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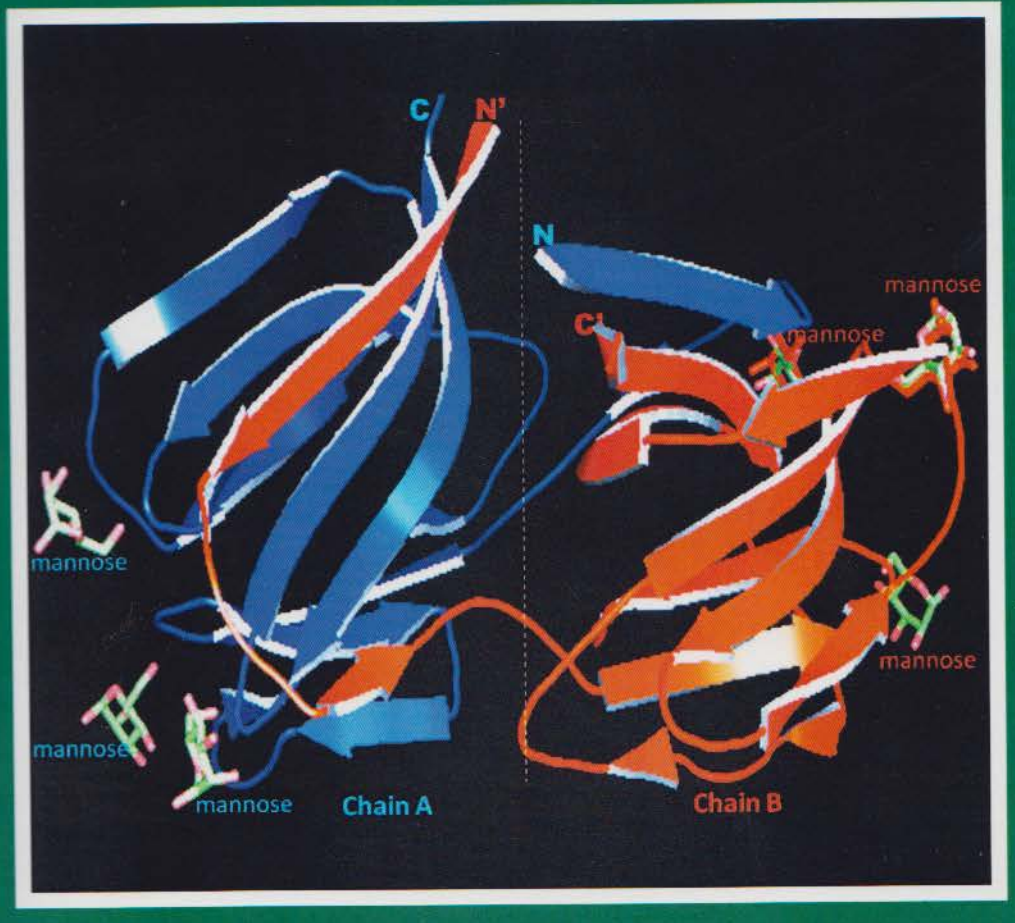

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Resveratrol and Its Analogs as Antitumoral Agents for Breast Cancer Treatment

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Abstract: Resveratrol (3,5,4'-tri-hydroxystilbene) (RSV), a naturally occurring phytoalexin, readily available in the diet, has gained interest as a non-toxic agent capable of displaying cancer-preventing and anti-cancer properties. Several studies, using both *in vitro* and *in vivo* models, have illustrated RSV capacity to modulate a multitude of signaling pathways associated with cellular growth and division, apoptosis, angiogenesis, invasion and metastasis. However, its clinical application is limited because of a low oral bioavailability with high adsorption but rapid metabolism and low tissue concentrations. Several chemical modifications to the backbone structure have been made for the purpose of improving pharmacokinetic parameters. One promising strategy involves the introduction of methoxylic or hydroxylic groups on the phenylic rings of RSV. Moreover, by replacing the alkene linker between the two aromatic rings with a heterocyclic system rigid analogs such as 2,3-thiazolidin-4-ones and 3-chloro-azetidin-2-ones that displayed higher cytotoxic activity and hence higher ability to inhibit *in vitro* breast cancer cell growth have been synthesized. *In vitro* studies have demonstrated, for some of these compounds, a greater bioaccessibility than RSV and more selective inhibitory effects on breast cancer cell growth. Further investigations, particularly *in vivo*, are required as next step to implicate these analogs as pharmacologic agents for a possible clinical anticancer application.

Keywords: Analogs, Antitumoral Agents, Breast Cancer, Cell Growth Inhibition, RSV, RSV Bioaccessibility.

1. INTRODUCTION

Cancer is a disease responsible for the death of millions of people every year. This disease may occur when the physiological balance between mitosis and apoptosis is altered, either by an increase in cell proliferation or a decrease in cell death. Surgery, radiotherapy and chemotherapy represent the standard treatment protocols for cancer. The goal of cancer chemotherapy is to promote cancer cell death without affecting normal cells, by inducing apoptosis or by direct toxicity. Often, inherent or acquired tumor drug resistance limits drug therapy efficacy aimed to block cancer progression [1]. It is known that cancer progression is the result of the interplay between several factors including genetic, environmental and dietary factors. In particular, environmental and/or dietary factors have a significant role in the incidence and development of breast cancer, the most frequent tumor and the major cause of cancer death among women [2]. Results from several studies support the hypothesis that intake of vegetables and fruits decreases breast cancer risk [3]. For example, the low incidence of breast cancer in Asian women has been linked to the consumption of a diet rich in soy products known to contain high amounts of phytoestrogens [4, 5]. These natural compounds have been demonstrated capable of mimicking or antagonizing estrogen effects on breast cancer growth [6-8]. During the last few years a growing interest for natural-product-based drug discovery for cancer therapy [9] has integrated new technologies, such as combinatorial synthesis [10] and high-throughput screening (HTS) [11, 12]. In particular, *in vitro* bioassays have been developed using both cellular (*i.e.* assessment of growth or cell death by different methods) [13-15] and molecular-based approaches (*i.e.* HTS against specific kinases or other enzymes) [16, 17].

