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Inhibitors of type 1 17β-hydroxysteroid dehydrogenase with reduced estrogenic activity: Modifications of the positions 3 and 6 of estradiol

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Abstract

Breast cancer is the second most frequent cancer affecting women. Among all endocrine therapies for the treatment of breast cancer, inhibition of estrogen biosynthesis is becoming an interesting complementary approach to the use of antiestrogens. The enzyme type 1 17β-hydroxysteroid dehydrogenase (17β-HSD) plays a critical role in the biosynthesis of estradiol catalyzing preferentially the reduction of estrone into estradiol, the most active estrogen. Consequently, this enzyme is an interesting biological target for designing drugs for the treatment of estrogen-sensitive diseases such as breast cancer. Our group has reported the synthesis and the biological evaluation of N-methyl, N-butyl 6β-(thiaheptamamide)estradiol as a potent reversible inhibitor of type 1 17β-HSD. Unfortunately, this inhibitor has shown an estrogen effect, thus reducing its possible therapeutic interest. Herein three strategies to modify the biological profile (estrogenicity and inhibitory potency) of the initial lead compound were reported. In a first approach, the thioether bond was replaced with a more stable ether bond. Secondly, the hydroxyl group at position 3, which is responsible for a tight binding with the estrogen receptor, was removed. Finally, the amide group of the side-chain was changed to a methyl group. Moreover, the relationship between the inhibitory potency and the configuration of the side-chain at position 6 was investigated. The present study confirmed that the 6β-configuration of the side chain led to a much better inhibition than the 6α-configuration. The replacement of the 3-OH by a hydrogen atom as well as that of the amide group by a methyl was clearly unfavorable for the inhibition of type 1 17β-HSD. Changing the thioether for an ether bond decreased by 10-fold the estrogenic profile of the lead compound while the inhibitory potency on type 1 17β-HSD was only decreased by 5-fold. This study contributes to the knowledge required for the development of compounds with the desired profile, that is, a potent inhibitor of type 1 l7β-HSD without estrogen-like effects.

Keywords: *Enzyme, 17β-hydroxysteroid dehydrogenase, inhibitor, steroid, estrogen, 17β-HSD*

Introduction

It is now well-recognized that circulating inactive steroids such as dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) are submitted to peripheral biotransformations that generate the active androgens, testosterone (T) and dihydrotestosterone (DHT), and estrogens, estrone (E_1) and estradiol (E_2), in their sites of action [1]. The last step in the biosynthesis of estrogens involves the reduction of E_1 into E_2 catalyzed by type 1 17β-hydroxysteroid dehydrogenase (17β-HSD) [2,3]. This enzyme is expressed in all classical steroidogenic tissues and in almost all peripheral tissues, including the skin and breast [4]. Furthermore, the reductive activity of the 17β-HSD is enhanced in ER^+ breast cancer cells [5–8]. Consequently, this enzyme is an attractive pharmacological target for the treatment of estrogen-sensitive diseases such as breast and endometrial cancers.

The design of 17β-HSD inhibitors has been attempted during the past decades, but without achieving progress significant enough to justify their

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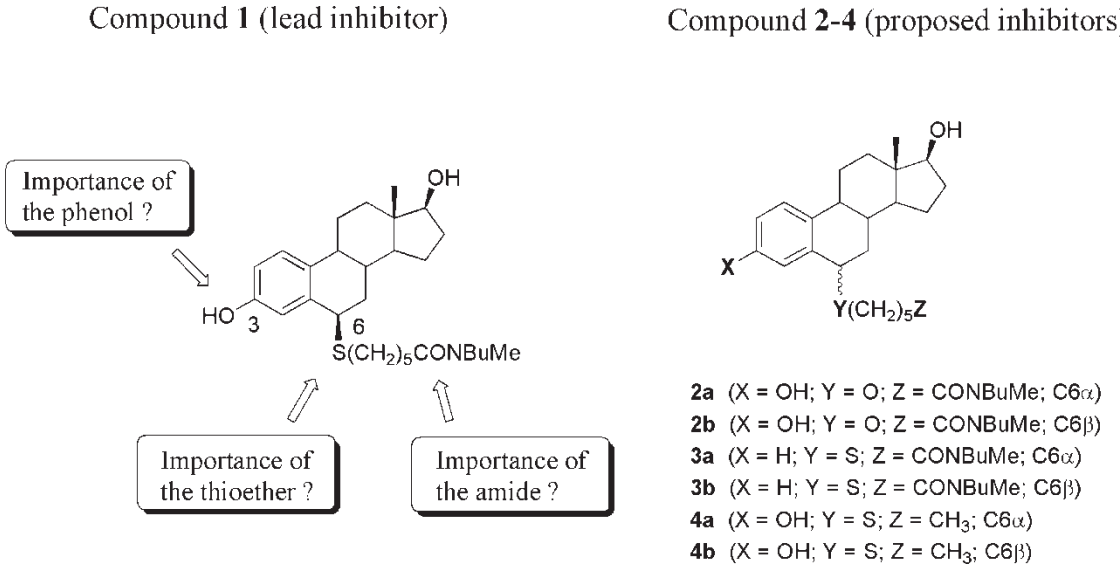


Figure 1. Chemical structure of the previously reported inhibitor **1** and of new inhibitors **2**–**4** of type 1 17β-HSD.

use in the treatment of estrogen-sensitive diseases [9–11]. In order to achieve a therapeutic benefit, an inhibitor of 17β-HSD would ideally have a K_i below the K_m of E_1 and, more importantly, should not display estrogenic activity. Our group has recently reported the synthesis and the inhibitory potency of a number of E_2 derivatives functionalized at position 6 [12]. Compound **1** (Figure 1) is a good inhibitor of the synthesis of E_2 from E_1, while the corresponding 6α-epimer is inactive. Such dependence upon the stereochemistry might indicate selective interactions between this type of compound and the enzyme. Unfortunately, an estrogenic activity was also observed for compound **1**, as illustrated by its proliferative activity on estrogen-sensitive cells in culture [13]. The instability of the thioether bond was hypothesized to be responsible for this observation. This phenomenon was also observed in the case of the 7α-(4-aminophenylthia)-androst-4-ene-3,17-dione, an aromatase inhibitor [14]. For compound **1**, the cleavage of the thioether bond probably provokes the formation of the fully estrogenic $\Delta^{6,7}$-E_2. Two strategies were developed to reduce the intrinsic estrogenic properties of this type of inhibitors (Figure 1). Firstly, the thioether bond was replaced by an ether bond, which should be much less likely to be modified by metabolizing enzymes. Secondly, the functionality at position 3 was modified to reduce, if not abolish, the binding of these inhibitors with the estrogen receptor. In addition, the importance of the amide function as well as the stereochemistry at position 6 was investigated. The chemical synthesis, inhibitory potency on type 1 17β-HSD and proliferative/antiproliferative activity on T47-D ER^+ cells of the target compounds (**2**–**4**) are reported in this paper.

