Synthesis and Biochemical Studies of 16- or 19-Substituted Androst-4-enes as Aromatase Inhibitors

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Androst-4-en-17-one derivatives [19-acetoxide **4**, 16-bromides **14** and **15**, 19,19-difluoride **18**, and (19*R*,*S*)-19-acetylenic alcohol **25**] and androst-4-en-17β-ol derivatives **3**, **5**, **10**, **12**, and **19** were synthesized and tested for their ability to inhibit aromatase in human placental microsomes. All the 17-oxo steroids, except compound **25** and 17,19-diol **3** of this series, were effective competitive inhibitors with apparent K_i's ranging from 170 to 455 nM. 19,19-Difluoro steroid **18** and 19-acetylenic alcohol **25**, a weak competitive inhibitor (K_i = 7.75 μM), caused a time-dependent, pseudo-first-order inactivation of aromatase activity with k_{inact}'s of 0.0213 and 0.1053 min^{-1} for compounds **18** and **25**, respectively. NADPH and oxygen were required for the time-dependent inactivation, and the substrate, androst-4-ene-3,17-dione, prevented it, but a nucleophile, L-cysteine, did not in each case. The results strongly suggest that aromatase would attack the 19-carbon of steroids **18** and **25**.

Aromatase is a unique cytochrome P-450 enzyme complex which catalyzes the synthesis of estrone and estradiol from 4-en-3-one androgens androst-4-ene-3,17-dione (androstenedione) and testosterone.[1] Although the details of the aromatization mechanism are still a subject of debate, it is generally believed that human placental aromatase carries out three sequential oxygenations of the androgen: the first two are sequential hydroxylations of the 19-methyl group to produce 19-hydroxy and 19,19-*gem*-diol intermediates, respectively.[1d,2] Dehydration of this *gem*-diol leads to the readily isolated 19-oxo intermediate.[3] In the third step, C-19[4] and 1β,2β-protons[5] are eliminated as formic acid and water, respectively, to produce the estrogen. However, it is now thought to be a substrate-dependent variation in stereospecificity of the proton loss at C-2.[6]

The production of estrogens throughout the aromatase pathway is important in the genesis of endometrial carcinoma in obese women[7] and in the growth of established estrogen-dependent breast cancer.[8] For this reason, the development of inhibitors of aromatase has been considered to be of major clinical and experimental importance. Present knowledge of the aromatization mechanism has led to development of a wide variety of suicide substrates of aromatase.[8] Known suicide substrates primarily have made use of the oxygenation of the 19-methyl group in the inactivation process. An aromatase suicide substrate, thus specifically oxygenated at this position, would lead to the additional suggestion of the mechanism proposed for the aromatase reaction.

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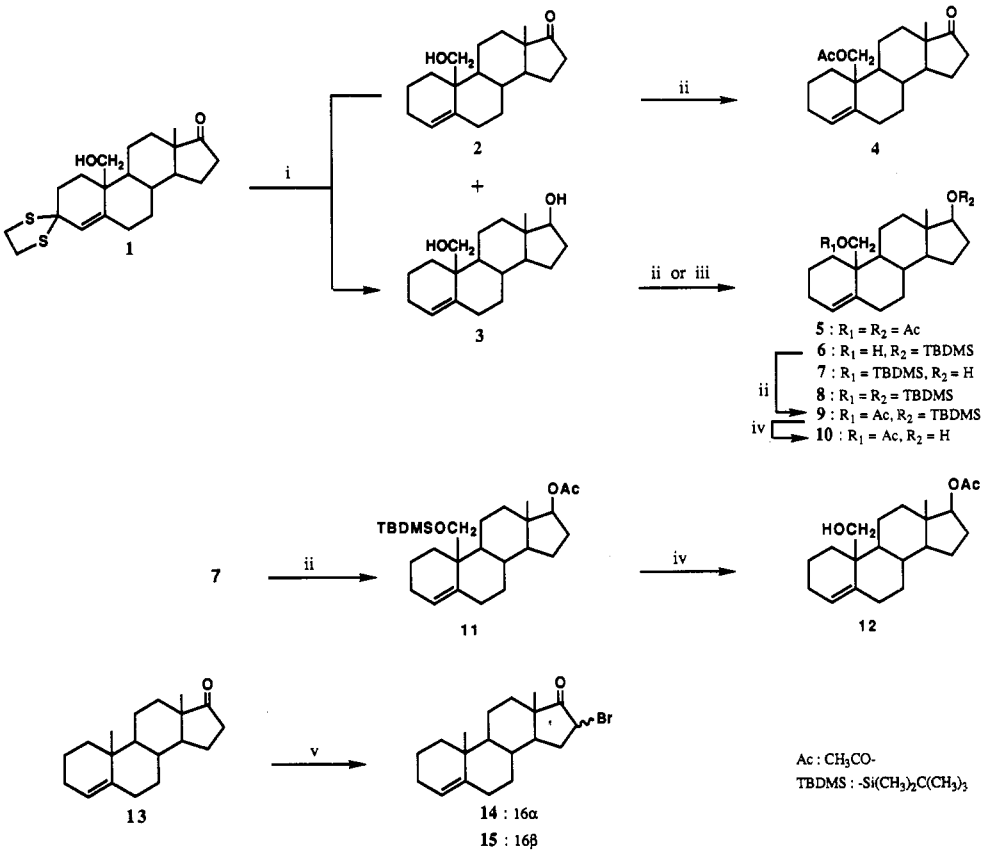
Scheme I[a]



