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Synthesis of 3′-methoxy-*E*-diethylstilbestrol and its analogs as tumor angiogenesis inhibitors

Qian Liu [a], Weiwei Jin [a], Yan Zhu [b], Jieyun Zhou [b], Min Lu [a], Qian Zhang [a,*]

[a] Department of Medicinal Chemistry, School of Pharmacy, Fudan University, Shanghai 201203, China
[b] Department of Reproductive Pharmacology, NPFPC Key Laboratory of Contraceptives and Devices, Shanghai Institute of Planned Parenthood Research, Shanghai 200032, China

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ABSTRACT

3′-Methoxy-*E*-diethylstilbestrol (**2**), with the structural and original similarities to 2-methoxyestradiol (2-ME2, **1**), was synthesized and screened against HUVEC and a series of human cancer cell lines including RL95-2, SKOV-3, MCF-7 and T-47D *in vitro*. The configuration of the title compound was determined *via* the single crystal X-ray diffraction of its benzoyl-ester derivative (**10**). The fact that 3′-methoxy-*E*-diethylstilbestrol and its analogues (**8** and **11**) showed potential antiangiogenesis and anti-tumor activities at a close level, whereas its ester derivative (**10**) did not display any cytotoxic activities on all the screening cell lines indicated that the core scaffold of 3′-methoxy-3,4-diphenylhexane and the exposed hydroxyl-groups in the structures are essential pharmacophores for their anti-tumor activities.

1. Introduction

The therapeutic use of 2-methoxyestradiol (2-ME2, **1**, Fig. 1), an endogenous metabolite of 17β-estradiol, against solid tumors has progressed into Phase II/III clinical trials. This compound has attracted considerable interest owing to its anti-proliferative [1] and anti-angiogenic activities [2,3] by a mechanism independent of the estrogen receptor. The transformation of 2-ME2 in humans involves the 2-hydroxylation of 17β-estradiol by hepatic cytochrome P450 and sequential 2-*O*-methylation by catechol *O*-methyltransferase [4–6]. Research on its mechanism of action has revealed that 2-ME2, as a multitargeted anti-tumor agent, can disrupt tubulin polymerization [7], downregulate hypoxia inducible factor-1α (HIF-1α) [8] and inhibit superoxide dismutases (SOD) [9], among other functions [10].

Interestingly, 3′-methoxy-*E*-diethylstilbestrol (Fig. 1), with a similar structure to 2-ME2 [11], was also reported to be an oxidative metabolite of diethylstilbestrol by Gottschlich Regina and coworkers in 1980. The compound was isolated from the fetus and placenta of 15-day pregnant Syrian golden hamsters after administration of diethylstilbestrol at a dose of 20 mg/kg and was identified only by mass fragmentography [12,13]. Diethylstilbestrol, a synthetic non-steroidal estrogen, was used clinically as a replacement for estradiol beginning in the 1940s. It was not until the 1970s that diethylstilbestrol was shown to cause fetal abnormalities during pregnancy; its metabolites, including 3′-methoxy-*E*-diethylstilbestrol, were also presumed to possess fetotoxicity. However, analysis of the preparation, chemical behavior and bioactivity of 3′-methoxy-*E*-diethylstilbestrol remains limited.

Here, we report the synthesis of 3′-methoxy-*E*-diethylstilbestrol and its anti-angiogenic and anti-tumor activities, along with its two analogs 3-(4-hydroxy-3-methoxyphenyl)-4-(4-hydroxyphenyl) hex-2-ene (**8**) and 3-(4-hydroxy-3-methoxyphenyl)-4-(4-hydroxyphenyl)hexane (3′-methoxy-*E*-diethyldihydrostilbestrol, **11**). Compared to that of diethylstilbestrol [14], the synthesis of 3′-methoxy-*E*-diethylstilbestrol was substantially more difficult due to the elimination of hydroxyl with β-H in **6**, the orientation of the double bond and the separation of these isomers.

2. Experimental

2.1. Chemistry

Melting points were measured on a SGW X-4 melting point apparatus and were uncorrected. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker-DPX (400 MHz) spectrometer, and values are expressed in ppm. ESI-MS spectra were recorded on an Agilent G1946D mass spectrometer, and EI-MS data were obtained using an HP5989A mass spectrometer. High-resolution mass spectra were determined using a Finnigan LTQ-ORBITRAP mass spectrometer with ESI as the ionization source. All chemicals and solvents were purchased from commercial sources

* Corresponding author. Tel.: +86 21 51980119.
E-mail address: zhangqian511@shmu.edu.cn (Q. Zhang).

