent screening for the inhibition of the TNFα-induced activation of NF-*k*B has been carried out on 75 *trans*-stilbenes, with various substituents, including nitrogenated groups and halogens [32].



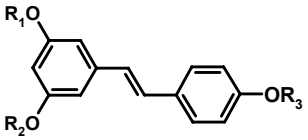
	R_1	R_2	R_3	R_4	R_5	R_6	R_7
4	H	OH	H	OH	H	H	H
5	H	OH	OH	H	H	H	H
6	H	OH	H	OH	H	H	H
7	H	H	OH	H	OH	H	H
8	H	H	OH	H	H	H	H
9	OCH_3	H	H	H	H	OH	H
10	OCH_3	H	H	H	H	H	OH
11	H	H	OC_2H_5	H	H	H	H
12	H	H	OCH_3	H	H	OCH_3	H

In this screening, a number of resveratrol analogues were more active than **1**, and the most promising compounds were subjected to a further evaluation of both antioxidant activity and inhibition of NF-*k*B activation. Among these compounds, the monoethoxystilbene **11** (IC_{50} = 0.3 μM) and the dimethoxystilbene **12** (IC_{50} = 0.6 μM) were significantly more effective inhibitors than resveratrol (IC_{50} = 20 μM), although lacking any antioxidant activity: this was confirmed by the other assays (see the following Sections) showing that the most potent inhibitors of the activation of NF-*k*B generally did not exhibit antioxidant activity.

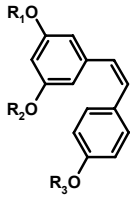
Trioxygenated analogues

The natural 3,5-dimethylated analogue of resveratrol, pterostilbene (**13**), found in some berries and grape varieties, has previously been reported as antioxidant and cancer chemopreventive [25,33]. In a more recent search for antiproliferative analogues of resveratrol with a longer half-life [34], it was found that **13**, when used in association with the flavonoid quercetin, caused a 56% growth inhibition of B16M-F10 melanoma cells, whereas separate administration of **13** and quercetin inhibited growth by 40% and 19%, respectively: thus, the authors suggested a possible additive/synergic effect. Pterostilbene has recently been evaluated as an antiproliferative and apoptosis-induction agent in comparison with **1** and two tetraoxygenated analogues (**42** and **44**, see below) [35]; **13** was barely active against the sensitive cell lines HL-60 (human myeloid leukaemia) and HUT-78 (human T lymphoma), but was able to induce apoptosis in two Fas-ligand resistant lymphoma cell

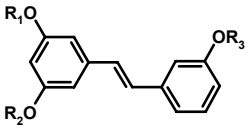
lines (HUT-78B1 and HUT-78B3) and in the multi drug-resistant (MDR) leukaemia cell lines HL-60R and K563-ADR, although being less potent than its 3'-hydroxy analogue **44**. In addition, **13** and **44** did not show any cytotoxicity towards normal haemopoietic stem cells.



	R_1	R_2	R_3
13	CH_3	CH_3	H
15	CH_3	CH_3	CH_3
21	H	H	CH_3
22	H	H	$COCH_3$
23	$COCH_3$	$COCH_3$	$COCH_3$
24	COC_3H_7	COC_3H_7	COC_3H_7
25	COC_7H_{15}	COC_7H_{15}	COC_7H_{15}
26	$COCH_3$	$COCH_3$	H
27	$COCH_3$	H	H
28	COC_3H_7	COC_3H_7	H
29	COC_3H_7	H	H
30	COC_7H_{15}	COC_7H_{15}	H
31	COC_7H_{15}	H	H
32	CH_3	H	CH_3
36	$COCH_3$	H	$COCH_3$
37	$COCH_3$	H	H



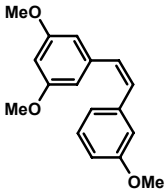
	R_1	R_2	R_3
14	CH_3	CH_3	H
16	CH_3	CH_3	CH_3
34	CH_3	H	CH_3



	R_1	R_2	R_3
17	CH_3	CH_3	CH_3
19	CH_3	CH_3	H

Although published in 2003, some reports on resveratrol analogues are the basis of more recent studies and are worth citing here. A series of tri- and tetra-substituted stilbenes (see also the Sections below) with *E* and *Z* configuration were tested as growth-inhibitors (IC_{50}) and apoptosis-inducers (AC_{50}) on HL-60 promyelocytic leukaemia cells [36]: all the *Z*-stilbenes were more active than their *E*-stereoisomers, with the exception of **2** (IC_{50} = 42 μM, AC_{50} > 200 μM), the activity of which was lower than that of **1** (IC_{50} = 5 μM, AC_{50} = 50 μM), which in turn was more active than *E*-pterostilbene (**13**), but less than *Z*-pterostilbene (**14**) (IC_{50} = 2 μM and AC_{50} = 5 μM). The cytotoxic and pro-apoptotic activities both diminished when **13** and **14** were tested as 4'-*O*-*t*-butyldimethylsilyl derivatives. In a further cytotoxicity evaluation of a series of synthetic stilbene analogues, including variously substituted stilbenes (see below), the *E*-3,5,4'-trimethoxystilbene (**15**), previously known as a natural product [37], proved one of the most active compounds, with IC_{50} values of 0.8 and 0.9 μg/mL towards A549 (lung) and Col2 (colon) cancer cells, respectively [38]. The *Z*-stereoisomer **16** was examined in a study focusing on human colon cancer Caco-2 cells, and exhibited a very potent anti-mitotic activity with 80% growth inhibition at 0.3 μM [39]: **16** was 100-fold more active than **15** and **1**, and inhibited tubulin polymerization in a dose dependent manner (IC_{50} = 4 μM), causing the cell cycle arrest at the G2-M phase transition. In addition, it reduced the depletion of polyamines and strongly inhibited the binding of radiolabeled colchicines to tubulin. These results prompted the authors to continue their studies on both **15** and **16**. Because the observed cytotoxicity of **16** was not related to a pro-apoptotic effect on Caco-2 cells, which express a mutated p53 gene, they investigated the pro-apoptotic effect of **16** on two human lymphoblastoid cell lines that differ in the status of their p53 gene: TK6 (expressing wild-type p53) and NH32 (p53-knockout cells) [40]. The results clearly demonstrated that **16** induces apoptosis regardless of the p53 status of the cells, although the mechanism remains to be clarified. The authors also suggested that the higher lipophylicity of **16** with respect to **1** may favour interaction with the cell membrane and consequently to its pro-apoptotic properties. Recently, a small library of resveratrol analogues was prepared (see also the Sections below) [41]. All compounds were tested against the HL-60 leukaemia cell line both for cytotoxic (IC_{50}) and pro-apoptotic activity (AC_{50}). The most potent analogue was again **16** (IC_{50} = 0.15 μM, AC_{50} = 0.24 μM). It was found that **16**, differently from **1** and other stilbenes, caused a decrease of cells in all phases of the cell cycle and a proportional increase of apoptotic cells. In this SAR study, two further trimethoxystilbenes with a different substitution pattern were evaluated, namely *E*-3,5,3'-trimethoxystilbene (**17**) and its *Z*-isomer (**18**), exhibiting IC_{50} values of 37 μM and 2.8 μM,

respectively. A further work of the same group [42] afforded a series of 3,5-dimethoxy analogues, including a number of 2-phenylnaphthalenes and terphenyls (not reported herein), which were evaluated for their antiproliferative and pro-apoptotic activities on sensitive leukaemia HL-60 cells, and MDR leukaemia (HL-60R and K562) cells. Among the stilbene-based analogues, compound **15** was the most potent, with an IC_{50} value of 2.5 μM on HL-60 cells, followed by compound **63** (see below); compounds **19**, **20** and the other below reported compounds showed either moderate activity or were less active than the natural leads **1** and **13**.