[a] Reagents: (i) Na, liquid NH_3, THF; (ii) Ac_2O, pyridine: (iii) TBDMS-Cl, imidazole, DMF; (iv) 3 M HCl, propan-2-ol, THF; (v) $CuBr_2$, MeOH.

We have previously reported that a C_{19} steroid having a unique structure, a 4-en-6-one system,[9] instead of the 4-en-3-one that is principally contained in known potent aromatase inhibitors and the substrate of this enzyme or having a 4-ene system,[10] efficiently blocks the aromatase activity in a reversible manner, even though there is no oxygen function at its C-3 position.

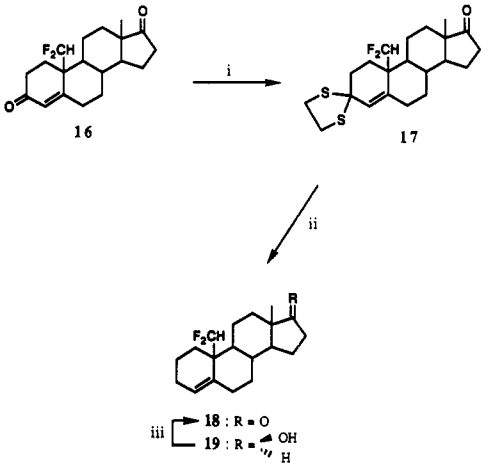
As a continuing study of the 4-ene steroids as aromatase inhibitors, we have investigated further structure requirements necessary for aromatase inhibition by 3-deoxyandrostendione derivatives. The studies described in this paper focus on the preparation and biochemical evaluation of 16- and 19-substituted analogues of androst-4-enes. A 17-carbonyl function is necessary for effective binding to the active site of aromatase, and the 19,19-difluoro compound **18** and 19-acetylenic alcohol **25** inactivated aromatase in a mechanism-based manner.

Results

Chemistry. Androst-4-ene-17β,19-diol (**3**) was obtained in 75% yield along with the 17-oxo compound **2** (15%) upon desulfurization of the known 3,3-ethylene dithioacetal **1**[10] with sodium metal–liquid NH_3 (Scheme I). Reaction of diol **3** with an excess of *tert*-butyldimethylsilyl (TBDMS) chloride in the presence of imidazole gave a mixture of 17-TBDMS ether **6** (19%) and its 19-silyl isomer **7** (14%) along with 17,19-bis-TBDMS ether **8** (62%). TBDMS ethers **6** and **7** are then separately acetylated with acetic anhydride–pyridine followed by treatment with 3 M HCl[11] to yield 19-acetate **10** and 17-acetate **12**, respectively.

Bromination of 3-deoxyandrostenedione (**13**) with $CuBr_2$ in MeOH (reflux, 9 h) according to the method[12] previously

Scheme II[a]



[a] Reagents: (i) $HS(CH_2)_2SH$, *p*-TsOH, AcOH; (ii) Na, liquid NH_3, THF; (iii) CrO_3, H_2SO_4, acetone.

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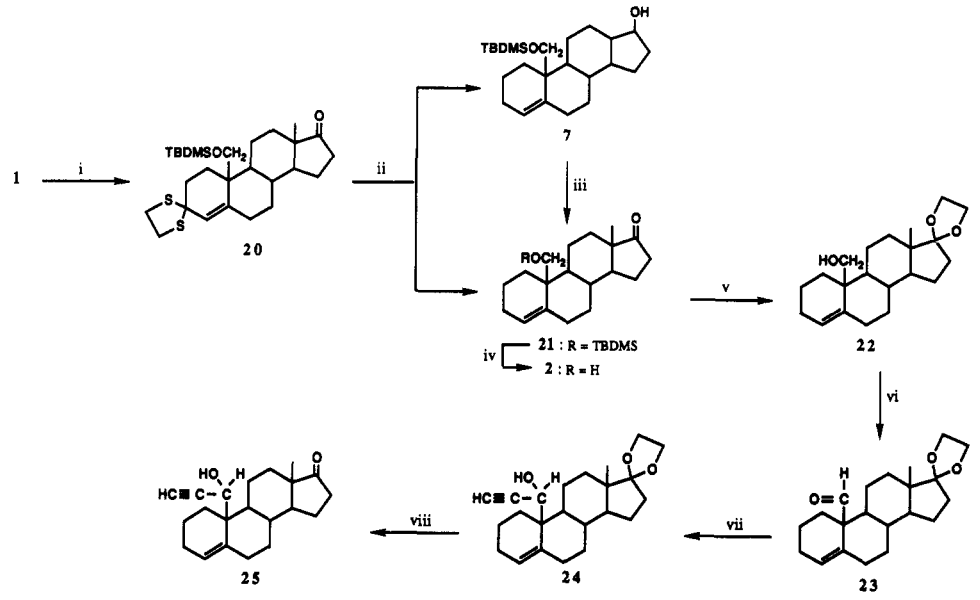
Scheme III[a]