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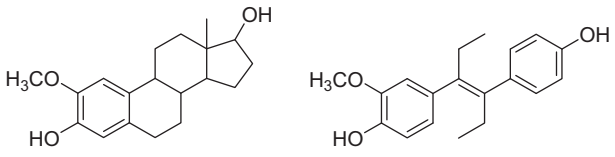


2-Methoxyestradiol, 1 **2**

Fig. 1. Structures of 2-ME2 and the title compound.

and were used without further purification unless otherwise noted. Column chromatography (CC) was carried out on silica gel (300–400 mesh, Qingdao Ocean Chemical Company, China), and thin-layer chromatography (TLC) analysis was carried out on silica gel GF254 glass plates (2.5 cm × 10 cm with a 250 μm silica layer, Qingdao Ocean Chemical Company, China).

*2.1.1. 1-(4-Hydroxy-3-methoxyphenyl)-2-(4-hydroxyphenyl)ethanone (**3**)*

A mixture of 2-methoxyphenol (8.1 g, 65 mmol) and 2-(4-hydroxyphenyl)acetic acid (9.93 g, 65 mmol) was dissolved in 30 mL of BF_3–Et_2O and was stirred continuously at 65 °C for 19 h. After cooling, the reaction was poured into 300 mL of ice water and was extracted with ethyl acetate (3 × 50 mL). The combined organic layer was washed with saturated brine (50 mL) and dried with anhydrous Na_2SO_4. Following removal of the solvent under vacuum, the crude solid was recrystallized from ethyl acetate, yielding a white powder as **3** (4.7 g, 27.8%). Melting point [m.p.]: 187–188 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.61 (1H, dd, J_1 = 1.96 Hz, J_2 = 8.22 Hz, 6′-H), 7.55 (1H, d, J = 1.96 Hz, 2′-H), 7.13 (2H, d, J = 8.60 Hz, 2″, 6″-H), 6.93 (1H, d, J = 8.22 Hz, 5′-H), 6.78 (2H, d, J = 8.60 Hz, 3″, 5″-H), 6.04 (1H, s, –OH), 4.52 (1H, s, –OH), 4.17 (2H, s, 2-H), 3.92 (3H, s, –OC**H**$_3$); EI-MS *m/z* (%): 258 (M^+, 4.61), 123(19.76), 151(100).

*2.1.2. 1-(4-Benzyloxy-3-methoxyphenyl)-2-(4-benzyloxyphenyl)ethanone (**4**)*

Benzyl bromide (1.5 g, 8.7 mmol) was added to a mixture of **3** (0.9 g, 3.5 mmol) and K_2CO_3 (1.0 g, 7.0 mmol) in acetone (20 mL). Following a 10 h reflux, the reaction was cooled and filtered to eliminate K_2CO_3, and the filtrate was concentrated under vacuum to yield a crude product of 1.53 g. A white solid was obtained *via* recrystallization from acetone as **4** (1.4 g, 88.2%). m.p.: 121–123 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.59–7.56 (1H, dd, J_1 = 1.96 Hz, J_2 = 7.43 Hz, 6′-H), 7.56 (1H, d, J = 1.96 Hz, 2′-H), 7.44–7.30 (10H, m, benzyl-H), 7.17 (2H, d, J = 8.61 Hz, 2″, 6″-H), 6.93 (2H, d, J = 8.61 Hz, 3″, 5″-H), 6.88 (1H, dd, J = 7.43 Hz, 5′-H), 5.21 (2H, s, –OC**H**$_2$–), 5.02 (2H, s, –OC**H**$_2$–), 4.15 (2H, s, 2-H), 3.92 (3H, s, –OC**H**$_3$); EI-MS *m/z* (%): 438 (M^+, 2.58), 241(57.58), 91(100).

*2.1.3. 1-(4-Benzyloxy-3-methoxyphenyl)-2-(4-benzyloxyphenyl)-butan-1-one (**5**)*

