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Synthesis and antimitotic activity of novel 2-methoxyestradiol analogs. Part III

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ABSTRACT

The syntheses and antimitotic activity of several novel analogs of 2-methoxyestradiol are described. Structural modifications include ring-D homologation, aromatization of the six-membered ring-D to a chrysine type molecule, and introduction of unsaturation in five-membered ring-D along with substitution of alkyl and ethynyl groups for the 17β-hydroxy function. Of nine analogs synthesized, five have demonstrated superior antiproliferative activities compared to 2-methoxyestradiol.

1. Introduction

2-Methoxyestradiol (2ME2), a natural metabolite of estradiol which has no estrogenic activity, was found to be a potent antitumor and antiangiogenic compound [1,2]. It is currently in clinical trials for treatment of a variety of cancers. Several structural modifications of 2ME2 were reported in the literature to obtain biologically more potent analogs. We have previously shown [3,4] that modification of ring-D by incorporation of additional unsaturation or introduction of an α-substituted functional group such as a 15α,16α acetonide significantly increases the biological activity *in vitro*. We also observed that in some cases protection of the 17β-hydroxyl function as a methoxy derivative resulted in compounds with increased activity [5]. It is speculated that the 17β-hydroxyl protected analogs of 2ME2 are not readily amenable to metabolic oxidation to the less active 17-oxo-analogs.

In continuation of our studies on the structure–activity relationship in 2ME2 series, we synthesized several novel ring-D-modified compounds. Structural modifications include the expansion of the five-membered ring-D to a six-membered structure (D-homo), aromatization of the six-membered ring-D to chrysine type molecules, and substituting the 17β-hydroxyl function in five-membered ring-D with alkyl and ethynyl functions. We now present their synthesis and evaluation of their cytotoxic activity in a variety of cell types.

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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 (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 Thermo-Nicolet model 370 FT-IR instrument equipped with an attenuated reflectance (ATR) accessory. 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-Methoxyestradiol was provided as a gift by EntreMed, Inc., 9640 Medical Center Drive, Rockville, MD.

2.1.1. *2-Methoxy-3-acetoxy-17β-hydroxyestra-1,3,5(10)-triene*

(2)

To a solution of 2-methoxyestradiol (**1**, 20 g, 58 mmol) in isopropanol (350 ml) was added sodium hydroxide solution (2 M, 100 ml, 400 mmol) and acetic anhydride (16 ml, 169 mmol). The reaction mixture was stirred at room temperature and monitored by TLC (5% acetone/CH_2Cl_2) which indicated a complete reaction after 2 h. The reaction was slowly quenched with methanol, diluted with water, and concentrated *in vacuo*. The residue was acidified with HCl (3 M) and extracted with ethyl acetate (3×). The organic fractions were washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo* to give the 3-acetate derivative (**2**, 24.4 g) as a white solid. mp = 148–149 °C; FT-IR (ATR) ν_{max}: 3459, 2926, 2859, 1758, and 1505 cm^{-1}.

NMR (300 MHz, $CDCl_3$), δ (ppm): 0.76 (s, 18-CH_3), 2.30 (s. 3-OAc), 3.80 (s, 2-OCH_3), 3.71 (t, J = 8.5 Hz, 17-H), 6.73 (s, 4-H), 6.90 (s, 1-H).

Anal. Calcd for $C_{21}H_{28}O_4$: C, 73.26; H, 8.19. Found: C, 73.13; H, 8.04.

2.1.2. *2-Methoxy-3-acetoxyestra-1,3,5(10)-trien-17-one*

(3)

Under nitrogen, the 17-hydroxy compound (**2**, 10 g, 29 mmol) was dissolved in 20 ml of acetone and chilled to 0 °C. Jones reagent was slowly added with stirring until the yellow–orange color persisted (18 ml). The reaction was stirred an additional 5 min then slowly quenched with isopropanol. The solution was concentrated *in vacuo*, diluted with water, and extracted with ethyl acetate (3×). The organic fractions were washed with water, brine, combined, dried over Na_2SO_4, and concentrated *in vacuo* to give the 17-ketone (**3**, 9.07 g, 91%) as a white solid. mp = 152 °C; FT-IR (ATR) ν_{max}: 2939, 1761, 1733, 1616, and 1508 cm^{-1}.NMR (300 MHz, $CDCl_3$), δ (ppm): 0.92 (s, 18-CH_3), 2.31 (s. 3-OAc), 3.81 (s, 2-OCH_3), 6.77 (s, 4-H), 6.90 (s, 1-H).

Anal. Calcd. for $C_{21}H_{26}O_4$: C, 73.66; H, 7.65. Found: C, 73.53; H, 7.67.

2.1.3. *2-Methoxy-3-hydroxyestra-1,3,5(10)-trien-17-one*

(4)

Under nitrogen, a solution of the 3-acetate derivative (3, 11.3 g, 33 mmol) in 1:1 THF/H_2O (100 ml) was treated with NaOH (2 M, 75 ml, 150 mmol) at room temperature for 1 h. Analysis by TLC (5% acetone/CH_2Cl_2) indicated a complete reaction. The reaction was cooled to 0 °C, quenched with 3 M HCl, concentrated *in vacuo*, and extracted with ethyl acetate (3×). The organic fractions were washed with water, brine, combined, dried over Na_2SO_4, and concentrated *in vacuo* to give 2-methoxyestrone (**4**, 10 g, 100%) as a white solid. mp = 189–190 °C (Lit. [6], mp = 189–191 °C); FT-IR (ATR) ν_{max}: 3359, 2928, 1720, 1588, and 1503 cm^{-1}. NMR (300 MHz, $CDCl_3$) δ (ppm): 0.93 (s, 18-CH_3), 3.87 (s, 2-OCH_3), 5.51 (s, 3-OH), 6.67 (s, 4-H), 6.80 (s, 1-H).

2.1.4. *2-Methoxy-3-methoxymethoxyestra-1,3,5(10)-trien-17-one*

(5)

Under nitrogen, 2-methoxyestrone (**4**, 9.2 g, 30 mmol) was dissolved in 60 ml of THF. *N*,*N*-Diisopropylethylamine (35 ml, 200 mmol) and chloromethyl methyl ether (12.5 ml, 160 mmol) were added and the reaction mixture stirred at 65 °C overnight. The reaction was cooled to room temperature, quenched with 20% NH_4Cl solution, and extracted with ethyl acetate (3×). The organic phase washed with water (3×), brine, dried over Na_2SO_4, and concentrated *in vacuo* to give the pure methoxymethyl ether (**5**, 9.39 g, 89%) as a white solid. mp = 117 °C; FT-IR (ATR) ν_{max}: 2928, 2854, 1731, 1606 and 1509 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 0.93 (s, 18-CH_3), 3.53 (s, 3-$OCH_2O\mathbf{CH_3}$) 3.87 (s, 2-OCH_3), 5.21 (s, 3-$O\mathbf{CH_2}OCH_3$), 6.85 (s, 4-H), 6.90 (s, 1-H). Anal. Calcd for $C_{21}H_{28}O_4$: C, 73.23; H, 8.19. Found: C, 73.09; H, 8.16.

2.1.5. *2-Methoxy-3-methoxymethoxy-17α-cyano-17β-trimethylsilyloxyestra-1,3,5(10)triene*

(6)

To a solution of the 3-methoxymethyl ether (**5**, 4.9 g, 14.3 mmol) in chloroform (40 ml) were added zinc iodide (20 mg, 0.063 mmol) and trimethylsilylcyanide (3.0 ml, 23.5 mmol) and the reaction mixture stirred at room temperature overnight. The reaction mixture was quenched with water, concentrated *in vacuo*, and extracted with ethyl acetate (3×). The organic phase was washed with water (3×), brine, dried over Na_2SO_4, and concentrated *in vacuo* to give 7.0 g of residue. Analysis by ^1H NMR showed an incomplete reaction. The residue was re-reacted for another 4 h, quenched and extracted as above and purified by flash column (2% acetone/CH_2Cl_2) to give 2.51 g of material. Analysis by ^1H NMR showed the loss of the 3-methoxymethyl ether.

In this material, the 3-hydroxyl function was re-protected by reaction with chloromethyl methyl ether and *N*,*N*-diisoproplyethylamine in THF at 65 °C overnight. The reaction was cooled to room temperature, quenched with 20% NH_4Cl,

and extracted with ethyl acetate (3×). The organic fractions were washed with water (3×), brine, dried over Na_2SO_4, and concentrated *in vacuo* to give the pure 3-methoxymethoxy-17α-cyano product (**6**, 2.78 g, 40%) as a yellow foam. mp = 63 °C; FT-IR (ATR) ν_{max}: 2929, 1608, and 1507 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 0.20 (s, 17B-OSiMe_3), 0.79 (s, 18-CH_3), 3.45 (s, 3-$OCH_2O\mathbf{CH_3}$) 3.8 (s, 2-OCH_3), 5.12 (s, 3-$O\mathbf{CH_2}OCH_3$), 6.74 (s, 4-H), 6.76 (s, 1-H). Anal. Calcd for $C_{25}H_{37}NO_4Si\cdot(2/5)H_2O$: C, 66.60; H, 8.45; N, 3.11. Found: C, 66.56; H, 8.17; N, 3.24.

2.1.6. *2-Methoxy-3-methoxymethoxy-17α-aminomethylestra-1,3,5(10)-trien-17β-ol* (*7*)

Under nitrogen, the 17α-cyano compound (**6**, 2.7 g, 6.26 mmol) in THF (40 ml) was slowly added to an ice cold solution of $LiAlH_4$ (1 M in THF, 12.5 ml, 12.5 mmol). After the addition was complete, the reaction was allowed to come to room temperature and stirred overnight. The reaction mixture was chilled to 0 °C, quenched with saturated sodium sulfate, then solid sodium sulfate. The resulting mixture was filtered through Celite and washed (3×) with THF. The solution was concentrated *in vacuo* and the residue extracted with ethyl acetate (3×). The organic phase washed with water (3×), brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column to give the pure 17α-aminomethyl (**7**, 1.72 g, 75%) as a yellow solid. mp = 114 - 115 °C; FT-IR (ATR) ν_{max}: 3350, 2918, 1607, and 1513 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 0.8 (s, 18-CH_3), 1.274 (d, J = 7.5 Hz, 1H), 3.40 (s, 3-$OCH_2O\mathbf{CH_3}$) 3.75 (s, 2-OCH_3), 5.08 (s, 3-$O\mathbf{CH_2}OCH_3$), 6.73 (s, 4-H), 6.75 (s, 1-H). Anal. Calcd for $C_{22}H_{33}NO_4$: C, 70.37; H, 8.86. Found: C, 70.22; H, 8.82.

