ELSEVIER

Steroids 67 (2002) 371–377

Steroids

Synthesis and receptor-binding examination of 16-hydroxymethyl-3,17-estradiol stereoisomers

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Received 27 March 2001; received in revised form 6 September 2001; accepted 12 September 2001

Abstract

The four 16-hydroxymethylestra-1,3,5(10)-triene-3,17-diol isomers were synthesized and tested in a radioligand-binding assay. The estrogen receptor recognizes these compounds, but their relative binding affinities are lower than 2.0% relative to that of the reference molecule estra-1,3,5(10)-triene-3,17β-diol. The affinities of the tested compounds for the androgen and progesterone receptors are very low (K_i> 100 μm and 1 μM, respectively). The prepared 16-hydroxymethylestra-1,3,5(10)-triene-3,17-diol isomers are therefore estrogen receptor-selective molecules.

Keywords: 16-Hydroxymethylestra-1,3,5(10)-triene-3,17-diol isomers; Estrogen receptor binding

1. Introduction

Breast cancer is the most frequently diagnosed cancer in women worldwide [1]. Since estrogens are known to play a role in the development of many breast cancers, a logical approach to the treatment of estrogen-sensitive breast cancer is the use of anti-estrogens that block the interaction of estrogens with their specific receptor. A new generation of steroidal compounds have recently been described as anti-estrogens. These compounds generally contain a long alkyl-amide side-chain at position 7α or 11β of an estradiol nucleus, this side-chain playing an essential role in the anti-estrogenic properties of the compounds [2–6]. Earlier examinations suggested that the estrogen receptor tolerates reasonably large substituents at positions 7α, 11β, 16α and 17α in estradiol [9,10]. Poirier et al. demonstrated that 16α-halogenopropylestradiol and 16α-bromoalkylamide-estradiol derivatives inhibit 17β-hydroxysteroid dehydrogenase, which is responsible for the transformation of the less potent estrone to the most potent estrogen, estradiol [7,8]. A number of estradiol derivatives have been synthesized and their relative binding affinities for estrogen receptors have been evaluated over the years [11]. The sites of the substituents are unequivocal; the steric structure often depends on the method of synthesis. As concerns the 16-substituted estrogens, usually the 16α-substituted-17β-hydroxy compounds have been studied. The biologic activity has generally not been studied for the whole isomer series.

In the 16 substituted 17-hydroxysteroids, the two chiral centres permit four stereochemical modifications. We set out to prepare the four possible isomers of 16-hydroxymethyl-3,17-estradiol and to subject them to receptor-binding examinations to obtain answers to the following questions: (1) How do the receptor-binding processes of the four isomers **3d, 4d, 5c** and **6d** differ? (2) How is the receptor-binding process influenced by the relative steric position of the 16-hydroxymethyl and 17-hydroxy groups?

2. Experimental

2.1. General

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. Specific rotations were measured with a POLAMAT-A (Zeiss-Jena) polarimeter on solutions in chloroform (*c* 1) and are given in units of 10^{-1} deg cm^2 g^{-1}. Elemental analyses were performed with a

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0039-128X/02/$ – see front matter
PII: S0039-128X-(01)00191-X

Perkin-Elmer CHN analyser model 2400. Thin-layer chromatography: silica gel 60, layer thickness 0.2 mm (Merck); solvent system (ss): (A) ethyl acetate/chloroform (10:90 v/v), (B) ethyl acetate/chloroform (15:85 v/v), (C) ethyl acetate/chloroform (50:50 v/v); detection with iodine or UV (365 nm) after spraying with 50% phosphoric acid and heating at 100–120°C for 10 min. Flash chromatography: silica gel 60, 40–63 μm. Column chromatography: Al_2O_3 (standarized according to Brockmann) with an activity of III-IV. The NMR spectra were recorded with a Bruker AMX-400 instrument. Chemical shifts (δ) are given in ppm, and coupling constants (J) in Hz.

*2.2. 3-Benzyloxyestra-1,3,5(10)-trien-17-one (**1b**)*

Sodium (5 g, 0.22 mol) was dissolved in methanol (250 ml) and 3-hydroxy-1,3,5(10)-trien-17-one (27 g, 0.1 mol) was added. The mixture was stirred and warmed slightly until the steroid had dissolved. After the addition of benzyl chloride (25 ml, 0.15 mol), the reaction mixture was heated at reflux for 6 h, and then poured into water. The precipitate was collected by filtration, washed with water and dried in a vacuum desiccator over P_2O_5. Crystallization from acetone gave **1b** as white crystals (32.4 g, 90%). Mp 127–128°C, $[\alpha]_D^{20}$ +132 (*c* 1 in chloroform) ([12] Mp 132–134°C, $[\alpha]_D$ + 122) (Found C, 83.15; H, 7.92. $C_{25}H_{28}O_2$ requires C, 83.29; H, 7.83%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 0.90(s, 3H, H-18), 2.89(m, 2H, H-6), 5.03(s, 2H, $C_6H_5CH_2$), 6.73(d, 1H, J = 2.6 Hz, H-4), 6.78(dd, 1H, J = 8.6 Hz, 2.6 Hz, H-2), 7.19(d, 1H, J = 8.6 Hz, H-1), 7.31–7.43(m, 5H, C_6H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 13.8(C-18), 21.5(C-15), 25.9, 26.5, 29.6, 31.6, 35.8(C-16), 38.3, 43.9, 47.9(C-13), 50.4(C-14), 69.9($C_6H_5CH_2$), 112.3(C-2), 114.9(C-4), 126.3(C-1), 127.3(2C, C-2′ and C-6′), 127.8(C-4′), 128.5(2C, C-3′ and C-5′), 132.3(C-10), 137.2(C-1′), 137.7(C-5), 156.8(C-3), 220.6(C-17).

