Anticancer Activity of Stilbene-Based Derivatives

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\[\text{stilbene-containing anticancer compounds}\]

resveratrol

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Stilbene is an abundant structural scaffold in nature, and stilbene-based compounds have been widely reported for their biological activity. Notably, (E)-resveratrol and its natural stilbene-containing derivatives have been extensively investigated as cardioprotective, potent antioxidant, anti-inflammatory, and anticancer agents. Starting from its potent chemotherapeutic activity against a wide variety of cancers, the stilbene scaffold has been subject to synthetic manipulations with the aim of obtaining new analogues with improved anticancer activity and better bioavailability. Within the last decade, the majority of new synthetic stilbene derivatives have demonstrated significant anticancer activity against a large number of cancer cell lines, depending on the type and position of substituents on the stilbene skeleton. This review focuses on the structure–activity relationship of the key compounds containing a stilbene scaffold and describes how the structural modifications affect their anticancer activity.

Introduction

Cancer prevention is currently one of the most widely researched areas. Because there are many mechanisms involved in the pathophysiology of cancer, different approaches are necessary. Decisive advances in cellular and molecular biology have led to the improvement of chemotherapeutic management of cancer, but further efforts to discover new anticancer agents to overcome resistance and toxicity concerns, are still necessary. Because natural products are one of the major sources for lead compounds, exhibiting various modes of cytostatic action,[1] in the last decades, interest has been focused on the use of natural products or their derivative with the aim of developing more efficacious chemotherapeutic agents.[2]

Recently, natural compounds with a stilbene backbone demonstrated promising activity in cancer prevention, targeting a wide variety of intracellular pathways.[3] Stilbene is a versatile scaffold, characterized by two aromatic rings linked by an ethylene bridge (Figure 1). Stilbenes are defense compounds produced by some plants in response to pathogen attack and other stresses. Stilbenes are abundant in natural products[4] with a variety of important biological activities.[5] Structurally, they are divided into Z-type and E-type based on the configuration of their central double bond; this can undergo Z/E isomerization, changing the overall configuration and decreasing the biological activity. In fact, photoisomerization is a problem that represents a common challenge in optimization work and most quantum chemistry calculation research has focused on its mechanisms[6] but has not been concerned with optimizing these compounds to improve their stability and maintain their biological activity.[7–9]

Stilbene containing compounds have drawn the attention of chemists and pharmacologists over the years mainly because of their important biological properties, such as antioxidant, hypolipidemic, antiviral, anti-inflammatory and anticancer.[10–14] Among these, resveratrol represents one of the most extensively studied and its antiproliferative, antioxidant,[15] anti-inflammatory and anticancer activities[16–18] have been largely reported.

To improve the cancer chemopreventive and/or therapeutic activities, as well as the bioavailability with respect to the parent drug, new stilbene derivatives have been designed, synthesized and tested on multiple cellular targets. These potential, new, chemotherapeutic agents have received much attention from the medicinal chemistry community. Starting from the scaffold of resveratrol, many analogues contain one or both aromatic rings differently substituted. There are clinically used drugs characterized by the presence of a stilbene nucleus and numerous hybrid derivatives have been tested on different biological targets.[14]

This review focuses on the anticancer properties associated with stilbene scaffolds and the structural aspect of the stilbene-containing derivatives based on their chemical structure, covering the most relevant papers from 2010 to the present.

Natural Anticancer Stilbenes

The stilbene moiety is a common structural grouping in nature. Hydroxylated stilbenes, among them (E)-resveratrol 1 (Figure 1), are abundant in nature and play a significant role...
in the prevention of coronary artery disease due to their antioxidan t and anti-inflammatory properties.[16] Resveratrol 1 (3,4',5-trihydroxy-trans-stilbene) is a naturally occurring phytoalexin present in grapes, red wine, peanuts, chocolate, and mulberries.

Resveratrol is only present in small amounts in plant sources and its isolation from plant sources is not suitable. It has been shown to possess antioxidant,[19] anti-inflammatory, antiplatelet, cancer preventative and anticancer properties.[17, 20–23] Although both Z and E isomers of resveratrol are found in nature, it is only the trans isomer that exhibits bioactivity and it has been reported that only the E form exhibits more potent activity than the corresponding Z form across biological screens including anticancer and antioxidant activities.[24]

Resveratrol shows antioxidant activity on molecular targets involved in tumor initiation, promotion and progression, disturbing progression through the block of the S and G2 phases of the cell cycle.[18] Its potential cancer chemoprevention is based on its striking inhibitory effects on cellular events. It also induces apoptosis and decreases angiogenesis through the suppression of fibroblast growth factor 2 (FGF-2) and vascular endothelial growth factor (VEGF),[26] classifying it as anticancer agent.[27]

In the last decade, numerous reviews have summarized the effects of resveratrol treatment on breast, colorectal, liver, pancreatic, and prostate cancers.[17, 18, 23a, 27, 28] Numerous in vitro studies have shown that resveratrol has multiple anticancer effects, protecting against both tumor initiation and cancer pro-
Some compounds structurally related to resveratrol are present in nature and also show a wide spectrum of biological activities. Pterostilbene (Figure 1) is a stilbene natural compound found primarily in blueberries and *Pterocarpus marsupium* heartwood. It is a dimethoxy derivative of resveratrol with similar biological activity, but with more potent antioxidant and anticancer activities. Also, it has a better bioavailability due to the presence of methoxy groups that increase lipophilicity. Trimethoxypterostilbene is a natural product isolated from *Virola elongata*. It is significantly more potent than its parent compound in inhibiting the growth of human cancer cells, antiangiogenic activity and with better oral bioavailability, longer half-life, greater plasma exposure, and lower clearance than resveratrol.