2.1.7. *2-Methoxy-3-methoxymethoxy-D-homoestra-1,3,5(10)-trien-17a-one* (*8*) *and 2-methoxy-3-methoxymethoxy-D-homoestra-1,3,5(10)-trien-17-one* (*9*)

Under nitrogen, the 17α-aminomethyl compound (**7**, 1.72 g, 4.58 mmol) was dissolved in 48 ml of 5:1 HOAc/H_2O and chilled to 0 °C. Sodium nitrite (1.67 g, 24.2 mmol) in 7 ml of H_2O was added dropwise over 1/2 h, the solution was then stirred an additional 3 h at 0 °C, then warmed to room temperature and stirred overnight. Analysis by TLC (5% i-prOH/CH_2Cl_2) indicated a complete reaction. The solvent was removed and the residue extracted with ethyl acetate (3×). The organic phase was washed with water (3×), brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column (4:1 ether/hexanes) to give the pure 17a and 17-ketone (**8**, 1.0 g, 61% and **9**, 90 mg, 5.5%, respectively) as white solids. mp (**8**) = 115 °C; FT-IR (ATR) ν_{max}: 2936, 2866, 1697, 1606 and 1505 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 1.116 (s, 18-CH_3), 3.502 (s, 3-$OCH_2O\mathbf{CH_3}$) 3.850 (s, 2-OCH_3), 5.183 (s, 3-$O\mathbf{CH_2}OCH_3$), 6.843 (s, 4-H), 6.855 (s, 1-H). Anal. Calcd for $C_{22}H_{30}O_4$: C, 73.71; H, 8.44. Found: C, 73.06; H, 8.29.

mp (**9**) = 104–105 °C; FT-IR (ATR) ν_{max}: 2935, 1707, 1608 and 1507 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 0.85 (s, 18-CH_3), 3.53 (s, 3-$OCH_2O\mathbf{CH_3}$) 3.86 (s, 2-OCH_3), 5.21 (s, 3-$O\mathbf{CH_2}OCH_3$), 6.86 (s, 4-H), 6.89 (s, 1-H). Anal. Calcd for $C_{22}H_{30}O_4$: C, 73.71; H, 8.44. Found: C, 73.31; H, 8.28.

2.1.8. *2-Methoxy-D-homoestra-1,3,5(10)-trien-3,17aβ-diol* (*10*) *and 2-methoxy-D-homoestra-1,3,5(10)-trien-3,17aα-diol* (*11*)

Under nitrogen, the 17a-ketone (**8**, 1.0 g, 2.79 mmol) was dissolved in 20 ml of THF and cooled to 0 °C. Lithium tri-*tert*-butoxyaluminum hydride solution (1 M in THF, 5.6 ml, 5.6 mmol) was added dropwise and the solution stirred at room temperature for 2.5 . The reaction was quenched with ethyl acetate, concentrated *in vacuo*, diluted with water, acidified with 1 M HCl, and extracted with ethyl acetate (3×). The organic phase was washed with water (3×), brine, dried over Na_2SO_4, and concentrated *in vacuo* to give a residue that was a mixture of the 17aβ and 17aα alcohols. The residue was separated by flash chromatography (4:1 ether/hexanes) and subsequently hydrolyzed with 6 M HCl at 60 °C. Trituration from ether/hexanes yielded the 17aβ-OH and 17aα-OH compounds, (**10**, 300 mg, 32%) and (**11**, 100 mg, 10%) as white solids. mp (**10**) = 138 °C; FT-IR (ATR) ν_{max}: 3503, 3384, 2927, 2860, 1589, and 1505 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 0.85 (s, 18-CH_3), 3.28 (dd, J_1 = 10.95 Hz, J_2 = 4.65 Hz, 17-H), 3.87 (s, 2-OCH_3), 6.64 (s, 4-H), 6.80 (s, 1-H). Anal. Calcd for $C_{20}H_{28}O_3\cdot(1/4)H_2O$: C, 74.85; H, 8.95. Found: C, 74.73; H, 8.88.

mp (**11**) = 146 °C; FT-IR (ATR) ν_{max}: 3561, 3499, 3271, 2937, 2863, 1608, and 1524 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 0.87 (s, 18-CH_3), 3.43 (m, 17-H), 3.87 (s, 2-OCH_3), 6.64 (s, 4-H), 6.81 (s, 1-H). Anal. Calcd for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.49; H, 8.82.

2.1.9. *2-Methoxy-D-homoestra-1,3,5(10)-trien-3,17β-diol* (*12*)

The 17-ketone (**9**, 90 mg, 0.251 mmol) in 10 ml of THF was reacted similarly with lithium tri-*tert*-butoxyaluminum hydride solution (1 M in THF, 0.5 ml, 0.5 mmol). Purification by flash chromatography (4:1 ether/hexanes), subsequent hydrolysis with 6 M HCl at 60 °C and trituration from ether/hexanes yielded the pure17β-OH compound (**12**, 36 mg, 43%) as a white solid. mp = 207 °C; FT-IR (ATR) ν_{max}: 3544, 3466, 3272, 2925, 1595, 1513 and 1501 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 1.10 (s, 18-CH_3), 3.86 (s, 2-OCH_3), 4.15 (m, 17-H), 6.64 (s, 4-H), 6.80 (s, 1-H). Anal. Calcd for $C_{20}H_{28}O_3\cdot(2/5)H_2O$: C, 74.22; H, 8.97. Found: C, 74.02; H, 8.66.

2.1.10. *2-Methoxy-3-methoxymethoxyestra-1,3,5(10)-trien-17β-ol* (*13*)

Under nitrogen, sodium borohydride (1.3 g, 34.35 mmol) in 1:1 EtOH:H_2O (65 ml) was added dropwise to a solution of the 17-ketone (**5**, 4.76 g, 13.74 mmol) in 1:1 THF:EtOH (250 ml) and stirred at room temperature for 1/2 h. Analysis by TLC (5% acetone/CH_2Cl_2) indicated a complete reaction. The reaction was chilled in an ice bath, quenched with acetic acid until acidic, then concentrated *in vacuo*. The residue was diluted with water, acidified with 6 M HCl, and extracted with ethyl acetate (3×). The organic phase was washed with water, brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash chromatography (5% acetone/CH2Cl_2) to give the 17-hydroxy compound (**13**, 4.74 g, 99%) as a yellow semisolid that still contained some solvent. FT-IR (ATR) ν_{max}: 3422, 2923, 2866, 1608 and 1507 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 0.79 (s, 18-CH_3), 3.51 (s, 3-$OCH_2O\mathbf{CH_3}$) 3.73 (t, J = 8.4 Hz,

17-H), 3.85 (s, 2-OCH_3), 5.19 (s, 3-O**CH_2**OCH_3), 6.84 (s, 4-H), 6.87 (s, 1-H). Anal. Calcd for $C_{21}H_{30}O_4 \cdot (1/6)H_2O$: C, 72.18; H, 8.75. Found: C, 72.09; H, 8.52.

2.1.11. *2-Methoxy-3-methoxymethoxyestra-1,3,5(10)-trien-17β-yl-tosylate* (**14**)

Under nitrogen, *p*-toluenesulfonic anhydride (97%, 5.5 g, 16.3 mmol) was added to a solution of the 17-hydroxy compound (**13**, 4.74 g, 13.7 mmol) in anhydrous pyridine (80 ml) at 0 °C and stirred for 2 h. Analysis by TLC (5% acetone/CH_2Cl_2) indicated a complete reaction. The reaction mixture was quenched with water and extracted with ethyl acetate (3×). The organic phase was washed with cold 2 M HCl, water, cold saturated $NaHCO_3$ solution, brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash column (2% acetone/CH_2Cl_2) and crystallization from acetone/hexanes afforded the 17-tosylate (**14**, 6.7 g, 95%) as a white solid. mp = 115 °C; FT-IR (ATR) ν_{max}: 2969, 2922, 2868, 1598, and 1515 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 0.83 (s, 18-CH_3), 2.46 (s, OTs-CH_2), 3.50 (s, 3-OCH_2O**CH_3**), 3.84 (s, 2-OCH_3), 4.36 (t, J = 8.1 Hz, 17-H), 5.18 (s, 3-O**CH_2**OCH_3), 6.79 (s, 4-H), 6.85 (s, 1-H), 7.34 (d, J = 3.9 Hz, OTs-Ar-H), 7.80 (d, J = 4.0 Hz, OTs-Ar-H). Anal. Calcd for $C_{28}H_{36}O_6S$: C, 67.17; H, 7.25; S, 6.40. Found: C, 67.02; H, 6.98; S, 6.35.

2.1.12. *2-Methoxy3-hydroxy-17-methylgona-1,3,5(10),13(17)-tetraene* (**15**)

Under nitrogen, the 17-tosylate derivative (**14**, 6.23 g, 12.5 mmol) in benzene (125 ml) was added dropwise to a solution of ethylmagnesium bromide (3M in ether, 53 ml, 159 mmol). Once the addition was complete, the ether was distilled off, the solution refluxed for 3 h, then stirred overnight at room temperature. The reaction was cooled to 0 °C, quenched with the dropwise addition of cold 2 M H_2SO_4, and extracted with ethyl acetate (3×). The organic phase was washed with cold saturated $NaHCO_3$ solution, water, brine, dried over Na_2SO_4 and concentrated *in vacuo*. Purification by flash column (2% acetone/CH_2Cl_2) and crystallization from acetone/hexanes afforded the17-methyl product (**15**, 2.86 g, 79%) as a white solid. mp = 108 °C; FT-IR (ATR) ν_{max}: 3444, 2911, 2855, 2832, 1591, and 1505 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 1.65 (s, 17-CH_3), 3.87 (s, 2-OCH_3), 5.42 (s, 3-OH), 6.64 (s, 4-H), 6.83 (s, 1-H). Anal. Calcd for $C_{19}H_{24}O_2$: C, 80.24; H, 8.51. Found: C, 79.86; H, 8.14.

