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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,