Piceatannol (Figure 1) is another naturally occurring hydroxy-stilbene found in grapes, berries, peanuts and sugar cane. It is synthesized in plants in response to fungal attack, ultraviolet exposure, and microbial infection. Also, piceatannol has been shown to exert various pharmacological effects such as anticancer, anti-inflammatory, antioxidant, antiaging, antiinflammatory and cardioprotective activities. Furthermore, the potency of piceatannol usually is greater than that of resveratrol.

Recently, a series of novel C- and O-prenylated piceatannols has been discovered from propolis in Kangaroo Island of South Australia. An example of synthetic prenylated tetrabenzylostilbenes is reported in Figure 1; they inhibited cell growth in the MTT antiproliferative assay against the leukemia K562 cell line in a very low micromolar range, depending on the position of prenyl group.

Some resveratrol metabolites are found as piceatannol in red wine, grapes, passion fruit, and white tea (Figure 1). For these compounds, similar biological effects are described. A C-geranylated resveratrol, the amorphastilbol (Figure 1), is the most active compound extracted from the *Robinia pseudocacia* var. *umbraclufer* (RPu).

Notably, the natural (Z)-stilbene combretastatin A-4 (CA-4, Figure 1), isolated from the bark of the African willow tree *Combretum caffrum* in 1982, shows biological activities. CA-4 belongs to a complex family of potent Z-stilbenoid depolymerizing agents and is a strong inhibitor of tubulin polymerization and promotes cancer cell death. Cytotoxic properties of its Z derivatives have been reviewed by several authors. In addition, CA-4 leads to rapid and selective vascular dysfunction in solid tumors by attacking endothelial cells within the tumor vasculature. Unfortunately, its Z double bond tends to isomerize to the more thermodynamic stable, but less depolymerizing active E configuration. To overcome this shortcoming, a large number of modifications for CA-4 have been made and some examples are reported below. In a paper published in 2001, Stivala et al. investigated whether antiproliferative properties are dependent on configuration of the molecule; surprisingly, both E or Z configured synthetic methylated stilbenes stop mitosis at the M phase, whereas only (E)-resveratrol blocks cells at the S phase.

Studies on colorectal cancer cell proliferation, conducted with a series of polymethoxystilbenes, led to the unexpected result that the strongest effect depends on Z stereochemistry. Computational docking confirmed that Z isomers, apart from (Z)-resveratrol and (Z)-tetramethoxystilbene of this series, can be incorporated into the colchicine site of tubulin and all Z isomers substantially overlap with the docked structure of CA-4. Even if resveratrol is a small molecule, structure requirements for biological activity have been studied. For instance, the presence of additional hydroxy groups, either at the ortho or para position, further increases the antioxidative activity of the compound, thanks to an intramolecular hydrogen bond that stabilizes the phenoxyl radicals. The hydroxy group at the 4'-position, however, contributes more to cytotoxic activity than the other hydroxy group, together with the E-configured isomer: the 4'-hydroxystyryl moiety is absolutely required for inhibition of cell proliferation. It is clear that the conjugated double bond between the two aromatic rings is an important structural feature in the inhibition of cancer cell proliferation; reduction of this bond dramatically decreases potency (100-fold), and the resonance property is also important for antioxidant effects.

Unfortunately, resveratrol possesses unfavorable pharmacokinetic properties. Various in vivo studies have shown that resveratrol is impaired by a short half-life, rapid clearance, and low bioavailability. Its bioavailability is low because it exhibits a high rate of metabolism by phase II enzymes, specifically sulfotransferases (SULTs) and glucuronyltransferases (GLUTs) and this fact significantly decreases its bioavailability in the circulatory system. Bioavailability of resveratrol could be improved through nanoformulations such as liposomes, polymeric nanoparticles, or cyclodextrins, which has been recently reviewed. However, interesting studies on its metabolites have been performed in vitro and in vivo on different types of tumors. Depending on the tumor type studied, some of these metabolites retain physiological activity even if at considerably high concentrations, they do not induce tumor cell death or prevent their proliferation in vitro. For example, five resveratrol sulfate metabolites were synthesized and assessed as cancer chemopreventive agents showing a relevant degree of activity with respect to the parent molecule.

