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RESEARCH PAPER

Synthesis of substituted 15β-alkoxy estrone derivatives and their cofactor-dependent inhibitory effect on 17β-HSD1

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ABSTRACT

17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) is a key enzyme in the biosynthesis of 17β-estradiol. Novel estrone-based compounds bearing various 15β-oxa-linked substituents and hydroxy, methoxy, benzyloxy, and sulfamate groups in position C3 as potential 17β-HSD1 inhibitors have been synthesized. In addition, *in vitro* inhibitory potentials measured in the presence of excess amount of NADPH or NADH were investigated. We observed substantial inhibitory potentials for several derivatives ($IC_{50} < 1\,\mu M$) and increased binding affinities compared to unsubstituted core molecules. Binding and inhibition were found to be cofactor-dependent for some of the compounds and we propose structural explanations for this phenomenon. Our results may contribute to the development of new 17β-HSD1 inhibitors, potential drug candidates for antiestrogen therapy of hormone-dependent gynecological cancers.

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Introduction

The 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1, EC 1.1.1.62) catalyzes the conversion of estrone (**1**) to highly active estrogen 17β-estradiol (**2**) (Scheme 1). Human 17β-HSD1 is expressed in tissues of female reproductive organs (such as placenta, ovarian follicles, mammary gland and uterus)[1]. The expression of 17β-HSD1 was shown to be elevated and to have prognostic significance in gynecological malignancies, e.g. in hormone-dependent breast cancer[2–4]. The enzyme is involved in progression of these diseases due to the increase of local 17β-estradiol (**2**) levels. Inhibition of the enzyme is able to control estrogen actions at the pre-receptor level; therefore, a suppression of the 17β-HSD1 activity has a significant therapeutic potential[5]. Numerous 17β-HSD1 inhibitors based on either steroidal or non-steroidal structures have been developed, but none of them have been introduced to the medical practice, so far[6–11].

Extensive earlier studies indicated that attachment of C15 substituents on the estrone scaffold might be a successful way for the synthesis of inhibitors against 17β-HSD1[12,13]. In addition to improving binding affinity to 17β-HSD1, appropriate side chains may confer selectivity towards 17β-HSD type 2[7,10,14]. 17β-HSD type 2 catalyzes inactivation of 17β-estradiol (**2**) to estrone (**1**) and is considered to be an important enzyme for the control of proliferation of breast cancer cells[15]. C15 substituents may also suppress inherent estrogenicity of the estrone core[9,10,12,16]. These features of C15 derivatives make this substitution strategy particularly attractive for the development of estrone-based 17β-HSD1 inhibitors.

It was shown that neither the presence of the phenolic hydroxyl group nor the hydrogen bonding of the C3 function is essential to the effective 17β-HSD1 binding of estrone derivatives[17]. This tolerance provides further options for the modulation of enzyme inhibition and other biological effects of the candidate compounds. Accordingly, several 3-methoxy analogues were tested as 17β-HSD1 inhibitors[12,13] presumably that they exert reduced estrogenicity compared to estrone possessing phenolic hydroxy group[18]. Estrone 3-sulfamate analogues in this series tend to show moderate 17β-HSD1 inhibition[13]. However, the sulfamate moiety may lead to an inhibitory effect against steroid sulfatase (STS), another enzyme playing a central role in 17β-estradiol biosynthesis. Such a dual inhibitory effect was recently proposed to be beneficial as it should result in a stronger suppression of estrogen biosynthesis compared to selective inhibition of 17β-HSD1[19]. Steroidal sulfamates may be delivered to the tumour by the carbonic anhydrase II, and evolve targeted actions[20].

In this paper, we report the synthesis and chemical characterization of new substituted 15β-alkoxy estrone derivatives. We also aimed to investigation 17β-HSD1 inhibitory potentials of these compounds, including comparison of their inhibitor potentials measured in the presence of NADPH or NADH.

Experimental

General

Melting points (mp) were determined on a Kofler block and are uncorrected. Specific rotations were measured in $CHCl_3$, or MeOH (*c* 1) at 25 C with a POLAMAT-A (Zeiss-Jena) polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. Elementary analysis data were determined with a PerkinElmer CHN analyzer model 2400. Reactions were monitored by TLC on Kieselgel-G (Merck Si 254 F) layers (0.25 mm thick); solvent systems (ss): (A) (ethyl acetate/CH_2Cl_2 (1:1 v/v), (B) acetone/toluene/hexane (30:35:35 v/v), (C) ethyl acetate/CH_2Cl_2 (5:95 v/v), (D) ethyl acetate. The spots were

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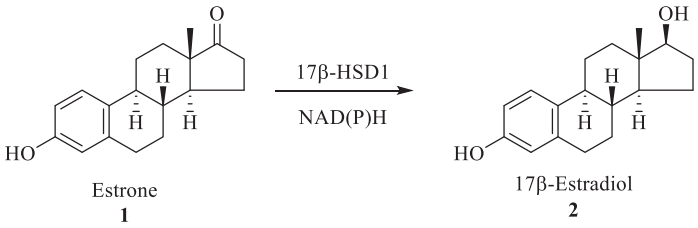


Scheme 1. Transformation of estrone to 17β-estradiol catalyzed by 17β-HSD1.

detected by spraying with 5% phosphomolybdic acid in 50% aqueous H_3PO_4. The R_f values were determined for the spots observed by illumination at 254 and 365 nm. Flash chromatography: silica gel 60, 40–63 μm. All solvents were distilled immediately prior to use. NMR spectra were recorded on a Bruker DRX 500 instrument at 500 (^{1}H NMR) or 125 MHz (^{13}C NMR). Chemical shifts are reported in ppm (δ scale), and coupling constants (J) in Hz. For the determination of multiplicities, the J-MOD pulse sequence was used.

Materials for enzyme experiments

Radiolabelled steroids [6,7-^{3}H(N)]estrone (S. A. = 52 Ci/mmol), was obtained from American Radiolabeled Chemicals (St. Louis, MO, USA). Non-radioactive estrone, 3-methyl-*O*-estrone, 3-benzyl-*O*-estrone and estrone-3-sulfamate (EMATE) standards and other chemicals and solvents of analytical grade purity were purchased from Sigma (St. Louis, MO, USA), from Fluka (Buchs, Switzerland) or Merck (Darmstadt, Germany).

Human placenta microsomal fraction and cytosol were produced and applied with the permission of the Human Investigation Review Board of the University of Szeged.

General procedure for the synthesis of 3-methoxy-, and 3-benzyloxy-15β-alkoxyestra-1,3,5(10)-trien-17-ones (8–12)

A solution of **6**[23] or **7**[12] (2 mmol) in CH_2Cl_2 (10 ml) and ethane-1,2-diol, propane-1,3-diol or butane-1,4-diol (15 ml), containing 5% aqueous sodium hydroxide (1 ml) was stirred at room temperature for 6 h. The reaction mixture, after the addition of CH_2Cl_2 (50 ml), was diluted with water (100 ml). The organic phase was separated, washed with water, dried over Na_2SO_4, and evaporated *in vacuo*. The residual product was purified by flash chromatography using ethyl acetate/CH_2Cl_2 in different proportions.

3-Methoxy-15β-(2′-hydroxy)ethoxy-estra-1,3,5(10)-trien-17-one (8)

Compound **6** (565 mg, 2 mmol) and ethane-1,2-diol (15 ml) were used for the synthesis as described in general procedure. The crude product was chromatographed on silica gel with dichloromethane/hexane (1:1 v/v) to yield pure **8** (580 mg, 84%). Mp: 139–140 °C; $R_f = 0.55$ (ss B); $[\alpha]_D^{20}$ + 54 (c 1 in $CHCl_3$). Found: C, 73.45; H, 7.98. $C_{21}H_{28}O_4$ (344.45) requires: C,73.23; H, 8.19%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.16 (s, 3H, 18-H_3), 2.92 (m, 2H, 6-H_2), 3.42 and 3.64 (2xm, 2x1H, linker H_2), 3.72 (m, 2H, linker OCH_2), 3.77 (s, 3H, 3-OCH_3), 4.22 (t, 1 H, $J = 5.3$ Hz, 15-H), 6.64 (d, 1H, $J = 2.2$ Hz, 4-H), 6.71 (dd, 1H, $J = 8.6$ Hz, $J = 2.2$ Hz, 2-H), 7.19 (d, 1H, $J = 8.6$ Hz, 1-H).^{13}C NMR (δ, ppm, $CDCl_3$):17.5 (C-18), 25.6, 26.2, 29.4, 32.5, 34.8, 43.1, 44.0, 47.2 (C-13), 54.4, 55.1 (3-OCH_3), 61.9 (CH_2-OH), 70.7 (linker CH_2), 75.1 (C-15), 111.4 (C-2), 113.8 (C-4), 126.0 (C-1), 132.0 (C-10), 137.6 (C-5), 157.5 (C-3), 219.4 (C-17).

3-Methoxy-15β-(3′-hydroxy)propoxy-estra-1,3,5(10)-trien-17-one (9)

Compound **6** (565 mg, 2 mmol) and propane-1,3-diol (15 ml) were used for the synthesis as described in general procedure. The crude product was chromatographed on silica gel with ethyl acetate/CH_2Cl_2 (1:99 v/v) to yield pure **9** (575 mg, 80%). Mp: 83–84 °C; $R_f = 0.50$ (ss B); $[\alpha]_D^{20}$ + 48 (c 1 in $CHCl_3$). Found: C, 73.92; H, 8.26%. $C_{22}H_{30}O_4$ requires: C, 73.71; H, 8.44%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.15 (s, 3H, 18-H_3), 2.94 (m, 2H, 6-H_2), 3.42 (m, 1H) and 3.74 (m, 3H): 2xlinker H_2, 3.77 (s, 3H, 3-OCH_3), 4.17 (t, 1 H, $J = 5.3$ Hz, 15-H), 6.64 (d, 1H, $J = 2.2$ Hz, 4-H), 6.71 (dd, 1H, $J = 8.6$ Hz, $J = 2.2$ Hz, 2-H), 7.19 (d, 1H, $J = 8.6$ Hz, 1-H).^{13}C NMR (δ, ppm, $CDCl_3$):17.4 (C-18), 25.7, 26.2, 29.4, 32.3, 32.6, 34.9, 42.8, 44.1, 47.2 (C-13), 54.2, 55.1 (3-OCH_3), 61.5 (CH_2-OH), 68.3 (linker CH_2), 75.1 (C-15), 111.4 (C-2), 113.8 (C-4), 126.1 (C-1), 131.9 (C-10), 137.7 (C-5), 157.5 (C-3), 219.5 (C-17).

3-Benzyloxy-15β-(2′-hydroxy)ethoxy-estra-1,3,5(10)-trien-17-one (10)

Compound **7** (717 mg, 2 mmol) and ethane-1,2-diol (15 ml) were used for the synthesis as described in general procedure. The crude product was chromatographed on silica gel with ethyl acetate/CH_2Cl_2 (30:70 v/v) to yield pure **10** (690 mg, 82%). Mp: 102–104 °C; $R_f = 0.45$ (ss B); $[\alpha]_D^{20}$ + 73 (c 1 in $CHCl_3$). Found: C, 76.95; 7.84. $C_{27}H_{32}O_4$ (420.54) requires: C, 77.11; H, 7.67%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.17 (s, 3H, 18-H_3), 2.91 (m, 2H, 6-H_2), 3.42 and 3.65 (2xm, 2x1H, linker H_2), 3.73 (m, 2H, linker OCH_2), 4.23 (t, 1 H, $J = 5.7$ Hz, 15-H), 5.04 (s, 2H, Bn-H_2), 6.74 (d, 1H, $J = 2.2$ Hz, 4-H), 6.79 (dd, 1H, $J = 8.6$ Hz, $J = 2.2$ Hz, 2-H), 7.19 (d, 1H, $J = 8.6$ Hz, 1-H), 7.32 (t, 1H, $J = 7.3$ Hz, 4-H of Bn), 7.39 (t, 2H, $J = 7.3$ Hz, 3-H and 5-H of Bn), 7.45 (d, 2H, $J = 7.3$ Hz, 2-H and 6-H of Bn).^{13}C NMR (δ, ppm, $CDCl_3$): 17.6 (C-18), 25.7, 26.2, 29.5, 32.7, 34.9, 43.1, 44.2, 47.2 (C-13), 54.5, 62.0 (CH_2-OH), 70.0 (linker CH_2), 70.8 (Bn-CH_2), 75.2 (C-15), 112.4 (C-2), 114.9 (C-4), 126.1 (C-1), 127.4 (2 C: C-2 and C-6 of Bn), 127.8 (C-4 of Bn), 128.5 (2 C: C-3 and C-5 of Bn), 132.4 (C-10), 137.2 (C-1 of Bn), 137.7 (C-5), 156.9 (C-3), 219.1 (C-17).