Materials and methods

Chemistry

General. Reagents were obtained from Sigma-Aldrich Canada Co. (Oakville, ON, Canada). Estrone (E_1) and estradiol (E_2) were supplied by Steraloids (Newport, RI, USA). Usual solvents were obtained from Fisher Scientific (Montréal, Qc, Canada) and were used as received. Anhydrous solvents were obtained from Aldrich in SureSeal bottles, which were conserved under positive argon pressure. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under argon. All anhydrous reactions were performed in oven-dried glassware under positive argon pressure. Flash chromatography was performed on E. Merck 60 230–400 mesh silica gel. Thin-layer chromatography was performed on 0.25 mm E. Merck silica gel 60 F_{254} plates and visualized by UV (254 nm) and/or cerium ammonium molybdate. Infrared (IR) spectra were recorded on a Perkin-Elmer series 1600 FT-IR spectrometer (Norwalk, CT, USA) and only significant bands were reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded at 300 MHz for ^1H and 75.5 MHz for ^{13}C on a Bruker AC/F300 spectrometer (Billerica, MA, USA). Only significant signals were reported in ^1H NMR while all signals were listed for ^{13}C NMR. As previously reported [15–17], several NMR signals of compounds bearing a tetrahydropyran (THP) or an amide group are duplicated. Low-resolution mass spectra were recorded on API-150ex apparatus (Foster City, CA, USA) equipped with a turbospray source. Epimeric mixtures were resolved with an HPLC system (Waters Associates, Milford, MA, USA) using a Delta Prep 4000 apparatus, a UV detector, a reverse-phase

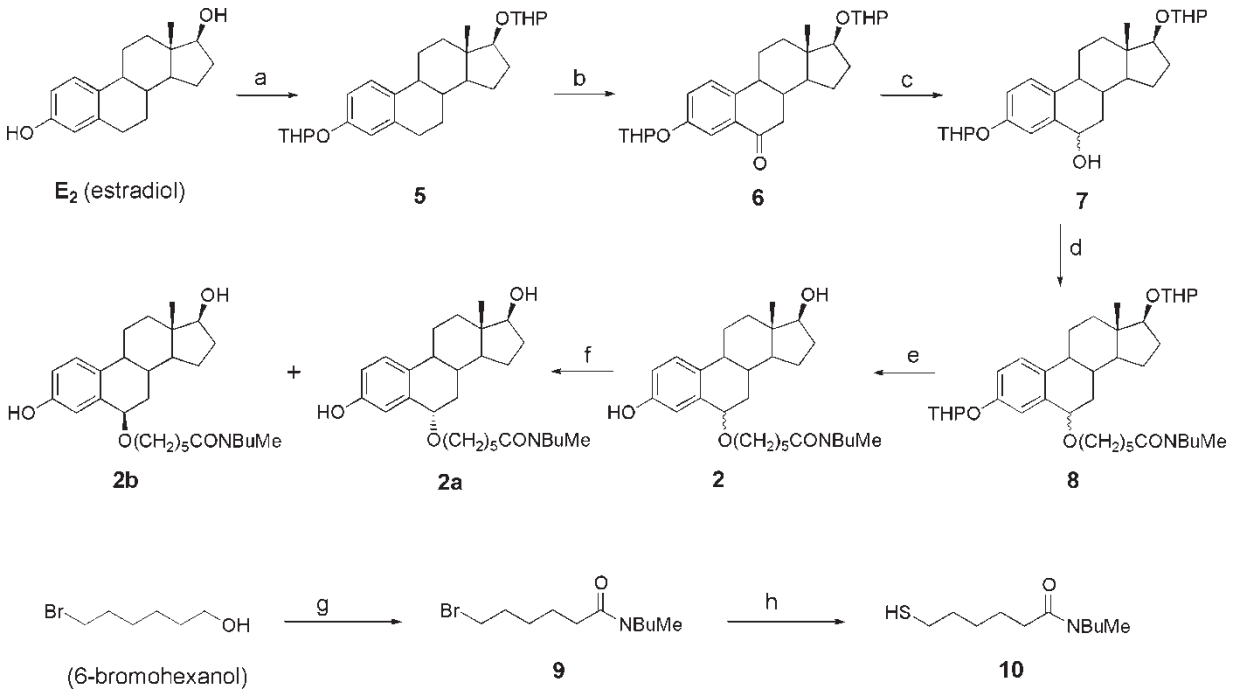
column (Nova-Pak HR C18, 40 × 100 mm) and the specified mixture of solvents.

*Synthesis of N-butyl, N-methyl 7-(3′,17′β-dihydroxy-1′,3′,5′(10′)-estratrien-6′α-yl)-7-oxoheptanamide (**2a**) and N-butyl, N-methyl 7-(3′,17′β-dihydroxy-1′,3′,5′(10′)-estratrien-6′β-yl)-7-oxoheptanamide (**2b**) (Scheme 1)*

*3,17β-bis(2′-tetrahydropyranyloxy)-1,3,5(10)-estratriene (**5**)*. To a solution of estradiol (994 mg; 3.7 mmol) in dry toluene (17 mL) at 0°C was added 3,4-dihydro-2H-pyran (3.4 mL; 37.3 mmol) followed by *p*-toluenesulfonic acid (60 mg; 0.37 mmol). The mixture was allowed to gradually reach room temperature and was stirred for 2 h. Then, a saturated $NaHCO_3$ solution was slowly added and the reaction mixture was poured into water. The crude material was extracted twice with EtOAc and twice with CH_2Cl_2. Each organic phase was washed separately with brine, then combined to be dried over $MgSO_4$. After evaporation of the solvent, the crude material was purified by flash chromatography (hexanes:Et_3N 98:2) to give 1.32 g (82%) of the (bis)protected compound **5**. All spectroscopic data were in agreement with the literature [18].

*3,17β-bis(2′-tetrahydropyranyloxy)-1,3,5(10)-estratrien-6-one (**6**)*. To a suspension of chromic anhydride (3.9 g; 39 mmol) in dry CH_2Cl_2 (30 mL) under argon was added 3,5-dimethylpyrazole (3.7 g; 39 mmol) and the resulting mixture was stirred at 25°C for 15 min. To the cooled (−20°C) resulting dark red solution, (bis)protected compound **5** (1.7 g; 3.9 mmol) dissolved in CH_2Cl_2 (10 mL) was added and the reaction mixture was stirred at −20°C for 2 h. Then, the slurry was filtered through a column of silica gel and the oxidized compound was eluted with a mixture of hexanes:EtOAc:Et_3N (95:5:2) to give 1.1 g (62%) of ketone **6**. The yield however dropped to 12% in a second assay using 6.4 g of compound **5**. All spectroscopic data were in agreement with the literature [18].

*3,17β-bis(2′-tetrahydropyranyloxy)-1,3,5(10)-estratrien-6(α/β)-ol (**7**)*. A solution of ketone **6** (455 mg; 1.0 mmol) in EtOAc (20 mL) was added to pre-reduced platinum dioxide (341 mg; 1.5 mmol) in EtOAc (20 mL) and shaken with hydrogen at atmospheric pressure. After 16 h, the catalyst was carefully removed by filtration on celite and the solution was evaporated to dryness. Purification by flash chromatography (hexanes:EtOAc 85:15) gave 324 mg (71%) of an epimeric mixture of alcohols **7**. White wax; IR (NaCl): 3410 (OH); ^{1}H NMR ($CDCl_3$): 0.79 and 0.81 (2s, 3H, 18-CH_3), 3.4–4.0 (m, 5H, CH_2O of THPs and 17α-CH), 4.65 (m, 1H, CHO of 17-THP), 4.80 (m, 1H, 6-CH), 5.43 (m, 1H, OCHO of 3-THP), 6.93 (d_{app}, J = 8.7 Hz, 1H, 2-CH), 7.17 (dd, J_1 = 8.7 Hz and J_2 = 4.3 Hz, 1H, 1-CH), 7.26 (s_{app}, 1H, 4-CH); ^{13}C NMR ($CDCl_3$): 11.69 (C-18), 18.75, 19.32, 19.85, 23.00, 23.12, 25.24, 25.5, 25.62, 26.23, 27.17, 28.73, 30.36, 31.08, 37.07, 37.62, 37.88, 38.07, 38.17, 42.75, 43.25,



Scheme 1. *Reagents and conditions*: (a) Dihydropyran, *p*-TSA (cat.), toluene, 25°C (82%); (b) CrO_3, CH_2Cl_2, 3,5-dimethylpyrazole, −20°C (62%); (c) H_2, PtO_2, EtOAc, 25°C (71%); (d) NaH, **9**, 80°C (89%); (e) *p*-TSA (cat.), MeOH, 25°C (82%); (f) HPLC separation; (g) *i*-BuOCOCl, NBu_3 then HNBuMe; (h) $SC(NH_2)_2$, EtOH, reflux, then NaOH.

