The final incubation mixtures were filtered through glass fiber filter mats by using a Skatron cell harvester. Filters were allowed to dry and 3.0 mL of Scintiverse E (Fisher Scientific) was added. After the mixture was shaken for 30 min, the radioactivity concentration was determined by an LKB RackBeta liquid scintillation counter. Data were analyzed by custom computer software, and compared, in selected cases, to that obtained from EDBA-LIGAND. Tissue protein levels were estimated by using the Folin reagent method of Lowry adapted to a Technicon Autoanalyzer I (Tarrytown, NY).

Acknowledgment. This research was supported by USPHS grants nos. NS-22899, MH-40537, MH-37404, training grant no. HL-07367, and the Department of Energy (grant no. DE-AC03-76SF00098). We thank Dr. A. T. Shulgin for helpful synthetic chemistry discussions.

Synthesis and Biological Activity of New Halo-Steroidal Antiestrogens

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Received August 31, 1990

Antiestrogen therapy is the most widely used endocrine manipulation for the treatment of breast cancer, especially in postmenopausal women. Unfortunately, the compounds presently available possess mixed agonistic/antagonistic activity, thus potentially limiting their therapeutic efficacy. Following the observations that an aliphatic chain at the 7α-position of 17β-estradiol does not prevent binding to the estrogen receptor while halogenation of estradiol can increase the affinity of its binding (expressed as RBA) to the estrogen receptor, we have synthesized a series of new steroidal antiestrogens (6–10) which possess both an 7α-undecanamide group and an halogen atom (Cl, Br, or I) at the 16α-position. The stereochemistry of these compounds was unambiguously established by high-field (400-MHz) nuclear magnetic resonance. Some of the compounds obtained possess potent in vivo antiestrogenic activity. At the low twice daily 3-μg dose, 16α-chloro 3,17β-diol amide, 16α-iodo 3,17β-diol amide, 16α-bromo 3,17β-diol amide, 16α-chloro 3,17α-diol amide, and 16α-bromo 3,17α-diol amide inhibit by 74, 63, 52, 35, and 60%, respectively, the estradiol-induced stimulation of uterine weight in ovariectomized Balb/c mice while 78–99% blockade of estradiol action is achieved at the 20-μg dose. These new antiestrogens show no estrogenic activity on uterine weight at the doses used while tamoxifen (2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-*N,N*-dimethylethanamine) shows full estrogenic activity and is only a weak partial antiestrogen in the same assay.

Introduction

Breast cancer is the most frequent cancer in women. In fact, one out of 11 women in North America suffers from breast cancer during her life time while more than 50 000 women die annually from this disease.[1] Unfortunately, the present therapies show positive results in only a proportion of cases and, when present, the positive response is of short duration.[2-4] Since the first evidence for a role of estrogens in breast cancer,[5] considerable attention has been given to the mechanisms involved.[2-4] Based on the well-known observations that the action of estrogens in target tissues requires binding to the estrogen receptor,[6] a logical approach for the treatment of estrogen-sensitive breast cancer is the use of a compound which could block the interaction of estrogens with their specific receptor. In fact, such compounds, called antiestrogens, are presently used with some success for the treatment of breast cancer.[4]

However, antiestrogens devoid of estrogenic activity and thus possessing the characteristics of pure antiestrogens have not yet been made available for clinical use. The nonsteroidal antiestrogen routinely used in the endocrine therapy of breast cancer, namely tamoxifen (2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-*N,N*-dimethylethanamine)[7,8] behaves as a mixed agonist/antagonist of estrogen action, thus limiting its therapeutic potential. Similarly, the benzothiophene derivatives LY117018[9] [6-hydroxy-2-(4-hydroxyphenyl)benzo[*b*]thien-3-yl)][4-[2-(1-pyrrolidinyl)ethoxy]phenyl]methanone and keoxifene (LY156758)[10] ([6-hydroxy-2-(4-hydroxyphenyl)benzo[*b*]thien-3-yl][4-(2-(1-piperidinyl)ethoxy)phenyl]methanone hydrochloride display significant uterotrophic activity in the rat. Recently, based on estradiol derivatives originally developed for purification of the estrogen receptor by affinity chromatography,[11] it has been observed that a series of 7α-alkyl derivatives of 17β-estradiol[12] display significant antagonistic activity in various systems, including the rat uterus and human breast cancer cells.[12,13]

On the other hand, Heiman et al.[14] and Fevig et al.[15] have reported that halogenation of the D ring of the steroid nucleus, especially at the 16α-position, results in compounds having affinities for the lamb and rat uterine estrogen receptors which are higher than 17β-estradiol itself. On the basis of this knowledge, we have synthesized a

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0022-2623/91/1834-1624$02.50/0

series of 7α-undecanamide-substituted 17β-estradiols with different halogens (Cl, Br, I) at C16 which show potent and pure antagonistic activity in the highly sensitive mouse uterine in vivo assay.

Experimental Section

General Procedure. Melting points were determined with a Gallenkamp melting point apparatus and are presented uncorrected. Infrared spectra were obtained on a Perkin-Elmer 1310 spectrophotometer. ^1H nuclear magnetic resonance spectra were obtained at 200 MHz with a VARIAN XL-200 from the Department of Chemistry, Université Laval, Québec, Canada or at 400 MHz (specified in parentheses) from Laboratoire Régional de RMN à Haut Champ, Université de Montréal, Montréal, Canada. Chemical shifts are reported in parts per million ppm units (δ) downfield from tetramethylsilane as internal reference. Mass spectra were obtained with a Micromass 16F spectrometer. Exact mass was provided by the Centre Régional de Spectrométrie de Masse, Département de Chimie, Université de Montréal, Montréal, Canada.

For column chromatography, silica gel (100-fold weight of product) (Merck, Kieselgel 60F254, 0.063–0.200 mm) was used, while for "flash chromatography", silica gel (Merck, Kieselgel, F60, 230 mesh ASTM) was used. For purification, high-performance liquid chromatography (HPLC) of each compound was performed on a Waters Model 510 liquid chromatograph with UV detector and with a 10-μm semipreparative reverse-phase C-18 column (μ-Bondapak C-18, 1.9 cm × 15 cm) with use of a mixture of acetonitrile, methanol, and water (65:5:30, v/v/v) as eluant. Combustion analyses (C, H, N) were performed by Galbraith Laboratories, Inc. (Knoxville, TN). For that purpose, the compounds were first chromatographed twice by HPLC with a 10 μm semipreparative reverse-phase C-18 column such as described above with the same solvent mixture and finally with a 10 μm semipreparative normal-phase cartridge column (μPORASIL 2.5 cm × 10 cm) with a mixture of ethyl acetate and hexane (35:65, v/v).