3-Benzyloxy-15β-(3′-hydroxy)propoxy-estra-1,3,5(10)-trien-17-one (11)

Compound **7** (717 mg, 2 mmol) and propane-1,3-diol (15 ml) were used for the synthesis as described in general procedure. The crude product was chromatographed on silica gel with ethyl acetate/CH_2Cl_2 (30:70 v/v) to yield pure **11** (742 mg, 78%). Mp: 144–146 °C; $R_f = 0.35$ (ss B); $[\alpha]_D^{25}$ + 88 (c 1 in $CHCl_3$). Found: C, 77.54; H, 8.02. $C_{28}H_{34}O_4$ (434.57) requires: C, 77.39; H, 7.89%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.15 (s, 3H, 18-H_3), 2.94 (m, 2H, 6-H_2), 3.36 (t, 2H, $J = 6.0$ Hz, linker H_2), 3.72 (m, 2H, linker H_2), 4.17 (t, 1 H, $J = 6.5$ Hz, 15-H), 5.03 (s, 2H, H_2 of Bn), 6.73 (d, 1H, $J = 3.0$ Hz, 4-H), 6.78 (dd, 1H, $J = 10.5$ Hz, $J = 3.0$ Hz, 2-H), 7.18 (d, 1H, $J = 10.5$ Hz, 1-H), 7.37 (m, 5H, 5x CH of Bn). ^{13}C NMR (δ, ppm, $CDCl_3$): 18.0 (C-18), 26.2, 26.6, 30.0, 32.8, 33.2, 35.4, 43.3 (linker CH_2), 44.7, 47.7 (C-13), 54.8, 62.1 (CH_2-OH), 68.9 (OCH_2), 70.4 (Bn-CH_2), 75.7 (C-15), 112.8 (C-2), 115.4 (C-4), 126.6 (C-1), 127.9 (C-2 and -6 of Bn), 128.3 (C-4 of Bn), 129.0 (C-3and C-5 of Bn), 132.8 (C-10), 138.0 (C-1 of Bn), 138.3 (C-5), 157.4 (C-3), 219.7 (C-17)

3-Benzyloxy-15β-(4′-hydroxy)butoxy-estra-1,3,5(10)-trien-17-one (12)

Compound **7** (717 mg, 2 mmol) and butane-1,4-diol (15 ml) were used for the synthesis as described in general procedure. The crude product was chromatographed on silica gel with ethyl

acetate/CH_2Cl_2 (1:1 v/v) to yield pure **12** (720 mg, 80%). Mp: 131–130 °C; $R_f = 0.30$ (ss B); $[\alpha]_D^{25}$ + 58 (*c* 1 in $CHCl_3$). Found: C, 77.48; H, 9.15. $C_{29}H_{36}O_4$ (448.59) requires: C, 77.64; H, 8.09%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.16 (s, 3H, 18-H_3), 3.34 (m, 2H, linker H_2, 3.64 (m, 2H, O-CH_2), 4.14 (t, 1 H, $J = 6.5$ Hz, 15-H), 5.03 (s, 2H, H_2 of Bn), 6.73 (d, 1H, $J = 3.0$ Hz, 4-H), 6.78 (dd, 1H, $J = 10.5$ Hz, 2-H), $J = 3.0$ Hz, 2H), 7.18 (d, 1H, $J = 10.5$ Hz, 1-H), 7.36 (m, 5H, 5x CH of Bn). ^{13}C NMR (δ, ppm, $CDCl_3$): 18.0 (C-18), 26.2, 26.8, 27.0, 30.0, 33.2, 35.3, 41.9, 43.7 (linker CH_2), 44.7, 47.7 (C-13), 55.0, 63.0 (CH_2OH), 69.9 (OCH_2), 70.5 (CH_2 of Bn), 75.2 (C-15), 112.8 (C-2), 115.4 (C-4), 126.6 (C-1), 126.6 (C-2 and C-6 of Bn), 127.8 (C-4 of Bn), 128.3 (C-3 and C-5 of Bn), 132.9 (C-10), 138.0 (C-1 of Bn), 138.3 (C-5), 157.3 (C-3), 220.2 (C-17).

3-Methoxy-15β-(carboxyl)methoxy-estra-1,3,5(10)-trien-17-one (13)

Compound **8** was dissolved in acetone (15 ml). Jones reagent (1 ml) was added while cooling with ice. The mixture was diluted with ice-water, and the precipitate was filtered off, washed with water and dried. The crude product was dissolved in CH_2Cl_2 and was chromatographed on silica gel with ethyl acetate/CH_2Cl_2 (25:75 v/v), yielding pure **13** (285 mg, 39%). Mp: 142–144 °C; $R_f = 0.32$ (ss B); $[\alpha]_D^{25}$ + 42 (*c* 1 $CHCl_3$). Found C, 70.54; H, 7.43. $C_{21}H_{26}O_5$ (358.43) requires: C, 70.73; H, 7.31%. ^{1}H NMR (δ, ppm, DMSO-d_6): 1.08 (s, 3H, 18-H_3), 2.82 (m, 2H, 6-H_2), 3.69 (s, 3H, 3-OCH_3), 4.05 (m, 2H, O-CH_2), 4.29 (t, 1 H, $J = 5.5$ Hz, 15-H), 6.63 (s, 1H, 4-H), 6.68 (dd, 1H, $J = 8.5$ Hz, $J = 2.0$ Hz, 2-H), 7.17 (d, 1H, $J = 8.5$ Hz, 1-H), 12.60 (brs, 1H, OH). ^{13}C NMR (δ, ppm, DMSO-d_6): 17.2 (C-18), 25.3, 25.4, 29.1, 32.4, 34.7, 42.5 (C-13), 43.7, 46.5, 53.2, 54.8 (3-OCH_3), 65.8 (O-CH_2), 74.9 (C-15), 111.4 (C-2), 113.5 (C-4), 126.0 (C-1), 131.8 (C-10), 137.4 (C-5), 157.1 (C-3), 171.9 (COOH), 218.8 (C-17).

3-Methoxy-15β-(2′-carboxyl)ethoxy-estra-1,3,5(10)-trien-17-one (14)

Compound **9** was dissolved in acetone (15 ml). Jones reagent (1 ml) was added during cooling with ice. The mixture was diluted with ice-water, and extracted with CH_2Cl_2. The organic phase was evaporated to dryness and subjected to chromatographic separation on silica gel in ethyl acetate/CH_2Cl_2 (1:1 v/v), yielding pure **14** (346 mg, 46%). Mp: 150–152 °C; $R_f = 0.30$ (ss B); $[\alpha]_D^{25}$ + 46 (*c* 1 in $CHCl_3$). Found: C, 71.15; H, 7.32. $C_{22}H_{28}O_5$ (372.46) requires: C, 70.94; H, 7.58%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.14 (s, 3H, 18-H_3), 2.91 (m, 2H, 6-H_2), 3.59 (m, 1H, 14-H), 3.80 (s, 4H, 2x linker H_2), 4.21 (t, 1 H, $J = 6.5$ Hz, 15-H), 6.66 (s, 1H, 4-H), 6.73 (dd, 1H, $J = 10.5$ Hz, $J = 3.0$ Hz, 2-H), 7.21 (d, 1H, $J = 10.5$ Hz, 1-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 18.0 (C-18), 26.2, 26.6, 29.9, 33.1, 35.2, 35.5, 43.3, 44.6 (linker CH_2), 47.7 (C-13), 54.9, 55.6 (3-OCH_3), 65.0 (linker CH_2), 75.4 (C-15), 111.7 (C-2), 114.5 (C-4), 126.6 (C-1), 132.6 (C-10), 138.3 (C-5), 158.0 (C-3), 177.2 (COOH), 220.1 (C-17).

3-Benzyloxy-15β-(2′-carboxyl)ethoxy-estra-1,3,5(10)-trien-17-one (15)

Compound **11** (435 mg, 1 mmol) was dissolved in acetone (10 ml) and Jones reagent (1 ml) was added while cooling with ice. The mixture was diluted with ice-water, the precipitate separating out was filtered, dried and recrystallized from CH_2Cl_2/hexane, yielding **15** (342 mg, 76%). Mp: 184–186 °C; $R_f = 0.30$ (ss B); $[\alpha]_D^{20}$ + 98 (*c* 1 in $CHCl_3$). (Found: C, 74.86; H, 7.35. $C_{28}H_{32}O_5$ (448.55) requires: C, 74.79; H, 7.19%). ^{1}H NMR (δ, ppm, $CDCl_3$): 1.15 (s, 3H, 18-H_3), 2.93 (m, 2H, 6-H_2), 3.60 (m, 1H, O-CH_2), 3.81 (m, 1H, O-CH_2), 4.21 (t, 1H, $J = 6.5$ Hz, 15-H), 5.06 (s, 2H, Bn-H_2), 6.75 (s, 1H, 4-H), 6.80 (dd, 1H, $J = 10.5$ Hz, $J = 3.0$ Hz, 2-H), 7.21 (d, 1H, $J = 10.5$ Hz, 1-H), 7.40 (m, -5H, 5x CH of Bn). ^{13}C NMR (δ, ppm, $CDCl_3$): 17.8 (C-18), 26.2, 26.6, 29.9, 33.2, 35.2, 35.5, 43.3 (linker CH_2), 44.6, 47.7 (C-13), 54.9, 65.0 (O-CH_2), 70.4 (Bn-CH_2), 75.4 (C-15), 112.6 (C-2), 115.5 (C-4), 126.6 (C-1), 127.9 (C-2 and C-6 of Bn), 128.3 (C-4 of Bn), 129.0 (C-3 and C-5 of Bn), 132.9 (C-10), 138.0 (C-1 of Bn), 138.4 (C-5), 157.3 (C-3), 177.3 (COOH), 220.1 (C-17).

3-Hydroxy-15β-(2′-carboxyl)ethoxy-estra-1,3,5(10)-trien-17-one (16)

Compound **15** (448 mg, 1 mmol) was dissolved in ethyl acetate (30 ml) and the solution containing Pd/C (300 mg, 10%) was hydrogenated under 5 bar for 6 h at room temperature. The solution was filtered off, the residue was crystallized from methanol to yield **16** (320 mg, 89%). Mp: 200–202 °C; $R_f = 0.2$ (ss B); $[\alpha]_D^{25}$ + 29 (*c* 1 in $CHCl_3$). Found C, 70.18; H, 7.45. $C_{21}H_{26}O_5$ requires: C, 70.37; H, 7.31%. ^{1}H NMR (δ, ppm, DMSO): 0.78 (s, 3H, 18-H_3), 2.52 (m, 2H, 6-H_2), 3.18 (m, 6H, 2x linker H_2), 3.90 (t, 1 H, $J = 6.5$ Hz, 15-H), 6.24 (d, 1H, $J = 2.5$ Hz, 4-H), 6.29 (dd, 1H, $J = 10.5$ Hz, $J = 2.5$ Hz, 2-H), 6.81 (d, 1H, $J = 10.5$ Hz, 1-H). ^{13}C NMR (δ, ppm, DMSO-d_6): 17.6 (C-18), 25.8, 26.0, 29.3, 32.7, 35.1, 35.4, 43.1 (linker CH_2), 43.9, 46.9 (C-13), 53.6, 65.1 (linker CH_2), 74.6 (C-15), 113.1 (C-2), 115.4 (C-4), 126.2 (C-1), 130.6 (C-10), 137.6 (C-5), 155.4 (C-3), 173.1 (COOH), 219.3 (C-17).

3-Benzyloxy-15β-(3′-carboxyl)propoxy-estra-1,3,5(10)-trien-17-one (17)

Compound **12** (448 mg, 1 mmol) was dissolved in acetone (10 ml) and Jones reagent (1 ml) was added while cooling with ice. The mixture was diluted with ice-water, the precipitate separating out was filtered, dried, and crystallized from acetone/hexane to yield **17** (390 mg, 84%). Mp: 138–140 °C; $R_f = 0.25$ (ss B); $[\alpha]_D^{25}$ + 57 (*c* 1 in $CHCl_3$). Found: C, 75.22; H, 7.67. $C_{29}H_{34}O_5$ (462.58) requires: C, 70.30; H, 7.41%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.14 (s, 3H, 18-H_3), 3.29 (m, 1H, O-CH_2), 3.56 (m, 1H, O-CH_2), 4.12 (t, 1 H, $J = 6.5$ Hz, 15-H), 5.02 (s, 2H, Bn-H_2), 6.73 (d, 1H, $J = 3.0$ Hz, 4-H), 6.77 (dd, 1H, $J = 10.5$ Hz, $J = 3.0$ Hz, 2-H), 7.18 (d, 1H, $J = 10.5$ Hz, 1-H), 7.36 (m, 5H, 5x CH of Bn). ^{13}C NMR (δ, ppm, $CDCl_3$): 18.0 (C-18), 25.5, 26.2, 26.8, 29.9, 31.3, 33.1, 35.3, 43.5 (linker CH_2), 44.7, 47.7 (C-13), 55.0, 68.6 (O-CH_2), 70.4 (Bn-CH_2), 75.3 (C-15), 112.8 (C-2), 115.4 (C-4), 126.6 (C-1), 127.8 (C-2 and C-6 of Bn), 128.3 (C-4 of Bn), 129.0 (C-3 and C-5 of Bn), 132.9 (C-10), 137.7 (C-1 of Bn), 138.3 (C-5), 157.3 (C-3), 179.5 (COOH), 220.2 (C-17).

3-Methoxy-15β-(2′-cyano)ethoxy-estra-1,3,5(10)-trien-17-one (18)

Compound **6** (282 mg, 1 mmol) was dissolved in CH_2Cl_2 (10 ml) and 3-hydroxypropionylnitrile (10 ml), containing 5% aqueous NaOH (1 ml), was stirred at room temperature for 8 h. CH_2Cl_2 (50 ml) was added to the reaction mixture and then it was diluted with water (100 ml). The organic phase was separated, washed with water, dried over Na_2SO_4, and evaporated *in vacuo*. The residual product was purified by flash chromatography using CH_2Cl_2 to yield **18** (305 mg, 86%). Mp: 197–200 °C; $R_f = 0.50$ (ss B); $[\alpha]_D^{25}$ + 63 (*c* 1 in $CHCl_3$). Found C, 74.92; H, 7.55. $C_{22}H_{27}NO_3$ (353.45) requires C, 74.76; H, 7.70%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.17 (s, 3H, 18-H_3), 2.60 (s 2H, linker H_2), 3.00 (m, 2H, 6-H_2), 3.65 (s, 3H, 3OCH_3), 3.77 (s, 2′H, linker H_2), 16-H_2), 4.23 (t, 1 H, $J = 6.4$ Hz, 15-H), 6.65 (s, 1H, 4-H), 6.71 (d, 1H, $J = 10.5$ Hz, 2-H), 7.19 (d, 1H, $J = 10.5$ Hz, 1-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 18.2 (C-18), 20.0, 26.4, 27.1, 30.1, 33.4, 35.4, 43.5, 44.9 (linker CH_2), 47.9 (C-13), 55.1 (3-OCH_3), 55.9, 65.0 (O-CH_2), 72.2 (C-15), 112.2 (C-2), 114.6 (C-4),

118.3 (CN), 126.8 (C-1), 132.7 (C-10), 138.5 (C-5), 158.3 (C-3), 219.2 (C-17).

