Synthesis and biologic activities of 11β-substituted estradiol as potential antiestrogens

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The effect of attachment of a dimethylaminoethoxy or a dimethylaminopropoxy group at the 11β-position of estradiol (E_2) on its relative binding affinity (RBA) to estrogen receptor (ER) and intrinsic biologic activity is described. The binding of 11β-[2-(N,N-dimethylamino)ethoxy]estra-1,3,5(10)-triene-3,17β-diol ***(4)*** *and 11β-[3-(N,N-dimethylamino)propoxy]estra-1,3,5(10)-triene-3,17β-diol* ***(5)*** *to the ER from immature rat uterine tissue was measured relative to that of [^{3}H]E_2 by a competitive binding assay. It was found that the 11β-substituted E_2 analogs have considerably lower RBA to ER than the corresponding parent compound. The intrinsic activity of compounds* ***4*** *and* ***5*** *were studied in terms of uterotrophic and antiuterotrophic activity. It was found that the uterotrophic activity of these compounds was drastically reduced compared with E_2. However, no antiuterotrophic activity was observed in these compounds at dosages ranging from 1 to 100 μg/rat/d.* (Steroids **55**:238–241, 1990)

Keywords: steroids; 11β-substituted estradiol; steroidal antiestrogens

Introduction

The triphenylethylene (TPE)-derived antiestrogens have an outstanding structural feature in that all of them have a polar functional group, such as a dialkylaminoethoxy or a glyceryl group, directly attached to the aromatic ring of TPE. The dialkylaminoethyl side chain has been shown to be essential for their antiestrogenic activity.[1] Substitution of the side chain by a methoxy group changes the biologic activity of tamoxifen from antiestrogenic to estrogenic.[2] On the other hand, attachment of the side chain to a weak estrogen, such as *gem*-dichlorodiphenylethylene, results in a compound with antiestrogenic activity.[3]

Based on the above facts, we proposed that attachment of the dialkylaminoethoxy side chain to estradiol may result in compounds having antiestrogenic activity.[4] In an earlier investigation, the effect of attachment of the side chain to the 17β-position of estradiol (E_2) was investigated. These results showed that the 17β-substituted estradiol analogs had decreased binding affinity to the estrogen receptor (ER), had very low estrogenic activity, and did not have any antiestrogenic activity. The aim of the present study is to investigate the effect of attachment of the dialkylaminoethoxy side chain at the 11β-position of E_2. In the following sections, the synthesis of 11β-dimethylaminoethoxy- and dimethylaminopropoxy-substituted E_2 is described, and the effects of these substituents on the relative binding affinity (RBA) and biologic activities of E_2, in terms of uterotrophic and antiuterotrophic activity, are presented.

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Experimental

Commercially available steroids were obtained from Steraloids (Wilton, NH, USA). All chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA), radiolabeled steroids from New England Nuclear (Boston, MA, USA), and biochemicals from Sigma Chemical Co. (St. Louis, MO, USA).

11β-[2-(N,N-Dimethylamino)ethoxy]estra-1,3,5(10)-triene-3-benzyloxy-17-ethyleneketal (2)

NaH (120 mg, 5 mmol) suspended in dry tetrahydrofuran (THF) (5 ml) was carefully added to a solution of **1** (400 mg, 0.95 mmol) in freshly distilled THF (50 ml). After refluxing for 1 hour, 2-(dimethylamino)ethyl chloride (330 mg, 3.0 mmol, freshly prepared from its