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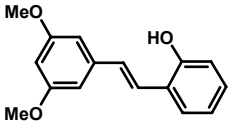

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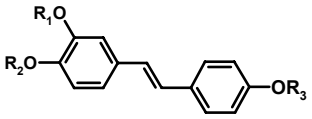
In a comparative study on the antiproliferative properties of **1** and seven analogues (including naphthalene-based compounds) [43], the three methylated analogues **13**, **15**, and **21** were tested against breast cancer cells MDA-MB-231: among these compounds, **15** was the most potent (IC_{50} = 1.2 μM), followed by **13** (IC_{50} = 10.0 μM). Interestingly, **21** (IC_{50} > 50 μM) proved less active than **1** (IC_{50} = 20.5 μM), and the authors argue that this is further evidence in favour of the previously reported essential role of the 4'-hydroxy group for the antiproliferative activity of resveratrol [44]. In this connection, we have recently evaluated the H-donating ability of the three hydroxyl groups in resveratrol through a laser flash-photolysis study. We found that the 4'-hydroxy appears to be the most reactive due to the high stability of the corresponding phenoxyl radical by conjugation with the rings [45].

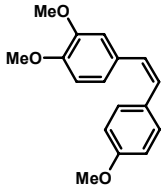
In a recently published cytotoxicity evaluation, a series of 17 resveratrol derivatives, including brominated stilbenoids (see miscellaneous compounds), were tested against the KB (human oral epidermoid carcinoma) tumour cell line [46]; compound **15** (IC_{50} = 7.40 μM), although proving more active than the anticancer drug 5-fluorouracil (IC_{50} = 13.4) and one of the most active compounds, was less active than the brominated analogue (**84**). Recently, **15** has been shown to exert potent anti-angiogenic activity [47], a property which appears to be highly promising in the search for new anticancer therapeutic agents because of the important role of neovascularisation in neoplasia [48]. In this study, **15** was evaluated for its capacity to affect different steps of the angiogenesis process in comparison with **1** and other analogues, including dihydroresveratrol and the partially methylated analogues **13** and **21**. Interestingly, hydrogenation of the central double bond in **1** or methylation only at the 4' position suppressed the antiangiogenic activity of resveratrol. The activity was significantly potentiated by methylation at the 3,5 positions and further methylation at 4' caused a 30 to 100 fold higher activity than **1**. It should also be mentioned that in a recent study on the inhibitory activity towards cytochromes P450 1A2 and 2E1 [49], compounds **13**, **15** and other monomethylated analogues showed comparable inhibitory activity towards CYP1A2, and were more active than **1**: this suggests that neither the number nor the position of methoxy groups are critical for the inhibition of CYP1A2, but their presence increases the inhibitory activity.

Employing standard chemical conversions and a chemo-enzymatic methodology for regioselective acylation of resveratrol, we have carried out the preparation of a small library of lipophilic analogues [50,51]. By regioselective acetylation catalysed by *Candida antarctica* lipase (CAL) in organic solvent, **1** afforded 4'-acetylresveratrol (**22**). CAL biocatalysed regioselective alcoholysis of 3,5,4'-triacetylresveratrol (**23**), 3,5,4'-tributanoyl-resveratrol (**24**) and 3,5,4'-trioctanoylresveratrol (**25**) gave the selectively acylated analogues **26 – 31** in good yields. Further resveratrol analogues were obtained through methylation (**15** and **32**) and hydrogenation reactions (these products are not reported here), whereas the 3,4,4'-trimethoxystilbene (**33**) was obtained by complete synthesis. These 18 compounds were evaluated as antiproliferative agents on androgen non-responsive human prostate tumour cells DU-145: most of the compounds showed either higher or comparable activity to that of **1** (GI_{50} = 24.09 μM), the most potent being **15** (GI_{50} = 2.92 μM), followed by the partially methylated analogue **32** (GI_{50} = 12.24 μM). Some acylated analogues (**23**, **24**, **28**, **29**) were slightly more active than **1**; hydrogenation caused only small variations in the activity, and this result seems in contrast with data recently reported by others [52]. More recently, we tested resveratrol, its analogues **15**, **32** and **33** and their *Z*-isomers **2**, **16**,

34 and **35** on a set of four human cancer cell lines: M-14 (human melanoma), LNCaP (androgen responsive human prostate tumour), DU-145 and KB [53]. The methylated analogues of **1** were more active than the natural lead compound in the large majority of bioassays. The most active compound was **16**, exhibiting antiproliferative activity comparable to that of the anticancer drug vinorelbine against DU-145 and LNCaP cells, and gave a GI_{50} value of 0.1 μM against KB cells. Some methylated *Z*-isomers displayed a higher activity than their relevant *E*-isomers, but a general rule stating that stilbenoids with a *Z* configuration of the double bond display a considerably higher antiproliferative activity than their *E*-isomers could not be established. In particular, **1** was more active than **2** towards all the tested cell lines.



	R_1	R_2	R_3
33	CH_3	CH_3	CH_3
38	H	H	H
41	H	H	CH_3



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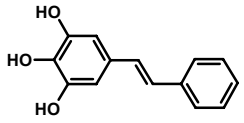
The use of levulinate and chloroacetate protecting groups under palladium-*N*-heterocyclic carbene (NHC) catalyzed decarbonylative coupling conditions allowed the synthesis of the selectively mono- and diacetylated analogues **22**, **26**, **36**, **37** [54]. These acetates, as well as a series of fluorinated resveratrol analogues (not reported here), were tested against HL-60 leukaemia cells: only compound **22** (IC_{50} = 17 μM) proved more active than **1**. However, the authors report that all the fluorinated analogues were toxic to the HL-60 and other cells, limiting their potential for future investigations.

As cited above, a parallel solution-phase synthesis was used to obtain a library of *E*-stilbenoids, evaluated as antiproliferative agents. Of these, compound **19** was the most potent against breast cancer cells MDA-MB-468 (GI_{50} = 0.96 μM) [31]. Its growth-inhibitory activity was approximately twice that of its 2'- and 4'-isomers. The activity of the most potent antiproliferative agents towards breast cancer cells was correlated to their ability to induce apoptosis. The authors suggested that the potency of the reported resveratrol analogues does not primarily reside in their growth-inhibitory activity, but rather in their selective pro-apoptotic properties.

The trihydroxystilbenes **38** and **39** were included in the above cited comparative study [30] on antioxidant and pro-apoptotic activity of **1** and other mono, di- and tryhydroxylated stilbenes. Both exhibited higher activities than **1**, confirming the importance of the *ortho*-dihydroxy moiety. In a more recent study, **39** rapidly induced apoptosis in Jurkat cells, unlike **1** [55]. It induced activation of caspase-8 and apoptosis by a Fas-associated death domain (FADD), protein-dependent mechanism that was unresponsive to resveratrol.



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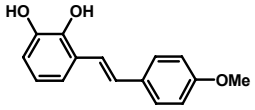

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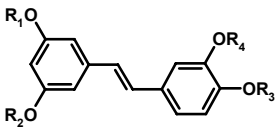
The above cited, very recent screening of 75 resveratrol analogues as antioxidants and inhibitors of the TNFα-induced activation of NF-*k*B [32] included some trioxygenated stilbenes that were more effective inhibitors than **1**. Among them, the stilbenes **40** and **41** were potent inhibitors, with IC_{50} values of 0.5 and 0.6 μM, respectively. As reported above, compounds incorporating an *ortho*-dihydroxy group were also good antioxidants, although inhibition of NF-*k*B is not necessarily related to the presence of either free hydroxyl groups or antioxidant activity.