44.34, 44.47, 49.38, 49.50, 61.79 and 62.68 (CH_2O of THP), 62.00 (CH_2O of THP), 69.90 (C-6), 84.02 and 86.49 (C-17), 96.19 and 96.47 (CHO of THP), 96.57 and 99.38 (CHO of THP), 114.79 and 114.98 (C-2), 115.69 and 115.75 (C-4), 126.26 (C-1), 133.37 (C-10), 140.81 (C-5), 155.42 (C-3); LRMS: calcd for $C_{29}H_{41}O_7$ $[M + HCO_2]^-$ 501.3, found 501.6 m/z.

N-butyl, N-methyl 7-(3′,17′β-dihydroxy-1′,3′,5′(10′)-estratrien-6′α/β-yl)-7-oxoheptanamide ***(2)***. To a mixture of alcohols **7** (133 mg; 0.29 mmol) and bromide **9** [19] (230 mg; 0.87 mmol) was added NaH 60% suspension in mineral oil (93 mg; 2.3 mmol) and the resulting slurry was heated at 80°C for 4 h. The reaction mixture was then cooled at room temperature and EtOAc was added followed by water. The crude compound was extracted three times with EtOAc and the combined organic layers were dried over $MgSO_4$, and evaporated to dryness. Purification by flash chromatography (hexanes:EtOAc 8:2) gave 165 mg (89%) of the epimeric mixture of ethers **8**. This compound (95 mg; 0.15 mmol) was then suspended in MeOH (5 mL) with a catalytic amount of *p*-toluenesulfonic acid (14 mg; 0.07 mmol) and the mixture was stirred at 25°C for 30 min. Then, water was added followed by a saturated $NaHCO_3$ solution and the crude compound was extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. Purification by flash chromatography (hexanes:EtOAc 1:1) gave 58 mg (82%) of the epimeric mixture of ethers **2**. The separation of isomers **2a** and **2b** from the epimeric mixture **2** was carried out by HPLC with a Guard-Pak cartridge (Nova-Pak HR C18, 40 × 10 mm) and a Prep-Pak cartridge (Nova-Pak HR C18, 40 × 100 mm). Compounds were eluted from a gradient using CH_3CN:H_2O:MeOH (25:50:25) at a flow rate of 15 mL/min.

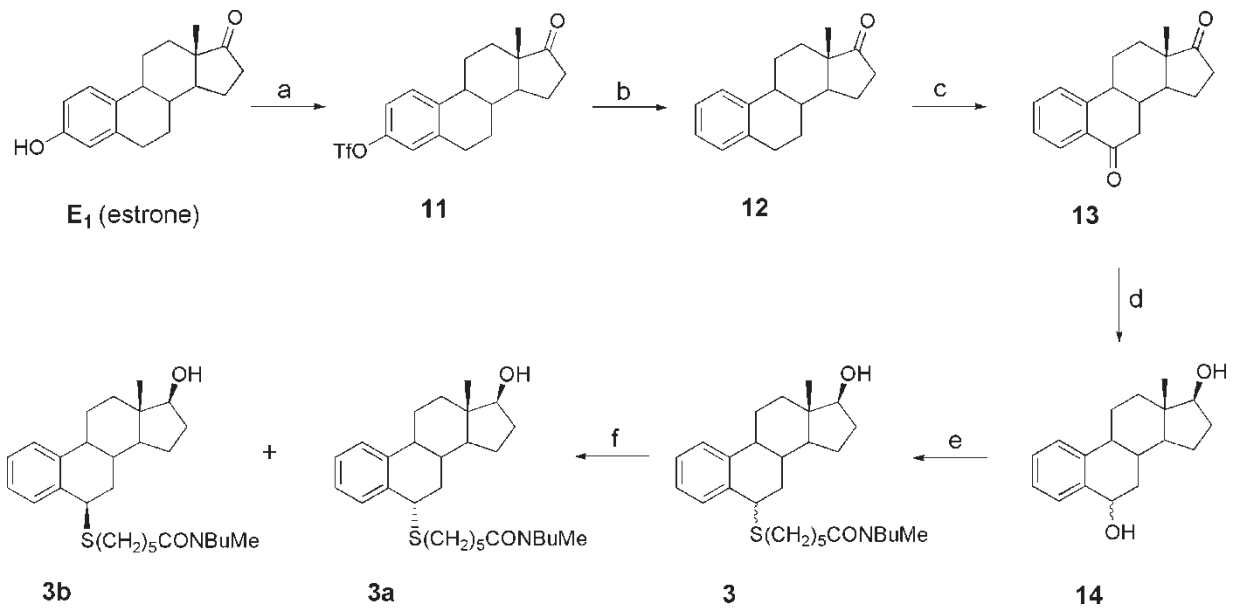
2b*(6β-major isomer)*. Amorphous white solid; IR (KBr): 3315 (OH, phenol and alcohol), 1623 (C=O, amide); ^{1}H NMR (acetone-d_6): 0.77 (s, 3H, 18′-CH_3), 0.90 and 0.94 (2t, J = 7.3 Hz, 3H, CH_3 of butyl), 2.34 (2t, J ~ 7 Hz, 2H, CH_2CO), 2.87 and 3.02 (2s, 3H, NCH_3), 3.34 (t, J = 7.4 Hz, 2H, NCH_2), 3.53 (m, 1H of CH_2O), 3.65 (m, 2H, 1H of CH_2O and 17′α-CH), 4.27 (dd, J_1 = 3.5 Hz and J_2 = 1.9 Hz, 1H, 6′-CHO), 6.68 (dd, J_1 = 8.5 Hz and J_2 = 2.6 Hz, 1H, 2′-CH), 6.91 (d, J = 2.5 Hz, 1H, 4′-CH), 7.13 (d, J = 8.5 Hz, 1H, 1′-CH), 8.37 (d, J = 5.2 Hz, OH); ^{13}C NMR (acetone-d_6): 11.64 (C-18′), 14.14 (CH_3 of butyl), 20.56, 20.65, 23.80, 25.55, 25.82, 26.97, 27.03, ~ 30.5 (under solvent peaks), 31.37, 32.87, 33.22, 33.43, 33.85, 35.52, 34.62, 37.70, 44.20, 44.86, 47.85, 50.17, 50.58, 68.99 (OCH_2), 75.39 (C-6′), 81.81 (C-17′), 115.62 (C-2′), 117.64 and 117.74 (C-4′), 126.54 (C-1′), 132.32 (C-10′), 138.86 (C-5′), 156.21 (C-3′), 172.99 (C=O); LRMS: calcd for $C_{29}H_{46}NO_4$ $[M + H]^+$ 472.3, found 472.6 m/z; HPLC purity: 98% (RT = 13.1 min, Nova-Pak C18, 3.9 × 150 mm, CH_3CN:H_2O:MeOH (40:55:5) at 1 mL/min flow rate).