[a] Reagents: (i) TBDMS-Cl, imidazole, DMF; (ii) Na, liquid NH_3, THF; (iii) CrO_3, H_2SO_4, acetone; (iv) 3 M HCl, propan-2-ol, THF; (v) $HO(CH_2)_2OH$, *p*-TsOH, toluene; (vi) PDC, CH_2Cl_2; (vii) HC≡CMgBr, THF; (viii) 10% H_2SO_4, acetone.

reported gave an approximate 3:2 mixture of 16α-bromo derivative **14** and its 16β-isomer **15** (Scheme I) in 40% yield. Reaction of a 3β-hydroxy-17-oxo steroid with a 5-ene or 5α-system with $CuBr_2$ under the same conditions affords essentially the corresponding 16α-bromide in excellent yield,[12] suggesting that a conformational transmission of distortion through the B–C–D rings might be in operation, although the detailed conformational analysis by X-ray crystallography is not available.

The known 19,19-difluoroandrostenedione (**16**)[13] was converted to 3,3-(ethylenedithio) acetal **17** according to the previous report[10] and desulfurization with sodium metal–liquid NH_3 yielded the desired product **18** (22%) and its 17β-reduced compound **19** (56%) (Scheme II). The 17β-alcohol **19** could be converted to the 17-ketone **18** by reaction with Jones reagent.

(19*R*/*S*)-19-Ethynyl-19-hydroxyandrost-4-en-17-one (**25**) was principally synthesized from the 19-alcohol **2** according to the method[14] previously reported for the synthesis of the 3-oxo derivative of compound **25** (Scheme III). Compound **2** was obtained from 3,3-(ethylenedithio) 19-alcohol **1** in a markedly improved yield (54%) as compared to a previous report,[10] through the protection of a 19-hydroxyl group of compound **1** with TBDMS and desulfurization of silyl ether **20** with sodium metal–liquid NH_3 followed by Jones oxidation and a subsequent acid treatment. Compound **2** was converted to acetal **22** which was oxidized with pyridinium dichromate followed by Grignard reaction with ethynylmagnesium bromide in THF at room temperature to yield alcohol **24** (41% from 19-alcohol **2**). ^1H NMR analysis of the product revealed that this is an ca. 60:40 mixture of diastereomers with the *S* and *R* configuration at C-19 [δ 4.80 and 4.82 ppm (19-C*H*OH)]. Assuming that the addition of the Grignard reagent to 19-oxo steroid **23** follows the same steric course as that to the 3,3-ethylenedioxo derivative of steroid **23**, one would expect the major product to have the *S* configuration at C-19.[2c,14] However, since the conformational preference of a 19-carbonyl group of compound **23** and the sterically preferred route of attack employed by the Grignard reagent are uncertain in the 3-deoxy steroid, the configuration of the major product is not clear. Compound **24** was converted to the desired compound **25** by acid treatment. The alcohol mixtures **24** and **25** could not be separated by attempted HPLC.

Table I. Aromatase Inhibition by Various Androst-4-enes[a]

compound	IC_{50}, μM	K_i, nM	inhibition
17-Oxo Steroid			
19-acetoxide **4**	4.0	289	competitive
16α-bromide **14**	4.1	228	competitive
16β-bromide **15**	5.9	455	competitive
19,19-difluoride **18**	1.2	443	competitive
19-ethynyl 19-alcohol **25**	62	7750	competitive
17β-Hydroxy Steroid			
19-hydroxide **3**	2.3	170	competitive
19-acetoxide **10**	44		
19-hydroxy 17-acetate **12**	100		
19,19-difluoride **19**	26		
19-acetoxy 17-acetate **5**	38		
3-deoxytestosterone	7.3	830	competitive
For Comparison			
3-deoxyandrostenedione (**13**)[b]	0.53	37	competitive
19-hydroxy-4-en-17-one **2**[b]	0.27	13	competitive
4-hydroxyandrostenedione	0.41		

[a] Substrate: 1 μM [1β-^{3}H]androstenedione; human placental microsomes: 40 μg protein. [b] Reference 10.

