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Structure-Activity Relationships of Nonisomerizable Derivatives of Tamoxifen: Importance of Hydroxyl Group and Side Chain Positioning for Biological Activity

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SUMMARY

The antiestrogen tamoxifen [(*Z*)-1(*p*-β-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene] is an effective anticancer agent against estrogen receptor (ER)-positive breast cancer. The alkylaminoethane side chain is essential for antiestrogenic activity, but the potency of the antiestrogen can be increased by *para* hydroxylation of the phenyl ring on carbon 1 of but-1-ene. This compound, 4-hydroxytamoxifen, is a metabolite of tamoxifen and has a very high binding affinity for ER [*J. Endocrinol.* **75**:305–316 (1977)] because the hydroxyl is located in the equivalent position as the 3-phenolic hydroxyl of 17β-estradiol. In this study, we have examined the relationship between the relative positions of the hydroxyl and the alkyl-aminoethane side chain and the pharmacological activity of the ligand. A fixed seven-membered ring derivative of the triphenylethylene was used to prevent isomerization. All compounds were tested, with and without 17β-estradiol, for their effects on the growth of estrogen-responsive T47D and MCF-7 human breast cancer cells *in vitro*. The growth of MDA-MB-231 ER-negative breast cancer cells was not affected by any of the compounds tested, at a concentration (1 μM) that had a profound estrogenic or antiestrogenic action in ER-positive cell lines. The relative binding affinity of the compounds was determined using rat uterine ER and was found to be consistent with the observed potencies *in vitro*. The compounds found to be antiestrogens *in vitro* were antiestrogenic against estradiol (0.08 μg daily) in the 3-day immature rat uterine weight test. All compounds were partial agonists *in vivo*. In general, the estrogenic and antiestrogenic results obtained *in vivo* were consistent with the potency estimates obtained with the breast cancer cells *in vitro*. The results of this extensive structure-activity relationship study demonstrate that the substitution for 4-hydroxytamoxifen appears to be optimal to produce a potent antiestrogen; all other substitutions produced either estrogenic compounds or less potent antiestrogens. The hydroxyl group appears to be critical to locate the alkylaminoethoxy side chain in the correct position in the steroid-binding site to block estrogen action. Novel antiestrogens were identified that could have been predicted based upon earlier drug-receptor models for the ER.

The antiestrogen TAM is the hormonal drug of choice for the treatment of ER-positive breast cancer (1). As a result of its efficacy and relative lack of toxicity, long term adjuvant use of TAM has been recommended recently for the treatment of breast cancer in postmenopausal patients (2). Although TAM is an effective agent for breast cancer treatment, patients eventually fail TAM therapy and ultimately present with hormone-resistant disease. However, it has been difficult to design more effective antihormonal agents to prevent the outgrowth of hormone-resistant breast cancer, because the precise mechanisms of TAM action are not known.

Structure-activity relationship studies of TAM and its metabolites have provided useful information regarding the areas of the TAM molecule that are critical for its action (3). Certain key areas of TAM are thought to be important for its ability to bind to the ER and to block estrogen action (1). The aminoethoxy side chain of the α′ ring of TAM is critical for its antiestrogenic activity (Fig. 1) (3). The addition of a 4-position hydroxyl group to the α ring of TAM, producing 4-OHT, greatly increases the binding affinity of 4-OHT for the ER, thereby increasing its potency (Fig. 1) (3–5). It has been proposed (3, 4) that the 4-hydroxyl group, analogous to the 3-hydroxyl group of 17β-estradiol, tightly anchors the ligand at the binding site of the ER.

Until recently, it has been impossible to study the relationship between the aminoethoxy side chain and the hydroxyl group of 4-OHT, because this compound readily isomerizes from its *cis* to its *trans* form. Compound isomerization alters

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ABBREVIATIONS: TAM, tamoxifen; ER, estrogen receptor; 4-OHT, 4-hydroxy-tamoxifen; f.r., fixed ring; HBSS, Hanks' balanced salt solution; RBA, relative binding affinity.

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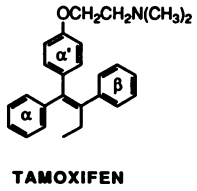

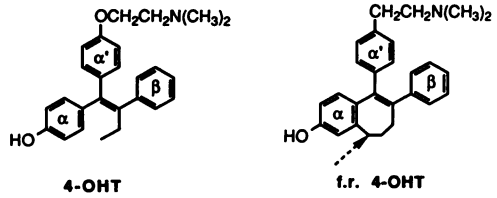

Fig. 1. Structures of TAM, 4-OHT, and f.r.-4-OHT. *Arrow*, area of the fused seven-membered ring.

