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Synthesis and anticancer activity of a hydroxytolan series

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ABSTRACT

This paper describes the development of novel anticancer poly-hydroxylated tolans. Based on structural similarity to resveratrol, a series of hydroxytolans were synthesized and evaluated for their antitumor capability against three tumor cell lines and one fibroblast cell line for selectivity comparisons. The 4,4′-dihydroxytolan (KST-201) exhibited the most significant anticancer activity with increased selectivity when compared to resveratrol and other hydroxytolans. Unlike resveratrol, KST-201 can boost hydrogen peroxide in tumor cells, which are often at high basal level of reactive oxygen species, to cause cell death by overwhelming the cellular tolerance of oxidative stress.

Resveratrol (RES), a phenolic phytoestrogen found concentrated in red wine, has drawn much attention for its protective effect against cardiovascular diseases.[1–3] This stilbene compound consists of two aromatic rings, with three hydroxyl groups at the 3, 5 and 4′ positions, linked by an ethylenic bridge (Fig. 1a). More bioactivities potentially beneficial to human health were discovered afterward such as anti-obesity, anti-aging, anti-inflammation and anticancer.[4–8] Inhibition of tumorigenesis of RES was first reported in a mouse skin cancer model.[6] Reactive oxygen species (ROS) activate survival-promoting signaling pathways that favor cancer progression.[9] RES can serve as an antioxidant to counteract ROS to shift the oxidative state to a reduced state in the cellular microenvironment.[10,11] However, this compound's relatively short half-life in vivo[12] and its tendency to assume the *cis*-isomer conformation[13] has necessitated the search to discover and synthesize more potent analogues or derivatives. Hydroxytolans that are structurally similar to resveratrol were designed and synthesized for biological activity studies. By changing the linker from a double bond to a triple bond, generating hydroxylated tolans, the *cis*- versus *trans*-conformation was eliminated. Despite the change in the linker, the compounds with the corresponding hydroxylation patterns in the stilbenes and tolan series have the same number of non-hydrogen atoms and bonds (NAB), a topological molecular descriptor used to quantify the chemical structure (Fig. 1b). It is an integer value that denotes the total number of non-hydrogen atoms and bonds in a molecule, which allows the molecules to be classified into topological isomer groups.[14]

Synthesis of the poly-hydroxylated tolan is outlined in Scheme 1. Briefly, the methoxy substituted arylethylyl trimethylsilane was synthesized from the reaction mixture of aryl methoxy substituted aryl iodide, $Pd(PPh_3)_2Cl_2$, cuprous iodide and trimethylsilylacetylene in diisopropylamine and purified by column chromatography.[15–17] The methoxy substituted arylacetylene was synthesized from the reaction mixture of the methoxy substituted arylethynyltrimethylsilane and potassium fluoride in methanol.[15–17] The purified product was then added to a solution of methoxy substituted aryl iodide, $Pd(PPh_3)_2Cl_2$, and cuprous iodide in diisopropylamine followed by column chromatography to elute out the methoxytolan.[15–17] The reaction mixture containing methoxytolan and boron tribromide in anhydrous methylene chloride was prepared to generate the hydroxytolan[18,19] (4,4′-dihydroxytolan: ^{1}H NMR (CDCl3, 300 Mz): δ ppm 9.82(s, 2H, 2-OH), 7.31(d, 4H, J = 8.7, Ar–H), 6.77(d, 4H, J = 8.7, Ar–H); 3,4′,5-trihydroxytolan: ^{1}H NMR (CDCl3, 300 Mz): δ ppm: 9.89(s, 1H, OH), 9.45(s, 2H, 2-OH), 7.33(d, 2H, J = 8.65, Ar–H), 6.78(d, 2H, J = 8.63, Ar–H), 6.31(d, 2H, J = 2.2, Ar–H), 6.23(d, 2H, J = 2.2, Ar–H; 3,3′,5,5′-tetrahydroxytolan: ^{1}H NMR (CDCl3, 300 Mz): δ ppm: 9.49(s, 4H, 4-OH), 6.33(d, 4H, J = 2.2, Ar–H), 6.25(t, 2H, J = 2.2,