Tetraoxygenated analogues

Piceatannol (**42**), known also as astringinin, is a naturally occurring analogue of resveratrol, bearing an additional OH group at C-3'. It has been found in sugar cane, berries, peanuts, grapes and other plants [6,25]. Piceatannol has a variety of biological properties, among them antioxidant and anti-tumor [25]. As reported in a recent review [26], the

antiproliferative effects of resveratrol on cancer cells has been thought to be the result of a metabolic conversion of **1** to **42** by cytochrome P450 1B1 (CYP1B1); this cytochrome is highly expressed in various cancerous tissues, but not in normal tissue. Recently, **42** showed interesting antiproliferative properties on HL-60 (leukaemia) and other cancer cells [56]. It proved to be a potent inducer of apoptosis in human SK-Mel-28 melanoma cells at 1 μM concentration [57] and induced apoptosis in NRP-154 (but not in DU-145) prostate cancer cells [58]. Six stilbenes, including **42** and rhaponticin (**43**), were investigated, together with five known flavonoids, for their cytotoxic and apoptosis-inducing activity against four human tumour cell lines (squamous cell carcinoma HSC-2, HSC-3, submandibular gland carcinoma HSG and leukaemia HL-60) [59] and some normal cell lines; **42** showed a higher tumour-specificity than **1** and **43**, that is it was more cytotoxic to tumour than to some normal cell lines. Recent results demonstrate a possible use of **42** as an adjuvant in cancer vaccines [60].



	R_1	R_2	R_3	R_4
42	H	H	H	H
43	Glc*	H	CH_3	H
44	CH_3	CH_3	H	H
47	H	H	CH_3	H
48	CH_3	CH_3	H	CH_3
49	CH_3	CH_3	CH_3	H
50	CH_3	CH_3	H	H
55	CH_3	CH_3	CH_3	CH_3
62	CH_3	CH_3	H	C_2H_5

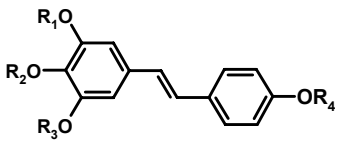
* Glc = glucosyl

Piceatannol and resveratrol were recognized as potential inhibitors of CNS-associated kinases through an *in silico* screening method: in *in vitro* kinase assays, **42** proved to be a potent inhibitor of CNS associated kinases CK2 and PKD and the purified CNS complex [61]. In a further recent work, both **1** and **42** were demonstrated to be less potent antiproliferative and apoptosis-inducer agents than their 3,5-methylated analogues, respectively **13** and **44** [35]. In particular, **44** was 50-97 times more potent than **1** in inducing apoptosis, not only in sensitive HL-60 leukaemia and human T-lymphoma (HUT-78) cell lines, but also in resistant lymphoma and leukaemia cell lines. Interestingly, **44** has a noticeable pro-poptotic activity against the MDR leukaemia cells, with an AC_{50} value of 5 μM (HL-60R) and 3.5 μM (K562-ADR).

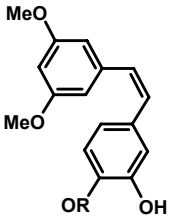
In a SAR study of six polyhydroxystilbenes evaluated for their pro/antioxidant and cytotoxic activity [62], compounds **42, 45** and **46** showed cytotoxic activity towards HL-60 (leukaemia) cells comparable to that of **1**, although they were less active than the hexahydroxystilbene **71** (see hexaoxygenated analogues). The most potent radical scavenger was **42**, but also the stilbenes **45** and **71**, bearing an *ortho*-dihydroxy group, were thousands-fold more effective than **1** and **46**. *Ortho*-hydroxystilbenes showed more than three-fold higher cytostatic activity than those compounds with a different substitution pattern, and their oxidation in a microsomal model system resulted in the formation of *ortho*-semiquinones; these intermediates undergo redox-cycling, thereby forming cytotoxic oxygen radicals. Thus, the authors suggest that the increased cytotoxicity of *ortho*-hydroxystilbenes is related to the presence of *ortho*-semiquinones formed during either metabolism or autoxidation. In a more recent work, **42** showed a protective effect against the DNA oxidative damage in leukaemic cells higher than that of **1** [63].

Rhapontigenin (**47**), a stilbene abundant in *Rheum undulatum* [64], is the 4'-*O*-methylated analogue of **42** and the main active metabolite of the glycoside rhaponticin. It was previously reported as a potent and highly selective inhibitor of human cytochrome P450 1A1 and 1B1 [25], and has recently been tested on HepG2 liver cancer cells and proved moderately active (IC_{50} = 115 μg/mL) [65]. In the above cited biological evaluation of a series of *E*- and *Z*-stilbenes [36], **47** (IC_{50} = 48 μM; AC_{50} > 200) was less cytotoxic and less of an apoptosis-inducer than **1** on HL-60 leukaemia cells; its analogue, **48**, was only slightly more active, whereas the analogues **49** and **50** were highly active (IC_{50} and $AC_{50} \leq 1$ μM). Their *Z*-stereoisomers **51** (IC_{50} = 0.03 μM; AC_{50} = 0.04 μM) and **52** (IC_{50} = 0.05 μM; AC_{50} = 0.1 μM) were even more active, showing IC_{50} and AC_{50} values in the nanomolar range; in addition, these compounds were active toward MDR HL-60R cells and their activity was higher than that of known anticancer drugs. In the same evaluation, the 3'-amino analogue of **51** (**76**, see miscellanous compounds) showed the same activity. The authors suggested that the main mechanism of cytotoxicity of **51** and **76** could be activation of apoptosis.

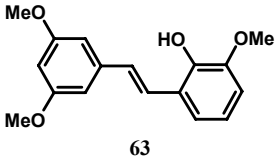
Another natural analogue of **1**, oxyresveratrol (**53**), is a major component of the heart-wood of *Artocarpus*



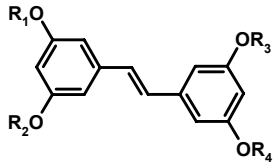
	R_1	R_2	R_3	R_4
45	H	H	H	H
61	CH_3	CH_3	CH_3	CH_3



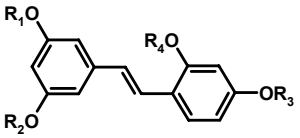
	R
51	CH_3
52	H



63



	R_1	R_2	R_3	R_4
46	H	H	H	H
56	CH_3	CH_3	CH_3	CH_3



	R_1	R_2	R_3	R_4
53	H	H	H	H
54	CH_3	CH_3	CH_3	CH_3
57	H	H	H	CH_3
58	CH_3	H	H	CH_3
59	CH_3	H	CH_3	H

lakoocha [66] and was also found in other plants [6]. It showed a lower cytotoxic activity (IC_{50} > 20 μg/mL) than **1** on both lung (A549) and colon (Col2) cancer cells. The related 3,5,2',4'-tetramethoxy derivative, **54**, was highly active, with IC_{50} values of 0.8 (A549) and 0.8 (Col2) μg/mL, whereas permethylrhapontigenin (**55**) and **56**, the latter with a different substitution pattern, were scarcely active, displaying IC_{50} values greater than 15 μg/mL [38]. Oxyresveratrol is also known as a potent inhibitor of tyrosinase [67], a key enzyme in biosynthesis of melanin pigments, and has potential as a skin depigmentation agent. Recently, some chemical conversions were carried out on **53** to evaluate the effect of these transformations on both the cytotoxic activity against the human cancer cell lines KB, BC (lung) and NCI-H187 (lung) and tyrosinase inhibition [52]: **53** proved to be non-cytotoxic. Interestingly, when **53** was hydrogenated, a more potent tyrosinase inhibitor, devoid of any cytotoxicity, was obtained. Complete or partial methylation of **53** afforded the methoxy analogues **54** and **57** – **59**; by photoisomerisation, **54** gave the *Z*-isomer **60**. Among these stilbenes, **57** was either scarcely active or inactive; **54**, **58** and **59** were moderately active (IC_{50} in the range 5.5 – 33.5 μM), whereas **60** was highly active, (IC_{50} = 0.3 μM on KB and NCI-H187; 1.0 μM on BC) showing a cytotoxicity higher than ellipticine. When hydrogenated, **54** was completely inactive and, thus, the authors concluded that the central double bond and the presence of methoxy groups in stilbene analogues are both required for cytotoxicity.