2.1.13. *2-Methoxy-3-methoxymethoxy-21-trimethylsilyl-19-norpregna-1,3,5(10),16-tetraene-20-yne* (**16a**) *and 2-methoxy-3-hydroxy-21-trimethylsilyl-19-norpregna-1,3,5(10),16-tetraene-20-yne* (**16b**)

Under nitrogen, a solution of trimethylsilylacetylene (1 ml, 7 mmol) in THF (10 ml) was cooled to −78 °C. Butyl lithium (1.6 M in hexanes, 4.4 ml, 7 mmol) was slowly added and the mixture stirred at −78 °C for 10 min, and then allowed to warm to 0 °C in an ice bath. After stirring at 0 °C for 1/2 h, the mixture was cooled again to −78 °C and transferred to a solution of the ketone (**5**, 1 g, 2.9 mmol) in 12 ml of 2:1 THF/benzene, also at −78 °C. The reaction mixture was stirred for 15 min then allowed to warm to room temperature. After stirring at room temperature for 2 h, TLC (sat. NH_4Cl aliquot, 2% acetone/CH_2Cl_2) indicated complete formation of the lithium intermediate. Methanesulfonyl chloride (1 ml, 4.77 mmol) was added followed 1/2 h later by pyridine (2 ml, 1.8 eq). The reaction mixture was then stirred overnight at room temperature. The reaction mixture was quenched with saturated $NaHCO_3$ solution and extracted with ethyl acetate (3×). The organic phase was washed with water, 1 M HCl (2×), water, brine, dried over Na_2SO_4, and concentrated *in vacuo* to give 1.6 g of a dark violet residue. The material was passed through silica gel (2% acetone/CH_2Cl_2) to give a crude mixture composed mainly of the 3-methoxymethyl (**16a**) and 3-hydroxy (**16b**) derivatives. This mixture was used directly for the next reaction.

2.1.14. *2-Methoxy-17-ethynylestra-1,3,5(10),16-tetraen-3-ol* (**17**)

Under nitrogen, the crude 21-trimethylsilyl mixture (**16**, 0.85 g, ~2 mmol) was dissolved in 15 ml of THF. Tetrabutylammonium fluoride (1 M/THF, 3.7 ml, 3.7 mmol) was added and the reaction stirred at room temperature for 2 h. The reaction was quenched with water and extracted with ethyl acetate (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo* to give 0.65 g of a brown foam. The residue was twice purified by flash column (1% acetone/CH_2Cl_2), then (7:3 CH_2Cl_2/hexanes) to give 120 mg of material. The material was twice crystallized from acetone/hexanes to give the pure product (**17**, 40 mg, 6.4%). mp = 202 °C; FT-IR (ATR) ν_{max}:3490, 3293, 2932, 1591, and 1504 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 0.895 (s, 18-CH_3), 1.549 (s, 17-CCH), 3.874 (s, 2-OCH_3), 6.158 (t, J = 2.55 Hz, 16-H), 6.658 (s, 4-H), 6.794 (s, 1-H). Anal. Calcd for $C_{21}H_{24}O_2$: C, 81.78; H, 7.84. Found: C, 81.73; H, 7.97.

2.1.15. *17,17a-Dimethyl-3-methoxy-D-homogona-1,3,5(10),13,15,17(17a)-hexaene* (**19**)

Compound (**19**) was prepared from mestranol (**18**) by reacting with formic acid as reported in the literature [11].

2.1.16. *17,17a-Dimethyl-3-hydroxy-D-homogona-1,3,5(10),13,15,17(17a)-hexaene* (**20**)

Under nitrogen, the 3-methoxy derivative (**19**, 1.0 g, 3.41 mmol) was dissolved in toluene (10 ml) and treated with DIBAL (1 M/toluene, 17 ml, 17 mmol) at 95 °C overnight. Analysis by TLC (5% acetone/CH_2Cl_2) showed a complete reaction. The reaction mixture was quenched with 1:1 ethanol/water, the resulting precipitate was dissolved with 5% HCl and extracted with ethyl acetate (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by flash column (2% acetone/CH_2Cl_2) gave the 3-hydroxy compound (**20**, 0.73 g, 76%) as a white solid. mp = 177 °C; FT-IR (ATR) ν_{max}: 3372, 2918, 2828, and 1607 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 2.186 (s, 17-H), 2.30 (s, 17α-H), 4.82, (s, 3-OH), 6.62 (d, J = 2.7 Hz, 4-H), 6.68 (dd, J_1 = 8.1 Hz, J_2 = 3.0 Hz, 2-H), 7.05 (d, J = 9.6 Hz, 1-H), 7.20 (d, J = 8.1 Hz, 15-CH_3), 7.28 (d, J = 8.4 Hz, 16-H). Anal. Calcd for

$C_{20}H_{22}O\cdot(1/10)CH_2Cl_2$: C, 84.15; H, 7.80. Found: C, 84.08; H, 7.86.

2.1.17. *17,17a-Dimethyl-3-methoxymethoxy-D-homogona-1,3,5(10),13,15,17(17a)-hexaene* (**21**)

Under nitrogen, the 3-hydroxy compound (**20**, 0.9 g, 3.23 mmol) was dissolved in 15 ml of dry THF. Chloromethyl methyl ether (1.25 ml, 16.5 mmol) and diisopropylethylamine (3.4 ml, 19.5 mmol) were added and the reaction mixture heated to 65 °C overnight. Analysis by TLC (2% acetone/CH_2Cl_2) showed a complete reaction. The reaction mixture was cooled to room temperature, quenched with 20% NH_4Cl solution, and extracted with ethyl acetate (3×). The organic phase was washed with water (3×), brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by flash column (1.5% acetone/CH_2Cl_2) gave the 3-methoxymethoxy ether product (**21**, 0.8 g, 77%) as a white solid. mp = 102 °C; FT-IR (ATR) ν_{max}: 2953, 2826, 1613 and 1575 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 2.16 (s, 17-H), 2.27 (s, 17α-H), 3.47 (s, 3-$OCH_2O\mathbf{CH_3}$), 5.18 (m, 3-$O\mathbf{CH_2}OCH_3$), 6.84 (d, J = 2.4 Hz, 4-H), 6.88 (dd, J_1 = 8.7 Hz, J_2 = 3.0 Hz, 2-H), 7.02 (d, J = 8.1 Hz, 1-H), 7.18 (d, J = 8.4 Hz, 15-CH_3), 7.30 (d, J = 8.4 Hz, 16-H). Anal. Calcd for $C_{22}H_{26}O_2\cdot(1/4)H_2O$: C, 80.82; H, 8.17. Found: C, 80.96; H, 7.97.

2.1.18. *17,17a-Dimethyl-2-hydroxy-3-methoxymethoxy-D-homogona-1,3,5(10),13,15,17(17a)-hexaene* (**22**)

Under nitrogen, the 3-methoxymethoxy ether (**21**, 0.8 g, 2.48 mmol) was dissolved in 20 ml of dry THF and chilled to -78 °C. To this was added sec-BuLi (1.4 M/cyclohexane, 3.5 ml, 4.9 mmol) such that the temperature did not exceed −65 °C and the reaction mixture was stirred at −78 °C for 3 h. Trimethyl borate (1.1 ml, 9.9 mmol) was then added such that the temperature did not exceed −65 °C and the mixture stirred for 1 h at −78 °C. The reaction mixture was quenched with 15 ml of 20% NH_4Cl solution, allowed to come to room temperature, and stirred for 1 h. Sodium perborate tetrahydrate (1.5 g, 9.7 mmol) was then added at such a rate that the temperature did not exceed 35 °C and the reaction mixture was stirred at room temperature overnight. The reaction mixture was quenched with water and extracted with ethyl acetate (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash column (8:2 CH_2Cl_2/hexanes) to give the 2-hydroxy compound (**22**, 0.26 g, 31%) as a white solid. mp = 145 - 146 °C; FT-IR (ATR) ν_{max}: 3354, 2949, 1618, and 1508 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 2.16 (s, 17-H), 2.27 (s, 17α-H), 3.50 (s, 3-$OCH_2O\mathbf{CH_3}$), 5.15 (m, 3-$O\mathbf{CH_2}OCH_3$), 6.85 (s, 1-H), 7.02 (d, J = 7.8 Hz, 15-CH_3), 7.17 (d, J = 8.4 Hz, 16-H). Anal. Calcd for $C_{22}H_{26}O_3\cdot(1/3)H_2O$: C, 76.73; H, 7.80. Found: C, 76.76; H, 7.59.

2.1.19. *17,17a-Dimethyl-2-methoxy-3-methoxymethoxy-D-homogona-1,3,5(10),13,15,17(17a)-hexaene* (**23**)

To a solution of the 2-hydroxy (**22**, 0.46 g, 1.36 mmol) in DMF (20 ml) was added potassium carbonate (1.8 g, 13 mmol) and tetrabutylammonium iodide (30 mg, .08 mmol) and the reaction mixture stirred for 15 min. Methyl iodide (1.9 ml, 30.5 mmol) was added and the mixture stirred at 45 °C for 4 h then stirred overnight at room temperature. Analysis by TLC (7:3 ether/hexanes) showed a complete reaction. The reaction mixture was quenched with water and extracted with ethyl acetate (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by flash column (7:3 ether/hexanes) gave the 2-methoxy derivative (**23**, 0.49 g, >100%) as a white foam. mp = 82 °C; FT-IR (ATR) ν_{max}: 2917, 2857, 1608 and 1514 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 2.17 (s, 17-H), 2.28 (s, 17α-H), 3.52 (s, 3-$OCH_2O\mathbf{CH_3}$), 3.89 (s, 2-OCH_3), 5.21 (m, 3-$O\mathbf{CH_2}OCH_3$), 6.94 (s, 4-H), 6.95 (s, 1-H), 7.03 (d, J = 8.7 Hz, 15-CH_3), 7.20 (d, J = 8.1 Hz, 16-H). Anal. Calcd for $C_{23}H_{28}O_3$: C, 78.38; H, 8.01. Found: C, 78.07; H, 8.08.

2.1.20. *17,17a-Dimethyl-2-methoxy-3-hydroxy-D-homogona-1,3,5(10),13,15,17(17a)-hexaene* (**24**)

Under nitrogen, the 3-methoxymethoxy ether (**23**, 0.46 g, 1.3 mmol) was hydrolyzed with 6 M HCl (15 ml) in THF (20 ml) at room temperature over the weekend. Analysis by TLC (7:3 ether/hexanes) indicated a complete reaction. The reaction mixture was quenched with water and extracted with ethyl aceate (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by flash column (1% acetone/CH_2Cl_2) and subsequent crystallization from ether gave the 3-hydroxy derivative (**24**, 0.35 g, 87%) as a white solid. mp = 177 °C; FT-IR (ATR) ν_{max}: 3511, 2920, 2841, 1592, and 1514 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 2.19 (s, 17-H), 2.30 (s, 17α-H), 3.91 (s, 2-OCH_3), 5.48 (s, 3-OH), 6.72 (s, 4-H), 6.91 (s, 1-H), 7.05 (d, J = 8.1 Hz, 15-CH_3), 7.22 (d, J = 7.8 Hz, 16-H). Anal. Calcd for $C_{21}H_{24}O_2$: C, 81.78; H, 7.84. Found: C, 81.05; H, 7.75.