A solution of NaH (0.15 g, 6.2 mmol) and **4** (1.4 g, 3.1 mmol) in toluene (40 mL) was refluxed for 30 min and was cooled to 25 °C, at which time bromoethane (1.3 g, 12.3 mmol) was added dropwise. This mixture was refluxed for 16 h, quenched with ice water, and extracted with ethyl acetate (3 × 20 mL). The combined organic fractions were washed with saturated brine (50 mL), dried with anhydrous Na_2SO_4, and filtered and concentrated under vacuum to yield a crude product (1.3 g) that was further purified by column chromatography (petroleum ether/ethyl acetate, 10:1) and crystallized from ethyl acetate to yield **5** (1.0 g, 71.0%). m.p.: 106 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.57–7.52 (2H, m, 2′, 6′-H), 7.42–7.28 (10H, m, benzyl-H), 7.23–7.18 (2H, d, J = 8.61 Hz, 2″, 6″-H), 6.98–6.88 (2H, d, J = 8.61 Hz, 3″, 5″-H), 6.82 (1H, d, J = 8.21 Hz, 5′-H), 5.19 (2H, s, –OC**H**$_2$–), 5.01 (2H, s, –OC**H**$_2$–), 4.42 (1H, t, J = 7.43 Hz, 2-H), 3.89 (3H, s, –OC**H**$_3$), 2.19–1.75 (2H, m, -C**H**$_2$CH$_3$), 0.88 (3H, t, J = 7.44 Hz, -C**H**$_3$); EI-MS *m/z* (%): 466 (M^+, 1.73), 241(59.46), 225(9.83), 91(100).

*2.1.4. 3-(4-Benzyloxy-3-methoxyphenyl)-4-(4-benzyloxyphenyl) hexan-3-ol (**6**)*

To a solution of **5** (560 mg, 1.20 mmol) in dried THF (40 mL) was added ethylmagnesium bromide (0.6 M in THF, 20 mL) at room temperature. Following stirring under a nitrogen atmosphere for 3 h at 35 °C, the mixture was poured into 50 mL of ice water. The aqueous layer was neutralized using 5 N HCl and was extracted with ethyl acetate (3 × 30 mL). The combined layer was washed with saturated brine (50 mL), dried with anhydrous Na_2SO_4, and the solvent was removed under vacuum to yield a crude product (650 mg) that was further purified by column chromatography (petroleum ether/ethyl acetate, 8:1) and crystallized from ethyl acetate to yield **6** (600 mg, 82.9%). m.p.: 90–91 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.46–7.28 (10H, m, benzyl-H), 6.85 (2H, d, J = 8.22 Hz, 2″, 6″-H), 6.79 (2H, d, J = 8.22 Hz, 3″, 5″-H), 6.80–6.76 (1H, d, J = 8.22 Hz, 5′-H), 6.71 (1H, d, J = 1.96 Hz, 2′-H), 6.67–6.64 (1H, dd, J_1 = 1.96 Hz, J_2 = 8.22 Hz, 6′-H), 5.12 (2H, s, –OC**H**$_2$–), 5.01 (2H, s, –OC**H**$_2$–), 3.78 (3H, s, –OC**H**$_3$), 2.75–2.69 (1H, m, 4-H), 2.18 (1H, s, 3-OH), 1.96–1.77 (2H, m, 5-H), 0.83–0.86 (2H, m, 2-H), 0.74 (3H, t, J = 7.44 Hz, 1-C**H**$_3$), 0.64 (3H, t, J = 7.44 Hz, 6-C**H**$_3$); ESI-MS *m/z* (%): 519 (M+23).

*2.1.5. 3-(4-Benzyloxy-3-methoxyphenyl)-4-(4-benzyloxyphenyl)hex-2-ene (**7**)*

H_2SO_4 (0.67 mL) was added dropwise to a solution of **6** (600 mg, 1.21 mmol) in 15 mL of acetone at room temperature and was stirred for 30 min. Following the addition of 20 mL of water, the aqueous layer was extracted with ethyl acetate (3 × 20 mL) and the combined organic fractions were washed with brine (50 mL) and dried with anhydrous Na_2SO_4. A crude product (500 mg) was obtained after removing the solvent under vacuum and was further purified using column chromatography (petroleum ether/ethyl acetate, 50:1) and crystallization from ethyl acetate to yield **7** (440 mg, 76%). m.p.: 87–89 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.45–7.25 (10H, m, benzyl-H), 7.10–6.99 (2H, d, J = 8.61 Hz, 2″, 6″-H), 6.87–6.85 (2H, d, J = 8.61 Hz, 3″, 5″-H), 6.78 (1H, d, J = 8.22 Hz, 5′-H), 6.35 (1H, dd, J_1 = 1.96 Hz, J_2 = 8.22 Hz, 6′-H), 6.19 (1H, d, J = 1.96 Hz, 2′-H), 5.60 (1H, q, J = 6.26 Hz, 2-H), 5.11 (2H, s, –OC**H**$_2$–), 5.03 (2H, s, –OC**H**$_2$–), 3.63 (3H, s, –OC**H**$_3$), 3.29 (1H, t, J = 7.43 Hz, 4-H), 2.18–1.74 (2H, m × 2, 5-H), 1.51 (3H, d, J = 6.26 Hz, 1-C**H**$_3$), 0.88 (3H, t, J = 7.43 Hz, 6-C**H**$_3$); ESI-MS *m/z* (%): 501 (M+23).