**Synthetic Anticancer Stilbenes**

Stilbene-based compounds have become of interest to chemists because of their range of different biological activities. New stilbene derivatives have been designed and synthesized in order to find resveratrol analogues with cancer chemopreventive and/or therapeutic activities superior to that of the parent compound.

Resveratrol derivatives, in which all or each individual hydroxy functions were selectively substituted with methyl groups or other substituents, and the double bond reduced or incorporated in more complex structures, were synthesized and the biological activity of these compounds was evaluated in order to prepare a novel compound with greater bioactivity.
and bioavailability than that of resveratrol. The synthesis and bioactivity evaluation of modified stilbene derivatives received much attention and interest.

To enhance resveratrol stability, bioavailability and uptake, different biocompatible drug delivery systems containing resveratrol have been developed, using polymeric nanoparticles, liposomes, cyclodextrins, solid lipid nanoparticles or yeast cell as carriers\(^{[\text{51}]}\) and tested in vitro\(^{[\text{52}]}\) and in vivo.\(^{[\text{53}]}\) Recently, resveratrol has been conjugated through phosphate bridge(s) to different lipophilic groups related to membrane lipids, such as cholesteryl or diacylglycerol moieties. These lipoconjugates were tested against human neuroblastoma SH-SYSY cells and proved to be significantly more active than resveratrol, improving its bioavailability.\(^{[\text{54}]}\)

The configuration of double bonds has been the subject of interest. As previously mentioned, both (Z)- and (E)-stilbene derivatives demonstrated anticancer properties in numerous experimental models in vitro and in vivo, but with different mechanisms. Unlike resveratrol that exhibits higher antiproliferative activity in the E configuration, some Z isomers of methoxylated stilbenes and related compounds have shown higher antiproliferative or anti-metastatic activity than their E isomers, but they isomerize during storage, administration and during metabolism in liver microsomes.\(^{[\text{55–57}]}\)

(Z)- and (E)-stilbene derivatives have been reviewed for their cytotoxic and antitubulin properties.\(^{[\text{40}]}\) SAR analysis and virtual screening conducted on a wide range of stilbene analogues have elucidated the importance of the Z configuration of the olefinic bridge for cytotoxicity and these studies were useful in the design of novel agents with improved antimitotic activity. For instance, tamoxifen (Figure 2) is a stilbene derivative currently used for the treatment of several types of breast cancer in women, and as a hormone treatment for male breast cancer.

![Figure 2. Structure of tamoxifen.](Image)

In recent years, structural modifications of naturally occurring stilbene compounds led to numbers of compounds with significant antitumor potential.\(^{[\text{58}]}\) In the next section, we summarize the most important studies of stilbene variously substituted derivatives, based on the main type of modification made to the stilbene scaffold.

**Polymethoxy stilbenes**

Efforts have been made to obtain synthetic analogues with an increased metabolic stability and potentially enhanced antitumor activity. Methoxylation of hydroxy groups is thought to prevent polyphenol metabolism and enhance stilbene bioactivity.\(^{[\text{59}]}\) Methoxylation has been suggested to significantly improve the antitumor potential of compounds.\(^{[\text{60}]}\) The greater the number of methoxy groups added, the better the antitumor activity of the compound.

Polymethoxy stilbenes and related compounds are a subgroup of resveratrol derivatives frequently more antiproliferative than the parent drug.\(^{[\text{61}]}\) with better antiangiogenic activity, VEGF and nuclear factor (NF)-κB inhibition,\(^{[\text{62}]}\) and inhibition of multidrug resistance (MDR) of tumor cells.\(^{[\text{63}]}\) Polymethoxystilbenes are able to interact with biomembrane models, undergo a different metabolic conversion and have a higher bioavailability than resveratrol.\(^{[\text{64}]}\) 3,5,4′-Trimethoxystilbene presented greater antitumor activity than resveratrol in Colo-205 tumor xenografts.\(^{[\text{62}]}\) To determine the role of the number of methoxy substituents and their position, double bond configuration, or the additional hydroxy groups on anticancer activity and define structure–activity relationships (SARs), many polymethoxylated resveratrol derivatives have been synthesized and tested.\(^{[\text{65}]}\) SAR studies conducted on methoxylated stilbenes revealed that 3,5-dimethoxy and 3,4,5-trimethoxy motifs are important to the pro-apoptotic activity while a 2-methoxy group in the stilbene skeleton confers selectivity against cancer cells by targeting cytochrome P450 (CYP1B1), a tumor-specific enzyme. These studies led to two important compounds depicted in Figure 3: 1 and 2. They are potent apoptosis-inducing agents, which act on microtubule polymerization.\(^{[\text{66}]}\) Compound 1 was found to exhibit superior availability than resveratrol in the colon\(^{[\text{67}]}\) and effectively decreased adrenoma load in Apc(Min -1 ) mice.\(^{[\text{68}]}\) Compound 3, a hybrid molecule of 1 and 2 (Figure 3), is a potent and selective apoptosis-inducing agent; it displayed a potent in vitro antimitogenic effect on colon cancer cells (Caco-2, HT-29, and SW1116). Moreover, compound 3 inhibited tumor growth in vivo in a colon cancer xenograft model.\(^{[\text{66}]}\)

