Synthesis, Structure Elucidation, and Biochemical Evaluation of 7α- and 7β-Arylaliphatic-Substituted Androst-4-ene-3,17-diones as Inhibitors of Aromatase

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The inhibition of aromatase, the cytochrome P450 enzyme complex responsible for the conversion of androgens to estrogens, may be useful for the endocrine treatment of breast cancer. Previously, several 7α-thio-substituted androstenediones have been shown to be potent inhibitors of aromatase. Recent research has focused on producing a more metabolically stable aromatase inhibitor by replacing the carbon–sulfur bond at the 7α-position with a carbon–carbon bond. The new inhibitors, 7α-arylaliphatic-substituted androst-4-ene-3,17-diones (**2**–**4**), have alkyl chains of varying length between the steroid and the aryl ring at the 7α-position. The desired targets were synthesized via a 1,6-conjugate addition of the appropriate cuprate to 17β-(*tert*-butyldimethylsiloxy)androsta-4,6-dien-3-one (**7**). The synthesis also resulted in the formation of the 7β-substituted diastereomers (**10**–**11** and **13**) as minor products. Initial assignments of the 7α-phenethyl and 7β-phenethyl diastereomers were made using highfield 1-D and 2-D NMR studies. The assignment of the diastereomers was confirmed using X-ray crystallography. These compounds were all good inhibitors of aromatase *in vitro* when assayed using microsomes isolated from human placenta. The 7α-substituted androst-4-ene-3,17-diones (**2**–**4**) were effective inhibitors with apparent K_is of 13–19 nM. The corresponding 17β-hydroxy analogs (**8** and **14**) and the 7β-substituted androstenediones (**13** and **16**) were less effective inhibitors with apparent K_is of 36–44 nM. Thus, a new series of 7α-arylaliphatic-substituted androst-4-ene-3,17-diones has been synthesized, and the compounds are potent competitive inhibitors of aromatase.

Breast cancer is the second major cause of mortality in women. A startling fact is that one out of every eight women in the United States will get breast cancer within her lifetime.[1] Due to the high incidence and the profound impact breast cancer has on women, it has become one of the most feared cancers. Numerous approaches to the treatment of breast cancer exist, including the use of endocrine treatment to manipulate a woman's hormones and hormonal responses. This approach is useful in cases in which the tumors are dependent upon hormones (i.e., estrogens) to grow. Inhibition of aromatase is an attractive approach to endocrine therapy. Aromatase is a cytochrome P450 enzyme complex which is responsible for the conversion of androgens to estrogens in the final step of the steroid biosynthetic pathway. Thus, by inhibiting this enzyme complex, the levels of estrogen in the body will be reduced, decreasing the levels of estrogen available for the tumor to use to continue its growth.

Aromatase inhibitors, such as 4-hydroxyandrostenedione and aminoglutethimide, have been shown to be clinically useful in the treatment of advanced breast cancer.[2–5] These drugs are examples of the two main types of aromatase inhibitors: steroidal[6] and nonsteroidal.[7] The main disadvantage of the nonsteroidal inhibitors can be their lack of specificity for the aromatase enzyme. Although steroidal inhibitors are more specific for the aromatase enzyme, they may have some other inherent hormonal activity. The structure activity relationship for steroidal aromatase inhibitors has been extensively investigated.[8,9] Research has shown that the C-19 methyl and the carbonyls at C-3 and C-17 are necessary for high affinity. Studies also indicate that a small group can be tolerated at C-1, C-4, and C-19 while bulky substituents can be tolerated at the 7α-position. 7α-Thio-substituted androstenediones, such as 7α-APTA (**1**, Figure 1), have been synthesized and evaluated for inhibition of aromatase in our laboratory.[10–12] Our results suggest that the aryl group at the 7α-position increases the affinity of the inhibitor for the enzyme.[10–12] 7α-APTA (**1**) has been shown to be a potent inhibitor of aromatase. The aryl ring is connected to the steroid nucleus by a carbon–sulfur bond, and this bond may be a possible site for metabolism *in vitro* and *in vivo*. Replacement of the carbon–sulfur bond at the 7α-position with a carbon–carbon bond may result in a more metabolically stable compound. Replacement of the sulfur with the carbon atom will also address the question of whether the thioether is important for the activity of 7α-APTA (**1**) as an inhibitor of aromatase.

This paper describes the synthesis of androstenediones which have an alkyl chain of varying length between the aryl ring and the steroid nucleus at the 7α-position (**2**–**4**). Structure elucidation of the 7α- and 7β-diastereomers produced, results from the inhibitor screen, and kinetic analysis of the new 7-substituted androgens are also described.

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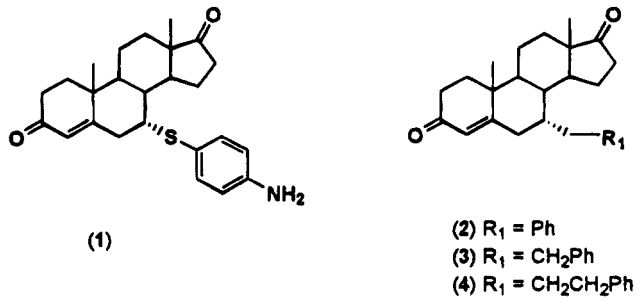
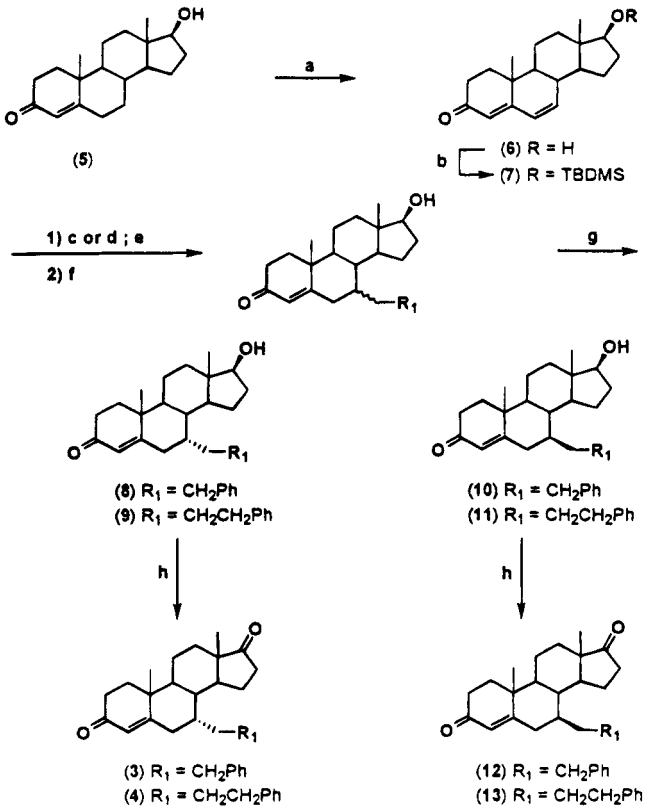


Figure 1. Structures of 7α-APTA and the 7α-arylaliphatic-substituted androstenediones.

Scheme 1[a]



[a] (a) Chloranil, *t*-BuOH, reflux; (b) TBDMSCl, imidazole, DMF; (c) phenethyl iodide, *t*-BuLi, ether, −78 °C; (d) phenpropyl iodide, *t*-BuLi, ether, −78 °C; (e) [CuI{(*n*-Bu)$_3$P}]$_4$, ether, −40 °C; (f) 6 N HCl, THF, room temperature; (g) separation (MPLC; SiO_2); (h) PCC; CH_2Cl_2, room temperature.

Chemistry

A logical approach to the synthesis of the desired 7α-arylaliphatic-substituted androgens would be by 1,6-conjugate addition.[13,14] Previous work has shown that alkyl chains can be introduced at the 7α-position via a 1,6-conjugate addition of the cuprate of a Grignard or lithium reagent to the dienone analog of testosterone.[15,16] The introduction of an aryl group at the 7α-position has only been reported for 19-nortestosterone analogs.[17]

The synthesis of the desired targets began with the oxidation of testosterone (**5**) using tetrachlorobenzoquinone to the known dienone **6** as shown in Scheme 1. The TBDMS-protected testosterone analog (**7**) was prepared from **6** in 88−92% yield and served as the starting point for the rest of the synthesis of the desired compounds. Previous attempts at the 1,6-conjugate addition reaction using other protecting groups (MOM ether, THP ether, and acetate) were unsuccessful.