2.1.21. *2-Methoxy-3-acetoxy-17-methylgona-1,3,5(10),13(17)-tetraene* (**25**)

Under nitrogen, the 3-hydroxy compound (**15**, 14.2 g, 50 mmol) was acetylated in pyridine (250 ml) with acetic anhydride (60 ml, 634.8 mmol) at room temperature overnight. Analysis by TLC (1% acetone/CH_2Cl_2) indicated a complete reaction. The reaction mixture was quenched with methanol and concentrated *in vacuo* to give the 3-acetate derivative (**25**, 17.5 g, 100%) as a yellow solid. This material was used in the subsequent reaction without further purification. mp = 91 °C; FT-IR (ATR) ν_{max}: 2924, 2837, 1762, 1615, and 1508 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 1.6 (s, 17-CH_3), 2.30 (s, 3-OAc), 3.80 (s, 2-OCH_3), 6.72 (s, 4-H), 6.92 (s, 1-H). Anal. Calcd for $C_{21}H_{26}O_3$: C, 77.27; H, 8.03. Found: C, 77.20; H, 7.96.

2.1.22. *(8s,4bS,8aR)-3-Methoxy-7-oxo-8-(3-oxobutyl)-5,6,8,9,10,4b,8a-heptahydrophenanthra-2-yl acetate* (**26**)

Under nitrogen, the 3-acetate derivative (**25**, 2.0 g, 6.1 mmol) in 3:1 dioxane/water (10 ml) was treated with a solution of OsO_4 (6.2 mg, 0.024 mmol) in *t*-BuOH (2 ml). Sodium periodate (0.52 g, 2.43 mmol) and pyridine (0.1 ml, 1.24 mmol) were added and the mixture was heated at 65–70 °C for 3 h and then stirred at room temperature overnight. Analysis by TLC (CH_2Cl_2) indicated an incomplete reaction. Additional OsO_4 in *t*-BuOH (3 mg in 1 ml, 0.012 mmol) was added and the reaction heated at 65–70 °C for 4 h. Analysis by TLC showed disappearance of the starting material. The reaction mixture was cooled to

room temperature, quenched with water, and extracted with EtOAc (3×). The organic fractions were washed with water (3×) and brine, combined, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash chromatography (5% acetone/CH_2Cl_2) gave compound (**26**, 0.9 g, 41%) as a white solid. mp = 111–112 °C; FT-IR (ATR) ν_{max}: 2950, 2863, 1760, 1704, 1615 and 1509 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 2.14 (s, 3-oxobutyl CH_3), 2.30 (s, 2-OAc), 3.80 (s, 3-OCH_3), 6.76 (s, 1-H), 6.89 (s, 4-H). Anal. Calcd for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.44; H, 7.38.

2.1.23. *(10bS,4aS,4bS)-8-Hydroxy-9-methoxy-3,4,5,6,11,12,10b,4a,4b-nonahydrochrysen-2-one* (**27**)

Under nitrogen, the diketone compound (**26**, 3 g, 8.4 mmol) was cyclized in methanol (100 ml) with 10% KOH solution (40 ml) at reflux for 2.5 h. Analysis by TLC (5% acetone/CH_2Cl_2) indicated a complete reaction. The reaction mixture was quenched with water, acidified with 10% HCl, concentrated *in vacuo*, and extracted with EtOAc (3×). The organic fractions were washed with water (3×) and brine, combined, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give the cyclized compound (**27**, 2.4 g, 99%) as a yellow solid. mp = 211–213 °C; FT-IR (ATR) ν_{max}: 3267, 2923, 2863, 1658, and 1505 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 3.86 (s, 9-OCH_3), 5.91 (s, 1-H) 6.64 (s, 7-H), 6.78 (s, 10-H). Anal. Calcd for $C_{19}H_{22}O_3$: C, 76.48; H, 7.43. Found: C, 76.48; H, 7.48.

2.1.24. *(10aS,10bS,4bS)-3-Methoxy-8-oxo-5,6,9,10,11,12,10a,10b,4b-nonahydrochrysen-2-yl acetate* (**28**)

Under nitrogen, the 3-hydroxy compound (**27**, 0.62 g, 2.0 mmol) was acetylated in pyridine (50 ml) with acetic anhydride (10 ml, 105.8 mmol) at room temperature overnight. Analysis by TLC (3% acetone/CH_2Cl_2) indicated a complete reaction. The reaction mixture was quenched with methanol and concentrated *in vacuo*. Purification by flash chromatography (3% acetone/CH_2Cl_2) and subsequent crystallization from ether gave the 2-acetate derivative (**28**, 0.36 g, 51%) as a white solid. mp = 139 °C; FT-IR (ATR) ν_{max}: 2916, 2860, 1759, 1665, 1615 and 1508 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 2.30 (s, 2-OAc), 3.81 (s, 3-OCH_3), 5.90 (s, 7-H) 6.76 (s, 1-H), 6.89 (s, 4-H). Anal. Calcd for $C_{21}H_{24}O_4 \cdot (1/5)H_2O$: C, 73.32; H, 7.15. Found: C, 73.37; H, 7.01.

2.1.25. *(4bS,10bR)-8-Hydroxy-3-methoxy-5,6,11,12,10b,4b-hexahydrochrysene-2-yl acetate* (**29**)

Under nitrogen, compound (**28**, 2.3 g, 5.3 mmol) in CH_3CN (150 ml) was aromatized with $CuBr_2$ (1.4 g, 6.3 mmol) at room temperature for 24 h. Analysis by TLC (5% acetone/CH_2Cl_2) indicated a complete reaction. The reaction mixture was quenched with water and extracted with EtOAc (3×). The organic fractions were washed with water (3×) and brine, combined, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash chromatography (5% acetone/CH_2Cl_2) and subsequent crystallization from ether gave the hexahydrochrysene derivative (**29**, 1.6 g, 70%) as a white solid. mp = 199–200 °C; FT-IR (ATR) ν_{max}: 3455, 2928, 2839, 1745, 1610, and 1503 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 2.33 (s, 2-OAc), 3.84 (s, 3-OCH_3), 5.13 (s, 8-OH), 6.59 (d, J = 2.7 Hz, 7-H), 6.62 (dd, J_1 = 8.25 Hz, J_2 = 3.0 Hz, 9-H), 6.80 (s, 1-H), 6.96 (s, 4-H), 7.20 (d, J = 8.4 Hz, 10-H). Anal. Calcd for $C_{21}H_{22}O_4$: C, 74.54; H, 6.55. Found: C, 74.11; H, 6.58.

2.1.26. *(4bS,10bR)-3-Methoxy-5,6,11,12,10b,4b-hexahydrochrysene-2,8-diol* (**30**)

Under nitrogen, the 3-acetate compound (**29**, 0.17 g, 0.5 mmol) was hydrolyzed in MeOH/H_2O (60 ml, 5:1) with potassium carbonate (1.9 g, 13.7 mmol) at room temperature for 2 h. Analysis by TLC (5% acetone/CH_2Cl_2) indicated a complete reaction. The reaction mixture was quenched with water and extracted with EtOAc (3×). The organic fractions were washed with water (3×) and brine, combined, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash chromatography (5% acetone/CH_2Cl_2) and subsequent crystallization from ether/hexanes gave compound (**30**, 130 mg, 88%) as a white solid. mp = 225 °C; FT-IR (ATR) ν_{max}: 3458, 3312, 2922, 2829, 1614, 1584, 1497, and 1463 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 3.89 (s, 3-OCH_3), 4.74 (s, 2-OH), 5.49 (8-OH), 6.63 (d, J = 3.0 Hz, 7-H), 6.68 (dd, J_1 = 8.4 Hz, J_2 = 3.0 Hz, 9-H), 6.71 (s, 1-H), 6.87 (s, 4-H), 7.25 (d, J = 8.4 Hz, 10-H). Anal. Calcd for $C_{19}H_{20}O_3 \cdot (1/2)H_2O$: C, 74.73; H, 6.93. Found: C, 74.82; H, 6.70.

2.1.27. *(4bS,10bR)-2,8-bis(Methoxymethoxy)-3-methoxy-5,6,11,12,10b,4b-hexahydrochrysene* (**31**)

Under nitrogen, the 2,8-diol derivative (**30**, 1 g, 3.4 mmol) in THF (100 ml) was treated with *N*,*N*-diisopropylethylamine (2.1 ml, 5.75 mmol) and chloromethyl methyl ether (6 ml, 79 mmol) and the reaction mixture stirred at 65–70 °C overnight. Analysis by TLC (5% acetone/CH_2Cl_2) indicated a complete reaction. The reaction mixture was cooled to room temperature, quenched with 20% NH_4Cl solution, concentrated *in vacuo*, and extracted with EtOAc (3×). The organic fractions were washed with water (3×) and brine, combined, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash chromatography (5% acetone/CH_2Cl_2) gave the dimethoxymethyl ether derivative (**31**, 1.1 g, 88%) as a low melting solid. mp = 83–84 °C; FT-IR (ATR) ν_{max}: 2908, 2834, 1606, and 1498 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 3.48 (s, 8-OCH_2OCH_3), 3.52 (s, 2-OCH_2OCH_3) 3.88 (s, 3-OCH_3), 5.16 (s, 8-OCH_2OCH_3), 5.21 (s, 2-OCH_2OCH_3), 6.84 (d, J = 2.4 Hz, 7-H), 6.88 (dd, J_1 = 8.4 Hz, J_2 = 2.7 Hz, 9-H), 6.92 (s, 1-H), 6.93 (s, 4-H). 7.30 (d, J = 8.4 Hz, 10-H). Anal. Calcd for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 71.68; H, 7.32.