![Figure 3. Polymethoxylated stilbenes 1–4.](Image)

A study carried out on four major metabolites of 1 highlighted the importance of trimethoxy substitutions and the methoxy group at the 4’-position for the antiproliferative activity.\(^{[\text{59}]}\) In fact, only the metabolite that retained this substitution pattern exhibited the ability to inhibit of HepG2 and MCF-7 cell proliferation with a similar mechanism of action as 1. This compound was found to potently inhibit the proliferation of a variety of cancer cells, including HeLa cervical cancer cells,
MCF-7 and MDA-MB-435/LCC6 breast cancer cells, HepG2 hep-atomica cells, LnCaP prostate and HT-29 colon cancer cells. Its analogue (E)-3,5,4’trimethoxystilbene was the most effective anticancer agents among the analogues investigated, and showed improved cytotoxicity compared to resveratrol itself.

Natural resveratrol methyl ethers are described to be more specific and potent inhibitors of cytochromes P450 (CYP450) Family 1 in comparison with the parent compound. Also, stilbene derivatives with methilithy substituent are selective and potent inhibitors of CYP1 enzymes. The isozymes CYP1A1 and CYP1B1, and a very low affinity to CYP1A2. This result was explained by molecular docking studies. The less electronegative sulfur atom with respect to the 4’-oxygen atom decreased toxicity toward HEK293 cells and enhanced the ability of the compound to activate human sirtuin-1.

4’-Methylthiostilbenes have also been reported as efficient inhibitors of NF-κB and AP-1, confirming the crucial role of this transcription factor in tumor pathogenesis in HaCaT keratinocytes. More recently, in a novel series of polymethoxy-trans-stilbenes in which the 3,4-dimethoxy motif was retained, compound 5 (Figure 4) was the most selective inhibitor of both CYP1B1 and CYP1A1, showing very low affinity (nanomolar range) toward CYP1A2. These enzymes are involved in the activation of potential carcinogenesis. Docking studies outlined the importance of the methoxy group in the 2’ position in 5; this compound showed the energetically favorable orientation into CYP1B1 pocket that allowed van der Waals and π–π stacking interactions.

Recently, Madadi and colleagues reported the synthesis of a series of (Z)-diarylacrylonitrile analogues of resveratrol; these compounds were evaluated against a panel of 60 human tumor cell lines. The most potent compound 6 (Figure 4) with GI50 values of <10 nm against almost all the cell lines in the human cancer cell panel, was also screened against the acute myeloid leukemia cell line, MV4-1. Its Z analogue 7 is more potent than 6 at inhibiting tubulin polymerization in MV4-11 cells; this fact was confirmed by molecular docking studies that indicated a common binding site for 6 and 7 on the αβ-tubulin heterodimer, with a slightly more favorable binding for 7 relative to 6, which is consistent with the results from microtubule depolymerization assays.

Compound 8 in Figure 4, was largely studied for its apoptotic activity in HL60 myeloblastic acute leukemia cell line inducing a partial block of cells in S phase. Subsequently, compound 8 was checked in an in vivo study in immunodeficient mice with HT-29 xenografts. In the same study, its 4’-amine analogues (Z)- and (E)-9 (Figure 4) were more potent. Also, the natural (E)-8 was found to be the most growth inhibitory against HT-29 and Caco-2 colon cancer cell lines in vitro, among all the stilbenes tested (IC50 values of 0.04 and 0.08 μM in HT-29 and Caco-2 cells, respectively). Generally, the Z analogues demonstrated greater activity than the E isomers in vitro, but the tumor growth inhibitory effect in vivo was different depending on type of substituent at the 4’ position (amino, ester, methoxy, halogen, hydroxy). The Z -amino analogue of 9, which showed only moderate activity in vitro, had the same effect as its E isomer in vivo due to its isomerization (Z/E).

These compounds decreased the percent of PCNA-labeled cells. As PCNA is an auxiliary protein of DNA polymerase 6 required for DNA synthesis during S phase, it is a useful marker in reporting cell proliferation. They also increased the levels of p27, a cyclin-dependent kinase inhibitor, that regulates the G0/S transition in the cell cycle.

Compounds 9 and 10 (Figure 4) were also tested for their activities on 12 anticancer targets, three cancer cell lines and in preliminary animal studies, together with a large library of 92 different compounds. Compound 9 inhibited ODC induction (IC50 of 1.3 μM), modestly inhibited NF-κB (IC50: 5.8 μM), induced quinone reductase (QR)1 by 1.9-fold, and inhibited aromatase with IC50 value of 0.31 μM. In the same study, 10 showed a better activity on aromatase inhibition with an IC50 of 0.04 μM. Preliminary absorption and metabolism studies were also performed.