Biochemical Properties. Reversible inhibition of aromatase activity in human placental microsomes by the 16-bromo- (**14** and **15**), 19,19-difluoro- (**18** and **19**), (19*R*/*S*)-19-ethynyl-19-hydroxy- (**25**), 19-hydroxy- (**3** and **12**), and 19-acetoxy (**4**, **5**, and **10**) androst-4-enes, synthesized in this study, and by 3-deoxytestosterone[15] was

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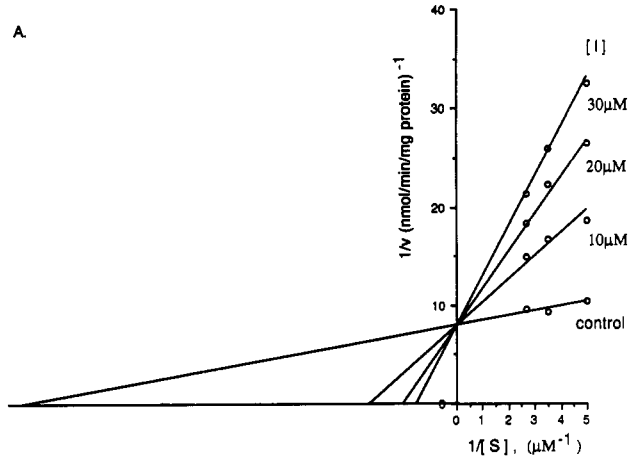

Figure 1. Lineweaver–Burk plot of inhibition of human placental aromatase by 19-acetylenic alcohol **25** with androstenedione as a substrate. Each point represents the mean of two determinations. The inhibition experiments with compounds **3**, **4**, **14**, **15**, and **18** and 3-deoxytestosterone gave essentially similar plots to Figure 1 (data not shown).

initially studied and the results are shown in Table I. In addition to the above compounds, 3-deoxyandrostenedione **13** and its 19-hydroxy derivative **2**[10] and 4-hydroxyandrostenedione,[16] potent aromatase inhibitors, are listed for comparison. Aromatase activity in the placental microsomes was determined by the radiometric method in which tritiated water released from [1β-^{3}H]-androstenedione into the incubation medium during aromatization was determined according to Siiteri and Thompson.[17] IC_{50}'s, the concentration required to give 50% inhibition of the enzyme activity, were initially obtained, and relatively potent inhibitors were further studied in order to characterize the nature of their interaction with the catalytic site of aromatase. Aromatization was measured at several inhibitor concentrations of the androstenedione substrate. The results of the studies were plotted in a typical Lineweaver–Burk plot. All the inhibitors examined exhibited clear cut competitive-type inhibition, and the apparent inhibition constants (K_i), an index of the affinity of the enzyme for the inhibitor, are determined by analysis of the Dixon plot. A Lineweaver–Burk plot of aromatase inhibition by 19-acetylenic alcohol **25** is shown in Figure 1. In these studies, the apparent K_m for androstenedione was found to be 60 ± 7 nM.

17-Oxo steroids **4**, **14**, **18**, and **25** were then tested for their abilities to cause a time-dependent inactivation of aromatase. The time-dependent inactivation was observed when 19,19-difluoride **18** and the (19*R*/*S*)-19-acetylenic alcohol **25** were incubated in the presence of NADPH in air (Figures 2 and 3), while 16-bromide **14** and 19-acetoxide **4**, at the concentrations employed (1, 5, and 10 μM), behaved only as a competitive inhibitor with no evidence of

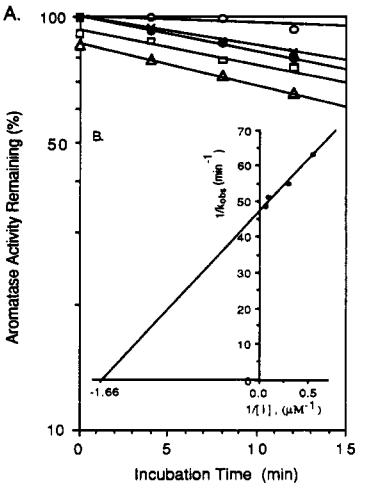

Figure 2. Time-dependent inactivation (A) and concentration-dependent inactivation (B) of human placental aromatase by 19,19-difluoro compound **18** in the presence of NADPH in air. Concentrations of the inhibitor: control (0 μM), ○; 2 μM, ×; 4 μM, ●; 20 μM, □; 40 μM, △. Each point represents the mean of two determinations.