In a study of pharmacokinetics in mice and anti-tumor properties on human colon cancer cells (HCA-7 and HT-29), the 3,4,5,4'-tetramethoxystilbene (**61**) was slightly more potent than **1** (IC_{50} in the range 6 - 26 μM) [68]. Interestingly, **61** showed a higher bioavailability compared to **1** in the small intestine and colon; in contrast to resveratrol, which is metabolized to either its sulphate or glucuronate conjugate, **61** underwent hepatic metabolic hydroxylation or single and double *O*-demethylation. The authors report also that, on the basis of unpublished results, **61** is devoid of any toxicity in rats when administered at single doses of up to 40 mg kg^{-1} (intravenous) or up to 400 mg kg^{-1} (oral). In this connection, it is worth mentioning here that **61** is currently under

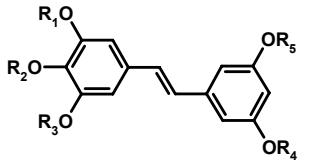
preclinical evaluation as a potential anti-tumor prodrug that undergoes metabolic activation by cytochrome P450 enzymes [69]. More recently, the same research group tested **61** as an inhibitor of adenomatous polyposis development in Apc^{Min+} mice, and found a 24% adenoma reduction produced by **61**, whereas **1** decreased development by 27% [70]. In this study, **61**, unlike **1**, proved to be ineffective in decreasing COX-2 expression, although reducing prostaglandin E2 (PGE-2) production; this latter event was probably mediated by metabolites of **61**. Another recent study has been carried out on **61** in comparison with **1**, employing both normal and cancer cell lines [71]; **61** was more potent than **1** against various cancer cell lines (WI38VA, IMR-90SV, HeLa, LNCaP, HT-29, and HepG2), but had almost no inhibitory effect on the growth of normal cells (WI38, IMR-90, BJ-T). When the two compounds were tested on normal human fibroblasts (WI38), both had little inhibitory effect up to 50-100 μM; on transformed fibroblasts (WI38VA), **61** completely inhibited the growth at 1-2 μM, whereas **1** was effective only at concentrations higher than 50 μM. Further analysis revealed that **61** causes a selective activation of the mitochondrial apoptotic pathway in WI38VA, but not in WI38 cells, and the authors suggest that this could be a major reason for the striking differential growth inhibitory effect of **61**.

In the above cited SAR study of 3,5-dimethoxy analogues tested on sensitive leukemia cells (HL-60) and MDR leukaemia cells (HL-60R and K562) [42], the tetraoxygenated stilbenes **62** and **63** were examined, the latter being significantly active against HL-60 cells (IC_{50} = 3.5 μM).

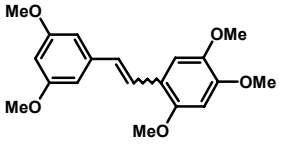
Pentaoxygenated analogues

In the above reported SAR study of polyhydroxystilbenes, the pentahydroxystilbene **64** was slightly more cytotoxic than **1** on HL-60 cells, and was clearly more effective as an antioxidant [62]. The above cited cytotoxicity evaluation of resveratrol analogues towards A549 (lung) and Col2 (colon) cancer cells [38] included four pentamethoxy derivatives, namely **65** and the stilbenes **66**, **67** and **68,** examined as *E*/*Z* mixtures.

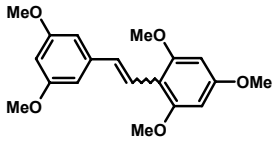
All these samples showed cytotoxic activity either comparable to or lower than that of **1**. Compound **65** and its *Z*-isomer **69** were recently tested against HL-60 leukemia cells in comparison with **1** and other methoxylated stilbenes [41]. The cytotoxic and pro-apoptotic activities of **69** (IC_{50} = 1.8 μM; AC_{50} = 2.6 μM) were approximately ten-fold higher than those of **65** (IC_{50} = 22 μM, AC_{50} = 42 μM). Compound **70**, the 4-demethylated analogue of **65**, was recently tested on sensitive and MDR leukaemia cells [42], but was scarcely active.



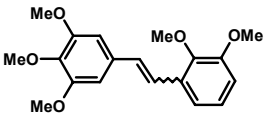
	R_1	R_2	R_3	R_4	R_5
64	H	H	H	H	H
65	CH_3	CH_3	CH_3	CH_3	CH_3
70	CH_3	H	CH_3	CH_3	CH_3



66



67



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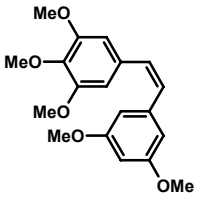

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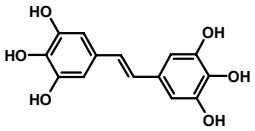
Hexaoxygenated analogues

As discussed above, the hexahydroxystilbene **71** showed the highest cytotoxic activity (IC_{50} = 4.2 μM) towards HL-60 cells when compared with **1** and other stilbenes bearing four (**42**, **45**, **46**) or five (**64**) hydroxy groups. Stilbenes with a pyrogallol and/or catechol groups, including **71**, exhibited an antiradical activity far higher than **1**, as confirmed by a further study [63] in which **71** exhibited a more potent protective effect against H_2O_2-induced DNA damage in leukaemia cells than either **1** or **42**. Due to its promising biological properties, **71** was studied in-depth in a more recent work focusing on its cytotoxic and biochemical effects on HL-60 leukaemia cells [72]. This compound induced apoptosis at concentrations significantly lower than **1** and the authors suggest that it could notably inhibit the activation of the nuclear transcription factor NF-*k*B; it arrested cells in the S phase of the cell cycle while depleting cells in the G2-M phase. In growth-inhibition experiments, **71** gave an IC_{50} value of 6.25 μM, whereas the value for **1** was 12

μM; addition of ascorbic acid decreased the IC_{50} value of **71** to 2 μM, thus indicating that protection of polyhydroxylated stilbenes by oxidative processes can enhance their anti-tumor properties. It is also worthy of note here that **71** exhibited synergistic effects when applied in combination with Ara-C, a first-line antileukaemic agent. Finally, the authors report that preliminary *in vivo* experiments indicate that **71** can be safely employed at therapeutic concentrations for nude mice.



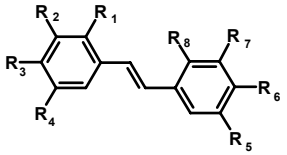
71

Miscellaneous stilbene-based compounds

In addition to the resveratrol analogues reported above, some stilbenes substituted with either nitrogen or halogen groups have recently been obtained, some of them possessing noticeable anti-tumor properties, and thus are worthwhile reporting here. A few methylated stilbenes have also been included in this section.

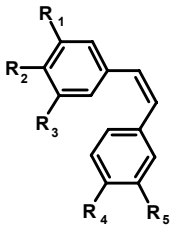
The above-cited series [36] of *E*- and *Z*-stilbenes include some amino-analogues of resveratrol and rhapontigenin [36]. Again, these *Z*-isomers were more active than the related *E*-isomers as antiproliferative and apoptosis-inducing agents towards HL-60 leukaemia cells. Actually, activities of the same order were observed for *E* and *Z* isomers of the pairs **72**/**73** and **74**/**75** (IC_{50} values in the range 4 – 80 μM), whereas **76** (IC_{50} = 0.03 μM; AC_{50} = 0.04 μM) was a potent apoptosis-inducing agent, and much more active than its *E*-isomer, **77** (IC_{50} = 4 μM; AC_{50} = 8 μM). Compound **76** and the above cited **51** showed identical activity, thus indicating that replacement of an OH group in C-3' with an NH_2 group does not affect the pro-apoptotic activity. These compounds were active against MDR HL-60 cells and showed activity higher than that of common anticancer drugs.