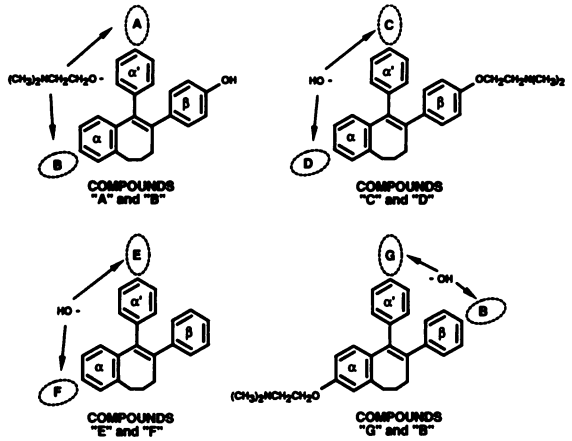

Fig. 2. Schematic drawings of compounds used in these studies. *Stippled ovals*, areas of test compounds where either an aminoethoxy side chain (compounds **A** and **B**) or a hydroxy group (compounds **C**, **D**, **G**, and **B**) is substituted.

the positioning of these groups, making it difficult to study their impact on biological activity (6, 7). Nonisomerizable f.r. derivatives of 4-OHT, which contain hydroxyl and aminoethoxy side chain groups in a variety of positions around the molecule, were recently synthesized (8, 9). The evaluation of compounds with the hydroxyl or aminoethoxy side chain groups repositioned around a core molecule in a systematic manner will extend the pharmacological database for prediction of antiestrogenic or estrogenic activity (3). This information will be useful for designing more effective antihormonal agents to combat hormone-sensitive and hormone-resistant disease and will be invaluable to molecular biologists studying the ER to understand the specific molecular events that program the ligand-receptor complex to initiate or block estrogen action. We have determined the effects of the test compounds (Fig. 2) on the growth of T47D and MCF-7 estrogen-responsive human breast cancer cells in culture. The RBA, using rat uterine ER, was established for each compound, and potential nonspecific antiproliferative activity was determined in the ER-negative cell line MDA-MB-231. The action of the compounds *in vitro* was compared with the pharmacological actions *in vivo*. The uterotrophic activity was determined in a 3-day immature rat uterine weight test, and the relative antiestrogenic activity was compared with that of 4-OHT for those compounds found to be partial agonists.

Materials and Methods

Cell culture. The T47D cell line (10, 11) used in these studies was originally obtained at passage 81 from the American Type Culture Collection (Rockville, MD). Cell stocks were cultured in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum (heat inactivated), 6 ng/ml bovine insulin (Sigma Chemical Co., St. Louis, MO), 100 units/ml penicillin, and 100 μg/ml streptomycin. All tissue culture reagents were obtained from GIBCO Laboratories (Grand Island, NY), unless otherwise stated. Cells were removed from flasks with 0.25% trypsin/EDTA solution for passage. Cell stocks were kept in T150 flasks (Corning, Park Ridge, IL) in a humidified atmosphere of 95% air/5% CO_2, at 37°. All experiments were conducted on cells between passages 97 and 191.

The MCF-7 cell line used in these studies was originally obtained from Dean Edwards at the Department of Medicine, Health Sciences Center at San Antonio (San Antonio, TX). Cell stocks were kept in minimal essential medium supplemented with 5% dextran-coated charcoal-stripped calf serum, 6 ng/ml bovine insulin (Sigma), 100 units/ml penicillin, and 100 μg/ml streptomycin. All tissue culture reagents were obtained from GIBCO, including calf serum, unless otherwise stated. Cell stocks were kept in 162-cm^2 flasks (Costar Corp., Cambridge, MA) in a humidified atmosphere of 95% air/5% CO_2, at 37°. All experiments were conducted on cells in continuous passage between passages 465 and 475.

In addition, MDA-MB-231 cells, a hormone-independent breast cancer cell line obtained from the American Type Culture Collection, were also used in parallel experiments with the MCF-7 cell line. All culture conditions were the same as previously described for MCF-7 cells, except for the replacement of dextran-coated charcoal-stripped calf serum with whole calf serum.

Hormones. 17β-Estradiol was purchased from Sigma. f.r. compounds (Figs. 1 and 2) were synthesized as described previously (8, 9). 4-OHT was obtained from ICI Pharmaceuticals (Macclesfield, England). All compounds were prepared in a concentrated form in 100% ethanol and diluted in cell culture medium. Final ethanol concentrations in the media never exceeded 0.2%.

Growth-response studies. T47D cells were plated into T150 flasks and incubated with phenol red indicator-free medium (12–15), containing 10% dextran-coated charcoal-stripped serum, for 10 days before being plated into 24-well dishes. Medium was changed every 3 days. On day 0 of the experiment, cells were plated into 24-well dishes at 1.0 × 10^5 cells/well. Medium containing compounds was added on day 1, and cells were allowed to grow for 6 days. Medium containing compounds was replaced on day 4. On day 7, cells were washed with complete HBSS and harvested for DNA assays. Cells were treated with 1 ml/well hypotonic calcium/magnesium-free HBSS and were sonicated for 12 sec/well with a Kontes ultrasonic cell disrupter. Samples (50–100 μl) were taken for DNA determinations. Assays for DNA were performed, according to the method of LaBarca and Paigen (16), by incubation of the samples with Hoechst dye 33258 (Calbiochem-Behring Corp., La Jolla, CA). Samples were analyzed on a SLM-Aminco Fluoro-Colorimeter III. All points for each DNA measurement represent a mean ± standard error of three or four sampled wells.