Compound 9 and its analogue with an additional methoxy group in 4-position, also displayed potent QR2 inhibitory activity (IC50 values of 1.7 and 0.27 μM, respectively) and their crystal structures in complex with QR2 have been determined. They bind to QR2 in an orientation similar to that of resveratrol: these two compounds interact with a direct hydrogen bond interaction of the amino group and this fact is most likely responsible for enhancing the QR2 inhibitory activity with respect to resveratrol (IC50 values of 5.1 μM).

Figure 4. Polymethoxylated stilbenes 5–10.
The inhibition of QR2 blocks the production of highly reactive species from some quinone substrates; this block is very important for the protection of cells.\(^\text{82}\) The 4'-aminostilbene scaffold was found to exhibit versatile biological activities including nitric oxide synthase inhibition, aromatase inhibition (IC\(_{50}\) 22 \(\mu\)M), and inhibition of tumor necrosis factor (TNF)-\(\alpha\)-induced NF-\(\kappa\)B activity.\(^\text{83}\) Larger studies carried out on (\(\E\))-9 (Figure 4) outlined the importance of the para- amino group on the 4'-stilbene benzene ring for aromatase inhibitory activity (IC\(_{50}\) 0.59 \(\mu\)M), and the introduction of an imidazole moiety on the double bond improved the activity greatly in 10 (IC\(_{50}\) 36 nm, Figure 4).\(^\text{84}\) An analogue of compound 8, compound 11 (Figure 5) with a methoxy group in the 3’-position was effective for inhibition without inducing cytotoxicity (IC\(_{50}\) of 0.16 \(\mu\)M), suggesting that this molecule could be used in combination with other inhibitors to block ABCG2 drug-efflux activity. ABCG2 are resistant cancer cells, which overexpress multidrug ABC (ATP-binding cassette) transporters in the plasma membrane. This alters the efficiency of chemotherapeutics by lowering their intracellular concentration.\(^\text{85}\) Compound 11 chemosensitized the growth of resistant ABCG2-transfected HEK293 cells at sub-micromolar concentrations.\(^\text{86}\) This effect is dependent on the number of methoxy substituents, from 1 to 4, and on their positions and the molecular mechanism of inhibition.

An extraordinarily high and wide spectrum of antiproliferative activities were observed for compounds 12 and 13 shown in Figure 5. This evaluation was carried out on four different human cancer cell lines (Colo-205, MDA-468, HT-29, and MGC80-3). This study indicated that the nature and the positions of substituents greatly affected the activity profile of these compounds, indeed isomers or derivatives had a narrow spectrum of activity.\(^\text{87}\)

Phenols 14 and 15 (Figure 5) are antiangiogenic agents acting on granulosa cell VEGF production.\(^\text{88}\) Compounds 14 and 15 also showed high antiproliferative activity toward Caco-2 and SHSY5Y cancer cells, with a Gl\(_{50}\) value of 3 \(\mu\)M.\(^\text{89}\)

Glucosides and galactosides of the active aglycones 14 and 15 lacked antiproliferative activity even if glucosides display antitumor activity or other promising biological properties\(^\text{90}\) and resveratrol-glucoside showed cytotoxic activity against human tumor cell lines. This fact could be due to poor hydrolysis by intracellular enzymes; they also showed \(\beta\)-glucosidase or \(\beta\)-galactosidase inhibition with IC\(_{50}\) values in the range of 170–350 \(\mu\)M, compared with the antidiabetic drug acarbose (IC\(_{50}\) = 65 \(\mu\)M).\(^\text{91}\)

Polymethoxy stilbenes with \(\alpha\),\(\beta\)-unsaturated amide tail were evaluated for anticancer, anti-inflammatory and COX-2 inhibition.\(^\text{92}\) Because COX-2 is overexpressed in many human cancers,\(^\text{93}\) selective COX-2 inhibition could represent an opportunity for both cancer prevention and therapy.\(^\text{94}\) Compounds 16, 17, and 18 (Figure 6) exhibited the most potent antitumor activity against MCF-7, A549 and B16F10 tumor cell lines and optimal COX-2 inhibition and selectivity compared with celecoxib. These data were confirmed by further crystallization studies and molecular docking that demonstrated a high binding potency of COX-2.\(^\text{95}\)

In recent work, a series of very simple stilbene derivatives showed a measurable inhibitory effect on angiogenesis, some of them to a higher degree than resveratrol. VEGF is an inducer of angiogenesis and an overexpression in the production of VEGF is implicated in various types of tumors.\(^\text{96}\) These compounds characterized by hydroxy groups free or protected by methyl or allyl groups in ortho positions (19–21, Figure 6),\(^\text{97}\) lead to a decrease in the production of VEGF by 50\% in HT-29 cells, which is more than the response observed with resveratrol. They may be considered a potentially valuable anticancer agent with a lower rate of being metabolized due the lack free hydroxy groups, as in resveratrol.