3-Benzyloxy-15β-(2′-cyano)ethoxy-estra-1,3,5(10)-trien-17-one (19)

Compound **7** (358 mg, 1 mmol) dissolved in CH_2Cl_2 (10 ml) and 3-hydroxypropionytrile (10 ml), containing 5% aqueous NaOH (1 ml), was stirred at room temperature for 8 h. After adding CH_2Cl_2 (50 ml) to the reaction mixture, the organic phase was separated, washed with water, dried over Na_2SO_4, and evaporated *in vacuo*. The residual product was purified by flash chromatography using ethyl acetate/CH_2Cl_2 (2.5/97.5 v/v) to yield **19** (346 mg, 80%). Mp: 183–185 °C; $R_f = 0.45$ (ss B); $[\alpha]_D^{25} + 54$ (*c* 1 in $CHCl_3$). Found C, 78.52; H, 7.42. $C_{28}H_{31}NO_3$ (429.55) requires: C, 78.29; H, 7.27%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.20 (s, 3H, 18-H_3), 3.54 (m, 1H, O-CH_2), 3.78 (m, 1H, O-CH_2), 4.27 (t, 1 H, $J = 6.5$ Hz, 15-H), 5.07 (s, 2H, Bn-H_2), 6.78 (s, 1H, 4-H), 6.81 (d, 1H, $J = 11.0$ Hz, 2-H), 7.22 (d, 1H, $J = 11.0$ Hz, 1-H), 7.41 (m, 5H, 5x CH of Bn). ^{13}C NMR (δ, ppm, $CDCl_3$): 18.0 (C-18), 19.8, 26.2, 26.8, 29.9, 33.2, 35.2, 43.3 (CN), 44.7, 47.7 (C-13), 54.9, 64.8 (O-CH_2), 70.4 (Bn-CH_2), 76.0 (C-15), 112.8 (C-2), 115.4 (C-4), 118.1 (CN), 126.6 (C-1), 127.9 (C-2 and C-6-of Bn), 128.3 (C-4-of Bn), 129.0 (C-3 and C-5 of Bn), 132.7 (C-10), 137.7 (C-1 of Bn), 138.4 (C-5), 157.4 (C-3), 219.1 (C-17).

3-Hydroxy-15β-(2′-cyano)ethoxy-estra-1,3,5(10)-trien-17-one (20)

Compound **19** (430 mg, 1 mmol) was dissolved in ethyl acetate (30 ml) and the solution containing Pd/C (300 mg, 10%) was hydrogenated under 5 bar pressure for 6 h, at room temperature. The reaction mixture was filtered off, evaporated *in vacuo* and crystallized from CH_2Cl_2/hexane to yield **20** (286 mg, 84%). Mp: 221–223 °C; $R_f = 0.25$ (ss A); $[\alpha]_D^{25} + 37$ (*c* 1 in MeOH). Found C, 74.62; H, 7.35. $C_{21}H_{25}O_3N$ (339.43) requires: C, 74.31; H, 7.42%. ^{1}H NMR (δ, ppm, DMSO-d_6): 1.07 (s, 3H, 18-H_3), 2.76 (m, 2H, 6-H_2), 3.33 (s, 3H, CN-H_2), 15-H), 3.70 (m, 1H, O-CH_2), 4.04 (m, 1H, O-CH_2), 4.21 (t, 1 H, $J = 6.5$ Hz, 15-H), 6.47 (d, 1H, $J = 3.0$ Hz, 4-H), 6.53 (dd, 1H, $J = 10.5$ Hz, $J = 3.0$ Hz, 2-H), 7.05 (d, 1H, $J = 10.5$ Hz, 1-H), 9.00 (brs, 1H, 3-OH). ^{13}C NMR (δ, ppm, DMSO-d_6): 17.2 (C-18), 18.5, 25.4, 25.7, 28.9, 32.3, 34.7, 42.7 (linker CH_2), 43.5, 46.5 (C-13), 53.1, 63.7 (O-CH_2), 74.5 (C-15), 112.7 (C-2), 115.0 (C-4), 119.2 (CN), 125.9 (C-1), 130.1 (C-10), 137.2 (C-5), 155.0 (C-3), 218.6 (C-17).

3-Sulfamoyloxy-15β-(2′-cyano)ethoxy-estra-1,3,5(10)-trien-17-one (21)

Compound **20** (340 mg, 1 mmol) was dissolved in dimethylformamide (20 ml), and 575 mg (5 mmol) sulfamoylchloride was added dropwise during cooling with ice. The reaction mixture was allowed to stand 6 h and then poured onto ice (300 g). The precipitate was filtered off and dried. The product was crystallized from ethyl acetate to yield **21** (360 mg, 86%). Mp: 78–80 °C; $R_f = 0.30$ (ss A); $[\alpha]_D^{25} + 52$ (*c* 1 in $CHCl_3$). Found: C, 60 55; H, 6.42. $C_{21}H_{26}N_2O_5S$ (418.51) requires: C, 60.27; H, 6.26%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.10 (s, 3H, 18-H_3), 3.45 (m, 1H, O-CH_2), 3.70 (m, 1H, O-CH_2), 4.19 (t, 1 H, $J = 7.0$ Hz, 15-H), 5.25 (s, 2H, NH_2), 6.99 (d, 1H, $J = 3.0$ Hz, 4-H), 7.02 (dd, 1H, $J = 10.5$ Hz, $J = 3.0$ Hz, 2-H), 7.23 (t, 1 H, $J = 10.5$ Hz, 1-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 17.9 (C-18), 19.8, 26.0, 26.4, 29.6, 33.0, 34.7, 43.2 (linker CH_2), 44.7, 47.6 (C-13), 54.8, 64.7 (OCH_2), 75.9 (C-15), 118.3 (CN), 119.5 (C-2), 122.5 (C-4), 127.0 (C-1), 139.3 (C-10), 139.4 (C-5), 148.5 (C-3), 219.3 (C-17).

3-Benzyloxy-15β-(2′-methoxy-2′-oxoethoxy)-estra-1,3,5(10)-trien-17-one (22)

Compound **7** (717 mg, 2 mmol) in CH_2Cl_2 (10 ml) and ethane-1,2 diol (15 ml), containing 5% aqueous sodium hydroxide (1 ml) was stirred at room temperature for 6 h. The reaction mixture was diluted with water (100 ml). The organic phase was separated, washed with water, dried and evaporated *in vacuo*. The crude 3-benzyloxy-15β-(2′-hydroxy)ethoxy-estra-1,3,5(10)-trien-17-one was dissolved in acetone (15 ml) and Jones reagent (1 ml) was added cooling with ice. The mixture was diluted with ice-water, the precipitate was filtered off, and dried. The crude 3-benzyloxy-15β-(carboxyl)methoxy-estra-1,3,5(10)-trien-17-one was dissolved in tetrahydrofurane (10 ml) and diethyl ether containing 1% diazomethane (50 ml) was added during cooling with ice. After standing 6 h, the solution was evaporated and the residue was chromatographed on silica gel with ethyl acetate/CH_2Cl_2 (2.5/97.5 v/v) to yield **22** (265 mg, 29%). Mp: 101–103 °C; $R_f = 0.55$ (ss C); $[\alpha]_D^{25} + 71$ (*c* 1 in $CHCl_3$). Found: C, 75.12; H, 7.35. $C_{28}H_{32}O_5$ (448.55) requires: C, 74.97; H, 7.19%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.21 (s, 3H, 18-H_3), 2.93 (m, 2H, 6-H_2), 3.76 (s, 3H, $COOCH_3$), 4.10 (m, 2H, O-CH_2), 4.36 (t, 1 H, $J = 6.5$ Hz, 15-H), 5.05 (s, 2H, Bn-H_2), 6.99 (s, 1H, 4-H), 6.76 (d, 1H, $J = 3.5$ Hz, 2-H), 7.21 (d, 1H, $J = 10.5$ Hz, 1-H), 7.38 (m, 5H, 5x CH of Bn). ^{13}C NMR (δ, ppm, $CDCl_3$): 16.9 (C-18), 25.2, 25.6, 29.0, 32.3, 34.3, 42.4, 43.9, 46.8 (C-13), 51.2, 54.1 (OCH_3), 66.0 (O-CH_2), 69.5 (Bn-CH_2), 75.2 (C-15), 111.8 (C-2), 114.5 (C-4), 125.6 (C-1), 126.9 (C-2 and C-6 of Bn), 127.3 (C-4-of Bn), 128.0 (C-3 and C-5 of Bn), 131.9 (C-10), 136.9 (C-1′), 137.5 (C-5), 156.4 (C-3), 170.2 (C = O), 218.4 (C-17).

3-Methoxy-15β-(3′-methoxy-3′-oxopropoxy)-estra-1,3,5(10)-trien-17-one (23)

Compound **14** (373 mg, 1 mmol) was dissolved in tetrahydrofuran (10 ml) and diethylether containing 1% diazomethane (50 ml) was added during cooling with ice. After standing 6 h, the solution was evaporated and the residue was crystallized from MeOH, to yield **23** (370 mg, 95%). Mp: 95–97 °C; $R_f = 0.58$ (ss C); $[\alpha]_D^{25} + 64$ (*c* 1 in $CHCl_3$). Found: C, 71.62; H, 8.04; $C_{23}H_{30}O_5$ (386.48) requires: C, 71.48; H, 7.82%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.13 (s, 3H, 18-H_3), 2.40 (m, 2H, linker H_2), 2.90 (m, 2H, 6-H_2), 3.59 (m, 2H, linker H_2) 3.69 (s, 3H, 3-OCH_3), 3.80 (s, 3H, $COOCH_3$), 4.20 (t, 1 H, $J = 6.5$ Hz, 15-H), 6.68 (s, 1H, 4-H), 6.74 (dd, 1H, $J = 10.5$ Hz, $J = 3.0$ Hz, 2-H), 7.21 (d, 1H, $J = 10.5$ Hz, 1-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 17.8 (C-18), 26.2, 26.6, 30.0, 33.2, 35.2, 35.6, 43.3, 44.6 (linker CH_2), 47.7 (C-13), 52.1, 54.9, 55.6 (3-OCH_3), 65.3 (O-CH_2), 75.3 (C-15), 111.9 (C-2), 114.3 (C-4), 126.6 (C-1), 132.6 (C-10), 138.3 (C-5), 158.1 (C-3), 172.4 (C = O), 219.9 (C-17).

3-Hydroxy-15β-(3′-methoxy-3′-oxopropoxy)-estra-1,3,5(10)-trien-17-one (24)

Compound **15** (448 mg, 1 mmol) was dissolved in tetrahydrofuran (10 ml) and diethyl ether containing 1% diazomethane (50 ml) was added during cooling with ice. After standing 6 h, the solution was evaporated and the residue was crystallized from MeOH, to yield crude 3-benzyloxy-15β-(3′-methoxy-3′-oxopropoxy)-estra-1,3,5(10)-trien-17-one. This compound was dissolved in ethyl acetate (30 ml) and the solution containing Pd/C (300 mg, 10%) was hydrogenated under 5 bar pressure for 6 h, at room temperature. The reaction mixture was filtered off, evaporated *in vacuo* and the residue was chromatographed on silica gel with ethyl acetate/CH_2Cl_2 (10:90 v/v) to yield **24** (196 mg, 52%). Mp: 140–142 °C; $R_f = 0.25$ (ss A); $[\alpha]_D^{20} + 51$ (*c* 1 in $CHCl_3$). Found: C, 71.08; H, 7.76.

$C_{22}H_{28}O_5$ (372.45) requires: C, 70.94; H, 7.58%. ^{1}H NMR (δ, ppm, DMSO-d_6): 0.99 (s, 3H, 18-H_3), 2.75 (m, 2H, 6-H_2), 3.34 (s, 4H, 2 x linker H_2), 3.58 (s, 3H, COO-H_3), 4.13 (t, 1 H, $J = 6.5$ Hz, 15-H), 6.48 (s, 1H, 4-H), 6.52 (dd, 1H, $J = 10.5$ Hz, $J = 3.0$ Hz, 2-H), 7.04 (d, 1H, $J = 10.5$ Hz, 1-H), 8.99 (brs, 1H, 3-OH). ^{13}C NMR (δ, ppm, DMSO-d_6): 18.0 (C-18), 26.3, 26.5, 29.9, 33.2, 35.6, 35.6, 43.4 (linker CH_2), 44.4, 47.4 (C-13), 52.1, 54.0, 65.3 (O-CH_2), 75.2 (C-15), 113.6 (C-2), 115.9 (C-4), 126.8 (C-1), 131.0 (C-10), 138.0 (C-5), 155.9 (C-3), 172.6 (C = O), 219.7 (C-17).

General procedure for the synthesis of 3-hydroxy-, and 3-benzyloxy-15β-(carboxamido)alkoxy-estra-1,3,5(10)-trienes with ammonium hydroxide, morpholine or N-cyclohexyl,N-methylamine (25–33)

To the solution of the corresponding 3-hydroxy-, or 3-benzyloxy-15β-(carboxyl)alkoxy-estra-1,3,5(10)-triene (1 mmol) in CH_2Cl_2 (20 ml) 0.2 ml (2 mmol) oxalyl chloride was added dropwise while cooling in ice under continuous stirring. The solution was allowed to stand at room temperature for 2 h. After evaporation *in vacuo* the residue was dissolved in CH_2Cl_2 (20 ml) and 4 mmol of the corresponding amine component was added while cooling in ice under continuous stirring. After 1 h, water (100 ml) was added and extracted with CH_2Cl_2 (2x 50 ml). The organic phase was washed with water, dried and evaporated. The residual material was chromatographed on a silica gel column with ethyl acetate/CH_2Cl_2 in different concentrations.