Introduction of the phenethyl or phenpropyl substituents was achieved by the addition of the appropriate cuprate reagent as shown in Scheme 1. The lithium reagent was generated *in situ* from the corresponding iodide by reaction with *t*-BuLi in ether at −78 °C. The cuprate was formed by reacting 1 equiv of tetrakis[iodo(tri-*n*-butylphosphine)copper(I)], ([CuI{(*n*-C_4H_9)$_3$P}]$_4$),[19] with the lithium reagent at −20 °C in ether.[20,21] This copper complex was chosen due to its solubility in ether, and prior experiments with other copper salts (CuI, CuCN, and CuBr·DMS) did not produce the desired 1,6-conjugate addition products. The steroid (0.2 equiv) in ether was then reacted with the cuprate at −40 °C for 3 h and quenched with argon-purged saturated ammonium chloride. It was important to stringently remove oxygen from the reaction system in order to obtain the desired 1,6-conjugate addition product. The conjugate addition resulted in a mixture of α- and β-diastereomers. After initial purification, the mixture of diastereomers was deprotected to the corresponding alcohols. Initial deprotection attempts using TBAF were not successful; however, 6 N HCl in THF proved to cleanly cleaved the silicon−oxygen bond giving the desired alcohols as a mixture of diastereomers. The diastereomers were then purified and separated using MPLC to the 17β-hydroxy-7α-substituted-androst-4-en-3-ones (**8** and **9**) and the 17β-hydroxy-7β-substituted-androst-4-en-3-ones (**10** and **11**).

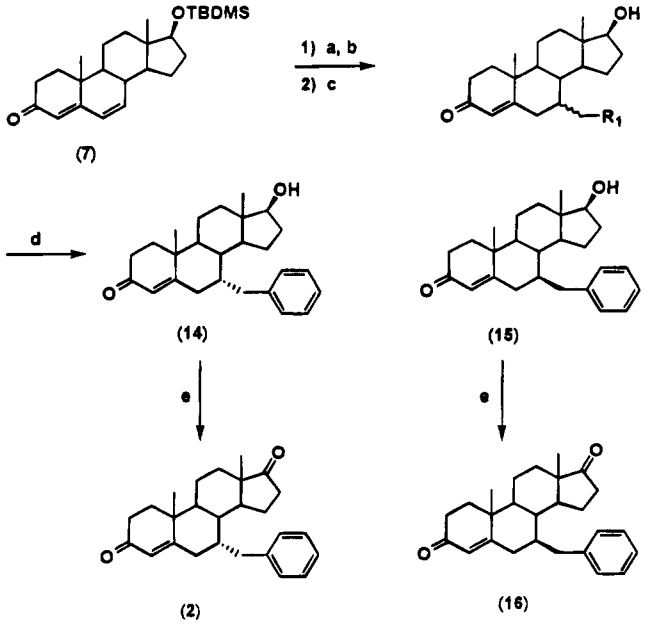
The 17β-hydroxy-7-phenethyl derivatives (**8** and **10**) were obtained in an overall yield of 71% as a 2:1 mixture of α:β diastereomers. NMR experiments described in the next section were used to differentiate between the α- and β-diastereomers; confirmation of the stereochemistry was obtained by X-ray crystallography. The 17β-hydroxy-7-phenpropyl derivatives (**9** and **11**) were obtained in an overall yield of 50% as a 1:1 mixture of α:β diastereomers. The testosterone derivatives (**8**−**11**) were then oxidized to the corresponding 7α-substituted androst-4-ene-3,17-dione derivatives (**3** and **4, 12** and **13**) using PCC in dry CH_2Cl_2 at room temperature in quantitative yield.

A similar approach was utilized for the introduction of the benzyl substituent as shown in Scheme 2. The major difference was that a Grignard formed from benzyl bromide and magnesium was used. The amount of the tetrakis[iodo(tri-*n*-butylphosphine)copper(I)] used was catalytic rather than equimolar. The use of less copper complex allowed for immediate deprotection of the TBDMS ether without any prior purification. A 33% overall yield of the 17β-hydroxy-7-benzyl-substituted analogs (**14** and **15**) was obtained as a 5:1 mixture of α:β diastereomers. The other main product from the synthesis resulted from 1,2-addition of the benzyl group. The diastereomers were purified and separated using MPLC. 7α- And 7β-benzyl-17β-hydroxyandrost-4-en-3-ones were oxidized to their corresponding androgens (**2** and **16**) using PCC in quantitative yield.

Structure Elucidation

High-field 1-D and 2-D NMR studies were utilized for the structure determination of the two 7-phenethylandrostenedione diastereomers. Initial assignments of the carbon and proton resonances for each diastereomer (**3** and **10**) were made using one-dimensional proton, DEPT, COSY, and C−H correlation experiments. The

Scheme 2[a]



[a] (a) Benzyl bromide, Mg, ether, room temperature; (b) [CuI{(n-Bu)$_3$P}]$_4$, ether, −40 °C; (c) 6 N HCl, THF, room temperature; (d) separation (MPLC; SiO_2); (e) PCC, CH_2Cl_2, room temperture.

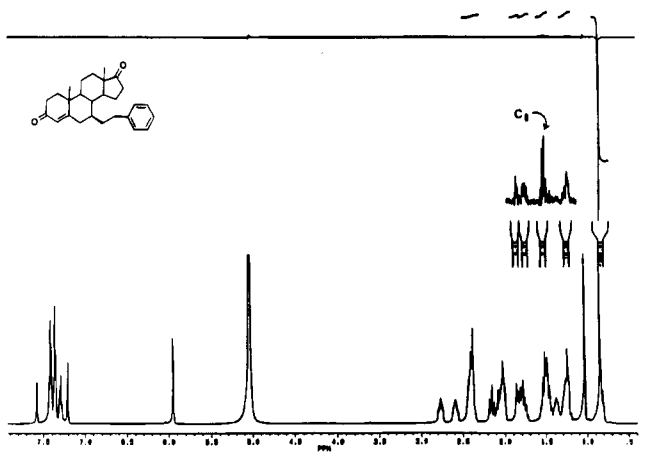


Figure 2. NOE difference spectrum for compound **12** with irradiation at the C-18 protons. The normal NOE spectrum is at the top, an enlargement of NOE spectrum between 1.1 and 2.2 ppm is in the middle, and the ordinary proton spectrum is at the bottom.

upfield region of the spectra for these steroids was complex, making many assignments difficult. Since the resonances of most of the protons which could aid in the differentiation of the two diastereomers were overlapped, NOE difference experiments were utilized. NOEs were measured after irradiation of the protons on the C-4, C-18, and C-19 positions, all of which were well-resolved within each spectrum.

These NOE difference spectra allowed for observation of the J couplings between the more important and useful protons. For example, in Figures 2 and 3 the irradiation of the protons on C-18 for each diastereomer (**3** and **12**) showed large NOEs to the C-8 proton along with other enhancements. On the basis of the structure of the β-diastereomer (**12**), it would be expected that the C-8 proton should have three axial−axial interactions with the C-9, C-14, and C-7α protons with coupling constants of about 10 Hz each. In fact, the experiment showed a doublet of doublets of doublets (ddd) which

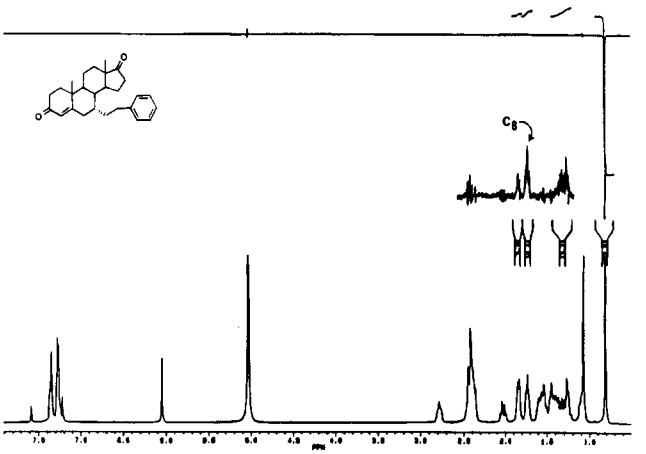


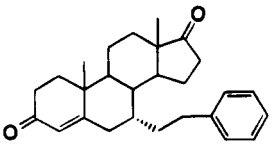
Figure 3. NOE difference spectrum for compound **3** with irradiation at the C-18 protons. The normal NOE spectrum is at the top, an enlargement of NOE spectrum between 1.2 and 2.7 ppm is in the middle, and the ordinary proton spectrum is at the bottom.