2a*(6α-minor isomer)*. Amorphous white solid; IR (KBr): 3300 (OH, phenol and alcohol), 1625 (C=O, amide); ^{1}H NMR (CD_3OD): 0.76 (s, 3H, 18′-CH_3), 0.92 and 0.96 (2t, J = 7.3 Hz, 3H, CH_3 of butyl), 2.39 (2t, J ~ 7 Hz, 2H, CH_2CO), 2.89 and 3.02 (2s, 3H, NCH_3), 3.35 (t, J = 7.2 Hz, 2H, NCH_2), 3.50 (m, 1H of CH_2O), 3.65 (m, 2H, 1H of CH_2O and 17′α-CH), 4.53 (dd, J_1 = 6.5 Hz and J_2 = 9.7 Hz, 1H, 6′-CHO), 6.62 (dd, J_1 = 8.5 Hz and J_2 = 2.7 Hz, 1H, 2′-CH), 6.91 (d, J = 2.7 Hz, 1H, 4′-CH), 7.09 (d, J = 8.5 Hz, 1H, 1′-CH); ^{13}C NMR (CD_3OD): 11.61 (C-18′), 14.17 (CH_3 of butyl), 20.91, 21.04, 24.00, 26.13, 26.48, 27.17, 27.44, 30.37, 30.65, 30.95, 31.64, 33.73, 33.88, 34.32, 36.03, 34.66, 37.89, 39.42, 44.33, 45.26, ~ 49.0 (under solvent peaks), 51.05, 68.89 (OCH_2), 78.45 (C-6′), 82.38 (C-17′), 115.13 (C-2′), 115.44 (C-4′), 127.05 (C-1′), 133.02 (C-10′), 139.73 (C-5′), 156.41 (C-3′), 175.45 (C=O); LRMS: calcd for $C_{29}H_{46}NO_4$ $[M + H]^+$ 472.3, found 472.0 m/z; HPLC purity: 92% (RT = 14.0 min, Nova-Pak C18, 3.9 × 150 mm, CH_3CN:H_2O:MeOH (40:55:5) at 1 mL/min flow rate).

Synthesis of N-butyl, N-methyl 7-(17′β-hydroxy-1′,3′,5′(10′)-estratrien-6′α-yl)-7-thiaheptanamide ***(3a)*** *and N-butyl, N-methyl 7-(17′β-hydroxy-1′,3′,5′(10′)-estratrien-6′β-yl)-7-thiaheptanamide* ***(3b)*** *(Scheme 2)*

3-(trifluoromethylsulfonyloxy)-1,3,5(10)-estratrien-17-one ***(11)***. This compound was synthesized as previously described and spectroscopic data were in agreement with the literature [20].

1,3,5(10)-estratrien-17-one ***(12)***. To a stirred solution of compound **11** (5.2 g; 12.9 mmol) in dry DMF (125 mL) under argon at 25°C were sequentially added Et_3N (7.2 mL; 51.7 mmol), formic acid (2.0 mL; 51.7 mmol), PPh_3 (678 mg; 2.6 mmol), and $Pd(OAc)_2$ (145 mg; 0.65 mmol). The reaction mixture was raised to 40°C. After 5 h, PPh_3 (300 mg; 1.2 mmol) and $Pd(OAc)_2$ (75 mg; 0.34 mmol) were added to the reaction mixture and the stirring was prolonged to 10 h. Then, the mixture was diluted with CH_2Cl_2 and brought to neutral pH with sequential washes of 5% aqueous HCl and water. The organic layers were dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography (hexanes:EtOAc 9:1) to give 2.8 g (85%) of the 3-desoxy-estrone (**12**). White solid; IR (NaCl): 1740 (C=O, ketone); ^{1}H NMR ($CDCl_3$): 0.92 (s, 3H, 18-CH_3), 2.94 (m, 2H, 6-CH_2), 7.16 (m, 3H, CH-aromatic), 7.31 (d, J = 6.5 Hz, 1H, CH-aromatic); ^{13}C NMR ($CDCl_3$): 13.82 (C-18),



Scheme 2. *Reagents and conditions*: (a) Tf_2O, pyridine, 0°C; (b) HCOOH, Et_3N, $Pd(OAc)_2$, PPh_3, DMF, 25°C (85%); (c) CrO_3, CH_2Cl_2, 3,5-dimethylpyrazole, −20°C (51%); (d) $LiAlH_4$, THF, −78°C (97%); (e) ZnI_2, **10**, $ClCH_2CH_2Cl$, 25°C (78%); (f) HPLC separation.

21.56 (C-15), 25.66 (C-11), 26.47 (C-7), 29.34 (C-6), 31.60 (C-12), 35.82 (C-16), 38.09 (C-8), 44.46 (C-9), 47.94 (C-13), 50.55 (C-14), 125.28 (C-1), 125.74 and 125.79 (C-2 and C-3), 129.03 (C-4), 136.43 (C-5), 139.69 (C-10), 220.76 (C-17); LRMS: calcd for $C_{18}H_{23}O$ $[M+H]^+$ 255.2, found 255.2 m/z; HPLC purity: 90% (RT = 20.5 min, Nova-Pak C18, 3.9 × 150 mm, CH_3CN:H_2O:MeOH (45:45:10) at 1 mL/min flow rate).

*1,3,5(10)-estratrien-6,17-dione (**13**)*. To a suspension of chromic anhydride (2.4 g; 24 mmol) in dry CH_2Cl_2 (45 mL) under argon was added 3,5-dimethylpyrazole (2.3 g; 24 mmol) and the resulting mixture was stirred at 25°C for 15 min. To the cooled (−20°C) resulting dark red solution, compound **12** (609 mg; 2.4 mmol) dissolved in CH_2Cl_2 (10 mL) was added and the reaction mixture was stirred at −20°C for 2 h. Then, the slurry was filtered through a short column of silica gel and the compound was eluted with CH_2Cl_2. The filtrate was concentrated and purified by flash chromatography (hexanes:EtOAc 9:1) to give 30 mg of the starting material **12** (18%) and 327 mg (51%) of the oxidized compound **13**. White solid; IR (NaCl): 1735 (C=O, ketone) and 1680 (C=O, conjugated ketone); ^{1}H NMR ($CDCl_3$): 0.92 (s, 3H, 18-CH_3), 2.88 (dd, J_1 = 16.6 Hz and J_2 = 3.3 Hz, 1H, 7β-CH), 7.35 (t_{app}, J = 7.4 Hz, 1H, 3-CH), 7.43 (d, J = 7.8 Hz, 1H, 1-CH), 7.56 (t_{app}, J = 7.1 Hz, 1H, 2-CH), 8.08 (d, J = 7.1 Hz, 1H, 4-CH); ^{13}C NMR ($CDCl_3$): 13.61 (C-18), 21.32 (C-15), 24.91 (C-11), 31.18 (C-12), 35.61 (C-16), 39.23 (C-8), 43.22 (C-7), 43.36 (C-9), 47.56 (C-13), 50.39 (C-14), 125.19 (C-1), 126.72 (C-3), 127.41 (C-4), 132.32 (C-5), 133.88 (C-2), 146.19 (C-10), 197.26 (C-6), 219.54 (C-17); LRMS: calcd for $C_{18}H_{21}O_2$ $[M+H]^+$ 269.2, found 269.2 m/z; HPLC purity: 99% (RT = 5.3 min, Nova-Pak C18, 3.9 × 150 mm, CH_3CN:H_2O:MeOH (45:45:10) at 1 mL/min flow rate).

