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Antihormonal potential of selected D-homo and D-seco estratriene derivatives

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ABSTRACT

Since many estrogen derivatives exhibit anti-hormone or enzyme inhibition potential, a large number of steroidal derivatives have been synthesised from appropriate precursors, in order to obtain potential therapeutics for the treatment of hormone-dependent cancers. In molecular docking studies, based on X-ray crystallographic analysis, selected D-homo and D-seco estratriene derivatives were predicted to bind strongly to estrogen receptor α (ERα), aromatase and 17,20 lyase, suggesting they could be good starting compounds for antihormonal studies. Test results *in vivo* suggest that these compounds do not possess estrogenic activity, while some of them showed weak anti-estrogenic properties. *In vitro* anti-aromatase and anti-lyase assays showed partial inhibition of these two enzymes, while some compounds activated aromatase. Aromatase activators are capable of promoting estrogen synthesis for treatment of pathological conditions caused by estrogen depletion, *e.g.* osteopenia or osteoporosis.

1. Introduction

Steroid hormones regulate many different physiological processes in humans, through all phases of life. Tissue-specific enzymes catalyze steroidogenesis, which starts with initial entry of cytosolic cholesterol into the mitochondrion, and then proceeds with conversion of cholesterol to the biologically active androgen, followed by estrogen hormones. Enzymes belonging to cytochrome P450 enzymes and hydroxysteroid dehydrogenases catalyze adrenal, ovarian, testicular, placental and other steroidogenic processes [1].

The reduction of circulating steroid hormones and/or blockade of steroid action in cancer tissues are still primary goals in the current strategy for breast- and prostate-cancer treatment [2]. In the treatment of androgen-dependent tumors, antiandrogens, as well as 17α-hydroxylase/C17,20 lyase (CYP17A1) and 5α-reductase inhibitors are the most promising therapeutics [2,3].

Since estrogen hormones are involved in the development and growth of breast tumors, estrogen deprivation remains a key therapeutic approach. Endocrine agents are designed to inhibit the production of estrogen or to block its action at the estrogen receptor [2,4]. For many years, tamoxifen, an antiestrogen that compete with estradiol for estrogen receptor, was the most important therapeutic in the hormonal therapy for all stages of postmenopausal patients with estrogen-receptor–positive cancers. However, tamoxifen does not completely prevent the action of endogenous estrogen, and this remaining partial estrogen agonist activity is probably responsible for some of its undesirable side effects, such as an increased risk of endometrial cancer.

On the other hand, the enzyme aromatase [5], which catalyzes the key and final step of estrogen synthesis, namely conversion of C19 steroids to estrogens, acts as a master switch in physiological and pathological processes. Apart from the fact that, unlike tamoxifen, aromatase inhibitors (AI) have no partial agonist activity, cancer cells could become resistant to this kind of therapy [6].

Thus, by combining these two breast cancer therapy approaches, the greatest benefit for patients can be realized [2,7–10].

Considering the partial agonistic effects of tamoxifen and the existence of breast cancer cells resistant to aromatase inhibitors, an important objective of researchers is to find new efficient strategies and/or more effective therapeutics for treating patients suffering from estrogen-dependent breast cancer, with no side effects or resistance.

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A broader project, directed towards preparation of potential antihormones and steroidogenesis enzymes inhibitors resulted in the synthesis of many D-modified steroidal compounds possessing promising antihormonal properties [11–18] Among others, 3-benzyloxy-17-hydroxy-16,17-secoestra-1,3,5(10)-trien-16-nitrile, was of special interest, as well as its corresponding 3-hydroxy derivative. Specifically, this compound showed practically complete loss of estrogenic activity, while displaying satisfactory antihormonal properties. On the other hand, its 3-hydroxy equivalent showed less antiestrogenic activity, with complete loss of estrogenic potency [11]. These facts prompted us to further pursue chemical transformations of this secocyanoalcohol, in order to study the influence of different modifications of the D-ring on the biological potential of new compounds.

2. Experimental

2.1. Synthesis and characterization of the newly synthesised compounds

2.1.1. General

Melting points were determined in open capillary tubes on a Büchi SMP apparatus and values are reported uncorrected. Infrared spectra (ν in cm^{-1}) were recorded in KBr pellets on a Perkin-Elmer M457 or Carl Zeiss Specord 75 spectrophotometer. NMR-spectra were taken on a Bruker AC 250E spectrometer operating at 250 Hz (proton) and 62.9 Hz (carbon), using standard Bruker software. The signals are reported in ppm downfield from a tetramethylsylane internal standard (δ 0.00); symbols s, d, dd and m denote singlet, doublet, double doublet and multiplet, respectively. Mass spectra were recorded on a Finnigan-Math 8230 instrument, using chemical ionization (*iso*-butane) techniques; the first number denotes *m/z* value, and the ion abundances are given in parentheses.