*2.3. 16-Hydroxymethylene-3-benzyloxyestra-1,3,5(10)-trien-17-one (**2a**)*

Sodium (2.5 g, 0.11 mol) was dissolved in methanol (120 ml), and the solvent was removed by distillation under vacuum to obtain $NaOCH_3$ free from methanol. 3-Benzyloxyestra-1,3,5(10)-trien-17-one (18 g, 0.05 mol) dissolved in anhydrous benzene (100 ml) was added to the homogenized $NaOCH_3$ powder. Freshly distilled ethyl formate (60 ml) was added dropwise and the mixture was stirred at room temperature for 2 h, and then at 50°C for 4 h. The reaction mixture was poured onto ice, and the aqueous phase was separated and acidified with dilute HCl to pH 3. The precipitate was collected by filtration, washed with water and dried in a vacuum desiccator over P_2O_5 (18.5 g, 95%).

*2.4. 16-Acetoxymethylene-3-benzyloxyestra-1,3,5(10)-trien-17-one (**2b**)*

Crude **2a** (7.76 g, 0.02 mol) was dissolved in a mixture of pyridine (10 ml) and acetic anhydride (10 ml), and the solution was allowed to stand at room temperature for 12 h. The reaction mixture was then poured onto a mixture of ice (300 g) and 5 ml of concentrated H_2SO_4. The precipitate was collected by filtration and recrystallized from a mixture of methanol and water (8.1 g, 94%). Mp 215–218°C, R_f = 0.90 (ss A); $[\alpha]_D^{20}$ +98 (*c* 1 in chloroform) (Found: C, 78.25; H, 6.95. $C_{28}H_{30}O_4$ requires: C, 78.11; H, 7.02%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 0.93(s, 3H, H-18), 2.23(s, 3H, CH_3CO), 2.90(m, 2H, H-6), 5.03(s, 2H, $C_6H_5CH_2$), 6.73(d, 1H, J = 2.6 Hz, H-4), 6.79(dd, 1H, J = 8.6 Hz, 2.6 Hz, H-2), 7.19(d, 1H, J = 8.6 Hz, H-1), 7.31–7.43(m, 5H, C_6H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 14.4(C-18), 20.6(CH_3CO), 25.1, 25.9, 26.7, 29.6, 31.4, 37.8, 38.3, 44.0, 48.0, 48.9(C-13), 69.9($C_6H_5CH_2$), 112.4(C-2), 114.9(C-4), 121.2(C-16), 126.2(C-1), 127.4(2C, C-2′ and C-6′), 127.8(C-4′), 128.5(2C, C-3′ and C-5′), 132.2(C-10), 137.2(C-1′), 137.6(C-5), 139.9(CHOAc), 156.9(C-3), 167.1(CH_3CO), 209.2(C-17).

*2.5. 16-Hydroxymethyl-3-benzyloxyestra-1,3,5(10)-trien-17-ol isomers (**3a**, **4a** and **5a**)*

Compound **2a** (19.4 g, 0.05 mol) was suspended in ethanol (500 ml), and KBH_4 (8.1 g, 0.15 mol) was added in small portions during cooling in ice. The reduction mixture was allowed to stand for 24 h, and was then acidified with dilute HCl. The resulting solution was poured onto ice (1000 g) and the precipitate was collected by filtration and dried in a vacuum desiccator over P_2O_5. The dried isomeric mixture of **3a**, **4a** and **5a** (18 g, 92%) was suspended in a mixture of dichloromethane (500 ml) and acetone (100 ml) in the presence of a catalytic amount of *p*-toluenesulfonic acid. The reaction mixture was kept at boiling temperature for 1 h, and was next neutralized with morpholine and evaporated to dryness. The residue obtained was then dissolved in chloroform (50 ml) and chromatographed on Al_2O_3. Chloroform/light petroleum (1:3) eluted **3c** (9.75 g; 49%). Mp 133–134°C, R_f = 0.80 (ss A); $[\alpha]_D^{20}$ +56 (*c* 1 in chloroform). (Found: C, 80.40; H, 8.32. $C_{29}H_{36}O_3$ requires: C, 80.52; H, 8.39%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 0.90(s, 3H, H-18), 1.36 an d 1.37(two s, 6H, $(CH_3)_2C$), 2.86(m, 2H, H-6), 3.56(dd, 1H, J = 11.9 Hz, 10.6 Hz, H-16), 3.69(dd, 1H, J = 10.6 Hz, 9.3 Hz, H-16), 3.78(d, 1H, J = 9.3 Hz, H-17), 5.05(s, 2H, $C_6H_5CH_2$), 6.73(d, 1H, J = 2.6 Hz, H-4), 6.79(dd, 1H, J = 8.6 Hz, 2.6 Hz, H-2), 7.21(d, 1H, J = 8.6 Hz, H-1), 7.32–7.45(m, 5H, C_6H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 13.3(C-18), 25.1 and 27.4(2C, $(CH_3)_2C$), 26.4, 27.7, 27.9, 29.8, 37.8, 38.3, 38.4, 43.9, 44.3(C-13), 49.7, 63.5(C-16′), 70.0($C_6H_5CH_2$), 79.2(C-17), 98.7($(CH_3)_2C$), 112.3(C-2), 114.9(C-4), 126.2(C-1), 127.4(2C, C-2′ and C-6′), 127.8(C-4′), 128.5(2C, C-3′ and C-5′), 133.0(C-10), 137.4(C-1′), 137.9(C-5), 156.8(C-3). Continued