For growth-response studies with the MCF-7 and MDA-MB-231 cell lines, approximately 1.4 × 10^4 cells were plated directly into 24-well dishes (Costar Corp., Cambridge, MA) and incubated for 24 hr with phenol red indicator-containing medium with 5% dextran-coated charcoal-stripped serum. On the following day, the medium was changed to phenol red indicator-free medium with the same concentration of serum, and subsequent changes were made for 2 more days. Twenty-four hours later, medium containing compounds was added and changed daily as the cells were allowed to grow for 5 days. On day 5, cells were harvested for DNA assays. Cells were treated with 1 ml/well hypotonic calcium/magnesium-free HBSS and sonicated for 20 sec with a Kontes

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ultrasonic cell disrupter. Samples (50 μl) were taken for DNA determinations as described above. All points for each DNA measurement represent a mean ± standard error of three sampled wells.

Inhibition of the binding of [^{3}H] to the ER. Uteri from immature rats were dissected free from adhering tissues, frozen, and stored at −70° until use. Cytosols (100,000 × *g* supernatants) were prepared and the assay was performed as previously described (17). The RBA was calculated from the following relationship: RBA = (molar concentration of estradiol for 50% inhibition/molar concentration of competitor for 50% inhibition) × 100.

Immature rat uterine weight test. To determine estrogenic activity, immature female rats (35–50 g of body weight) were randomly allocated into groups (six rats/group), and estradiol or test compounds were administered (subcutaneously in 0.1 ml of peanut oil) on 3 consecutive days. In antiestrogenic tests, estradiol (0.08 μg daily) was administered with varying daily doses of test compounds on 3 consecutive days. Control group received peanut oil alone. On day 4, the rats were killed by stunning and cervical dislocation, and the uteri were dissected out, cleaned of adhering fat, blotted dry of intrauterine fluid, and weighed wet on a torsion balance.

Results

Nonisomerizable triphenyl ring compounds were used to determine the relationship between the hydroxyl and aminoethoxy side chain constituents of 4-OHT (Figs. 1 and 2). All of the compounds tested contain a fused seven-membered ring, which prevents their isomerization around the central double bond (Fig. 1).

Compounds **A** and **B** both contain a hydroxyl group substituted *para* to the β ring of the triphenyl ring structure (Fig. 2). Compound **A** contains an aminoethoxy side chain *para* in the α′ ring, whereas compound **B** contains an aminoethoxy side chain *para* in the α ring (Fig. 2). Compounds **C** and **D** both have an aminoethoxy side chain group substituted *para* to the β ring. Compound **C** contains a hydroxyl group *para* in the α′ ring, whereas compound **D** contains a hydroxyl group *para* in the α ring (Fig. 2).

All compounds were tested for their effects on the growth of T47D cells in culture. 17β-Estradiol (EC_{50} = 3 × 10^{-12} M) stimulated the growth of T47D cells 4-fold over control (Fig. 3A). Compound **A**, compound **C**, and f.r.-*trans*-4-OHT had little agonist activity, whereas compound **B** and compound **D** were partial agonists. Compound **B** was approximately 10 times more potent than compound **D** at stimulating the growth of T47D cells.

Compounds **E** and **F** (referred to as f.r.-*trans*-metabolite E and f.r.-*cis*-metabolite E, respectively, in Ref. 18) lack an aminoethoxy side chain substitution (Fig. 2). These compounds contain a hydroxyl group substitution either *para* in the α′ ring (compound **E**) or *para* in the α ring (compound **F**). Compound **E** and compound **F** were tested against compounds **C** and **D** to determine the effect on biological activity of the addition of an aminoethoxy side chain *para* in the β ring (Fig. 3B). Compound **F** was a full agonist in this system (EC_{50} = 6 × 10^{-11} M), whereas related compound **E** was only a weak partial agonist (Fig. 3B). The addition of an aminoethoxy side chain to compound **F**, producing compound **D**, decreased the potency and activity of the compound, making it a partial agonist. Surprisingly, addition of the aminoethoxy side chain to compound **E**, producing compound **C**, abolished any agonist activity.

Compounds that lacked any agonist activity (f.r.-4-OHT, compound **A**, and compound **C**) were then tested in combination with 17β-estradiol (0.1 nM) to determine whether these