*2.1.6. 3-(4-Hydroxy-3-methoxyphenyl)-4-(4-hydroxyphenyl)hex-2-ene (**8**) and 3-(4-hydroxy-3-methoxyphenyl)-4-(4-hydroxyphenyl) hexane (3′-methoxy diethyldihydrostilbestrol, **11**)*

To a solution of **7** (440 mg, 0.92 mmol) in CH_2Cl_2 (5 mL) and MeOH (12 mL), Pd/C (5%, 0.3 g) and ammonium formate (464 mg, 7.38 mmol) were added in turn. The reaction was stirred at room temperature and was monitored by TLC until the substrate was no longer visible. The mixture was filtered to recover Pd/C, and the filtrate was poured into water (50 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic fractions were washed with saturated brine (50 mL), and dried with anhydrous Na_2SO_4. Following removal of the solvent by concentration under vacuum, a mixture of **8** and **11** (253.2 mg) was obtained that was separated *via* column chromatography (petroleum ether/ethyl acetate, 50:1) and was purified by crystallization from ethyl acetate to yield **8** and **11**, respectively. For **8** (111.1 mg, 40.5%), m.p.: 102–104 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.97–6.95 (2H, d, J = 8.60 Hz, 2″, 6″-H), 6.79–6.77 (1H, d, J = 7.83 Hz, 5′-H), 6.72–6.70 (2H, d, J = 8.60 Hz,

3″, 5″-H), 6.38–6.36 (1H, dd, J_1 = 1.56 Hz, J_2 = 7.83 Hz, 6′-H), 6.13 (1H, d, J = 1.56 Hz, 2′-H), 5.60 (1H, q, J = 6.66 Hz, 2-H), 5.46 (1H, s, –OH); 4.63 (1H, s, –OH), 3.69 (3H, s, –OC**H**$_3$), 3.30–3.26 (1H, t, J = 7.44 Hz, 4-H), 1.78–1.64 (2H, m, 5-H), 1.50 (3H, d, J = 6.66 Hz, 1-CH3), 0.89–0.85 (3H, t, J = 7.04 Hz, 6-CH3); ESI-MS *m/z* (%): 297 (M-1); HR-MS (ESI+), calculated for $C_{19}H_{23}O_3$ $[M+H]^+$: 299.16472, was found to be 299.16439.

For **11** (130.0 mg, 47.1%), m.p.: 148–150 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.03–7.01 (2H, d, J = 8.56 Hz, 2″, 6″-H), 6.87–6.85 (1H, d, J = 7.95 Hz, 5′-H), 6.80–6.77 (2H, d, J = 8.56 Hz, 3″, 5″-H), 6.68–6.65 (1H, dd, J_1 = 1.84 Hz, J_2 = 7.95 Hz, 6′-H), 6.60 (1H, d, J = 1.84 Hz, 2′-H), 5.48 (1H, s, –OH), 4.65 (1H, s, –OH), 3.88 (3H, s, –OC**H**$_3$), 2.46–2.43 (2H, m, 3, 4-H), 1.43–1.23 (4H, m, 2, 5-H), 0.57–0.52 (6H, t, J = 7.33 Hz, 1, 6-C**H**$_3$); EI-MS *m/z* (%): 301 (M^+, 1.29), 165(100); HR FTMS (ESI+), calculated for $C_{19}H_{25}O_3$ $[M+H]^+$: 301.18037, was found to be 301.17996.

2.1.7. 3-(4-Hydroxy-3-methoxyphenyl)-4-(4-hydroxyphenyl)hex-3-ene (**9**)