HCl salt) dissolved in THF (3 ml) was added to the reaction in three portions over a period of 4 hours. The reaction was then refluxed for an additional 4 hours. After inactivation of NaH by the addition of methanol, the reaction mixture was poured into ice water and extracted with chloroform/methanol (9:1). The organic phase was dried over $MgSO_4$, and the solvent was removed under reduced pressure. Chromatography over silica gel ($CHCl_3$/methanol, 9:1) afforded 295 mg (0.61 mmol, 64% yield) of the desired compound (**2**) as an oil. ^1H nuclear magnetic resonance (NMR) ($CDCl_3$) δ 1.08 (s, 3H, C-18-C$\underline{H}_3$), 2.12 [s, 6H, C-11-OCH$_2$CH$_2$N(C$\underline{H}_3$)$_2$], 2.36 (t, 2H, J = 6.22 Hz, C-11-OCH$_2$C$\underline{H}_2$N(CH$_3$)$_2$), 3.90 (s, 4H, C-17-OCH$_2$CH$_2$O-), 4.31 (m, 1H, C-17α-H), 5.02 (s, 2H, C$_6$H$_5$C$\underline{H}_2$), 6.69 to 7.25 (m, 3H, C-1H, C-2H, C-4H), 7.36 (m, 5H, C$_6\underline{H}_5$CH$_2$); infrared (IR) (KBr) 2,931 (aliphatic CH), 1,616, 1,581 (aromatic C=C) cm^{-1}.

11β[3-(N,N-Dimethylamino)propoxy]estra-1,3,5(10)-triene-3-benzyloxy-17-ethyleneketal (**3**)

This compound was prepared in the same way as **2**. ^1H NMR ($CDCl_3$) δ (s, 3H, C-18-C$\underline{H}_3$), 2.06 [s, 6H, C-11-OCH$_2$CH$_2$-N(C$\underline{H}_3$)$_2$], 2.32 [t, 2H, J = 6.22 Hz, C-11-OCH$_2$CH$_2$C$\underline{H}_2$N(CH$_3$)$_2$)], 3.62 [t, 2H, J = 6.22 Hz, C-11-OC$\underline{H}_2$CH$_2$CH$_2$H(CH$_3$)$_2$], 3.90 (s, 4H, C-17-OCH$_2$CH$_2$O-), 4.31 (m, 1H, C-17α-H), 5.02 (s, 2H, C$_6$H$_5$C$\underline{H}_2$), 6.69 to 7.25 (m, 3H, C-1H, C-2H, C-4H), 7.36 (m, 5H, C$_6\underline{H}_5$CH$_2$); IR (KBr) 2,931 (aliphatic CH), 1,616, 1,581 (aromatic C=C) cm^{-1}.

11β-[2-(N,N-Dimethylamino)ethoxy]estra-1,3,5(10)-triene-3,17β-diol (**4**)

To a solution of **2** (250 mg, 0.51 mmol) in 10 ml methanol was added 2 ml of 0.4 M HCl; the mixture was refluxed for 15 minutes. After cooling and neutralization with KOH, the reaction mixture was added to a solution of $NaBH_4$ (200 mg, 2 mmol) in 20 ml of THF/methanol (2:1) and kept at room temperature for 20 minutes. After removal of the solvent under reduced pressure, the residue was dissolved in a solution of methanol (20 ml) containing glacial acetic acid (0.5 ml) and 50 mg of 5% Pd/C. The reaction mixture was shaken under hydrogen gas (40 psi) for 12 hours, filtered, and evaporated under reduced pressure. The crude product was dissolved in $CHCl_3$ and washed with 10% Na_2CO_3. After the organic phase was dried over $MgSO_4$ and the solvent removed under reduced pressure, the crude product was recrystallized from methanol, giving 165 mg of **4** (0.46 mmol, 91% yield). Melting point 218 to 220 C; ^1H NMR (CD_3OD) δ 0.97 (s, 3H, C-18-C$\underline{H}_3$), 2.12 [s, 6H, C-11-OCH$_2$CH$_2$N(C$\underline{H}_3$)$_2$], 4.26 (m, 1H, 17α-H), 6.48 to 6.50 (m, 2H, C-2H, C-4H), 7.06 (d, 1H, C-4H); IR (KBr) 2,931 (aliphatic CH), 1,616, 1,581 (aromatic C=C) cm^{-1}.