Ultracentrifugation was performed on a Beckman L5-65 ultracentrifuge. Radioactivity was determined on a Beckman LS 3821 liquid scintillation counter with Formula-963 (New England Nuclear, Boston) used as scintillation fluid. All chemicals were reagent grade and were purchased from Aldrich Chemical Company (Milwaukee, WI), or from Sigma Chemical Company (St. Louis, MO). The compound ICI 164384 was a gift from the Imperial Chemical Industries PLC Pharmaceutical Division, Alderley Park, Cheshire, UK, or synthesized in our laboratory. Commercially available steroids were obtained from Steraloids (Wilton, NH). The 16α-halo-17α,β-estradiols were synthesized in our laboratory as described.[14-17]

Synthesis. Synthesis of 16α-Chloro, 16α-Bromo, and 16α-Iodo 7α-Alkylamide Derivatives (Figure 1). *N*-*n*-Butyl-*N*-methyl-11-(3′,17′-diacetoxyestra-1′,3′,5′(10′)16′-tetraen-7′α-yl)undecanamide (3). To 11-[3′-(benzoyloxy)-17′-oxoestra-1′,3′,5′(10′)-trien-7′α-yl]undecanoic acid (1) (3.94 g, 7.22 mmol), prepared as described,[12] dissolved in anhydrous CH_2Cl_2 (100 mL), and cooled at −10 °C, was added tributylamine (2.18 mL, 9.15 mmol) and isobutyl chloroformate (1.30 mL, 10.0 mmol). The solution was stirred for 35 min and methylbutylamine (13 mL, 109.7 mmol) was added. The mixture was warmed to room temperature and stirred for 1 h. Afterward, CH_2Cl_2 was added and the organic phase was washed with 1 N HCl, water, saturated sodium bicarbonate solution, and finally with water and dried with anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel. Elution with a mixture of EtOAc/hexane (1.5:8.5 v/v) yielded *N*-*n*-butyl-*N*-methyl-11-(3′-(benzoyloxy)-17′-oxoestra-1′,3′,5′(10′)-trien-7′α-yl)undecanamide (4.25 g, 96%) as a colorless oil; IR ν_{max} (neat) 1750, 1725 and 1640 cm^{-1}. The above described benzoyloxy amide (341 mg, 0.54 mmol) was dissolved in methanol (10 mL) and cooled at 0 °C. Then, 2 N NaOH (5 mL) was added and the mixture was stirred for 60 min at 0 °C. The solution was neutralized with 1 N HCl and extracted with CH_2Cl_2. The organic phase was dried with anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel. Elution with a mixture of EtOAc/hexane (3:7 v/v) yielded *N*-*n*-butyl-*N*-methyl-11-(3′-hydroxy-17′-oxoestra-1′,3′,5′(10′)-trien-7′α-yl)undecanamide (2) (284 mg, 97%) as a colorless oil which was mentioned but not described in reference:[18] ^1H NMR δ ($CDCl_3$) 0.91 (s, 3 H, 18′-CH_3), 2.76 app (d, 1 H, J = 16.3 Hz, part of ABX system, 6′-H), 2.96 and 2.98 (2 s, 3 H, NCH_3), 3.27 and 3.38 (2 t_{app}, 2 H, J = 7.5 Hz, NCH_2), 6.63 (br s, 1 H, 4′-H), 6.70 (br d, 1 H, J = 8.5 Hz, 2′-H), 7.12 (d, 1 H, J = 8.4 Hz, 1′-H); IR ν_{max} (neat) 3270, 1730, 1615 cm^{-1}; MS m/e 523 (M^+, 100), 508 ($M^+ - CH_3$, 32), 142 ($C_2H_4CON(CH_3)C_4H_9^+$, 47). The ketone amide 2 (163 mg, 0.50 mmol) was dissolved in isopropenyl acetate (10 mL). *p*-Toluenesulfonic acid (44 mg) was then added and the solution was distilled to about two-thirds of the original volume within 7 h and was then stirred at reflux for 12 h. Afterward, the solution was cooled in an ice/water bath and extracted with 50 mL of cooled ether. The ether was washed with cooled saturated sodium bicarbonate and water. The organic phase was dried with anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The residue was filtered through alumina (15 mm × 50 mm alumina Woehlm neutral, activity II) by using a mixture of benzene/diethyl ether (3:7 v/v) as eluant. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel. Elution with a mixture of EtOAc/hexane (1:4 v/v) yielded *N*-*n*-butyl-*N*-methyl-11-(3′,17′-diacetoxyestra-1′,3′,5′(10′),16′-tetraen-7′α-yl)undecanamide (3) (244 mg, 80%) as a colorless oil: ^1H NMR δ ($CDCl_3$) 0.92 (s, 3 H, 18′-CH_3), 0.92 and 0.95 (2 t, 3 H, J = 7.0 Hz, $N(CH_2)_3CH_3$), 2.18 (s, 3 H, 17′-$OCOCH_3$), 2.28 (s, 3 H, 3′-$OCOCH_3$), 2.76 app (d, 1 H, J = 16.1 Hz, part of ABX system, 6′-H), 2.90 and 2.96 (2 s, 3 H, NCH_3), 3.26 and 3.35 (2 t_{app}, 2 H, J = 7.6 Hz, NCH_2), 5.52 (m, 1 H, 16′-H), 6.80 (br s, 1 H, 4′-H), 6.85 (dd, 1 H, J_1 = 9.1 Hz and J_2 = 3.0 Hz, 2′-H), 7.27 (d, 1 H, J = 9.1 Hz, 1′-H); IR ν_{max} (neat) 1750, 1635, 1200 cm^{-1}; MS m/e 607 (M^+, 2), 565 ($M^+ - COCH_2$, 100), 550 ($M^+ - COCH_2 - CH_3$, 13), 523 ($M^+ - 2COCH_2$, 45), 142 ($C_2H_4CON(CH_3)C_4H_9^+$, 55), 129 ($C_4H_9(CH_3)NCOCH_3^+$, 38), 114 ($C_4H_9(CH_3)NCO^+$, 60), 86 ($C_4H_9(CH_3)N^+$, 25); exact mass calcd for $C_{38}H_{57}O_5N$ 607.4239, found 607.4234.

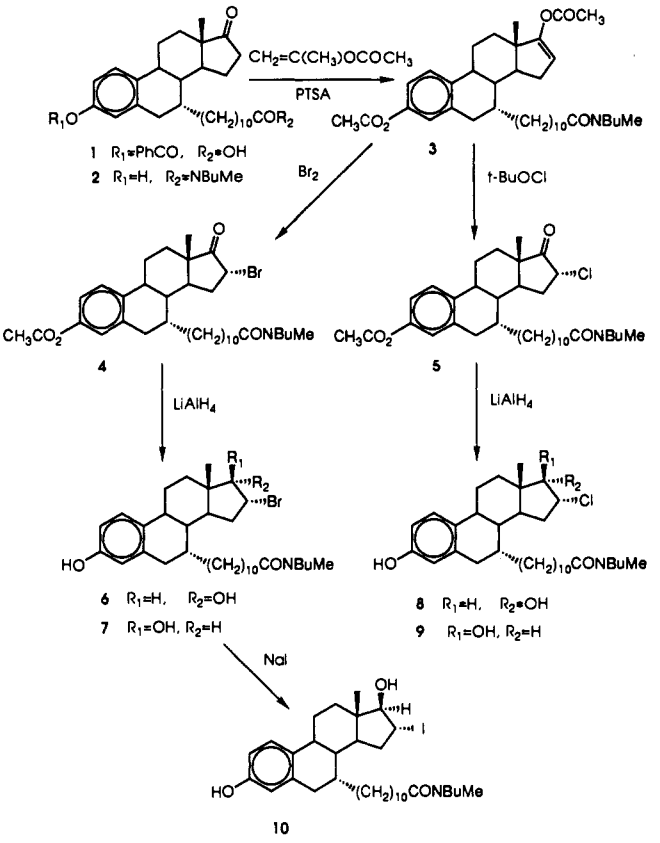

Figure 1. Preparation of 16α-chloro, 16α-bromo, and 16α-iodo derivatives.