In the recent study of variously substituted stilbenes tested against HL-60 leukaemia cells [41], the nitrostilbenes **78** – **80** were examined, showing, respectively, IC_{50} values of 35, 10 and 2.5 μM, this last value being lower than that of resveratrol (IC_{50} = 5 μM). In addition, the 4-hydroxy-3',4'-dimethylstilbene (**81**) was synthesized and assayed, giving an IC_{50} value of 3.5 μM. In the above cited evaluation of resveratrol analogues towards A549 (lung) and Col2 (colon) tumour cell lines [38], the brominated analogue **82** was more active than **1**, showing IC_{50} values of 4.7 (lung) and 1.6 μg/mL (colon), but the most potent compound was its *Z*-isomer **83**, with IC_{50} values of 0.01 mg/mL towards both cell lines. The mechanism of action of **83** was later investigated [73]. It induced arrest at the G2-M phase of the cell cycle at an early stage and



	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
72	H	OCH_3	H	OCH_3	H	NH_2	H	H
74	H	OH	H	OH	H	OCH_3	NH_2	H
77	H	OCH_3	H	OCH_3	H	OCH_3	NH_2	H
78	H	OCH_3	H	OCH_3	H	NO_2	H	H
80	H	H	OH	H	H	NO_2	H	H
81	H	H	OH	H	H	CH_3	CH_3	H
82	H	OCH_3	OCH_3	OCH_3	H	Br	H	H
84	H	OH	H	OH	H	$O(CH_2)_2Br$	H	H
85	H	$O(CH_2)_2Br$	H	OH	H	OH	H	H
86	H	$O(CH_2)_2Br$	H	$O(CH_2)_2Br$	H	$O(CH_2)_2Br$	H	H
87	H	OCH_3	H	OCH_3	Cl	H	H	OH
88	H	OH	H	H	H	H	H	F
89	OH	H	H	H	H	F	F	H
90	H	OH	H	H	H	F	F	H
91	H	H	$N(CH_3)_2$	H	H	H	H	H
92	H	H	OCH_3	H	H	H	H	F
93	H	H	OCH_3	H	H	H	F	H
94	H	H	OCH_3	H	H	H	Cl	H
95	H	H	OCH_3	H	H	H	H	Cl
96	H	H	OCH_3	H	H	H	CH_3	H
97	H	H	OCH_3	H	H	CH_3	H	H

subsequently increased in the sub-G1 phase DNA contents in a time-dependent manner, indicating induction of apoptosis. Also, the cited synthesis of 17 resveratrol derivatives [46] included some brominated stilbenoids, which were more active than **1** towards the KB tumour cell line. In particular, compound **84** (IC_{50} = 3.9 μM), bearing one brominated side chain at C-4', was more active than the anticancer drug 5-fluorouracil (IC_{50} = 13.4 μM). Further brominated analogues with noticeable activity were **85** (IC_{50} = 10.7 μM) and **86** (IC_{50} = 14.0 μM), bearing respectively one (at C-3) and three (at C-3, C-5, C-4') brominated chains. Substitution of the methoxy groups in **16** with $O(CH_2)_2Br$ groups caused a reduction of the activity in compounds **85** and **86** and an enhancement in compound **84**.



	R_1	R_2	R_3	R_4	R_5
73	OCH_3	H	OCH_3	NH_2	H
75	OH	H	OH	OCH_3	NH_2
76	OCH_3	H	OCH_3	OCH_3	NH_2
79	OCH_3	H	OCH_3	NO_2	H
83	OCH_3	OCH_3	OCH_3	Br	H

In a SAR study of a series of 3,5-dimethoxy analogues tested on sensitive and MDR leukaemia cells [42], the chlorinated derivative **87** and a further bis-alkylated stilbene (not reported) showed little activity. Among the 30 monohydroxylated *E*-stilbene analogues obtained through a parallel solution-phase synthesis, 15 fluorinated compounds were synthesized and evaluated against the HCT-116 (colon) and MDA-MB-468 (breast) cancer cell lines. Amongst them, the most potent compounds against the more sensitive line MDA-MB-468 were **88** (GI_{50} = 1.4 μM), **89** (GI_{50} = 1.1 μM) and **90** (GI_{50} = 1.6 μM) [31].

Finally, a very recent screening of 75 stilbenes (including compounds only remotely related to resveratrol) tested as inhibitors of the TNFα-induced activation of NF-*k*B led, as reported above [32], to a restricted list of twelve compounds. Amongst these, compound **91** (IC_{50} = 0.15 μM), bearing a dimethylamino group, was one of the most potent inhibitors, approximately 100-fold more active than **1** (IC_{50} = 20 μM). An identical activity was exhibited by compound **92**, which has a methoxy group and a fluorine atom as substituents. Interestingly, the isomer **93** (IC_{50} = 1.0 μM), as well as the chlorinated and methylated analogues **94** – **97** (IC_{50} in the range 0.8 – 1.5 μM) were significantly less active.

Conclusions

The recent literature on stilbene-based resveratrol analogues has been reviewed, and a total of 94 compounds are reported (see structures **4** – **97**), selected either for their promising anti-tumor properties or for comparative purposes in SAR studies. As a general outline, these recent literature data confirm the previously reported observation that minimal modification in the nature and position of the substituents on the stilbene nucleus may cause large variations in biological activity and, more specifically, in the compounds anti-tumor properties. Among the polyhydroxylated stilbenes, it has been firmly established that those with either a catechol or pyrogallol moiety are by far better radical scavengers than **1** or other analogues lacking an *ortho*-dihydroxy group; this property was also shown to be related to pro-apoptotic activity. Some authors suggest that the increased cytotoxicity of *ortho*-hydroxystilbenes is related to the presence of *ortho*-semiquinones formed during either metabolism or autoxidation. The essential role of the 4'-hydroxy group for the antiproliferative activity of resveratrol has been claimed by some authors, but there are a number of stilbenes that exhibit potent antiproliferative activity in which the 4'-hydroxy group is either substituted or absent; important examples are compounds **11**, **12**, **15**, **19** and **54**. A plausible hypothesis is that the anti-tumor properties of stilbenoids with free hydroxy groups reside in a different mechanism of action with respect to those where the hydroxy groups are blocked, as in polymethoxystilbenes.

The importance of the central double bond has been confirmed: with few exceptions, dihydrostilbenes are either less active than the unsaturated related compounds, or devoid of any activity. This is in agreement with observations made on hydrogenated combretastatin analogues, suggesting an entropic penalty associated with conformationally free derivatives [22].

In the large majority of cases where pairs of *E*- and *Z*-isomers were evaluated for either cytotoxic or pro-apoptotic activity, the *Z*-isomers proved significantly more active than their *E* analogues; a striking difference was observed for the *E*/*Z* couples **15**/**16**, **54**/**60**, **49**/**51**, **50**/**52** and **65**/**69**, all bearing two or three methoxylated groups in ring A, but also for the amino-analogues **77**/**76** and the bromo-analogues **82**/**83**. Previous reports document well that *Z*-stilbenes are more potent microtubule interactors than their *E*-isomers [74]; nevertheless, the antiproliferative/apoptotic activity ratio between the *E*/Z isomers reported here has wide variations and in some cases both either have comparable activities or the *E*-isomer may be even more active, as for *E* and *Z*-resveratrol. Thus, a general rule stating that stilbenoids with a *Z* configuration of the double bond display a considerably higher antiproliferative activity than their *E*-isomers cannot be considered as established. The structural analogy of methoxylated *Z*-stilbenoids with combretastatin A4 (**3**), as well as the similarities of biological properties observed in some cases, like the inhibition of tubulin polymerisation, prompts the hypothesis that, at least in part, these two families of related stilbenoids may share a similar mechanism of action, for instance, a preferential interaction with a receptor. Nevertheless, the observation that *Z*-resveratrol has a low activity has been highlighted by some authors, who argued that the interpretation of the available data is not straightforward.