appeared as an apparent quartet with couplings of 10.1 Hz. The NOE enhancement of the proton on C-8 for compound **12** was 9.8% and was one of four NOE's observed after irradiation of the protons on C-18 (Figure 2). On the other hand, the C-8 proton of the α-diastereomer (**3**) should exhibit two axial−axial interactions with the C-9 and C-14 protons and one possible axial−equatorial interaction with the C-7β proton. The NOE difference spectrum showed an apparent triplet (dd) for the C-8 proton with a coupling constant of 9.8 Hz (Figure 3), consistent with the two axial−axial coupling interactions predicted. However, the axial−equatorial interaction was not seen even at 500 Mhz. The NOE enhancement of the C-8 proton for compound **3** was 10.4% and was one of three NOE's observed upon the irradiation of the proton on C-18. Similar results were obtained when the irradiation of the protons on C-19 of the two diastereomers were conducted. Confirmation of the initial assignment of the C-8 proton was obtained by COLOC experiments on both the α- and β-diastereomers (**3** and **12**).

Irradiation of the proton on C-4 produced results which were consistent with the assignments made from the irradiation of the protons on C-18. Irradiation of the proton on C-4 produced a single enhancement in both diastereomers which corresponds to the α-proton on the C-6 carbon. In the β-diastereomer (**12**), the enhancement of the 6α-proton was 7.6%, and it appeared as a doublet of doublets with coupling constants of 5 and 14.5 Hz. These coupling constants were in agreement with the axial-equatorial interaction between the 6α- and 7α-protons and a geminal interaction between the 6α- and 6β-protons, as expected for the structure of the β-diastereomer (**12**). In the α-diastereomer (**3**), the enhancement of the 6α-proton was 7.3% and showed a doublet with a coupling constant of 14.5 Hz. This coupling constant results from the geminal interaction between the 6α- and the 6β-protons. However, while the assigned structure of the α-diastereomer suggests a possible equatorial−equatorial interaction, it was not observed even at 500 Mhz.

On the basis of these NMR experiments, the assignment of the structure of the two diastereomers of the 7-phenethyl-substituted androgens was made (Tables 1 and 2). In order to confirm the diastereomer NMR

Table 1. NMR Analysis of Compound **3**



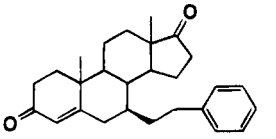
carbon	^{13}C chemical shift	^{1}H chemical shift
C-1	36.06 (t)	1.48
		1.81
C-2	36.26 (t)	2.41
		2.41
C-3	197.96 (s)	–
C-4	126.40 (d)	6.04
C-5	168.83 (s)	–
C-6	34.46 (t)	2.41 (d), J = 14.5 Hz
		2.41
C-7	38.51 (d)	1.68
C-8	35.36 (d)	1.70 (t), J = 9.8 Hz
C-9	46.56 (d)	1.07
C-10	38.92 (s)	–
C-11	20.82 (t)	1.25
		1.42
C-12	31.68 (t)	1.22
		1.78
C-13	47.63 (s)	–
C-14	46.56 (d)	1.39
C-15	21.31 (t)	1.28
		1.55
C-16	35.67 (t)	1.97
		2.41
C-17	219.29 (s)	–
C-18	13.54 (q)	0.76
C-19	17.84 (q)	1.02
C-1′	27.87 (t)	1.37
		1.52
C-2′	33.73 (t)	2.41
		2.66 (ddd), J = 5.0, 9.3, 13.8 Hz
ArH	126.33 (d)	7.25 (m)
ArH	128.87 (d)	7.19–7.36 (m)
ArH	129.05 (d)	7.19–7.36 (m)
ArH	142.91 (s)	–

assignments, crystals of the major diastereomer (**3**) were grown and submitted for X-ray diffraction analysis. The structure from the X-ray crystal analysis can be found in Figure 4. The crystal structure confirms that the NMR assignment of the major diastereomer as the α-diastereomer was correct. Thus, the major diastereomer produced in the synthesis was the desired 7α-phenethylandrogen.

Biochemistry

7α-Arylaliphatic-substituted androst-4-ene-3,17-diones (**2**–**4**), the corresponding testosterone analogs (**8** and **14**), and the 7β-arylaliphatic-substituted androst-4-ene-3,17-diones (**12** and **16**) were tested *in vitro* using human placental microsomes. The activity of aromatase in the placental microsomes was determined by a radiometric assay developed by Sitteri and Thompson[22] in which the tritium from [1β-^{3}H]androst-4-ene-3,17-dione was released as tritiated water during aromatization. The amount of $^{3}H_2O$ released during the assay was used as an index of estrogen production. Initially an inhibitor screening assay was performed in which two concentrations of inhibitor and a single concentration of androstenedione were assayed. The amount of inhibition was compared to a control in which there was no inhibitor present. The results of the inhibitor screening assay are shown in Table 3.

Table 2. NMR Analysis of Compound **12**



carbon	^{13}C chemical shift	^{1}H chemical shift
C-1	35.92 (t)	1.50
		1.64
C-2	34.42 (t)	2.40
		2.40
C-3	198.36 (s)	–
C-4	123.50 (d)	5.95 (d), J = 1.5 Hz
C-5	169.66 (s)	–
C-6	38.94 (t)	2.18 (ddd), J = 1.9, 12.2, 14.3 Hz
		2.40 (dd), J = 5.0, 14.5 Hz
C-7	41.88 (d)	1.38
C-8	39.68 (d)	1.56 (q), J = 10.1 Hz
C-9	54.45 (d)	0.79
C-10	38.43 (s)	–
C-11	21.29 (t)	1.28
		1.52
C-12	32.19 (t)	1.25
		1.68
C-13	48.70 (s)	–
C-14	52.06 (d)	1.30
C-15	25.82 (t)	1.60
		2.01
C-16	36.24 (t)	2.12
		2.40
C-17	219.71 (s)	–
C-18	14.33 (q)	0.86
C-19	17.45 (q)	1.06
C-1′	38.03 (t)	1.54
		2.09
C-2′	33.26 (t)	2.59 (ddd), J = 5.9, 10.9, 13.5 Hz
		2.76 (ddd), J = 5.2, 11.2, 13.4 Hz
ArH	126.37 (d)	7.29 (m)
ArH	128.93 (d)	7.30–7.42 (m)
ArH	128.98 (d)	7.30–7.42 (m)
ArH	143.06 (s)	–

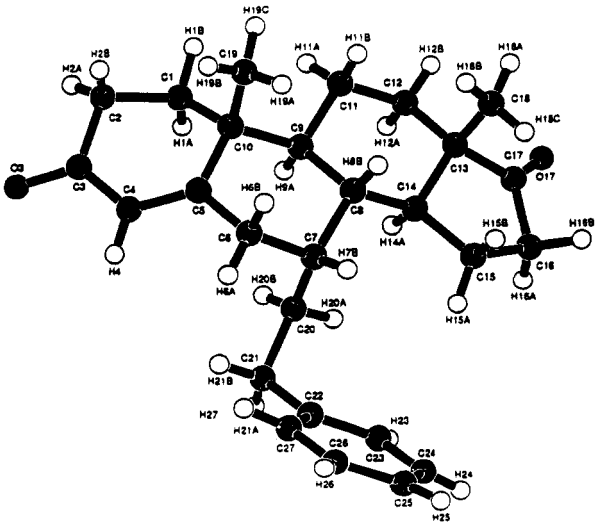
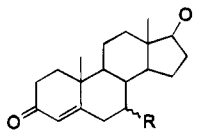


Figure 4. Ball and stick drawing of the X-ray crystal structure for compound **3**.

