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Novel Aza-resveratrol analogs: Synthesis, characterization and anticancer activity against breast cancer cell lines

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ABSTRACT

Novel Aza-resveratrol analogs were synthesized, structurally characterized and evaluated for cytotoxic activity against MDA-MB-231 and T47D breast cancer cell lines, which exhibited superior inhibitory activity than parent resveratrol compound. The binding mechanism of these compounds with estrogen receptor-α was rationalized by molecular docking studies which indicated additional hydrogen binding interactions and tight binding in the protein cavity. Induction of Beclin-1 protein expression in breast cancer cell lines after treatment with newly synthesized resveratrol analogs indicated inhibition of growth of these cell lines through autophagy. The study highlighted the advantage of introducing the imino-linkage in resveratrol motif in enhancing the anticancer potential of resveratrol suggesting that these analogs can serve as better therapeutic agents against breast cancer and can provide starting point for building more potent analogs in future.

In spite of extensive research on cancer and its cellular pathways, target identification and drug development, the disease still remains the major cause of death in economically developed countries as well as in developing countries.[1] Amongst all cancers, breast cancer is the most common type of cancer affecting more than one million women and accounting for the highest mortality worldwide.[2,3] It is estimated that there have been 229,060 new cases diagnosed of breast cancer in USA in 2012 and 39,920 deaths resulting due to the disease.[4] Even though large number of breast cancers express estrogen receptor (ER) and progesterone receptor (PR)[5] and respond to therapy with hormones or aromatase inhibitors, there is a group of patients (12–17%) that do not respond to such treatment due to lack of ER, PR as well as the Human Epithelial Receptor (Her-2/neu) (ErbB2). These are known as triple-negative breast cancers (TNBC) which represent a highly aggressive sub-type of breast cancer that is difficult to treat.[6,7]

Among the phytochemicals that have been investigated to treat breast cancer resveratrol stands out as unique because its chemical space resembles mammalian estrogen 17β-estradiol as shown in Figure 1 and hence is anticipated to interfere with the functions of 17β-estradiol based on space considerations.[8] It is, therefore, not surprising to have been implicated in reducing incidence of hormone-dependant cancers including breast and prostate.[9] These compounds possess ability to modulate the biological responses of estrogens generally by binding to ER.[10] Their structural similarities with the estrogens include phenolic rings which are crucial for binding to ER-α and ER-β receptors, and the hydroxyl groups at specific positions.[11] We have recently summarized the possible mechanism of actions of these phytoestrogens in prevention of breast cancer.[12]

Resveratrol is a naturally occurring stilbene phytoestrogen that exists in various food and beverages and is known to possess wide variety of biological properties including antioxidant, anti-inflammatory and anticancer activities. The compound is known to modulate several signaling kinases (Raf, Src, MAPK, PKD, PKCd), transcription factors (p53/p21, IkB kinase/NF-kB), and other targets (TRAIL/DR4 + 5, Fas/CD95, PI3K/Akt) leading to cancer cell death.[13] Being a phytoestrogen, the anticancer activities of resveratrol against breast cancer have attracted interest of many researchers including our group. The compound has been shown to inhibit EGF-mediated migration and expression of MED28, which is a subunit of the mammalian mediator complex for transcription and matrix metalloproteinase MMP-9 in MDA-MB-231 cells.[14] Recently

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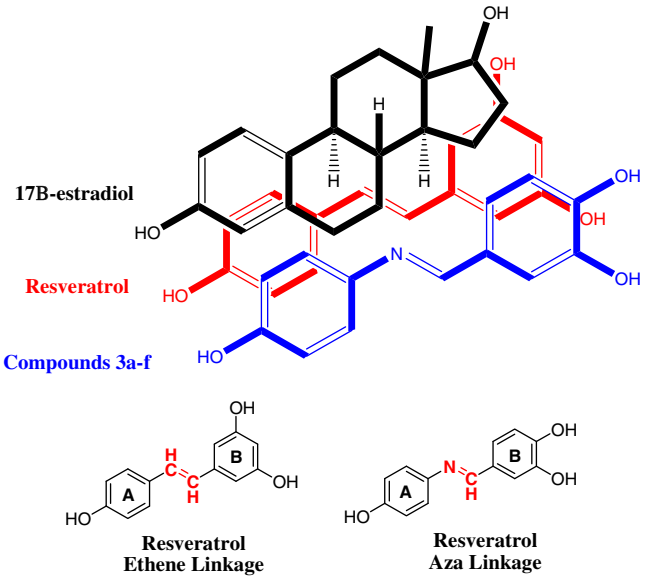

Figure 1. Structural similarities between 17β-estradiol, Aza-resveratrol and compounds **3a–f**.