elution with chloroform yielded **4a** together with **5a**. The chloroform solution was evaporated to dryness, and the residue was dissolved in hot acetone. From this solution, the bulk of the **4a** crystallized out as hard crystals (6.7 g, 37%). Mp 151–153°C, R_f = 0.35 (ss B); $[\alpha]_D^{20}$ +56 (*c* 1 in chloroform). (Found: C, 79.65; H, 8.14. $C_{26}H_{32}O_3$ requires: C, 79.56; H, 8.22%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 0.90(s, 3H, H-18), 1.36 and 1.37(two s, 6H, $(CH_3)_2C$), 2.86(m, 2H, H-6), 3.56(dd, 1H, *J* = 11.9 Hz, 10.6 Hz, H-16), 3.69(dd, 1H, *J* = 10.6 Hz, 9.3 Hz, H-16), 3.78(d, 1H, *J* = 9.3 Hz, H-17), 5.05(s, 2H, $C_6H_5CH_2$), 6.73(d, 1H, *J* = 2.6 Hz, H-4), 6.79(dd, 1H, *J* = 8.6 Hz, 2.6 Hz, H-2), 7.21(d, 1H, *J* = 8.6 Hz, H-1), 7.32–7.45(m, 5H, C_6H_5) ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 13.3(C-18), 25.1 and 27.4(2C, $(CH_3)_2C$), 26.4, 27.7, 27.9, 29.8, 37.8, 38.3, 38.4, 43.9, 44.3(C-13), 49.7, 63.5(C-16′), 70.0($C_6H_5CH_2$), 79.2(C-17), 98.7($(CH_3)_2C$), 112.3(C-2), 114.9(C-4), 126.2(C-1), 127.4(2C, C-2′ and C-6′), 127.8(C-4′), 128.5(2C, C-3′ and C-5′), 133.0(C-10), 137.4(C-1′), 137.9(C-5), 156.8(C-3). The mother liquor was evaporated and subjected to chromatographic separation on a silica gel column with *tert*-butyl methyl ether. **4a** eluted first (1.3 g, 7%). Continued elution resulted in **5a** (0.85 g, 4.7%). Mp 128–130°C, R_f = 0.30 (ss B); $[\alpha]_D^{20}$ +46. (Found: C, 79.60; H, 8.10. $C_{26}H_{32}O_3$ requires: C, 79.56; H, 8.22%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 0.75(s, 3H, H-18), 2.85(m, 2H, H-6), 3.68(d, 1H, *J* = 1.6 Hz, H-17), 3.66(dd, 1H, *J* = 10.5 Hz, 8.8 Hz) and 3.75(dd, 1H, *J* = 10.5 Hz, 6.5 Hz): H-16a, 5.03(s, 2H, $C_6H_5CH_2$), 6.72(d, 1H, *J* = 2.7 Hz, H-4), 6.78(dd, 1H, *J* = 8.6 Hz, 2.7 Hz, H-2), 7.22(d, 1H, *J* = 8.6 Hz, H-1), 7.31–7.44(m, 5H, C_6H_5) ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 17.7(C-18), 26.0, 28.0, 29.0, 29.8, 32.0, 38.6, 43.4, 44.9(C-13), 48.8, 52.0, 66.5(C-16′), 70.0($C_6H_5CH_2$), 82.4(C-17), 112.3(C-2), 114.9(C-4), 126.3(C-1), 127.4(2C, C-2′ and C-6′), 127.8(C-4′), 128.5(2C, C-3′ and C-5′), 132.9(C-10), 137.3(C-1′), 137.9(C-5), 156.8(C-3).

Compound **3c** (8.64 g, 0.02 mol) was dissolved in 96% ethanol (150 ml) in the presence of a catalytic amount of *p*-toluenesulfonic acid. The reaction mixture was allowed to stand at room temperature for 6 h, and was then diluted with water. The crystalline substance was collected by filtration and was recrystallized from chloroform/light petroleum to obtain **3a** (7.5 g, 95%). Mp 151–153°C, R_f = 0.40 (ss B); $[\alpha]_D^{20}$ + 45. (Found: C, 79.31; H, 8.35. $C_{26}H_{32}O_3$ requires C, 79.56; H, 8.22%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 0.85(s, 3H, H-18), 3.65(m, 1H) and 3.84(m, 1H): H-16a, 3.94(d, 1H, *J* = 9.8 Hz, H-17), 5.02(s, 2H, $C_6H_5CH_2$), 6.71(d, 1H, *J* = 2.6 Hz, H-4), 6.77(dd, 1H, *J* = 8.6 Hz, 2.6 Hz, H-2), 7.19(d, 1H, *J* = 8.6 Hz, H-1), 7.30–7.42(m, 5H, C_6H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 12.2(C-18), 26.3, 27.5, 27.8, 29.7, 37.7, 38.1, 41.9, 43.9, 44.3(C-13), 49.1, 64.7(C-16′), 70.0($C_6H_5CH_2$), 83.0(C-17), 112.3(C-2), 114.9(C-4), 126.3(C-1), 127.4(2C, C-2′ and C-6′), 127.8(C-4′), 128.5(2C, C-3′ and C-5′), 132.8(C-10), 137.3(C-1′), 137.9(C-5), 156.8(C-3).

*2.6. 16-Acetoxymethyl-3-benzyloxyestra-1,3,5(10)-trien-17-acetate isomers (**3b**, **4b** and **5b**)*

2.6.1. (General procedure)