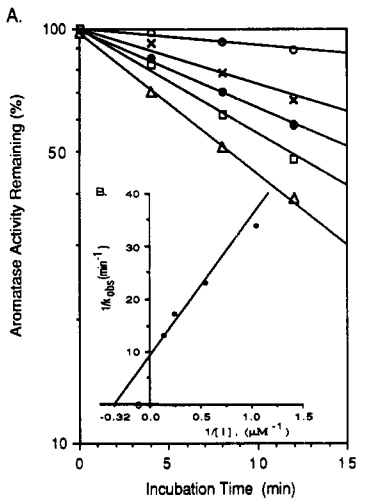

Figure 3. Time-dependent inactivation (A) and concentration-dependent inactivation (B) of human placental aromatase by 19-acetylenic alcohol **25** in the presence of NADPH in air. Concentrations of the inhibitor: control (0 μM), ○; 1 μM, ×; 2 μM, ●; 5 μM, □; 10 μM, △. Each point represents the mean of two determinations.

enzymatic generation of reactive substance (data not shown). Pseudo-first-order kinetics were obtained during the first 12 min of the incubation of inhibitors **18** and **25** when the kinetic data were analyzed according to Kitz and Wilson[18] (Figures 2B and 3B). Double-reciprocal plots of k_{obs} versus inhibitor concentration yielded k_{inact}'s of 0.0213 and 0.1053 min^{-1} and K_i's of 600 nM and 3.1 μM, respectively, for inhibitors **18** and **25**.

NADPH and oxygen were essential for the time-dependent activity loss by inhibitor **18** or **25**. The substrate androstenedione blocked the inactivation, while a nucleophile, L-cysteine, had no significant effect on it, in each case (Figure 4).

Discussion

3-Deoxyandrostenedione (**13**) is a potent competitive inhibitor of aromatase (K_i = 37 nM), and an introduction

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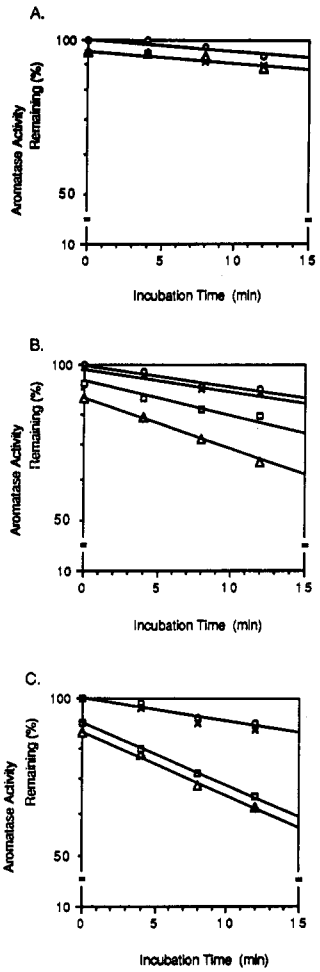

Figure 4. Inactivation of human placental aromatase by 19,19-difluoro compound **18** under various conditions: (A) In the absence of NADPH (△) or in N_2 atmosphere (×), the inhibitor at concentration of 40 μM failed to produce the inactivation. Control sample contained no inhibitor (○). (B) Androstenedione at various concentrations (0 μM, △; 1 μM, □; 2 μM, ×) was incubated with aromatase, the inhibitor (40 μM), and NADPH in air and protected the enzyme from inactivation. Control sample contained no inhibitor (○). (C) In the presence (□) or absence (△) of L-cysteine (0.5 mM), a pseudo-first-order inactivation of aromatase by the inhibitor (40 μM) was observed. Control sample with (×) and without (○) L-cysteine contained no inhibitor. Each point represents the mean of two determinations. The inactivation experiments with compound **25** in the absence of NADPH, in N_2 atmosphere, and in the presence of L-cysteine or androstenedione gave essentially similar results to Figure 4 (data not shown).

of a hydroxy group at C-19 of it provides enhanced affinity for the active site,[10] although its opposite effect is observed in the androstenedione series.[1d,19] This suggests that the C-3 oxygen of steroid having a 4-en-3-one system does not play a critical role in steroid binding, and that the 3-deoxy steroids presumably approach the active site in a different way from the natural substrate and intermediate, androstenedione and its 19-hydroxy derivative, during the aromatization process. Analogs of compound **13**, having a substituent at C-16 or C-19, were synthesized as potential reversible or enzyme-activated inhibitors of aromatase.

17-Alcohols (3-deoxytestosterone and compounds **3**, **10**, and **19**) had a lower binding affinity to aromatase when compared with their respective 17-oxo analogues **13**, **2**, **4**, and **18** [K_i = 830 vs 37 nM (3-deoxytestosterone vs **13**), 170 vs 13 nM (**3** vs **2**); IC_{50} = 44 vs 4.0 μM (**10** vs **4**), 26 vs 1.2 μM (**19** vs **18**)] (Table I). The results show that a 17-carbonyl function of 3-deoxy steroid plays an important role in steroid binding to the active site. A similar relationship of the affinity is reported for the 3-oxo steroid series.[20] An introduction of a hydroxy group at C-19 of 3-deoxytestosterone expectedly enhanced the affinity (K_i = 830 vs 170 nM). On the other hand, acetylation of 19-alcohols **2** and **3** markedly decreased the affinity [K_i = 13 vs 289 nM (**2** vs **4**); IC_{50} = 2.3 vs 44 μM (**3** vs **10**)], while acetylation of 19-hydroxy-17-acetate **12** increased it [**12** vs **5** (100 vs 38 μM)]. 16α-Bromo compound **14** and its 16β-isomer **15** are relatively potent competitive inhibitors of aromatase with K_i's of 228 and 455 nM. With respect to the stereochemical selectivity of the aromatase inhibition, analogous results have been reported for 6-bromo[21] and 6-bromoacetoxy[22] steroids.