A variety of methoxystilbenes (and one ethoxystilbene) has recently been reported: these analogues showed, in many cases, either potent antiproliferative, pro-apoptotic activity or strong inhibition of TNFα-induced activation of NF-*k*B. (see for instance **11**, **12**, **15, 19**, **49**, **54** and related *Z*-isomers). Substitution with larger groups generally afforded analogues with either lower or comparable activity, but it is worthy of mention that the brominated derivative **84** was more active than 5-fluorouracil towards KB cells. In the most potent analogues, close to the nanomolar range of activity, a 3,5-dimethoxy or a 3,4,5-trimethoxy moiety (ring A) is generally present, but minimal structural differences cause significant difference in activity. For instance, it is interesting to compare **13** with either **15** or **19**, the latter with **17**, and **48** with **49**. In contrast, it is rather surprising that **51** and **76** exhibit an identical activity. Globally considered, polymethoxystilbenes appear as a sub-group of great interest among the resveratrol analogues; these analogues are worthy of a deeper evaluation also in relation to their potential anti-angiogenic properties. In addition, *in vivo* studies indicate that methoxystilbenes undergo different metabolic conversion and have a higher bioavailability with respect to resveratrol.

The potent activity of some amino- and halogenated stilbenes, and in particular **76**, **91** and **92** is undoubtedly worthy of attention, but the toxicity of these compounds to normal cells has rarely been evaluated and some of them have been indicated as being too toxic to justify future investigations.

In conclusion, the synthesis and evaluation of stilbene-based resveratrol analogues has proved to be a highly active field of research and has recently afforded compounds with cytotoxic and pro-apoptotic activity in the nanomolar range. Nevertheless, the exact structural determinants to optimize the anti-tumor properties of these compounds and details of their mechanism of action remain to be clarified. Future studies on either previously reported or new analogues will certainly be useful for a better understanding of the biological activity of the stilbenoid class, and hopefully may afford either new anti-tumor compounds or adjuvants of the anticancer drugs in current clinical use.

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References

[1] Renaud S, de Lorgeril M. (**1992**) Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet*, ***339***, 1523-1526.

[2] Takaoka MJ. (**1940**) Of the phenolic substances of white hellebore (*Veratrum grandiflorum* Loes. fil.). *Journal of the Faculty of Science Hokkaido Imperial University*, ***3***, 1-16.

[3] Nonomura S, Kanagawa H, Makimoto A. (**1963**) Chemical constituents of Polygonaceous plants. I. Studies on the components of Ko-jo-kon (*Polygonum cuspidatum* Sieb. et Zucc.). *Yakugaku Zasshi*, ***83***, 988-990.

[4] Jeandet P, Bessis R, Sbaghi M, Meunier P, Trollat P. (**1995**) Resveratrol content of wines of different ages: relationship with fungal disease pressure in the vineyard. *American Journal of Enology and Viticulture*, ***46***, 1-4.

[5] Baur JA, Sinclair DA. (**2006**) Therapeutic potential of resveratrol: the *in vivo* evidence**.** *Nature Reviews Drug Discovery*, ***5***, 493-506.

[6] Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S, Takada Y. (**2004**) Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Research*, ***24***, 2783-2840.

[7] Burjonroppa S, Fujise K. (**2006**) Resveratrol as cardioprotective agent: evidence from bench and bedside. In *Resveratrol in Health and Disease,* Aggarwal BB, Shishodia S. (Eds). Taylor & Francis, London, 539-555.

[8] Signorelli P, Ghidoni R. (**2005**) Resveratrol as an anticancer nutrient: molecular basis, open questions and promises. *Journal of Nutritional Biochemistry*, ***16***, 449-466.

[9] Delmas D, Lançon A, Colin D, Jannin B, Latruffe N. (**2006**) Resveratrol as a chemopreventive agent: a promising molecule for fighting cancer. *Current Drug Targets*, ***7***, 423-442.

[10] Aggarwal BB, Shishodia S. (**2006**) Oxidative Stress and Disease vol 20. *Resveratrol in Health and Disease.* 1-712 pp**.** Taylor & Francis, London.

[11] Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CWW, Fong HHS, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM. (**1997**) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*, ***275***, 218-220.

[12] Fulda S, Debatin KM. (**2006**) Resveratrol modulation of signal transduction in apoptosis and cell survival: a mini-review. *Cancer Detection and Prevention*, ***30***, 217-223.

[13] Aggarwal BB, Shishodia S. (**2006**) Molecular targets of dietary agents for prevention and therapy of cancer. *Biochemical Pharmacology*, ***71***, 1397-1421.

[14] Jazirehi AR, Bonavida B. (**2006**) Resveratrol as a sensitizer to apoptosis-inducing stimuli. In *Resveratrol in Health and Disease*. Aggarwal BB, Shishodia S. (Eds). Taylor & Francis, London, 399-421.

[15] Wenzel E, Somoza V. (**2005**) Metabolism and bioavailability of *trans*-resveratrol. *Molecular Nutrition & Food Research*, ***49***, 472-481.

[16] Gorham J. (**1995**) *The Biochemistry of the Stilbenoids*. Chapman & Hall, London.

[17] Mattivi F, Remiero F, Korhammer S. (**1995**) Isolation, characterization, and evolution in red wine vinification of resveratrol monomers. *Journal of Agricultural and Food Chemistry*, ***43***, 1820-1823.

[18] Ingham JL. (**1976**) 3,5,4'-Trihydroxystilbene as a phytoalexin from groundnuts (*Arachis hypogaea*). *Phytochemistry*, ***15***, 1791-1793.

[19] Sarker SD, Whiting P, Dinan L. (**1999**) Identification and ecdysteroid antagonist activity of three resveratrol trimers (suffruticosols A, B and C) from *Paeonia suffruticosa*. *Tetrahedron*, ***55***, 513-24.

[20] Hadfield JA, Gaukroger K, Hirst N, Weston AP, Lawrence NJ, McGown AT. (**2005**) Synthesis and evaluation of double bond substituted combretastatins. *European Journal of Medicinal Chemistry*, ***40***, 529-541.

[21] Tozer GM, Kanthou C, Baguley BC. (**2005**) Disrupting tumour blood vessels. *Nature Reviews Cancer*, ***5***, 423-435.

[22] Tron GC, Pirali T, Sorba G, Pagliai F, Busacca S, Genazzani AA. (**2006**) Medicinal chemistry of combretastatin A4: Present and future directions. *Journal of Medicinal Chemistry*, ***49***, 3033-3044.

[23] Ovesná Z, Horváthová-Kozics K. (**2005**) Structure-activity relationship of *trans*-resveratrol and its analogues. *Neoplasma*, ***52***, 450-455.

[24] Zhou B, Liu Z-L. (**2005**) Bioantioxidants: from chemistry to biology. *Pure and Applied Chemistry*, ***77***, 1887-1903.

[25] Roupe KA, Remsberg CM, Yáñez JA, Davies NM. (**2006**) Pharmacometrics of stilbenes: seguing towards the clinic. *Current Clinical Pharmacology*, ***1***, 81-101.