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Bromination. Enol diacetate amide **3** (244 mg, 0.40 mmol) was brominated according to a modification of the method of Johnson and Johns.[19] To a stirred acetic acid (10 mL) solution of this compound was added dropwise within 10 min at room temperature a solution of bromine (1 equiv) and sodium acetate (1.5 equiv) in acetic acid and water (40:1 v/v). During the course of this reaction, a red coloration appeared and disappeared. The mixture was then poured in ice/water (100 mL) containing sodium bisulfite (about 5 mg) and extracted with ether. The organic phase was washed with a saturated sodium bicarbonate solution and water and dried with anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel with a mixture of EtOAc/hexane (1:4 v/v) yielded *N*-*n*-butyl-*N*-methyl-11-(16′α-bromo-3′-acetoxy-17′-oxoestra-1′,3′,5′(10′)-trien-7′α-yl)undecanamide (**4**) (201 mg, 78%) as a colorless oil: ^{1}H NMR δ($CDCl_3$) 0.94 (s, 3 H, 18′-CH_3), 2.28 (s, 3 H, 3′-$OCOCH_3$), 2.82 app (d, 1 H, J = 16.4 Hz, part of AB*X* system, 6′-H) 2.90 and 2.96 (2 s, 3 H, NCH_3), 3.24 and 3.35 (2 t_{app}, 2 H, J = 7.7 Hz, NCH_2), 4.58 (t, 1 H, J = 3.6 Hz, 16′β-H), 6.82 (br s, 1 H, 4′-H), 6.88 (dd, 1 H, J_1 = 8.0 Hz and J_2 = 4.0 Hz, 2′-H), 7.29 (d, 1 H, J = 8.0 Hz, 1′-H): IR ν_{max} (neat) 1750, 1745 (shoulder), 1200 cm^{-1}; MS *m/e* 644 (M^+, 7), 565 (M^+ - Br, 77), 522 (M^+ - Br - $COCH_2$, 55), 142 ($C_2H_4CON(CH_3)C_4H_9^+$, 67), 114 ($C_4H_9(CH_3)NCO^+$, 66), 88 (100).

Chlorination. Enol diacetate amide **3** (131 mg, 0.22 mmol) was chlorinated with *tert*-butyl hypochlorite [prepared from *tert*-butyl alcohol (4 mL) and Javel water (Javex 6.1%, 50 mL)] according to the method described by Heiman et al.[14].

Then, to this compound, dissolved in 5 mL of acetone, was added a solution of sodium acetate (2.6 equiv) in acetic acid and water (1:11.3 v/v) before treatment with *tert*-butyl hypochlorite (1 equiv). The clear solution was warmed to 55 °C and stirred for 1 h. Afterward, the solvent was evaporated to dryness. The residue was dissolved in ether (100 mL) and water was then added (20 mL). The organic phase was washed with water, dried with anhydrous $MgSO_4$ and evaporated to dryness. The residue was purified by chromatography on silica gel by using a mixture of EtOAc/hexane (3:7 v/v), giving the *N*-*n*-butyl-*N*-methyl-11-(16′α-chloro-3′-acetoxy-17′-oxoestra-1′,3′,5′(10′)-trien-7′α-yl)undecanamide (**5**) (115 mg, 89%) as a colorless oil: ^{1}H NMR δ ($CDCl_3$) 0.92 and 0.95 (2 t, 3 H, J = 7.0 Hz, $N(CH_2)_3CH_3$), 0.96 (s, 3 H, 18′-CH_3), 2.28 (s, 3 H, 3′-$OCOCH_3$), 2.80 app (d, 1 H, J = 16.6 Hz, part of AB*X* system, 6′-H), 2.90 and 2.96 (2 s, 3 H, NCH_3), 3.24 and 3.35 (2 t_{app}, 2 H, J = 7.4 Hz, NCH_2), 4.46 (d, 1 H, J = 6.6 Hz, 16′β-H), 6.82 (br s, 1 H, 4′-H), 6.86 (dd, 1 H, J_1 = 9.1 Hz and J_2 = 2.6 Hz, 2′-H), 7.29 (d, 1 H, J = 9.1 Hz, 1′-H); IR ν_{max} (neat) 1750, 1640, 1205 cm^{-1}; MS *m/e* 601, 599 (M^+, 24, 68), 142 ($C_2H_4CON(CH_3)C_4H_9^+$, 100), 114 ($C_4H_9(CH_3)NCO^+$, 93).

General Procedure for Reduction of Chloro and Bromo Derivatives. The reduction of the 17-ketone was carried out with $LiAlH_4$ according to an modified method of Heiman et al.[14] The stirred solution of halo ketone amide in anhydrous tetrahydrofuran (THF) (10 mL) under argon was chilled to −70 °C with 2-propanol/dry ice bath. A solution of 1.0 M of lithium aluminum hydride (2 equiv) was then added dropwise. After 30 min, the reaction was allowed to return slowly at 0 °C for 5 min before it was quenched with the dropwise addition of a mixture of THF/EtOAc (5 mL) (1:1 v/v) and acidified to pH ~4 with (10%) HCl. The mixture was stirred for 5 min at room temperature and then extracted with EtOAc. The organic phase was washed with water, dried on anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was a two-component mixture (as shown by TLC) which was separated by chromatography on silica gel.

By using the above described general procedure followed by chromatography carried out with a mixture of EtOAc/hexane (3:7 v/v), the 16α-bromo ketone amide **4** (295 mg, 0.46 mmol) yielded the following compounds in order of increasing polarity.

***N*-*n*-Butyl-*N*-methyl-11-(16′α-bromo-3′,17′α-dihydroxyestra-1′,3′,5′(10′)-trien-7′α-yl)undecanamide (6)** (63 mg, 21%): a colorless oil; analytical sample was obtained by HPLC purification; ^{1}H NMR δ ($CDCl_3$, 400 MHz) 0.81 (s, 3 H, 18′-CH_3), 0.93 and 0.96 (2 t, 3 H, J = 7.3 Hz, $N(CH_2)_3CH_3$), 2.79 (2 H, $J_{6,6}$ = 16.6 Hz and $J_{6,7}$ = 4.7 Hz, Δδ = 24.34 Hz, AB*X* system, 6′-H), 2.94 and 2.99 (2 s, 3 H, NCH_3), 3.27 (dd, J = 7.7 and 7.5 Hz) and 3.31–3.44 (m, 2 H, NCH_2), 3.66 (dd, 1 H, $J_{17,17}$-OH = 1.4 Hz and $J_{17,16}$ = 4.3 Hz, 17′β-H), 4.68 (dt, 1 H, $J_{16,17}$ = 4.3 Hz and $J_{16,15}$ = 9.7 Hz, 16′β-H), 6.60 (d, 1 H, J = 2.4 Hz, 4′-H), 6.65 (dd, 1 H, J_1 = 8.5 and J_2 = 2.5 Hz, 2′-H), 7.14 (d, 1 H, J = 8.5 Hz, 1′-H); IR ν_{max} (neat) 3300, 1615, 1495 cm^{-1}; MS *m/e* 605, 603 (M^+, 17), 523 (M^+ - HBr, 81), 142 ($C_2H_4CON(CH_3)C_4H_9^+$, 100), 114 ($C_4H_9(CH_3)NCO^+$, 97); exact mass calcd for $C_{34}H_{54}O_3N^{79}Br$ 603.3289, found 603.3304. Anal. Calcd: C, 67.53; H, 9.00; N, 2.32. Found: C, 66.62, H, 9.48; N, 2.10.