2.1.2. 3-Benzyloxy-17-oxa-D-homo-estra-1,3,5(10)-triene-16-on (**2**)

Secocianoalcohol **1** (1 g, 2.67 mmol) was dissolved in benzene (5 mL) and *p*-toluenesulfonic acid (0.64 g, 3.37 mmol) was added. The reaction mixture was refluxed for 4 h. The catalyst was filtered off and the solvent was evaporated to dryness. The crude 3-benzyloxy-D-homo derivative **2** (0.96 g, 95.71%) was purified by column chromatography on silica gel (100 g, toluene-ethyl acetate /2:1/), giving 0.55 g (57.30%) of analytically pure 3-benzyloxy-17-oxa-D-homo-estra-1,3,5(10)-triene-16-on (**2**, mp. 162 °C).

IR spectrum: 3060, 2980–2960, 1750, 1620, 1250, 1050,760, 740, 705

^{1}H NMR spectrum ($CDCl_3$): 1.05 (s, 3H, CH_3, C_{18}); 2.22 (2d, 3H, $1H_{15}$ and 2 protons from the skeleton; J_{gem} = 18.61 Hz, $J_{15a,14}$ = 12.79 Hz); 2.85 (m, 3H, $1H_{15}$ 2 protons from the skeleton); 3.98 (d, 1H, H_{17a}, J_{gem} = 10.69 Hz); 4.05 (d, 1H, H_{17a}); 5.05 (s, 2H, O-C**H**$_2$-C_6H_5); 6.74–7.47 (group of signals, 8H, aromatic protons).

^{13}C NMR-spectrum ($CDCl_3$): 14.98 (CH_3, C_{18}); 69.80 (O-**C**H_2-C_6H_5); 81.07 (C_{17a}); 156.87 (C_3); 170.64 (C=O).

Mass spectrum: 378 (9; $(M+2)^+$); 377 (30; $(M+1)^+$); 92 (19; $(C_7H_8)^+$); 91 (100; $(C_7H_7)^+$).

Anal. Calcd. for $C_{25}H_{28}O_3$: C, 79.78; H, 7.45. Found: C, 80.03; H, 7.64.

2.1.3. 3-Benzyloxy-16,17-secoestra-1,3,5(10)-triene-16,17-diol (**3**)

Compound **2** (0.52 g, 1.38 mmol) was dissolved under heating in a mixture of methanol and dichloromethane (2:1, 30 mL). To the cooled solution $NaBH_4$ (0.42 g, 11 mmol) was added stepwise. The reaction mixture was stirred for 20 min at room temperature and then refluxed for 40 min. After cooling, the resulting solution was acidified with dilute HCl (1:4) to pH 5. The obtained suspension was extracted with dichloromethane (3 × 30 mL), collected extracts were washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The crude 3-benzyloxy D-seco-diol **3** (0.43 g, 81.13%) was purified by column chromatography on silica gel (50 g, toluene-ethyl acetate /2:1/), giving 0.32 g (74.42%) of 3-benzyloxy-16,17-secoestra-1,3,5(10)-triene-16,17-diol (**3**) in form of a pale yellow oil, which after crystallization from the mixture dichloromethane-*n*-hexane gave white crystals of **3** (mp. 141 °C).

IR spectrum: 3500–3300, 2980–2900, 1630, 1515, 1300, 1270, 1050

^{1}H NMR spectrum ($CDCl_3$): 0.71 (s, 3H, CH_3, C_{18}); 2.87 (d, 2H, H_{15}, J_{gem} = 3.72 Hz); 3.10 (d, 1H, H_{17a}, J_{gem} = 11.78 Hz); 3.48 (s, 2H, **H**O—C_{17} and **H**O—C_{16}); 3.59 (m, 1H, H_{17b}); 3.70 (d, 1H, H_{16a}, J_{gem} = 11.77 Hz); 3.91 (m, 1H, H_{16b}); 5.04 (s, 2H, O-C**H**$_2$-C_6H_5); 6.47–6.72 (group of signals, 8H, aromatic protons).