Compound **3a**, **4a** or **5a** (3.9 g, 0.01 mol) was dissolved in a mixture of pyridine (10 ml) and acetic anhydride (10 ml) and the solution was allowed to stand at room temperature for 12 h. The mixture was then diluted with water and the precipitate was collected by filtration and recrystallized from methanol. **3b** (4.7 g, 98%). Mp 100–101°C, R_f = 0.80 (ss A); $[\alpha]_D^{20}$ + 37. (Found: C, 75.78; H, 7.55. $C_{30}H_{36}O_5$ requires C, 75.60; H, 7.61%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 0.86(s, 3H, H-18), 2.03 and 2.08(two s, 6H, two CH_3CO), 2.88(m, 2H, H-6), 4.03(dd, 1H, *J* = 11.1 Hz, 7.5 Hz) and 4.13(dd, 1H, *J* = 11.1 Hz, 7.0 Hz): H-16a, 4.90(d, 1H, *J* = 10.1 Hz, H-17), 5.03(s, 2H, $C_6H_5CH_2$), 6.72(d, 1H, *J* = 2.6 Hz, H-4), 6.78(dd, 1H, *J* = 8.6 Hz, 2.6 Hz, H-2), 7.19(d, 1H, *J* = 8.6 Hz, H-1), 7.31–7.44(m, 5H, C_6H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 12.9(C-18), 20.9(2C, CH_3CO), 26.1, 27.3, 29.3, 29.7, 37.4, 37.6, 37.9, 43.6(C-13), 43.7, 48.7, 65.3(C-16′), 69.9($C_6H_5CH_2$), 81.6(C-17), 112.3(C-2), 114.8(C-4), 126.3(C-1), 127.4(2C, C-2′ and C-6′), 127.8(C-4′), 128.5(2C, C-3′ and C-5′), 132.6(C-10), 137.3(C-1′), 137.8(C-5), 156.8(C-3), 170.9(2C, CH_3CO). **4b** (4.65 g, 97%). Mp 131–132°C, R_f = 0.75 (ss A); $[\alpha]_D^{20}$ + 3. (Found: C, 75.72; H, 7.66. $C_{30}H_{36}O_5$ requires: C, 75.60; H, 7.61%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 0.86(s, 3H, H-18), 2.05 and 2.08(two s, 6H, two CH_3CO), 2.86(m, 2H, H-6), 4.07(dd, 1H, *J* = 10.9 Hz, 6.7 Hz) and 4.13(dd, 1H, *J* = 11.3 Hz, 6.7 Hz): H-16a, 4.75(d, 1H, *J* = 8.1 Hz, H-17), 5.03(s, 2H, $C_6H_5CH_2$), 6.72(d, 1H, *J* = 2.6 Hz, H-4), 6.78(dd, 1H, *J* = 8.6 Hz, 2.6 Hz, H-2), 7.19(d, 1H, *J* = 8.6 Hz, H-1), 7.31–7.44(m, 5H, C_6H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 12.6(C-18), 20.9 and 21.0(2C, CH_3CO), 26.0, 27.1, 27.4, 29.6, 36.8, 38.3, 40.2, 43.7, 44.3(C-13), 48.6, 66.7(C-16′), 69.9($C_6H_5CH_2$), 83.7(C-17), 112.3(C-2), 114.8(C-4), 126.3(C-1), 127.4(2C, C-2′ and C-6′), 127.8(C-4′), 128.5(2C, C-3′ and C-5′), 132.5(C-10), 137.3(C-1′), 137.8(C-5), 156.7(C-3), 170.8 and 171.0(2C, CH_3CO). **5b** (4.55 g, 95%). Mp 79–82°C, R_f = 0.50 (ss A); $[\alpha]_D^{20}$ + 51. (Found: C, 75.55; H, 7.78. $C_{30}H_{36}O_5$ requires: C, 75.60; H, 7.61%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 0.85(s, 3H, H-18), 2.06 and 2.07(two s, 6H, two CH_3CO), 2.85(m, 2H, H-6), 4.17(dd, 1H, *J* = 11.0 Hz, 7.2 Hz) and 4.22(dd, 1H, *J* = 11.0 Hz, 7.3 Hz): H-16a, 4.71(d, 1H, *J* = 2.0 Hz, H-17), 5.03(s, 2H, $C_6H_5CH_2$), 6.72(d, 1H, *J* = 2.7 Hz, H-4), 6.78(dd, 1H, *J* = 8.6 Hz, 2.7 Hz, H-2), 7.19(d, 1H, *J* = 8.6 Hz, H-1), 7.30–7.43(m, 5H, C_6H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 17.0(C-18), 20.9 and 21.1(2C, CH_3CO), 25.9, 27.9, 29.3, 29.7, 32.2, 38.5, 43.4, 44.6(C-13), 45.7, 49.9, 66.5(C-16′), 69.9($C_6H_5CH_2$), 83.5(C-17), 112.3(C-2), 114.8(C-4), 126.3(C-1), 127.4(2C, C-2′ and C-6′), 127.8(C-4′), 128.5(2C, C-3′ and C-5′), 132.6(C-10), 137.3(C-1′), 137.8(C-5), 156.8(C-3), 170.4 and 171.1(2C, CH_3CO).

2.7. 16α-Acetoxymethyl-3-benzyloxyestra-1,3,5(10)-trien-17β-ol (**4c**)

Compound **4a** (20 g, 0.05 mol) was dissolved in pyridine (80 ml), and acetic anhydride (5 ml, 0.05 mol) in pyridine (40 ml) was added dropwise during cooling with ice. The reaction mixture was allowed to warm up to room temperature, stirred for 3 h, then poured onto a mixture of ice and H_2SO_4, and extracted with chloroform. The chloroform solution was washed with $NaHCO_3$ solution and then with water, dried and evaporated. The residual oil was chromatographed on silica gel with ethyl acetate/chloroform (10:90), yielding pure **4b** (2.4 g, 10%). Continued elution resulted in **4c** (14.4 g, 65%). Mp 91–93°C, R_f = 0.50 (ss B); $[\alpha]_D^{20}$ + 49. (Found: C, 77.25; H, 7.95. $C_{28}H_{34}O_4$ requires: C, 77.39, H, 7.89%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 0.84(s, 3H, H-18), 2.09(s, 3H, C*H*$_3$CO), 2.85(m, 2H, H-6), 3.46(d, 1H, *J* = 7.6 Hz, H-17), 4.14(dd, 1H, *J* = 10.7 Hz, 7.0 Hz) and 4.18(dd, 1H, *J* = 10.7 Hz, 6.7 Hz): H-16a, 5.04(s, 2H, $C_6H_5CH_2$), 6.72(d, 1H, *J* = 2.6 Hz, H-4), 6.78(dd, 1H, *J* = 8.6 Hz, 2.6 Hz, H-2), 7.20(d, 1H, *J* = 8.6 Hz, H-1), 7.30–7.44(m, 5H, C_6H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 11.8(C-18), 21.0(*C*H$_3$CO), 26.1, 27.2, 27.4, 29.7, 36.7, 38.5, 42.9, 43.9, 44.1(C-13), 48.7, 67.7(C-16′), 69.9($C_6H_5CH_2$), 84.7(C-17), 112.3(C-2), 114.8(C-4), 126.3(C-1), 127.4(2C, C-2′ and C-6′), 127.8(C-4′), 128.5(2C, C-3′ and C-5′), 132.7(C-10), 137.3(C-1′), 137.9(C-5), 156.7(C-3), 171.3(CH$_3$*C*O). Methanol eluted the unchanged starting compound **4a** (3.8 g, 19%).