Incorporation of two fluorine atoms or an acetylenic alcohol group into the 19-position of 3-deoxy steroids significantly lowered the binding ability for aromatase in each case compared to the corresponding parent compounds [K_i = 37 vs 443 nM (**13** vs **18**), 13 nM vs 7.75 μM (**2** vs **25**); IC_{50} = 7.3 vs 26 μM (3-deoxytestosterone vs **19**)]. Substitution of these groups for a proton would cause significant structure perturbation, but the affinities of inhibitors **18** and **25** for the enzyme are still higher than those previously reported for the corresponding 3-oxo steroids.[13,23] Both the 19,19-difluoro compound **18** and (19*R*/*S*)-19-acetylenic alcohol **25** inactivated the aromatization of androstenedione in a time-dependent, pseudo-first-order manner in the presence of NADPH in air. A double-reciprocal plot of the apparent rate constants for inactivation versus the concentration of each steroid was linear and gave the apparent K_i and overall rate constant for inactivation (k_{inact}), respectively. This indicates formation of a dissociable enzyme–inhibitor complex followed by unimolecular inactivation. The similarities of the apparent K_i's with those obtained from the competition experiments described in Table I suggests that the initial binding of the inhibitors to the enzyme is rate-limiting. The inactivation rates of the inhibitors are comparable to

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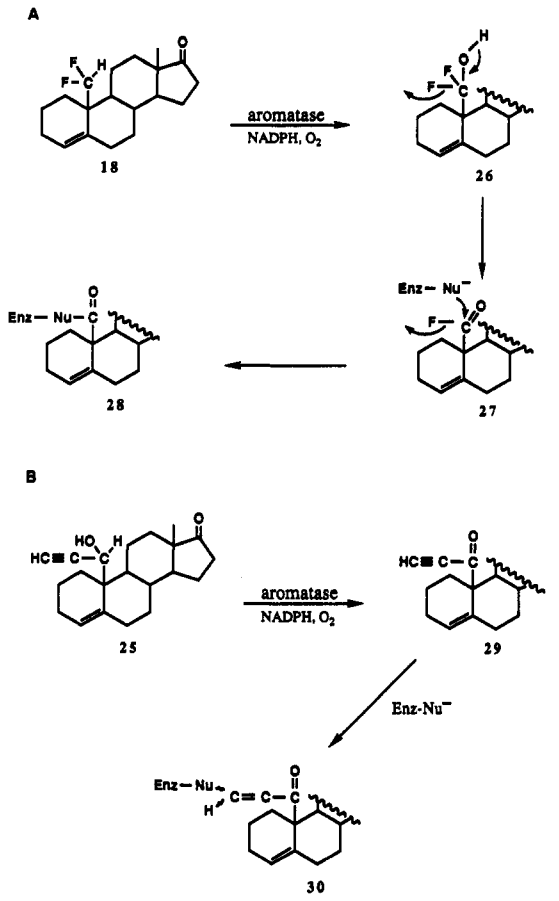
those previously reported for the corresponding 3-oxo derivatives.[13,23] The rate of inactivation decreased when the substrate androstenedione was included in the incubation mixture. In the nucleophile protection experiments, L-cysteine failed to protect aromatase from inactivation by the inhibitors. Thus, covalent-bond formation between the enzyme and the reactive intermediate appears to occur rapidly at the active site, therefore, preventing diffusion of the activated inhibitor, a reactive electrophile, into the surrounding media. Acetylenic alcohol **25** is a mixture of the 19*S* and 19*R* diastereomers. Diastereomeric 19-acetylenic alcohol derivatives of androstenedione can be separated by HPLC[14] and the 19*R* diastereomer has a much higher affinity for aromatase than the other stereoisomer.[23] Moreovere, only the 19*S* diastereomer inactivates the enzyme in a suicide manner. On the other hand, when a double bond at C-6 is introduced into the isomers, both diastereomers irreversibly inactivate the enzyme.[8d] Thus, further study is necessary for elucidation of the stereochemical specificity of aromatase inhibition by compound **25**.

