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Synthesis and antimitotic activity of novel 2-methoxyestradiol analogs☆

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Abstract

The syntheses and antimitotic activity of several novel 2-methoxyestradiol analogs are described. Structural modifications investigated include introduction of additional unsaturation in rings B and D; inversion at C-13; and substitution at the C-2, C-15, C-16, and C-7α positions. Of 15 analogs synthesized, 2 have demonstrated superior biological activities compared to 2-methoxyestradiol.

Keywords: Steroids; Estrogens; 2-Methoxyestradiol; Antiangiogenesis; Antimitotic activity

1. Introduction

The sequential biochemical hydroxylation and methylation of the natural hormone estradiol (E2, **1**, Fig. 1) gives rise to the endogenous mammalian metabolite 2-methoxyestradiol (2-ME2, **2**, Fig. 1) [1]. 2-Methoxyestradiol is a natural metabolite of estrogen devoid of uterotropic or estrogenic activity in vivo. Recent studies [2] have shown that 2-ME2 inhibits the cellular machinery involved in replicating cancer cells, specifically microtubules. In addition, 2-ME2 has been demonstrated to act as an antiangiogenic agent that prevents the growth of new blood vessels required to nourish tumors [3]. Initiation of either of these events will cause tumors to shrink but the combination of effects may provide significant advantages over current anticancer therapies. The mechanism of action of 2-ME2 involves disruption of cellular microtubules leading to mitotic arrest and initiation of apoptosis. Preclinical studies with 2-ME2 reveal that it is orally active, inexpensive to produce and, in contrast to most antitumor agents, exhibits no overall toxicity at therapeutically effective doses. Both NCI and private sector sponsored Phase I clinical trials have been initiated to determine if 2-ME2 is safe for use in humans. Preliminary results indicate no occurrence of life-threatening toxicities and no maximal tolerated dose level was achieved. Subsequently, Phase II clinical trials have been initiated to determine if 2-ME2 is effective against multiple myeloma and prostate cancer.

The biological activities of 2-ME2 are generating considerable excitement because of its efficacy without toxicity. Because 2-ME2 is a promising drug for cancer therapy, work has begun on second-generation derivatives with superior properties, including better oral availability and different chemosensitivity profiles.

Recently [4–8], several synthetic analogs of 2-ME2 have been developed, some of which exhibit increased antitumor activity compared to the parent 2-methoxyestradiol. All of the new compounds synthesized involve structural modifications in ring A or ring B of 2-ME2. Our continuing interest in the field of estrogen metabolites [9–15] prompted us to devise a new efficient approach to synthesize 2-ME2 based upon the regioselective zirconium tetrachloride-mediated Fries rearrangement carried out on estradiol diacetate [16]. Subsequently, we have conceived and carried out the synthesis and biological testing of a series of novel 2-ME2 analogs. Structural modifications investigated include introduction of additional unsaturation in rings B and D; inversion at C-13; and substitution at the C-2, C-15, C-16, and C-7α positions. Of 15 analogs synthesized and tested, 2 have demonstrated potentially superior biological effects compared to 2-ME2.

2. Experimental

2.1. Chemistry

Melting points were determined on a Thomas–Hoover apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a General Electric GE-300

☆ The compounds described in this communication are the subject of a pending U.S. patent.

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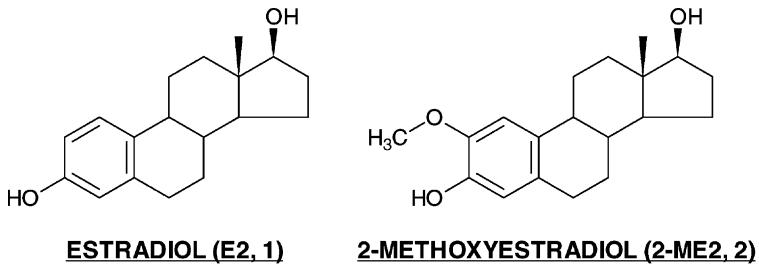

Fig. 1. Estradiol and 2-methoxyestradiol.

(300 MHz) spectrometer as deuterochloroform ($CDCl_3$) solutions using tetramethylsilane (TMS) as an internal standard ($\delta = 0$) unless noted otherwise. Infrared spectra were recorded on a Perkin-Elmer model 1600 FT-IR instrument equipped with a diffuse reflectance accessory using a KBr matrix. Combustion analyses were performed by Midwest Microlabs Ltd. (Indianapolis, IN). 'Flash column' chromatography was performed on 32–64 μM silica gel obtained from EM Science, Gibbstown, New Jersey. 'Dry column' chromatography was performed on 70–230 mesh silica gel, also obtained from EM Science. Thin-layer chromatography (TLC) analyses were carried out on silica gel GF (Analtech) glass plates (2.5 cm × 10 cm with 250 μM layer and prescored). Most chemicals and solvents were analytical grade and used without further purification. Commercial reagents were purchased from Aldrich Chemical Company (Milwaukee, WI). 2-Methoxyestrone was purchased some time ago from Organon Inc., W Orange, New Jersey.

*2.2. 3-(Benzyloxy)-2-methoxyestra-1,3,5(10)-trien-17-one (**4**)*

A solution of 3-(benzyloxy)-2-methoxyestra-1,3,5(10)-trien-17β-ol [16] (**3**, 8.1 g, 20.64 mmol) in acetone (300 ml) was cooled to 0 °C in an ice bath and treated dropwise with Jones reagent with stirring until a yellow color persisted. At that point, the reaction mixture was stirred at 0 °C for another 5 min and then was treated dropwise with isopropanol until a green color persisted. The resulting green suspension was diluted with water (500 ml) and extracted with methylene chloride (3×). The organic fractions were washed with water (1×), saturated sodium bicarbonate solution (1×), and brine (1×). The organic fractions were filtered through anhydrous sodium sulfate, combined and concentrated in vacuo. The residue was crystallized from methanol to give the pure product (**4**, 4.9 g, 60.8%): mp = 154–156 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 2929, 1732, and 1605 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.92 (s, 18-CH_3), 3.86 (s, 2-OCH_3), 5.11 (s, benzyl CH_2), 6.638 (s, 4-H), 6.84 (s, 1-H), 7.29–7.46 (m, benzyl aromatic). Analysis calculated for $C_{26}H_{30}O_3 \cdot 1/5H_2O$: C, 79.24; H, 7.77. Found: C, 79.13; H, 7.76.

*2.3. 17,17-Ethylenedioxy-2-methoxyestra-1,3,5(10)-trien-3-ol 3-benzyl ether (**5**)*

Under nitrogen, triethylorthoformate (3.8 ml, 22.8 mmol), ethylene glycol (2.5 ml, 44.8 mmol) and toluenesulfonic acid monohydrate (0.1 g, 0.53 mmol) were added to a solution of the 17-keto steroid (**3**, 3.5 g, 6.96 mmol) in methylene chloride (35 ml). The reaction mixture was stirred at room temperature for 16 h. After that time, analysis by TLC (CH_2Cl_2) indicated a complete reaction. The reaction mixture was diluted with methylene chloride (100 ml) and washed with saturated sodium bicarbonate solution (1×), water (1×), and brine (1×). The organic fractions were filtered through anhydrous sodium sulfate, combined and concentrated in vacuo to give 3.8 g of a clear oil. This material was purified by flash chromatography (CH_2Cl_2) followed by crystallization from methanol to give the pure 17-ketal (**5**, 3.28 g, 84.2%): mp = 87–88 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 2940 and 1606 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.89 (s, 18-CH_3), 3.86 (s, 2-OCH_3), 3.89–3.98 (m, ketal CH_2's), 5.10 (s, benzyl CH_2), 6.62 (s, 4-H), 6.85 (s, 1-H), 7.29–7.46 (m, benzyl aromatic). Analysis calculated for $C_{28}H_{34}O_4$: C, 77.39; H, 7.89. Found: C, 77.10; H, 7.86.