***N*-*n*-Butyl-*N*-methyl-11-(16′α-bromo-3′,17′β-dihydroxyestra-1′,3′,5′(10′)-trien-7′α-yl)undecanamide (7)** (170 mg, 50%): a colorless oil; analytical sample was obtained by HPLC purification; ^{1}H NMR δ ($CDCl_3$, 400 MHz) 0.80 (s, 3 H, 18′-CH_3), 0.93 and 0.96 (2 t, 3 H, J = 7.3 Hz,N ($CH_2)_3CH_3$), 2.80 (2 H, $J_{6,6}$ = 16.4 Hz and $J_{6,7}$ = 4.6 Hz, Δδ = 24.34 Hz, AB*X* system, 6′-H), 2.94 and 2.99 (2 s, 3 H, NCH_3), 3.27 (dd, J = 7.7 Hz and 7.5 Hz) and 3.31–3.45 (m, 2 H, NCH_2), 4.02 (dd, 1 H, $J_{17,17}$-OH = 3.7 Hz and $J_{17,16}$ = 6.1 Hz, 17′α-H), 4.15 (ddd, 1 H, $J_{16,15}$ = 10.2 Hz, $J_{16,17}$ = 6.1 Hz and $J_{16,15}$ = 2.9 Hz, 16′β-H), 6.61 (d, 1 H, J = 2.5 Hz, 4′-H), 6.66 (dd, 1 H, J_1 = 8.4 Hz and J_2 = 2.5 Hz, 2′-H), 7.12 (d, 1 H, J = 8.4 Hz, 1′-H); IR ν_{max} (neat) 3320, 1610, 1490 cm^{-1}; MS *m/e* 605, 603 (M^+, 29), 523 (M^+ - HBr, 100), 142 ($C_2H_4CON(CH_3)C_4H_9^+$, 70), 114 ($C_4H_9(CH_3)NCO^+$, 60); exact mass calcd for $C_{34}H_{54}O_3N^{79}Br$ 603.3289, found 603.3289. Anal. Calcd: C, 67.53; H, 9.00; N, 2.32. Found: C, 66.58; H, 8.88; N, 2.33.

By using the above described general procedure followed by chromatography carried out with a mixture of EtOAc/hexane (4:6 v/v), the 16α-chloro ketone amide **5** (55 mg, 0.092 mmol) gave the following compounds in order of increasing polarity.

***N*-*n*-Butyl-*N*-methyl-11-(16′α-chloro-3′,17′α-dihydroxyestra-1′,3′,5′(10′)-trien-7′α-yl)undecanamide (8)** (15 mg, 29%): a colorless oil; analytical sample was obtained by HPLC purification; ^{1}H NMR δ ($CDCl_3$, 400 MHz) 0.79 (s, 3 H, 18′-CH_3), 0.93 and 0.96 (2 t, 3 H, J = 7.3 Hz, $N(CH_2)_3CH_3$), 2.80 (2 H, $J_{6,6}$ = 17.1 Hz and $J_{6,7}$ = 4.5 Hz, Δδ = 24.34 Hz, AB*X* system, 6′-H), 2.94 and 2.99 (2 s, 3 H, NCH_3), 3.26 (dd, J_1 = 7.6 Hz and J_2 = 7.4 Hz) and 3.32–3.43 (m, 2 H, NCH_2), 3.71 (d, 1 H, J = 4.5 Hz, 17′β-H), 4.63 (ddd, 1 H, $J_{16,15}$ = 10.2 Hz, $J_{16,17}$ = 4.5 Hz and $J_{16,15}$ = 3.9 Hz, 16′β-H), 6.50 (d, 1 H, J = 24 Hz, 3′-OH), 6.60 (d, 1 H, J = 2.5 Hz, 4′-H), 6.66 (dd, 1 H, J_1 = 8.4 Hz and J_2 = 2.5 Hz, 2′-H), 7.14 (d, 1 H, J = 8.5 Hz, 1′-H); IR ν_{max} (neat) 3300, 1615, 1495 cm^{-1}; MS *m/e* 561, 559 (M^+, 40, 100), 523 (M^+ - HCl, 20), 142 ($C_2H_4CON(CH_3)C_4H_9^+$, 44), 114 ($C_4H_9(CH_3)NCO^+$, 37); exact mass calcd for $C_{34}H_{54}O_3N^{35}Cl$ 559.3785, found 559.3821. Anal. Calcd: C, 72.89, H, 9.72; N, 2.50. Found: C, 72.61; H, 10.03; N, 2.46.

***N*-*n*-Butyl-*N*-methyl-11-(16′α-chloro-3′,17′β-dihydroxyestra-1′,3′,5′(10′)-trien-7′α-yl)undecanamide (9)** (30 mg, 55%): a colorless oil; analytical sample was obtained by HPLC purification; ^{1}H NMR δ ($CDCl_3$, 400 MHz) 0.81 (s, 3 H, 18′-CH_3), 0.93 and 0.96 (2 t, 3 H, J = 7.3 Hz, $N(CH_2)_3CH_3$), 2.78 (2 H, $J_{6,6}$ = 16.2 Hz and $J_{6,7}$ = 4.5 Hz, Δδ = 24.34 Hz, AB*X* system, 6′-H), 2.94 and 2.99 (2 s, 3 H, NCH_3), 3.27 (dd, J_1 = 7.6 Hz and J_2 = 7.5 Hz) and 3.31–3.45 (m, 2 H, NCH_2), 3.86 (dd, 1 H, $J_{17,17}$-OH = 3.4 Hz and $J_{17,16}$ = 5.9 Hz, 17′α-H), 4.11 (ddd, 1 H, $J_{16,15}$ = 10.8 Hz, $J_{16,17}$ = 5.9 Hz and $J_{16,15}$ = 2.5 Hz, 16′β-H), 6.56 (d, 1 H, J = 19.7 Hz, 3′-OH), 6.61 (d, 1 H, J = 2.5 Hz, 4′-H), 6.66 (dd, 1 H, J_1 = 8.4 Hz and J_2 = 2.6 Hz, 2′-H), 7.12 (d, 1 H, J = 8.4 Hz, 1′-H); IR ν_{max} (neat) 3320, 1615, 1490 cm^{-1}; MS *m/e* 561, 559 (M^+, 38, 100), 523 (M^+ - HCl, 16), 142 ($C_2H_4CON(CH_3)C_4H_9^+$, 80), 114 ($C_4H_9(CH_3)NCO^+$, 76); exact mass calcd for $C_{34}H_{54}O_3N^{35}Cl$ 559.3785, found 559.3825. Anal. Calcd: C, 72.89; H, 9.72; N, 2.50. Found: C, 72.44; H, 9.74; N, 2.62.