Kinetic studies were performed in which inhibitors were evaluated at concentrations ranging from 0 to 150 nM. The enzyme assays were run under initial velocity conditions of low product formation by limiting the

Table 3. Inhibition Screen for Various 7α- and 7β-Substituted Androgens



compound	R	functionality of 17-position	% inhibition 100 nM[a]	% inhibition 250 nM[a]
2	α-benzyl	ketone	64.33	81.53
3	α-phenethyl	ketone	66.14	81.57
4	α-phenpropyl	ketone	62.84	81.83
14	α-benzyl	β-hydroxy	59.44	72.46
8	α-phenethyl	β-hydroxy	45.79	56.67
16	β-benzyl	ketone	46.69	68.86
12	β-phenethyl	ketone	55.69	67.82
7α-APTA[b]	α-(4-aminophenyl))thio	ketone	70.59	83.89

[a] Each value represents the average of five or six samples. The percent inhibition was calculated with respect to an incubation which was without any inhibitor present. [b] See ref 10.

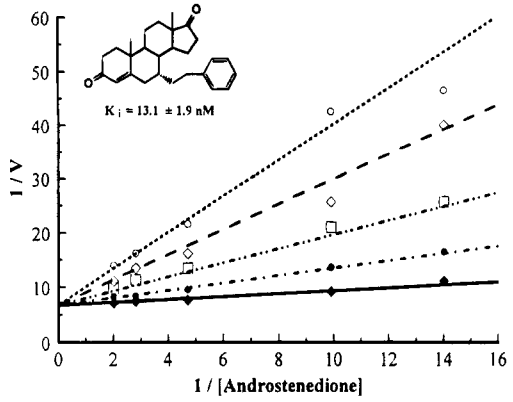


Figure 5. Lineweaver–Burk plot of aromatase inhibition by compound **3**. Various concentrations of androstenedione (70–500 nM) were incubated with microsomal enzyme preparations at inhibitor concentrations of 0 nM (◆), 20 nM (●), 50 nM (□), 100 nM (◇), or 150 nM (○). Velocity is expressed as nmol product per μg protein per minute and 1/[androstenedione] values have units of μmol^{-1}. Each point represents the average of three determinations with less than 10% variation from the mean.

enzyme concentration. During these kinetic assays, the inhibitor concentration was held constant while the concentration of androstenedione was varied from 70 to 500 nM. Each substrate concentration was run in triplicate, and the results were plotted in a typical Lineweaver–Burk plot as 1/velocity vs 1/[androstenedione]. Analysis of the data by weighted regression analysis[23] provided apparent K_is for the various analogs. The results of the assay for compound **3** are shown in the Lineweaver–Burk plot in Figure 5. The apparent K_is, the apparent K_ms, and the K_m/K_i ratios for compounds (**2–4, 8, 12, 14,** and **16**) are shown in Table 4. Each of the inhibitors demonstrated competitive inhibition as determined from the Lineweaver–Burk plots and V_{max} intercepts.

Results and Discussion

The synthesis of the desired 7α-arylaliphatic-substituted androstenediones was achieved via a 1,6-conjugate addition. Control of the reaction conditions using the appropriate copper salt (such as $[CuI\{(n\text{-}Bu)_3P\}]_4$), solvent, and protecting group was important for successful reactions. The use of high-field 1-D and 2-D NMR experiments allowed for the assignment of the 7α- and 7β-phenethyl androstenediones produced from the synthesis (Tables 1 and 2). The assignments made from the NMR studies were confirmed by X-ray crystallography.

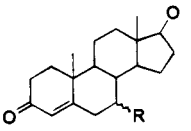
The compounds tested were all good inhibitors of aromatase as can be seen in the results from the inhibitor screening and kinetic assays (Tables 3 and 4). The apparent K_is of the synthesized compounds ranged from 13 to 45 nM. The 7α-arylaliphatic-substituted androstenediones (**2–4**) were potent inhibitors of aromatase with apparent K_is between 13 and 19 nM. These 7α-substituted androstenediones (**2–4**) were equipotent to the known inhibitor 7α-APTA (**1**) whose apparent K_i was 18 nM.[10] The 7α-arylaliphatic-substituted testosterone analogs (**8** and **14**) and the 7β-arylaliphatic-substituted androstenediones (**12** and **16**) were also good inhibitors which exhibit binding to aromatase equal to that of the natural substrate androstenedione, but they are not as potent as the 7α-arylaliphatic-substituted androstenediones.

These new inhibitors support the known structure–activity relationship of steroidal aromatase inhibitors. It was clearly shown that the carbonyl at the C-17 was better than the 17β-hydroxyl group for optimum binding to the enzyme. Previous studies[9] reported that a bulky group can be tolerated at the 7α-position and that an aryl group on the 7α-side chain can enhance activity, this was supported by the new 7α-arylaliphatic-substituted androstenediones (**2–4**). A variation of the chain length between the steroid and the aryl group (one, two, or three methylene units) were incorporated to determine whether or not the distance between the steroid and aryl group could influence binding to the enzyme. The differences between the inhibitors with the various chain lengths was not statistically significant, indicating all three distances can be tolerated in the pocket of the enzyme. As previously indicated, the 7β-arylaliphatic-substituted androstenediones (**12** and **16**) were good inhibitors of aromatase, probably because the alkyl chain allows for the steroid to adopt an appropriate conformation which can still fit in the pocket of the enzyme. Finally, the sulfur atom in the side chain was not essential for activity as an inhibitor of aromatase.

Experimental Section

General Procedures. The steroids used were purchased from Steraloids (Wilton, NH) and used as received. Chemicals used were purchased from Aldrich Chemical Co. (Milwaukee, WI) and were used as purchased unless otherwise noted. The purity of the compounds purchased was checked by TLC. All air-sensitive reactions were performed under an inert atmosphere, and the glassware was base-washed and dried overnight at 120 °C. Ether was dried over lithium aluminum hydride and freshly distilled prior to use. CH_2Cl_2 was dried over calcium hydride and distilled prior to use. Silica gel was purchased from E. Merck (Darmstadt, Germany). Basic alumina oxide was purchased from Fischer Scientific (Fair Lawn, NJ). TLC plates were purchased from Scientific Adsorbants (Atlanta, GA). Melting points were determined on a Thomas-Hoover capillary melting point apparatus and were uncorrected. The ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker FTNMR (250 MHz) spectrophotometer unless otherwise noted. The NMRs were obtained using the residual $CHCl_3$ as a reference unless otherwise noted. All high-field NMR experiments were run on a 11.75 T instrument at The Ohio State University Chemical Instrumentation Center by

Table 4. Kinetic Values for the Inhibition of Aromatase by Various 7α- and 7β-Substituted Androgens



compound	R	functionality at 17-position	app K_m, nM[a]	app K_i, nM[a]	K_m/K_i
2	α-benzyl	ketone	29.3 ± 6.4	18.9 ± 4.3	1.55
3	α-phenethyl	ketone	40.0 ± 6.2	13.1 ± 1.9	3.05
4	α-phenpropyl	ketone	36.7 ± 7.3	16.5 ± 3.2	2.22
14	α-benzyl	β-hydroxy	45.5 ± 5.2	39.5 ± 4.9	1.15
8	α-phenethyl	β-hydroxy	34.0 ± 5.2	36.0 ± 6.0	0.94
16	β-benzyl	ketone	46.4 ± 4.8	44.5 ± 5.2	1.04
12	β-phenethyl	ketone	40.0 ± 5.0	40.2 ± 5.6	1.00
7α-APTA[b]	(4-aminophenyl)thio	ketone	63	18	3.5

[a] Apparent K_m, apparent K_i, and SE values were calculated by weighted regression analysis.[25] [b] See ref 10.

Dr. Chuck E. Cottrell. The experiments were run using residual pyridine as the reference. Infrared spectra were recorded as either neat (liquid or oil) or a KBr pellet (solids) on a Laser Precision Analytical RFX spectrophotometer. Mass spectra were performed at The Ohio State University Chemical Instrumentation Center and were determined using electron impact on either a VG 70-2505, a Nicolet FTMS-2000, or a Finnigan MAT-900 mass spectrometer unless otherwise noted. Elemental analyses were performed by Oneida Research Services (Whitesboro, NY).