*1,3,5(10)-estratrien-6(α/β)-17β-diol (**14**)*. To a cooled solution of dione **13** (148 mg; 0.55 mmol) in dry THF (10 mL) at −78°C was added a 1.0 M solution of $LiAlH_4$ in THF (1.4 mL; 1.4 mmol) and the reaction was stirred for 1 h at −78°C. Then, acetone was added followed by sodium sulfate decahydrate. The resulting slurry was allowed to stir overnight, filtered through celite and the solvent was evaporated under reduced pressure. Purification by flash chromatography (hexanes:EtOAc 7:3) gave 146 mg (97%) of alcohols **14**. White solid; IR (KBr): 3320 (OH, alcohols); ^{1}H NMR (CD_3OD) (only major isomer reported): 0.77 (s, 3H, 18-CH_3), 3.66 (t, J = 8.6 Hz, 1H, 17α-CH), 4.78 (dd, J_1 = 10.3 Hz and J_2 = 6.7 Hz, 1H, 6-CH), 7.16 (m, 2H, 2-CH and 3-CH), 7.26 (m, 1H, 1-CH), 7.51 (m, 1H, 4-CH); ^{13}C NMR (CD_3OD) (only major isomer reported): 11.66 (C-18), 24.01 (C-15), 27.32 (C-11), 30.68 (C-16), 37.99 (C-12), 38.77 (C-7), 39.54 (C-8), 44.25 (C-13), 46.23 (C-9), 50.88 (C-14), 70.36 (C-6), 82.38 (C-17), 126.03 (C-1), 126.95 (C-2), 128.09 (C-3), 128.51 (C-4), 141.00 (C-5), 141.30 (C-10); LRMS: calcd for $C_{18}H_{25}O_2$ $[M+H]^+$ 273.2, found 273.2 m/z; HPLC purity: 85% (only major isomer) (RT = 2.5 min, Nova-Pak C18, 3.9 × 150 mm, CH_3CN:H_2O:MeOH (45:45:10) at 1 mL/min flow rate).

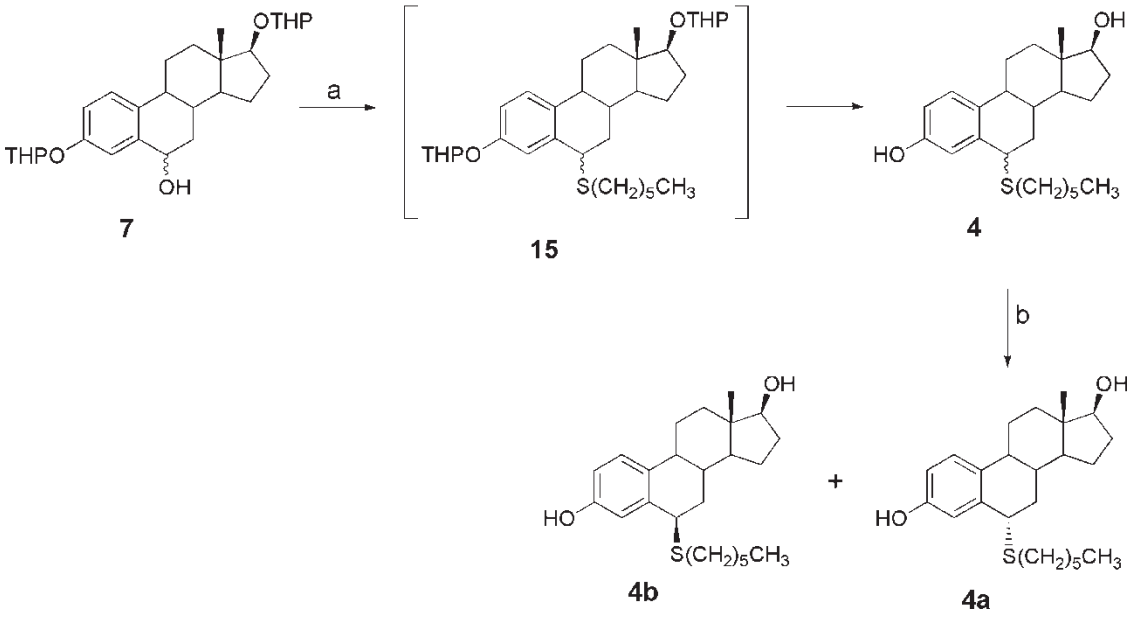
*N-butyl, N-methyl 7-(17′β-hydroxy-1′,3′,5′(10′)-estratrien-6′α/β-yl)-7-thiaheptanamide (**3**)*. To a solution of the diol **14** (140 mg; 0.51 mmol) and thiol **10** [19] (223 mg; 1.02 mmol) in dry 1,2-dichloroethane (10 mL) was added zinc iodide (328 mg; 1.02 mmol) and the mixture was stirred at 25°C for 3 h. The reaction was quenched with water, and the reaction mixture extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over $MgSO_4$, and solvent evaporated under reduced pressure. The residue was purified by chromatography (hexanes:EtOAc 1:1) to give 188 mg (78%) of thioethers **3**. The separation of isomers **3a** and **3b** from the epimeric mixture **3** was carried out by HPLC with a Guard-Pak cartridge (Nova-Pak HR C18, 40 × 10 mm) and a Prep-Pak cartridge (Nova-Pak HR C18, 40 × 100 mm). Compounds were eluted using CH_3CN:H_2O:MeOH (50:40:10) at 15 mL/min flow rate.

***3b** (6β-isomer)*. White amorphous solid; IR (NaCl): 3400 (OH, alcohol) and 1632 (C=O, amide); ^{1}H NMR ($CDCl_3$): 0.84 (s, 3H, 18′-CH_3), 0.94 (t, J = 7.2 Hz, 3H, CH_3 of butyl), 2.35 (t, J = 7.3 Hz, 2H, CH_2CO), 2.60 (2m, 2H, CH_2S), 2.95 (s, 3H, NCH_3), 3.30 (m, 2H, NCH_2), 3.75 (t, J = 8.3 Hz, 1H, 17′α-CH), 4.16 (d_{app}, J = 3.4 Hz, 1H, 6′-CHS), 7.16 (m, 2H, 2′-CH and 3′-CH), 7.26 (m, 1H, 1′-CH), 7.33 (m, 1H, 4′-CH); ^{13}C NMR ($CDCl_3$): 11.21 (C-18′), 13.83 (CH_3 of butyl), 20.01, 23.01, 24.98, 25.87, 28.83, 29.54, 30.59, 32.36 (SCH_2), 32.89, 33.20, 36.75, 38.77, 39.52, 43.52, 44.46, 44.84 (C-6′), 49.50, 81.82 (C-17′), 125.40 (C-1′), 125.71 (C-2′), 126.99 (C-3′), 130.85 (C-4′), 136.31 (C-5′), 140.22 (C-10′), 173.00 (C = O); LRMS: calcd for $C_{29}H_{46}NO_2S$ $[M + H]^+$ 472.3, found 472.5 m/z; HPLC purity: 96% (RT = 15.3 min, Nova-Pak C18, 3.9 × 150 mm, CH_3CN:H_2O:MeOH (50:40:10) at 1 mL/min flow rate).

***3a** (6α-isomer)*. White amorphous solid; IR (NaCl): 3400 (OH, alcohol) and 1630 (C=O, amide); ^{1}H NMR ($CDCl_3$): 0.75 (s, 3H, 18′-CH_3), 0.94 (t, J = 7.3 Hz, 3H, CH_3 of butyl), 2.95 (s, 3H, NCH_3), 3.31 (m, 2H, NCH_2), 3.74 (t, J = 8.5 Hz, 1H, 17′α-CH), 4.12 (t_{app}, J = 8.6 Hz, 6′-CHS), 7.17 (m, 2H, 2′-CH and 3′-CH), 7.27 (m, 1H, CH), 7.62 (m, 1H, 4′-CH); ^{13}C NMR ($CDCl_3$): 10.93 (C-18′), 13.85 (CH_3 of butyl), 19.99, 23.10, 25.02, 25.80, 28.85, 29.22, 29.50 (SCH_2), 30.50, 32.66, 36.46, 39.02, 43.07, 43.37, 43.60 (C-6′), 50.19, 81.71 (C-17′), 124.94 (C-1′), 125.90 (C-2′), 126.61 (C-3′), 129.29 (C-4′), 136.74 (C-5′), 141.63 (C-10′), 173.47 (C=O); LRMS: calcd for $C_{29}H_{46}NO_2S$ $[M + H]^+$ 472.3, found 472.4 m/z; HPLC purity: 93% (RT = 17.5 min, Nova-Pak C18, 3.9 × 150 mm, CH_3CN:H_2O:MeOH (50:40:10) at 1 mL/min flow rate).