Iodination. Under an argon atmosphere, a mixture of 16α-bromo diol amide **7** (55 mg, 0.091 mmol) and dry sodium iodide (10 equiv) in fresh ethyl methyl ketone (25 mL) was refluxed in darkness during 12 h. Afterward, the solvent was evaporated, water was added, and the product was extracted with EtOAc. The organic phase was washed with 5% sodium thiosulfate and water, dried with anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was purified by chromatography on silica gel carried out with a mixture of EtOAc/hexane (1:1 v/v), giving the following compounds in order of increasing polarity: *N*-*n*-butyl-*N*-methyl-11-(3′-hydroxy-3′,17′-oxoestra-1′,3′,5′(10′)-trien-7′α-yl)undecanamide (**2**) (3 mg, 5%), as a colorless oil, followed by a mixture of starting material and iodo compound (52:48). The

(19) Johnson, W. S.; Johns, W. F. *J. Am. Chem. Soc.* **1957**, *79*, 2005.

HPLC separation afforded *N*-*n*-butyl-*N*-methyl-11-(16′α-iodo-3′,17′β-dihydroxyestra-1′,3′,5′(10′)-trien-7′α-yl)undecanamide (**10**) (21 mg, 36%) as a colorless oil: ^{1}H NMR δ ($CDCl_3$, 400 MHz) 0.78 (s, 3 H, 18′-CH_3), 0.93 and 0.96 (2 t, 3 H, J = 7.3 Hz, N-$(CH_2)_3CH_3$), 2.79 (2 H, $J_{6,6}$ = 16.5 Hz and $J_{6,7}$ = 4.4 Hz, Δδ = 24.34 Hz, ABX system, 6′-H), 2.94 and 2.99 (2 s, 3 H, NCH_3), 3.27 (dd, J_1 = 7.6 Hz and J_2 = 7.5 Hz) and 3.32–3.44 (m, 2 H, NCH_2), 4.09–4.17 (m, 2 H, 16′β-H and 17′α-H), 6.60 (d, 1 H, J = 2.4 Hz, 4′-H), 6.65 (dd, 1 H, J_1 = 8.4 Hz and J_2 = 2.4 Hz, 2′-H), 7.13 (d, 1 H, J = 8.4 Hz, 1′-H); IR ν_{max} (neat) 3310, 1610, 1490 cm^{-1}; MS m/e 651 (M^+, 8), 523 (M^+ – HI, 100), 508 (M^+ – HI – CH_3, 38), 142 ($C_2H_4CON(CH_3)C_4H_9^+$, 54), 114 ($C_4H_9(CH_3)NCO^+$, 49); exact mass calcd for $C_{34}H_{54}O_3NI$–HI 523.4028, found 523.4028. Anal. Calcd for $C_{34}H_{54}O_3NI$: C, 62.66; H, 8.35; N, 2.15. Found: C, 62.23; H, 8.08; N, 2.37.

Introduction of Fluorine at 16α-Position. *N*-*n*-Butyl-*N*-methyl-11-(16′β-hydroxy-3′-acetoxy-17′-oxoestra-1′,3′,5′(10′)-trien-7′α-yl)undecanamide (11). An oven-dried flask was placed under a dry-air atmosphere and charged with bis(trifluoroacetoxy)iodobenzene (1.38 g, 3.22 mmol). The solid was dissolved in 30 mL of dry CH_3CN (distilled from CaH_2). Enol diacetate amide **3** (1.92 g, 3.16 mmol) dissolved in 5.5 mL of dry CH_2Cl_2 was added. The reaction mixture was stirred at room temperature and followed by TLC. After 25 h, the solvent was removed under reduced pressure and the residue was dissolved in THF (about 30 mL) and 30 mL of 10% aqueous ethanol was added. The reaction mixture was stirred at room temperature for 12 h. The solvent was then removed under reduced pressure and the residue was purified by flash chromatography on silica gel. Elution with the mixture of EtOAc/hexane (1:1, v/v) yielded the *N*-*n*-butyl-*N*-methyl-11-(16′β-hydroxy-3′-acetoxy-17′-oxoestra-1′,3′,5′(10′)-trien-7′α-yl)undecanamide (**11**) (852 mg, 46% from enol diacetate amide **3**) as a colorless oil: ^{1}H NMR δ ($CDCl_3$, 400 MHz) 0.99 (s, 3 H, 18′-CH_3), 0.92 and 0.95 (2 t, 3 H, J = 7.3 Hz, $N(CH_2)_3CH_3$), 2.29 (s, 3 H, 3′-$OCOCH_3$), 2.88 app (d, 1 H, J = 17.4 Hz part of ABX system, 6′-H), 2.91 and 2.96 (2 s, 3 H, NCH_3), 3.25 and 3.36 (2 t_{app}, 2 H, J = 7.4 Hz, NCH_2), 4.05 (t, 1 H, J = 1.9 Hz, 16′α-H), 6.81 (d, 1 H, J = 2.4 Hz, 4′-H), 6.86 (dd, 1 H, J_1 = 8.0 and J_2 = 2.4 Hz, 2′-H), 7.27 (d, 1 H, J = 7.9 Hz, 1′-H): IR ν_{max} (neat) 3360, 1750, 1740, 1620, 1200 cm^{-1}; MS m/e 581 (M^+, 32), 142 ($C_2H_4CON(CH_3)C_4H_9^+$, 76), 88 (100).

***N*-*n*-Butyl-*N*-methyl-11-[16′β-[(*p*-tolylsulfonyl)oxy]-3′-acetoxy-17′-oxoestra-1′,3′,5′(10′)-trien-7′α-yl]undecanamide (12).** Ketol **11** (35 mg, 0.060 mmol) was dissolved under an argon atmosphere in triethylamine (3 mL) with 4-(dimethylamino)-pyridine (1.7 mg, 0.01 mmol). After dissolution, *p*-toluenesulfonyl chloride (460 mg, 2.4 mmol) was added in four portions every 10 h. The reaction was stirred at room temperature for 48 h. The reaction was quenched with water (20 mL) and extracted with EtOAc. The organic phase was washed two times with HCl and water and finally dried with anhydrous $MgSO_4$. The solvent was removed under reduced pressure. The residue was purified by chromatography on silica gel. Elution with a mixture of EtOAc/hexane (4:6 v/v) yielded the *N*-*n*-butyl-*N*-methyl-11-[16′β-[(*p*-tolylsulfonyl)oxy]-3′-acetoxy-17′-oxoestra-1′,3′,5′(10′)-trien-7′α-yl]undecanamide (**12**) (24 mg, 55%) as a colorless oil: ^{1}H NMR δ ($CDCl_3$) 0.96 (s, 3 H, 18′-CH_3), 2.28 (s, 3 H, 3′-$OCOCH_3$), 2.46 (s, 3 H, 16′β-$OSO_2C_6H_4CH_3$), 2.82 app (d, 1 H, J = 16.6 Hz, part of ABX system, 6′-H), 2.90 and 2.96 (2 s, 3 H, NCH_3), 3.24 and 3.35 (2 t_{app}, 2 H, J = 7.3 Hz, NCH_2), 4.67 (t, J = 8.0 Hz, 16′α-H), 6.81 (br s, 1 H, 4′-H), 6.88 (dd, 1 H, J_1 = 8.0 Hz and J_2 = 4.1 Hz, 2′-H), 7.29 (d, 1 H, J = 8.0 Hz, 1′-H), 7.36 (d, J = 7.7 Hz) and 7.87 (d, J = 8.4 Hz, 4 H, 16′β-$OSO_2C_6H_4CH_3$); IR ν_{max} (neat) 1750, 1635, 1365, 1205, 1170 cm^{-1}; MS m/e 580 (M^+ – $OSO_2C_6H_4CH_3$, 4), 565 (M^+ – $OSO_2C_6H_4CH_3$–CH_3, 48), 523 (M^+ – $OSO_2C_6H_4CH_3$–CH_3–$COCH_2$, 31), 142 ($C_2H_4CON(CH_3)C_4H_9^+$, 82), 88 (100).