*2.4. 17,17-Ethylenedioxy-2-methoxyestra-1,3,5(10)-trien-3-ol (**6**)*

Under nitrogen, ammonium formate (0.5 g, 7.9 mmol) and palladium on charcoal (10%, 0.5 g) were added to a solution of the 3-benzyl ether (**5**, 0.5 g, 1.15 mmol) in methanol (10 ml) and THF (5 ml). The reaction mixture was then stirred overnight at room temperature. After that time, analysis by TLC (CH_2Cl_2) indicated a complete reaction. The mixture was diluted with methylene chloride (50 ml), filtered through Celite, and concentrated in vacuo. The residue was taken up in methylene chloride, washed with water (2×) and brine (1×), filtered through anhydrous sodium sulfate, combined and concentrated in vacuo. The residue was crystallized from methanol to give the pure product (**6**, 0.33 g, 83.3%): mp = 150–151 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 3441, 2932, and 1619 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.89 (s, 18-CH_3), 3.85 (s, 2-OCH_3), 3.88–3.98 (m,

ketal CH_2's), 5.53 (br.s, OH), 6.63 (s, 4-H), 6.79 (s, 1-H). Analysis calculated for $C_{21}H_{28}O_4 \cdot 1/10MeOH$: C, 72.90; H, 8.23. Found: C, 72.77; H, 8.09.

2.5. 17,17-Ethylenedioxy-2-methoxyestra-1,3,5(10)-trien-3-ol acetate (7)

Under nitrogen, acetic anhydride (25 ml, 265 mmol) was added to a solution of the ketal (**6**, 4.5 g, 13.06 mmol) in dry pyridine (25 ml, 310 mmol). The reaction mixture was stirred overnight at room temperature in the dark. After that time, methanol (50 ml) was added and solvents removed in vacuo. The treatment with methanol was repeated and the residue obtained crystallized from methanol to give the pure 3-acetate (**7**, 4.85 g, 96%): mp = 166–167 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 2933, 1765, and 1615 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.885 (s, 18-CH_3), 2.30 (s, OAc), 3.801 (s, 2-OCH_3), 3.88–3.98 (m, ketal CH_2's), 6.73 (s, 4-H), 6.90 (s, 1-H). Analysis calculated for $C_{23}H_{30}O_5 \cdot 1/6MeOH$: C, 71.01; H, 7.89. Found: C, 71.04; H, 7.81.

2.6. 17,17-Ethylenedioxy-16α-bromo-2-methoxyestra-1,3,5(10)-trien-3-ol acetate (8)

Under nitrogen, solid phenyltrimethylammonium tribromide (4.92 g, 13 mmol) was added to a solution of the 3-acetate (**7**, 4.6 g, 11.9 mmol) in dry THF (100 ml) cooled to −5 °C in an ice-salt bath. The reaction mixture was stirred at 2 °C overnight. After that time, saturated sodium bicarbonate solution (50 ml) was added and the mixture was extracted with ethyl acetate (3×). The organic fractions were washed with saturated sodium bicarbonate solution (2×), sodium thiosulfate solution (10%, 1×), and ice-cold water (3×). The organic fractions were dried over sodium sulfate, filtered and concentrated in vacuo to give 6.5 g residue. This material was crystallized from methanol to give the pure 16-bromo compound (**8**, 2.97 g, 54%): mp = 210–212 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 2918, 1766, and 1616 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.92 (s, 18-CH_3), 2.30 (s, OAc), 3.80 (s, 2-OCH_3), 3.92–4.31 (m, ketal CH_2's), 4.55 (dd, J_1 = 10.4 Hz, J_2 = 4.4 Hz, 16-H), 6.73 (s, 4-H), 6.87 (s, 1-H). Analysis calculated for $C_{23}H_{29}BrO_5$: C, 59.36; H, 6.28. Found: C, 59.51; H, 6.30.

2.7. 17,17-Ethylenedioxy-2-methoxyestra-1,3,5(10),15-tetraen-3-ol (9)

Under argon, freshly cut potassium metal (0.77 g, 19.7 mmol) was added to *tert*-butanol (33 ml) and the mixture heated to reflux until all of the metal had reacted. The mixture was cooled slightly and the excess *tert*-butanol was removed under a slight vacuum. Xylene (70 ml) was added and distilled off three times followed by a fourth addition of xylene (70 ml). A solution of the 16-bromo compound (**8**, 1.07 g, 2.3 mmol) in dry xylene (100 ml) was concentrated to 50 ml to remove any moisture. After cooling to room temperature, the steroid solution was added to the potassium *t*-butoxide solution and the mixture heated to reflux for 17 h. The reaction mixture was cooled to room temperature, poured into water, and extracted with ethyl acetate (3×). The organic fractions were washed with water (3×), combined, dried over sodium sulfate, filtered, and concentrated in vacuo to give 1.01 g residue. This material was dissolved in benzene and filtered through a small Florisil column. The elute was concentrated in vacuo to give 0.73 g residue. This material was crystallized from methanol to give the pure tetraene (**9**, 0.64 g, 87%): mp = 161–162 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 3432, 2935, 2900, and 1619 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.97 (s, 18-CH_3), 3.87 (s, 2-OCH_3), 3.96–4.03 (m, ketal CH_2's), 5.43 (s, OH), 5.75 (dd, J_1 = 6.2 Hz, J_2 = 3.4 Hz, 15-H), 6.26 (dd, J_1 = 6.2 Hz, J_2 = 1.2 Hz, 16-H), 6.65 (s, 4-H), 6.79 (s, 1-H). Analysis calculated for $C_{21}H_{26}O_4 \cdot 1/2MeOH$: C, 72.04; H, 7.87. Found: C, 72.16; H, 7.78.

2.8. 2-Methoxyestra-1,3,5(10),15-tetraen-3-ol-17-one (10)

Under nitrogen toluenesulfonic acid monohydrate (0.1 g, 0.53 mmol) was added to a solution of the 17-ketal (**9**, 1.88 g, 5.5 mmol) in acetone (120 ml) and water (20 ml). The reaction mixture was stirred at room temperature for 1.5 h, diluted with cold water (150 ml), and extracted with benzene (3×). The organic fractions were washed with saturated sodium bicarbonate solution (2×) and brine (3×), dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give 1.78 g residue. This material was purified by chromatography followed by crystallization from methylene chloride/hexanes to give the pure 17-ketone (**10**, 1.05 g, 64%): mp = 212–214 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 3332, 2930, and 1696 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 1.12 (s, 18-CH_3), 3.87 (s, 2-OCH_3), 5.46 (s, OH), 6.09 (dd, J_1 = 6.0 Hz, J_2 = 3.2 Hz, 16-H), 6.678 (s, 4-H), 6.79 (s, 1-H), 7.64 (dd, J_1 = 6.0 Hz, J_2 = 1.1 Hz, 15-H). Analysis calculated for $C_{19}H_{22}O_3$: C, 76.48; H, 7.43. Found: C, 76.42; H, 7.27.