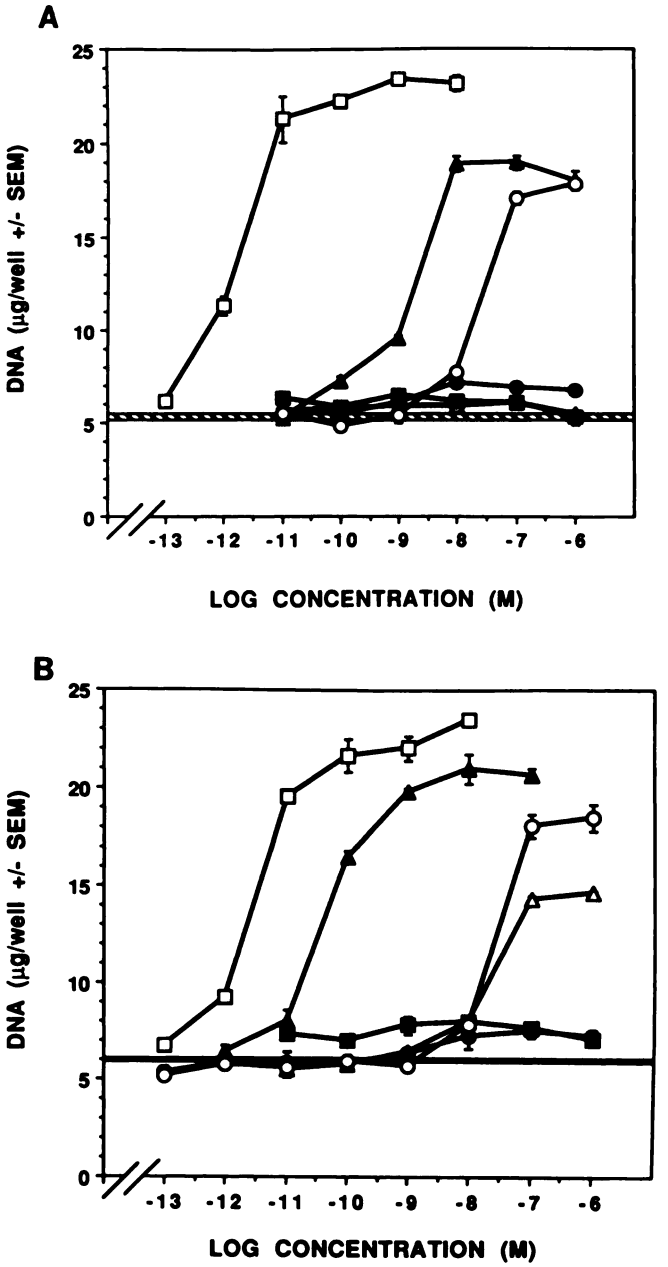

Fig. 3. Growth response of T47D cells to increasing concentrations of test compounds. T47D cells were deprived of estrogen for 10 days, and 1 × 10^5 cells/well were plated into 24-well tissue culture dishes. Cells were harvested after 6 days of treatment with compounds, and the amount of total DNA/well was determined. A, □, 17β-Estradiol; △, compound **A**; ▲, compound **B**; ●, compound **C**; ○, compound **D**; ■, f.r.-4-OHT; ▧, control. B, □, 17β-Estradiol; ▲, compound **F**; △, compound **E**; ●, compound **C**; ○, compound **D**; ■, f.r.-4-OHT; ▧, control.

compounds were antagonists (Fig. 4). As expected, f.r.-4-OHT was a potent antiestrogen (IC_{50} = 3 × 10^{-11} M). Both compounds **A** and **C** were antiestrogenic and completely inhibited the stimulation of cell growth by 17β-estradiol (0.1 nM) (Fig. 4). Compound **A** (IC_{50} = 8 × 10^{-9} M) was more potent than compound **C** (IC_{50} = 3 × 10^{-8} M) at inhibiting 17β-estradiol-stimulated cell growth.

The results from the concentration-response experiments with T47D cells were confirmed using MCF-7 cells (ER positive). A full concentration-response curve for compound **B**, with and without estradiol (0.1 nM), demonstrated (Fig. 5) that

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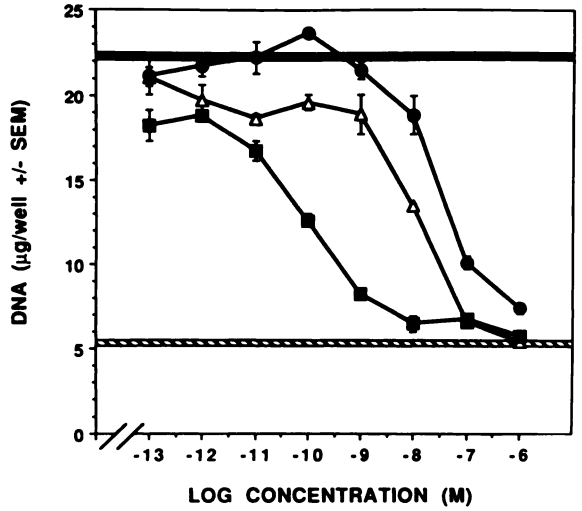

Fig. 4. Inhibition of 17β-estradiol-stimulated cell growth by compound **A**, compound **C**, and f.r.-4-OHT at increasing concentrations. T47D cells were deprived of estrogen for 10 days, and 1 × 10^5 cells/well were plated into 24-well tissue culture dishes. Cells were harvested after 6 days of treatment with compounds, and the amount of total DNA/well was determined. ■, f.r.-*trans*-4-OHT plus 17β-estradiol (0.1 nM); △, compound **A** plus 17β-estradiol (0.1 nM); ●, compound **C** plus 17β-estradiol (0.1 nM); ■, 17β-estradiol (0.1 nM); ▩, control.