2.8. 16α-Acetoxymethyl-3-benzyloxyestra-1,3,5(10)-trien-17-one (**7**)

Compound **4c** (10.8 g, 0.025 mol) was dissolved in acetone (200 ml) and Jones reagent was added dropwise during cooling with ice. The reaction mixture was poured onto ice (500 g) and extracted with chloroform. The chloroform solution was washed with water, dried and evaporated. The substance obtained was crystallized from acetone/light petroleum to give pure **7** (10.2 g, 94%). Mp 111–113°C, R_f = 0.70 (ss B); $[\alpha]_D^{20}$ + 98. (Found: C, 77.64; H, 7.28. $C_{28}H_{32}O_4$ requires: C, 77.75; H, 7.46%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 0.97(s, 3H, H-18), 2.05(s, 3H, C*H*$_3$CO), 2.89(m, 2H, H-6), 4.21(dd, 1H, *J* = 11.0 Hz, 6.8 Hz) and 4.34(dd, 1H, *J* = 11.0 Hz, 4.5 Hz): H-16a, 5.04(s, 2H, $C_6H_5CH_2$), 6.74(d, 1H, *J* = 2.6 Hz, H-4), 6.79(dd, 1H, *J* = 8.6 Hz, 2.6 Hz, H-2), 7.20(d, 1H, *J* = 8.6 Hz, H-1), 7.32–7.44(m, 5H, C_6H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 14.4(C-18), 20.9(*C*H$_3$CO), 25.7 (2C), 26.5, 29.5, 31.4, 38.2, 44.0, 44.1, 48.5(C-13), 48.6, 64.1(C-16′), 69.9($C_6H_5CH_2$), 112.3(C-2), 114.9(C-4), 126.3(C-1), 127.4(2C, C-2′ and C-6′), 127.8(C-4′), 128.5(2C, C-3′ and C-5′), 132.1(C-10), 137.2(C-1′), 137.7(C-5), 156.8(C-3), 170.9(CH$_3$*C*O), 218.2(C-17).

2.9. 16α-Acetoxymethyl-3-benzyloxyestra-1,3,5(10)-trien-17α-ol (**6c**)

$LiAlH_4$ (3.8 g, 0.1 mol) was suspended in anhydrous diethyl ether (200 ml), and *tert*-butanol (28.25 ml, 0.3 mol) was added carefully during stirring and cooling with salt-ice. Compound **7** (8.64 g, 0.02 mol) dissolved in diethyl ether (150 ml) was added dropwise to the suspension. The reaction mixture was stirred for 1 h and was then decomposed by the careful addition of water (200 ml) during stirring and cooling. It was next acidified with dilute HCl. The organic phase was separated and the aqueous phase was extracted with diethyl ether. The substance obtained on washing, drying and evaporation of the solvent was subjected to chromatographic separation on silica gel with *tert*-butyl methyl ether/light petroleum (40:60). **6c** eluted first (1.42 g, 16%). Mp 58–60°C, R_f = 0.55 (ss B); $[\alpha]_D^{20}$ + 40. (Found: C, 77.51; H, 7.71. $C_{28}H_{34}O_4$ requires: C, 77.39; H, 7.89%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 0.78(s, 3H, H-18), 2.08(s, 3H, C*H*$_3$CO), 2.88(m, 2H, H-6), 3.76(d, 1H, *J* = 5.0 Hz, H-17), 4.15(dd, 1H, *J* = 11.0 Hz, 6.1 Hz) and 4.33(dd, 1H, *J* = 11.0 Hz, 9.5 Hz): H-16a, 5.04(s, 2H, $C_6H_5CH_2$), 6.72(d, 1H, *J* = 2.6 Hz, H-4), 6.78(dd, 1H, *J* = 8.6 Hz, 2.6 Hz, H-2), 7.22(d, 1H, *J* = 8.6 Hz, H-1), 7.32–7.44(m, 5H, C_6H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 17.3(C-18), 21.1(*C*H$_3$CO), 26.0, 27.9(2C), 29.8, 31.1, 38.9, 40.4, 43.5, 46.3(C-13), 46.8, 64.8(C-16′), 69.9($C_6H_5CH_2$), 79.3(C-17), 112.3(C-2), 114.8(C-4), 126.3(C-1), 127.4(2C, C-2′ and C-6′), 127.8(C-4′), 128.5(2C, C-3′ and C-5′), 133.0(C-10), 137.3(C-1′), 138.0(C-5), 156.7(C-3), 171.5(CH$_3$*C*O). Further elution gave **4c** (5.34 g, 61%).