Biochemicals were purchased from Sigma Chemical Co. (St. Louis, MO) and used as received. Centrifugation was performed on a Sorvall RC2-B centrifuge and a Beckman L5-50B Ultracentrifuge at 4 °C. [1β-^{3}H]Androst-4-ene-3,17-dione with a specific activity of 24.1 Ci/mmol was purchased from Dupont/New England Nuclear (Boston, MA) and used as purchased. Radioactive samples were detected on a Beckman LS6800 scintillation counter using Budget Solve purchased from Research Products International Corp. (Mount Prospect, IL) as the counting solution; counting efficiencies were 30–35%.

17β-[(*tert*-Butyldimethylsilyl)oxy]androsta-4,6-dien-3-one (7). According to the general procedure of Corey,[24] 17β-hydroxyandrosta-4,6-dien-3-one (**6**) (2.0 g, 70 mmol), *tert*-butyldimethylsilyl chloride (TBDMSCl; 1.6 g, 10.6 mmol), and imidazole (1.3 g, 21.6 mmol) were dissolved in dry DMF and then stirred for 16 h at room temperature. The reaction mixture was poured into H_2O (200 mL), and an off-white precipitate was collected and purified by column chromatography (SiO_2, EtOAc/hexanes, 1:4) to yield colorless crystals (2.47 g, 88%): mp 114.5–115.5 °C; IR (KBr) 2929, 2860, 1668, 1624, 1589, 1466, 1257, 1082, 887, 833, 755 cm^{-1}; ^{1}H NMR δ −0.012 (s, 6H, Me_2Si), 0.77 (s, 3H, C_{18}), 0.86 (s, 9H, t-BuSi), 1.09 (s, 3H, C_{19}), 3.57 (m, 1H, C_{17}), 5.64 (s, 1H, C_4), 6.08 (s, 2H, C_6–C_7); ^{13}C NMR δ −4.8, −4.5, 11.2, 16.3, 18.1, 20.4, 23.1, 25.8, 30.9, 34.0, 34.1, 36.2, 36.8, 37.8, 44.3, 48.0, 51.1, 81.3, 123.7, 127.9, 140.5, 163.6, 199.2; MS *m/z* (M^+) calcd 400.2798, obsd 400.2784. Anal. ($C_{25}H_{40}O_2Si$) C, H, N.

17β-Hydroxy-7α- and -17β-phenethylandrost-4-en-3-one (8 and 10). Phenethyl iodide (2.79 g, 12.02 mmol) in ether was cooled to −78 °C and reacted with *t*-BuLi (14.5 mL, 24.65 mmol), turning the reaction bright yellow. After 30 min, the phenethyllithium was transferred by cannula to a flask containing [CuI{(n-Bu)$_3$P}]$_4$ (4.75 g, 12.00 mmol) in ether at −20 °C. After 10 min, the reaction mixture was cooled to −40 °C and stirred for another 30 min. **7** (0.62 g, 1.55 mmol) in ether was then added, and the reaction mixture was stirred at −40 °C for 3 h. The reaction was quenched with degassed saturated aqueous NH_4Cl, and the mixture was extracted with ether. The organic layer was washed with H_2O and then brine, dried over $MgSO_4$, and concentrated *in vacuo* to leave a yellow oil. The majority of impurities were removed by flash column chromatography (SiO_2, EtOAc/Hex/Et_3N, 1:4:0.05) yielding a clear oil (710 mg) as a 2:1 mixture of α/β-diastereomers. The material was used directly in the next step.

The mixture was dissolved in THF and stirred at room temperature with 6 N HCl (15 mL) for 2 h. The reaction mixture was diluted with H_2O and extracted with ether. The organic layer was washed with H_2O until it was neutral and then washed with brine. After drying over $MgSO_4$, the solvent was removed *in vacuo* to yield a clear oil. Purification using flash column chromatography (SiO_2, EtOAc/Hex, 2:3) gave a mixture of the α- and β-diastereomers (435 mg, 71%), which was further purified by MPLC (SiO_2, EtOAc/hexane, 3:7) to give the separate diastereomers.

17β-Hydroxy-7α-phenethylandrost-4-en-3-one (8): mp 186–187 °C; IR (KBr) 3450, 3024, 2968, 2945, 2914, 2873, 1657, 1450, 1437, 1417, 1381, 1336, 1074, 700 cm^{-1}; ^{1}H NMR δ 0.75 (s, 3H, C_{18}), 1.20 (s, 3H, C_{19}), 3.60 (dd, 1H, C_{17}), 5.79 (s, 1H, C_4), 7.10–7.29 (m, 5H, ArH); ^{13}C NMR δ 10.9, 18.1, 20.9, 22.7, 27.6, 30.4, 33.8, 34.1, 36.0, 36.3, 36.6, 38.8, 39.1, 42.9, 46.1, 47.3, 81.6, 125.9, 126.0, 128.4, 142.2, 169.5, 198.9; MS *m/z* (M^+) calcd 392.2715, obsd 392.2716. Anal. ($C_{27}H_{36}O_2$·0.25H_2O) C, H, N.

17β-Hydroxy-7β-phenethylandrost-4-en-3-one (10): IR (KBr) 3429, 2943, 2873, 1734, 1718, 1676, 1660, 1616, 1496, 1454, 1437, 1419, 1385, 1362, 1342, 1271, 1230, 1190, 1132, 1111, 1070, 1059, 1020, 750, 700 cm^{-1}; ^{1}H NMR δ 0.78 (s, 3H, C_{18}), 1.17 (s, 3H, C_{19}), 3.56 (t, 1H, J = 8.4 Hz, C_{17}), 5.71 (s, 1H, C_4), 7.12–7.29 (m, 5H, ArH); ^{13}C NMR δ 11.4, 17.7, 21.4, 27.2, 30.7, 32.9, 34.0, 35.9, 36.8, 37.2, 38.4, 39.1, 40.1, 42.8, 44.2, 51.4, 54.4, 81.1, 123.0, 125.9, 128.2, 128.4, 142.2, 170.4, 199.2; MS *m/z* (M^+) calcd 392.2715, obsd 392.2723.

7α-Phenethylandrost-4-ene-3,17-dione (3). **8** (235 mg, 0.6 mmol) in dry CH_2Cl_2 (20 mL) was stirred at room temperature with pyridinium chlorochromate (PCC; 325 mg, 1.5 mmol) for 1½ h. The reaction was quenched with ether, and the mixture was filtered through a mixture of Celite and SiO_2 and washed thoroughly with ether. The solvent was removed *in vacuo*, and the crude product was recrystallized from acetone and hexane to yield a colorless solid (232 mg, quant): mp 162–163 °C; IR (KBr) 2945, 2891, 1738, 1672, 1616, 1496, 1454, 1375, 1331, 1269, 1238, 1219, 1188, 1053, 1012, 731, 700, 650 cm^{-1}; ^{1}H NMR δ 0.87 (s, 3H, C_{18}), 1.21 (s, 3H, C_{19}), 5.81 (s, 1H, C_4), 7.10–7.29 (m, 5H, ArH); ^{13}C NMR δ 13.5, 18.1, 20.6, 21.1, 27.5, 31.2, 33.6, 34.0, 35.2, 35.6, 36.0, 36.4, 38.7, 38.8, 46.7, 47.2, 47.5, 126.1, 126.3, 128.4, 128.5, 141.9, 168.6, 198.7, 219.8; MS *m/z* (M^+) calcd 390.2558, obsd 390.2552. Anal. ($C_{27}H_{34}O_2$·0.25H_2O) C, H, N.

7β-Phenethylandrost-4-ene-3,17-dione (12). **10** (70 mg, 0.17 mmol) in dry CH_2Cl_2 (6 mL) was stirred at room temperature with PCC (90 mg, 0.39 mmol) for 1½ h. The reaction was quenched with ether, and the mixture was filtered through a Celite and SiO_2 mixture and thoroughly washed with ether. The solvent was removed *in vacuo* and the product crystallized form acetone and hexane to yield a colorless solid (69 mg, quant): mp 197–198 °C dec; IR (neat) 3024, 2943, 1738, 1676, 1618, 1496, 1454, 1419, 1362, 1269, 1230, 1190, 1028, 860, 752, 703 cm^{-1}; ^{1}H NMR δ 0.91 (s, 3H, C_{18}), 1.18 (s, 3H, C_{19}), 5.74 (s, 1H, C_4), 7.14–7.31 (m, 5H, ArH); ^{13}C NMR δ 14.4, 17.7, 21.2, 25.6, 31.7, 33.2, 34.0, 35.8, 36.1, 37.8, 38.4, 39.0, 39.8, 41.8, 48.6, 52.3, 54.5, 123.4, 126.1, 128.3,

128.6, 142.0, 169.4, 198.9, 220.0; MS *m/z* (M^+) calcd 390.2558; obsd 390.2552. Anal. ($C_{27}H_{34}O_2 \cdot 0.75H_2O$) C, H, N.