*Synthesis of 7-(3′,17′β-dihydroxy-1′,3′,5′(10′)-estratrien-6′α-yl)-7-thiaheptane (**4a**) and 7-(3′,17′β-dihydroxy-1′,3′,5′(10′)-estratrien-6′β-yl)-7-thiaheptane (**4b**) (Scheme 3)*

*7-(3′,17′β-Dihydroxy-1′,3′,5′(10′)-estratrien-6′α/β-yl)-7-thiaheptane (**4**)*. To a solution of alcohol **7** (100 mg; 0.22 mmol) and hexanethiol (48 μL; 0.33 mmol) in dry 1,2-dichloroethane (10 mL) was added zinc iodide (210 mg; 0.66 mmol) and the mixture was stirred at 25°C for 4 h. The reaction was quenched with water, and the reaction mixture extracted with CH_2Cl_2. The combined organic



Scheme 3. *Reagents and conditions*: (a) ZnI_2, $HS(CH_2)_5CH_3$, $ClCH_2CH_2Cl$, 25°C (97%); (b) HPLC separation.

extracts were washed with brine and dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc 8:2) to give 83 mg (97%) of thioethers **4**. The separation of isomers **4a** and **4b** from the epimeric mixture **4** was carried out by HPLC with a Guard-Pak cartridge (Nova-Pak HR C18 (40 × 10 mm) and a Prep-Pak cartridge (Nova-Pak HR C18, 40 × 100 mm) using CH_3CN:H_2O:MeOH (45:45:10) at a flow rate of 15 mL/min.

4b *(6β-isomer)*. White amorphous solid; IR (KBr): 3340 (OH, phenol and alcohol); ^{1}H NMR ($CDCl_3$): 0.84 (s, 3H, 18′-CH_3), 0.90 (t, J = 6.5 Hz, 3H, CH_3 of hexyl), 3.75 (t, J = 8.2 Hz, 1H, 17′α-CH), 4.09 (d_{app}, J = 3.8 Hz, 1H, 6′-CHS), 6.68 (dd, J_1 = 8.6 Hz and J_2 = 2.6 Hz, 1H, 2′-CH), 6.83 (d, J = 2.6 Hz, 1H, 4′-CH), 7.16 (d, J = 8.5 Hz, 1H, 1′-CH); ^{13}C NMR ($CDCl_3$): 11.22 (C-18′), 14.05 (CH_3 of hexyl), 22.57, 23.01, 26.13, 28.67, 29.70, 30.61, 31.47, 32.51 (SCH_2), 32.93, 33.46, 36.72, 43.54, 44.33 (C-6′), 49.39, 81.87 (C-17′), 114.43 (C-2′), 116.89 (C-4′), 126.68 (C-1′), 132.71 (C-10′), 137.81 (C-5′), 153.27 (C-3′); LRMS: calcd for $C_{25}H_{37}O_4S$ $[M + HCO_2]^-$ 433.2, found 433.0 m/z; HPLC purity: 98% (RT = 10.7 min, Nova-Pak C18, 3.9 × 150 mm, CH_3CN:H_2O:MeOH (50:40:10) at 1 mL/min flow rate).

4a *(6α-isomer)*. White amorphous solid; IR (KBr): 3340 (OH, phenol and alcohol); ^{1}H NMR ($CDCl_3$): 0.76 (s, 3H, 18′-CH_3), 0.87 (t, J = 6.5 Hz, 3H, CH_3 of hexyl), 3.73 (t, J = 8.3 Hz, 1H, 17′α-CH), 4.04 (t_{app}, J = 8.6 Hz, 1H, 6′-CHS), 4.08 (br, 1H, OH), 6.67 (dd, J_1 = 8.6 Hz and J_2 = 2.6 Hz, 1H, 2′-CH), 7.14 (d, J = 8.6 Hz, 1H, 1′-CH), 7.17 (d, J = 2.5 Hz, 1H, 4′-CH); ^{13}C NMR ($CDCl_3$): 10.96 (C-18′), 14.01 (CH_3 of hexyl), 22.50, 23.06, 26.11, 28.75, 29.41, 29.56 (SCH_2), 30.52, 31.43, 36.44, 36.56, 39.27, 43.12, 43.72 (C-6′), 49.94, 81.76 (C-17′), 113.74 (C-2′), 115.63 (C-4′), 126.27 (C-1′), 133.98 (C-10′), 138.34 (C-5′), 153.62 (C-3′); LRMS: calcd for $C_{24}H_{35}O_2S$ $[M - H]^-$ 387.2, found 387.4 m/z; HPLC purity: 98% (RT = 12.5 min, Nova-Pak C18, 3.9 × 150 mm, CH_3CN:H_2O:MeOH (50:40:10) at 1 mL/min flow rate).

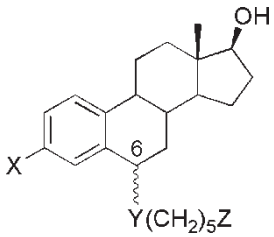
Inhibition of type 1 17β-HSD (Table I)

The enzymatic assay with a crude preparation of type 1 17β-HSD was performed as previously described [21]. Briefly, human embryonic kidney (HEK)-293 cells (American Type Culture Collection, Rockville, MD, USA) transfected with cDNA encoding for the enzyme [22] were sonicated to free the enzyme that was used as the enzymatic pool without further purification. The enzymatic assay was performed as follows: a stock solution was first prepared containing the radiolabeled substrate [^{14}C]-E_1 (0.1 μM), NADH (1 mM) in a phosphate buffer (pH 7.4, 50 mM KH_2PO_4, ethylenediaminetetraacetic acid (EDTA) 1 mM, 20% glycerol). For the assay, 890 μL of the stock solution and 10 μL of a solution of inhibitor (EtOH) were added in a tube. The reaction was started by adding 100 μL of a solution of crude enzyme prepared as indicated above. The mixture was incubated for 1 h at 37°C, and the reaction was stopped by adding an excess of unlabeled E_1 and E_2. Steroids were extracted with diethyl ether and solvent was removed under vacuum. The residue was dissolved in CH_2Cl_2, spotted on a silica gel plate (TLC, 20 × 20 × 0.2 cm, Kieselgel 60 F_2) and eluted with CH_2Cl_2:EtOAc (9:1). Less polar E_1 and more polar E_2 were identified on TLC as two rows of spots visible under UV light. Radioactivity signals associated to [^{14}C]E_1 and [^{14}C]E_2 were detected and quantified using a Phosphor Imager (Sunny Vale, CA, USA). The percent of transformation of [^{14}C]E_1 into [^{14}C]E_2 was calculated as follows: % trans. = 100 × {[^{14}C]E_2 cpm)/([^{14}C]E_1 (cpm) + [^{14}C]E_2 (cpm)}. Consequently, percent of inhibition = [(% trans. of control − % trans. of compound)/(% trans. of control)] × 100. When several concentrations of an inhibitor were used in the enzymatic assay, an inhibition curve was plotted using the percentage of transformation versus the concentration of inhibitor. From this inhibition curve, the IC_{50} value was calculated by a computer using an unweighted iterative least-squares method for 4-parameters logistic curve fitting (DE_{50} program, CHUL Research Center, Québec, Canada).