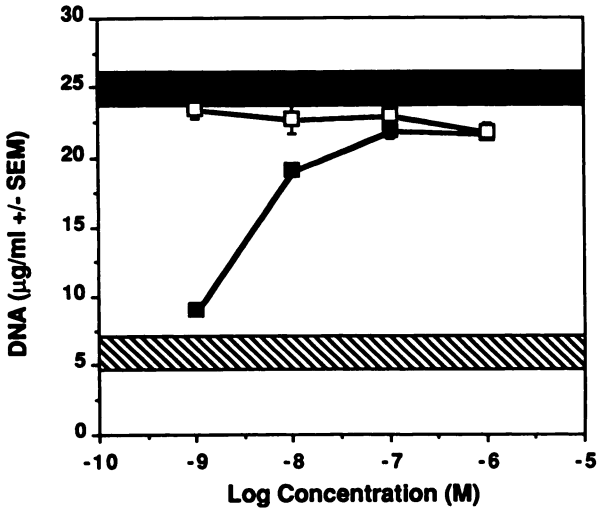

Fig. 5. Growth response of MCF-7 cells to increasing concentrations of compound **B**. MCF-7 cells (1.4 × 10^4 cells/well) were plated in 24-well tissue culture dishes and deprived of estrogen. Cells were harvested after 5 days of treatment with various concentrations of compound **B**, with (□) or without (■) estradiol (0.1 nM), or estradiol alone (■). Controls (▩) were cultured in medium alone. Results represent DNA levels/well, with three determinations/concentration.

the estrogenic properties were equivalent in both MCF-7 and T47D (Fig. 3A) cell lines. All compounds were then tested, alone or with estradiol (0.1 nM), at two concentrations (10 nM and 100 nM) to determine their estrogenic or antiestrogenic activity. A single concentration (1 µM) of compounds was used to determine non-ER-mediated cytotoxic effects on the breast cancer cell line MDA-MB-231 (ER negative).

Compounds **A** and **C** were weak antiestrogens, compared with 4-OHT or its f.r. derivative (Fig. 6A), whereas compounds **F** and **B** were more potent estrogens, compared with compounds **D** and **E** (Fig. 6B). Each of the responses was consistent with the results predicted from the concentration-response curves using T47D cells. None of the compounds produced a cytotoxic effect with the MDA-MB-231 cells, indicating that the antiproliferative effects on the cells were produced via an ER-mediated mechanism.

The RBA for each of the compounds was determined for rat uterine ER. 4-OHT and its f.r. derivative had the highest RBAs, relative to estradiol, and, consistent with the cell culture data, the antiestrogenic compounds **A** and **C** had low RBAs (Table 1). Similarly, estrogenic compound **F** had a high RBA, whereas compounds **B**, **D**, and **E** had lower RBAs (Table 1). Although pharmacological potency can be estimated using the RBAs (except that compounds **B** and **D** had reversed RBAs, compared with their biological potencies in cell culture), the pharmacological properties cannot be determined. The estrogenic and antiestrogenic activity was compared in the rat uterine weight test.

Compounds **E**, **F**, **B**, and **D** were estrogenic *in vivo* and increased uterine weight in a dose-related manner. Compound **F** was the most potent (Fig. 7), and compounds **E**, **B**, and **D** had potencies approximately 5–10% that of compound **F**. The antiestrogens, 4-OHT, f.r.-4-OHT, compound **A**, and compound **C**, were partial agonists (Fig. 8A), compared with the estrogenic test compounds (Fig. 8B), and displayed antiestrogenic potencies (Figure 8B) consistent with the potencies noted with the ER-positive breast cancer cells.

Discussion

Structure-activity relationship studies have provided valuable information about the features of ER ligands that are important for agonist or antagonist activity (17–22). However, studies of this nature have been difficult, because many hydroxylated triphenylethylenes are unstable and readily isomerize between their *cis* and *trans* forms (6, 7). As a result of isomerization, it has been impossible to determine the relationship between the hydroxyl and aminoethoxy side chain constituents of 4-OHT, a potent antiestrogenic metabolite of TAM (4). Recently, nonisomerizable analogues of 4-OHT have been synthesized (7, 8), and related compounds that contain the hydroxyl and aminoethoxy groups in a variety of positions around the molecule have now been used to determine the influence of the positioning of these constituents on the biological activity of the ligand.

We have determined the estrogenic and antiestrogenic properties of the compounds with estrogen-responsive T47D and MCF-7 breast cancer cells *in vitro* and confirmed the results *in vivo* using the immature rat uterine weight test. There are numerous reports on the use of MCF-7 cells (6, 23–25) and uterine weight tests (5, 26) to evaluate structure-activity relationships, and we have previously characterized (11, 18, 27) our particular line of T47D cells for studies of estrogen and antiestrogen action.