As an extension of this work, Martí-Centelles and colleagues reported a new series of variously substituted stilbene derivatives with hydroxy, methoxy groups and amine and/or amide (Figure 7).\(^\text{98}\) These new synthetic stilbene analogues were evaluated on VEGF production and on the expression of the telomerase reverse transcriptase (hTERT) gene, involved in the process of telomerase activation, an enzyme that has been detected in ~90\% of all malignant tumors. Also, the inhibition of expression of c-Myc, an oncogene involved in the transcription factor for hTERT, was measured. Among 39 stilbene derivatives, compound 22 (Figure 7) showed cytotoxic values in the low nanomolar range for HT-29 and MCF-7 cancer cell lines and it showed a large cytotoxic activity for HEK293 tumor cell line. It inhibited VEGF secretion and the expression of the telomerase related hTERT and c-Myc genes at similarly low concentrations.

![Figure 5. Polymethoxylated stilbenes 11–15.](image-url)
Highly substituted $E$-configured stilbenes were compared with (Z)-CA-4 for their antitumor activity against eight human cancer cell lines and showed cytotoxic activity in the micromolar range. Notably, compound 23 (Figure 7) that is configurationally stable, confirmed that Z configuration seems to be not essential for a high cytotoxicity. In fact, combretastatins with a Z-configured alkene bond, possessed a high tendency to undergo Z/E isomerization in vitro with accompanying decreasing loss of cytotoxicity.\[43\] A very interesting role of stilbene derivatives in chemotherapy was reported by Reddy et al.\[93\] They synthesized and tested a series of stilbene derivatives variously functionalized with different groups such as methyl, methoxy, methylenedioxy, 3,4,5-trimethoxy, chloro, fluoro, and bromo groups at different positions. These compounds contain an important nitrovinyl side chain that has been described to be able to bind and inhibit human telomerase with resulting in pro-apoptotic activity.\[94\] Almost all compounds demonstrated significant antiproliferative activity against the four cancer cell lines tested. In particular, compounds 24 and 25 (Figure 7), with a methyl or methoxy group, respectively, at the 4'-position of the aromatic ring, showed antiproliferative activity in the picomolar range, inhibition of tubulin assembly in high percentage, and induced cell-cycle arrest at the G2/M phase. This fact was explained by docking studies that demonstrated that the position of the trimethoxyphenyl group of compounds 24 and 25 exhibited a binding mode similar to that of the trimethoxy ring of colchicine, a natural compound that inhibits microtubule polymerization.\[93\]

As a further development of studies on nitrovinyl styrene derivatives, compounds 26 and 27 (Figure 7) having 3,4-(1,3-dioxolane) and 3,4-difluoro groups, respectively, exhibited IC50 values below 10 μM against four cancer cell lines, and inhibited tubulin polymerization by 62 and 55%, respectively, at a concentration of 5 μM.\[95\] Also for these compounds, molecular docking analysis demonstrated that they occupied colchicine binding site in tubulin.

Halogenated stilbenes

In 2009, Moran et al. prepared a series of trans-fluorinated analogues of resveratrol, some with hydroxy groups and others with protected hydroxy, amino, ether and/or nitro groups.\[96\] These compounds were assayed in lung cancer and melanoma cell lines. Among them, compound 28 (Figure 8) displayed greater potency than that of the parent compound resveratrol.
This was further evaluated on a panel of 60 cell lines by the National Cancer Institute under the Development Therapeutic Program (DTP), showing broad-spectrum anticancer activity against leukemia, colon, lung, breast, melanoma, prostate, ovarian, central nervous system and renal cancer lines.

A panel of fluorinated \(N,N\)-dialkylaminostilbenes, in particular in ortho position to the stilbene double bond, inhibited the Wnt pathway downstream of \(\beta\)-catenin, and repressed colorectal cancer cell proliferation. Wnt/\(\beta\)-catenin signaling is an important pathway in development and tumorigenesis,\(^{97}\) and blocking Wnt signaling is an attractive approach for colorectal cancer chemoprevention and therapeutics. Compound (E)-29 (Figure 8) inhibited Wnt signaling at nanomolar levels with respect to resveratrol and pterostilbene, which exhibited an inhibition in the micromolar range.\(^{98}\) In this study, the substitution of an aromatic ring with heterocyclic and polyaromatic systems or the substitution of the \(N,N\)-dimethylamino with other alkyl or aryl groups led to a loss of activity.

### Heteroaromatic stilbenes

There are reported studies carried out on derivatives of resveratrol in which one or both of the aromatic rings are replaced with a heteroaromatic one. In a recent study of anticancer stilbenes conducted on a series of derivatives of difluorinated \(N,N\)‘-dialkylaminostilbenes (FIDAS agents),\(^{99}\) \(2',6'\)-dihalostyrylanilines 29, pyridines 30 and pyrimidines 31 (Figure 8) were tested for their in vitro activity against LS174T cells showing cytotoxicity in the low nanomolar range on the proliferation of colon and liver cancer cells. They also inhibited the catalytic subunit of methionine S-adenosyltransferase-2 (MAT2A). This result confirmed the important correlation between the expression of MAT and human colorectal carcinoma.\(^{99}\) This study led to key findings: aniline and 2-aminopyridine derivatives possess lower IC\(_{50}\) values in the inhibition than that of the 5-aminopyridine and the 2-aminopyrimidine compounds. The most active FIDAS agents possessed either 2,6-difluorostyryl or 2-chloro-6-fluorostyryl subunits, and small N-alkyl groups, specifically the \(N\)-methylamino or the \(N,N\)-dimethylamino groups, in a para orientation relative to the 2,6-dihalostyryl subunit (Figure 8).\(^{100}\)