2.9. 2-Methoxyestra-1,3,5(10),15-tetraen-3,17β-diol (11)

Under nitrogen, solid lithium aluminum hydride (0.15 g, 3.95 mmol) was added to a solution of the 17-ketone (**10**, 0.06 g, 0.2 mmol) in dry ether (60 ml) cooled to −5 °C in an ice-salt bath. The reaction mixture was stirred at −5 °C for 1 h then quenched cautiously with dropwise addition of water. The mixture was neutralized with 5% H_2SO_4, diluted with water and extracted with ethyl acetate (3×). The organic fractions were washed with saturated sodium bicarbonate solution (2×) and water (3×), combined, dried over

sodium sulfate, filtered and concentrated in vacuo to give 0.073 g residue. This material was triturated with ether to give the pure 17-ol (**11**, 0.055 g, 91%): mp = 191–193 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 3506, 3241, 2938, and 1607 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.89 (s, 18-CH_3), 3.86 (s, 2-OCH_3), 4.40 (br.s, 17-H), 5.45 (s, OH), 5.72 (ddd, J_1 = 5.9 Hz, J_2 = 3.3 Hz, J_3 = 1.1 Hz, 16-H), 6.03 (m, 15-H), 6.66 (s, 4-H), 6.79 (s, 1-H). Analysis calculated for $C_{19}H_{24}O_3 \cdot 1/3H_2O$: C, 74.49; H, 8.11. Found: C, 74.35; H, 8.05.

*2.10. 2-Methoxy-3,17-diacetoxyestra-1,3,5(10),14,16-pentaene (**12**)*

Under nitrogen, toluenesulfonic acid monohydrate (0.1 g, 0.53 mmol) was added to a solution of 2-methoxyestra-1,3,5(10),15-tetraen-3-ol-17-one (**10**, 0.25 g, 0.84 mmol), isopropenyl acetate (5 ml, 45.15 mmol) and acetic anhydride (5 ml, 53 mmol) and the mixture was heated to reflux for 6 h. At the end of that time, analysis by TLC (2% acetone in CH_2Cl_2) indicated a complete reaction. The reaction mixture was cooled to room temperature, poured into ice water (~100 ml) and stirred for 1 h. The mixture was extracted with methylene chloride (3×). The organic fractions were washed with water (1×), saturated sodium bicarbonate solution (1×), and water (1×), filtered through anhydrous sodium sulfate, combined and concentrated in vacuo. The residue was crystallized from methanol to give the pure diacetate (**12**, 0.259 g, 80.8%): mp = 191–194 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 2931, 1763, and 1615 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 1.11 (s, 18-CH_3), 2.23 (s, OAc), 2.31 (s, OAc), 3.81 (s, 2-OCH_3), 5.87 (dd, J_1 = 2.6 Hz, J_2 = 1.5 Hz, 15-H), 6.16 (d, J = 2.6 Hz, 16-H), 6.79 (s, 4-H), 6.90 (s, 1-H). Analysis calculated for $C_{23}H_{26}O_5 \cdot 1/20MeOH$: C, 72.09; H, 6.88. Found: C, 72.02; H, 6.81.

*2.11. 2-Methoxyestra-1,3,5(10),14-tetraen-3,17β-diol (**13**)*

A solution of the diacetate (**12**, 0.08 g, 0.21 mmol) in ethanol (5 ml) and THF (3 ml) was cooled to 0 °C in an ice bath. A solution of sodium borohydride (0.05 g, 1.32 mmol) in ethanol/water (10:3, 5 ml) was cooled to 0 °C in an ice bath and added to the steroid solution. The reaction mixture was stirred at 0 °C for 1 h, then allowed to warm to room temperature and stirred overnight. After that time, the reaction mixture was diluted with water (20 ml) and neutralized with glacial acetic acid. The organic solvents were removed in vacuo under a stream of nitrogen, the residue was diluted with water (50 ml) and extracted with methylene chloride (3×). The organic fractions were washed with water (2×), brine (1×), filtered through sodium sulfate, combined and concentrated in vacuo. The residue was triturated with ether to give the pure product (**13**, 0.035 g, 56%): mp = 169–171 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 3499, 3185, 2920, and 1606 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 1.02 (s, 18-CH_3), 3.86 (s, 2-OCH_3), 4.10 (t, J = 8.4 Hz), 5.21 (m, 15-H), 6.66 (s, 4-H), 6.81 (s, 1-H). Analysis calculated for $C_{19}H_{24}O_3 \cdot 2/5H_2O$: C, 74.19; H, 8.13. Found: C, 74.04; H, 8.01.

*2.12. 2-Methoxyestra-1,3,5(10),15-tetraen-3,17β-diol 3,17-diacetate (**14**)*

Under nitrogen, acetic anhydride (30 ml, 318 mmol) was added to a solution of 2-methoxyestra-1,3,5(10),15-tetraene-3,17β-diol (**11**, 1.15 g, 3.83 mmol) in dry pyridine (35 ml, 434 mmol). The reaction mixture was stirred overnight at room temperature. Solvents were azeotropically removed in vacuo using benzene (2×). The residue was crystallized from methanol to give the pure diacetate (**14**, 1.07 g, 72.4%): mp = 164–165 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 2934, 1757, 1735, and 1613 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.88 (s, 18-CH_3), 2.11 (s, 17-OAc), 2.30 (s, 3-OAc), 3.80 (s, 2-OCH_3), 5.38 (t, J = 3.5 Hz, 17-H), 5.70 (ddd, J_1 = 6 Hz, J_2 = 3.2 Hz, J_3 = 1.4 Hz, 16-H), 6.09 (m, 15-H), 6.75 (s, 4-H), 6.88 (s, 1-H). Analysis calculated for $C_{23}H_{28}O_5 \cdot 1/10MeOH$: C, 71.57; H, 7.38. Found: C, 71.52; H, 7.46.

*2.13. 2-Methoxyestra-1,3,5(10)-trien-3,15ξ,16ξ, 17β-tetrol 3,17-diacetate (**15**)*

Under nitrogen, a solution of the Δ^{15}-steroid (**14**, 27.84 g, 72.4 mmol) in dry benzene (315 ml) was added to a solution of osmium tetroxide (20.5 g, 80.6 mmol) in benzene (520 ml) and pyridine (56 ml). The reaction mixture was stirred mechanically at room temperature for 4 h, and then left without stirring at room temperature for 64 h. The reaction mixture was diluted with benzene (510 ml) and methanol (940 ml). Aqueous solutions of potassium bicarbonate (123 g/720 ml) and sodium sulfite (123 g/720 ml) were added and the mixture was stirred at room temperature for 4 h. The reaction mixture was filtered and extracted with ethyl acetate (3×). The organic fractions were washed with cold brine (4×), combined, and concentrated in vacuo. Water was removed azeotropically in vacuo using benzene to give 32.3 g residue as crude product. This material was not characterized or further purified and was carried on to the next step.