Overall, the pharmacological classification of the compounds was consistent for each of the estrogen-responsive assay systems. The compounds all interacted with the ER, and only minor differences in potency were noted between studies *in vivo* and *in vitro*. There was no evidence that the compounds produced nonspecific cytotoxicity with MDA-MB-231 cells at a concentration of 1 µM, which exceeded the concentrations used to describe the concentration-response relationships for MCF-7 and T47D cells. We conclude that the compounds exert

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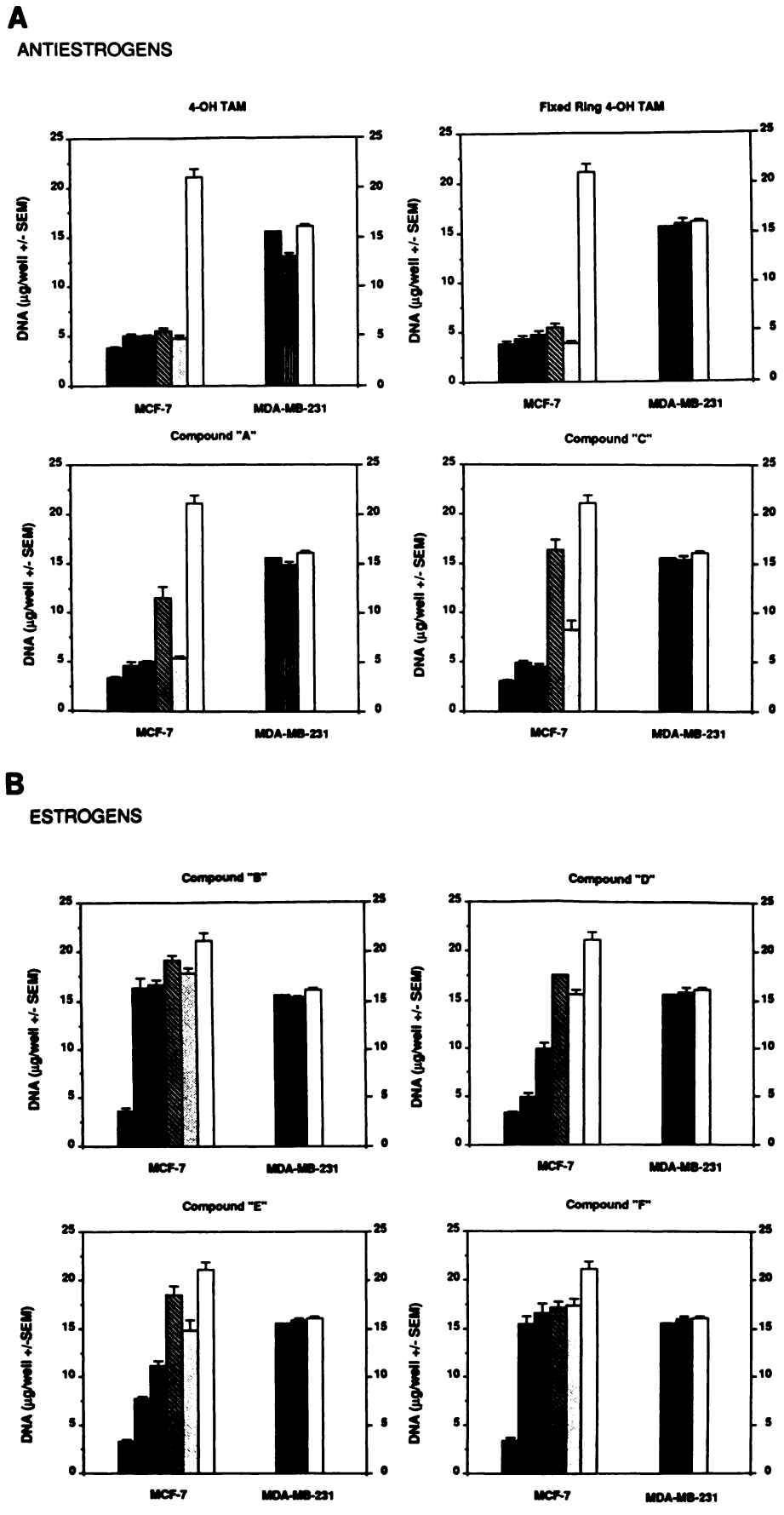

Fig. 6. Growth response of MCF-7 cells or MDA-MB-231 cells to antiestrogenic (A) or estrogenic (B) compounds. MCF-7 cells were cultured with two concentrations, 10 nM (■) and 100 nM (■), of compounds, with [10 nM plus estradiol (■) or 100 nM plus estradiol (■)] or without estradiol (0.1 nM) (□). ■, Control. MDA-MB-231 cells were incubated with 1 µM compounds (■) and compared with control (■) or estradiol (0.1 nM) (□).

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TABLE 1

RBA of compounds to inhibit specific [^{3}H]estradiol binding to rat uterine cytosol

The technical details and calculation of RBAs are described in Materials and Methods.

Compounds	RBA
Estradiol	100
4-OHT	75
f.r.-4-OHT	63
Compound A	29
Compound B	1
Compound C	5
Compound D	9
Compound E	2
Compound F	52

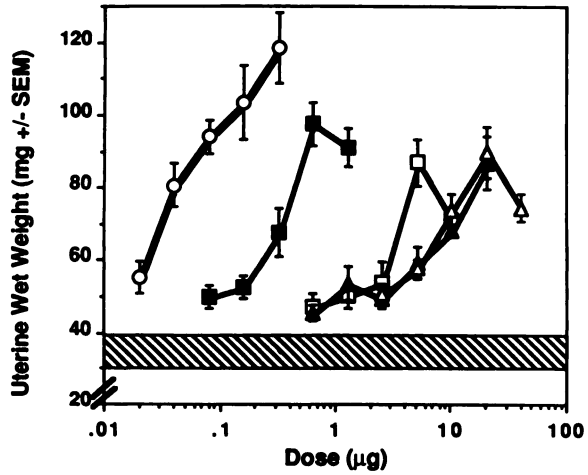

Fig. 7. Uterotrophic activity of the estrogen-like test compounds in the 3-day immature rat uterine weight test. Compounds [estradiol, (○), compound F (■), compound E (□), compound B (▲), compound D (△)] were injected subcutaneously daily, in 0.1 ml of peanut oil, on 3 consecutive days. Controls (■) received peanut oil alone. Animals were killed on day 5, and the uteri were weighed wet on a torsion balance. Results are mean ± standard error for six animals/group.

their control on breast cancer cell replication through an ER-mediated mechanism.