3-Benzyloxy-15β-(3′-amino-3-oxopropoxy)-estra-1,3,5,(10)-trien-17-one (25)

Compound **15** (448 mg, 1 mol) was used for synthesis as described in general procedure. The amine component was ammonium hydroxide solution (20 ml). The crude product was chromatographed on silica gel with ethyl acetate/CH_2Cl_2 (1:1 v/v) to yield **22** (238 mg, 53%). Mp: 183–185 °C; $R_f = 0.55$ (ss C); $[\alpha]_D^{25}$ + 61 (*c* 1 in $CHCl_3$). Found: C, 75.36; H, 7.26. $C_{28}H_{33}NO_4$ (447.57) requires: C, 75.14; H, 7.43%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.26 (s, 3H, 18-H_3), 2.30 (m, 2H, linker H_2), 2.96 (m, 2H, 6-H_2),3.60 (m, 2H, linker H_2), 4.43 (t, 1 H, $J = 6.0$ Hz, 15-H), 5.07 (s, 2H, Bn-H_2), 6.78 (s, 1H, 4-H), 6.83 (dd, 1H, $J = 10.5$ Hz, $J = 3.0$ Hz, 2-H), 7.24 (d, 1H, $J = 10.5$ Hz, 1-H), 7.38 (m, 5H, 5x CH of Bn). ^{13}C NMR (δ, ppm, $CDCl_3$): 15.5 (C-18), 22.0, 23.9, 24.2, 27.8, 30.9, 33.2, 33.8, 40.9, 41.9 (linker CH_2), 45.9 (C-13), 50.1, 51.8, 64.7 (linker CH_2), 73.9 (C-15), 112.2 (C-2), 114.6 (C-2), 125.8 (C-1), 123.0 (C-10), 126.8 (C-2 and C-6 of Bn), 127.1 (C-4 of Bn), 129.0 (C-3 and C-5 of Bn), 137.4 (C-5), 155.8 (C-3), 171.8 (C = O), 220.1 (C-17).

3-Hydroxy-15β-(3′-amino-3′-oxopropoxy)-estra-1,3,5(10)-trien-17-one (26)

Compound **25** (448 mg, 1 mmol) was dissolved in ethyl acetate (30 ml) and the solution containing Pd/C (300 mg, 10%) was hydrogenated under 5 bar pressure for 6 h, at room temperature. The reaction mixture was filtered off and evaporated *in vacuo*. The residue was chromatographed on silica gel column with ethyl acetate/CH_2Cl_2 (1:1 v/v) to yield **26** (308 mg, 86%). Mp: 124–126 °C; $R_f = 0.20$ (ss A); $[\alpha]_D^{25}$ + 54 (*c* 1 in MeOH). Found: C, 70.38; H, 7.85. $C_{21}H_{27}NO_4$ (357.44) requires: C, 70.56; H, 7.61%. ^{1}H NMR (δ, ppm, DMSO-d_6): 1.03 (s, 3H, 18-H_3), 2.77 (m, 2H, 6-H_2), 3.33 (s, 2H, linker H_2), 3.47 (m, 1H, O-CH_2), 3.68 (m, 1H, O-CH_2), 4.11 (t, 1 H, $J = 6.5$ Hz, 15-H), 6.47 (d, 1H, $J = 3.5$ Hz, 4-H), 6.52 (dd, 1H, $J = 10.5$ Hz, $J = 3.5$ Hz, 2-H), 6.78 (brs, 1H, NH_2), 7.04 (d, 1H, $J = 10.5$ Hz, 1-H), 7.22 (brs, 1H, NH_2), 7.90 (brs, 1H, 3-OH). ^{13}C NMR (δ, ppm, DMSO-d_6): 16.5 (C-18), 24.7, 24.9, 28.3, 31.7, 34.0, 35.3, 42.0, 42.8 (linker CH_2), 45.8 (C-13), 52.5, 64.4 (linker CH_2), 73.5 (C-15), 112.0 (C-2), 114.2 (C-2), 125.1 (C-1), 129.5 (C-10), 136.5 (C-5), 154.3 (C-3), 171.6 (C = O), 218.3 (C-17).

3-Benzyloxy-15β-(2′-morpholino-2′-oxoethoxy-estra-1,3,5(10)-trien-17-one (27)

3-Benzyloxy-15β-(carboxyl)methoxy-estra-1,3,5(10)-triene (434 mg, 1 mmol) was used for the synthesis as described in general procedure. The crude steroidal carbonyl chloride was dissolved in CH_2Cl_2 (20 ml), and morpholine (0.35 ml, 4 mmol) was added dropwise during cooling with ice under continuous stirring. After 1 h, water (100 ml) was added, and extracted with CH_2Cl_2 (2 x 50 ml). The organic solution was washed with water, dried, and evaporated. The residual material was chromatographed on silica gel column with ethyl acetate/CH_2Cl_2 (25:75 v/v) to yield **27** (432 mg, 85%). Mp: 121–123 °C; $R_f = 0.45$ (ss D); $[\alpha]_D^{25}$ + 37 (*c* 1 in MeOH). Found: C, 74.14; H, 7.53. $C_{31}H_{37}NO_5$ (503.63) requires: C, 73.93; H, 7.41%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.16 (s, 3H, 18-H_3), 3.58 (m, 8H, 4x morpholine H_2), 4.08 (d, 1H, $J = 13.0$ Hz, O-CH_2), 4.20 (d, 1H, $J = 13.0$ Hz, O-CH_2), 4.35 (t, 1 H, $J = 5.5$ Hz, 15-H), 5.04 (s, 2H, Bn-H_2), 6.75 (s, 1H, 4-H), 6.80 (dd, 1H, $J = 8.5$ Hz, $J = 2.5$ Hz, 2-H), 7.20 (d, 1H, $J = 8.5$ Hz, 1-H), 7.32 (t, 1H, $J = 7.5$ Hz, Bn 4-H), 7.38 (t, 2H, $J = 7.5$ Hz, Bn 3- and 5-H), 7.43 (d, 2H, $J = 7.5$ Hz, Bn-2- and 6-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 17.5 (C-18), 25.6, 26.3, 29.5, 32.7, 34.8, 42.1, 43.0, 44.3, 45.9 (C-13), 47.2, 54.4, 66.7 (morpholine CH_2), 66.7 (morpholine CH_2), 69.1 (O-CH_2), 69.9 (Bn-CH_2), 76.0 (C-15), 112.4 (C-2), 114.8 (C-4), 126.2 (C-1), 127.4 (C-2 and C-6 of Bn), 127.8 (C-4 of Bn), 128.5 (C-3 and C-5 of Bn), 132.1 (C-10), 137.1 (C-1 of Bn), 137.5 (C-5), 156.9 (C-3), 167.5 (C = O), 218.7 (C-17).

3-Hydroxy-15β-(carboxmorpholydo)methoxy-2′-morpholino-2′oxoethoxy)-estra-1,3,5(10)-trien-17-one (28)

Compound **27** (503 mg, 1 mmol) was dissolved in ethyl acetate (30 ml) and the solution containing Pd/C (448 mg, 10%) was hydrogenated under 5 bar pressure for 6 h at room temperature. The reaction mixture was filtered off, evaporated *in vacuo*, and the residue was crystallized from MeOH to yield **28** (350 mg, 69%). Mp: 215–220 °C; $R_f = 0.40$ (ss D); $[\alpha]_D^{25}$ + 54 (*c* 1 in MeOH). Found: C, 73.07; H, 7.92. $C_{25}H_{33}NO_4$ (411.53) requires: C, 72.96; H, 8.08%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.14 (s, 3H, 18-H_3), 3.51 (s, 2H, linker H_2), 3.58 (m, 2H, 2x H_2 of morpholine), 3.60 (m 2H, 2x H_2 of morpholine), 4.14 (t, 1 H, $J = 6.5$ Hz, 15-H), 6.60 (d, 1H $J = 2.5$ Hz, 4-H), 6.65 (dd, 1H, $J = 10.5$ Hz, $J = 3.0$ Hz, 2-H), 7.12 (d, 1H, $J = 10.5$ Hz, 1-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 18.0 (C-18), 26.0, 26.2, 26.7, 29.9, 30.1, 33.1, 35.4, 42.5, 43.7, 44.5, 46.4, 47.7 (C-13), 54.9, 66.6 (linker CH_2), 67.3 (linker CH_2), 75.2 (C-15), 113.4 (C-2), 115.8 (C-4), 126.7 (C-1), 132.1 (C-10), 138.2 (C-5), 154.2 (C-1), 172.0 (linker C = O), 220.5 (C-17).

3-Benzyloxy-15β-(3′-morpholino-3′oxopropoxy)-estra-1,3,5(10)-trien-17-one (29)

Compound **15** (448 mg, 1 mmol) was used for synthesis as described in general procedure. The crude steroidal carbonyl chloride was dissolved in CH_2Cl_2 (20 ml), and morpholine (0.35 ml, 4 mmol) was added dropwise during cooling with ice under continuous stirring. After 1 h, water (100 ml) was added, and extracted with CH_2Cl_2 (2x 50 ml). The organic solution was washed with water, dried, and evaporated. The residual material was

chromatographed on silica gel column with ethyl acetate/CH_2Cl_2 (2.5:97.5 v/v) to yield **29** (372 mg, 72%). Mp: 128–130 °C; $R_f = 0.42$ (ss D); $[\alpha]_D^{25}$ + 37 (*c* 1 in $CHCl_3$). Found: C, 74.46; H, 7.72. $C_{32}H_{39}NO_5$ (517.66) requires: C, 74.24; H, 7.59%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.12 (s, 3H, 18-H_3), 2.89 (m, 2H, 6-H_2), 3.51(m, 2H, linker H_2), 4.20 (t, 1H, $J = 6.5$ Hz, 15-H), 5.04 (s, 2H, Bn-H_2), 6.74 (d, 1H, $J = 3.5$ Hz, 4-H), 6.79 (dd, 1H, $J = 10.5$ Hz, $J = 3.5$ Hz, 2-H), 7.19 (d, 1H, $J = 10.5$ Hz, 1-H), 7.31 (t, 1 H, $J = 8.5$ Hz, Bn 4-H), 7.38 (t, 2H, $J = 8.5$ Hz, Bn 3- and 5-H), 7.43 (d, 2H, $J = 8.5$ Hz, Bn 2- and 6-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 17.9 (C-18), 26.2, 26.6, 30.0, 33.1, 33.7, 35.4, 42.4 (morpholine CH_2), 43.5 (linker CH_2), 44.6, 46.5 (morpholine CH_2), 47.7 (C-13), 54.9, 66.2 (O-CH_2), 67.1 (morpholine CH_2), 67.3 (morpholine CH_2), 70.4 (Bn-CH_2), 75.5 (C-15), 112.8 (C-2), 115.4 (C-4), 126.6 (C-1), 127.8 (C-2 and C-6 of Bn), 128.3 (C-4 of Bn), 129.0 (C-3 and C-5 of Bn), 132.9, 137.9 (C-1′), 138.2 (C-5), 157.3 (C-3), 169.9 (C = O), 219.9 (C-17).

3-Sulfamoyloxy-15β-(3′-morpholino-3′-oxopropoxy)-estra-1,3,5(10)-trien-17-one (30)

Compound **29** (517 mg, 1 mmol) was dissolved in ethyl acetate (30 ml) and the solution containing Pd/C (300 mg, 10%) was hydrogenated under 5 bar pressure for 6 h, at room temperature. The reaction mixture was filtered off and evaporated *in vacuo*. The residue was dissolved in dimethylformamide (20 ml), and 575 mg (5 mmol) sulfamoyl chloride was added dropwise during cooling with ice. The reaction mixture was allowed to stand 6 h and then poured onto ice (300 g). The precipitate separated out was filtered off, and subjected to chromatographic separation on silica gel column with ethyl acetate/CH_2Cl_2 (1:1 v/v) to yield **30** (240 mg, 45%). Mp: 104–108 °C; $R_f = 0.35$ (ss D); $[\alpha]_D^{25}$ + 17 (*c* 1 in $CHCl_3$). Found: C, 59.43; H, 6.54. $C_{25}H_{34}N_2O_7S$ (506.61) requires: C, 59.27; H, 6.76%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.12 (s, 3H, 18-H_3), 2.74 (m, 2H, linker CH_2), 4.46 (m, 4H, 2x H_2 of morpholine), 3.56 (m, 2H, O-CH_2), 3.61 (m, 4H, 2x H_2 of morpholine), 3.82 (t, 1 H, $J = 6.5$ Hz, 15-H), 5.42 (s, 2H, NH_2), 6.78 (d, 1H, $J = 3.5$ Hz, 4-H), 7.20 (dd, 1H, $J = 10.0$ Hz, $J = 3.5$ Hz, 2-H), 7.30 (t, 1 H, $J = 10.0$ Hz, 1-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 17.8 (C-18), 26.1, 26.7, 30.5, 33.4, 33.9, 35.5, 42.2 (morpholine CH_2), 43.4 (linker CH_2), 45.1, 46.7 (morpholine CH_2), 47.5 (C-13), 55.8, 64.3 (O-CH_2), 66.7 (morpholine CH_2), 67.5 (morpholine CH_2), 75.7 (C-15), 112.5 (C-2), 115.1 (C-4), 126.8 (C-1), 133.0, 140.5 (C-5), 159.1 (C-3), 170.9 (C = O), 220.2 (C-17).