Robinson's group[24] has confirmed the mechanism of inactivation of aromatase by 19,19-difluoroandrostenedione with the 19-^{3}H-labeled inhibitor; aromatase attacks the 19-carbon of the difluoro compound to generate an electrophilic acyl fluoride, resulting in covalent modification of the enzyme's active site. For the inactivation by the 19-acetylenic alcohol derivative of androstenedione, Covey's[23] group has reasoned that an acetylene-containing substituent may be metabolized by aromatase to generate a conjugated ketone. The reactive ketone would be an efficient Michael acceptor and may well be attacked by an active site nucleophile leading to covalent modification of the enzyme. On the other hand, sice there is not intermolecular kinetic isotope effect on the rate of inactivation by 19,19-dideuterio-19-ethynylandrostenedione, Johnston's group[25] has proposed that the inactivation may involve oxygen insertion in the ethynyl substituent to generate the highly reactive oxirene species which could covalently bind to the prosthetic heme. However, it has not directly been determined whether or not the oxirene mechanism is involved in the inactivation by the acetylenic alcohol derivative. Then, based on the structural similarities of inhibitors **18** and **25** to the 19,19-difluoro and acetylenic alcohol derivatives of androstenedione, it is presumed that compounds **18** and **25** would inactivate aromatase most likely via the mechanisms similar to those previously described for the corresponding 3-oxo derivatives (Scheme IV, mechanisms A and B); both of the 3-deoxy steroids are presumably hydroxylated at C-19 by aromatase. During the course of the present study, Cole et al.[26] have reported that a 3-deoxy-2,4-diene androgen analogue having a 19-hydroxy or 19-carbonyl function is found to serve as a substrate for the enzyme, respectively, while the 2,4-diene without an oxygen function at C-19 is not aromatized to 3-deoxyestrogen. This shows that the 2,4-diene having a 19-methyl is not hydroxylated at C-19 by the enzyme. Considering this along with the present results, it is suggested that in the aromatase reaction of

Scheme IV



3-deoxyandrogen series, the first hydroxylation at C-19 would take place only on a steroid having a 4-ene structural feature. In a preliminary study using large-scale incubation experiments (15 mg of protein of human placental microsomes, 0.17 μmol of 3-deoxy steroid **2** or **13**, 6 mg of NADPH, 10 mL of 67 mM phosphate buffer, 37 °C, 30 min), the transformation of these steroids to 3-deoxyestrone and 3-deoxyestradiol could not be detected by GC-MS (detection limit: ca. 500 pg each). 3-Ketone enolization plays a key role in the aromatase third step.[6,27] However, compounds **2** and **13** do not have a structural feature which is chemically enolized toward C-2. This may be one of reasons why these are not converted to an A-ring aromatic steroid by aromatase.

In conclusion, we synthesized various 16- and 19-substituted analogues of both 3-deoxyandrostenedione and 3-deoxytestosterone and studied their interactions with human placental aromatase. A 17-carbonyl function is essential for tight binding to the active site. 19,19-Difluoro steroid **18** and 19-acetylenic alcohol **25** have been incubated with the placental microsomes, resulting in inactivation of aromatase in a suicide manner. Our experiments suggest that both compounds would be activated by attack at the 19-carbon as part of the inactivation process, although there is no definite evidence.

Experimental Section

Chemistry. General Methods. Melting points were determined on a Yanagimoto melting point apparatus and are un-

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corrected. IR spectra were recorded on Shimadzu IR-430 and Perkin Elmer FT-IR 1725X spectrophotometers. ^{1}H NMR spectra were obtained in $CDCl_3$ solutions with JEOL PMX 60 (60 MHz) and JEOL GX 400 (400 MHz) spectrometers using tetramethylsilane (δ = 0.00) or $CHCl_3$ (δ = 7.26, for TBDMS derivatives) as an internal standard, and mass spectra (MS) were obtained with a JEOL JMS-DX 303 spectrometer. Optical rotation measurements were done on a JASCO DIP-360.

Androst-4-ene-17β,19-diol (3). A solution of 3,3-ethylenedithio steroid **1** (640 mg, 1.77 mmol) in THF (13 mL) was added to a solution of sodium metal (1.35 g) in 90 mL of liquid NH_3. The reaction mixture was stirred for 1 h. After removing most of the ammonia and carefully adding 10 mL of MeOH and 30 mL of cold water, the mixture was acidified with 5% HCl and then the product was extracted with AcOEt, washed with a 5% $NaHCO_3$ solution and water, and dried (Na_2SO_4). The oily product was purified by column chromatography (silica gel, 50 g). Elution with hexane–AcOEt (3:1) gave 19-hydroxyandrost-4-en-17-one (**2**) (78 mg, 15%): mp 155–156 °C (from aceteone) (lit.[10] mp 154–156 °C).

Elution with hexane–AcOEt (2:1) gave the more polar product **3** (385 mg, 75%): mp 99–101 °C (colorless prisms, from acetone); ^{1}H NMR (60 MHz) δ 0.77 (3 H, s, 18-Me), 3.53 (1 H, d, J = 10 Hz, 19-H_a), 3.63 (1 H, m, 17α-H), 3.97 (1 H, d, J = 10 Hz, 19-H_b), 5.70 (1 H, m, 4-H); IR (KBr) 3450 (OH) cm^{-1}. Anal. ($C_{19}H_{30}O_2$) C, H.