2.1.28. *(10bS,4bR)-2,8-bis(Methoxymethoxy)-9-methoxy-5,6,11,12,10b,4b-hexahydrochrysen-3-ol* (**32**)

Under nitrogen, the dimethoxymethyl ether derivative (**31**, 0.5 g, 1.3 mmol) in anhydrous THF (10 ml) at −78 °C was treated with the dropwise addition of *sec*-BuLi (1.4 M/cyclohexane, 1.8 ml, 2.52 mmol) and the reaction stirred for 3 h. Trimethyl borate (0.6 ml, 5.4 mmol) was added dropwise and the reaction mixture stirred for 20 min. The reaction mixture was warmed to 0 °C and quenched with 20% NH_4Cl solution (10 ml) and stirred at room temperature for 1 h. Sodium perborate tetrahydrate (0.8 g, 5.2 mmol) was added and the reaction

mixture stirred at room temperature overnight. The mixture was extracted with EtOAc (3×), the organic fractions were washed with water (3×) and brine, combined, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash chromatography (5% acetone/CH_2Cl_2) gave a mixture of the starting material (0.14 g) and the 16-hydroxy derivative (**32**, 0.31 g, 84%), which crystallized from ether/hexanes as a white solid. mp = 117–119 °C; FT-IR (ATR) ν_{max}: 3440, 2909, 2838, 1608, and 1502 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 3.50 (s, 2-OCH_2OCH_3), 3.52 (s, 8-OCH_2OCH_3) 3.87 (s, 9-OCH_3), 5.15 (s, 2-OCH_2OCH_3), 5.21 (s, 8-OCH_2OCH_3), 5.99 (3-OH), 6.86 (s, 1-H), 6.91 (d, J = 8.4 Hz, 4-H), 6.92 (s, 7-H), 6.98 (s, 10-H). Anal. Calcd for $C_{23}H_{28}O_6$: C, 69.98; H, 7.05. Found: C, 69.83; H, 6.99.

2.1.29. *(4bS,10bR)-2,8-bis(Methoxymethoxy)-3,9-dimethoxy-5,6,11,12,10b,4b-hexahydrochrysene* *(**33**)*

Under nitrogen, the 16-hydroxy derivative (**32**, 0.5 g, 1.24 mmol) in DMF (20 ml) was methylated with methyl iodide (5 ml, 80.3 mmol) in the presence of potassium carbonate (2 g, 14.5 mmol) and tetrabutyl ammonium iodide (20 mg) at room temperature overnight. Analysis by TLC (3% acetone/CH_2Cl_2) indicated an incomplete reaction. Additional methyl iodide (5 ml) was added and the reaction mixture stirred another 2 h. Analysis by TLC indicated no further progress. The reaction mixture was quenched with brine and extracted with EtOAc (3×). The organic fractions were washed with brine, combined, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash chromatography (3% acetone/CH_2Cl_2) gave the dimethoxy compound (**33**, 0.38 g, 73%) as a yellow solid. mp = 151–153 °C; FT-IR (ATR) ν_{max}: 2944, 2913, 2827, 1607, and 1510 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 3.53 (s, 2,8-OCH_2OCH_3), 3.89 (s, 3,9-OCH_3), 5.22 (s, 2,8-OCH_2OCH_3), 6.94 (s, 1,4,7,10-H). Anal. Calcd for $C_{24}H_{30}O_6$: C, 69.55; H, 7.30. Found: C, 69.56; H, 7.23.

2.1.30. *(4bS,10bR)-3,9-Dimethoxy-5,6,11,12,10b,4b-hexahydrochrysene-2,8-diol* *(**34**)*

Under nitrogen, the dimethoxymethyl ether derivative (**33**, 0.5 g, 1.2 mmol) in THF (30 ml) was hydrolyzed with 6M HCl (10 ml) at room temperature overnight. Analysis by TLC (3% acetone/CH_2Cl_2) indicated a complete reaction. The reaction mixture was quenched with water and extracted with EtOAc (3×). The organic fractions were washed with water (3×) and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash chromatography (5% acetone/CH_2Cl_2) and subsequent crystallization from MeOH/EtOAc gave compound (**34**, 0.16 g, 40%) as a white solid. mp = 295 °C (dec); FT-IR (ATR) ν_{max}: 3511, 3346, 2958, 2909, 2841, 1622, and 1500 cm^{-1}. NMR (300 MHz, $(CD_3)_2SO$), δ_H (ppm): 3.73 (s, 3,9-OCH_3), 6.50 (s, 1,7-H), 6.88 (s, 4,10-H). Anal. Calcd for $C_{20}H_{22}O_4 \cdot (1/2)H_2O$: C, 72.60; H, 6.85. Found: C, 72.65; H, 6.67.

2.1.31. *2-Methoxy-3-acetoxyestra-1,3,5(10),14-tetraen-17β-ol* *(**36**)*

To a solution of compound (**35**, 0.75 g, 2.5 mmol) [3] in isopropanol (20 ml) was added 2 M sodium hydroxide (15 ml, 30 mmol) and acetic anhydride (0.7 ml, 7.4 mmol) and the reaction mixture stirred at room temperature for 2 h. Analysis by TLC (5% acetone/CH_2Cl_2) indicated a complete reaction. The reaction was slowly quenched with methanol, diluted with water, concentrated *in vacuo* and extracted with ethyl acetate (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo* to give the 3-acetate derivative (**36**, 0.82 g, 96%) as a white solid. mp = 84 °C; FT-IR (ATR) ν_{max}: 3425, 2927, 2843,1757, 1615, and 1508 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 1.03 (s, 18-CH_3), 2.31 (s, 3-OAc), 3.81 (s, 2-OMe), 4.11 (t, J = 8.2 Hz, 17-H), 5.22 (s, 15-H), 6.77 (s, 4-H), 6.93 (s, 1-H). Anal. Calcd for $C_{21}H_{26}O_4$: C, 73.66; H, 7.65. Found: C, 73.77; H, 7.59.

2.1.32. *2-Methoxy-3-acetoxyestra-1,3,5(10),14-tetraen-17-one* *(**37**)*

Under nitrogen, the 17-hydroxy compound (**36**, 0.82 g, 2.4 mmol) was dissolved in 20 ml of acetone and chilled to 0 °C. Jones reagent was slowly added with stirring until the yellow–orange color persisted (~3 ml). The reaction was stirred an additional five minutes then slowly quenched with isopropanol. The solution was concentrated *in vacuo*, diluted with water, and extracted with ethyl acetate (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo* to give the 17-ketone (**37**, 0.82 g, 100%) as a yellow solid. mp = 133 °C; FT-IR (ATR) ν_{max}: 2966, 2938, 1762, 1734, 1615 and 1508 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 1.18 (s, 18-CH_3), 2.31 (s, 3-OAc), 3.81 (s, 2-OMe), 5.63 (s, 15-H), 6.79 (s, 4-H), 6.89 (s, 1-H). Anal. Calcd for $C_{21}H_{24}O_4 \cdot (1/10)H_2O$: C, 73.70; H, 7.13. Found: C, 73.81; H, 7.06.

2.1.33. *2-Methoxy-3-acetoxyestra-1,3,5(10),14-tetraene-17,17-ethylenethioketal* *(**38**)*

To a solution of the 17-ketone compound (**37**, 0.8 g, 2.4 mmol) in acetic acid (35 ml) was added ethanedithiol (1.9 ml, 22.6 mmol) and boron trifluoride diethyl etherate (1.9 ml, 15.4 mmol) and the reaction mixture stirred at room temperature for ninety minutes. Analysis by TLC (5% acetone/CH_2Cl_2) indicated a complete reaction. The reaction was quenched with water and extracted with ether (3×). The organic phase was washed with saturated $NaHCO_3$ solution (2×), water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification by flash chromatography (CH_2Cl_2) gave the thioketal derivative (**38**, 0.7 g, 71%) as a white foam. mp = 123 °C; FT-IR (ATR) ν_{max}: 2926, 2857, 2835, 1762, 1614, and 1508 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 1.12 (s, 18-CH_3), 2.31 (s, 3-OAc), 3.15-3.37 (m, 17-thioketal CH_2's) 3.82 (s, 2-OMe), 5.47 (s, 15-H), 6.77 (s, 4-H), 6.94 (s, 1-H). Anal. Calcd for $C_{23}H_{28}O_3S_2$: C, 66.31; H, 6.77; S, 15.39. Found: C, 66.33; H, 6.83; S, 15.62.

2.1.34. *2-Methoxyestra-1,3,5(10),14,16-pentaen-3-yl-acetate* *(**39**)*

The thioketal compound (**38**, 0.68 g, 1.63 mmol) in acetone (20 ml) was added to a mixture of deactivated Raney nickel (5 g) in acetone (50 ml) and the mixture refluxed for 8 h. Analysis by TLC (3% acetone/CH_2Cl_2) indicated a complete reaction. The reaction mixture was filtered through Celite,

and concentrated *in vacuo*. The residue was purified by flash chromatography (1:1 ether/hexanes) to give the $\Delta^{14,16}$ derivative (**39**, 60 mg, 9.3%) as a white solid. mp = 88 °C. FT-IR (ATR) ν_{max}: 2924, 2856, 1762, 1614 and 1509 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 1.08 (s, 18-CH_3), 2.31 (s, 3-OAc), 3.81 (s, 2-OMe), 5.92 (s, 15-H), 6.33 (dd, J_1 = 4.8 Hz, J_2 = 1.8 Hz, 16-H), 6.38 (d, J = 2.4 Hz, 17-H), 6.79 (s, 4-H), 6.92 (s, 1-H). Anal. Calcd for $C_{23}H_{28}O_3 \cdot (1/2)H_2O$: C, 75.65; H, 7.56. Found: C, 75.60; H, 7.58.

2.1.35. *2-Methoxy-3-hydroxyestra-1,3,5(10),14,16-pentaene* (*40*)

To a solution of the 3-acetate compound (**39**, 50 mg, 0.15 mmol) in 90% methanol/water (20 ml) was added potassium carbonate (0.2 g, 1.45 mmol) and the reaction mixture stirred at room temperature for 2 h. Analysis by TLC (1:1 ether/hexanes) indicated a complete reaction. The reaction mixture was acidified with 0.5 M HCl and extracted with ether (3×). The organic phase was washed with, water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by flash chromatography (1:1 ether/hexanes) to give the 3-hydroxy compound (**40**, 44 mg, 100%) as a clear oil. FT-IR (ATR) ν_{max}: 3549, 2923, 2854, 1592, and 1505 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ (ppm): 1.08 (s, 18-CH_3), 3.87 (s, 2-OMe), 5.92 (s, 15-H), 6.33 (dd, J_1 = 5.1 Hz, J_2 = 1.8 Hz, 16-H), 6.37 (d, J = 2.4 Hz, 17-H), 6.70 (s, 4-H), 6.82 (s, 1-H). Anal. Calcd for $C_{19}H_{22}O_2 \cdot (1/4)H_2O$: C, 79.55; H, 7.91. Found: C, 79.51; H, 7.92.