Estrogen Receptor Binding Assay. Apparent affinities of the synthesized compounds for the estrogen receptor were determined by competition binding with [^{3}H]estradiol to the rat uterine cytosol receptor according to Asselin and Labrie.[20] The incubations were performed at 25 °C for 3 h and nonspecific binding was determined by using an excess (1000 nM) of radioinert estradiol. The apparent affinities are expressed as relative binding affinity (RBA = 100 × ED_{50} estradiol/ED_{50} tested compound, where ED_{50} is the concentration which inhibits [^{3}H]estradiol binding by 50%).

Mouse Uterine Weight Assay. The antiestrogenic activity of the synthesized compounds was measured in vivo by inhibition of 17β-estradiol-induced stimulation of uterine weight in adult female ovariectomized Balb/c mice (body weight = 19–20 g) sacrificed five days after ovariectomy. The indicated compounds dissolved in ethanol were injected subcutaneously in the appropriate groups in 0.9% (w/v) sodium chloride and 1% (w/v) gelatin at appropriate concentrations in a total volume of 0.2 mL. The injections were given twice daily, starting on the day of ovariectomy for a total of nine injections. Estradiol was injected at the dose of 0.01 μg in 0.2 mL, twice daily, starting on the morning after ovariectomy for a total of eight injections. After sacrifice, the uteri were rapidly removed, freed from fat and connective tissue, and weighted. Results are the means ± SEM of 9–10 mice per group.

Results

Chemistry. The 16α-halosteroids were prepared by a modification of the procedure of Johnson and Johns[19] with use of an enol acetate as intermediate (Figure 1). Thus, enol diacetate amide **3** was stereospecifically chlorinated with *tert*-butyl hypochlorite in acetone buffered with sodium acetate, acetic acid, and water to give 16α-chloro ketone amide **5**. The same enol diacetate amide **3** was stereospecifically brominated with bromine in acetic acid to give 16α-bromo ketone amide **4**. All chloro and bromo derivatives were reduced specifically (without affecting the amide function) with 2 equiv of lithium aluminum hydride at low temperature (–70 °C) to an epimeric mixture of 17-alcohols which were separated by chromatography on silica gel.

The following 16α-chloro derivatives were then obtained: 17β-alcohol amide **9** and 17α-alcohol amide **8** as well as 16α-bromo derivatives (17β-alcohol amide **7** and 17α-alcohol amide **6**). The bromo diol amide **7** was refluxed for 12 h in 2-butanone with sodium iodide under equilibrium conditions to yield 16α-iodo 3,17β-diol amide **10**. When the reflux time was extended to 24 h, ketone **2** (probably formed by epoxide closing and rearrangement) was obtained as a major product. With a 12-h reflux, this reaction was only partial, thus yielding only 5% of ketone **2** while, as shown by HPLC separation, about 50% of the reaction product was the iodine compound. It is interesting to mention that, in the case of synthesis of 16α-iodoestradiol, the side reaction leading to the 17-ketone was not observed (results not shown).

For the 16α-fluoro derivatives, the approach described by Kiesewetter[16] for the synthesis of 16α-fluoro-3,17β-dihydroxyestra-1,3,5(10)-triene consisting in a stereospecific displacement of 16β-triflate (obtained from the 16-en-17-acetate) by the fluoride ion was attempted for the 7α-alkylestradiol series. Unfortunately, this method, applied to the 16β-hydroxy ketone amide **11**, did not yield the fluoro amide. On the other hand, the tosyl derivative **12** was obtained but the displacement of this leaving group could not be achieved with different fluorine salts (e.g. n-Bu_4NF, KF, CsF).

For biological applications, it is well known that the stereochemistry of each asymmetric center has primordial importance, especially when located near the active group of the molecule. The chemical structure of the new compounds was determined by NMR analysis (400 MHz) and compared with the corresponding compounds without side chain and with data from the literature. The chemical shift and coupling constant of the hydrogens located on carbons 16 and 17 are reported in Table I for every 16α-haloestradiol derivative. In general, for the proton located on

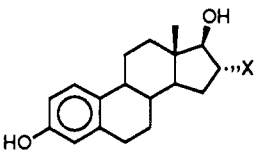
(20) Asselin, J.; Labrie, F. *J. Steroid Biochem.* 1978, *9*, 1079.

Table I. ^{1}H NMR Analysis for Protons H–C17 and H–C16[d]

16α-halo-steroid	literature:[a] δ (ppm), sys,[c] J (Hz) H–C17	literature:[a] δ (ppm), sys,[c] J (Hz) H–C16	observed:[b] δ (ppm), sys,[c] J (Hz) H–C17	observed:[b] δ (ppm), sys,[c] J (Hz) H–C16
fluoro 3,17β-diol	3.86, br d, 28.2	4.96, dm, 46	3.84, dt, 28.6, 4.6	4.96, dm, 47.1
chloro 3,17β-diol	3.60, m	4.10, m	3.60, dd, 5.3, 6.1	4.09, ddd, 10.1, 6.1, 2.3
chloro 3,17β-diol amide			3.86, dd, 3.4, 5.9	4.11, ddd, 10.8, 5.9, 2.5
chloro 3,17α-diol	3.53, m	4.60, m	3.54, dd, 5.1, 4.8	4.62, ddd, 10.3, 4.8, 4.0
chloro 3,17α-diol amide			3.71, d, 4.5	4.63, ddd, 10.2, 4.5, 3.9
bromo 3,17β-diol	3.50, t, 5	4.72, m	3.76, dd, 5.6, 6.3	4.14, ddd, 10.0, 6.3, 2.6
bromo 3,17β-diol amide			4.02, dd, 3.7, 6.1	4.15, ddd, 10.2, 6.1, 2.9
bromo 3,17α-diol	3.50, t, 5	4.72, m	3.49, dd, 5.4, 4.8	4.70, ddd, 10.1, 4.8, 3.8
bromo 3,17α-diol amide			3.66, dd, 1.4, 4.3	4.68, dt, 4.3, 9.7
iodo 3,17β-diol	4.15, m	4.15, m	3.86, dd, 6.2, 5.7	4.07, ddd, 10.5, 5.7, 3.3
iodo 3,17β-diol amide			4.09–4.17, m	4.09–4.17, m

[a] DMSO, F and I at 60 MHz, Cl and Br at 90 MHz (refs 14, 16, and 17). [b] DMSO for compounds without amide, $CDCl_3$ others, 400 MHz for all. [c] Abbreviations are as follows: br d, broad doublet; dd, doublet of doublet; ddd, doublet of doublet of doublet; dt, doublet of triplet; m, multiplet. [d] Note: H–C16 for 16β-F-, 16β-Br-, or 16β-I-17β-estradiol in NMR are given respectively: 4.90–5.08, 4.57, and 4.75 (refs 14, 16, and 28).