^{13}C NMR-spectrum ($CDCl_3$): 15.92 (CH_3, C_{18}); 30.52 (C_{15}); 64.04 (C_{16}); 69.90 (O-**C**H_2-C_6H_5); 70.01 (C_{17}); 156.67 (C_3).

Mass spectrum: 380 (20; M^+); 147 (37); 91 (100; $(C_7H_7)^+$).

Anal. Calcd. for $C_{25}H_{32}O_3$: C, 78.90; H, 8.47. Found: C, 79.24; H, 8.14.

2.1.4. 3-Hydroxy-17-oxa-D-homo-estra-1,3,5(10)-triene-16-on (**4**) and 16,17-secoestra-1,3,5(10)-triene-3,16,17-triol (**5**)

To the solutions of the appropriate 3-benzyloxy derivative (**2**, 0.30 g, 0.80 mmol, *i.e.* **3**, 0.25 g, 0.67 mmol) in dichloromethane-methanol mixture (1:1, 3 mL), 10% Pd/C (0.15 g) was added. The suspensions were stirred at room temperature for 5, *i.e.* 3 h under an atmosphere of hydrogen. After removal of catalyst, the reaction mixtures were evaporated to dryness, yielding 0.22 g (95.65%), i.e. 0.11 g (56.60%) of crude 3-hydroxy-derivatives **4**, *i.e.* **5**. The crude product **4** was purified by column chromatography on silica gel (40 g, toluene-ethyl acetate /2:1/), whereby 0.18 g (81.81%) of analytically pure 3-hydroxy-17-oxa-D-homo-estra-1,3,5(10)-triene-16-on (**4**, mp. 205 °C) was obtained. On the other hand, flash chromatography of crude **5** on silica gel (*n*-hexane–acetone /3:2/), yielded 0.08 g (41.88%) of 16,17-secoestra-1,3,5(10)-triene-3,16,17-triol (**5**, mp. 201 °C) in form of colorless crystals.

2.1.5. Compound **4**

IR spectrum: 3500–3300, 2970, 1730, 1300, 1270, 1050

^{1}H NMR spectrum (DMSO-d6): 0.92 (s, 3H, CH3, C18); 2.20 (m, 4H, $1H_{15}$ and 3 protons from the skeleton); 2.72 (m, 3H, $1H_{15}$ and 2 protons from the skeleton); 3.95 (2d, 2H, H_{17a}); 6.45 (d, 1H, H-4, $J_{4,2}$ = 2.58 Hz); 6.53 (dd, 1H, H-2, $J_{2,1}$ = 8.36 Hz, $J_{2,4}$ = 2.59 Hz); 7.06 (d, 1H, H-1, $J_{1,2}$ = 8.48 Hz); 9.03 (s, 1H, **H**O=C_3).

^{13}C NMR-spectrum (DMSO-d_6): 14.61 (CH_3, C_{18}); 80.15 (C_{17a}); 155.04 (C_3); 170.11 (C=O).

Mass spectrum: 288 (21; $(M+2)^+$); 287 (44; $(M+1)^+$); 286 (100; M^+); 259 (16); 172 (67); 159 (23); 145 (21); 133 (18); 107 (16).

Anal. Calcd. for $C_{18}H_{22}O_3$: C, 75.49; H, 7.74. Found: C, 75.24; H, 7.56.

2.1.6. Compound **5**

IR spectrum: 3500–3300, 2970, 1630, 1600, 1515, 1380, 1300, 1270, 1050.

^{1}H NMR spectrum (DMSO-d_6): 0.65 (s, 3H, CH_3, C_{18}); 3.02 (dd, 1H, C_{17a}, J_{gem} = 11.0 Hz, $J_{H,OH}$ = 6.51 Hz); 3.32 (m, 2H, $1H_{16}$ and $1H_{17}$); 3.45 (m, 1H, H_{16b}); 4.47 (t, 1H, **H**O=C_{17}, J = 6.21 Hz); 4.72 (t, 1H, **H**O=C_{16}, J = 4.78 Hz); 6.42 (d, 1H, H-4, $J_{4,2}$ = 2.51 Hz); 6.50 (dd, 1H, H-2, $J_{2,1}$ = 8.43 Hz, $J_{2,4}$ = 2.58 Hz); 7.05 (d, 1H, H-1, $J_{1,2}$ = 8.50 Hz); 9.00 (s, 1H, **H**O=C_3).

^{13}C NMR-spectrum (DMSO-d_6): 15.96 (CH_3, C_{18}); 29.99 (C_{15}); 62.20 (C_{16}); 69.24 (C_{17}); 154.88 (C_3).