2.2. *Biological assays*

Biological assays were carried out by EntreMed Inc., Rockville MD.

2.3. *Cell cultures*

Human umbilical vein endothelial cells (HUVEC) were obtained from Clonetics (San Diego, CA), MDA-MB-231, and U87-MG cells were obtained from ATCC (American Type Culture Collection, Manassas, VA). HUVEC cultures were maintained for up to five passages in EGM (Endothelial Growth Medium) containing bovine brain extract (Clonetics) and 1× antibiotic-animycotic (BioWhittaker, Walkersville, MD). MDA-MB-231, and U87-MG cells were maintained in DMEM/F12 (1:1) containing 10% (v/v) fetal bovine serum (Hyclone Laboratories, Logan, UT) and 1× antibiotic–antimycotic.

2.4. *In vitro inhibition of proliferation*

Proliferation assays were performed by evaluating detection of DNA synthesis by the use of the 5-bromo-2′-deoxyuridine (BrdU) cell proliferation colorimetric ELISA kit from Roche

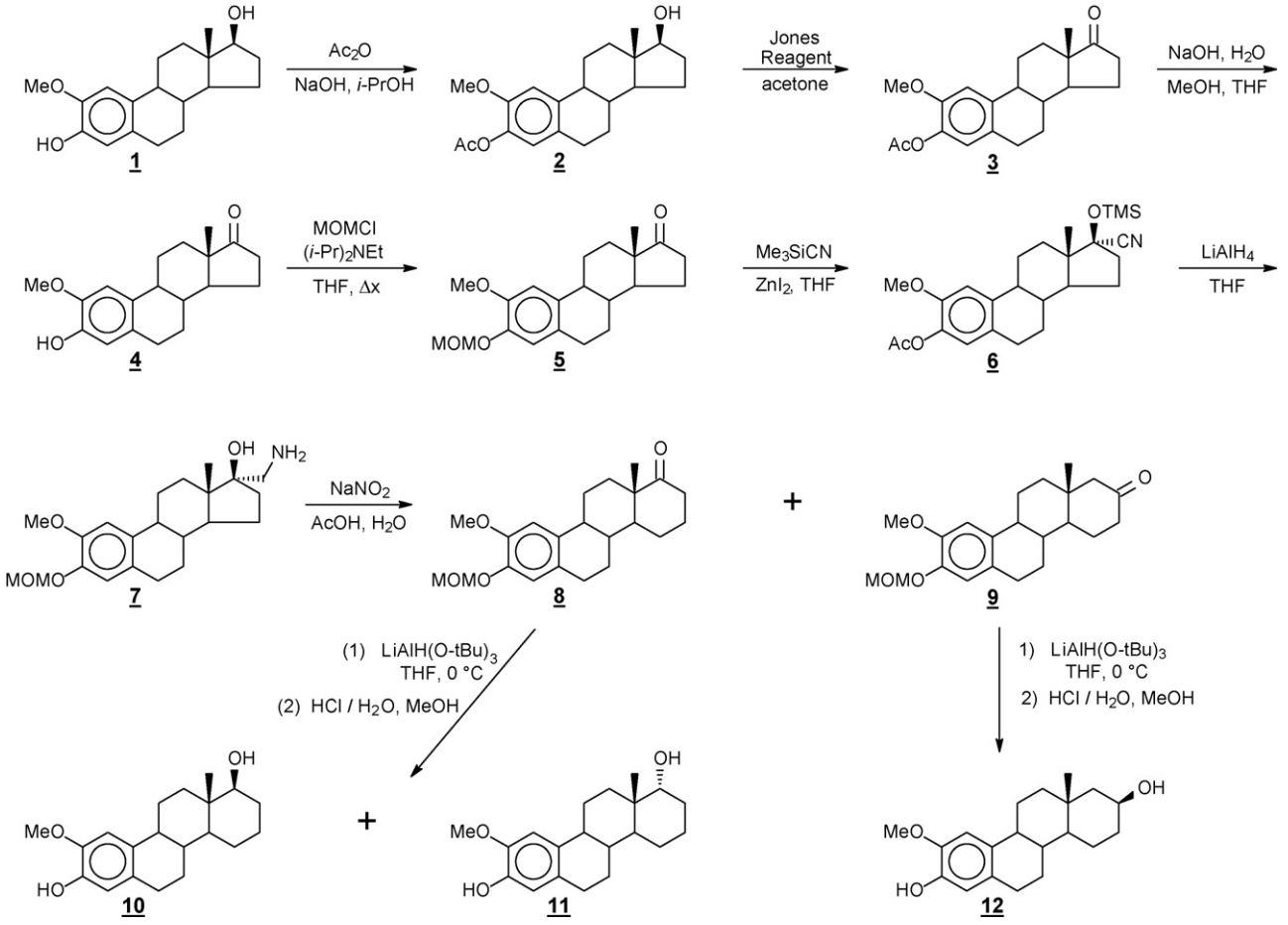


Fig. 1 – Synthesis of 2-methoxy-D-homoestrogens.

(Indianapolis, IN) according to the manufacturer's instructions. The cells were seeded at 1,000 cells/well (MDA-MB-231 and U87-MG cells, anti-tumor activity) or 3,000 cells/well (HUVEC, anti-angiogenic activity) in a 96 well plate, allowed to attach for 24 h and then exposed to the compound to be tested for 48 h. IC50 values and standard deviation (S.D.) were calculated for each assay using Sigma Plot (Systat Software Inc., San Jose, CA). Reported IC50 values are the mean and S.D. of a minimum of three separate assays.

2.5. *Computational analysis*

All calculations were carried out using the MM_3 forcefield [×] as implemented by PCMODEL (Version 9.1, Serena Software, Bloominton, IN). After initial input of basic starting geometries, the conformational space of each compound was explored by means of the GMMX routine (Global Energy Minimization) in PCMODEL using the MM_3 forcefield, and an energy window of 20 kcal/mol. In determining the global minima, all degrees of conformational freedom were considered including ring conformations and rotatable bond orientations. The overlap of energy minimized structures depicted in Fig. 6 was obtained by deleting the hydrogen atoms of the minimized structures and superimposing the A-ring carbon atoms of each analog with those of 2ME2 using the RMS Fit algorithm as implemented in Alchemy III (Tripos Inc., St. Louis, MO).

3. Results and discussion

3.1. *Chemistry*

The syntheses of the D-homo analogs of 2ME2 are outlined in Fig. 1 and are based on the Tiffeneau–Demjanov rearrangement as carried out by Avery et al. [7]. Reaction of 2-methoxyestradiol (**1**) with sodium hydroxide and acetic anhydride in isopropanol selectively gave the 3-acetate compound (**2**) in quantitative yield. Oxidation of (**2**) using Jones reagent in acetone at 0 °C afforded the 17-ketone derivative (**3**) in 91% yield. Hydrolysis of this material with sodium hydroxide in THF/methanol gave 2-methoxyestrone (**4**) in quantitative yield. Treatment of compound (**4**) with chloromethyl methyl ether and diisopropylethylamine in THF at 65 °C overnight afforded the 3-methoxymethoxy ether (**5**) in 89% yield. This material was then reacted with zinc iodide and trimethylsilylcyanide in chloroform at room temperature overnight. Analysis by ^{1}H NMR indicated an incomplete reaction. The residue was re-reacted for 4 h and purified by flash column. Analysis of the recovered material by ^{1}H NMR indicated the loss of the 3-methoxymethyl ether. The residue was re-protected by reaction with chloromethyl methyl ether and diisopropylethylamine at 65 °C overnight to give (**6**) in 40% overall yield. Reduction of the 17α-cyano group of (**6**) was carried out with $LiAlH_4$ in THF to give the 17α-aminomethyl-derivative (**7**) in 75% yield. Reaction of compound (**7**) with sodium nitrite in aqueous acetic acid gave compounds (**8** and **9**) in 61% and 5.5% respectively. Treatment of compound (**8**) with lithium tri-*tert*-butoxyaluminum hydride solution in THF at 0 °C followed by hydrolysis with HCl gave products (**10** and **11**) in 32% and 10%, respectively. Finally compound (**12**) was produced from (**9**) in 43% yield using the same conditions.

Table 1 – Selected proton chemical shifts for 10, 11, and 12

Compound	18-CH_3	H–C–OH
10	0.85	3.28
11	0.87	3.43
12	1.10	4.15

Stereochemistry for compounds (**10**), (**11**) and (**12**) were assigned based on the NMR chemical shifts for the 18-methyls and 17a-protons or 17-protons. This data is summarized in Table 1. According to Bhacca and Williams [8], axial protons attached to hydroxyl-substituted carbons resonate at

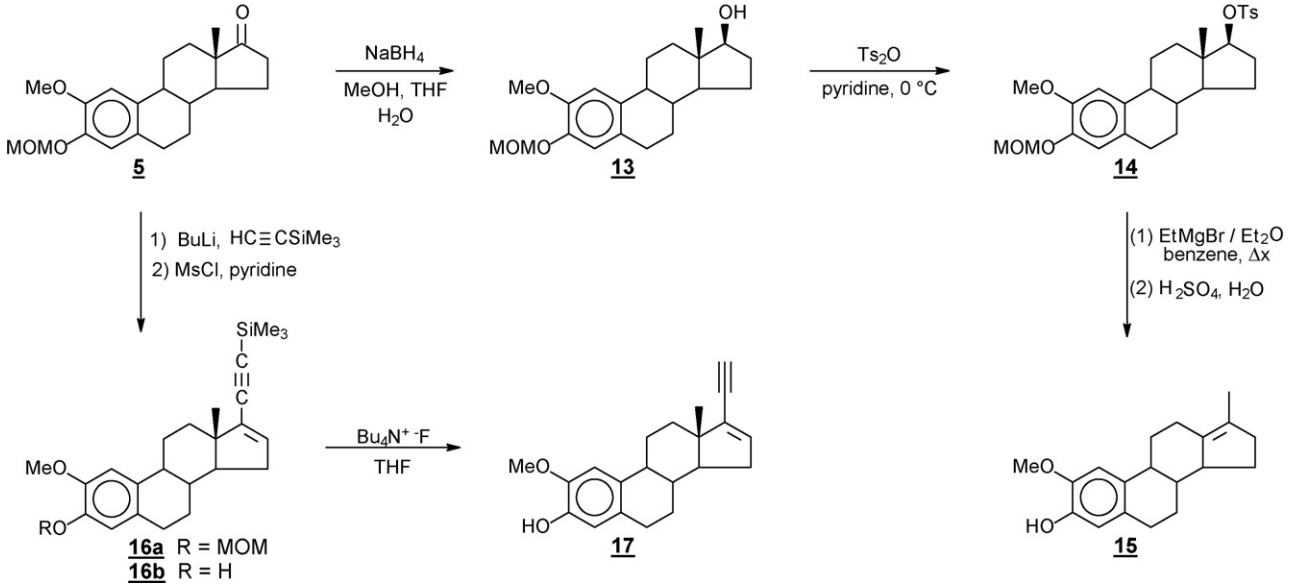


Fig. 2 – Syntheses of 2-methoxy-17-methyl and 17-ethynyl steroids with unsaturated ring-D.