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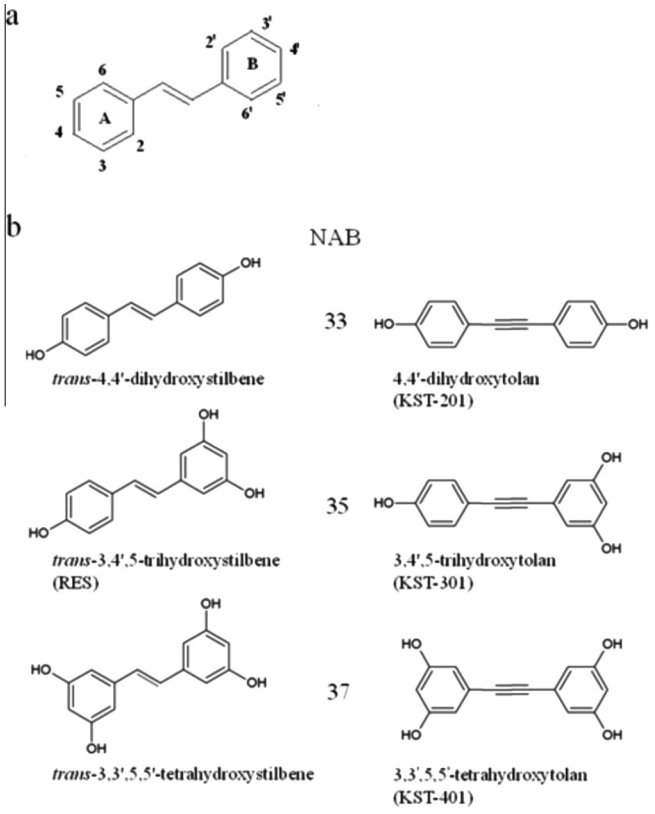
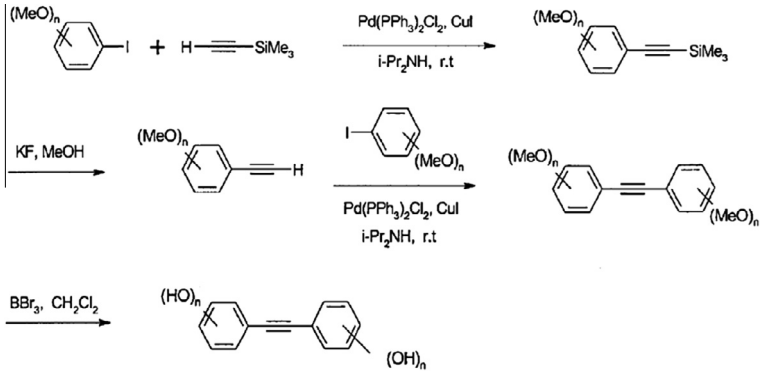


Figure 1. (a) This RES inspired stilbene platform consists of two aromatic rings connected by a double bond linker. Hydroxyl groups may be attached on both aromatics rings (ring A at positions 2–6 and ring B at positions 2′–6′, respectively). (b) The chemical structures of stilbenes and tolan series with matching NAB (number of non-hydrogen atoms and bonds).

Ar–H). All hydroxytolan derivatives were fully characterized by H NMR and column chromatography. The purity of all derivatives was >96% as determined by established HPLC methods. Resveratrol was purchased from Sigma Aldrich with >95% pure without further purification. Compound solubility was found to be ~3 mg/100 ml in water and the log*P* and pK_a values were previously determined and were found to be KST-201 (log*P* = 1.85) and KST-301 (log*P* = 2.30) compared to RES (log*P* = 1.85).[20] The log*P* and pK_a data were used to determine the logD values (data not shown) indicating that cellular uptake must be mediated by a carrier or transporter to undergo active transport or facilitated diffusion or be taken up by an endocytic mechanism. Studies are ongoing to determine a more exact route of cellular uptake.[20]

Cell lines used in this study were cultured in McCoy's 5a medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum and 50 μg/ml gentamycin sulfate at 37 °C with 5% CO_2, and all the reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA) if not specifically mentioned. The cytotoxicity of RES and three hydroxytolans 4,4′-dihydroxytolan (KST-201), 3,4′,5-trihydroxytolan (KST-301) and 3,3′,5,5′-tetrahydroxytolan (KST-401) were assessed against a battery of human tumor cell lines including androgen independent prostate cancer (DU-145), bladder cancer (T-24), ovarian cancer (MDAH-2774), and human foreskin fibroblasts (MHRF) which served as the normal cell control. KST series were prepared by dissolving in ethanol then diluting to desired concentrations in McCoy's 5a media supplemented with 10% fetal bovine serum (FBS, Gibco) and 50 μg/ml gentamycin sulfate (Sigma, St. Louis, MO). Final ethanol concentrations are ≪0.1%. RES or each hydroxytolan was added to 96-well tissue culture plates in serial two-fold dilutions. After incubation for 24, 48 or 72 h, an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-diphenyltetrazolium bromide assay was performed to assess cell viability, dose–response curves were generated and 50% cytotoxic doses (CD_{50}) were calculated to evaluate the compound's anticancer activity. The results are summarized in Table 1.[21]

To first compare the anticancer activity of RES with each KST compound, the ratio of RES CD_{50} to the KST CD_{50} (RES CD_{50}/KST CD_{50} = Δ) was calculated at each time point against each cell line. KST-201 appeared much more active than RES on both prostate cancer DU-145 and bladder cancer T-24 at all tested time points (Δ > 2 at 24, 48 and 72 h). On ovarian cancer MDAH-2774, the RES/KST-201 CD_{50} ratio was slightly lower than 2 (Δ = 1.7) at 24 h but increased after a 48 or 72 h treatment (Δ > 2). These data confirmed that KST-201 was a more effective anticancer agent than RES. In contrast, both KST-301 and KST-401 did not consistently exhibit higher degrees of anticancer activity in comparison to RES. On DU-145 cells, the RES/KST-301 CD_{50} ratios were lower than 2 throughout all the time points, and KST-401 showed a greater ratio (Δ > 2) only after the cells were treated for 72 h. Both KST-301 and KST-401 appeared more potent on inhibiting T-24 cell viability during the first 24 h, but this activity became inappreciable after 48 or 72 h. The anti-MDAH-2774 effect of both compounds was also not significant compared to RES (Δ < 2) at all tested time points. However, when the susceptibility of normal fibroblast MHRF was assessed, all KST compounds were less harmful than RES (Δ < 1).



Scheme 1. Synthesis of poly-hydroxylated tolan.