3-Benzyloxy-15β-(4′-morpholino-4′oxobutoxy)-estra-1,3,5(10)-trien-17-one (31)

Compound **17** (462 mg, 1 mmol) was used for the synthesis as described in general procedure. The crude steroidal carbonyl chloride was dissolved in CH_2Cl_2 (20 ml), and morpholine (0.35 ml, 4 mmol) was added dropwise during cooling with ice under continuous stirring. After 1 h, water (100 ml) was added, and extracted with CH_2Cl_2 (2 x 50 ml). The organic solution was washed with water, dried and evaporated *in vacuo*. The residual material was chromatographed on silica gel column with ethyl acetate/CH_2Cl_2 (30:70 v/v) to yield **31** (810 mg, 76%). Mp: 135–138 °C; $R_f = 0.42$ (ss D); $[\alpha]_D^{25}$ + 20 (*c* 1 in $CHCl_3$). Found: C, 74.87; H, 7.43. $C_{33}H_{41}NO_5$ (531.68) requires: C, 74.55; H, 7.77%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.14 (s, 3H, 18-H_3), 1.91 (m, 4H, 2 x morpholine H_2), 2.37 (m, 4H, 2 x morpholine H_2), 3.36 (t, 2H, $J = 6.5$ Hz, O-CH_2), 4.15 (t, 1 H, $J = 6.5$ Hz, 15-H), 5.04 (s, 2H, Bn-H_2), 6.73 (d, 1H, $J = 3.5$ Hz, 4-H), 6.79 (dd, 1H, $J = 10.5$ Hz, $J = 3.5$ Hz, 2-H), 7.20 (d, 1H, $J = 10.5$ Hz, 1-H), 7.36 (m, 5H, 5x CH of Bn). ^{13}C NMR (δ, ppm, $CDCl_3$): 18.0 (C-18), 26.0, 26.2, 26.7, 30.0, 33.1, 34.7, 35.4, 42.3 (morpholine CH_2), 43.7 (linker CH_2), 44.6, 46.3 (morpholine CH_2), 47.7 (C-13), 54.9, 67.0 (morpholine CH_2), 67.3 (morpholine CH_2), 69.0 (O-CH_2), 70.4 (Bn-CH_2), 75.1 (C-15), 112.8 (C-2), 115.4 (C-4), 126.6 (C-1), 127.8 (C-2 and C-6 of Bn), 128.3 (C-4′), 129.0 (C-3 and C-5 of Bn), 132.9 (C-10), 137.9 (C-1 of Bn), 138.1 (C-5), 157.4 (C-3), 171.6 (C = O), 220.0 (C-17).

3-Hydroxy-15β-(4′-morpholino-4′-oxobutoxy)-estra-1,3,5(10)-trien-17-one (32)

Compound **31** (531 mg, 1 mmol) was dissolved in ethyl acetate (30 ml) and the solution containing Pd/C (300 mg, 10%) was hydrogenated under 5 bar pressure for 6 h, at room temperature. The reaction mixture was filtered off, evaporated *in vacuo* and crystallized from MeOH to yield **32** (390 mg, 88%). Mp: 80–84 °C; $R_f = 0.35$ (ss D); $[\alpha]_D^{25}$ + 46 (*c* 1 in $CHCl_3$). Found: C, 70.58; H, 8.12. $C_{26}H_{35}NO_5$ (441.57) requires: C, 70.72; H, 7.99%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.14 (s, 3H, 18-H_3), 3.33 (t, 2H, $J = 6.0$ Hz, linker H_2), 3.48 (m, 4H, 2x morpholine H_2), 361 (m, 4H, 2x morpholine H_2), 4.14 (t, 1 H, $J = 6.5$ Hz, 15-H), 6.60 (d, 1H $J = 2.5$ Hz, 4-H), 6.65 (dd, 1H, $J = 10.5$ Hz, $J = 3.0$ Hz, 2-H), 7.12 (d, 1H, $J = 10.5$ Hz, 1-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 18.0 (C-18), 26.0, 26.2, 26.7, 29.9, 30.1, 33.1, 35.4, 42.5, 43.7, 44.5, 46.4, 47.7 (C-13), 54.9, 66.6 (linker CH_2), 67.3 (linker CH_2), 69.0 (linker CH_2), 75.2 (C-15), 113.4 (C-2), 115.8 (C-4), 126.7 (C-1), 132.1 (C-10), 138.2 (C-5), 154.2 (C-1), 172.0 (linker C = O), 220.5 (C-17).

3-Methoxy-15β-(2′N-cyclohexyl,N-methyl-2′oxoethoxy)-estra-1,3,5(10)-trien-17-one (33)

Starting from 3-methoxy-15β-(carboxyl)methoxy-estra-1,3,5(10)-trien-17-one (**13**) 358 mg (1 mmol) was used for synthesis as described in general procedure. The crude steroidal carbonyl chloride was dissolved in CH_2Cl_2 (20 ml), and *N*-cyclohexyl, *N*-methylamine (4.5 ml, 4 mmol) was added dropwise during cooling with ice under continuous stirring. After 1 h, water (100 ml) was added, and extracted with CH_2Cl_2 (2 x 50 ml). The organic solution was washed with water, dried, and evaporated. The residual material was chromatographed on silica gel column with ethyl acetate/CH_2Cl_2 (25:75 v/v) to yield **33** (385 mg, 84%). Mp: 53–58 °C (foam); $R_f = 0.50$ (ss B); $[\alpha]_D^{25}$ + 61 (*c* 1 in $CHCl_3$). Found: C, 73.92; H, 8.82. $C_{28}H_{39}NO_4$ (453.61) requires: C, 74.14; H, 8.67%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.18 (s, 3H, 18-H_3), 3.30 (s, 3H, N-CH_3), 3.77 (s, 3H, 3-OCH_3), 4.12 (m, 2H, O-CH_2), 4.39 (m, 1H, 15-H), 6.65 (s, 1H, 4-H), 6.71 (d, 1H, $J = 11.0$ Hz, 2-H), 7.19 (d, 1H, $J = 11.0$ Hz, 1-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 17.9 (C-18), 25.8, 26.0, 26.2, 27.6, 29.3, 29.9, 30.0, 31.3, 33.2, 35.4, 43.5, 43.8 (C-13), 44.8, 47.7, 53.0, 55.0, 55.6, 69.9 (O-CH_2), 75.7 (C-15), 112.0 (C-2), 114.3 (C-4), 126.6 (C-1), 132.5 (C-10), 138.1 (C-5), 158.1 (C-3), 166.3 (C = O), 219.5 (C-17).

General procedure for the synthesis of 3-benzyloxy-15β-(carbamoyloxy)alkoxy-estra-1,3,5(10)-trien-17-one (34–40)

To the solution of 3-benzyloxy-15β-(2′-hydroxy)ethoxy-estra-1,3,5(10)-trien-17-one (**10**) or 3-benzyloxy-15β-(3′-hydroxy)propoxy-estra-1,3,5(10)-trien-17-one (**11**) mmol) in CH_2Cl_2 (30 ml) containing 0.2 ml triethylamine, 4 mmol of the corresponding alkyl or aryl isocyanate was added under continuous stirring. After the addition of the reagent the reaction mixture was heated at reflux for 1 h, poured into water (100 ml), and then extracted with CH_2Cl_2 (2x 30 ml). The organic phase was washed with $NaHCO_3$ solution,

water, dried and evaporated *in vacuo*. The residual material was chromatographed on a silica gel column with ethyl acetate/CH_2Cl_2 in different concentrations.

3-Benzyloxy-15β-(2′-cyclohexylcarbamoyloxy)ethoxy-estra-1,3,5(10)-trien-17-one (34)

Compound **10** (420 mg, 1 mmol) was used for the synthesis as described in general procedure. The reagent was cyclohexyl isocyanate (4 mmol). The crude product was chromatographed with ethyl acetate/CH_2Cl_2 (2.5:97.5 v/v) to yield **34** (430 mg, 78%). Mp: 96–98 °C; $R_f = 0.55$ (ss B); $[\alpha]_D^{25}$ + 39 (*c* 1 in $CHCl_3$). Found: C, 74.65; H, 8.14. $C_{34}H_{43}NO_5$ (545.71) requires: C, 74.83; H, 7.94%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.16 (s, 3H, 18-H_3), 3.65 (m, 2H, 16-H_2), 3.68 (m, 1H, OCH_2), 4.19 (m, 3H, OCH_2), 4.55 (d, 1H, $J = 7.5$ Hz, 15-H), 5.03 (s, 2H, Bn-H_2), 6.73 (d, 1H, $J = 3.0$ Hz, 4-H), 6.78 (dd, 1H, $J = 10.5$ Hz, $J = 3.0$ Hz, 2-H), 7.19 (d, 1H, $J = 10.5$ Hz, 1-H), 7.37 (m, 5H, 5x CH of Bn). ^{13}C NMR (δ, ppm, $CDCl_3$): 17.9 (C-18), 24.8, 25.2, 25.9, 26.2, 30.0, 33.2, 33.8, 35.2, 43.7 (O-CH_2), 44.6, 47.8 (C-13), 50.2, 53.8, 55.0, 64.0, 68.3 (O-CH_2), 70.4, 70.5 (Bn-CH_2), 75.4 (C-15), 112.7 (C-2), 115.4 (C-4), 126.6 (C-1), 127.8 (C-2 and C-6 of Bn), 127.8 (C-4 of Bn), 129.0 (C-3 and C-5 of Bn), 133.0 (C-10), 137.8 (C-1′), 138.3 (C-5), 157.3 (C-3), 220.0 (C-17).

3-Hydroxy-15β-(2′-cyclohexylcarbamoyloxy)ethoxy-estra -1,3,5(10)-trien-17-one (35)

Compound **34** (545 mg, 1 mmol) was dissolved in ethyl acetate (30 ml) and the solution containing Pd/C (300 mg, 10%) was hydrogenated under 5 bar for 6 h, at room temperature. The reaction mixture was filtered off and evaporated *in vacuo*. The residual material was chromatographed on silica gel column with ethyl acetate/CH_2Cl_2 (25:75 v/v) to yield **35** (390 mg, 85%). Mp: 95–96 °C; $R_f = 0.40$ (ss D); $[\alpha]_D^{25}$ + 51 (*c* 1 in $CHCl_3$). Found: C, 71.37; H, 8.02. $C_{27}H_{37}NO_5$ (455.60) requires: C, 71.18; H, 8.19%.1H NMR (δ, ppm, $CDCl_3$): 1.12 (s, 3H, 18-H_3), 1. 22 (m, 4H, 2x H_2 of cyclohexyl), 1. 56 (m, 4H, 2x H_2 of cyclohexyl), 3.62 (m, 2H, 16-H_2), 3.66 (m, 2H, O-CH_2), 4.28 (m, 2H, O-CH_2), 4.55 (d, 1H, $J = 7.5$ Hz, 15-H), 6.61 (d, 1H, $J = 3.0$ Hz, 4-H), 6.71 (dd, 1H, $J = 10.5$ Hz, $J = 3.0$ Hz, 2-H), 7.10 (d, 1H, $J = 10.5$ Hz, 1-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 17.9 (C-18), 24.6, 25.4, 25.9, 25.9, 26.1, 30.1, 33.3, 33.7, 35.4, 44.1 (O-CH_2), 44.8, 48.9 (C-13), 50.4, 54.0, 55.0, 64.2, 68.4 (O-CH_2), 70.5, 75.6 (C-15), 112.7 (C-2), 115.5 (C-4), 126.8 (C-1), 133.3 (C-10), 138.6 (C-5), 157.5 (C-3), 220.0 (C-17).

3-Benzyloxy-15β-(2′-t-butylcarbamoyloxy)ethoxy-estra-1,3,5(10)-trien-17-one (36)

Compound **10** (420 mg, 1 mmol) was used for the synthesis as described in general procedure. The reagent was *t*-butyl isocyanate (4 mmol). The crude product was chromatographed with ethyl acetate/CH_2Cl_2 (2.5:97.5 v/v) to yield **36** (265 mg, 51%). Mp: 206–207 °C; $R_f = 0.45$ (ss B); $[\alpha]_D^{25}$ +8 (*c* 1 in $CHCl_3$). Found: C, 73.82; H, 8.15. $C_{32}H_{41}NO_5$ (519.67) requires: C, 73.95; H, 7.95%.1H NMR (δ, ppm, $CDCl_3$): %) ^{1}H NMR (δ, ppm, $CDCl_3$): 1.15 (s, 3H, 18-H_3), 1.42 (s, 9H, *t*-Bu), 3.62 (m, 2H, O-CH_2), 3.81 (m, 2H, O-CH_2), 4.21 (t, 1 H, $J = 7.0$ Hz, 15-H), 5.12 (s, 2H, Bn-H_2), 6.62 (d, 1H, $J = 3.5$ Hz, 4-H), 6.80 (dd, 1H, $J = 11.0$ Hz, $J = 3.5$ Hz, 2-H), 7.19 (d, 1H, $J = 11.0$ Hz, 1-H), 7.40 (m, 5 H, 5x CH of Bn). ^{13}C NMR (δ, ppm, $CDCl_3$): 13 C NMR (δ, ppm, $CDCl_3$): 18.2 (C-18), 26.0, 26.5, 29.3 (3 x CH_3 of *t*-Bu), 29.6, 33.0, 35.1, 41.9, 43.9 (O-CH_2), 44.7, 48.0 (C-13), 55.0, 56.7 (quaterner C of *t*-Bu), 64.9 (O-CH_2), 67.1 (O-CH_2), 70.3 (Bn-CH_2), 75.3 (C-15), 112.8 (C-2), 115.2 (C-4), 124.4 (C-1), 126.8 (C-2 and C-6 of 3-Bn), 128.1 (C-4 of 3-Bn), 129.5 (C-3 and C-5 of 3-Bn), 133.1 (C-10), 138.0 (C-1 of 3-Bn), 133.5 (C-5), 158.1 (C-3), 220.0 (C-17).

3-Benzyloxy-15β-(2′-phenylcarbamoyloxy)ethoxy-estra-1,3,5(10)-trien-17-one (37)

Compound **10** (420 mg, 1 mmol) was used for the synthesis as described in general procedure. The reagent was phenyl isocyanate (4 mmol). The crude product was chromatographed with ethyl acetate/CH_2Cl_2 (25:75 v/v) to yield **37** (310 mg, 57%). Mp: 73–76 °C (foam); $R_f = 0.65$ (ss B); $[\alpha]_D^{25}$ + 35 (*c* 1 in $CHCl_3$). Found: C, 75.83; H, 7.12. $C_{34}H_{37}NO_5$ (539.66) requires: C, 75.67; H, 6.91% ^{1}H NMR (δ, ppm, $CDCl_3$): 1.16 (s, 3H, 18-H_3), 3.60 (m, 1H, O-CH_2), 3.73 (m, 1H, O-CH_2), 4.21 (t, 1 H, $J = 6.5$ Hz, 15-H), 4.30 (m, 2H, O-CH_2), 5.02 (s, 2H, Bn-H_2), 6.64 (s, 1H, NH), 6.67 (d, 1H, $J = 3.0$ Hz, 4-H), 6.77 (dd, 1H, $J = 10.5$ Hz, $J = 3.0$ Hz, 2-H), 7.06 (t, 1H, $J = 9.0$ Hz, 4H of N-Ph), 7.17 (d, 1H, $J = 10.5$ Hz, 1-H), 7.34 (m, 9 H, 5x CH of Bn and 4x CH of N-Ph). ^{13}C NMR (δ, ppm, $CDCl_3$): 18.0 (C-18), 26.2, 26.7, 29.9, 33.2, 35.2, 41.8, 43.7 (O-CH_2), 44.6, 47.8 (C-13), 55.0, 64.7 (O-CH_2), 68.1 (O-CH_2), 70.4 (Bn-CH_2), 75.5 (C-15), 112.8 (C-2), 115.4 (C-4), 121.1 (C-4″ of N-Ph), 124.1 (C-1), 126.6 (C-2′ and C-6′), 127.9 (C-4′), 128.3 (C-2 and C-6 of 3Bn), 129.0 (C-3 and C-5 of 3Bn), 129.5 (C-3 and C-5 of N-Ph), 132.9 (C-10), 137.7 (C-1 of 3-Bn), 132.8 (C-5), 154.8 (C-1), 157.3 (C-3), 220.0 (C-17).