Resveratrol (3,5,4'-tri-hydroxystilbene) (RSV) is a natural phytoalexin present in medicinal plants, grape skin, peanuts, red wine and other foods that are commonly consumed as part of the human diet [18, 19]. Data from basic research and preclinical studies disclosed the broad range of advantageous biological actions possessed by RSV [20]. RSV is produced by some spermatophytes, such as grapevines, and protects the plant from injury, ultraviolet irradiation and fungal attack [21]. It exists in *cis*- and *trans*- isomeric forms (Fig. **1**) but the *cis*-isomer has never been identified in grape extract [21, 22].

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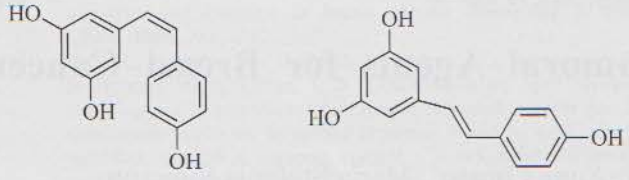


Fig. (1). Structure of *cis* (**I**) and *trans* (**II**) Resveratrol.

This natural compound exerts multiple beneficial properties including antioxidant activity, modulation of lipid and lipoprotein metabolism, platelet aggregation inhibition, vasodilator, anticancer, chemopreventive, anti-proliferative, proapoptotic, cardioprotective and estrogenic activity [21] by modulation of multiple pathways such as mTOR, ERK and PI3K/Akt [23-25]. RSV antioxidant properties [26] have been attributed to its capability to reduce copper-catalyzed oxidation [27] and to inhibit LDL [28] and membrane lipids peroxidation [29]. Some observations have highlighted the protective effects of RSV against atherosclerosis by decreasing intracellular concentration in apo B, cholesterol esters, and triglycerides secretion rate [30]. Furthermore, the chemopreventive effect of RSV is thought to relay on the inhibition of quinone reductase-2 activity, allowing up-regulation in the expression of enzymes involved in antioxidative and detoxificative reactions, improving cell resistance to oxidative stress [31]. *In vitro* studies have demonstrated that RSV is able to inhibit formation of thromboxane B2 and lipoxygenase products, substances involved in inflammatory processes, such as chemotactic factors formation and platelet aggregation [32, 33]. In particular, it has been reported that RSV exerts anti-inflammatory effects by inhibiting cyclooxygenase-1 and -2 expression [34] and catalytic activity [35]. The cardiovascular effects of grape products, particularly those of *trans*-RSV, can relay on nitric oxide-dependent and -independent vasodilatation [36, 37]. RSV also increases the activity of SIRT1 (a member of the sirtuin family of nicotinamide adenine dinucleotide-dependent deacetylases), resulting in improved cellular stress resistance and longevity [23, 38]. In particular, RSV displays important neuroprotective effects on animal models of Parkinson's disease and prevents free-radical-mediated damage of neuronal cells through the activation of a SIRT1 pathway [39]. Chemopreventive effects of RSV might also depend on the suppression of human CYP1A1 and CYP1B1 gene expression [40]; these genes encode for enzymes involved in the metabolic activation of several pro-carcinogens and in the catabolism of several xenobiotic compounds [41, 42].

Several reports confirm that RSV inhibits *in vitro* all three phases of tumor development: initiation, promotion, and progression [43] of various cancer types such as B-cell lymphoma [44], T-cell lymphoma [45], melanoma [46] prostate [47], colon [48], pancreatic [49], gastric [50], ovarian [51], endometrial [52], liver [53] and breast cancer [54]. However, contradictory effects on the inhibition of breast cancer cell proliferation have been reported. High doses of RSV suppressed the growth of estrogen receptor-α (ERα)-positive MCF-7 [55, 56], T47D [57], KPL-1 [58] and ERα-negative MDA-MB-231, MKL-F [56, 58, 59] breast cancer cells, while a low dose of RSV potentiated MCF-7 and T47D proliferation [60, 61]. These results suggest that depending on the cell type and on the used dose, RSV could contribute to the activation of different signaling pathways involved in breast cancer cell proliferation and gene expression regulation [62-64]. The anti-proliferative effects of RSV on breast cancer are dependent on its concentrations but appear to be independent from the estrogen receptor status [59]. RSV anti-proliferative properties are consequent to cell cycle arrest and/or apoptosis [49, 65]. Lanzilli *et al.* have demonstrated that in MCF7 cells RSV treatment caused an S-phase arrest with a concomitant reduction in telomerase activity followed by apoptosis [66]. It should be noted that RSV's ability to induce apoptosis depends on the tumor cell type [56]. Moreover, different doses can induce different effects on several tumor cells; for example it has been shown that 10-30 μM of RSV can induce cell cycle arrest or cancer cell growth inhibition [67] while higher doses (>100 μM) can induce death by apoptosis [68, 69].