Table II. Relative Binding Affinities of Compounds without Side Chain to the Estrogen Receptor from Immature Rat Uterus

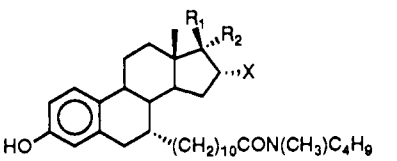


16α-substituted-17β-estradiol

X	RBA[a]
H	100
F	35
Br	88
Cl	61
I	56

[a] RBA = 100 × (ED_{50} estradiol/ED_{50} compound).

Table III. Relative Binding Affinities of New Antiestrogens to the Cytosolic Estrogen Receptor from Immature Rat Uterus



compd	R_1	R_2	X	RBA[a]
ICI 164384	OH	H	H	1.2
7	OH	H	Br	1.2
9	OH	H	Cl	1.2
10	OH	H	I	1.2
8	H	OH	Cl	1.1
6	H	OH	Br	1.1

[a] See Table II for RBA definition.

carbon 16, we have observed (400 MHz), a complex system, namely doublet of doublet of doublet which gave a higher coupling constant of about 10 Hz compared with H–C15 (pseudoaxial), another of about 6 Hz compared with H–C17α or 4–5 Hz compared with H–C17β, and the last one, about 4 Hz relative to H–C15 (pseudoequatorial) when the alcohol is located at the 17α-position or 2–3 Hz when the alcohol is at the 17β-position. However, for the 16α-bromo 3,17α-diol amide, we have observed a doublet of triplet while for 16α-iodo 3,17β-diol amide, the system was too complicated for proper interpretation (multiplet). In the case of the proton located on carbon 17, the system was a doublet of doublet except for the 16α-chloro 3,17α-diol amide which gave a doublet. In general, coupling constants between H–C17 and OH–C17 were lower when the side chain was present, probably due to an interaction between OH–C17 and the amide function.

Relative Binding Activity. The apparent affinity of the compounds (without the side chain) for the estrogen receptor was determined in vitro by using cytosol from immature rate uterus and is expressed in relative binding affinity (RBA) (Table II). It can be seen that the apparent affinity decreases when the 16α-proton of 17β-estradiol is substituted by a halogen in the following order: bromine, chlorine, iodine, and fluorine. We then measured the RBA of each new compound bearing the 7α-substituted chain (Table III). It can be seen that all compounds, in the total uterine cytosol assay used, have an apparently similar and low ability to displace [^{3}H]estradiol from the estrogen receptor. It can be noticed that compounds 8 and 6 which have an alcohol at the 17α-position, have a similar apparent affinity.

Biological Activity. Since in vivo changes in uterine weight offer a more significant evaluation of the biological activity of potential antiestrogens, we have measured the antiestrogenic activity of the new compounds in vivo with the mouse uterine weight assay and have compared these data with those obtained with tamoxifen and ICI 164384. The inhibition achieved with the doses of 3 μg and 20 μg (twice daily) are shown in Table IV. A more precise assessment of the pure antiestrogenic activity of 9 is illustrated in Figure 2. It can be seen in this figure that a 60% reversal of E_2-induced uterine weight is already achieved with the twice daily dose of 1 μg of compound 9 with a further decrease in uterine weight at higher doses and a complete reversal at the 20-μg dose. On the other hand, as illustrated in Figure 3, tamoxifen, at the same doses, has full estrogenic activity. Moreover, only a small and partial (30%) antiestrogenic activity is achieved with

Table IV. Inhibition of Estradiol-Induced Stimulation of Uterine Weight in Ovariectomized Balb/c Mice by Antiestrogens at the Twice Daily Doses of 3 and 20 μg

compd	inhibition %[a] 3 μg	inhibition %[a] 20 μg
tamoxifen	7 ± 8(NS)[b]	3 ± 15
ICI 164384	40 ± 9**,[c]	86 ± 10**
6	60 ± 12**	78 ± 5**
8	35 ± 6**	82 ± 4**
10	63 ± 7**	89 ± 6**
9	74 ± 7**	99 ± 6**
7	52 ± 12**	79 ± 8**

[a] Inhibition of estradiol-stimulated uterine weight (UW) by test compounds (3 μg and 20 μg per injection) is calculated according to the following equation:

$$\% \text{ of inhibition} = 100 - [100 \times (\text{UW test compound} - \text{UW control})/(\text{UW } E_2 - \text{UW control})]$$

[b] NS, nonsignificant (Duncan–Kramer[33]). [c] $p < 0.01$.

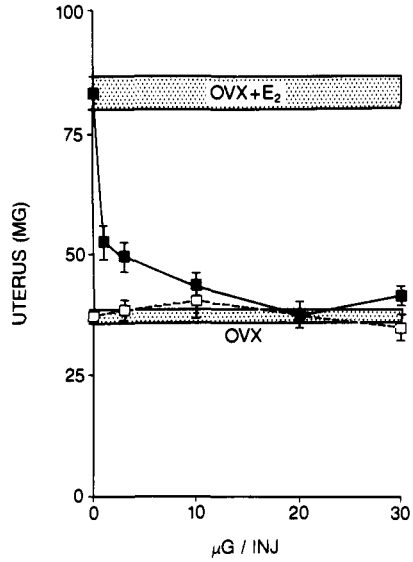


Figure 2. Effect of the indicated doses of compound **9** injected twice daily for 4.5 days on uterine weight in adult female ovariectomized Bald/c mice (OVX) in the presence (■—■) or absence (□- -□) of treatment with 17β-estradiol.

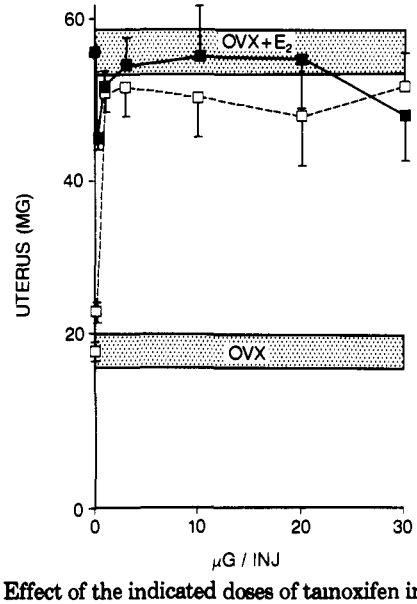


Figure 3. Effect of the indicated doses of tamoxifen injected twice daily for 4.5 days on uterine weight in adult female ovariectomized Balb/c mice (OVX) in the presence (■—■) or absence (□- -□) of treatment with 17β-estradiol.

tamoxifen at the 1-μg dose, while at all other doses the potent intrinsic estrogenic activity of the compound prevents any reversal of the E_2-induced stimulation of uterine weight.

At the twice daily 3-μg dose, compounds **10** and **7** caused 63 ± 7 and 52 ± 12% inhibition of E_2-induced uterine weight, respectively, while a 40 ± 9% inhibition was obtained with the unsubstituted C-16 estradiol derivatives. It can also be seen in Table IV that the 17α-alcohol amide compounds **8** and **6** caused 60 ± 12% and 35 ± 6% inhibitions, respectively, at the same 3-μg dose.