Pyridine and pyrimidine stilbenes were investigated for their cytotoxicity and their ability to inhibit the production of VEGF and the activation of telomerase in two tumor cell lines (HT-29 and MCF-7) and one non-tumor cell line (HEK293). Pyridine stilbenes substituted at C4 and an \(ortho\) or \(meta\) methoxy group in the benzene ring, such as compound 32 (Figure 9), showed an action similar to resveratrol.\(^{101}\)

In a recent study, heteroaromatic analogues of 1 were evaluated for their anticancer activity against a panel of 60 human cancer cell lines (NCI-60 panel) at a concentration of \(10^{-5}\) \(M\).\(^{102}\) In these compounds, the 4-methoxyphenyl moiety in compound 1 was replaced with a heterocyclic ring such as indole, benzo[\(f\)]furan, benzothiazole, and benzothiophene and the methoxy substitution pattern was also varied. The combination of (E)-3,4,5-trimethoxystyryl moiety and benzothiazole or benzothiophene, respectively (compounds 33 and 34, Figure 9), exhibited the most potent growth inhibition against most of the tested cancer cell lines and this strongest binding interaction at the colchicine-binding site on tubulin was studied by molecular modeling studies.

A cyano group was introduced to compound 35 at the olefinic bridge. Both \(E\) and \(Z\) isomers showed nanomolar growth inhibition in a NCI-60-cell line screen.\(^{103}\) As an extension of this study, a series of \(\beta\)-quinolyl cyanostilbene analogues that incorporate 3,4,5-trimethoxyphenyl or 3,5-dimethoxyphenyl, were also synthesized and tested as anticancer agents against an extensive panel of human cancer cell lines (compound 36, Figure 9). These compounds exhibited the most potent growth inhibition in the micromolar range, against most of the human cancer cell lines in the panel.\(^{104}\)

Heteroaromatic stilbene derivatives are also hybrid molecules,\(^{41,105}\) that combine the anticancer properties of the stilbene moiety with that of a counterpart with documented ability to modulate one or several pathways relevant for cancer pathogenesis.\(^{106}\) Polypharmacological compounds represent an innovative strategy to obtain a new chemical entity with multitarget activities.\(^{107}\)

In recent work by Yan and colleagues,\(^{108}\) the antiproliferative activity of a series of hybrid compounds of resveratrol was studied. These compounds combine the pharmacophores of resveratrol and ebselen, a selenium-containing compound that was described for its antiproliferative activity as well as for other biological activities, such as inhibition of cyclooxygenases, lipoxygenases and thioredoxin reductase (TrxR).\(^{109}\)

![Figure 9. Heteroaromatic stilbenes 32–36.](image-url)
TrxR plays an essential functional role in cancer therapy because it is overexpressed in tumor cells. So, a series of benzo-selenazole-stilbene hybrids were synthesized and the antiproliferative activity, TrxR inhibitory effect, and ability to increase the intracellular ROS levels against four human cancer cell lines were evaluated. Compound 37 (Figure 10) induced G2/M cell-cycle arrest and apoptosis of the human liver carcinoma Bel-7402 cell line and exhibited the best TrxR inhibitory activity. This data confirmed the influence of methoxy groups on blocking the cell cycle as previously reported for resveratrol derivatives.[44] This fact could explain how oxidative stress, related to TrxR inhibitory activity, is able to induce apoptosis in cancer cells.

![Figure 10. Hybrid stilbenes 37–39.](Image)

Recently, (E)-stilbene has been conjugated with the 3H-quinazolin-4-one, a natural bioactive scaffold with many documented biological activities, including anticancer activity.[110] The nucleus of quinazolinone was substituted at the N1-position with alkyl, aryl or benzyl groups, while no structural change was made on the stilbene core bound in the 2-position. These compounds were tested for their cytotoxic activity on three human cancer cell lines including MCF-7, MDA-MB-231, and T-47D using the MTT assay. Compounds with N1-alkyl, N1-aryl and N1-benzyl substituents and N1-sec-butyl derivative 38 (Figure 10) showed the best profile of activity with an IC50 value of <5 μM for all tested cell lines. In this case, stilbene has certainly contributed positively to the activity of what was previously reported for structural analogues of 2-aryl quinazoliones.[111]

In a recent study, a series of (E)-styrylbenzimidazoles 39 (Figure 10) showed an interesting inhibitory activity against epoxide hydrolase (sEH) in the range of 1.7–2.6 μM on four cancer cell lines.[112] Epoxide hydrolase hydrolyses the epoxyeicosatrienoic acids which is critical for primary tumor growth and metastasis in mouse models of cancer.[113] Promoting metastasis by triggering secretion of VEGF by the endothelium. A compound with an ortho-trifluoromethyl on the aromatic ring was docked in the binding pocket of the catalytic domain of sEH confirming its potent sEH inhibition and antiproliferative activities.