2.10. 16-Acetoxymethyl-3-benzyloxyestra-1,3,5(10)-trien-17α-acetate (**6b**)

Compound **6c** (2.17 g, 0.005 mol) was dissolved in a mixture of pyridine (5 ml) and acetic anhydride (5 ml) and the solution was allowed to stand at room temperature for 12 h. The mixture was then diluted with water and the precipitate was collected by filtration and recrystallized from methanol to obtain **6b** (2.30 g, 96%). Mp 106–108°C, R_f = 0.85 (ss A); $[\alpha]_D^{20}$ + 67. (Found: C, 75.58; H, 7.72. $C_{30}H_{36}O_5$ requires: C, 75.60; H, 7.61%). NMR (400 MHz, $CDCl_3$): δ ppm 0.86(s, 3H, H-18), 2.02 and 2.08(two s, 6H, two C*H*$_3$CO), 2.85(m, 2H, H-6), 4.05(dd, 1H, *J* = 10.6 Hz, 7.1 Hz) and 4.10(dd, 1H, *J* = 10.6 Hz, 9.0 Hz): H-16a, 5.03(s, 2H, $C_6H_5CH_2$), 5.10(d, 1H, *J* = 5.5 Hz, H-17), 6.72(d, 1H, *J* = 2.5 Hz, H-4), 6.78(dd, 1H, *J* = 8.6 Hz, 2.5 Hz, H-2), 7.19(d, 1H, *J* = 8.6 Hz, H-1), 7.31–7.44(m, 5H, C_6H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 16.7(C-18), 20.9 and 21.0(2C, *C*H$_3$CO), 25.8, 27.9, 28.1, 29.8, 31.6, 38.5, 38.9, 43.4, 46.0(C-13), 48.1, 64.0(C-16a′), 69.9($C_6H_5CH_2$), 80.7(C-17), 112.3(C-2), 114.8(C-4), 126.3(C-1), 127.4(2C, C-2′ and C-6′), 127.8(C-4′),

128.5(2C, C-3′ and C-5′), 132.6(C-10), 137.3(C-1′), 137.8(C-5), 156.8(C-3), 170.9(2C, CH_3CO).

2.11. 16α-Hydroxymethyl-3-benzyloxyestra-1,3,5(10)-trien-17α-ol (6a)

Compound **6b** (0.47 g, 0.001 mol) was dissolved in methanol (25 ml) containing $NaOCH_3$ (54 mg, 1 mmol) and the solution was allowed to stand for 24 h. It was then diluted with water, and the white precipitate was collected by filtration and recrystallized from ethanol to obtain **6a** (0.36 g, 91%). Mp 139–141°C, R_f = 0.45 (ss B); $[\alpha]_D^{20}$ + 81. (Found: C, 79.68; H, 8.12. $C_{26}H_{32}O_3$ requires: C, 79.56; H, 8.22%). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 0.78(s, 3H, H-18), 2.85(m, 2H, H-6), 3.74(dd, 1H, *J* = 11.0 Hz, 8.3 Hz) and 3.89(dd, 1H, *J* = 11.0 Hz, 4.5 Hz): H-16a, 3.94(d, 1H, *J* = 5.5 Hz, H-17), 5.04(s, 2H, $C_6H_5CH_2$), 6.72(d, 1H, *J* = 2.6 Hz, H-4), 6.78(dd, 1H, *J* = 8.6 Hz, 2.6 Hz, H-2), 7.22(d, 1H, *J* = 8.6 Hz, H-1), 7.32–7.44(m, 5H, C_6H_5). ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 17.3(C-18), 26.0, 27.5, 28.0, 29.9, 31.3, 39.1, 41.8, 43.5, 46.3(C-13), 47.6, 63.6(C-16′), 69.9($C_6H_5CH_2$), 81.9(C-17), 112.3(C-2), 114.8(C-4), 126.3(C-1), 127.4(2C, C-2′ and C-6′), 127.8(C-4′), 128.5(2C, C-3′ and C-5′), 132.9(C-10), 137.3(C-1′), 138.0(C-5), 156.7(C-3).

2.12. 16-Hydroxymethylestra-1,3,5(10)-triene-3,17-diol isomers (3d, 4d, 5c and 6d)

2.12.1. (General procedure)

Compound **3a**, **4a**, **5a** or **6a** (590 mg, 1.5 mmol) was dissolved in ethanol (50 ml) in an autoclave, and Pd/C (50 mg) was added to the solution, which was then stirred at room temperature for 3 h at 20 bar H_2 pressure. The reaction mixture was filtered, the filtrate was evaporated and the product obtained was crystallized from a mixture of ethanol/water. **3d** (430 mg, 94%). Mp 279–282°C, R_f = 0.45 (ss C). (Found: C, 75.38; H, 8.72. $C_{19}H_{26}O_3$ requires: C, 75.46; H, 8.67%). ^{1}H-NMR (400 MHz, DMSO-d_6): δ ppm 0.69(s, 3H, H-18), 2.70(m, 2H, H-6), 3.30(m, 1H, H-16a), 3.67(m, 2H, H-16a and H-17), 4.13(dd, 1H, *J* = 5.9 Hz, 4.6 Hz, 16-CH_2OH), 4.59(d, 1H, *J* = 4.4 Hz, 17-O*H*), 6.43(d, 1H, *J* = 2.4 Hz, H-4), 6.50(dd, 1H, *J* = 8.4 Hz, 2.4 Hz, H-2), 7.03(d, 1H, *J* = 8.4 Hz, H-1), 8.97(s, 1H, 3-O*H*). **4d** (425 mg, 93%). Mp 297–298°C, ([13] 277°C dec.), R_f = 0.45 (ss C). (Found: C, 75.53; H, 8.58. $C_{19}H_{26}O_3$ requires: 75.46; H, 8.67%). ^{1}H-NMR (400 MHz, DMSO-d_6): δ ppm 0.71(s, 3H, H-18), 2.70(m, 2H, H-6), 3.18(dd, 1H, *J* = 7.6 Hz, 5.1 Hz, H-17), 3.32(m, 1H) and 3.54(m, 1H): H-16a, 4.39(t, 1H, *J* = 5.0 Hz, 4.6 Hz, 16-CH_2OH), 4.43(d, 1H, *J* = 5.1 Hz, 17-O*H*), 6.43(d, 1H, *J* = 2.5 Hz, H-4), 6.50(dd, 1H, *J* = 8.4 Hz, 2.5 Hz, H-2), 7.03(d, 1H, *J* = 8.4 Hz, H-1), 8.94(s, 1H, 3-O*H*). **5c** (410 mg, 90%). Mp 235–238°C, R_f = 0.40 (ss C). (Found: C, 75.38; H, 8.50. $C_{19}H_{26}O_3$ requires: C, 75.46; H, 8.67%). ^{1}H-NMR (400 MHz, DMSO-d_6): δ ppm 0.71(s, 3H, H-18), 2.70(m, 2H, H-6), 3.18(dd, 1H, *J* = 7.6 Hz, 5.1 Hz, H-17), 3.32(m, 1H) and 3.54(m, 1H): H-16a, 4.39(t, 1H, *J* = 5.0 Hz, 4.6 Hz, 16-CH_2OH), 4.43(d, 1H, *J* = 5.1 Hz, 17-O*H*), 6.43(d, 1H, *J* = 2.5 Hz, H-4), 6.50(dd, 1H, *J* = 8.4 Hz, 2.5 Hz, H-2), 7.03(d, 1H, *J* = 8.6 Hz, H-1), 8.92(s, 1H, 3-O*H*).