11β-[3-(N,N-Dimethylamino)propoxy]estra-1,3,5(10)-triene-3,17β-diol (**5**)

This compound was prepared in the same way as **4**: mp 160 to 162 C; ^1H NMR ($CDCl_3$) δ 0.96 (s, 3H, C-18-C$\underline{H}_3$), 2.12 [s, 6H, C-11-OCH$_2$CH$_2$CH$_2$N(C$\underline{H}_3$)$_2$], 4.23 (m, 1H, C-17α-H), 6.48 to 6.61 (m, 2H, C-2H, C-4H), 7.01 (d, 1H, J = 7.1 Hz, C-4H); IR (KBr) 2,945 (aliphatic CH), 1,614, 1,580 (aromatic C=C) cm^{-1}.

Estrogen receptor-binding assay

Estrogen receptor from immature female Sprague-Dawley rats was isolated as described previously.[5] Aliquots (150 μl) of the diluted cytosol (3.5 mg/ml) were added to each tube (12 mm × 75 mm) containing [1,2,4,6-^{3}H]estradiol (50 μl; 4 nM; specific activity, 91 Ci/mmol) and assay solution of test compounds or nonradioactive estradiol (50 μl, concentration varied from 0 nM to 3 μM at nine levels). Each concentration of the assay solution was run in duplicate, and the final volume of each mixture was 250 μl. After incubation for 3 hours at 4 C, 300 μl DCC solution (dextran-coated charcoal solution: 10 mM Trizma base, 1.0 mM EDTA, 250 mM sucrose, 0.05% dextran, 0.5% charcoal) was added to each incubation tube, vortexed, and allowed to stand in ice for 15 minutes with occasional shaking. After centrifugation at 2,000 × *g* for 5 minutes at 0 C, 200 μl of the supernatant was pipetted into vials containing 2 ml of scintillation solution (0.055% PPO, 0.001% POPOP, 66.7% toluene, 33.3% Triton X-100) and the radioactivity (cpm) was determined. The RBA of each test compound was determined by comparing the concentration of test compound required to reduce the specific binding of tritium-labeled estradiol to ER by 50% to the concentration of unlabeled estradiol required to achieve the same reduction.

Uterotrophic and antiuterotrophic assay

For uterotrophic and antiuterotrophic studies, compounds were prepared in absolute ethanol and were diluted 1:9 with olive oil immediately before injection; the desired dosage was administered in 0.1 ml oil/ethanol. Groups of immature rats (22 days of age; Biolab Co., St. Paul, MN, USA) received subcutaneous injections for 3 days, and animals were killed 24 hours after the last injection. The uteri were excised, slit longitudinally, blotted, and weighed.

Results and discussion

The synthesis of 11β-dialkylaminoalkoxy-substituted estradiol was accomplished as outlined in Figure 1. Alkylation of the 11β-hydroxy group in **1** (prepared as described in ref. 6) was achieved by treatment with NaH and the corresponding dialkylaminoalkylchloride in dry THF, giving compounds **2** and **3**. The 17-ketal group in **2** and **3** was then removed by refluxing in methanol containing HCl. Without further purification, the resulting 17-ketone was reduced to a 17β-

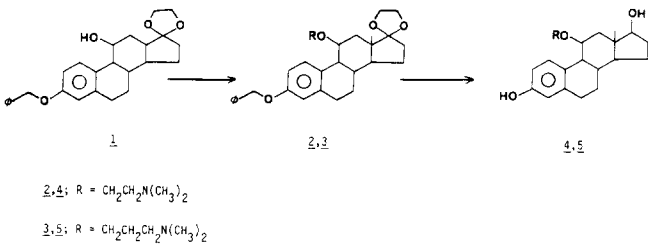

Figure 1 Synthesis of 11β-substituted estradiol.

hydroxy group by $NaBH_4$. Subsequent removal of the 3-benzyl group by hydrogenolysis with Pd/C in hydrogen gas afforded the final compounds **4** and **5**.