(Z)-stilbenes

Although Z and E isomers of resveratrol occur in nature, the Z form is not biologically active. However, the introduction of methoxy groups at key positions on the Z form results in more potent anticancer properties, as also described in the introduction.

In a study conducted by Mazué and colleagues, a series of Z and (E)-trimethoxy and tetramethoxystilbenes were synthesized and tested for proliferation inhibition of the SW480 cell line.[46] Most of the synthetic methoxylated derivatives (E or Z) stopped mitosis at the M phase while (E)-resveratrol inhibited cells in the S phase. The number of methyl groups present in these molecules is crucial for determining the inhibitory properties and Z stereoisomers were found to have the strongest effect. Docking studies showed that almost all of the docked structures of (Z)-polymethoxy isomers substantially overlap the docked structure of CA-4, taken as reference compound, apart from (Z)-resveratrol and (Z)-tetramethoxystilbene, but not most of the E isomers. The most powerful molecule is compound (Z)-40 (Figure 11), which showed a very interesting decrease in metastatic mouse melanoma B16F10 cells, non-metastatic B16F1 cells, and immortalized melanocytes all of which were isolated from C57BL/6 mice.[61]

New 2-benzyl α,β-unsaturated indanones were studied as tubulin assembly inhibitors.[9] Compound 41 (Figure 11) displayed good antiproliferative activities with GI50 values in the micromole to sub-micromole range on five human cancer cell lines, using an MTT assay. This compound demonstrated that the three-methoxy groups on the 4,5,6-position of the indane moiety are very important for the inhibition of tubulin polymerization in the HeLa cell line, arresting cell-cycle progression at the G2/M phase in a concentration-dependent manner; this is in agreement with most of the antimitotic agents.

Vinyl hydroxamic acids linked to (Z)-stilbene were described as orally available histone deacetylases (HDAC) inhibitors.[114] Most of the 42 tested compounds showed a good HDAC inhibition (IC50 < 100 nM) and good antiproliferative activity in three different cancer cell lines (HCT-116, NCI-H-460, and U251) with GI50 ranging from 0.01 μM to 3 μM. In Figure 11, two of the best vinyl hydroxamic acids (42 and 43) are depicted as examples.[106]

The most active compound 44 (Figure 11) of a series of 28 (Z)- and (E)-styrylbenzoxazolones, containing 3,4,5-trimethoxy-tyril fragment at position 6 of benzoxazolone, showed the highest antiproliferative effect against HepG2, EA.hy926, and K562 cancer cell lines and on additional cell lines, with IC50 values similar to or better than that of CA-4. It induced mitotic arrest in the G2/M phase like CA-4, as confirmed by X-ray studies.[24]

The combretastatin-based hybrid 45 (Figure 11) and its analogues were tested in vitro for potential antitumor and cytotoxic activities on HeLa tumor cell line and non-tumor Vero cell line. This derivative showed a high potential antitumor activity with an IC50 value in the low micromolar range.[115]
Z-constrained stilbenes, analogues of CA-4, were synthesized and tested for their ability to inhibit tumor cell growth on the HT-29 and SKOV3 cancer cell lines. In vitro and in vivo, the best activity was detected for compound 46, Figure 11. Studies of Z/E isomerization with UV/Vis spectrophotometric analysis have also been performed for some of these compounds.[97]

Conclusions

Stilbene is an extensively explored aromatic scaffold of novel agents implicated in a wide variety of pathophysiological conditions, for its antioxidant, antiaging, anti-inflammatory, anti-diabetic, cardioprotective, and anticancer properties. Natural stilbenes such as resveratrol and combretastatin, as well as their synthetic analogues, are of great interest to medicinal chemists. In the last decades, different studies have been devoted to the development of stilbene-based compounds with potential clinical utility in the treatment of a variety of different tumors.

This review summarizes the main natural and synthetic compounds containing stilbene scaffolds and describes how the structural modifications affect their anticancer activity. This collection aims to provide an important insight into stilbene-containing compounds providing a useful tool to design new anticancer agents with potential activity on the different pathways involved in cancer therapy.

Abbreviations

RAF: rapidly accelerated fibrosarcoma; MAPK: mitogen-activated protein kinase; SAR: structure–activity relationship; MAT2A, methionine S-adenosyl transferase-2; VEGF: vascular endothelial growth factor; hTERT: telomerase reverse transcriptase; QR2: quinone reductase 1; FGF-2: fibroblast growth factor 2; SULTs: sulfotransferases; GLUTs: glucuronyltransferases; sEH: epoxide hydrolase; NF-κB: nuclear factor-κB.

Acknowledgements

This study was supported by local grants from the University “G. d’Annunzio” of Chieti, Italy.

Conflict of interest

The authors declare no conflict of interest.

Keywords: anticancer agents · antiproliferation · resveratrol · stilbene analogues