*2.14. 2-Methoxyestra-1,3,5(10)-trien-3,15α,16α, 17β-tetrol tetraacetate (**16a**) and 2-methoxyestra-1,3, 5(10)-trien-3,15β,16β,17β-tetrol tetraacetate (**16b**)*

Acetic anhydride (100 ml, 1.06 mol) was added to a solution of the crude mixture (**15**, 32.3 g, assume 72.4 mmol) in dry pyridine (100 ml, 1.24 mol) and the mixture was stirred overnight at room temperature. Solvents were removed in vacuo under a stream of nitrogen with solvent residues being removed azeotropically using methanol (2×) to give

37 g crude product. This material was combined with 39 g of crude product obtained from a separate batch to give a total amount of 76 g crude product as an isomer mixture. This material was resolved by stepwise crystallization (methanol) and dry column chromatography (ether) to give the pure 15α,16α-isomer (**16a**, 43.08 g, 59.2%): mp = 168–171 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 2930, 1758, 1738, and 1613 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.95 (s, 18-CH_3), 2.04 (s, OAc), 2.08 (s, OAc), 2.09 (s, OAc), 2.30 (s, 3-OAc), 3.80 (s, 2-OCH_3), 5.01 (d, J = 6.6 Hz, 17-H), 5.16 (dd, J_1 = 14.7 Hz, J_2 = 8.4 Hz, 15-H), 5.40 (dd, J_1 = 8.4 Hz, J_2 = 6.6 Hz, 16-H), 6.75 (s, 4-H), 6.88 (s, 1-H). Analysis calculated for $C_{27}H_{34}O_9$: C, 64.53; H, 6.82. Found: C, 64.63; H, 6.84. And the 15β,16β-isomer (**16b**, 11.78 g, 16.2%): mp = 169–171 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 2939, 1743, and 1612 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 1.09 (s, 18-CH_3), 2.03 (s, OAc), 2.06 (s, OAc), 2.06 (s, OAc), 2.31 (s, 3-OAc), 3.81 (s, 2-OCH_3), 4.72 (d, J = 7.4 Hz, 17-H), 5.39 (t, J = 6.3 Hz, 15-H), 5.50 (t, J = 7.4 Hz, 16-H), 6.750 (s, 4-H), 6.88 (s, 1-H). Analysis calculated for $C_{27}H_{34}O_9$: C, 64.53; H, 6.82. Found: C, 64.73; H, 6.80.

*2.15. 2-Methoxyestra-1,3,5(10)-trien-3,15α,16α,17β-tetrol (**17a**)*

Under nitrogen, a solution of potassium carbonate (6.0 g, 43.4 mmol) in water (330 ml) was added to a solution of the tetraacetate (**16a**, 10.0 g, 19.9 mmol) in methanol (1800 ml). The reaction mixture was stirred overnight at room temperature. Glacial acetic acid (5.2 ml, 90.48 mmol) was added and the solvents removed in vacuo. The residue was crystallized from methanol/water to give the purified tetrol (**17a**, 6.1 g, 91.7%): mp = 228–230 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 3342, 2930, and 1589 cm^{-1}; NMR (300 MHz, $CDCl_3$ + D_6DMSO + D_2O), δ (ppm): 0.81 (s, 18-CH_3), 3.52 (d, J = 5.7 Hz, 17-H), 3.85 (s, 2-OCH_3), 3.94 (m, 15- and 16-H), 6.64 (s, 4-H), 6.78 (s, 1-H). Analysis calculated for $C_{19}H_{26}O_5$: C, 68.24; H, 7.84. Found: C, 68.29; H, 8.00.

*2.16. 2-Methoxyestra-1,3,5(10)-trien-3,15β,16β,17β-tetrol (**17b**)*

Following the same procedure given for the preparation of **17a**, the tetraacetate (**16b**, 10.0 g, 19.9 mmol) in methanol (1800 ml) was hydrolyzed with potassium carbonate (6.0 g, 43.4 mmol) in water (330 ml) to give the pure tetrol (**17b**, 3.87 g, 58.2%): mp = 224–225 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 3526, 3398, 3266, 2938, and 1621 cm^{-1}; NMR (300 MHz, $CDCl_3$ + D_6DMSO + D_2O), δ (ppm): 0.92 (s, 18-CH_3), 3.42 (d, J = 7.2 Hz, 17-H), 3.85 (s, 2-OCH_3), 4.12 (t, J = 6.9 Hz, 16-H), 4.26 (dd, J_1 = 6.9 Hz, J_2 = 5.1 Hz, 15-H), 6.63 (s, 4-H), 6.79 (s, 1-H). Analysis calculated for $C_{19}H_{26}O_5$·1/10MeOH: C, 67.95; H, 7.88. Found: C, 67.93; H, 7.79.

*2.17. 2-Methoxyestra-1,3,5(10)-trien-3,15α,16α,17β-tetrol 15,16-acetonide (**18a**)*

Under nitrogen, a solution of the tetrol (**17a**, 0.15 g, 0.45 mmol) in acetone (10 ml) was treated with perchloric acid (70%, one drop). The reaction mixture was stirred at room temperature overnight. Analysis by TLC (5% acetone in CH_2Cl_2) indicated a complete reaction. The mixture was quenched with saturated sodium bicarbonate solution (1 ml) and solvent removed in vacuo under a stream of nitrogen. The residue was extracted with ethyl acetate (3×). The organic fractions were washed with saturated sodium bicarbonate solution (1×), water (1×), and brine (1×), combined, dried over sodium sulfate, filtered and concentrated in vacuo. The residue was crystallized from ether/hexanes to give the purified acetonide (**18a**, 0.16 g, 95%): mp = 155–157 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 3534, 3208, 2934, and 1594 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.89 (s, 18-CH_3), 1.32 (s, acetonide CH_3), 1.52 (s, acetonide CH_3), 3.78 (br.s, 17-H), 3.86 (s, 2-OCH_3), 4.43 (t, J = 8 Hz, 16-H), 4.51 (dd, J_1 = 8 Hz, J_2 = 4.4 Hz, 15-H), 5.45 (s, OH), 6.65 (s, 4-H), 6.78 (s, 1-H). Analysis calculated for $C_{22}H_{30}O_5$·1/4hexane: C, 71.27; H, 8.53. Found: C, 71.35; H, 8.70.

*2.18. 2-Methoxyestra-1,3,5(10)-trien-3,15β,16β,17β-tetrol 15,16-acetonide (**18b**)*

Following the same procedure given for the preparation of **18a**, the tetrol (**17b**, 0.1 g, 0.299 mmol) was reacted with perchloric acid (70%, one drop) in acetone (10 ml) overnight at room temperature. Identical workup gave after crystallization from ether/hexanes the pure acetonide (**18b**, 0.064 g, 57.2%): mp = 188–191 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 3574, 3516, 2927, 1621, and 1594 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 1.04 (s, 18-CH_3), 1.34 (s, acetonide CH_3), 1.52 (s, acetonide CH_3), 3.44 (m, 17-H), 3.86 (s, 2-OCH_3), 4.53 (t, J = 6.1 Hz, 16-H), 4.64 (dd, J_1 = 6.1 Hz, J_2 = 4.5 Hz, 15-H), 5.44 (OH), 6.65 (s, 4-H), 6.78 (s, 1-H). Analysis calculated for $C_{22}H_{30}O_5$·1/5H_2O: C, 69.89; H, 8.10. Found: C, 69.88; H, 8.15.

*2.19. 2-Methoxyestra-1,3,5(10),7-tetraen-3,17β-diol (**20**)*

Under nitrogen, a solution of L-selectride in THF (1 M, 0.081 ml, 0.081 mmol) was added dropwise to a solution of 2-methoxy-3-hydroxyestra-1,3,5(10),7-tetraen-17-one [15] (**19**, 0.008 g, 0.027 mmol) in dry THF (1.0 ml) cooled to 0 °C in an ice bath. The reaction mixture was allowed to warm to room temperature and stirred for 45 min. Methanol (three drops) followed by methanolic KOH (3%, three drops) were added and the mixture was cooled to 0 °C in an ice bath. Hydrogen peroxide solution (30%, five drops) was added and the mixture was allowed to warm to room temperature. The reaction mixture was diluted with water, acidified with HCl, and extracted with methylene chloride

(3×). The organic fractions were washed with water (1×) and brine (1×), filtered through sodium sulfate, combined and concentrated in vacuo to give 0.006 g residue. This material was crystallized from ether/hexanes to give the purified product (**20**, 0.0037 g, 45.9%): NMR (300 MHz, $CDCl_3$), δ (ppm): 0.66 (s, 18-CH_3), 3.38 (m, 17-H), 3.87 (s, 2-OCH_3), 5.40 (t, $J = 1.65$ Hz, 7-H), 6.66 (s, 4-H), 6.69 (s, 1-H).