[26] King RE, Bomser JA, Min DB. (**2006)** Bioactivity of resveratrol. *Comprehensive Reviews in Food Science and Food Safety*, ***5***, 65-70.

[27] Celimene CC, Smith DR, Young RA, Stanosz GR. (**2001**) In vitro inhibition of *Spaeorpsis sapinea* by natural stilbenes. *Phytochemistry*, ***56***, 161-165.

[28] Roupe KA, Fukuda C, Yáñez JA, Halls S, Davies NM. (**2005**) Pinosylvin: Method of analysis, anti-cancer activity and metabolism. *The AAPS Journal*, *7(S2) Abstract T3261.*

[29] Cai Y-J, Fang J-G, Ma L-P, Yang L, Liu Z-L. (**2003**) Inhibition of free radical-induced peroxidation of rat liver microsomes by resveratrol and its analogues. *Biochimica et Biophysica Acta*, ***1637***, 31-38.

[30] Cai Y-J, Wei Q-Y, Fang J-G, Yang L, Liu Z-L, Wyche JH, Han Z. (**2004)** The 3,4-dihydroxyl groups are important for *trans*-resveratrol analogs to exhibit enhanced antioxidant and apoptotic activities. *Anticancer Research*, ***24***, 999-1002.

[31] Lion CJ, Matthews CS, Stevens MFG, Westwell AD. (**2005**) Synthesis, antitumor evaluation, and apoptosis-inducing activity of hydroxylated (*E*)-stilbenes. *Journal of Medicinal Chemistry*, ***48***, 1292-1295.

[32] Heynekamp JJ, Weber WM, Hunsaker LA, Gonzales AM, Orlando RA, Deck LM, Vander Jagt DL. (**2006**) Substituted *trans*-stilbenes, including analogues of the natural product resveratrol, inhibit the human tumor necrosis factor alpha-induced activation of transcription factor nuclear factor kappaB. *Journal of Medicinal Chemistry*, ***49***, 7182-7189.

[33] Rimando AM, Cuendet M, Desmarchelier C, Mehta RG, Pezzuto JM, Duke SO. (**2002**) Cancer chemopreventive and antioxidant activities of pterostilbene, a naturally occurring analogue of resveratrol. *Journal of Agricultural and Food Chemistry*, ***50***, 3453-3457.

[34] Ferrer P, Asensi M, Segarra R, Ortega A, Benlloch M, Obrador E, Varea MT, Asensio G, Jorda L, Estrela JM. (**2005**) Association between pterostilbene and quercetin inhibits metastatic activity of B16 melanoma**.** *Neoplasia*, ***7***, 37-47.

[35] Tolomeo M, Grimaudo S, Di Cristina A, Roberti M, Pizzirani D, Meli M, Dusonchet L, Gebbia N, Abbadessa V, Crosta L, Barucchello R, Grisolia G, Invidiata F, Simoni D. (**2005**) Pterostilbene and 3'-hydroxypterostilbene are effective apoptosis-inducing agents in MDR and BCR-ABL-expressing leukemia cells. *International Journal of Biochemistry & Cell Biology*, ***37***, 1709-1726.

[36] Roberti M, Pizzirani D, Simoni D, Rondanin R, Baruchello R, Bonora C, Buscemi F, Grimaudo S, Tolomeo M. (**2003**) Synthesis and biological evaluation of resveratrol and analogues as apoptosis-inducing agents. *Journal of Medicinal Chemistry*, ***46***, 3546-3554.

[37] MacRae WD, Towers GHN. (**1985**) Non-alkaloidal constituents of *Virola elongate* bark. *Phytochemistry*, ***24***, 561-566.

[38] Lee SK, Nam KA, Hoe YH, Min H-Y, Kim E-Y, Ko H, Song S, Lee T, Kim S. (**2003**) Synthesis and evaluation of cytotoxicity of stilbene analogues. *Archives of Pharmacal Research*, ***26***, 253-257.

[39] Schneider Y, Chabert P, Stutzmann J, Coelho D, Fougerousse A, Gosse F, Launay J-F, Brouillard R, Raul F. (**2003**) Resveratrol analog (*Z*)-3,5,4'-trimethoxystilbene is a potent anti-mitotic drug inhibiting tubulin polymerization. *International Journal of Cancer*, ***107***, 189-196.

[40] Schneider Y, Fischer B, Coelho D, Roussi S, Gosse F, Bischoff P, Raul F. (**2004**) (*Z*)-3,5,4'-Tri-*O*-methylresveratrol induces apoptosis in human lymphoblastoid cells independently of their p53 status. *Cancer Letters*, ***211***, 155-161.

[41] Simoni D, Roberti M, Invidiata FP, Aiello E, Aiello S, Marchetti P, Baruchello R, Eleopra M, Di Cristina A, Grimaudo S, Gebbia N, Crosta L, Dieli F, Tolomeo M. (**2006**) Stilbene-based anticancer agents: Resveratrol analogues active toward HL60 leukemic cells with a non-specific phase mechanism. *Bioorganic & Medicinal Chemistry Letters*, ***16***, 3245–3248.

[42] Roberti M, Pizzirani D, Recanatini M, Simoni D, Grimaudo S, Di Cristina A, Abbadessa V, Gebbia N, Tolomeo M. (**2006**) Identification of a terphenyl derivative that blocks the cell cycle in the G_0-G_1 phase and induces differentiation in leukemia cells. *Journal of Medicinal Chemistry*, ***49***, 3012-3018.

[43] Minutolo F, Sala G, Bagnacani A, Bertini S, Carboni I, Placanica G, Prota G, Rapposelli S, Sacchi N, Macchia M, Ghidoni R. (**2005**) Synthesis of a resveratrol analogue with high ceramide-mediated proapoptotic activity on human breast cancer cells. *Journal of Medicinal Chemistry*, ***48***, 6783-6786.

[44] Matsuoka A, Takeshita K, Furuta A, Ozaki M, Fukuhara K, Miyata N. (**2002**) The 4'-hydroxy group is responsible for the *in vitro* cytogenetic activity of resveratrol. *Mutation Research*, ***521***, 29-35.

[45] Petralia S, Spatafora C, Tringali C, Foti MC, Sortino S. (**2004**) Hydrogen atom abstraction from resveratrol and two lipophilic derivatives by tert-butoxyl radicals. A laser flash photolysis study. *New Journal of Chemistry*, ***28***, 1484-1487.

[46] Ruan B-F, Huang X-F, Ding H, Xu C, Ge H-M, Zhu H-L, Tan R-X. (**2006**) Synthesis and cytotoxic evaluation of a series of resveratrol derivatives. *Chemistry & Biodiversity*, ***3***, 975-981.

[47] Belleri M, Ribatti D, Nicoli S, Cotelli F, Forti L, Vannini V, Stivala LA, Presta M. (**2005**) Antiangiogenic and vascular-targeting activity of the microtubule-destabilizing *trans*-resveratrol derivative 3,5,4'-trimethoxystilbene. *Molecular Pharmacology*, ***67***, 1451–1459.

[48] Bhowmick N, Neilson EG, Moses HL. (**2004**) Stromal fibroblasts in cancer initiation and progression. *Nature*, ***432***, 332-337.

[49] Mikstacka R, Rimando AM, Szalaty K, Stasik K, Baer-Dubowska W. (**2006**) Effect of natural analogues of *trans*-resveratrol on cytochromes P4501A2 and 2E1 catalytic activities. *Xenobiotica*, ***36***, 269-285.

[50] Nicolosi G, Spatafora C, Tringali C. (**2002**) Chemo-enzymatic preparation of resveratrol derivatives. *Journal of Molecular Catalysis B: Enzymatic*, ***16***, 223-229.