3-Benzyloxy-15β-(2′-4″-chlorophenylcarbamoyloxy)ethoxy-estra-1,3,5(10)-trien-17-one (38)

Compound **10** (420 mg, 1 mmol) was used for the synthesis as described in general procedure. The reagent was 4-chlorophenyl isocyanate (4 mmol). The crude product was chromatographed with ethyl acetate/CH_2Cl_2 (25:75 v/v) to yield **38** (380 mg, 66%). Mp: 103–106 °C; $R_f = 0.62$ (ss B); $[\alpha]_d 25 + 32$ (*c* 1 in $CHCl_3$) . Found: C, 71.38; H, 6.55. $C_{34}H_{35}ClNO_5$ (574.11) requires: C, 71.13; H, 6.32%. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.18 (s, 3H, 18-H_3), 3.60 (m, 1H, O-CH_2), 3.76 (m, 1H, O-CH_2), 4.23 (t, 1 H, $J = 7.0$ Hz, 15-H), 4.33 (m, 2H, O-CH_2), 5.06 (s, 2H, Bn-H_2), 6.70 (s, 2H, 4-H, NH), 6.81 (dd, 1H, $J = 11.0$ Hz, $J = 3.0$ Hz, 2-H), 7.21 (d, 1H, $J = 11.0$ Hz, 1-H), 7.37 (m, 9H, 5x CH of 3-Bn and 6′-H, 4x CH of N-Ph). ^{13}C NMR (δ, ppm, $CDCl_3$): 18.0 (C-18), 26.2, 26.7, 27.5, 29.9, 33.2, 35.2, 42.2, 43.7 (O-CH_2), 44.6, 47.8 (C-13), 55.0, 62.4, 64.8, 68.1 (O-CH_2), 70.4 (Bn-CH_2), 75.6 (C-15), 112.8 (C-2), 115.3 (C-4), 118.5 (C-4 of 3-Bn), 126.6 (C-1), 127.9 (C-2 and C-6 of 3-Bn), 128.3 (C-2 and C-6 of N-Ph), 129.0 (C-3 and C-5 of 3-Bn), 129.3 (C-3 and C-5 of N-Ph),129.5 (C-4 of N-Ph), 157.3 (C-3), 160.1 (C = O), 219.9 (C-17).

3-Benzyloxy-15β-(3′-butylcarbamoyloxy)propoxy-estra-1,3,5(10)-trien-17-one (39)

Compound **11** (434 mg, 1 mmol) was used for the synthesis as described in general procedure. The reagent was *n*-butyl isocyanate (4 mmol). The crude product was chromatographed with ethyl acetate/CH_2Cl_2 (2.5:97.5 v/v) to yield **39** (460 mg, 86%). Mp: 101–103 °C; $R_f = 0.40$ (ss B); $[\alpha]_D^{25}$ + 58 (*c* 1 in $CHCl_3$). Found: C, 74. 43; H, 8.39. $C_{33}H_{43}NO_5$ (533.70) requires: C, 74.27; H, 8.12%). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.88 (t, 3H, $J = 6.5$ Hz, $(CH_2)_3$-H_3), 11.1 (s, 3H, 18-H_3, 2.88 (m, 2H, 6-H_2), 3.12 (d, 1H, $J = 8.0$ Hz, NH-CH_2), 3.29 (m, 1H, O-CH_2), 3.55 (m, 1H, O-CH_2), 4.09 (m, 3H, O-CH2, 15-H), 4.57 (brs, 1H, NH), 4.99 (s, 2H, Bn-H_2), 6.70 (d, 1H, $J = 3.0$ Hz, 4-H), 6.74 (dd, 1H, $J = 11.0$ Hz, $J = 3.0$ Hz, 2-H), 7.15 (d, 1H, $J = 11.0$ Hz, 1-H), 7.33 (m, 5H, 5x CH of Bn). ^{13}C NMR (δ, ppm, $CDCl_3$): 13.6 $(CH_2)_3$-CH_3), 17.4 (C-18), 19.7, 25.6, 26.2, 29.4, 29.6, 31.9, 32.6, 34.7, 40.6,

43.0, 44.1, 47.1 (C-13), 54.4, 61.7 (O-CH_2), 65.7 (O-CH_2), 69.8 (Bn-CH_2), 74.6 (C-15), 112.2 (C-2), 114.8 (C-4), 126.0 (C-1), 127.3 (C-2 and C-6 of Bn), 127.7 (C-4 of Bn), 128.4 (C-3 and C-5 of Bn), 132.4 (C-10), 137.2 (C-1 of Bn), 137.7 (C-5), 156.4 (C = O), 156.8 (C-3), 219.5 (C-17).

3-Hydroxy-15β-(3′-n-butylcarbamoyloxy)propoxy-estra-1,3,5(10)-trien-17-one (40)

Compound **39** (533 mg, 1 mmol) was dissolved in ethyl acetate (30 ml) and the solution containing Pd/C (300 mg, 10%) was hydrogenated under 5 bar for 6 h, at room temperature. The reaction mixture was filtered off and evaporated *in vacuo*. The residual material was chromatographed on silica gel column with ethyl acetate/CH_2Cl_2 (25:75 v/v) to yield **40** (290 mg, 65%). Mp: 65–70 °C (foam); $R_f = 0.42$ (ss D); $[\alpha]_D^{25} + 63$ (*c* 1 in $CHCl_3$). Found: C, 70.27; H, 8.63. $C_{26}H_{37}NO_5$ (443.58) requires: C, 70.40; H, 8.41%. ^{1}H NMR (δ, ppm, $CDCl_3$): 0.91 (s, 3H, $J = 6.5$ Hz, NH-$(CH_2)_2$-H_3), 1.15 (s, 3H, 18-H_3), 3.13 (m, 2H, 6-H_2), 3.20 (t, 2H, $J = 6.5$ Hz, N-$H_{2)}$, 3.33 (t 2H, $J = 6.0$ Hz, linker H_2) 4.75 (s, 1H, 15-H), 6.17 (s, 1H, 4-H), 6.64 (dd, 1H, $J = 10.5$ Hz, $J = 3.5$ Hz, 2-H), 7.11 (d, 1H, $J = 10.5$ Hz, 1-H). ^{13}C NMR (δ, ppm, $CDCl_3$): 11.6 (NH-$(CH_2)_2$-CH_3), 13.9, 18.0 (C-18), 23.6, 26.2, 26.7, 29.8, 30.1, 33.1, 35.3, 43.2 (C-13), 43.6 (linker CH_2), 44.6, 47.8 (linker CH_2), 55.0, 62.5 (linker CH_2), 66.2 (linker CH_2), 75.2 (C-15), 113.3 (C-2), 115.8 (C-4), 126.6 (C-1), 132.1 (C-10), 138.4 (C-5), 154.5 (C-3), 220.8 (C-17).

Measurement of inhibition of 17β-HSD1

Our previously published methods were used for the measurement of 17β-HSD1 inhibition[21,22]. In brief, human placental cytosol was incubated as enzyme source with 1 μM [^{3}H]-labelled estrone substrate at 37 °C. The cofactor, either NADH or NADPH, was used in an excess concentration of 100 μM. The buffer medium consisted of 0.1 M HEPES (pH = 7.3), 1 mM EDTA, and 1 mM dithiotreitol. The substrate was added to the incubate in 10 μl of a 25 v/v% propylene glycol in HEPES buffer solution, whereas test compounds were applied in 10 μl of dimethyl sulfoxide solution.

After an incubation time of 2.5 min, the enzymatic reaction was stopped and the product 17β-estradiol was isolated by TLC. Radioactivity of the 17β-estradiol (**2**) formed was measured by means of liquid scintillation counting. Test compounds were usually applied in 10 μM concentration, whereas concentrations of 0.1–50 μM were used during determination of IC_{50} values. The inhibitor effect was assessed with relative conversion results calculated in comparison to non-inhibited controls (100%). IC_{50} results were calculated by using unweighted iterative least squares logistic curve fitting by means of the "absolute IC_{50} calculation" function of the GraphPad Prism 4.0 (GraphPad Software, Inc., San Diego, CA, USA). The IC_{50} of unlabelled estrone (**1**) was measured as reference. The relative inhibitory potential (RIP) values of the test compounds were calculated by using reference IC_{50} data measured with the corresponding cofactor: RIP = IC_{50} of test compound/IC_{50} of unlabelled estrone (**1**).

Results and discussion

Synthetic studies

To prepare novel substituted 15β-alkoxy steroids, 3-methoxy-estra-1,3,5(10),15-tetraen-17-one (**6**), and 3-benzyloxy-estra-1,3,5(10),15-tetraen-17-one (**7**) were chosen as starting compounds[12,23]. The synthetic strategy for the preparation of the different type of compounds is illustrated in Scheme 2.

Treatment of Δ^{15}-17-one compound **6** with aqueous potassium hydroxyde in methanol afforded 3,15β-dimethoxy-estra-1,3,5(10)-trien-17-one via 1,4 addition in practically quantitative yield. On the basis of an earlier observation from W. S. Johnson and W. F. Johns[23], we extended this 1,4-addition process to 1,2-ethanediol, 1,3-propanediol and 1,4-butanediol to receive from compound **6** the corresponding 3-methoxy-15β-(2′-hydroxy)ethoxy-, and 3-methoxy-15β-(3′-hydroxy)propoxy-estra-1,3,5(10)-triene-17-ones (**8** and **9**), from compound **7** the 3-benzyloxy-15β-(2′-hydroxy)ethoxy-, 3-benzyloxy-15β-(3′-hydroxy)propoxy-, and 3-benzyloxy-15β-(4′-hydroxy)butoxy-estra-1,3,5(10)-trien-17-ones (**10**–**12**). The addition of different nucleophiles is highly stereospecific, giving 15β substituted estranes in all cases[12,23]. Jones oxidation of these compounds (**8**–**12**) furnished the corresponding 3-methoxy-, and 3-benzyloxy-15β-(carboxyl)-alkoxy-estra-1,3,5(10)-trien-17-one derivatives (**13**–**17**). The 1,4-addition process of compounds **6** and **7** with 3-hydroxypropionitrile afforded the corresponding 3-methoxy-, and 3-benzyloxy-15β-(2′-cyano)ethoxy-estra-1,3,5(10)-trien-17-ones (**18**, **19**). Cleavage of the 3-benzyloxy group of **19** yielded **20**, which reacted with sulfamoyl chloride to yield **21**. Esterification of 15β-(carboxyl)alkoxy derivatives by diazomethane yielded the corresponding methyl esters **22**–**24**. The 15β-(carboxyl)alkoxy compounds were reacted with oxalyl chloride to give carboxylic acid chloride which, upon reaction with ammonium hydroxide, morpholine or *N*-cyclohexyl, *N*-methylamine yielded the corresponding carboxamides **25**–**33**. The 3-benzyloxy-15β-(2′-hydroxy)ethoxy- and 3-benzyloxy-15β-(3′-hydroxy)propoxy-estra-1,3,5(10)-triene-17-ones (**10** and **11**) reacted with different alkyl- and aryl isocyanates to furnish alkyl- and aryl urethane derivatives **34**–**40**.