*2.20. 2-Methoxyestra-1,3,5,7,9-pentaen-3,17β-diol (**22**)*

Under nitrogen, a solution of L-selectride in THF (1 M, 0.09 ml, 0.09 mmol) was added dropwise to a solution of 2-methoxy-3-hydroxyestra-1,3,5,7,9-pentaen-17-one [15] (**21**, 0.009 g, 0.03 mmol) in dry THF (1.0 ml) cooled to 0 °C in an ice bath. The reaction mixture was allowed to warm to room temperature and stirred for 45 min. Methanol (four drops) followed by methanolic KOH (3%, four drops) were added and the mixture was cooled to 0 °C in an ice bath. Hydrogen peroxide solution (30%, five drops) was added and the mixture was allowed to warm to room temperature. The reaction mixture was diluted with water, acidified with HCl, and extracted with methylene chloride (3×). The organic fractions were washed with water (1×) and brine (1×), filtered through sodium sulfate, combined and concentrated in vacuo to give 0.011 g residue. This material was crystallized from ether/hexanes to give the purified product (**22**, 0.0066 g, 73%): NMR (300 MHz, $CDCl_3$), δ (ppm): 0.71 (s, 18-CH_3), 3.96 (m, 17-H), 4.03 (s, 2-OCH_3), 7.06 (d, $J = 8.25$ Hz, 6-H), 7.18 (s, 4-H), 7.26 (s, 1-H), 7.50 (d, $J = 8.25$ Hz, 7-H).

*2.21. 2-Methoxy-13α-estra-1,3,5(10)-trien-2-ol-17-one (**24**)*

Under nitrogen, a mixture of 2-methoxyestrone (**23**, 1.0 g, 3.33 mmol) and 1,2-phenylenediamine (0.6 g, 5.55 mmol) in glacial acetic acid (10 ml) was heated to reflux for 4 h. The mixture was allowed to cool to room temperature and poured into ice-water (∼400 ml). The resulting precipitate was collected by filtration, washed with water and air dried to give 0.9 g crude product as an off-white solid. This material was combined with that obtained from a second 1 g batch, and the total crude material (1.8 g) was purified via flash chromatography (3% acetone in CH_2Cl_2) to give 1.4 g of a white solid indicated by NMR to consist of the expected 13α-product (**24**) plus 5–10% starting material (**23**). This material was suspended in ethanol (50 ml). Girard's Reagent P (1.5 g, 8.0 mmol) and glacial acetic acid (1 ml, 17.4 mmol) were added and the mixture was heated to reflux under nitrogen for 1.5 h. The reaction mixture was allowed to cool to room temperature, poured into half saturated sodium bicarbonate solution (350 ml) and extracted with methylene chloride (3×). The organic fractions were washed with water (2×), filtered through Na_2SO_4, combined and concentrated in vacuo to give 1.18 g crude product (**24**) as a white solid indicated by NMR to contain none of the 13β-isomer.

For purposes of characterization and biological testing, 100 mg of this material was crystallized from methanol/water to give the pure 13α-product (**24**, 0.065 g): mp = 179–180.5 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 3442, 2922, 2874, 2860, 1724, 1709, 1611, and 1587 cm^{-1}. (Note: an unusual occurrence of 17-ketone absorbance showing up as two peaks.) NMR (300 MHz, $CDCl_3$), δ (ppm): 1.06 (s, 18-CH_3), 3.84 (s, 2-OCH_3), 5.46 (s, OH), 6.62 (s, 4-H), 6.75 (s, 1-H). Analysis calculated for $C_{19}H_{24}O_3 \cdot 1/10H_2O$: C, 75.52; H, 8.07. Found: C, 75.43; H, 8.06.

*2.22. 2-Methoxy-13α-estra-1,3,5(10)-trien-3,17β-diol (**25**) and 2-methoxy-13α-estra-1,3,5(10)-trien-3,17α-diol (**26**)*

A solution of the 13α-isomer (**24**, 1.0 g, 3.33 mmol) in methanol (30 ml) and THF (25 ml) was cooled to 0 °C in an ice bath and treated portionwise with solid $NaBH_4$ (0.25 g, 6.6 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 3 h. Acetone (∼2 ml) was added followed by ice-water (∼10 ml). The mixture was poured into ice-water (100 ml) and the resulting precipitate collected by filtration, washed with water and air-dried. The crude product mixture was separated via flash chromatography (10% acetone in toluene) to give the less polar 17β-isomer (**25**, 0.53 g, 52.6%) as a white solid: mp = 164–166 °C (MeOH/H_2O); FT-IR (KBr, diffuse reflectance) ν_{max}: 3373, 3166, 2944, 2901, 1614, 1598, and 1516 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.95 (s, 18-CH_3), 3.81 (t, $J = 3$ Hz, 17-H), 3.84 (s, 2-OCH_3), 5.46 (s, OH), 6.61 (s, 4-H), 6.75 (s, 1-H). Analysis calculated for $C_{19}H_{26}O_3$: C, 79.68; H, 9.15. Found: C, 79.49; H, 9.25. And the more polar 17α-isomer (**26**, 0.31 g, 30.8%) as a white solid: mp = 150–152 °C (MeOH/H_2O); FT-IR (KBr, diffuse reflectance) ν_{max}: 3533, 3316, 2915, 2874, 1623, 1593, and 1506 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.93 (s, 18-CH_3), 3.84 (s, 2-OCH_3), 4.18 (t, $J = 8.4$ Hz, 17-H), 5.60 (br.s, OH), 6.61 (s, 4-H), 6.79 (s, 1-H). Analysis calculated for $C_{19}H_{26}O_3$: C, 79.68; H, 9.15. Found: C, 79.73; H, 9.02.

*2.23. 2-Acetylestra-1,3,5(10)-trien-3,17β-diol (**28**)*

Under nitrogen, a suspension of 2-acetylestra-1,3,5(10)-trien-3,17β-diol 17-acetate [16] (**27**, 0.5 g, 1.4 mmol) in methanol (100 ml) was treated with 1N KOH (4 ml, 4 mmol). Water (10 ml) was added and the reaction mixture was stirred overnight at room temperature. After that time, analysis by TLC (5% acetone in CH_2Cl_2) of a small aliquot made acidic indicated ∼90% reaction. The reaction mixture was heated to reflux for 30 min, after which time analysis by TLC indicated a complete reaction. The solvents were removed in vacuo, the residue diluted with water (100 ml) and glacial acetic acid (0.6 ml, 7 mmol) was added. The resulting suspension was extracted with methylene chloride (3×). The organic fractions were washed with water (3×),

filtered through Na_2SO_4, combined and concentrated in vacuo to give 0.46 g yellow foam as residue. This material was crystallized from ether to give the pure diol (**28**, 0.406 g, 92%): mp = 192–195 °C; FT-IR (KBr, diffuse reflectance) ν_{max}: 3477, 2972, 2937, 2913, 2867, 1634, 1607, and 1570 cm^{-1}; NMR (300 MHz, $CDCl_3$), δ (ppm): 0.80 (s, 18-CH_3), 2.60 (s, acetyl), 3.75 (t, J = 8.4 Hz, 17-H), 6.69 (s, 4-H), 7.61 (s, 1-H), 12.04 (s, 3-OH). Analysis calculated for $C_{20}H_{26}O_3 \cdot 1/5Et_2O$: C, 75.88; H, 8.57. Found: C, 75.92; H, 8.42.