To a solution of **8** (240 mg, 0.80 mmol) in 6 mL of acetic acid was added 48% HBr (13.2 mL). The reaction was stirred at room temperature for 48 h to reach equilibrium and was poured into 100 mL water and extracted with ethyl acetate (3 × 20 mL). The combined organic layer was washed with brine (50 mL) and dried with anhydrous Na_2SO_4. The solvent was removed under vacuum to yield a crude product (235 mg), which was purified *via* column chromatography (petroleum ether/ethyl acetate, 7:1) and crystallization from ethyl acetate to yield **9** (120 mg, 50.0%, **8** was recovered at 120 mg). m.p.: 87–89 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.07 (2H, d, J = 8.22 Hz, 2″, 6″-H), 6.91 (1H, d, J = 8.59 Hz, 5′-H), 6.83 (2H, d, J = 8.22 Hz, 3″, 5″-H), 6.69 (1H, s, 2′-H), 6.56 (1H, d, J = 8.59 Hz, 6′-H), 5.55 (1H, s, –OH), 4.76 (1H, s, –OH), 3.91 (3H, s, –OC**H**$_3$), 2.13 (4H, q, J = 6.65 Hz, 2, 5-H), 0.78 (6H, t, J = 6.65 Hz, 1, 6-C**H**$_3$); ESI-MS *m/z* (%): 297 (M−1).

2.1.8. 3′-Methoxy-E-diethylstilbestrol dibenzoate (**10**)

Benzoyl chloride (103.6 mg, 0.74 mmol) was added to a mixture of **9** (100 mg, 0.335 mmol) and K_2CO_3 (74 mg, 0.536 mmol) in CH_2Cl_2 (10 mL) at room temperature. The reaction mixture was refluxed for 3 h, filtered, and concentrated under vacuum. The *E*-configuration isomer was separated by column chromatography (petroleum ether/ethyl acetate, 40:1) and recrystallized from ethyl acetate (114 mg, 67.0%). m.p.: 164–165 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.22–8.25 and 7.51–7.55 (4H × 2, m × 2, benzoyl-2, 3, 5, 6-H), 7.65 (2H, dd, J_1 = 8.4 Hz, J_2 = 12.0 Hz, benzoyl-4-H), 7.25–7.29 (2H, d, J = 8.4 Hz, 2″, 6″-H), 7.23–7.25 (2H, d, J = 8.4 Hz, 3″, 5″-H), 7.16 (1H, dd, J = 7.6 Hz, 5′-H), 6.84 (2H, dd, J_1 = 1.6 Hz, J_2 = 8.4 Hz, 2, 6-H, 3-ArH), 3.85 (3H, s, –OC**H**$_3$), 2.20 (4H, m, 2, 5-C**H**$_2$), 0.83 (6H, m, 1, 6-C**H**$_3$); ESI-MS *m/z* (%): 507 (M + 1); HR-MS (ESI+), calculated for $C_{33}H_{31}O_5$ $[M+H]^+$: 507.21715, was found to be 507.21642.

2.1.9. 3′-Methoxy-E-diethylstilbestrol (**2**)

To a solution of **10** (114 mg, 0.225 mmol) in 8 mL of CH_2Cl_2/EtOH (1/1) was added 30% NaOH (8 mL). The reaction was refluxed for 12 h, neutralized with 5 N HCl and extracted with ethyl acetate (3 × 30 mL). The combined organic layer was washed with brine (50 mL) and dried with anhydrous Na_2SO_4, and the solvent was removed under vacuum, yielding a crude product (650 mg) that was purified *via* column chromatography (pure CH_2Cl_2) and crystallized from ethyl acetate to yield **2** (42 mg, 62.7%). m.p.: 102–106 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.07 (2H, d, J = 8.61 Hz, 2″, 6″-H), 6.91 (1H, d, J = 7.83 Hz, 5′-H), 6.83 (2H, d, J = 8.61 Hz, 3″, 5″-H), 6.69 (1H, s, 2′-H), 6.56 (1H, d, J = 7.83 Hz, 6′-H), 5.53 (1H, s, –OH), 4.76 (1H, s, –OH), 3.94 (3H, s, –OC**H**$_3$), 2.13 (4H, q, J = 6.65 Hz, 2, 5-C**H**$_2$), 0.78 (6H, t, J = 6.65 Hz, 1, 6-C**H**$_3$); ESI-MS *m/z* (%): 297 (M-1); HR-MS (ESI+), calculated for $C_{19}H_{23}O_3$ $[M+H]^+$: 299.16472, was found to be 299.16440.