Proliferative/antiproliferative cell assays (Figure 2)

The procedure for this assay has been previously described [23] The T-47D human breast cancer cells were obtained from American Type Culture Collection (HTB 133, Rockville, MD, USA) at passage 86 and routinely grown in phenol-free RPMI-1640 medium supplemented with 1 nM E_2, 2 mM L-glutamine, 1 mM sodium pyruvate, 15 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), 100 IU penicillin/mL, 50 μg streptomycin sulfate/mL and 10% fetal bovine serum (FBS). Cells in their late logarithmic growth phase were harvested with 0.1% pancreatin and resuspended in the appropriate medium containing 50 ng bovine insulin/mL and 5% FBS treated twice with dextran-coated charcoal to remove endogenous steroids. Cells were plated in 24-well Falcon plastic culture plates at an initial density of 7.5 × 10^3/2 cm^2/well and allowed to adhere to the surface of the plates for 72 h. Thereafter, medium was replaced with fresh medium containing the indicated concentrations of compounds diluted from a 1 μM stock solution in 99% redistilled ethanol in the presence or absence of E_2. Control cells received only the ethanolic vehicle. Cells were incubated for 10 days with medium changes at 2- or 3-day intervals. Cell number was

Table I. [1]H NMR chemical shift (δ) and coupling constant (J) observed for the C6-proton of compounds **1**–**4** and their inhibitory effects on type 1 17β-HSD.



No. (C6-α or β side chain)	X	Y	Z	NMR data for C6-H δ (ppm)	NMR data for C6-H J (Hz)	Inhibition of type 1 17β-HSD[a] at 0.1 μM (%)	Inhibition of type 1 17β-HSD[a] at 1.0 μM (%)	Inhibition of type 1 17β-HSD[a] IC_{50} (μM)
1 (β)	OH	S	CONBuMe	4.11 (dd)	5 and 6	69	92	0.16 ± 0.05
1 (α)[b]	OH	S	CONBuMe	4.05 (dd)	7 and 11	—	—	—
2b (β)	OH	O	CONBuMe	4.27 (dd)	1.9 and 3.5	31	73	0.8 ± 0.1
2a (α)	OH	O	CONBuMe	4.53 (dd)	6.5 and 9.7	NI[c]	NI	NI
3b (β)	H	S	CONBuMe	4.16 (d_{app})	3.4	14	38	>1.0
3a (α)	H	S	CONBuMe	4.12 (t_{app})	8.0	NI	NI	NI
4b (β)	OH	S	CH_3	4.09 (d_{app})	3.8	12	30	>10
4a (α)	OH	S	CH_3	4.04 (t_{app})	8.6	NI	NI	NI
$\mathbf{E_1}$	—	—	—	—	—	71	92	0.09 ± 0.01
$\mathbf{E_2}$	—	—	—	—	—	—	—	>10

[a]For the reduction of [^{14}C]E_1 (0.1 μM) into [^{14}C]E_2 using NADH as cofactor. [b]NMR data from reference 12. [c]NI: no enzyme inhibition observed.

determined by measurement of DNA content as previously described [24].

Results

Chemical synthesis

The reported thioether derivatives, exemplified by **1**, were synthesized from 6-hydroxy-E_2 [13]. Consequently, *O*-alkylation of **7** benzylic alcohols should deliver the desired ether analogues **2a** and **2b** after THP hydrolysis **7** (Scheme 1). The choice of reagents to affect these changes warrants some comment. Direct hydroxylation of the C6-benzylic position has been recently achieved by exploiting selective metalation using a superbase produced from lithium diisopropylamide and potassium 1,1-dimethylpropoxide (LIDAKOR) [18,25]. However, this method is reported to yield mainly the 6α-OH epimer over the 6β-OH epimer (ratio 9:1). The latter epimer being of greater interest for the inhibition of type 1 17β-HSD, an alternative two step procedure was adopted. The C6-benzylic oxidation [26,27] followed by platinum-catalyzed hydrogenation of the resulting ketone ultimately gave the best results. Although the use of pyridinium chlorochromate adsorbed in celite was reported to oxidize efficiently the E_2-diacetate to the corresponding C6-ketone [28], this former substrate was not optimal for the designed sequence of transformations requiring protecting groups shuffling. Therefore, a chromic anhydride-3,5-dimethylpyrazole complex was used to give ketone **6** from bis-tetrahydropyranyl-E_2 (**5**). Metal hydride reduction of ketone **6** has been already demonstrated to give solely the 6α-OH compound, while catalytic hydrogenation using platinum oxide usually produces a slight excess of the 6β-OH epimer [29–31]. Ketone **6** was submitted to these reductive conditions to give the mixture of epimeric alcohols **7**. The *O*-alkylation of this mixture of alcohols using $Br(CH_2)_5CONBuMe$ (**9**) [19] required optimization. It was found that this alkylation did not proceed efficiently when diluted sodium alkoxide solutions were prepared in THF or DMF (25°C or 80°C) by standard procedures. A suspension of the alcohols **7**, bromide **9** and NaH was then gently heated neat at 80°C to produce the expected ethers **8** in good yield. Subsequent deprotection by cleavage of THP groups and HPLC separation gave the pure target ethers **2a** and **2b**.

For the synthesis of **3a** and **3b**, the phenol of E_1 was converted into the corresponding triflate **11** [20], which was used for the palladium-mediated reduction [32] with triethylammonium formate as the hydride source to give the 3-deoxy-E_1 (**12**) (Scheme 2). The C6-benzylic position of **12** was oxidized similarly as described above to give the diketone **13**. The reduction of both carbonyl groups of **13** was performed simultaneously with $LiAlH_4$, at −78°C to

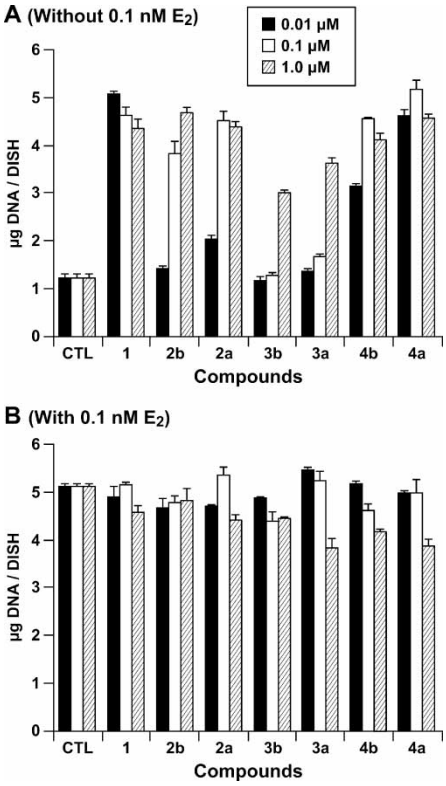


Figure 2. Effect of three concentrations of the newly synthesized inhibitors **2**–**4** on the basal (**A**) and E_2 (0.1 nM)-induced (**B**) growth of T-47D cells in culture. Results are expressed as mean ± SEM of triplicates.

give a mixture of C6-epimeric alcohols **14**. The stereoselectivity at position 17 (only 17β-OH) was ensured by the reduction of the corresponding ketone at low temperature and the presence of the β-oriented C18-methyl. Unlike the control of the stereochemistry at C17, the C6 center stereochemistry was unconsequential. Indeed, the subsequent synthesis of thioethers **3** using zinc iodide and the mercaptan side-chain **10**, obtained from **9** (Scheme 1) [19], is known to proceed regioselectively on allylic or benzylic alcohol by a S_Nl type mechanism [33] generating two isomers (6α and 6β). Separation of the epimers was achieved by HPLC to give pure **3a** and **3b**.

Finally, compounds **4a** and **4b** were synthesized to evaluate the importance of the amide group as the pharmacophore responsible for a specific interaction with the enzyme. The third approach consisted in simply replacing the tertiary amide by a methyl group. The intermediate **7** was submitted to zinc-mediated displacement using hexanethiol, giving directly thioethers **4** (Scheme 3). Indeed, the THP groups were removed under the reaction conditions used for thioether formation. Separation of the epimers by HPLC produced **4a** and **4b**.