2.24. Biological assays

2.24.1. Inhibition of proliferation

The sulforhodamine B (SRB) assay was used to evaluate the antiproliferative activity of 2-ME2 and 2-ME2 derivatives in the MDA-MB-435 and SK-OV-3 cell lines [17,18]. Cells were plated into 96-well plates and allowed to grow and attach for 24 h followed by addition of the test substances or vehicle controls. The cells were incubated with drugs for 48 h and then the cellular protein was fixed, stained, and concentration determined by absorbance at 560 nm. Log dose–response curves were constructed for each experiment and the IC_{50} for inhibition of proliferation determined.

2.24.2. Microtubule depolymerizing activity

The effects of the parental compound and derivatives on cellular microtubule depolymerization were determined by indirect immunofluorescence techniques in rat aortic smooth muscle A-10 cells. Microtubules were visualized using a β-tubulin antibody as previously described [19]. Three viewers determined the percent microtubule loss for each treatment concentration. The data were averaged and plotted as percent microtubule loss versus drug concentration and the EC_{50}s for microtubule depolymerization calculated from the log dose–response curves.

3. Results and discussion

3.1. Chemistry

The syntheses of compounds **2**, **3**, **19**, **21**, **27**, and **29** were described previously [15,16]. The syntheses of the Δ^{15}- and Δ^{14}-derivatives (**11** and **13**), the tetrols (**17a** and **17b**), and the acetonides (**18a** and **18b**) are outlined in Fig. 2 and are based upon well known synthetic methodologies [20–22].

Jones oxidation of the previously synthesized [16] benzyl ether (**3**) gave the 17-ketone (**4**) in 60.8% yield. Ketalization of (**4**) followed by catalytic transfer hydrogenation using ammonium formate [23] to remove the benzyl ether gave the 3-ol-17 ketal compound (**6**) in 70.1% overall yield. Acetylation of (**6**) followed by bromination using phenyltrimethylammonium tribromide gave the 16α-bromo-3-acetate (**8**) in 51.8% overall yield. Subsequent dehydrobromination of (**8**) using potassium *tert*-butoxide in refluxing xylene followed by acid catalyzed ketal exchange of (**9**) in acetone gave the Δ^{15}-17-one derivative (**10**) in 56% overall yield. Lithium aluminum hydride reduction of (**10**) to the Δ^{15}-17β-ol derivative (**11**) was successfully carried out in 91% yield using ether as solvent at −5 °C.

Following the procedure of Rasmusson and Arth [21], the Δ^{15}-17-ketone (**10**) was converted to the dienyl acetate (**12**) in 80.8% yield by reaction with isopropenyl acetate and toluenesulfonic acid monohydrate in acetic anhydride at reflux. The subsequent sodium borohydride reduction of this material gave the Δ^{14}-17β-ol derivative (**13**) in 55.7% yield.

Starting from the Δ^{15}-3,17β-diol (**11**), the syntheses of the 3,15α,16α,17β-tetrol (**17a**) and the 3,15β,16β,17β-tetrol (**17b**) were carried out following the procedure of Nambara et al. [22]. Diacetylation of (**11**) was carried out in 72.4% yield followed by reaction with osmium tetroxide in pyridine/benzene to give a mixture of the 15,16-diols (**15**). This mixture was converted to the corresponding tetraacetates which could be separated by chromatography and crystallization to give the 3,15α,16α,17β-tetraacetate (**16a**) in 59.2% yield and the 3,15β,16β,17β-tetraacetate (**16b**) in 16.2% yield. The assignment of the 15,16-configuration was based upon proton chemical shift data for the C-18 methyl group and the 17α-proton. Compound **16b** has greater deshielding of the 18-methyl (δ = 1.093) compared to **16a** (δ = 0.945) and compound **16a** has increased deshielding of the 17α-H (δ = 5.012) compared to **16b** (δ = 4.724). Subsequent mild base hydrolysis of the tetraacetates gave the corresponding 3,15α,16α,17β-tetrol (**17a**) and the 3,15β,16β,17β-tetrol (**17b**) in 91.7 and 58.2% yields, respectively. Conversions of the tetrols (**17a** and **17b**) to the corresponding acetonides (**18a** and **18b**) were carried out by reaction with acetone and catalytic perchloric acid in 95 and 57.2% yields, respectively.

The equine estrogen derivatives 2-methoxyestra-1,3,5(10),7-tetraen-3,17β-diol (**20**) and 2-methoxyestra-1,3,5,7,9-pentaene-3,17β-diol (**22**) were obtained from the previously synthesized [15] 17-ketones (**19** and **21**) by reduction with L-selectride in THF at 0 °C in 45.9 and 73% yields, respectively, as shown in Fig. 3. Having only small quantities of starting materials, these products were only characterized by NMR.

The syntheses of the 13α-compounds (**24**, **25**, and **26**) were carried out following the procedure of Schönecker et al. [24] and is outlined in Fig. 4. Reaction of 2-methoxyestrone (**23**) with 1,2-phenylenediamine in glacial acetic acid at reflux gave a 70% yield of the 13α-derivative (**24**) contaminated with 5–10% starting material (**23**). This material was purified by reaction with Girard's Reagent P which selectively reacts with the 13β-starting material. The overall yield of the purified 13α-ketone (**24**) was 59%. Sodium borohydride reduction of the 13α-ketone (**24**) gave a chromatographically separable mixture of the 17β- and 17α-alcohols (**25** and **26**) in 52.6 and 30.8%, respectively. The assignment of configuration at C-17 was based upon comparison