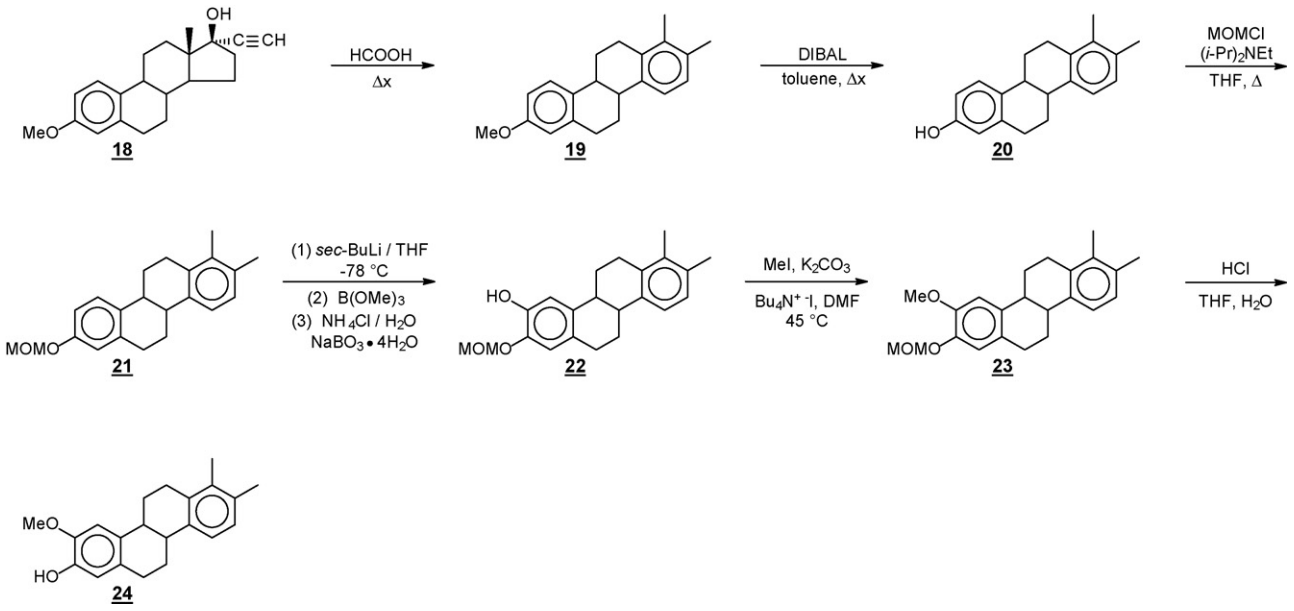


Fig. 3 – Synthesis of dimethylhexahydrochrysine analog.

higher field than the corresponding equatorial protons in the epimeric alcohol. As can be seen from Table 1, this observation is consistent with the assignment of 17aβ-OH for compound (**10**) and 17aα-OH for compound (**11**). These assignments agree with those made by Hillisch et al. [9] for the corresponding compounds as the 3-sulfamate derivatives. The assignment of 17β-OH for compound (**12**) is based on the strong downfield shift of the 18-methyl group due to 1,3-diaxial interaction with the 17β-axial OH.

The syntheses of D-ring unsaturated 2ME2 analogs (**13–17**) are outlined in Fig. 2. The 17-ketone (**5**) was converted to the 17β-hydroxy derivative (**13**) by reduction with sodium borohydride in 99% yield. Compound (**13**) was then treated with *p*-toluenesulfonic anhydride in pyridine at 0° C to give the 17β-tosylate product (**14**) in 95% yield. This material was then subjected to a Miescher–Kägi rearrangement following the procedure of Engel et al. [10] by refluxing with ethyl magnesium bromide in benzene to give the 18-nor-13(17)-ene derivative (**15**) in 79% yield.

Compound (**5**) was treated with trimethylsilylacetylene and butyl lithium at −78 °C in THF/benzene then stirred at room temperature for 2 h. Once TLC confirmed the formation of the lithium intermediate, methanesulfonyl chloride was added followed 0.5 h later with pyridine and the mixture stirred at room temperature overnight. Subsequent extraction and purification gave a crude mixture indicated by NMR to be composed mainly of the 3-methoxymethyl ether (**16a**) and the 3-hydroxy compound (**16b**) which were used directly for the next reaction. This material was then treated with tetrabutylammonium fluoride at room temperature for 2 h. Multiple purifications by flash column and subsequent crystallization from acetone/hexanes gave the product (**17**) in 6.4% yield. Apparently, the tetrabutylammonium fluoride reagent reacts with the free phenol of compound (**16b**) to generate HF which can potentially react with the acid sensitive ene-yne function to give byproducts. In retrospect, a better preparation of compound (**17**) could be achieved by isolation and purification of (**16a**) prior to removal of the silyl group.

The synthesis of the dimethylhexahydrochrysine analog (**24**) is outlined in Fig. 3. Compound (**19**) was prepared as described by Vincze et al. [11] by refluxing mestranol (**18**) in formic acid. This material was converted to the free hydroxy derivative (**20**) in 76% yield by reaction with DIBAL in toluene at 95 °C overnight. Treatment of (**20**) with chloromethyl methyl ether and *N*,*N*-diisopropylethylamine at 65 °C overnight afforded the 3-methoxymethoxy ether (**21**) in 77% yield. Introduction of the 2-methoxy-group was carried out following the procedure of Paaren et al. [12] whereby metallation of compound (**21**) was achieved by reaction with *n*-BuLi in THF at −65 °C. Subsequent addition of trimethyl borate and sodium perborate tetrahydrate gave the 2-hydroxy compound (**22**) in 31% yield. Methylation of compound (**22**) with methyl iodide in DMF in the presence of potassium carbonate and tetra-n-butyl ammonium iodide at 45 °C for 4 h afforded the 2-methoxy derivative (**23**) in quantitative yield. Hydrolysis of compound (**23**) in THF with 6M HCl at room temperature for 48 h followed by crystallization from ether gave compound (**24**) in 87% yield.

The syntheses of the 2,8-dihydroxyhexahydrochrysene derivatives (**30** and **34**) are outlined in Fig. 4. The 18-nor-13(17)-ene derivative (**15**) was treated with acetic anhydride in pyridine at room temperature overnight to give the 3-acetate derivative (**25**) in quantitative yield. Oxidative cleavage of the 13–17 double bond was carried out following the procedure of Wenshang et al. [13] using a catalytic amount of OsO_4 in dioxane/water/t-BuOH in the presence of excess sodium periodate and pyridine to give the diketone derivative (**26**) in 41% yield. Robinson annulation of diketone (**26**) following the procedure of Engle et al. [14] was accomplished by refluxing in methanol with 10% KOH to give compound (**27**) in quantitative yield. Compound (**27**) was converted to the 3-acetate derivative (**28**) in 51% yield by reaction with acetic anhydride in

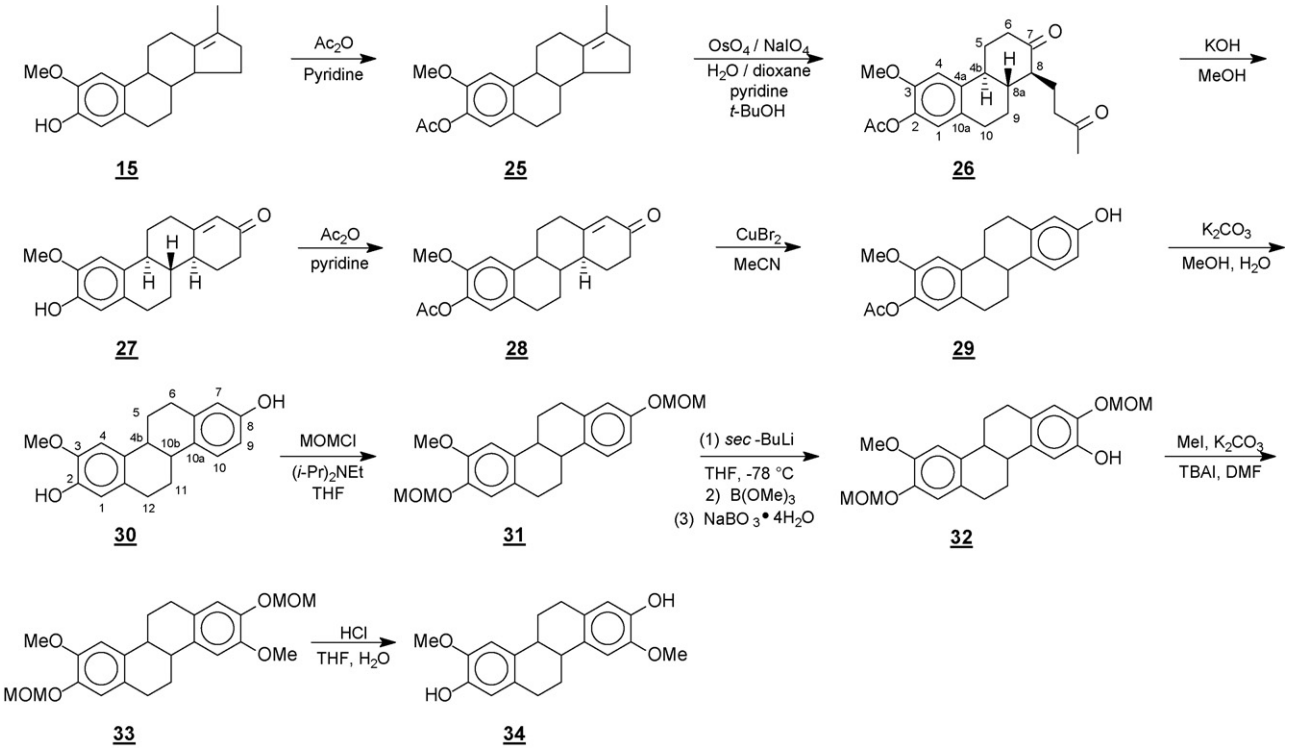


Fig. 4 – Syntheses of 2,8-dihydroxyhexahydrochrysene derivatives.