2.13. Receptor binding assay

The new compounds were evaluated for their ability to competitively inhibit the binding of [^{3}H]estra-1,3,5(10)-triene-3,17β-diol (155 Ci mmol^{-1}) to estrogen, of [^{3}H]ORG-2058 (33 Ci mmol^{-1}) to progesterone and of [^{3}H]dihydrotestosterone (112 Ci mmol^{-1}) to androgen receptors in cytosol prepared from rabbit uteri (estrogen and progesterone receptors) and rat prostate (androgen receptors). All of the tritiated ligands were purchased from Amersham, UK. All subsequent steps were carried out at 4°C. The minced tissues were homogenized in 6–10 volumes of 10 mM Tris-HCl buffer (pH 7.4) containing 1.5 mM EDTA (ethylenediaminetetraacetic acid), 0.5 mM DTT (dithiothreitol (DTT)), 10 mM Na_2MoO_4 (estrogen and progesterone receptors), or in 10 mM Tris-HCl buffer (pH: 7.4) containing 0.25 M sucrose and 15 mM Na_2MoO_4 (androgen receptors) [16]. The homogenate was centrifuged at 105 000× *g* for 60 min and the final supernatant (cytosol) was used for competitive binding. Aliquots of 100 109 ml cytosol were incubated overnight at 4°C with tritiated steroids in the presence or absence of synthetic derivatives. Unbound steroids were removed by treatment with dextran-coated charcoal (10% charcoal-Norit A and 0.1% dextran T-70 in the assay buffer). After incubation for 10 min at 4°C, the samples were centrifuged at 1500× *g* for 10 min, and the radioactivity of the supernatant was determined in a Wallac 1409 scintillation counter. The percentage of radioligand bound in the presence of competitor as compared with that bound in its absence was plotted against the concentration of unlabeled steroid. The molar concentration of the steroid competitor that reduced the radioligand binding by 50% (IC_{50}) and the K_i value (inhibition constant) were calculated with GraphPad 2.0 software. All assays were carried out at least three times in duplicates.

3. Results and discussion

3.1. Synthetic studies

The hydroxy group of estrone (**1a**) was protected with a benzyl group, which is easily removed by hydrogenolysis at

the end of the reaction sequence. Treatment of 3-benzyloxyestra-1,3,5(10)-trien-17-one (**1b**) with NaOMe and ethyl formate gave **2a** in a yield of 94%. The reduction of **2a** with KBH_4 in ethanol led to **3a**, **4a** and **5a** in a ratio of 50:45:5 in 92% yield.

The simultaneous development of the two chiral centers permits formation of the four isomers **3a**, **4a**, **5a** and **6a**. However, only three isomers (**3a**, **4a** and **5a**) were detected on thin-layer chromatography. The mixture of the isomers was reacted with acetone in dichloromethane in the presence of a catalytic amount of *p*-toluenesulfonic acid. **3a** was converted to the corresponding cyclic acetonide derivative **3c**, whereas the mixture of **4a** and **5a** could easily be separated by flash chromatography on Al_2O_3. The mixture of **4a** and **5a** was dissolved in acetone, and **4a** crystallized as a hard crystalline product. **5a** was separated from the mother liquor by flash chromatography on silica gel with *tert*-butyl methyl ether. Since reduction of the 17-ketone function in steroids normally yields a 17β-hydroxy group (with a few exceptions [14]), isomers **3a** and **4a**, present in larger quantities, are expected to have different configurations at C-16. In the NMR spectrum of the diacetate **3b**, the doublet at 4.90 ppm, due to coupling of the protons on C-16 and C-17 with a coupling constant of 10.1 Hz, confirms the β,β arrangement of the substituents on C-16 and C-17. The *cis* arrangement is supported by the selective acetonide building. The α,β arrangement of the C-16 and C-17 substituents in **4b** is indicated by the lower coupling constant (8.1 Hz) of the doublet at 4.75 ppm. The debenzylated compound **4d** is known in the literature [13]. The β,α arrangement of the C-16 and C-17 substituents in **5b** is indicated by the lowest coupling constant (2.0 Hz) of the doublet appearing at 4.71 ppm; these values are in good agreement with earlier observations on 16-hydroxymethyl-3-methoxyestra-1,3,5(10)-trien-17-ol isomers [15].

Since availability of the complete series of isomers would permit a number of interesting comparative examinations, we wished to prepare the unknown fourth isomer **6a**, which contains functional groups with the configuration 16α, 17α.

The primary hydroxy group in 16α-hydroxymethyl-3-benzyloxyestra-1,3(10)-trien-17β-ol (**4a**) was acetylated with acetic anhydride, and the product **4c** was oxidized by the Jones method to 16α-acetoxymethyl-3-benzyloxyestra-1,3(10)-trien-17-one (**7**). Reduction of **7** with lithium tri-*tert*-butoxyaluminum hydride afforded the isomers 16α-acetoxymethyl-3-benzyloxyestra-1,3,5(10)-trien-17β-ol (**4c**) and 16α-acetoxymethyl-3-benzyloxyestra-1,3,5(10)-trien-17α-ol (**6c**) in a ratio of 4:1. These isomers (**4c** and **6c**) are separable by flash chromatography. The repeated Jones oxidation of **4c** yields **7**, which returns to the reduction process. The deacetylation of **4c** by the Zemplen method produces the 16α-hydroxymethyl-3-benzyloxyestra-1,3,5(10)-trien-17α-ol (**4a**).