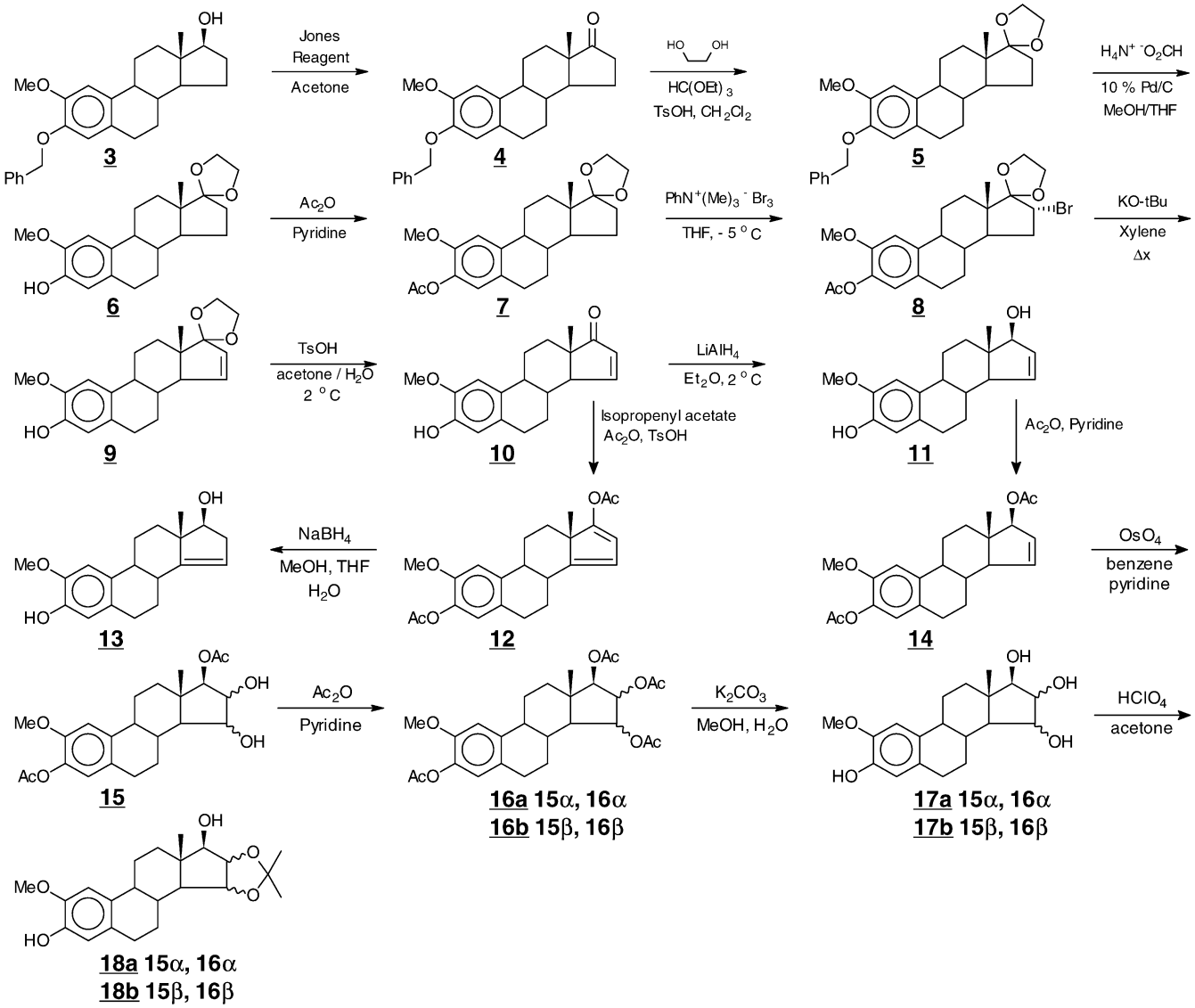

Fig. 2. Syntheses of 2-methoxyestradiol analogs.

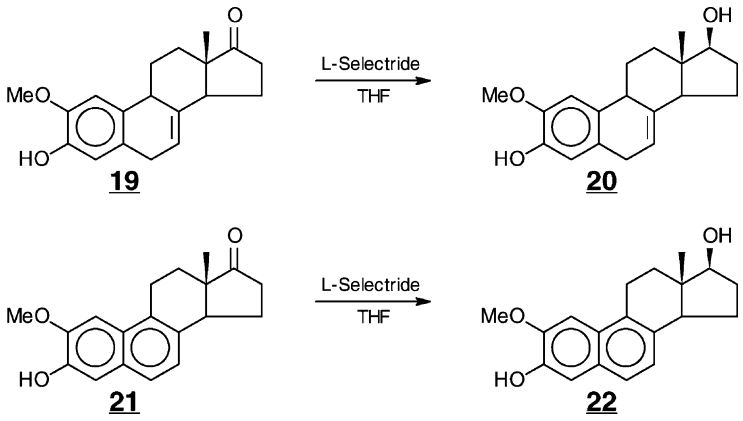

Fig. 3. Syntheses of equine estrogen derivatives.

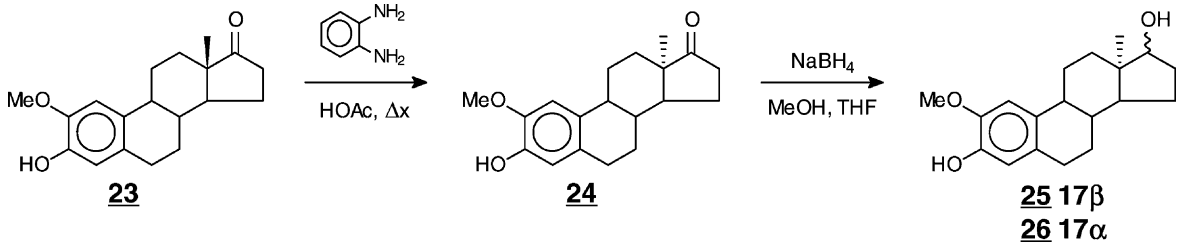

Fig. 4. Syntheses of 13α-analogs.

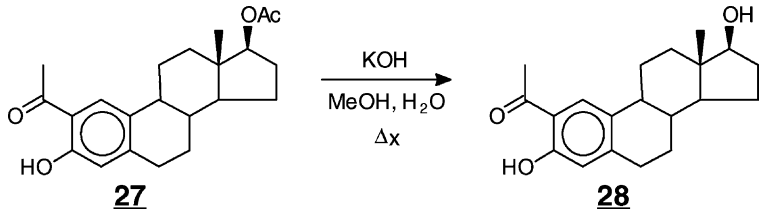

Fig. 5. Synthesis of 2-acetyl analog.

of the chemical shifts of the 17-proton with those obtained by Schönecker et al. [24] for the 17α- and 17β-isomers of 13α-estradiol 3-methyl ether.

The synthesis of the 2-acetyl-3,17β-diol derivative (**28**) is shown in Fig. 5 and was carried out by base hydrolysis of the previously synthesized [16] 3-acetate (**27**).

3.2. Biological activity

The compounds tested for biological activity are shown in Fig. 6.

The IC_{50}s for inhibition of proliferation were generated in two cancer cell lines. The MDA-MB-435 cells are derived from a breast carcinoma and SK-OV-3 is an ovarian carcinoma cell line. The data are presented in Table 1. The most potent analog is the Δ^{14} analog (**13**) and in these cell lines it is 23 and 12 times more potent than the parental compound, 2-ME2 (**2**). The Δ^{15}-compound (**11**) is approximately 1.3–3.3-fold more potent than the parental compound. In the MDA-MB-435 cell line, the Δ^{7}-compound (**20**) is slightly less potent than the reference compound and almost 3-fold less potent towards SK-OV-3 cells. The 15α,16α-acetonide derivative (**18a**) is 0.34–0.38 as potent as the reference compound.

The effects of the derivatives on cellular microtubule depolymerization were determined using rat aortic smooth

Table 1
IC_{50} for inhibition of proliferation

Compound	Inhibition of proliferation IC_{50} (μM)		EC_{50} for microtubule depolymerization in A-10 cells (μM)
	MDA-MB-435	SK-OV-3	
2	1.38 ± 0.10	1.79 ± 0.24	7.5
11	0.86 ± 0.11	1.37 ± 0.26	6.5
13	0.06 ± 0	0.15 ± 0.07	0.3
17a	47.4 ± 4.45	85.8 ± 16.0	>150
17b	265 ± 71.4	>200	>150
18a	3.66 ± 1.17	5.16 ± 0.99	17.4
18b	136 ± 24.1	N.D.	N.D.
19	7.85 ± 5.73	18.1 ± 3.49	N.D.
20	1.97 ± 0.40	4.49 ± 0.18	N.D.
21	105 ± 36.3	>200	150
22	10.5 ± 2.80	28.3 ± 1.86	>150
23	36.3 ± 30.4	32.1 ± 3.96	>150
24	60.4 ± 28.2	114 ± 27.8	>150
25	16.9 ± 3.87	56.3 ± 6.15	>150
26	22.6 ± 1.70	65.3 ± 8.13	>150
28	26.3 ± 1.61	29.7 ± 5.15	>150
29	6.03 ± 1.38	6.27 ± 0.40	45.0

Three or four experiments were conducted and the IC_{50} for each experiment was determined. The values presented are the means ± S.D. EC_{50} for microtubule depolymerization is the concentration of compound that causes 50% loss of cellular microtubules in A-10 cells. N.D.: experiment not carried out.