RSV's beneficial effects observed *in vitro* are limited by its short biological half-life and rapid metabolism and elimination [70, 71]. Therefore, fewer studies have investigated RSV's anticancer effects *in vivo* [72]. In humans and rodents, RSV is metabolized through three major pathways [71], then, despite administration of high doses of RSV, it might be insufficient to achieve the systemic concentrations required for cancer prevention [73]. Following intestinal absorption, *trans*-RSV and its glucoside are converted into glucuronide and sulphate metabolites (Fig. **2**) by enterocytes and hepatocyte through the action of uridine 5'-diphospho-glucuronosyltransferases and sulphotransferases, respectively [71]. Extremely rapid sulfate conjugation by intestine and liver appears to be the rate-limiting step in RSV's bioavailability [71]. In addition, intestinal microflora likely catalyzes hydrogenation of the RSV aliphatic double bond. Even though systemic RSV bioavailability is very low, active RSV metabolites can accumulate in the gastrointestinal tract. As a consequence, only for those tumors that can come into direct contact with RSV (gastrointestinal tumors), there are consistent evidences for its anticancer action, while for other cancers the evidences are uncertain [24].

Currently, there is a major interest in developing new formulations with improved RSV bioavailability together with potent anticancer activity. This review summarizes the current literature on syntheses of new and more powerful RSV analogs that display remarkable promises as potent chemopreventive agents against breast cancer.

2. ESTROGEN-LIKE ACTIONS OF RSV ON BREAST CANCER: *IN VITRO* AND *IN VIVO* STUDIES

Initial studies tried to link RSV effects to its phytoestrogenic character, derived from a structural similarity to diethylstilbesterol and from its capability of binding and activating both ERα and ERβ [60]. The most

studies evaluated RSV interactions with ERs alone or in the presence of 17β-estradiol (E2), the main physiological ligand of ERs. Conclusion from these studies are conflicting and depend on the breast cancer cell line used for the study, particularly to the ER and associated co-activators expression pattern [74, 75]. However, RSV exerts an action on both ER+ and ER- breast cancer cells [55, 59, 76]. In ERα+ and ERβ+ MCF-7 mammary cancer cells, RSV exhibits estrogenic and even superestrogenic (when combined with E2) properties [60], but also antiestrogenic activities in the presence of E2 [59, 75]. It was concluded that RSV acts as an agonist on mammary cells containing ERα, while exhibits antagonistic activity on ERβ [77]. However, it is now generally established that RSV acts as a mixed agonist/antagonist at low concentrations, while it acts as a pure anti-estrogen at higher concentrations and combined with the E2 [75, 78]. Nakagawa *et al.* showed that low concentrations of RSV increase cell proliferation of ER+ (KPL-1, ≤ 22 μM; MCF-7, ≤ 4 μM) human breast cancer cell lines whereas high concentrations (≥ 44 μM) suppress cell growth of ER- MKL-F breast cancer cells by activation of an apoptotic mechanism [58]. These results were confirmed by other studies using MCF7 and MDA-MB-231 cells, where RSV inhibited proliferation, activated proapoptotic effects in a dose and time-dependent manner [56]. Specifically, in MDA-MB-231, RSV regulated expression of G1/S and G2/M markers, without affecting expression of tumor suppressors p21Cip1/WAF1, p27Kip1 and p53, leading these cells to death by a non-apoptotic process. In MCF-7, RSV produced a significant increase in expression and in kinase activities of G1/S and G2/M regulators, such as p21Cip1/WAF1, p27Kip1 and p53. These events resulted in cell-cycle arrest at the S phase followed by apoptosis [56]. Another study comparing ER+ and ER- cells showed a different mechanism involved in the anti-proliferative effects of RSV. In ER+ MCF-7 cells, RSV antagonized E2-dependent stimulation of an estrogen responsive element reporter gene construct and inhibited mRNA expression of progesterone receptor [79]. Whereas on ER- MDA-MB-468 cells anti-proliferative effects occurred by decreasing the expression of transforming growth factor-alpha (TGFα), plasma cell derived growth factor (PCDGF) and insulin-like growth factor I receptor (IGFIR) [79].

In vivo studies of the estrogenic effects of RSV are limited [80], and the majority of those conducted thus far were unable to confirm the ERα agonistic effects observed in *in vitro* studies. Rather, RSV appears to have pure anti estrogenic effects at high doses [74, 80, 81]. In fact, RSV has been shown to inhibit the formation of carcinogen-induced preneoplastic mammary lesions and tumors in rodent models [74]. Previous studies performed in rats aimed to investigate whether RSV was an estrogen agonist on reproductive and non-reproductive estrogen target tissues. RSV administration in rats (1-100 μg/day) had no effect on uterine growth and differentiation, body weight, serum cholesterol and radial bone growth. These doses were not tested in combination with E2, while only 1000 μg/day of RSV was combined, and prevented estrogen-dependent serum cholesterol lowering effect. The study concluded that RSV exerted no estrogen