It appears that the substitution of an alkyl aminoethoxy side chain in the β ring area greatly decreases both the activity and the potency of the ligand. Compound **D** was much less effective than compound **F** at stimulating cell growth (Fig. 3). Previous studies have shown that large substitutions in the 17α-position of estradiol greatly decreased the estrogenic potency of the compound (28). It is probable that substitutions in this area hinder the folding of the ER protein around the ligand, which in turn may decrease the ability of the ER-ligand complex to stimulate the transcription of target genes.

Surprisingly, the addition of an aminoethoxy side chain to the β ring of compound **E** [producing compound **C** (Fig. 2)] converts the compound from a weak estrogen to a weak antiestrogen (Figs. 3B and 4). Based on previous models (3, 22), we propose that the antiestrogenic activity of compound **C** is not a direct result of substitution of the aminoethoxy side chain in the area analogous to the 17-position of estradiol. In previous studies (28) using estrogenic ligands, large substitutions at the 17α-position of estradiol decreased the potency of the compounds but did not convert them to antiestrogens. We propose that the antiestrogenic activity of compound **C** may result from a reorientation of the compound, placing its α' ring hydroxyl

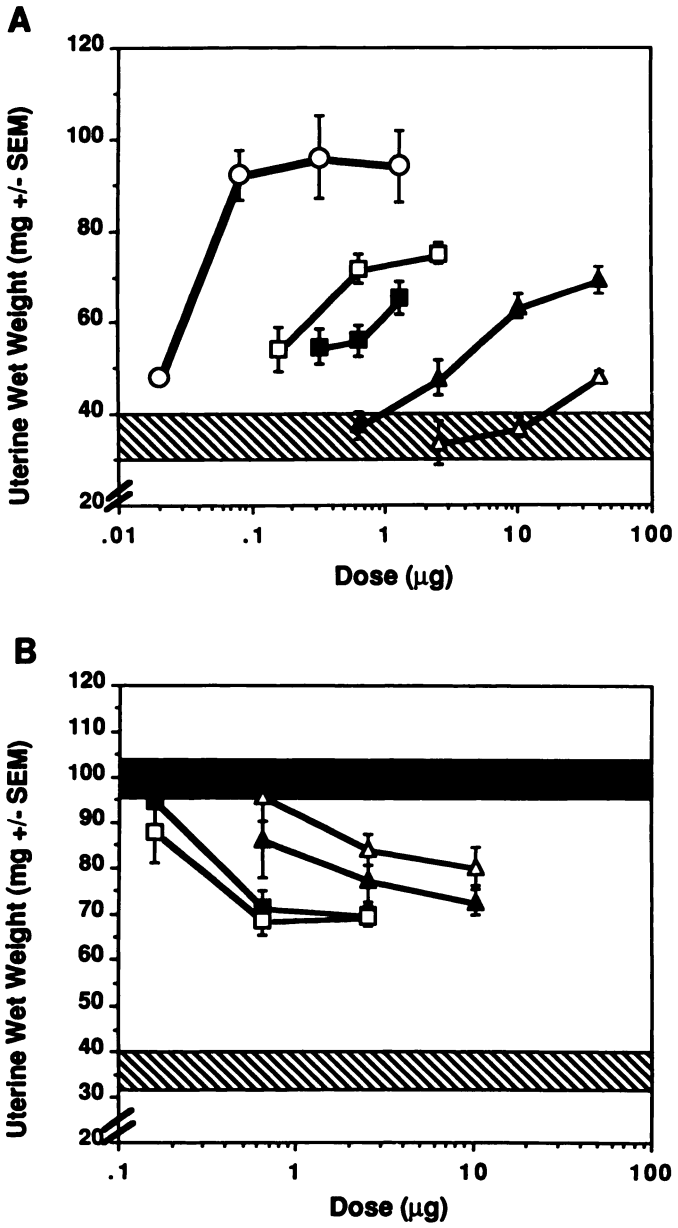

Fig. 8. The partial uterotrophic (A) and antiuterotrophic (B) activity of test compounds in the 3-day immature rat uterine weight test. A, Compounds [4-OHT (□), f.r.-4-OHT (■), compound A (▲), and compound C (△)] were administered alone and compared with estradiol (○). B, Compounds [4-OHT (□), f.r.-4-OHT (■), compound A (▲), and compound C (△)] were administered with estradiol (■) (0.08 μg daily). In both studies, control (■) was the administration of peanut oil injection vehicle alone. The method was as described in the legend to Fig. 7. Results are mean ± standard error for six animals/group.

group in a position analogous to that of the 3-hydroxyl of 17β-estradiol (Fig. 9). This reorientation would position the aminoethoxy side chain group of compound **C** in a position analogous to the 11β-position of 17β-estradiol. The known antiestrogen RU 39411 (28, 29) has a phenyl ring *para*-substituted with an aminoethoxy side chain at the 11β-position of estradiol (Fig. 9). Clearly, this is an area that can influence estrogenic action by preventing the correct folding of the ER around the ligand. The structural similarities between compound **C** and RU 39411 suggest that they may exert their antiestrogenic activity through a similar mechanism.