Inhibitory potentials of the C15 estrone derivatives towards 17β-HSD1

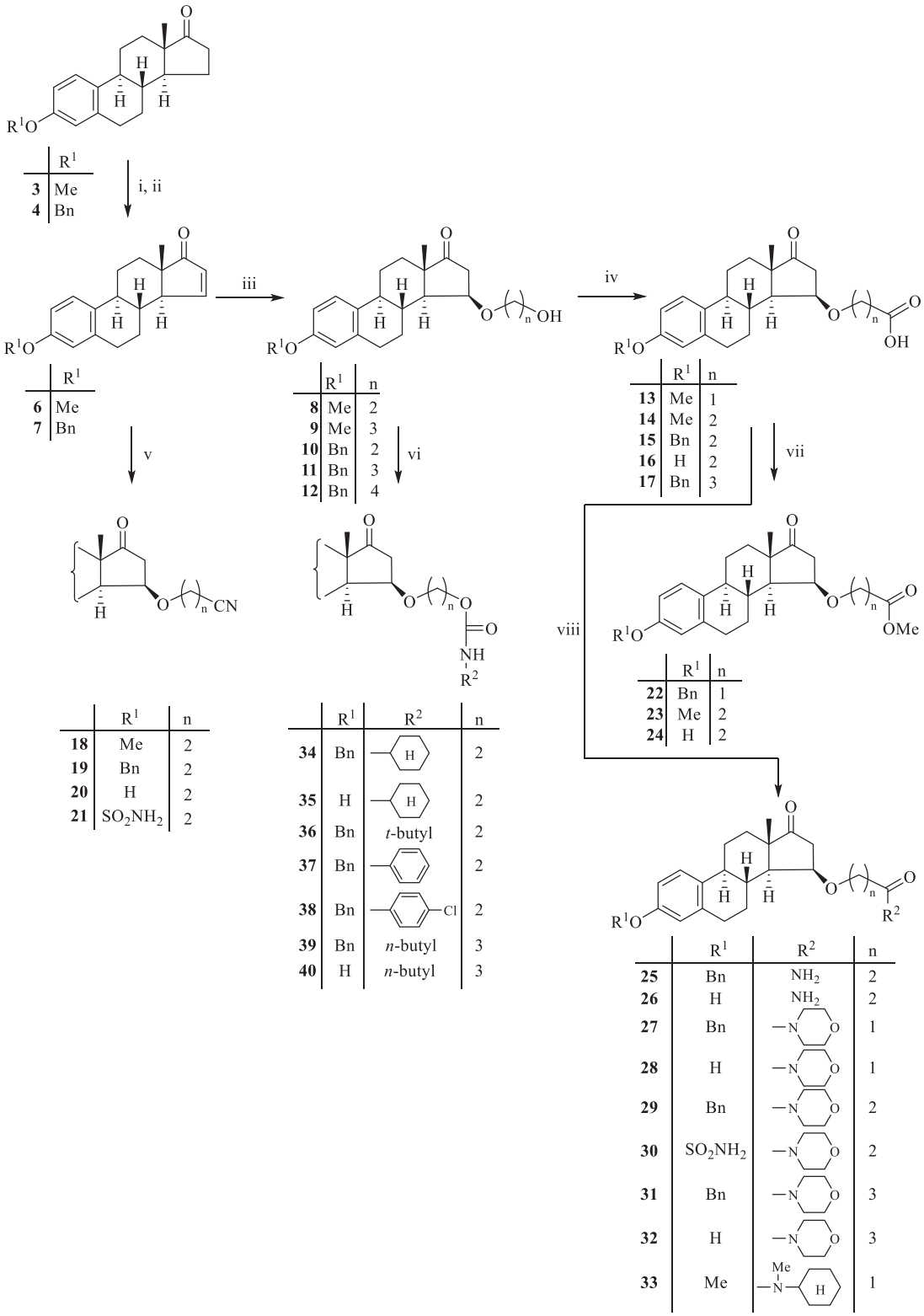
Ligand-based approach and the role of reference compounds

Experimental testing and biochemical analysis of the inhibition of novel compounds provide a feasible way for the development of inhibitors against 17β-HSD1. This ligand-based approach may also give valuable information on the molecular basis of substrate binding and catalytic mechanisms[24].

In our present experiments, thirty substituted 15β-alkoxy estrone derivatives possessing hydroxy, methoxy, benzyloxy and sulfamate groups in position C3 were investigated. *In vitro* conversion of estrone (**1**) to 17β-estradiol (**2**) was measured and cofactors, either NADPH or NADH were present in excess amounts in the incubations. Inhibitory effects were evaluated first by relative conversion measured at 10 μM test concentration. For more potent inhibitors IC_{50} values were determined. Inhibitory potentials and binding affinities were evaluated in comparison to those of natural substrate estrone (**1**) and the parent unsubstituted core molecules of the inhibitors, including 3-methoxy- and 3-benzyloxy-estrone (**3** and **4**) and estrone-3-sulfamate (**5**) (EMATE). Comparative evaluation of inhibitory potentials measured with different cofactors was performed on the basis of RIP parameters which were calculated in comparison to substrate estrone (Table 1).

17β-HSD1 inhibition results of the test compounds

Results obtained with NADPH. In the NADPH supplemented incubations compounds **18**, **20**, and **40** were found to be the most potent inhibitors (Table 2). Their IC_{50} values are below 1 μM



Scheme 2. Reagents and conditions: (i) and (ii)[12,23]; (iii) CH_2Cl_2, hydroxyl alcohol, NaOH; (iv) acetone, Jones reagent; (v) CH_2Cl_2, hydroxyl alkylnitrile, NaOH; (vi) CH_2Cl_2, TEA, hydroxyl alkyl-, or arylisocyanate; (vii) THF, diethylether, CH_2N_2; (viii) CH_2Cl_2, oxalyl chloride, amines.

(0.42–0.64 μM), indicating inhibitory effects similar to those of the unsubstituted parent compounds estrone (**1**) and 3-methyl-*O*-estrone (**3**) (0.63 and 0.77 μM, respectively). Compounds **11**, **21**, **22**, and **25** proved to be effective inhibitors displaying IC_{50} close to 1 μM (0.78–1.5 μM). Three compounds of this group (**11**, **22**, **25**) are derivatives of 3-benzyl-*O*-estrone, and they exert substantially stronger inhibition in comparison to the unsubstituted core itself. The IC_{50} value was found to be 2.7 μM for another 3-benzyloxy compound (**9**), and that shows a somewhat weaker effect, but still an improved inhibition when compared to the parent molecule

(**4**). IC_{50} values of **23**, **24**, and **26** were found also in the micromolar range (2.2–5.1 μM). These results, however, indicate decreased potentials compared to the unsubstituted estrone (**1**) and 3-methyl-*O*-estrone (**3**) cores. Relative conversions measured for the 10 μM test concentration of the other compounds in the presence of NADPH were higher than 50%. These results mean IC_{50} values higher than 10 μM and reveal a weak inhibitory effect against the 17β-HSD1.

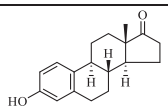
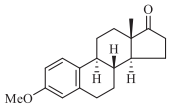
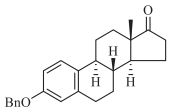
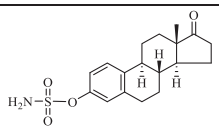
Results obtained with NADH. In experiments performed with NADH in excess, an outstanding inhibitory potential was measured for 3-hydroxy compound (**35**) (IC_{50} = 0.38 μM). Two other 3-hydroxy compounds (**20** and **40**) were found to be somewhat less potent, and their IC_{50} values (3.2 and 1.4 μM, respectively) reflect similar effects as the unsubstituted estrone. The 3-benzyloxy compound (**17**) and the 3-sulfamate compound (**21**) also displayed medium strengths with IC_{50} values 3.5 μM and 4.0 μM, respectively. The result of **17** indicates an improved inhibition compared to the basic molecule 3-benzyl-*O*-estrone (**4**). Compounds **24**, **28**, and **33** displayed moderate inhibitions with IC_{50} values between 5.6 and 7.0 μM. Other compounds exerted weak inhibition when NADH was applied as cofactor. In these cases, relative conversions were not suppressed below 50% at the 10 μM test concentration (IC_{50}>10 μM).

Results with NADPH versus NADH. Results measured with NADPH or NADH showed different tendencies for some of the test compounds when the inhibitory effects of the C15 derivatives were compared to those of their unsubstituted core compounds. Using the RIP values, derivative **35** exerted similar effect in the presence of NADPH than estrone (**1**) (RIP = 0.66 and 1.0, respectively), but this compound had a strong 5-fold increase in inhibitory potential, when NADH was applied (RIP = 0.19). Derivative **18** also displayed a maintained effect with NADPH (RIP = 0.89), but showed diminished potential with NADH (RIP = 10) in comparison to its unsubstituted core 3-methyl-*O*-estrone (**3**). The latter displayed RIP values 1.2 with NADPH and 2.1 with NADH). C15 substituents increased the inhibitory potentials in the case of 3-benzyloxy compounds **11**, **22**, and **25** when NADPH was applied (RIP = 1.2–2.4), but maintained effects were measured for these derivatives in the presence of NADH (RIP > 5.0). Compound **17** had an opposite behavior, that is, it showed improved inhibition with NADH (RIP = 1.8) compared to the unsubstituted core 3-benzyl-*O*-estrone (**4**) ((RIP (NADPH) > 15 and RIP (NADH) > 5)). On the other hand, **20** and **40** showed retained potentials with both cofactors compared to their unsubstituted core estrone (**1**) with RIP parameters close to 1 (0.70–1.6). For some other compounds derivatized at C15, however, decreased inhibitory effects could be observed when either NADPH or NADH was applied.

Biomedical evaluation of the inhibitory potentials obtained with NADPH

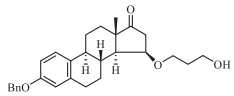
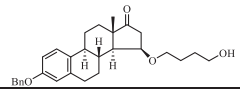
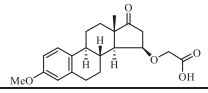
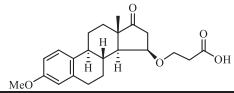
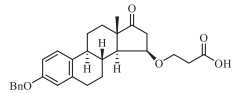
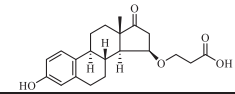
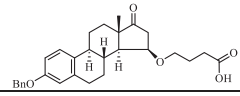
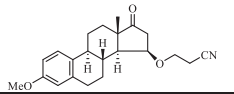
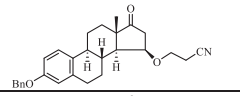
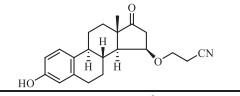
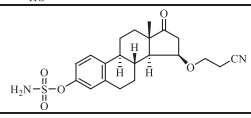
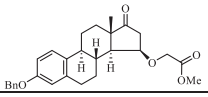
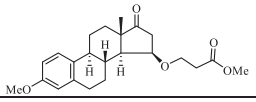
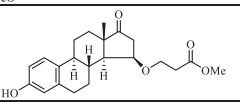
Literature background. Estrane-based inhibitors, as C15-substituted derivatives are assumed to occupy the substrate binding site of 17β-HSD1. Side chains are capable of establishing further contacts to the enzyme than the substrate molecule itself and, in this way, they may modulate binding affinity and inhibitory potential this way[5,7,9,25,26]. Messinger et al. analysed the X-ray structure of the 17β-HSD1[27] and identified a hole in the proximity of the enzyme's active site, which is composed of flexible amino acids Ser222, Leu219 and Met193 as well as Tyr218, Leu96, and Gly198[12]. The hole shows its opening towards the environment of C15 of the steroidal backbone, and thought to be able to accommodate side chains with appropriate length, spacer unit, and capping group. This finding inspired the Messinger group to synthesize numerous C15-substituted estrone derivatives as presumed 17β-HSD1 inhibitors and they have identified several

Table 1. Reference parameters of *in vitro* 17β-HSD1 inhibition of the unsubstituted compounds.

Nr.	Structure	17β-HSD1 inhibition in the presence of NADPH			17β-HSD1 inhibition in the presence of NADH		
		IC_{50} ± SD (μM)	Rel. conv. (%) at 10μM ± SD	RIP	IC_{50} ± SD (μM)	Rel. conv. (%) at 10μM ± SD	RIP
1		0.63 ± 0.11		1.0	2.0 ± 0.18		1.0
3		0.77 ± 0.29		1.2	4.2 ± 1.6		2.1
4		>10	52 ± 2	> 15	>10	52 ± 5	> 5
5		0.98 ± 0.71		1.6	2.6 ± 1.6		1.3

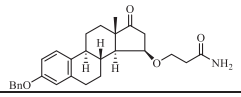
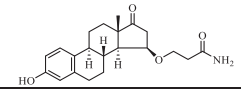
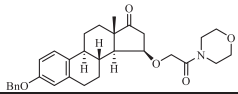
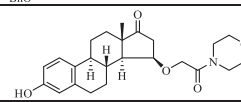
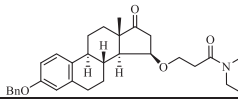
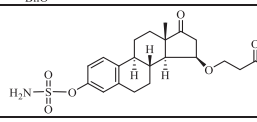
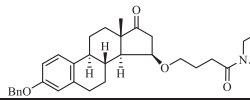
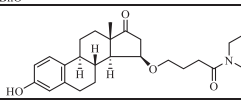
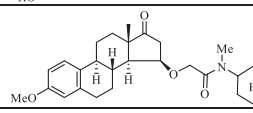
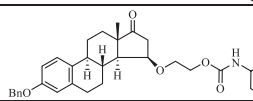
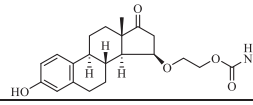
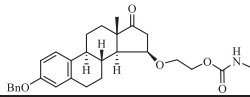
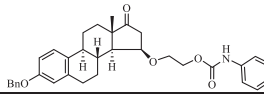
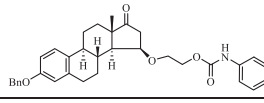
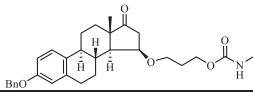
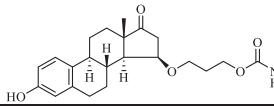
IC_{50}: concentration which decreases the enzyme activity to 50%.
Relative conversions (Rel. conv., control incubation with no inhibition is 100%) measured in the presence of 10 μM concentration of the compound tested. Mean ± SD, $n = 3$.
RIP: relative inhibitory potential compared to estrone; SD: standard deviation.

Table 2. *In vitro* 17β-HSD1 inhibition of the C15 derivatized test compounds.

Nr.	Structure	17β-HSD1 inhibition in the presence of NADPH			17β-HSD1 inhibition in the presence of NADH		
		IC_{50} ± SD (μM)	Rel. conv. (%) at 10μM ± SD	RIP	IC_{50} ± SD (μM)	Rel. conv. (%) at 10μM ± SD	RIP
11		0.78 ± 0.30		1.2	> 10	76 ± 7	> 5.0
12		2.7 ± 1.6		4.3	> 10	77 ± 5	> 5.0
13		> 10	90 ± 9	> 15	> 10	78 ± 7	> 5.0
14		> 10	88 ± 9	> 15	> 10	76 ± 3	> 5.0
15		> 10	78 ± 6	> 15	10 ± 3		5.1
16		> 10	76 ± 15	> 15	9.7 ± 4.5		4.9
17		> 10	87 ± 4	> 15	3.5 ± 1.0		1.8
18		0.56 ± 0.37		0.89	20 ± 9.0		10
19		> 10	65 ± 4	> 15	> 10	83 ± 1	> 5.0
20		0.64 ± 0.18		1.01	3.2 ± 1.5		1.6
21		1.1 ± 0.14		1.7	4.0±1.5		2.0
22		1.5 ± 0.50		2.4	> 10	60 ± 3	> 5.0
23		5.1 ± 2.3		8.1	> 10	78 ± 2	> 5.0
24		2.2 ± 0.73		3.5	7.0 ± 4.2		3.5

(*Continued*)

Table 2. Continued.