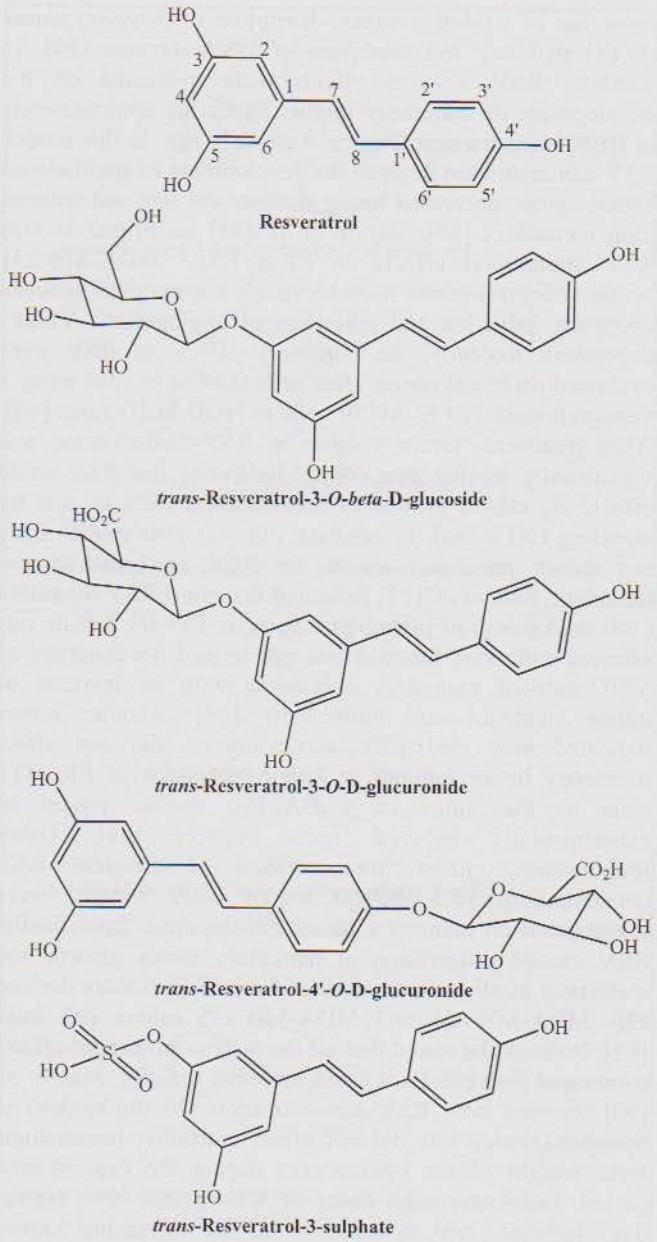


Fig. (2). *trans*-Resveratrol and its main metabolites.

agonistic action but could act as an estrogen antagonist [80]. Other studies examined RSV effects on breast tumor models. It has been reported that RSV (10-100 mg/kg/day) inhibited the early stages of *N*-methyl-*N*-nitrosourea (MNU) [74] or 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary carcinogenesis in female Sprague-Dawley rats [82]. In the same animal tumor model, diet enrichment with high doses of RSV decreased tumor number in rats and delayed tumor development after initiation at postnatal day 50 [83]. Similarly, in DMBA-induced mammary carcinogenesis of rat, Chatterjee *et al.* [84] showed that RSV supplementation in the diet decreased mammary tumor incidence after DMBA exposure. In addition, analyzing mammary tissue after animal sacrifice, the authors demonstrated that RSV reduced cell proliferation and induced apoptosis through the

reduction of oxidative stress, disruption of 5-lypoxygenase (LOX) pathway and inhibition of DNA damage [84]. In addition, RSV *in vivo* effects were evaluated on the development of mammary tumors appearing spontaneously in HER-2/neu transgenic mice at an early age. In this model, RSV administration delayed the development of spontaneous breast cancer, decreased tumor number and size and reduced lung metastases [85]. Garvin *et al.* [81] confirmed *in vivo* RSV antitumoral effects on ER α ERβ+ MDA-MB-231 breast xenograft tumor model through a synergism between apoptosis induction and inhibition of angiogenesis VEGF-dependent. Recently, the inhibitory effects of RSV were evaluated on breast cancer stem cells (CSCs) *in vivo*, using a xenograft model of SUM159 cells in NOD/SCID mice [86]. After treatment, tumor volume in RSV-treated mice was significantly smaller than control indicating that RSV could effectively inhibit growth of breast cancer cells *in vivo* by targeting CSCs [86]. In contrast, other *in vivo* studies have not shown promising results for RSV on breast cancer treatment. Sato *et al.* [87] indicated that short RSV treatment (100 mg/kg/day) of prebubertal Sprague-Dawley female rats affected endocrine function and accelerated development of MNU-induced mammary carcinoma with an increase of tumor incidence and multiplicity [87]. Another report indicated how oral RSV administration did not affect mammary tumor number or tumor metastasis of ER- 4T1 cells to the lungs in a BALB/c murine model of experimentally induced breast cancer [88]. Using immunocompromised mice, effects of different RSV concentrations (0.5, 5, 50 mg/kg body weight) were investigated on mammary tumor development. Specifically, RSV caused progression of mammary tumor growth and metastasis at all concentrations examined in tumors derived ER- MDA-MB-231 and MDA-MB-435 cancer cell lines [89]. It should be stated that all the *in vivo* anticancer effects mentioned above did not cause systemic toxicity. Juan *et al.* [90] reported how RSV administration (20 mg/kg/day) to Sprague-Dawley rats did not affect mortality, hematologic tests, weight of the vital organs during the experimental period. Only very high doses of RSV (1000-3000 mg/kg/day) induced renal toxicity in rats by increasing kidney weight and renal lesions, such as an increased incidence and severity of nephropathy [91]. Thus, a moderate RSV uptake displays no systemic toxicity and appears to show a chemopreventive effect *in vivo* by inhibiting the early stages of breast tumorogenesis.