2.2. X-ray crystal structural determination of **10**

Single crystals of **10** were cultured in ethyl acetate, and a colorless crystal with dimensions of 0.17 mm × 0.15 mm × 0.10 mm was selected and sealed in a capillary. The capillary was mounted on a Bruker SMART APEX CCD area detector diffractometer equipped with graphite-monochromatic MoKα radiation (λ = 0.71073 Å) for the preliminary examination and data collection at 293(2) K. Out of a total of 14376 reflections collected in the range of $1.73° \leqslant \theta \leqslant 26.01°$, 5406 were independent, with R_{int} = 0.0570. The structure was solved directly using the SHE-LXL-97 program and was refined by a full-matrix least-squares procedure with anisotropic thermal parameters for all non-hydrogen atoms, and the hydrogen atoms were located geometrically. Structural analysis demonstrated that **10**, $C_{33}H_{30}O_5$ (Mr = 506.57), is of the monoclinic space group P2(1)/n, with a = 9.722(4) Å, b = 14.962(5) Å, c = 19.088(7) Å, α = 90°, β = 96.195(5)°, γ = 90°, V = 2760.3(17) Å^3, Z = 4, D_c = 1.219 mg/m^3, μ = 0.081 mm^{-1}, F(000) = 1072, the final R = 0.0470 and R_w = 0.1091 ($w = 1/[\sigma^2(Fo^2) + (0.0614P)^2 + 0.0000P]$ where $P = (Fo^2 + 2Fc^2)/3$), $(\Delta/\rho)_{max}$ = 0.196, $(\Delta/\rho)_{min}$ = −0.172 e/Å^3, $(\Delta/\sigma)_{max}$ = 0.000 and S = 0.899.

2.3. Biological assays

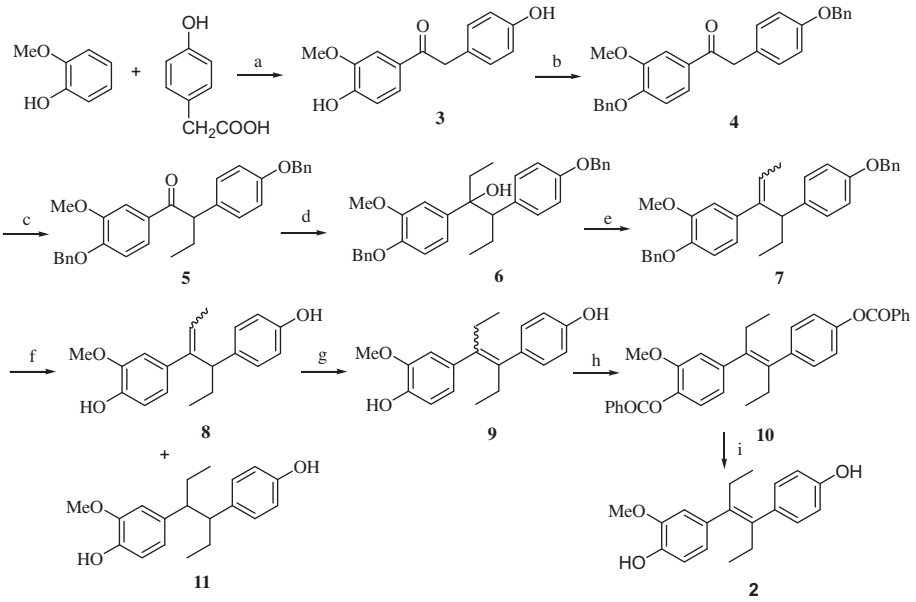
HUVEC (human umbilical vein endothelial cells), RL95-2 (human uterine epithelial carcinoma cells), SKOV-3 (human ovary carcinoma cells), MCF-7 (human breast adenocarcinoma cells) and T-47D (human breast adenocarcinoma cells) cell lines were cultured in media appropriate for each cell type, supplemented with 10% fetal bovine serum, in a humidified atmosphere of 5% CO_2 at 37 °C. Cell suspensions were plated in 96-well plates at a density of 8 × 10^4 cells mL^{-1}. One microliter of each test compound solution at various concentrations in DMSO was added to each well, and the culture was incubated for 48 h at 37 °C in a 5% CO_2 incubator. The incubation medium was maintained at a near-physiological pH of 7.3–7.4. Plates were incubated 1–4 h at 37 °C in the presence of 10 μL of cell counting kit-8 (cck-8) solution per well. Absorbance was measured at 450 nm. Experiments were performed in triplicate, and the results are presented as the half-maximal inhibitory concentration (IC_{50}, shown in Table 2).

3. Results and discussion

3.1. Chemistry

As described in Scheme 1, 2-methoxyphenol was condensed with 2-(4-hydroxyphenyl)acetic acid in the presence of BF_3–Et_2O to provide a skeleton of 1,2-diphenylethanone (**3**). The phenyl-hydroxyls of **3** were protected with benzyl groups, and two ethyl chains were introduced sequentially *via* substitution of the carbonyl α-H with bromoethane and, by the Grignard reaction, of ethylmagnesium bromide with the carbonyl group, with yields of 71.0% and 82.9%, respectively, to produce **6**.