17β-Hydroxy-7α- and -7β-phenpropylandrost-4-en-3-one (9 and 11). Phenpropyl iodide (2.5 g, 10.2 mmol) in ether was reacted with *t*-BuLi (12.3 mL, 20.9 mmol), turning the reaction white. After 30 min, the phenpropyllithium was transferred via cannula to a flask containing $[CuI\{(n\text{-}Bu)_3P\}]_4$ in ether at −40 °C for 20 min and then **7** (0.65 g, 1.62 mmol) in ether was added. The reaction was stirred at −40 °C for $2^1/_2$ h. The reaction was quenched with degassed saturated aqueous NH_4Cl, and the mixture was extracted with ether. The organic layer was washed with H_2O and then brine, dried over $MgSO_4$, and the concentrated *in vacuo* to yield a yellow oil. Removal of the major impurities was achieved using flash chromatography (SiO_2, EtOAc/Hex/Et_3N, 1:4:0.05) to yield an oil (630 mg) as a mixture of α- and β-diastereomers, which was then used directly in the next step.

The oil was dissolved in THF (25 mL) and stirred with 6 N HCl (10 mL) at room temperature for $2^1/_2$ h. The reaction mixture was diluted with H_2O and extracted with ether. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification using flash column chromatography (SiO_2, EtOAc/hexane, 1:4) yielded a mixture of diastereomers (310 mg, 49.2%). Further purification using MPLC (SiO_2, EtOAc/Hex, 3:7) gave the two separated diastereomers.

17β-Hydroxy-7α-phenpropylandrost-4-en-3-one (9): mp 157−158 °C; IR (KBr) 3456, 2945, 2870, 1662, 1618, 1496, 1448, 1437, 1417, 1385, 1356, 1336, 1273, 1236, 1225, 1194, 1140, 1109, 1076, 1020, 949, 748, 698 cm^{-1}; ^1H NMR δ 0.77 (s, 3H, C_{18}), 1.18 (s, 3H, C_{19}), 3.63 (t, 1H, J = 8.4 Hz, C_{17}), 5.68 (s, 1H, C_4), 7.12−7.28 (m, 5H, ArH); ^{13}C NMR δ 10.9, 18.1, 21.0, 22.8, 24.8, 29.3, 30.4, 36.0, 36.1, 36.3, 36.5, 36.7, 38.7, 39.1, 42.9, 46.2, 47.3, 81.8, 125.8, 126.0, 128.28, 128.32, 142.3, 169.5, 198.9; MS *m/z* (M^+) calcd 406.2872, obsd 406.2877. Anal. ($C_{28}H_{38}O_2 \cdot 0.75H_2O$) C, H, N.

17β-Hydroxy-7β-phenpropylandrost-4-en-3-one (11): IR (neat) 3427, 3024, 2943, 1660, 1618, 1383, 1362, 1344, 1311, 1271, 1232, 1192, 1132, 1111, 1093, 1049, 1030, 1022, 752, 700, 665 cm^{-1}; ^1H NMR δ 0.75 (s, 3H, C_{18}), 1.13 (s, 3H, C_{19}), 3.54 (t, 1H, J = 8.4 Hz, C_{17}), 5.68 (s, 1H, C_4), 7.13−7.30 (m, 5H, ArH); MS *m/z* (M^+) calcd 406.2872, obsd 406.2887.

7α-Phenpropylandrost-4-ene-3,17-dione (4). 9 (160 mg, 0.39 mmol) in dry CH_2Cl_2 (15 mL) was stirred at room temperature with PCC (195 mg, 0.91 mmol) for $1^1/_2$ h. The reaction was quenched with ether, and the mixture was filtered through a Celite and SiO_2 mixture and thoroughly washed with ether. The solvent was removed *in vacuo*, yielding a colorless oil. Crystallization from acetone/hexane gave an off-white solid (158 mg, quant): mp 85−86 °C; IR (neat) 2943, 2889, 2860, 1740, 1672, 1616, 1496, 1452, 1375, 1269, 1236, 1219, 1190, 1053, 1012, 752, 700 cm^{-1}; ^1H NMR δ 0.88 (s, 3H, C_{18}), 1.19 (s, 3H, C_{19}), 5.71 (s, 1H, C_4), 7.12−7.28 (m, 5H, ArH); ^{13}C NMR δ 13.3, 17.8, 20.4, 21.0, 24.3, 25.1, 28.9, 31.0, 33.7, 35.3, 35.6, 35.7, 36.3, 38.4, 38.5, 46.5, 47.0, 47.2, 125.6, 125.9, 128.1, 141.8, 168.5, 198.3, 219.5; MS *m/z* (M^+) calcd 404.2715, obsd 404.2710. Anal. ($C_{27}H_{34}O_2 \cdot 0.50H_2O$) C, H, N.

17β-Phenpropylandrost-4-ene-3,17-dione (13). 11 (190 mg; 0.47 mmol) in dry CH_2Cl_2 (18 mL) was stirred at room temperature with PCC (250 mg, 1.16 mmol) for $1^1/_2$ h. The reaction was quenched with ether, and the mixture was filtered through a Celite and SiO_2 mixture and thoroughly washed with ether. The solvent was removed *in vacuo*, yielding a colorless oil: IR (neat) 3026, 2941, 2890, 2860, 1739, 1676, 1618, 1496, 1454, 1433, 1362, 1342, 1271, 1230, 1190, 1028, 916, 731, 702 cm^{-1}; ^1H NMR δ 0.87 (s, 3H, C_{18}), 1.14 (s, 3H, C_{19}), 5.70 (s, 1H, C_4), 7.15−7.30 (m, 5H, ArH); ^{13}C NMR δ 14.2, 17.5, 20.9, 25.1, 28.2, 31.5, 33.8, 34.8, 35.6, 35.8, 35.9, 38.2, 38.9, 39.4, 41.9, 48.4, 51.9, 54.2, 123.0, 125.7, 128.2, 128.3, 141.9, 169.7, 198.8, 220.1; MS *m/z* (M^+) calcd 404.2715, obsd 404.2721.

7α- and 7β-Benzyl-17β-hydroxyandrost-4-en-3-one (14 and 15). Benzylmagnesium bromide was formed *in situ* from benzyl bromide (2.2 mL, 18.50 mmol) and magnesium turnings (0.45 g, 18.51 mmol) in ether using ethylene dibromide to initiate the reaction. The Grignard was then transferred via cannula to an addition funnel and slowly added to a flask containing a mixture of $[CuI\{(n\text{-}Bu)_3P\}]_4$ (0.12 g, 0.30 mmol) and **7** (0.80 g, 2.00 mmol) in ether (25 mL) over 45 min at −20 °C. The reaction mixture was stirred at −20 °C for 50 min, the reaction was then quenched with degassed saturated aqueous NH_4Cl, and the resulting mixture was extracted with ether. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, and concentrated *in vacuo* to yield a yellow oil.

The yellow oil was dissolved in THF (40 mL) and stirred at room temperature with 6 N HCl (15 mL) for $1^1/_2$ h. The reaction was diluted with H_2O and extracted with ether. The organic layer was washed with H_2O until it was neutral and then washed with brine. The mixture was dried over $MgSO_4$, and solvent was removed *in vacuo* to yield a yellow waxy solid. Purification using flash column chromatography (SiO_2, EtOAc/hexane, 2:3) yielded a mixture of the α- and β-diastereomers (250 mg, 33%). At this stage the two diastereomers were separated using MPLC (SiO_2, EtOAc/hexane, 3:7).