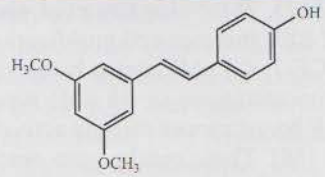
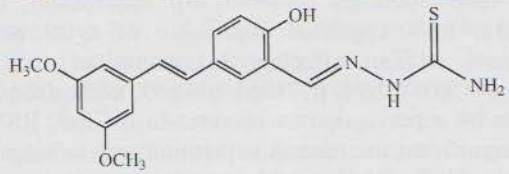
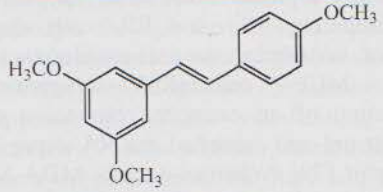
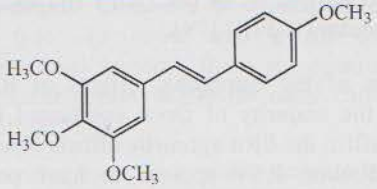
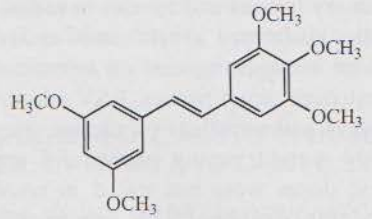
3. BIOLOGICAL EFFECTS OF RSV DERIVATIVES ON BREAST CANCER CELLS

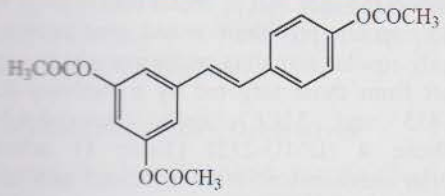
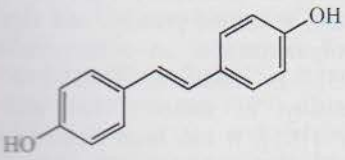
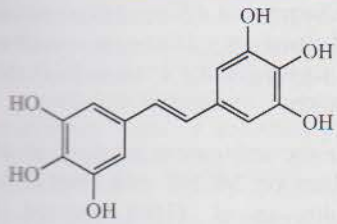
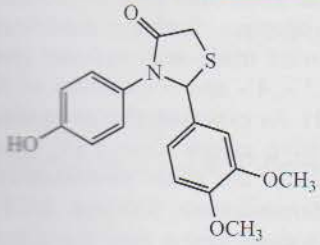
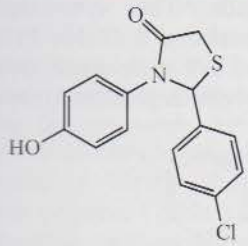
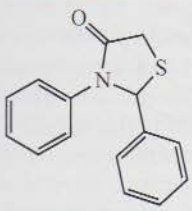
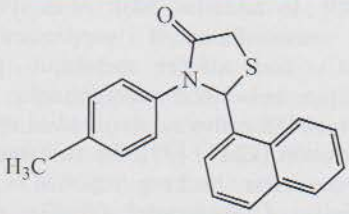
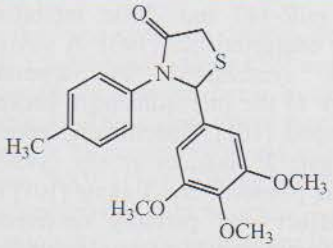
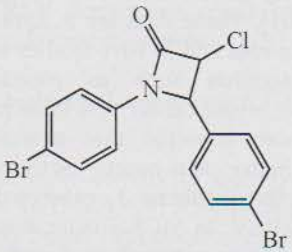
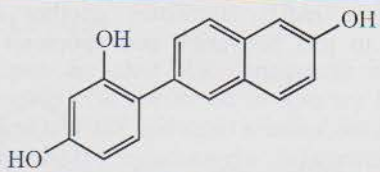
Several clinical trials have focused on characterizing the pharmacokinetics and metabolism of RSV, reporting how the chemical structure of RSV, which contains three free hydroxyl groups, makes it susceptible to extensive phase-II conjugation reactions *in vivo* [70]. Human studies demonstrated the potential drawbacks of the poor bioavailability of RSV [70]. To improve the pharmacokinetic properties and extend its cancer-protecting activity, several synthetic analogues have been prepared and tested in *in vitro* models [92].

3.1. Methoxylated RSV Derivatives

Numerous reports have supported the conclusion that the substitution of the hydroxyl groups present on phenylic moiety with methoxylic groups (Table **1**) substantially enables the anti-proliferative and apoptosis-inducing activities of RSV [92, 93]. In particular, the number and position of methoxy groups based on the RSV structure significantly influence these activities [94]. Pterostilbene **1**, the natural dimethylated analog of RSV, has higher oral bioavailability and enhanced potency than RSV [95].

Table 1. Resveratrol derivatives and analogs.