The RBA data of the 11β-substituted estradiols (Table 1) indicate that attachment of a N,N-dimethylaminoethoxy (DMAEE) or a N,N-dimethylaminopropoxy (DMAPE) group at the 11β-position of estradiol gave compounds with substantially reduced binding affinity to the ER. Compounds **4** and **5** have a binding affinity of 1.2 and 2.6% that of estradiol, respectively. When tested for their uterotrophic and antiuterotrophic activities (Figure 2), both DMAEE and DMAPE showed a dose response relationship with respect to their uterotrophic activities. Furthermore, both compounds were found not to antagonize the uterotrophic activity induced by estrone, indicating that these compounds have no antiestrogenic activities.

Our interest in this study was based on the assumption that the 11β-position of E_2 is one of the positions where structural modification would lead to compounds which have strong binding affinity to the ER.[8] When the molecular models of estradiol and 4-hydroxytamoxifen are superimposed in such a way that the A ring in estradiol overlaps with the α ring in 4-hydroxytamoxifen, and the 3-phenolic group in estradiol aligns with the 4-hydroxy group in 4-hydroxytamoxifen, the phenyl ring which contains the dialkylammonoethoxy side chain seems to extend from the 11β-position of estradiol.[9] This notion was further supported by the results obtained from the work of several investigators[10–12] who synthesized a number of 11β-substituted estradiol analogs. Many of these compounds have strong binding affinity to ER and possess potent estrogenic activity, suggesting that there is a relatively large-sized pocket in the ER around the 11-position of estradiol. On the other hand, when the 11β-position was substituted with a more polar group, such as an hydroxy or an aziridinylmethyl group, the relative binding affinities of the resulting compounds were substantially reduced, even though these substitutions have a much smaller size than the alkyl substitution, indicating the hydrophobic nature of the pocket in ER around the 11β-position of estradiol.

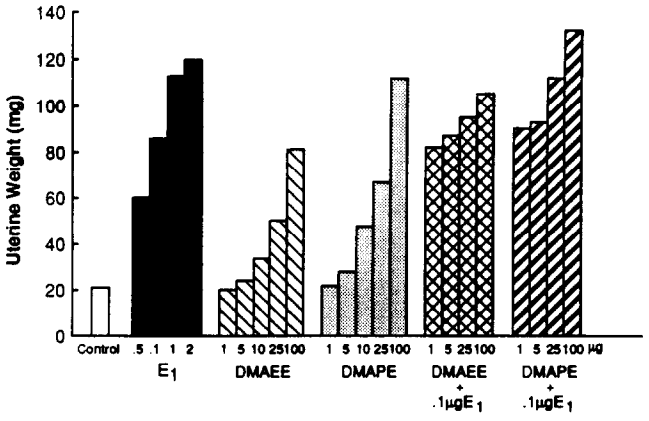

Figure 2 Effects of DMAEE, DMAPE, and E_1, alone or in various combinations, on uterine weight. Immature 22-day-old rats were injected with the indicated daily dose of compound(s) once daily for 3 days, and uterine weight was determined 24 hours after the last injection. Values are the mean ± SEM, with 10 uteri per determination.

Table 1 Relative binding affinities of 11β-substituted estradiol to estrogen receptor-immature rat uterus

4, DMAEE

5, DMAPE

Compound	RBA
Estradiol	100.0
11β-Hydroxy E_2	7.0*
Tamoxifen	6.0
4	1.6
5	2.6

* See ref. 7.

The fact that our studies showed that both DMAEE and DMAPE have reduced binding to the ER is not too surprising since earlier investigations[7] showed that oxygenation at the 11β-position of E_2 results in compounds with decreased binding affinities (Table 1). However, the results obtained from this study do not seem to support earlier observations which show that attachment of a dialkylaminoethoxy group to an estrogen results in compounds having antiestrogenic activity.[3] While the exact reasons for these results are not fully understood, experiments designed to attach the dialkylaminoethyl group to a high affinity estrogen, such as the 11β-hydroxymethyl E_2, may help explain the lack of antiestrogenicity of the compounds discussed in this study.

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