Unexpectedly, the dehydration of **6** with H_2SO_4 in acetone yielded only the olefinic product with the double bond located at the 2-position instead of yielding the predicted product following Sayzteff's rule, that is 3-(4-benzyloxy-3-methoxyphenyl)-4-(4-benzyloxyphenyl)hex-3-ene. Despite altering the dehydration reagents, solvents, and the reaction temperature, the non-Sayzteff product remained the predominant product of this elimination [15]. We attribute this result to steric effects and to the charged



Scheme 1. Synthetic route to 3′-methoxy-*E*-diethylstilbestrol. Reagents and conditions: (a) BF_3–Et_2O, 65 °C, 19 h; (b) BnBr, K_2CO_3, acetone, reflux, 10 h; (c) EtBr, NaH, toluene, reflux, 16 h; (d) EtMgBr, THF, 35 °C, 3 h; (e) H_2SO_4, acetone, room temperature, 30 min; (f) HCO_2NH_4, 5% Pd/C, CH_2Cl_2/MeOH, room temperature, 1 h; (g) 48% HBr, acetic acid, room temperature, 48 h; (h) benzoyl chloride, K_2CO_3, CH_2Cl_2, reflux, 3 h; (i) 30% NaOH, CH_2Cl_2, EtOH, reflux, 12 h.

OH_2^+ leaving group that forms under acidic reaction conditions, which would prefer to follow Hofmann's rule.

The shift of the olefinic bond from the 2-position to the 3-position was achieved following the de-protection of **7**. The hydrogenation of the benzyl-ether, utilizing ammonium formate as the hydrogen source and being catalyzed by Pd/C in dichloromethane and methanol, yielded two products: 3-(4-hydroxy-3-methoxyphenyl)-4-(4-hydroxyphenyl)hexane (**11**), as a thoroughly reduced product at 47.1%, and 3-(4-hydroxy-3-methoxyphenyl)-4-(4-hydroxyphenyl)hex-2-ene (**8**). The latter compound (**8**) reacted with HBr (48%) in acetic acid to reach an equilibrium of 2-ene (**8**) and 3-ene (**9**), which could subsequently be separated by column chromatography to obtain equal amounts of each compound. This reaction is proposed to occur in a two-step manner: first, the electrophilic addition of HBr to the double bond and, second, the β elimination of bromide. However, separating *Z*- and *E*-isomers (approximately 1:3 by ^{1}H NMR) from the mixture of **9** was problematic. To overcome this difficulty, esterization of **9** was performed by benzoyl chloride in the presence of K_2CO_3 to obtain the derivative of the *E*-isomer (**10**), of which the configuration was identified by X-ray diffraction. The *E*-isomer was hydrolyzed further to produce 3′-methoxy-*E*-diethylstilbestrol (**2**) with a yield of 62.7%.

However, **2** is likewise unstable in solution and can partially tautomerize to the 2-ene form. One milligram of **2** was dissolved in 1 mL of methanol at room temperature for 24 h, and the relative amount of each isomer was detected by HPLC, with a ratio of approximately 79:21 (**2**:**8**).

The X-ray structure and selected bond lengths and angles for compound **10** are given in Fig. 2 and Table 1, respectively. It can

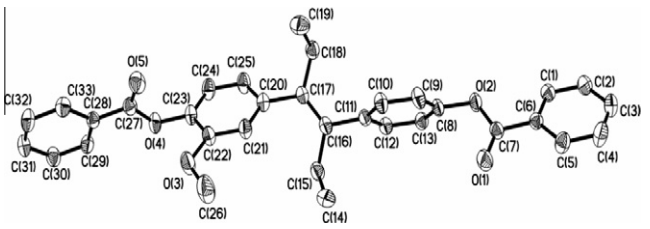


Fig. 2. X-ray crystal structure of **10**.

Table 1
Selected bond lengths and angles for compound **10**.