Chemical structures	References
 **3,5-dimethoxy-4'-hydroxy-*E*-stilbene (pterostilbene) (1)**	[95-101]
 **pterostilbene-isothiocyanate conjugate (2)**	[101]
 ***trans*-3,5,4'-trimethoxystilbene (3)**	[103, 104]
 ***trans*-3,4,5,4'-tetramethoxystilbene (DMU-212) (4)**	[93, 105, 106]
 ***trans*-3,5,3',4',5'-pentamethoxystilbene (5)**	[94]

 *trans*-3,5,4′-triacetyl-stilbene (6)	[104]
 *trans*-4,4′-dihydroxy-stilbene (DHS) (7)	[109-112]
 *trans*-3,3',4,4',5,5'-hexahydroxystilbene (8)	[113]
 2-(3,4-dimethoxyphenyl)-3-(4-hydroxyphenyl)-thiazolidin-4-one (9)	[116]
 2-(4-chlorophenyl)-3-(4-hydroxyphenyl)thiazolidin-4-one (10)	[116]
 2,3-diphenylthiazolidin-4-one (11)	[116]
 2-(naphthalen-1-yl)-3-*p*-tolylthiazolidin-4-one (12)	[116]
 2-(3,4,5-trimethoxyphenyl)-3-*p*-tolylthiazolidin-4-one (13)	[116]
 1,4-bis-(4-bromophenyl)-3-chloroazetidin-2-one (14)	[119]
 3-chloro-4-(4-chlorophenyl)-1-(4-iodophenyl)azetidin-2-one (15)	[119]
 4-(6-hydroxy-2-naphthyl)-1,3-benzenediol (HS-1793) (16)	[120, 121]

Pterostilbene **1** was more effective than RSV in inducing cycle arrest and the mithocondrial apoptotic pathway in breast cancer cells, probably because substitution of an hydroxyl with a methoxyl group increases lipophilicity and consequently bioavailability [96]. It has been demonstrated that pterostilbene **1** has an additive inhibitory effect on breast cancer cells when combined with tamoxifen, most likely by increasing cell apoptosis [97]. Interestingly, pterostilbene **1** simultaneously induced apoptosis, cell cycle arrest and cyto-protective autophagy in both Bcap-37 and MCF-7 breast

cancer cell lines [98]. In addition, Mak *et al.* [99] have demonstrated that pterostilbene **1** suppresses tumor enrichment in CSCs and affects metastatic potential activated by M2-tumor-associated macrophages (TAMs) modulating NF-KB/miR488 pathway involved in epithelial-to-mesenchymal transition (EMT) [99]. To an extent higher than RSV, pterostilbene **1** has also been reported to increase expression and activity of Argonaute2 (Ago2), a central RNA interference (RNAi) component. Ago2 allows an increase in the expression of a number of tumor-suppressive miRNAs, including miR-143 and -200c, inhibiting breast cancer stem cell-like characteristics [100]. A novel class of hybrid compounds synthesized by appending an isothiocyanate moiety to the pterostilbene **1** backbone, has recently been developed [101]. Specifically, pterostilbene-isothiocyanate conjugate **2**, induces greater cytotoxicity in breast cancer cells than peterostilbene **1** alone [101] (Table **1**). Interestingly, this effect was partially reversed, in the presence of peroxisome proliferator-activated receptor gamma (PPARγ) antagonists, suggesting that pterostilbene-isothiocyanate anticancer effects are mediated by activation of PPARγ pathway [101]. These data are in agreement with findings from several *in vivo* and *in vitro* studies showing the ability of PPARγ agonists such as rosiglitazone or troglitazone to decrease breast cancer cell lines proliferation [102]. It has also been reported that trimetoxystilbene derivatives show a better anti-cancer activity. Another derivative, 3,5,4′-trimethoxystilbene **3**, exhibits better anti-invasive activities than RSV. In MCF-7 cells, this compound reverses EMT by decreasing mesenchymal markers, such as snail, slug, and vimentin through PI3K/Akt and Wnt/β-catenin pathways and restores epithelial-like characteristics, such as E-cadherin expression [103]. In another work, it has been compared *trans*-RSV bioefficacy with its derivatives, trimethoxy-RSV (*trans*-3,5,4'-trimethoxystilbene) **3** and triacetyl-RSV (*trans*-3,5,4'-triacetylstilbene) **6** (Table **1**) in both ERα+ MCF-7 and ERα- MDA-MB-231 breast cancer cells [104]. Using combined *in silico* and biochemical approaches was demonstrated that RSV and triacetyl-RSV **6**, binding to integrin αvβ3, activate ERK and/or p38 kinase pathways, leading to p53 activation, cell cycle arrest, and finally DNA repair. Differently, trimethoxy-RSV **3** after binding to integrin αvβ3, stimulates another MAPK signaling leading to p53 activation and apoptosis [104]. These results support the idea that in breast cancer cells RSV and triacetyl-RSV regulate proliferation and gene expression by utilizing largely similar signaling pathways which appear relatively distinct from those targeted by trimethoxy-RSV. In MDA-MB-435 and MCF7 cells (*trans*)-3,4,5,4'-tetramethoxystilbene **4** (DMU-212) (Table **1**) activates different molecular mechanisms with increased anti-tumor effects over RSV [93]. This compound induced predominantly G2/M arrest whereas RSV caused G0/G1 arrest in both cell lines. In addition, it reduced more than RSV expression of anti-apoptotic proteins and significantly increased tubulin polymerization, an event unaffected by RSV treatment [93]. In addition, DMU-212 showed improved bioavailability in mouse liver and plasma compared with RSV [105]. It has been demonstrated that DMU-212 escapes glucuronidation reactions because of its methoxy groups and is metabolized *in vivo* to four major metabolites (*E*)-3'-hydroxy-3,4,5,4'-tetramethoxystilbene or DMU-214, (*E*)-4'-hydroxy-3,4,5-trimethoxystilbene or DMU-281, (*E*)-4-hydroxy-3,5,4'-trimethoxystilbene or DMU-291, and (*E*)-3-hydroxy-4,5,4'-trimethoxystilbene or DMU-807 (Fig. **3**) [105].