Discussion

Since the affinities of 16α-halo-17β-estradiols reported in the literature[14-17] are variable, depending upon the assay conditions used,[21] we report, in Table III, data where all RBA values for 16α-halo-17β-estradiols have been measured in the same experiment. Among the steroidal estrogens bearing an halogen on the D ring at C-16, we have observed that 16α-bromo-17β-estradiol has the highest affinity for the estrogen receptor, thus confirming the earlier report of Longcope et al.[22] and Heiman et al.[14] Under our conditions, the iodo group gave a RBA value of 56 which is different from the values reported by McElvany[24] (100) and Longcope[22] (160). Except for the fluoro compound which had the weakest affinity, the introduction of an halogen (Cl, Br, and I) substituent at the 16α-position does not dramatically modify the interaction between the steroid and the estrogen receptor. Such a substituent, however, could have major protective effects from metabolic degradation under in vivo conditions especially 17β-hydroxysteroid dehydrogenase,[25-27] 16α-steroid hydroxylase,[28] and steroid 15α-hydroxylase[29] activities.

The present data also show that the RBA values for new antiestrogens (Table III), as observed with other 7α-alkyl-substituted compounds[13,30] are low. These apparently low binding values are probably related to the high level of hydrophobicity of these compounds and their affinity for various other proteins present in the cytosol, thus leaving a minimal amount of free compound to interact with the estrogen receptor under the in vitro conditions used. It has in fact been found that the nonspecific binding of [^{3}H]ICI 164384 was 95% of total binding when measured in crude cytosolic preparations of the uterine estrogen receptor.[30] Such data clearly illustrate the serious limitations of binding studies performed with crude preparations of the estrogen receptor.

The aim of the present study was the discovery of novel antiestrogens having pure and potent antiestrogenic activity. It is well known that the most valid assays for such compounds are under in vivo conditions. The combination of chemical modifications of 16α-chloro-, 16α-bromo-, or 16α-iodo-17β-estradiol with the 7α side chain (alkylamide) led to highly potent new compounds having pure antiestrogenic activity (Table IV). In fact, the new compounds **6–10** can be considered as pure estrogen antagonists with no agonistic activity at any dose used. At the low 3-μg dose, the 16α-chloro compound **9** already shows a 74 ± 7% reversal of the stimulatory effect of E_2 on mouse uterine weight while a complete reversal (99 ± 6%) is achieved with the 20-μg dose. The antiestrogen is thus the most potent antiestrogen described so far.

The present data also show that the configuration β for the alcohol at carbon 17 is not absolutely necessary for antagonistic activity. Thus, compound **6** shows an antiestrogenic activity which is similar to that of its epimer, **7**. Such a change at carbon 17 could have a major influence

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on the metabolism of the compounds and their potential long-term therapeutical efficacy. The present data clearly suggest that factors other than affinity for the estrogen receptor play a major role in determining the potency of antiestrogen compounds. The factors pertain to absorption, transport,[32] and especially metabolism at various sites, including the site of intracellular action. The availability of these new halo-steroidal antiestrogens should be useful for a better understanding of estrogen action at the molecular and cellular level and for the development of an improved therapy of breast and endometrial cancer.

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Acknowledgment. The authors wish to thank Mesdames Karine Andryanczyk and France Lêtourneau, as well as Messrs Gilles Leblanc, Shengmin Li, and Jacques Paquet for their technical assistance, and Mrs. Patricia Pouliot and Elaine Leclerc for their secretarial help in the preparation of this manuscript. This work was supported in part by the Medical Research Council of Canada to the MRC Group in Molecular Endocrinology, the Fonds FCAR, and the Fonds de la Recherche en Santé du Québec (FRSQ) and Endorecherche.

Registry No. **1**, 55592-11-5; **2**, 98013-89-9; **3**, 131811-72-8; **4**, 131811-74-0; **5**, 131811-73-9; **6**, 131811-53-5; **7**, 131811-52-4; **8**, 131811-55-7; **9**, 131811-54-6; 10, 131811-56-8; **11**, 132463-56-0; **12**, 132463-57-1; methylbutylamine, 110-68-9; *N*-*n*-butyl-*N*-methyl-11-[3′-(benzoyloxy)-17′-oxoestra-1′,3′,5′(10′)-trien-7′α-yl]undecanamide, 131811-95-5.

Synthesis and Anthelmintic Activity of 3′-Benzoylurea Derivatives of 6-Phenyl-2,3,5,6-tetrahydroimidazo[2,1-*b*]thiazole†

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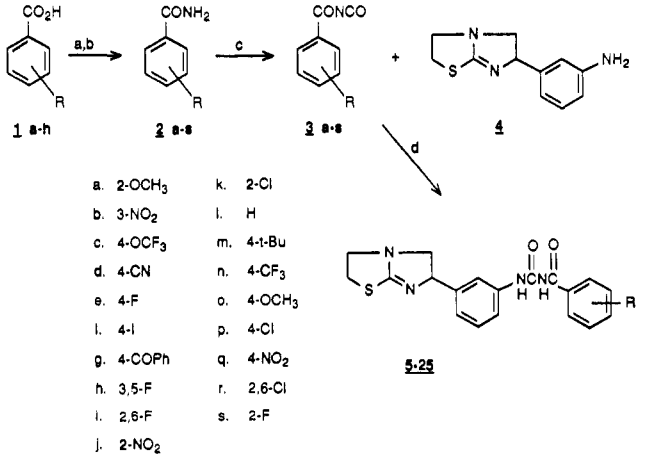
Reaction of 3-amino derivatives of the nematocides tetramisole and levamisole with variously substituted benzoylisocyanates gave a series of benzoylureas I which were tested for activity against helminths and ectoparasites. Compounds bearing 2,6-difluoro and 4-trifluoromethyl substituents had potent nematocidal activity in both mice and sheep. No antiectoparasitic activity was observed.

Parasitic infestations in food-producing animals are economically important in modern agriculture. The development of resistance to several important classes of veterinary anthelmintics[1,2] requires the production of more effective agents with a broad spectrum of activity. Traditionally, endo- and ectoparasitic infections have been treated as separate disease states requiring the administration of different pharmacologic agents. More recently there has been an increased interest in discovering therapeutic anthelmintics which possess both endoparasitic and ectoparasitic activity. The most notable compound to belong to this class is ivermectin which was introduced to the marketplace in 1981. This compound is extremely potent against both nematode and arthropod parasites.[3] These "endectosides" have the potential to be more efficient and economical in the field.

We have attempted to modify the well-known nematocide tetramisole (II)[4] in an effort to obtain a broad-spectrum anthelmintic with antiectoparasitic activity. We were encouraged by the report that certain isothiourea analogues of tetramisole (III) have an increased spectrum of activity and are effective against cestodes and trematodes as well as nematodes.[5] It is also well known that diacylureas such as diflubenzuron (IV), a benzoylurea which inhibits chitin synthesis, are effective for the control of arthropods.[6] We now report on the synthesis and anthelmintic activity of

Scheme I[a]



[a] (a) Oxalyl chloride, CH_2Cl_2, DMF (cat.), reflux; (b) NH_4OH, EtOAc; (c) oxalyl chloride, dichlorethane, reflux; (d) CH_3CN, 70 °C.

† Contribution #815 from the Institute of Organic Chemistry.
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