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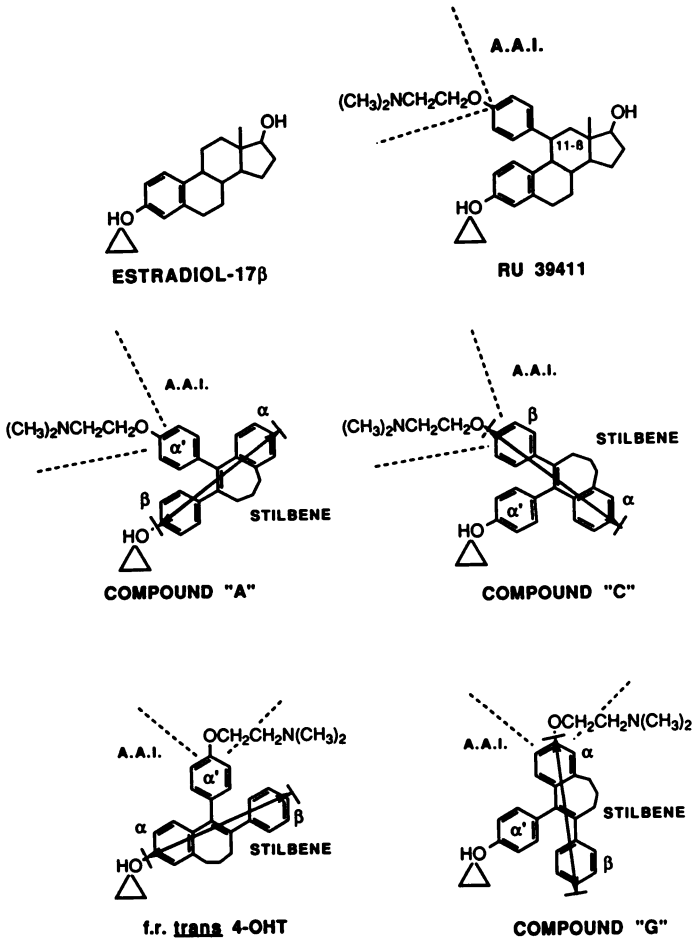

Fig. 9. Comparison of the structures of 17β-estradiol, RU 39411, compound **A**, compound **C**, f.r.-4-OHT, and compound **G**. The hydroxy groups of compounds **A**, **C**, and **G** are oriented with regard to the 3-hydroxyl of 17β-estradiol. Δ, High affinity binding site of the ER, which interacts with the phenolic hydroxyl group of the ligand. *A.A.I.*, area of antiestrogenic influence. *Double-headed arrows* emphasizes the stilbene-like structure.

It is of interest to note that compound **G** (Fig. 2) (referred to as *cis*-f.r.-4-OHT in Ref. 18) is also antiestrogenic in this cell culture system ($IC_{50} = 4 \times 10^{-8}$ to 2×10^{-7} M) (18). Based on early studies (6), it was possible that this isomer would be estrogenic, due to the lack of the aminoethoxy side chain on the α' ring. However, reorientation of this molecule with respect to its hydroxyl group could explain its weak antiestrogenic properties (Fig. 9). If the compound interacts with the binding site of the ER through its hydroxyl group, its unsubstituted phenyl ring, which forms a stilbene structure, could be positioned across the area optimally occupied by a stilbene-like structure (Fig. 9, compare with f.r.-*trans*-4-OHT). The steric hindrance produced by this ring might be expected to produce a compound with low affinity for the ER protein.

A similar argument could be developed to explain the observation that compound **A** is more potent than compound **C** and has a higher RBA (Table 1). The orientation of compound **A** at the ligand binding site could involve a favorable stilbene-like structure that occupies the site, whereas compound **C** has a less favorable fit, with the stilbene across the hypothetical binding cleft (Fig. 9).

We conclude from these studies that the positioning of the hydroxyl and aminoethoxy side chain groups is critical for determining both the activity and the potency of the triphenyl compounds. If the ligand is anchored to the ligand binding site at the α ring of a triphenylethylene, the area of the β ring should be unhindered to produce optimal potency. Substitution of the α' ring with an alkylaminoethoxy side chain is optimal for antiestrogenic activity if the α ring has a 4-hydroxyl group. If the α' ring has a 4-hydroxyl group, then either α or β rings can contain a 4-alkylaminoethoxy side chain to produce an antiestrogen. These findings will be useful in predicting the molecular mechanisms involved in the folding of the ligand-ER complex, once the ER protein has been purified, bound by ligand, and subjected to X-ray crystallography.

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