[51] Cardile V, Lombardo L, Spatafora C, Tringali C. (**2005**) Chemo-enzymatic synthesis and antiproliferative activity of resveratrol analogues. *Bioorganic Chemistry*, ***33***, 22-33.

[52] Likhitwitayawuid K, Sornsute A, Sritularak B, Ploypradith P. (**2006**) Chemical transformations of oxyresveratrol (*trans* -2,4,3′,5′-tetrahydroxystilbene) into a potent tyrosinase inhibitor and a strong cytotoxic agent. *Bioorganic & Medicinal Chemistry Letters*, ***16***, 5650-5653.

[53] Cardile V, Chillemi R, Lombardo L, Sciuto S, Spatafora C, Tringali C. (**2007**) Antiproliferative activity of methylated analogues of *E*- and *Z*-resveratrol. *Zeitschrift für Naturforschung C*, ***62 c***, in press.

[54] Andrus MB, Liu J. (**2006**) Synthesis of polyhydroxylated ester analogs of the stilbene resveratrol using decarbonylative Heck couplings. *Tetrahedron Letters*, ***47***, 5811-5814.

[55] Wang Y, Wang B, Cheng J, Yang L, Liu Z-L, Balan K, Pantazis P, Wyche JH, Han Z. (**2005**) FADD-dependent apoptosis induction in Jurkat leukemia T-cells by the resveratrol analogue, 3,4,5-trihydroxy-*trans*-stilbene. *Biochemical Pharmacology*, ***69***, 249-254.

[56] Roupe K, Fukada C, Halls S, Teng XW, Davies NM. (**2004)** Anti-cancer activity, pharmacokinetics and metabolism of piceatannol *in vitro* and *in vivo*. *Journal of Pharmacy & Pharmaceutical Sciences*, ***7***, 75-76.

[57] Larrosa M, Tomas-Barberan FA, Espin JC. (**2004**) The grape and wine polyphenol piceatannol is a potent inducer of apoptosis in human SK-Mel-28 melanoma cells. *European Journal of Nutrition*, ***43***, 275-284.

[58] Barton BE, Karras JG, Murphy TF, Barton A, Huang HFS. (**2004)** Signal transducer and activator of transcription 3 (STAT3) activation in prostate cancer: Direct STAT3 inhibition induces apoptosis in prostate cancer lines. *Molecular Cancer Therapeutics*, ***3***, 11–20.

[59] Chowdhury SA, Kishino K, Satoh R, Hashimoto K, Kikuchi H, Nishikawa H, Shirataki Y, Sakagami H. (**2005**) Tumor-specificity and apoptosis-inducing activity of stilbenes and flavonoids. *Anticancer Research*, ***25***, 2055-2063.

[60] Takei M, Umeyama A, Arihara S, Matsumoto H. (**2005**) Effect of piceatannol in human monocyte-derived dendritic cells *in vitro*. *Journal of Pharmaceutical Sciences*, ***94***, 974-982.

[61] Füllbeck M, Huang X, Dumdey R, Frommel C, Dubiel W, Preissner R. (**2005**) Novel curcumin- and emodin-related compounds identified by *in silico* 2D/3D conformer screening induce apoptosis in tumor cells. *BMC Cancer*, ***5***, 97, doi: 10.1186/1471-2407-5-97

[62] Murias M, Jaeger W, Handler N, Erker T, Horvath Z, Szekeres T, Nohl H, Gille L. (**2005**) Antioxidant, prooxidant and cytotoxic activity of hydroxylated resveratrol analogues: structure-activity relationship. *Biochemical Pharmacology*, ***69***, 903-912.

[63] Ovesná Z, Kozics K, Bader Y, Saiko P, Handler N, Erker T, Szekeres T. (**2006**) Antioxidant activity of resveratrol, piceatannol and 3,3',4,4',5,5'-hexahydroxy-*trans*-stilbene in three leukaemia cell lines. *Oncology Reports*, ***16***, 617-624.

[64] Matsuda H, Tomohiro N, Hiraba K, Harima S, Ko S, Matsuo K, Yoshikawa M, Kubo M. (**2001**) Study on anti-Oketsu activity of rhubarb II. Anti-allergic effects of stilbene components from *Rhei undulati Rhizoma* (dried rhizome of *Rheum undulatum* cultivated in Korea). *Biological & Pharmaceutical Bulletin*, ***24***, 264-267.

[65] Roupe KA, Helms GL, Halls SC, Yáñez JA, Davies NM. (**2005**) Preparative enzymatic synthesis and HPLC analysis of rhapontigenin: applications to metabolism, pharmacokinetics and anti-cancer studies. *Journal of Pharmacy & Pharmaceutical Sciences*, ***8***, 374-386.

[66] Likhitwitayawuid K, Sritularak B, Benchanak K, Lipipun V, Mathew J, Schinazi RF. (**2005**) Phenolics with antiviral activity from *Millettia erythrocalyx* and *Artocarpus lakoocha*. *Natural Product Research*, ***19***, 177-182.

[67] Kim YM, Yun J, Lee C-K, Lee H, Min KR, Kim Y. (**2002**) Oxyresveratrol and hydroxystilbene compounds: Inhibitory effect on tyrosinase and mechanism of action. *Journal of Biological Chemistry*, ***277***, 16340-16344.

[68] Sale S, Verschoyle RD, Boocock D, Jones DJL, Wilsher N, Ruparelia KC, Potter GA, Farmer PB, Steward WP, Gescher AJ. (**2004**) Pharmacokinetics in mice and growth-inhibitory properties of the putative cancer chemopreventive agent resveratrol and the synthetic analogue *trans*-3,4,5,4'-tetramethoxystilbene. *British Journal of Cancer*, ***90***, 736-744.

[69] Potter GA, Butler PC, Ruparelia KC, Ijaz T, Wilsher NC, Wanogho E, Tan HL, Hoang TTV, Stanley LA, Burke MD. (**2002**) DMU212: a novel CYP1B1 activated anticancer prodrug. *British Journal of Cancer*, ***86***, S117.

[70] Sale S, Tunstall RG, Ruparelia KC, Potter GA, Steward WP, Gescher AJ. (**2005**) Comparison of the effects of the chemopreventive agent resveratrol and its synthetic analog *trans* 3,4,5,4'-tetramethoxystilbene (DMU-212) on adenoma development in the Apc^{Min+} mouse and cyclooxygenase-2 in human-derived colon cancer cells. *International Journal of Cancer*, ***115***, 194-201.

[71] Gosslau A, Chen M, Ho C-T, Chen KY. (**2005**) A methoxy derivative of resveratrol analogue selectively induced activation of the mitochondrial apoptotic pathway in transformed fibroblasts. *British Journal of Cancer*, ***92***, 513-521.

[72] Horvath Z, Murias M, Saiko P, Erker T, Handler N, Madlener S, Jaeger W, Grusch M, Fritzer-Szekeres M, Krupitza G, Szekeres T. (**2006**) Cytotoxic and biochemical effects of 3,3',4,4',5,5'-hexahydroxystilbene, a novel resveratrol analog in HL-60 human promyelocytic leukemia cells. *Experimental Hematology*, ***34***, 1377-1384.

[73] Lee E-J, Min H-Y, Park HJ, Chung H-J, Kim S, Han YN, Lee SK. (**2004**) G2/M cell cycle arrest and induction of apoptosis by a stilbenoid, 3,4,5-trimethoxy-4'-bromo-*cis*-stilbene, in human lung cancer cells. *Life Sciences*, ***75***, 2829-2839.

[74] Gaukroger K, Hadfield JA, Lawrence NJ, Nolan S, McGown AT. (**2003**) Structural requirements for the interaction of combretastatins with tubulin: how important is the trimethoxy unit? *Organic & Biomolecular Chemistry*, ***1***, 3033-3037.