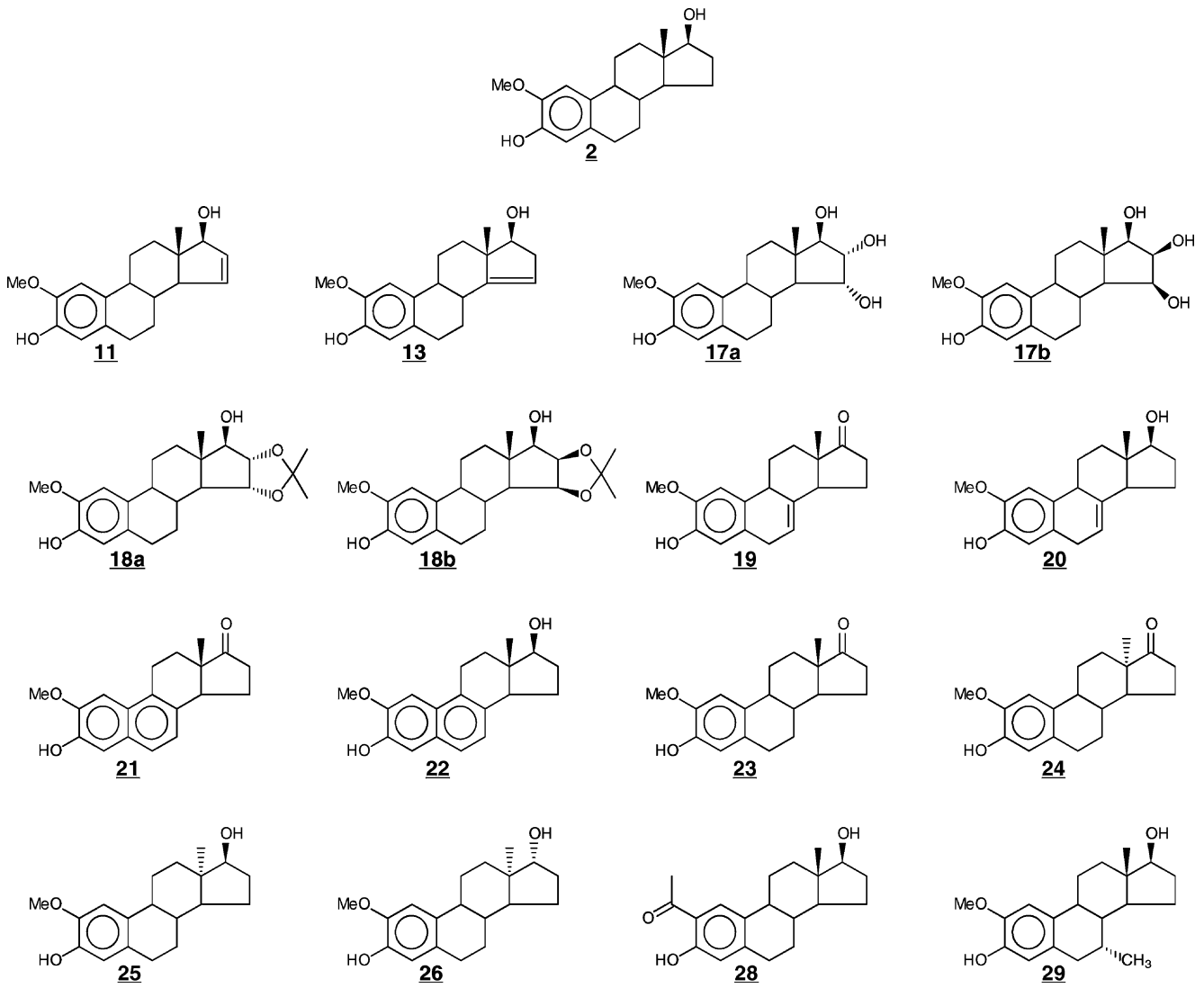

Fig. 6. Compounds tested for biological activity.

muscle A-10 cells. This data is also shown in Table 1. Consistent with the inhibition of cellular proliferation data, both (**11**) and (**13**) were more potent than the reference compound for causing microtubule depolymerization. Compound (**18a**) was 0.43 as potent as 2-ME2, consistent with its antiproliferative effects. The results suggest that the new derivatives and 2-ME2 share the same intracellular target, tubulin.

3.3. *Structure–activity relationships*

Prior testing of 2-ME2 variants for inhibition of tubulin polymerization [25] and inhibition of endothelial cell proliferation [3] would seem to indicate that free hydroxyl groups at the 3- and 17-positions are required for potent biological activity. The compounds previously tested include 2-methoxyestrone, 2,3-dimethoxyestradiol, and 2-hydroxyestradiol 3-methyl ether. From the data we present in Table 1 it can be seen that the 17-hydroxy analogs (**2**, **20**, **22**, **25**, and **26**) are more active than their 17-oxo counterparts (**23**, **19**, **21**, and **24**), thus supporting the generalization for the free 17-OH group being required for potent activity. However, an exception to this generalization is 2-methoxyestrone-3-*O*-sulfamate which was shown by Purohit et al. [7] to be 10 times as active as 2-ME2 against human breast cancer cells.

Other structural modifications on 2-ME2 carried out by Cushman et al. [4,5] have focused primarily on variations at the 2- and 6-positions of the parent compound. The authors conclude that the optimum 2-substituent for cytotoxic activity appears to be an unbranched chain containing three atoms chosen from the second row of the periodic table, with activity increasing with increased electron density adjacent to the aromatic ring. This generalization is supported by the decreased cytotoxicity we observed for compound **28** with the decreased electron density of the carbonyl carbon being adjacent to the aromatic ring. Concerning the 6-position,

most modifications carried out by Cushman decreased cytotoxic activity by varying degrees with only the 6-oximino derivatives being more active than the parent compound. The introduction of a double bond at the 6-position for the 2-ethoxy analog led to a 90-fold decrease in cytotoxic activity. This is in contrast to the equipotent activity we observed for compound **20** with the double bond at the 7-position. The total aromatization of the ring B in compound **22** led to decreased cytotoxic activity, but not as dramatic as that seen by Cushman for the Δ^6 analog.

Outside of 2-methoxyestrone, the only other ring D modified 2-ME2 analogs previously tested for antimitotic activity are 2-methoxyestriol and 2-methoxyethynylestradiol both of which proved to be inactive with regard to inhibition of tubulin polymerization [25]. The data for compounds **17a**, **17b**, **18a**, and **18b** seem to indicate cis-substitution at the 15,16-positions leads to decreased activity with the β-substituted analogs being much less active than the α. The inversion of configuration at the C-13 position in compounds **24**, **25**, and **26** also leads to decreased cytotoxic activity. The introduction of additional unsaturation in ring D for compounds **11** and **13** gives the most interesting results and leads to a modest to dramatic increase in cytotoxicities relative to 2-ME2. Whether these structural modifications lead to an increased binding affinity towards tubulin or perhaps a decreased rate of metabolic inactivation remains to be investigated.

Detailed biological testing of the most promising analogs will continue. Currently, compounds **11**, **13**, and **18a** are being evaluated in murine models for toxicity and antitumor activity in head-to-head tests with 2-ME2.

Acknowledgments

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