25		1.1 ± 0.42		1.7	12 ± 4		6.2
26		4.2±1.8		6.7	11 ± 5		5.5
27		17 ± 6.5		> 15	> 10	65 ± 2	> 5.0
28		> 10	67 ± 4	> 15	5.6 ± 2.2		2.8
29		> 10	58 ± 5	> 15	> 10	71 ± 6	> 5.0
30		12 ± 3		18	13±5.4		6.4
31		> 10	78 ± 9	> 15	> 10	81 ± 8	> 5.0
32		12 ± 4		> 15	13 ± 6		6.7
33		> 10	61 ± 2	> 15	7.0 ± 0.7		3.5
34		21 ± 15		33	> 10	68 ± 6	> 5.0
35		0.42 ± 0.33		0.66	0.38 ± 0.22		0.19
36		> 10	79 ± 5	> 15	> 10	87 ± 3	> 5.0
37		> 10	67 ± 6	> 15	> 10	76 ± 5	> 5.0
38		> 10	80 ± 5	> 15	> 10	79 ± 10	> 5.0
39		> 10	57 ± 1	> 15	> 10	77±3	> 5.0
40		0.45 ± 0.23		0.71	1.4 ± 0.9		0.70

IC_{50}: concentration which decreases the enzyme activity to 50%.
Relative conversions (Rel. conv, control incubation with no inhibition is 100%) measured in the presence of 10 μM concentration of the compound tested. Mean ± SD, $n = 3$.
RIP: relative inhibitory potential compared to estrone; SD: standard deviation.

compounds with high potential, displaying IC_{50} values in the low nanomolar range[12,13].

Further studies also established that 17β-HSD1 accomplished complex processes in ligand binding sites[11,28]. That is, the enzyme protein could change its conformation depending on the inhibitor molecule offered[12]. These mechanisms indicate that a very small change in inhibitor structure can make large differences in the course of binding to the enzyme[12]. Inhibitor studies, therefore, may give ambiguous picture with regard to the binding properties of the compounds and complete structure–activity relations could be revealed sometimes scarcely[9,11,12].

17β-HSD1 can use either NADPH or NADH as hydride donor for the estrone (**1**) to 17β-estradiol (**2**) transformation. NADPH, *in vivo,* seems to be the prevailing cofactor in the process[29,30]. It is, therefore, reasonable to make biomedical evaluation of inhibitor candidates according to their potentials exerted in NADPH supplemented medium[22].

Discussion of test compounds. Among the test compounds, we investigated four 15β-(2′-cyano)ethoxy derivatives. Three of them, the 3-methoxy-, 3-hydroxy-, and 3-sulfamate compounds (**18, 20**, and **21**, respectively) proved to be potent inhibitors. These results indicate that 15β-(2′-cyano)ethoxy substituent can be a beneficial side chain concerning 17β-HSD1 inhibitory effect of estrone derivatives possessing different functionalities in their C3 position.

Four 3-benzyloxyestrone derivatives exerted substantial inhibitory effects against 17β-HSD1. Two compounds with 15β-(3′-hydroxy)propoxy-, and 15β-(4′-hydroxy)butoxy side chain (**11** and **12** respectively), as well as the 15β-(1′-methoxycarbonyl)methoxy derivative **22** and 15β-(2′-carbonylamido)ethoxy derivative **25** were found to be potent inhibitors. Potentials observed for these compounds are interesting, since very few effective 3-benzyl-*O*-estrone derivatives have been published in the literature[9,13,21].

Some of the compounds tested display structural similarities to C15 derivatized estrone-based compounds published earlier as 17β-HSD1 inhibitor candidates[12,13] and interesting conclusions can be arrived at by comparison our inhibitors with their counterparts. In previous studies 15β-propanolyl and 15β-pentanolyl substituents were found to be beneficial substituents on estrone concerning 17β-HSD1 inhibition[12,13]. In our case, the corresponding 15β-(3′-hydroxy)propoxy- and 15β-(4′hydroxy)butoxy-substituted 3-benzyl-*O*-estrone **11** and **12** also displayed considerable inhibitions. We found that the 3-methyl-*O*-estrone compound bearing a C15 substituent with a cyclohexyl capping group **33** was a poor inhibitor. In Messingers' experiments, however, its non-oxa analogue exerted potent inhibition[12,13]. A related cyclohexyl derivative of estrone **35**, on the other hand, showed also a strong inhibitory effect in our tests. The Messinger group found efficient 17β-HSD1 inhibitors of estrone and 3-methyl-*O*-estrone compounds that bore 15β-substituents containing a morpholino capping group and long chain with 3–5 methylene units[12,13]. In comparison, 15β-oxy-coupled morpholino compounds in our tests displayed only weak inhibitions. The comparison of oxa-coupled derivatives with earlier non-oxa analogues indicates the importance of the C15 linker unit in the 17β-HSD1 inhibitory effect of estrone derivatives. Benchmarking of recent results against earlier data is not easy, since different studies may be performed with different methodologies and reference inhibition parameters may be missing in previous studies. Our best inhibitors, nevertheless, may be estimated to be equipotent with some of related earlier C15 derivatives of estrone compounds and non-oxa analogues studied by Messinger et al[12,13].

Our investigations reveal that C15 substituents of the tested estrogen derivatives have decisive influence in the binding to 17β-HSD1. These results further indicate that remote fragments on position C3 also determine affinity of the investigated inhibitor molecules. C15 substituents of the test compounds show high variety in their chemical nature. Compounds found to be potent inhibitors also possess diverse side chains and it seems difficult to identify chain length, capping groups or spacer units which can be definitely beneficial in 17β-HSD1 binding. C15 substituents of the compounds can be, however, regarded as side chains both long and flexible. Considering these common features, we may assume that binding may be promoted by accommodation of these side chains in the binding hole of 17β-HSD1 that exists in proximity of C15 positions of ring D as described by Messinger et al[12].

Our experiments identified several potent 17β-HSD1 inhibitors among 15β-derivatized estrone-based compounds tested. The results demonstrate definitive influence of C15 substituents as well as crucial role of different functionalities in position C3. Structural diversity of the test compounds makes difficult to give complete structure–activity relationship conclusions, nevertheless, several interesting observations can be established from the inhibition data set.

Cofactor dependence of the 17β-HSD1 inhibition, a comparative evaluation

Literature background. Both cofactor molecules, NADPH and NADH, bind to 17β-HSD1 in an extended conformation, with the nicotinamide moiety pointing towards the active site of the enzyme. Nicotinamide is relatively flexible in the complex and the major interactions between cofactor and enzyme occur at the adenine dinucleotide phosphate part[30]. Most of these interactions are common for both NADPH and NADH[25,27,31]. A major difference between the two cofactors is, however, that the 2′-phosphate group of NADPH is stabilized mainly through hydrogen bonds with residues Ser11 and Arg37[27,31], whereas the free hydroxyl groups on adenosine ribose of non-phosphorylated cofactor NADH may form hydrogen bonds with Ser11, but not with Arg37[31]. When a cofactor binds to 17β-HSD1, structural changes are induced in the area of the substrate binding site as well. An otherwise disordered loop, which are composed of residues 189–200, may adopt a specific conformation to accommodate more space for the cofactor in the active centre[25,27]. Structural differences between the holo form and the apo form of the enzyme may modify interactions of ligands bound in the substrate binding site. For instance, side chain of a potent inhibitor 3-[3′,17′β-dihydroxyestra-1′,3′,5′(10′)-trien-16′β-methyl]-3-benzamide occupies a different position in the ternary inhibitor complex, which is different from that in the binary complex[25]. Differences in binding of substrate or inhibitor ligands may also occur when different cofactors are complexed. The NADPH-bound holoenzyme exerts higher affinity to the substrate estrone than the NADH-bound complex[29,30]. In an earlier study we observed highly different inhibitory potential and binding affinity of various D-secoestrones depending on which cofactor (either NADPH or NADH) was applied in the *in vitro* experiments[22].

Discussion of test compounds, NADPH versus NADH. In our experiments, substantial inhibitory potentials and increased binding affinities were observed in presence of both cofactors. However, some of the compounds exerted different inhibitory potentials towards 17β-HSD1 complexed to NADPH- or NADH-complexed

17β-HSD1. Substrate estrone displays different IC_{50} values depending on the cofactor applied and RIP parameters reflect more reliably the cofactor-dependent differences in the inhibitor binding.

RIP parameters demonstrate 3–8 fold stronger binding with NADH than with NADPH for estrone derivatized with a 15β-(1′-morpholinocarbonyl)methoxy- side chain (**28**), for 3-methoxyestrone and estrone derivatized with 15β-(1′-*N*-methyl,cyclohexylamino carbonyl)methoxy- and 15β-(2′-cyclohexylcarbamoyloxy)ethoxy chains (**33** and **35**), as well for 3-benzyl-*O*-estrone derivative possessing a 15β-(3′-carboxylic)propoxy substituent (**17**). Binding of 3-benzyl-*O*-estrone compounds substituted with a 15β-(3′-hydroxy)propoxy, 15β-(1′-methoxycarbonyl)methoxy, or a 15β-(2′-aminocarbonyl)ethoxy side chain (**11**, **22**, and **25)** and 3-methyl-*O*-estrone bearing a 15β-(2′-cyano)methoxy side chain (**18**) display similar (2–11 fold) cofactor preference in terms of RIP values, but these derivatives favour binding to the NADPH bound enzyme.

Cofactor-dependent affinities of some of the investigated compounds indicate that binding capabilities of the binding hole may be different depending on the nature of cofactor the enzyme complexed with. The binding hole suitable to accommodate C15 side chains is known to be formed by amino acids Leu96, Met193, Gly198, Tyr218, Leu219, and Ser222[12]. Residues Met193 and Gly198 are also constituents of the disordered loop (amino acids 189–200) which adopt a specific conformation following cofactor binding[25,27]. The presence or absence of 2′-phosphate in the cofactors causes differences in the structure of the holoenzymes. Furthermore, the area of the substrate binding site may also be affected in a different way in these complexes[25,27,31]. We may also assume that binding of NADPH or NADH modifies the conformation of the loop of residues 189–200 differently. Joint residues Met193 and Gly198 of the two structural elements transmit this difference from the loop to the binding hole, and these processes induce different positioning and binding capabilities of the binding hole in the holoenzyme variants. Hosting certain C15 side chains, therefore, might be, therefore, favoured or unfavoured in the binding hole altered differently upon binding of phosphorylated or unphosphorylated cofactors.

In our earlier investigation, potent 17β-HSD1 inhibitory effects of ring D modified seco-oxime and seco-alcohol estrones were identified[22]. In that case we assumed that polar functionalities of short side chains of compounds studied might establish hydrophilic interactions or hydrogen bonds towards suitable amino acids of the enzyme present in close proximity of ring D region. Binding affinity of certain compounds in that series also displayed strong cofactor dependence and this phenomenon indicated that complexation with NADPH or NADH furnished different conformations to enzyme residues involved in the interactions.

In our present study we identified another group of compounds, C15 derivatized estrones, which may exert cofactor-dependent inhibition towards the 17β-HSD1. Structural features and binding mechanisms of side chains in the region of ring D differ to a great extent for those compounds investigated in our earlier report and for those presented in this study. Cofactor dependence of inhibitor binding, however, could be observed in both series. These results suggest that binding of the phosphorylated or the unphosphorylated cofactor may exert different influence on more areas and structural elements of the substrate binding site.

Early studies assigned NADH as a catalytic cofactor of 17β-HSD1 and numerous *in vitro* inhibition tests have been performed with this recognition. (See corresponding references in our earlier work[22].) Later, however, it became accepted that NADPH might be the prevalent partner of 17β-HSD1 in its main *in vivo* function in the catalysis of the estrone–17β-estradiol conversion[22,29,30]. Our present results obtained with C15 estrone derivatives support that *in vitro* potentials obtained with the two cofactors may differ substantially for certain inhibitor compounds. Data measured in the presence of NADPH are more relevant in inhibitor optimization and in lead selection, but NADH results could be valuable in understanding of the mechanism the inhibition of 17β-HSD1.

Conclusions

17β-HSD1 inhibitory potential of 15β-oxa-coupled estrone derivatives possessing hydroxy, methoxy, benzyloxy and sulfamate functionalities in position C3 has been investigated. Thirty inhibitor candidates were tested via *in vitro* radioincubations. We found several potent 17β-HSD1 inhibitors and the results demonstrated that potent inhibitory effect could be achieved with both various C15 substituents, and different C3 functional groups. We identified four 3- benzyloxyestrone derivatives (**11**, **22**, **25**), which exerted substantial inhibitory effect. We also found that 15β-(2′-cyano)ethoxy was a beneficial substituent of compounds bearing different functionalities in position C3 (**18**, **19**, **20**, and **21**). A comparison with earlier non-oxa analogues indicates that beyond the effect of capping groups and spacer units, there might be a strong influence of the presence or absence of oxygen in the 15β linker on the inhibitory potential.

Some of the compounds displayed considerable difference in binding affinities towards 17β-HSD1 complexed with NADPH or NADH. It is reasonable to assume that side chains of the potent compounds can be accommodated in the binding hole of 17β-HSD1 existing in proximity of the C15 position of ring D of the steroidal ligands[12]. This binding hole shares Met193 and Gly198 with a loop element which is known to adopt a specific conformation upon cofactor binding[25,27]. We suppose that conformation of this loop may be different in NADPH- or NADH-complexed 17β-HSD1. Structural differences can be forwarded by the joint amino acids inducing different positioning and binding capabilities of the binding hole. Further structural investigations (e.g., molecular docking studies) may confirm mechanisms involved in binding of our inhibitor compounds and different binding affinities of the test compounds exerted towards the holoenzyme variants.

Our investigations provide valuable data on binding processes of the enzyme and may contribute to the development of new 17β-HSD1 inhibitors, as novel drug molecules acting on enzyme level.

Disclosure statement

The authors declare no conflict of interest.

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