In order to define the anti-cancer mechanism of DMU-212 and its metabolites on MCF-7 and HepG2 hepatoma cells, Androutsopoulos *et al.* [106] showed that the trimethoxy substitutions along with the presence of a methoxy group at position 4' are necessary for retaining the activity of DMU-212. With the goal of obtaining more potent anticancer agents, further modifications to pentametoxystilbene were made and methoxy groups were placed on the 3-, 5-, 3'-, 4'- and 5'-positions of the phenyl rings of RSV (Table **1**). As expected, this compound showed superior anti-proliferative effects than RSV, pterostilbene and trimetoxystilbene, in the order pentametoxystilbene> trimetoxystilbene >pterostilbene> RSV on MCF-7 breast cancer cells in both a dose- and a time-dependent manner [94]. These results support the assumption that the presence and position of methoxy groups on the stilbene scaffold of RSV are relevant to the cytotoxic ability of these corresponding compounds. The cytotoxic effects of 3,5,3′,4′,5′-pentamethoxystilbene **5** (Table **1**) on MCF-7 cells depend on G1 phase cell cycle arrest, down-regulation of cyclin D1/D3/E and cyclin-dependent kinase (CDK)2/4/6

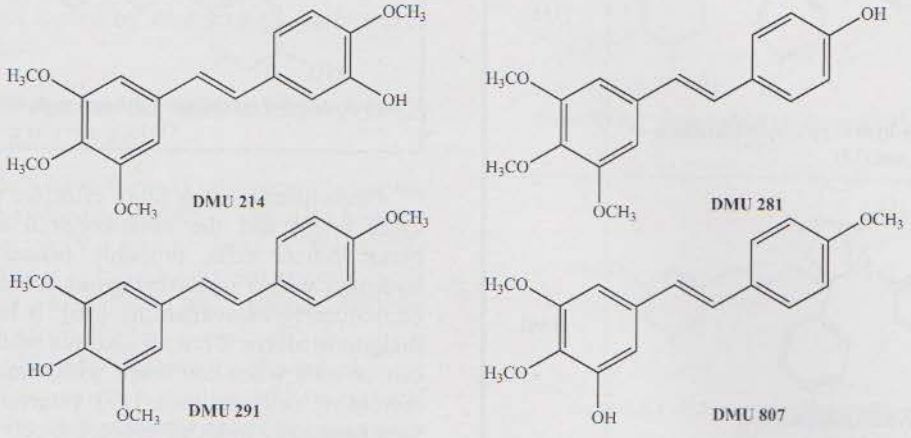


Fig. (3). DMU-212 metabolite structures.

and up-regulation of cyclin-dependent kinase inhibitors (CKIs) including p15 p15INK4B, p16 INK4A, p21Cip1/WAF1, and p27 Kip1 [94].

3.2. Hydroxylated RSV Derivatives

It has also been reported that introduction of additional hydroxyl groups increases RSV biological activity [107]. The 4'-hydroxystyryl moiety is absolutely required for RSV anti-proliferative activity and DNA polymerase inhibition [108] (Table **1**). The introduction of two hydroxyl groups at the 4 and 4' positions, resulting in the RSV analog 4,4'-dihydroxy-*trans*-stilbene **7** (DHS), increases the antioxidant and anti-estrogenic activities [109]. This compound acts as a specific ER ligand [110] and inhibited normal human fibroblasts cell proliferation with higher efficiency and through a mechanism different from RSV [111]. A further study showed that 4,4'-dihydroxy-*trans*-stilbene **7** (DHS) is more effective than RSV in suppressing fibroblasts cell transformation, as well as anchorage-dependent and -independent MCF-7 cell growth through an up-regulation of p53 and E-cadherin expression together with reduction in metalloproteinase-2 and -9 activities [112]. The activity of another RSV derivative, 3,3',4,4',5,5'-hexahydroxystilbene **8**, was investigated in ZR-75-1, MDA-MB-231 and T47D human breast cancer cells; it caused cell growth inhibition through apoptosis, activating caspase-8 only in MDA-MB-231 cells, and caspase-3 and caspase-9 in all three tested cell lines. These latter events were associated with increased p53 levels and mitochondrial superoxide dismutase down regulation [113].