The four 16-hydroxymethyl-3-benzyloxyestra-1,3,5(10)-triene-17-ol isomers (**3a**, **4a**, **5a** and **6a**) were deprotected by hydrogenolysis in the presence of Pd/C to afford the triols **3d**, **4d**, **5c** and **6d**, which are suitable for receptor-binding examinations.

Table 1
Inhibition constant (K_i) values of estra-1,3,5(10)-triene-3,17β-diol (reference compound) and newly synthesized estradiol derivatives (**3d**, **4d**, **5c** and **6d**) on uterine estrogen (ER), androgen (AR) and progesterone (PR) receptors. The following radioligands were used: 5 nM [^{3}H]estra-1,3,5(10)-triene-3,17β-diol (ER), 5 nM [^{3}H]ORG 2058 (PR) and 5 nM [^{3}H]dihydrotestosterone (AR). The relative binding affinity values on the ER were calculated according to the following equation: RBA = $K_i^{estradiol}/K_i$*

K_i [nM]				
Compound	ER	AR	PR	RBA
Estradiol	1.19 ± 0.53	>100 000	1532 ± 102	100
3d	82.32 ± 6.53	660.9 ± 58.9	3398 ± 249.5	1.44
4d	215.3 ± 47.6	834.2 ± 127.3	>100 000	0.62
5c	814.4 ± 79.5	>100 000	>100 000	0.14
6d	213.3 ± 35.7	>100 000	6242 ± 567	0.44

3.2. Radioligand-binding assays

The binding affinities of estra-1,3,5(10)-triene-3,17β-diol and its derivatives **3d**, **4d**, **5c** and **6d** for the estrogen, progesterone and androgen receptors were determined by radioligand-binding assay. Specifically bound [^{3}H]estra-1,3,5(10)-triene-3,17β-diol was readily displaced from the rabbit uterine estrogen receptor by estra-1,3,5(10)-triene-3,17β-diol and **3d**.

The remaining three derivatives (**4d**, **5c** and **6d**) exhibit lower affinities for the estrogen receptor, but only the K_i value of **5c** is significantly lower than the others. The inhibition constant (K_i) values and relative binding affinities (RBA) for the estrogen receptor are listed in Table 1. 16β-Hydroxymethylestra-1,3,5(10)-triene-3,17β-diol (**3d**) is the most potent estradiol derivative in this series (K_i: 82.32 nM), but its affinity is about two orders of magnitude lower than that measured for estradiol. Fevig et al. reported that 16α-hydroxymethylestra-1,3,5(10)-triene-3,17β-diol (**4d**) binds to the estrogen receptor with low affinity as compared to estra-1,3,5(10)-triene-3,17β-diol and has an RBA value of 2.4 [13]. Our study indicated that this compound has a K_i value of 191.7 nM and an RBA of 0.62 on rabbit uterine cytosol, relative to estra-1,3,5(10)-triene-3,17β-diol. The differences between the RBA values stem from the fact that the two groups used different uterine cytosol from different species (rat or lamb [13] and rabbit in the present work). The K_i values of 16α-hydroxymethylestra-1,3,5(10)-triene-3,17α-diol (**6d**) and 16β-hydroxymethylestra-1,3,5(10)-triene-3,17α-diol (**5c**) are higher than 200 nM (268.2 nM and 863.3 nM, respectively). The 16-hydroxymethylestra-1,3,5(10)-triene-3,17-diol isomers **3d**, **4d**, **5c** and **6d** possess less affinity toward the estrogen receptor than estra-1,3,5(10)-triene-3,17β-diol, because this region of the receptor tolerates groups of moderate size and polarity [13,

17]. The tested compounds inhibit [^{3}H]ORG 2058 binding to the progesterone receptor only in the micromolar range. The finding, that the tested compounds bind to the androgen and progesterone receptors with very low affinity is surely because these compounds contain a phenolic function and not a 3-keto function, which forms hydrogen bonds with Gln^{711} and Arg^{752} in the case of the androgen receptor [18,19] and with Gln^{725} and Arg^{766} in the case of the progesterone receptor [19,20]. It is interesting that **3d** and **4d** bind to the androgen receptor with affinities (K_i: 660.9 nM and 834.2 nM, respectively) more than 150 times higher than those for the other compounds ($K_i > 100$ 109 mM). In this case, the 17β-hydroxy group in estra-1,3,5(10)-triene-3,17β-diol, **3d** and **4d** forms hydrogen bonds with the side-chain of Asn^{705} and Thr^{877} while the 17α-hydroxy group in **5c** and **6d** can not. Moreover, the 16-hydroxymethyl group in **3d** and **4d** can form another hydrogen bond, which increases the affinity of these compounds toward the androgen receptor, while estra-1,3,5(10)-triene-3,17β-diol, which lacks a 16-hydroxymethyl group possesses a much lower affinity than those of **3d** and **4d**, but significantly higher than those of **5c** and **6d**, which contain a 17α-hydroxy group.

In summary, four 16-hydroxymethylestra-1,3,5(10)-triene-3,17-diol isomers were prepared and tested in radioligand-binding assays. The estrogen receptor recognizes these compounds, but their RBA values are lower than 2.0% as compared with that of the reference molecule estradiol. The affinities of the tested agents for the androgen and progesterone receptors are very low ($K_i > 100$ μM and 1 μM, respectively); the prepared 16-hydroxymethyl-3,17-estradiol isomers are therefore estrogen receptor-selective molecules.

Acknowledgments

Financial support by the Hungarian National Research Foundation (OTKA grant T 032265) and by the Hungarian Board of Education (FKFP grant 0110/2000) is gratefully acknowledged.

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