The exact configuration at position C6 of compounds **2**–**4** was established by ^{1}H NMR spectroscopy after a carefully performed HPLC separation of each mixture of epimers (Table I). The 6α-proton signal of the 6β-epimer was observed as a sharp doublet ($J \sim 2-4$ Hz) because of its pseudo-equatorial relationship with the C-7 protons. On the other hand, the corresponding resonance in the 6α-epimer appeared as a triplet ($J \sim 7-10$ Hz) because of the pseudo-axial position of the 6β-proton with the C-7 protons [12,31].

Biological activity

The inhibition of the transformation of [^{14}C]E_1 into [^{14}C]E_2 by the newly synthesized compounds **2**–**4** was tested with an homogenate of HEK-293 cells transfected with 17β-HSDl-cDNA (Table I). Three points can be highlighted from these results. First, there is a strong dependence of the inhibitory potency on the orientation of the side-chain. In all series, the 6β-configured compounds **2b**, **3b** and **4b** showed inhibitory effects on type 1 17β-HSD, but the 6α-configured compounds **2a**, **3a** and **4a** did not decrease the reduction of E_1 into E_2. Second, removing either the 3-OH (compound **3b**) or the amide group (compound **4b**) from the initial lead structure (compound **1**) significantly decreased (10 to 100-fold) the inhibitory potency of the corresponding compounds. Third, changing the thioether bond (compound **1**) for an ether (compound **2b**) also decreased the effectiveness of the inhibitor, but the effect was quite less drastic (5-fold) than the other two modifications.

Afterwards, the effect of three concentrations of inhibitors **2**–**4** (0.01, 0.1 and 1.0 μM) was investigated on basal and E_2-induced cell proliferation in estrogen-sensitive breast tumor T-47D cells (Figure 2). Cells were incubated with inhibitors for 10 days, in the absence or presence of 0.1 nM E_2, to obtain the optimal amplitude of the mitogenic effect, which was determined by measurement of DNA content. In the first experiment (Figure 2A), compounds were incubated in the absence of E_2 to evaluate their intrinsic estrogenicity. The lead compound **1** displayed strong estrogen-like effects even at the lowest concentration (0.01 μM). The same proliferative effect was observed for compounds that do not contain the amide moiety (**4a** and **4b**). On the other hand, compounds without hydroxyl group at position 3 (**3a** and **3b**) displayed estrogenicity only at a concentration of 1.0 μM. In contrast with the strong estrogenic effect observed at 0.01 μM for compound **1**, no significant estrogen-like effect was observed for compound **2b** at 0.01 μM. Despite the fact that compound **2b** did show estrogenicity at higher concentrations (0.1 and 1.0 μM), changing the thioether bond with an ether decreased the estrogenic activity of lead inhibitor **1** approximately 10-fold. In the second experiment (Figure 2B), inhibitors

were incubated in the presence of 0.1 nM E_2 to evaluate their ability to reverse the proliferation induced by E_2. As can be seen, no inhibitor displayed significant antiestrogenic activity.

Discussion

The predominant role of type 1 17β-HSD in the regulation of E_2 concentration in peripheral tissues has led to a growing interest for this particular enzyme as a biological target for drugs designed to treat estrogen-dependent diseases. Inhibition of type 1 17β-HSD should be a valuable complementary approach to antiestrogens for the treatment of breast cancer. However, inhibitors must not display estrogenic activity. Consequently, studies aimed at the determination of important structural features of inhibitors without estrogenicity are worthwhile. The present paper has described three modifications made on the structure of a previously described estrogenic inhibitor of type 1 17β-HSD in order to reduce its intrinsic estrogen-like effect.

Lead compound **1**, namely N-methyl, N-butyl 6β-(thiaheptanamide)-E_2, resulted from a prelimary study performed to determine the optimal thioalkylamide side-chain length at position 6 of E_2. The best alkyl spacer group was then established to be composed of five methylene groups between the sulfur atom and the amide group. Moreover, it was shown that the 6β-epimer was more active than the corresponding 6α-epimer [12]. However, kinetic data did not clearly indicate whether compound **1** was a competitive, non competitive or uncompetitive inhibitor (unpublished results). In addition, the structural requirements for 3-OH, the thioether bond, and the amide have not been investigated so far. Consequently, this kind of inhibitor exemplified by compound **1** must be further characterized to clearly establish the importance of these three chemical groups for the inhibitory potency.

The present paper has confirmed the 6β-configuration of the side-chain as a major structural requirement for inhibition of type 1 17β-HSD by this kind of compound. The amide group (CONBuMe) has also been shown to play an important role. In fact, compounds without the amide group (**4b** and E_2) showed very low inhibitory potency on type 1 17β-HSD. However, further studies are needed to discriminate whether it is the carbonyl or the N-methyl,N-butyl amide that is responsible for the inhibitory activity. The relative importance of the phenol group might suggest that inhibitor **1** as well as **2b** bind to the substrate binding domain. Indeed, X-ray analysis of the crystallized enzyme revealed that the 3-OH of E_1 forms two important hydrogen bonds with His-221 and Glu-282 of the enzyme [34,35]. The absence of such interactions led to the poor inhibitory potency observed for compound **3b**.

Replacing the thioether with an ether bond has provided puzzling information. The inhibitor containing the ether bond at position 6β (compound **2b**) still potently inhibited type 1 17β-HSD, but was 5-fold less active than the lead compound **1** having the thioether bond. We first hypothesized that the strong estrogenic effect displayed by compound **1** on cells in culture could be the result of a cleavage of the thioether bond under conditions of biological evaluation (10-day incubation in intact T-47D cells). However, it was unlikely that this cleavage occurred under conditions used for the enzymatic test (homogenated cells incubated 2 h at pH 7.4). It was indeed confirmed by the dependence of the inhibitory potency on the presence of the thioalkylamide side-chain (**1** vs E_2). Estrogenic effect of compound **2b** at 0.1 μM revealed that the stability of the thioether or ether was not the main issue in determining the estrogenicity of lead compound **1**. The difference in inhibitory potency and estrogenicity of compounds **1** and **2b** might be the result of several factors, which are not related to the thioether or ether bond stability. The thioether bond is longer than the corresponding ether, the latter of which should slightly shorten the entire side-chain. Therefore, the resulting side-chain does not provide the same effect as the previously found optimal side-chain [12]. On the other hand, the ether bond could have introduced a dipole close to the steroid nucleus, creating an unfavorable interaction with the substrate binding site. This hypothesis could also provide an explanation for the decreased estrogenicity displayed by compound **2b** compared to that of compound **1**. The data suggest that 6-alkylamide-E_2 derivatives, in opposition to 7-alkylamide-E_2 [36], bind to the estrogen receptor in a way that does not prevent the signal transduction for proliferative activity.

It is noteworthy that the keto-steroid E_1 is a better substrate for the reductive activity of type 1 17β-HSD than the corresponding hydroxy-steroid E_2. This study described inhibitors with a l7β-hydroxyl group because the lead compound **1**, which was investigated and characterized previously, belongs to this category. It is therefore obvious that, after the SAR study presented in this paper, the next step should be to synthesize the 17-ketone analogue of compound **1**. Improvement should be observed both in increasing inhibitory potency and reducing estrogenic activity. This modification would be even more attractive on these 6-thioalkylamide-E_2 derivatives than on the more characterized 16-alkylamide-E_2 derivatives [37,38] because of the absence of potential stereochemical scrambling on 16-alkyl-17-keto steroids.

In summary, the E_2 nucleus was modified at positions 3 and 6 using convenient synthetic transformations. The effects of these modifications were studied on two biological parameters, namely: inhibition of the reductive activity of type 1 17β-HSD and proliferative/antiproliferative activities on estrogen-sensitive T-47D

cells. Although this study did not result in a significant improvement of the previously reported inhibitor, it has provided interesting data that will be helpful for the design of future inhibitors of type 1 17β-HSD without estrogenic-like effects.

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