7α-Benzyl-17β-hydroxyandrost-4-en-3-one (14): mp 184−185 °C dec; IR (KBr) 3429, 2947, 1663, 1614, 1454, 1437, 1346, 1273, 1238, 1223, 1078, 1057, 1016, 949, 910, 733, 700, 646, 621 cm^{-1}; ^1H NMR δ 0.82 (s, 3H, C_{18}), 1.20 (s, 3H, C_{19}), 3.70 (t, 1H, J = 8.3 Hz, C_{17}), 5.72 (s, 1H, C_4), 7.05−7.29 (m, 5H, ArH): ^{13}C NMR δ 10.9, 18.0, 20.9, 22.9, 30.4, 32.0, 34.0, 35.7, 36.1, 36.4, 38.8, 38.9, 43.1, 46.3, 47.0, 81.6, 126.0, 126.6, 128.4, 129.1, 140.8, 169.4, 198.8; MS *m/z* (M^+) calcd 378.2559, obsd 378.2561. Anal. ($C_{27}H_{36}O_2$) C, H, N.

7β-Benzyl-17β-hydroxyandrost-4-en-3-one (15): mp 169−171 °C; IR (KBr) 3431, 2949, 2920, 2883, 1660, 1618, 1452, 1435, 1363, 1342, 1275, 1232, 1038, 1030, 910, 732, 702, 646 cm^{-1}; ^1H NMR δ 0.82 (s, 3H, C_{18}), 1.13 (s, 3H, C_{19}), 3.60 (t, 1H, J = 8.5 Hz, C_{17}), 5.47 (s, 1H, C_4), 7.06−7.28 (m, 5H, ArH); ^{13}C NMR δ 11.4, 17.7, 21.5, 27.9, 30.7, 33.9, 35.9, 36.8, 38.4, 38.6, 41.3, 42.1, 44.3, 44.4, 51.4, 54.3, 81.1, 123.0, 126.0, 128.4, 140.3, 170.13, 199.0; MS *m/z* (M^+) calcd 378.2559, obsd 378.2549. Anal. ($C_{26}H_{34}O_2 \cdot 0.50H_2O$) C, H, N.

7α-Benzylandrost-4-ene-3,17-dione (2). 14 (190 mg, 0.5 mmol) in dry CH_2Cl_2 (14 mL) was stirred at room temperature with PCC (290 mg, 1.35 mmol) for $1^1/_2$ h. The reaction was quenched with ether, and the mixture was filtered through a mixture of Celite and SiO_2 and washed thoroughly with ether. The solvent was removed *in vacuo* and the product crystallized from acetone and hexane to yield a colorless solid (187 mg, quant): mp 250−252 °C dec; IR (KBr) 2945, 2889, 2862, 1738, 1672, 1610, 1601, 1271, 1194, 1051, 1011, 949, 883, 750, 742, 714, 704 cm^{-1}; ^1H NMR δ 0.94 (s, 3H, C_{18}), 1.22 (s, 3H, C_{19}), 5.73 (s, 1H, C_4), 7.07−7.30 (m, 5H, ArH); ^{13}C NMR δ 13.5, 18.0, 20.6, 21.4, 31.3, 32.1, 34.0, 35.57, 35.6, 36.0, 38.3, 38.5, 38.8, 46.9, 48.0, 47.6, 126.2, 126.8, 128.5, 129.1, 140.3, 168.5, 198.6, 219.5; MS *m/z* (M^+) calcd 376.2402, obsd 376.2405. Anal. ($C_{27}H_{34}O_2$) C, H, N.

7β-Benzylandrost-4-ene-3,17-dione (16). 15 (80 mg, 0.21 mmol) in dry CH_2Cl_2 (10 mL) was stirred at room temperature with PCC (140 mg, 0.65 mmol) for $1^1/_2$ hours. The reaction was quenched with ether, and the mixture was filtered through a Celite and SiO_2 mixture and thoroughly washed with ether. The solvent was removed *in vacuo* and the product crystallized from acetone and hexane to yield a colorless solid (79 mg, quant): mp 169−170 °C dec; IR (KBr) 2970, 2962, 2935, 2906, 2885, 2850, 1743, 1676, 1668, 1618, 1452, 1431, 1402, 1385, 1269, 1228, 1190, 876, 742, 700 cm^{-1}; ^1H NMR δ 0.96 (s, 3H, C_{18}), 1.15 (s, 3H, C_{19}), 3.39 (dd, 1H, J = 2.1, 13.7 Hz), 5.51 (s, 1H, C_4), 7.11−7.31 (m, 5H, ArH); ^{13}C NMR δ 14.4, 17.7, 21.2, 26.2, 31.7, 33.9, 35.8, 36.1, 38.3, 38.4, 40.5, 42.3, 43.1, 48.7, 52.3, 54.4, 123.4, 136.3, 128.5, 128.9, 139.8, 169.1, 198.7, 219.9; MS *m/z* (M^+) calcd 376.2402, obsd 376.2394. Anal. ($C_{27}H_{34}O_2 \cdot 0.25H_2O$) C, H, N.

X-ray Crystallographic Analysis. Single crystals of **3** were obtained from isopropyl ether by slow evaporation. The X-ray crystallographic analysis was carried out at the Hauptman-Woodward Medical Research Institute using an Enraf-Nonius CAD4 diffractometer. The structure was solved by direct methods[25] and expanded using Fourier techniques.[26] A

Table 5. Single X-ray Crystallographic Analysis of **3**

empirical formula	$C_{27}H_{34}O_2$
formula weight	390.56
crystal color, habit	clear, rectangular
crystal dimensions, mm	0.380 × 0.200 × 0.150
crystal system	monoclinic
lattice type	*P*
lattice parameters	Å = 10.500 (3)
a, Å	10.835 (1)
c, Å	10.558 (3)
β, deg	114.33 (1)
volume, Å	1094.5 (4)
space-group	$P2_1$ (#4)
Z value	2
density (calcd), g/cm^3	1.185
F_{000}	424.00
solution	direct methods (SIR88)
refinement	full-matrix least-square
temperature, °C	20.0
radiation	Cu Kα (λ = 1.541 78 Å)
$2\theta_{max}$, deg	149.9
reflections measured	total: 7668 unique: 3596 (R_{int} = 6.85)
number of observations	2326 ($I > 3.000\sigma(I)$)
residuals: R, R_w	0.064, 0.067
reflection/parameter ratio	8.88

summary of parameters for data collection and refinement are provided in Table 5.

Biochemical Methods. Preparation of Placental Microsomes. Human term placentas were processed immediately upon delivery from The Ohio State University Hospitals at 4 °C. Large blood vessels, connective tissue, and blood clots were removed, and the tissue was cut into small pieces and thoroughly rinsed with saline. The tissue was homogenized in a cold warring blender with two parts tissue to one part homogenizing buffer (0.05 M sodium phosphate, 0.25 M sucrose, and 0.04 M nicotinamide at pH = 7.0). The homogenate was centrifuged at 10000*g* for 30 min. The debris was discarded, and the supernatant was centrifuged at 11000*g* for 30 min. The debris was discarded again, and the supernatant was centrifuged at 120000*g* for 60 min. The supernatant was discarded, and the microsomal pellet was resuspended in buffer (0.1 M sodium phosphate at pH = 7.4) and centrifuged at 120000*g* for 60 min. The resuspension and centrifugation was then repeated. The microsomes were divided up and stored at −80 °C until needed. The protein concentrations were determined by the method of Lowery.[27]

Inhibitor Screening Assay. The amount of inhibition was determined by following the tritium released as ^3H_2O from [1β-^{3}H]androst-4-ene-3,17-dione and used it as an index of estrogen formation.[22] [1β-^{3}H]Androst-4-ene-3,17-dione (200 000−250 000 dpm, 70 nM) and a concentration of inhibitor (100 or 250 nM) were preincubated with propylene glycol (100 μL) and the NADPH regeneration system (1.8 mM β-NADP$^+$, 2.85 mM glucose-6-phosphate, and 1.5 units of glucose-6-phosphate dehydrogenase) at 37 °C for 5 min. Placental microsomes suspended in a 0.1 M sodium phosphate buffer, pH = 7.0, and warmed to 37 °C were added to the incubation to make a final volume of 3.6 mL (which contained a total of 30−50 μg of protein). The assay was quenched after 15 min by the addition of $CHCl_3$ (5.0 mL). The samples were vortexed (15 s) and centrifuged for 10 min. The water layer was then extracted two more times with $CHCl_3$. Aliquots of water (1.0 mL) were mixed with scintillation cocktail (5.5 mL), and the amount of radioactivity was determined. The amount of inhibition was determined as a percent of the control which was incubated without any inhibitor. Assays were all run in triplicate and repeated at least twice for each inhibitor.