Bond	Dist. (Å)	Bond	Dist. (Å)
C(10)–C(11)	1.384(2)	C(16)–C(17)	1.337(2)
C(11)–C(12)	1.386(2)	C(17)–C(18)	1.508(3)
C(11)–C(16)	1.510(2)	C(17)–C(20)	1.508(2)
C(14)–C(15)	1.525(3)	C(18)–C(19)	1.523(3)
C(15)–C(16)	1.522(3)		
Angle	*(°)*	*Angle*	*(°)*
C(11)–C(10)–C(9)	121.45(17)	C(17)–C(16)–C(11)	121.81(15)
C(11)–C(10)–H(10A)	119.3	C(17)–C(16)–C(15)	125.97(15)
C(10)–C(11)–C(12)	118.09(15)	C(11)–C(16)–C(15)	112.22(15)
C(10)–C(11)–C(16)	121.22(15)	C(16)–C(17)–C(20)	122.85(16)
C(12)–C(11)–C(16)	120.62(15)	C(16)–C(17)–C(18)	123.10(15)
C(16)–C(15)–C(14)	113.22(15)	C(20)–C(17)–C(18)	114.04(16)
C(16)–C(15)–H(15A)	108.9	C(17)–C(18)–C(19)	113.70(15)
C(16)–C(15)–H(15B)	108.9	C(17)–C(18)–H(18A)	108.8
C(14)–C(15)–H(15A)	108.9	C(17)–C(18)–H(18B)	108.8
C(14)–C(15)–H(15B)	108.9	C(19)–C(18)–H(18A)	108.8

be concluded that the double bond is oriented between atom C16 and C17, based on the bond distances of C15–C16, C16–C17 and C17–C18, and on the bond angles of C16 and C17. As shown in Fig. 2, the C16–C17 double bond adopts a *trans*-configuration, and neither the phenyl plane of C8–C13 nor that of C20–C25 is co-planar with the olefinic bond, indicating that it is not conjugated with either of the two attached benzene rings of **10**.

3.2. Biological evaluation

Compounds **8**, **11**, **2** and **10** were evaluated *in vitro* against HUVEC, RL95-2, SKOV-3, MCF-7 and T-47D cell lines using 2-ME2 as a reference. The 50% inhibition concentration data (IC_{50}, listed in Table 2) showed that all of the compounds tested except for **10** possess potential anti-proliferative activity at a concentration approximately 5- to 10-fold lower than that of 2-ME2. Compound **10** was not cytotoxic to any of the cell lines tested (IC_{50} > 100 μM), likely due to the esterization of the two OH-groups by benzoyls, which is consistent with the SAR findings for 2-ME2 that, in addition to the 2-methoxy group, modifications at both the 3- and 17-positions

Table 2
Anti-proliferation activities *in vitro* of **8**, **11**, **2** and **10**.

Compounds	IC_{50} (μM)				
	HUVEC	RL95-2	SKOV-3	MCF-7	T-47D
8	38.8	25.82	22.98	40.31	45.37
11	38.2	40.75	41.76	52.24	47.48
2	58.3	55.02	73.93	66.44	46.57
10	>100	>100	>100	>100	>100
2-ME2	8.59	4.11	2.48	>100	4.73

are important determinants of drug activity [16]. Additionally, clinical results have shown that 2-ME2 has low bioavailability due to its rapid metabolism by the conjugation of both 3- and 17-hydroxyl moieties to form glucuronides, and by the oxidation of the 17-hydroxyl group to estrone [17]. Consequently, we considered that the mechanism of action of the synthesized compounds **8**, **11** and **2**, which all possess similar scaffolds to 2-ME2 and bear the same hydroxyl- and methoxy-groups at corresponding positions, could be similar to that of 2-ME2.

However, the bioactivity data of **2** cannot be supposed to wholly reflect the natural activity of this compound due to the tautomerization of **2**–**8** (at approximately 25%) in methanol as detected by HPLC. Our data showing that compounds **8** and **11** are slightly more potent than **2** indicates that the natural anti-proliferative activity of **2** may be lower than the data suggest. Compared to **2**, the structures of **8** and **11** are more flexible owing to the single bond between C3 and C4 that enables a conformational adjustment to better adapt to the target molecule. It can therefore be presumed that the double bond of *E*-3′-methoxystilbestrol (**2**) is not essential for its anti-tumor activity.

4. Conclusion

3′-Methoxy-*E*-diethylstilbestrol (**2**), a non-steroidal analog of 2-ME2, was synthesized along with 3-(4-hydroxy-3-methoxyphenyl)-4-(4-hydroxyphenyl)hex-2-ene (**8**) and 3-(4-hydroxy-3-methoxyphenyl)-4-(4-hydroxyphenyl)hexane (3′-methoxydihydrodiethylstilbestrol, **11**). The geometric configuration of **2** was determined by single-crystal X-ray diffraction of its benzoyl-ester derivative (**10**). We conclude from the preliminary bioactivity data that the core scaffold of 3′-methoxy-3,4-diphenylhexane and the exposed hydroxy groups in this type of structure are essential pharmacophores for their anti-angiogenic and anti-tumor activities.

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