3.3. Other RSV Analogs

3.3.1. 2,3-thiazolidin-4-one RSV Derivatives

It has been reported that *trans*-stilbene RSV derivatives exert chemopreventive properties and display non-specific effects on many biological targets [114]. Mayhoub *et al.* [115] described innovative derivatives characterized by replacement of RSV stilbene ethylenic bridge with a 1,2,4-thiadiazole heterocycle and modification of the substituents on the two aromatic rings, producing RSV derivatives with enhanced potencies and selectivity on aromatase and NF-kB inhibition and quinone reductase-1 induction [115]. Contemplating this approach that keeps the geometry of aromatic rings relatively unchanged and similar to RSV *cis* stilbene template, a library of 2,3-diaryl-4-thiazolidinone derivatives was prepared (Table **1**), with a thiazolidin-4-one nucleus connecting two aromatic rings which increased structural rigidity [116]. Some of these compounds showed stronger inhibitory effects than RSV on ER+ MCF-7 and ER- SKBR3 human breast cancer cell growth. Particularly, 2-(3,4-dimethoxyphenyl)-3-(4-hydroxyphenyl)- thiazolidin-4-one **9**, 2-(4-chlorophenyl)-3-(4-hydroxyphenyl) thiazolidin-4-one **10** compounds and 2,3-diphenylthiazolidin-4-one **11**, 2-(naphthalen-1-yl)-3-*p*-tolylthiazolidin-4-one **12**, 2-(3,4,5-trimethoxyphenyl)-3-*p*-tolylthiazolidin-4-one **13**, displayed potent cytotoxic activity against MCF-7 and SKBR3 cells, suggesting that these molecules could influence the biological action of different estrogen receptors [116]. In particular, in ER+ MCF-7 cells compounds 2-(3,4-dimethoxyphenyl)-3-(4-hydroxyphenyl)- thiazolidin-4-one **9**, 2-(4-chlorophenyl)-3-(4-hydroxyphenyl) thiazolidin-4-one **10** could interfere with ERα-dependent pathway, while in ER- and GPER+ SKBR3 cells compounds 2,3-diphenylthiazolidin-4-one **11**, 2-(naphthalen-1-yl)-3-*p*-tolylthiazolidin-4-one **12** and 2-(3,4,5-trimethoxyphenyl)-3-*p*-tolylthiazolidin-4-one **13** could antagonize GPER-dependent pathways [116] known to be involved in E2-dependent SKBR3 cell growth [117, 118].

3.3.2. 3-Chloro-azetidin-2-one RSV Derivatives

Using the same synthetic strategy described by Mayhoub *et al.* [115] 2,3-thiazolidin-4-one RSV analogs were synthesized by connecting an azetidin-2-one nucleus to two aromatic rings (Table **1**). These derivatives have increased structural rigidity, major bioaccessibility and more potent antitumoral activity than RSV [119]. Among all tested compounds 1,4-bis(4-bromophenyl)-3-chloroazetidin-2-one **14** and 3-chloro-4-(4-chlorophenyl)-1-(4-iodophenyl)azetidin-2-one **15** inhibited proliferation in a dose dependent manner in both estrogen dependent MCF-7 and SKBR3 cell lines suggesting that these RSV derivatives could be potentially active on different breast cancer subtypes [119].

3.3.3. 4-(6-hydroxy-2-naphthyl)-1,3-benzenediol RSV Analog

The anticancer activity of 4-(6-hydroxy-2-naphthyl)-1,3-benzenediol **16** (HS-1793) RSV analog has been evaluated in FM3 murine breast cancer cells. In this cellular model, HS-1793 induced apoptosis or inhibited cell proliferation at a dose (3-25 μM) lower than that required for using RSV (300 μM). Apoptosis was activated through a mitochondrial pathway characterized by cytochrome c, apoptosis inducing factor (AIF) and Endo G release [120]. Recently, anti-proliferative and apoptotic effects of HS-1793 were investigated in MCF-7 (wild-type p53) and MDA-MB-231 (mutant p53) cells. In the study authors emphasized the different apoptotic mechanisms observed in the two cell lines: induction of p53/p21WAF1/CIP1-dependent apoptosis in MCF-7 cells, exhibition of p53-independent apoptosis in MDA-MB-231 cells [121].

CONCLUSION

Despite the clear RSV anticancer effects *in vitro*, its beneficial effects confirmed *in vivo* are limited by its short biological half-life and rapid metabolism and elimination. To improve the pharmacokinetic properties of RSV several synthetic derivatives have been synthesized and tested in *in vitro* breast cancer models. Several reports indicated that introduction of methoxylic groups on the phenylic rings of RSV substantially enables the anti-proliferative and apoptosis-inducing activities of RSV on breast cancer cells. Likewise, additional hydroxylic groups on the aromatic portions of RSV or replacing the alkene linker between the two aromatic rings with a heterocyclic system, have generated libraries of new analogs that displayed higher cytotoxic activity and hence higher ability to inhibit *in vitro* breast cancer cell growth. Then, the ability of some analogs to exhibit greater bioaccessibility *in vitro* than RSV and to exert selective inhibitory effects on breast cancer cell growth

open new perspectives for these derivatives as new therapeutic agents for breast cancer treatment. However, further *in vivo* studies are required in order to evaluate bioavailability and to suggest some derivatives for a possible clinical anticancer application.

LIST OF ABBREVIATIONS

CSCs	=	Cancer stem cells
CYP1A1	=	Cytochrome P450, family 1, subfamily A, polypeptide 1
CYP1B1	=	Cytochrome P450, family 1, subfamily B, polypeptide 1
DMBA	=	7,12- dimethylbenz[a]anthracene
E2	=	17β-estradiol
EMT	=	Epithelial-to-mesenchymal transition
ER-	=	Estrogen receptor negative
ER+	=	Estrogen receptor positive
ERK	=	Extracellular-signal-regulated kinases
ERs	=	Estrogen receptors
ERα	=	Estrogen receptor alfa
ERβ	=	Estrogen receptor beta
GPER	=	G protein-coupled estrogen receptor 1
LDL	=	Low density lipoprotein
MAPK	=	Mitogen-activated protein kinases
MNU	=	N-methyl-N-nitrosourea
mTOR	=	Mammalian target of rapamycin
NOD/SCID	=	Nonobese diabetic/severe combined immunodeficiency
PI3K	=	Phosphoinositide 3-kinase
RNA	=	Ribonucleic acid
RSV	=	Resveratrol
VEGF	=	Vascular endothelial growth factor

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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