pyridine. Aromatization of compound (**28**) to give the chrysene derivative (**29**) was carried out in 70% yield, following the procedure of Rao et al. [15] using copper II bromide in acetonitrile. Hydrolysis of compound (**29**) in methanol/water with potassium carbonate gave the 2,8-diol derivative (**30**) in 88% yield. The diol (**30**) was converted to the dimethoxymethyl ether (**31**) in 88% yield, by reaction with chloromethyl methyl ether and *N*,*N*-diisopropylethylamine in THF at 65 °C. Introduction of a methoxy-group at the C-9 position was carried out following the procedure of Paaren et al. [12] as outlined above for compound (**22**) to give the 8-hydroxy compound (**32**) in 84% yield. O-Methylation of compound (**32**) was carried out following the procedure used for the preparation of compound (**23**) to afford the 3,9-dimethoxychrysine derivative (**33**) in 73% yield. Finally, hydrolysis of compound (**33**) in THF with 6M HCl followed by subsequent chromatographic purification and crystallization gave the 3,9-dimethoxyhexahydrochrysene-2,8-diol derivative (**34**) in 40% yield.

The syntheses of compounds (**35–40**) are outlined in Fig. 5. The 3,17β-dihydroxy-Δ^{14} derivative (**35**) was prepared as previously described [3]. This material was selectively acetylated with acetic anhydride in isopropanol in the presence of 2 M NaOH to give the 3-acetate compound (**36**) in 96% yield. Treatment of (**36**) with Jones reagent in acetone at 0 °C afforded the 17-ketone derivative (**37**) in quantitative yield. The 17-ketone (**37**) was converted to the 17-thioketal (**38**) in 71% yield by reaction with ethanedithiol and boron trifluoride diethyl etherate in acetic acid at room temperature for ninety minutes. This material was then refluxed with deactivated Raney nickel in acetone to give the $\Delta^{14,16}$ derivative (**39**) in 9.3% yield. Simple base hydrolysis of (**39**) with potassium carbonate in

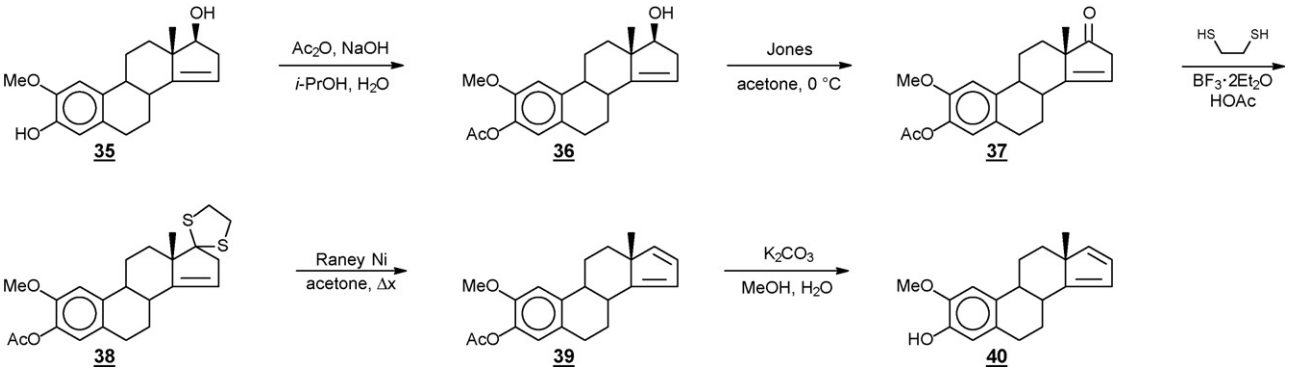
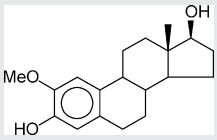
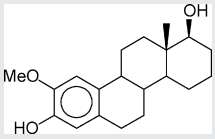
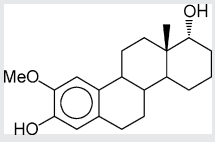
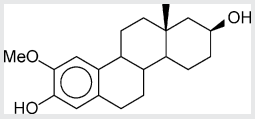
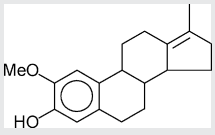
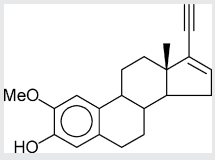
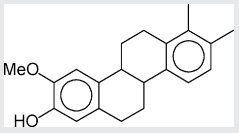
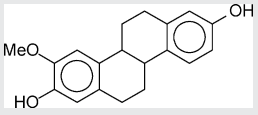
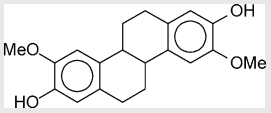
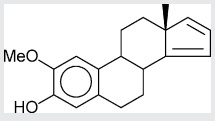


Fig. 5 – Synthesis of 2-methoxyestra-1,3,5(10),14,15-pentaen-3-ol.

Table 2 – IC_{50} values for inhibition of proliferation

Compound		Inhibition of cell proliferation IC_{50} (μM) ± S.D.		
Structure	No.	HUVEC	MDA-MB-231	U87-MG
	**1**	0.68 ± 0.15	0.69 ± 0.14	1.48 ± 0.62
	**10**	2.04 ± 0.51	7.25 ± 0.78	>100
	**11**	31.90 ± 28.98	24.75 ± 1.91	>100
	**12**	0.39 ± 0.13	0.26 ± 0.05	>100
	**15**	5.53 ± 1.00	19.11 ± 4.16	14.33 ± 10.26
	**17**	0.20 ± 0.10	0.66 ± 0.01	0.24 ± 0.01
	**24**	1.14 ± 0.52	0.22 ± 0.00	0.94 ± 0.04
	**30**	0.19 ± 0.01	0.18 ± 0.11	0.10 ± 0.01
	**34**	>10	n.d.[a]	>10
	**40**	0.31 ± 0.04	0.49 ± 0.40	0.59 ± 0.24

[a] Not done.

methanol/water gave the 3-hydroxy compound (**40**) in quantitative yield.

3.2. *Biological activity*

The IC_{50} values for inhibition of proliferation were generated in three cell types: human umbilical vein endothelial cells (HUVEC), human breast carcinoma cells (MDA-MB-231) and human gliomablastoma cells (U87-MG). The data are presented in Table 2. The IC_{50} value is the concentration at which cell proliferation is inhibited by 50%.

Simple D-homologation to give compounds (**10**), (**11**) and (**12**) resulted in total loss of activity in the U87-MG cell line for all of these compounds. Compounds (**10**) and (**11**), with the

Fig. 6 – A-ring superimposed MM3 minimized structures for compounds 2ME2 (red), 15 (orange), 17 (green), 24 (blue), 30 (cyan), 34 (purple), and 40 (black). Hydrogen atoms are omitted for clarity.

D-ring hydroxyl function most resembling that of the parent 2ME2 show much less activity in the HUVEC and MDA-MB-231 cell lines compared to 2ME2 with the 17aβ-OH (**10**) showing more activity than the 17aα-OH (**11**). Interestingly, when the D-ring hydroxyl group is at the 17β-position in compound (**12**), the activity is about twice that observed for 2ME2 in both the HUVEC and the MDA-MB-231 cell lines.

The chrysene analogs (**24**), (**30**), and (**34**) vary in biological activity depending on the type and position of substituents on ring-D. The dimethylhexahydrochrysine analog (**24**) is 0.6, 3.1, and 1.6 times as active as 2ME2 in the HUVEC, MDA-MB-231, and U87-MG cell lines respectively. It is notable that compound (**24**) is comparable to 2ME2 in biological activity despite the lack of a hydroxyl or polar group on ring-D. The chrysene analog (**30**) is the most active compound in this series, exhibiting 3.6, 3.8, and 14.8 times the antiproliferative activity of 2ME2. However, introduction of an additional methoxy-group at the nine-position to give the symmetrical chrysene derivative (**34**) results in a complete loss of biological activity.

The remaining new analogs (**15**, **17**, and **40**) share three common characteristics. The D-ring of all three of these compounds are five-membered, lack oxygen functionality, and have additional unsaturation. As can be seen from Table 2, compounds (**17**) and (**40**) are slightly more active than 2ME2 in all three cell lines whereas compound (**15**) is much less active, being 0.123, 0.036, and 0.103 times the activity of 2ME2 in the HUVEC, MDA-MB-231, and U87-MG cell lines, respectively.

While the various ring-D modifications carried out here can increase activity compared to 2ME2, clearly other factors play a role. In particular, the loss of activity for compound (**15**) was at first somewhat puzzling. A comparison of the MM3 minimized molecular structures of compounds 2ME2, **15**, **17**, **24**, **30**, **34**, and **40** is shown in Fig. 6 whereby the A-ring of compounds **15**, **17**, **24**, **30**, **34**, and **40** are superimposed on the A-ring of 2ME2 via the best root mean square fit of the A-ring carbon atoms. As can be seen from this comparison, the active compounds (**17**, **24**, **30** and **40**) have a D-ring that is either in line with that of 2ME2 or projects towards the α-side of the molecule. In contrast, the less active compound (**15**) has a D-ring that projects above that of 2ME2, towards the β-side of the molecule. Prior investigation has suggested that substituents on the β-side of the D-ring decrease antiproliferative activity [5]. In the case of compound (**15**) the $\Delta^{13,17}$ bond projects the entire D-ring towards the β-face of the molecule which could explain its decreased antiproliferative activity. The exception to this model is compound (**34**) which has no biological activity and whose D-ring projects to the α-side similar to the other two chrysene analogs (**24**) and (**30**). The reasons for the inactivity of compound (**34**) are unknown, although increased steric bulk of the additional methoxy group could be an explanation. Further investigations in this area are needed to clarify this.

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