it was reported to inhibit EGF-induced epithelial to mesenchymal cell transformation in MCF-7 cells.[15] The compound has been found to induce apoptosis mediated by activation of MAP kinase pathways,[16] modulation of phosphorylated Akt and caspase-9 in MCF-7 cells,[17] upregulation of ERK1/2 and suppression of Bcl-2 expression in MDA-MB-231 cells[18], and activation of the p53 in T47D breast cancer cell lines.[19] Further, it has been suggested that resveratrol-induced caspase-3 activation is required for PARP degradation and induction of apoptosis in MDA-MB-231 cells.[20] It has been shown that resveratrol can induce accumulation of COX-2 which facilitates apoptosis in MCF-7 and MDA-MB-231 breast cancer cells.[21] The compound is found to exert its anticancer effects in animal models of breast cancer-induced by 7,12-dimethylbenz-(a)-anthracene,[22] as well as *N*-methyl-*N*-nitrosourea.[23] It has also been demonstrated that resveratrol-induced activation of Sirt1 and inhibition of survivin expression elicits a more profound inhibitory effect on BRCA-1 (breast cancer type-1 susceptibility protein) mutant cells than on Brca-1-wild-type cancer cells both in vitro and in vivo.[24] The combination of resveratrol with quercetin, and catechin has been found to reduce primary tumor growth of breast cancer xenografts in a nude mouse model,[25] while significant lowering of tumor growth, decreased angiogenesis, and increased apoptotic index in MDA-MB-231 (ERα−, ERβ+) tumors has been found in resveratrol-treated nude mice.[26]

Resveratrol has been very effective as an antiproliferative agent in vitro but its poor bioavailability and rapid metabolism limits its use in vivo studies as the preferred anticancer agent. In humans, it is readily metabolized in the liver by phase II drug metabolizing enzymes to water soluble glucuronide and sulfate metabolites, accounting for its urine excretion.[27] Resveratrol has a plasma half-life of 8–14 min in humans.[27] In rodents, only 1.5% of resveratrol reaches to the plasma compartment.[28] Resveratrol can also bind to DNA and in presence of Cu^{2+} ions and can induce DNA strand breaks. In complex with Cu^{2+}, resveratrol reduces Cu^{2+} to Cu^{+} while the emerging oxidized resveratrol products further enhance the genotoxicity.[29] Gao et al. have observed strong antiproliferative effect of resveratrol in vitro on 32Dp210 leukemia cells but when they inoculated these leukemia cells into mice and treated with 8 mg/kg body weight of resveratrol, no anti-leukemic effect was detected. Even higher doses of resveratrol were able to protect only a negligible number of mice from leukemia.[30] This week in vivo response is thought to be due to poor bioavailability and rapid metabolism of the compound.

These problems can be addressed adequately by synthesis of resveratrol analogs. and few analogs of resveratrol have been synthesized by substitutions of hydroxy-,[31] methoxy-,[32] fluoro-,[33] acetate-,[34] methyl ether-[35] groups either on 'A' or 'B' ring. Some of these have been found to exert potent anticancer activity than resveratrol. Recently, Roberti and group have reported a potent and apoptosis inducing analog of resveratrol bearing 3,4-dihydroxy substitution against HL-60 leukemia cell line,[31] while Murias et al. have shown that resveratrol bearing trihydroxyl pharmacophores exhibited COX-2 inhibition at nanomolar range.[36] Cai et al. have shown that the 3,4-hydroxy substitution in 'A' ring of resveratrol significantly enhance the apoptosis inducing ability of resveratrol[36a] These studies seem to indicate that incorporation of the polyhydroxyl substitution on resveratrol may help in enhancing its cytotoxic and apoptosis inducing ability. Hence, in the present work we have synthesized some novel analogs of resveratrol (Fig. 1) by maintaining 3,4-dihydroxy substitution on the A ring while varying the substituents at C-4 position on the B ring along with inclusion of Aza functionality in the conjugated system.

The compounds **3a–f** were synthesized following the scheme shown in Figure 2 by condensing equimolar amount of 3,4-dihydroxy benzaldehyde (**1**) with respective 4-substituted anilines (**2a–f**) in absolute ethanol in presence of catalytic amount of concd. HCl at 60 °C. The resulting reaction product was washed with cold ethanol and purified by Silica gel column chromatography. All synthesized compounds are shades of pale yellow to reddish brown.

The newly synthesized analogs (**3a–f**) of resveratrol were characterized by UV, IR, NMR and Mass Spectroscopy (Table 1). The infra-red spectra of compounds **3a–f** exhibited a characteristic broad band in the range of 3400–3462 cm^{-1} due to hydroxyl

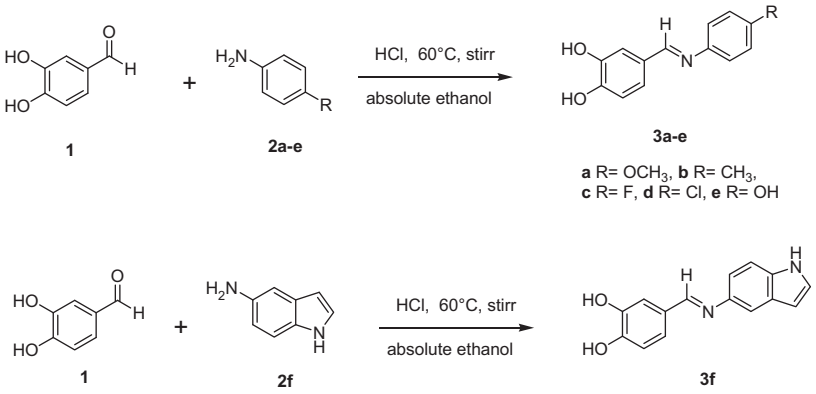

Figure 2. Synthetic scheme for Aza-resveratrol analogs.