Competitive Inhibition Studies. The procedures used are similar to those previously reported by Brueggemeier et al.[12] Various concentrations of [1β-^{3}H]androst-4-ene-3,17-dione (180 000−250 000 dpm, 70−500 nM) and a concentration of inhibitor (0−150 nM) were preincubated with propylene glycol (50 μL) and the NADP regeneration system (1.8 mM β-NADP$^+$, 2.85 mM glucose-6-phosphate, and 1.1 units of glucose-6-phosphate dehydrogenase) at 37 °C for 5 min. Placental microsomes suspended in a 0.1 M sodium phosphate buffer, pH = 7.0, and warmed to 37 °C were added to the incubation to make a final volume of 2.0 mL (which contained a total of 5 to 15 μg protein). The assay was quenched after 15 min by the addition of $CHCl_3$ (5.0 mL). The samples were vortexed (15 s) and centrifuged for 10 min. The water layer was then extracted two more time with $CHCl_3$. Aliquots of water (0.5 mL) were mixed with scintillation cocktail (5.0 mL), and the amount of radioactivity was determined. Assays were all run in triplicate, and blanks were determined by incubation of boiled microsomes. Results were analyzed by a weighted regression analysis computer program.[23]

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Supporting Information Available: Tables of atomic coordinates, anisotropic displacement parameters, bond lengths, bond angles, and torsion angles for the crystal structure of compound **3** (13 pages). Ordering information is given on any current masthead page.

References

(1) *Cancer Facts and Figures*; American Cancer Society: 1994.
(2) Brodie, A. M. H. Aromatase Inhibitors in the Treatment of Breast Cancer. *J. Steroid. Biochem. Mol. Biol.* **1994**, *49* (4−6), 281−287.
(3) Brodie, A. M. H.; Santen, R. J. Aromatase and its Inhibitors in Breast Cancer Treatment-Overview and Perspective. *Breast Cancer Res. Treat.* **1994**, *30*, 1−6.
(4) Coombes, R. L.; Goss, P. E.; Dowsett, M.; Gazet, J. C.; Brodie, A. M. H. 4-Hydroxyandrostenedione Treatment of Postmenopausal Patients with Advanced Breast Cancer. *Lancet* **1984**, No. 2, 1237−1239.
(5) Goss, P. E.; Coombes, R. L.; Powles, T. J.; Dowsett, M.; Brodie, A. M. H. Treatment of Advanced Postmenopausal Breast Cancer with Aromatase Inhibitor, 4-Hydroxyandrostenedione − Phase 2 Report. *Cancer Res.* **1986**, *46*, 4823−4826.
(6) Brueggemeier, R. W. Aromatase Inhibitors − Mechanisms of Steroidal Inhibitors. *Breast Cancer Res. Treat.* **1994**, *30*, 31−42.
(7) Bossche, H. V.; Moereels, H.; Koymans, L. M. H. Aromatase Inhibitors − Mechanisms for Non-Steroidal Inhibitors. *Breast Cancer Res. Treat.* **1994**, *30*, 43−55.
(8) Marsh, D. A.; Brodie, H. J.; Garrett, W.; Tsai-Marris, C. H.; Brodie, A. M. H. Aromatase Inhibitors. Synthesis and Biological Activity of Androstenedione Derivatives. *J. Med. Chem.* **1985**, *28*, 788−795.
(9) Brueggemeier, R. W. Biochemical and Molecular Aspects of Aromatase. *J. Enzyme Inhib.* **1990**, *4*, 101−111.
(10) Brueggemeier, R. W.; Snider, C. E.; Counsell, R. E. Substituted C_{19} Analogs as Inhibitors of Estrogen Biosynthesis. *Cancer Res.* **1982**, *42*, 3334s−3337s.
(11) Brueggemeier, R. W.; Katlic, N. E. Effects of the Aromatase Inhibitor 7α-(4′-Amino) phenylthio-4-androstene-3,17-dione in MCF-7 Human Mammary Carcinoma Cell Culture. *Cancer Res.* **1987**, *47*, 4548−4551.
(12) Brueggemeier, R. W.; Li, P. K.; Snider, C. E.; Darby, M. V.; Katlic, N. E. 7α-substituted Androstenediones as Effective *In Vitro* and *In Vivo* Inhibitors of Aromatase. *Steroids* **1987**, *2*, 163−178.
(13) Modi, S. P.; Gardner, J. O.; Milowsky, A; Wierzba, M.; Forgione, L.; Mazur, P.; Solo, A. J. Conjugate Addition of Grignard Reagents to Enones and Dieneones. *J. Org. Chem.* **1989**, *54*, 2317−2321.
(14) Normant, J. F. Organocopper(I) Compounds and Organocuprates in Synthesis. *Synthesis.* **1972**, 63−80.
(15) Grunwell, J. F.; Benson, H. D.; Johnston, J. O.; Petrow, V. Antiprogestatiional Agents. The Synthesis of 7-Alkyl Steroidal Ketone with Anti-Implantational and Antidecidual Activity. *Steroids* **1976**, *27* (6), 759−770.
(16) Solo, A. J.; Caroli, C.; Darby, M. V.; McKay, T.; Slaunwhite, W. D.; Hebborn, P. 7α-Alkyltestosterone Derivatives: Synthesis and Activity as Androgen and as Aromatase Inhibitors. *Steroids* **1982**, *40* (6), 603−614.
(17) Nique, F.; Nedelec, L.; Bouton, M. M.; Phillibert, D. New 7-Aryl-3-Oxygenated Oestrane Derivatives for Treating Hormone Dependent Cancers and Hypofolliculina. European Patent 0280618 A1, 957−989.

(18) Bailey, W. F.; Punzalan, E. R. Convenient General Method for the Preparation of Primary Alkyllithiums by Lithium-Iodine Exchange. *J. Org. Chem.* **1990**, *55*, 5404–5406.
(19) Kauffman, G. B.; Teter, L. A. Tetrakis[Iodo(Tri-n-Butylphosphine) Copper(I)] and Iodo(2,2′-Bipyridine)-(Tri-n-Butylphosphine) Copper (I). *Inorg. Synth.* **1961**, *7*, 9–12.
(20) House, H. O.; Respess, W. L.; Whitesides, G. M. The Chemistry of Carbanions. XII. The Role of Copper in the Conjugate Addition of Organometallic Reagents. *J. Org. Chem.* **1966**, *31*, 3128–3141.
(21) House, H. O.; Fischer, W. F., Jr. The Chemistry of Carbanions. XVII. The Addition of Methyl Organometallic Reagents to Cyclohexenone Derivatives. *J. Org. Chem.* **1968**, *33* (3), 949–956.
(22) Siiteri, P. K.; Thompson, E. A. Studies on Human Placental Aromatase. *J. Steroid Biochem.* **1975**, *6*, 317–322.
(23) Cleland, W. W. The Statistical Analysis of Enzyme Kinetic Data. *Method Enzymol.* **1979**, *63*, 103–138.
(24) Corey, E. J.; Venkateswarlu, A. Protection of Hydroxyl Groups as tert-Butyldimethylsilyl Derivatives. *J. Am. Chem. Soc.* **1972**, *94*, 6190–6191.
(25) Burla, M. C.; Camalli, M.; Cascarano, G.; Giacovazzo, C.; Polidori, G.; Spagna, R.; Veterbo, D. SIR88-A Direct-Methods Program for the Automatic Solution of Crystal Structure. *J. Appl. Crystallogr.* **1989**, *22* (4), 389–393.
(26) Beurskens, P. T.; Admiraal, G.; Beurskens, G.; Bosman, W. P.; Garcia-Grand, S.; Gould, R. O.; Smits, J. M. M.; Smykalla, C. *The DIRDIF Program System*; Technical Report of the Crystallography Laboratory, University of Nijmegen: The Netherlands, 1992.
(27) Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.

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