19-Acetoxyandrost-4-en-17-one (4). Compound **2** (20 mg, 0.069 mmol) was acetylated with acetic anhydride (0.1 mL)–pyridine (0.2 mL) (room temperature, overnight). After usual workup, the crude product was purified by preparative TLC, giving **4** as an oil (15 mg, 65%): ^{1}H NMR (60 MHz) δ 0.90 (3 H, s, 18-Me), 2.01 (3 H, s, 19-OCOMe), 4.13 and 4.47 (1 H each, d, J = 12 Hz, 19-H), 5.57 (1 H, m, 4-H); exact mass found 270.1978; calcd for $C_{19}H_{26}O$ (M^+ – 60) 270.1983.

17β,19-Diacetoxyandrost-4-ene (5). Compound **3** (20 mg, 0.069 mmol) was acetylated as above and the crude product obtained was purified by preparative TLC, yielding **5** as an oil: ^{1}H NMR (400 MHz) δ 0.80 (3 H, s, 18-Me), 2.03 and 2.04 (3 H each, s, 17- and 19-OCOMe), 4.12 and 4.41 (1 H each, d, J = 11.2 Hz, 19-H), 4.57 (1 H, dd, J = 7.8 and 9.3 Hz, 17α-H), 5.51 (1 H, br s, 4-H); exact mass found 314.2272, calcd for $C_{21}H_{30}O_2$ (M^+ – 60) 314.2821.

Reaction of Compound 3 with *tert*-Butyldimethylsilyl Chloride. Compound **3** (1.85 g, 6.38 mmol) and imidazole (652 mg, 9.57 mmol) were dissolved in 40 mL of DMF, and TBDMS-Cl (1.44 g, 9.57 mmol) was added to this solution and the mixture was stirred at room temperature for 1 day. After this time, another 1.44 g of the reagent was added and the stirring was continued for an another day. The mixture was poured into water (100 mL) and extracted with AcOEt (500 mL × 2). The combined organic phase was washed with water, dried (Na_2SO_4), and evaporated to give an oily product. Purification of the oil by column chromatography (silica gel, 200 g; hexane–AcOEt, 95:5) and recrystallization gave the silylated products **6**–**8**, respectively.

17β-(*tert*-Butyldimethylsiloxy)androst-4-en-19-ol (6): yield 19% (484 mg); mp 121–122 °C (colorless needles, from acetone); ^{1}H NMR (400 MHz) δ –0.01 and 0.00 (3 H each, s, 17-OSiMe_2), 0.71 (3 H, s, 18-Me), 0.87 (9 H, s, 17-OSi(Me$_2$)CMe_3), 3.52 (1 H, d, J = 10.3 Hz, 19-H_a), 3.53 (1 H, t, J = 8.3 Hz, 17α-H), 3.95 (1 H, d, J = 10.3 Hz, 19-H_b), 5.72 (1 H, br s, 4-H); IR (KBr) 3300 (OH) cm^{-1}. Anal. ($C_{25}H_{44}O_2Si$) C, H.

19-(*tert*-Butyldimethylsiloxy)androst-4-en-17β-ol (7): yield 14% (363 mg); mp 105.5–106 °C (colorless needles, from MeOH); ^{1}H NMR (400 MHz) δ 0.00 and 0.01 (3 H each, s, 19-OSiMe_2), 0.74 (3 H, s, 18-Me), 0.85 (9 H, s, 19-OSi(Me$_2$)CMe_3), 3.59 (1 H, dd, J = 8.3 and 8.8 Hz, 17α-H), 3.65 and 3.77 (1 H each, d, J = 9.8 Hz, 19-H), 5.37 (1 H, br s, 4-H); IR (KBr) 3400 (OH) cm^{-1}. Anal. ($C_{25}H_{44}O_2Si$) C, H.

17β,19-Bis(*tert*-butyldimethylsiloxy)androst-4-ene (8): yield 62% (2.05 g); mp 91–92 °C (colorless plates, from acetone); ^{1}H NMR (400 MHz) δ –0.01, 0.00, 0.025, and 0.036 (3 H each, s, 17- and 19-OSiMe_2), 0.72 (3 H, s, 18-Me), 0.87 and 0.88 (9 H each, s, 17- and 19-OSi(Me$_2$)CMe_3), 3.52 (1 H, dd, J = 7.8 and 8.8 Hz, 17α-H), 3.67 and 3.78 (1 H each, d, J = 10.0 Hz, 19-H), 5.39 (1 H, br s, 4-H). Anal. ($C_{31}H_{58}O_2Si_2$) C, H.

17β-(*tert*-Butyldimethylsiloxy)-19-acetoxyandrost-4-ene (9). Compound **6** (151 mg, 0.373 mmol) was acetylated with acetic anhydride (0.75 mL)–pyridine (1.5 mL) as above. A solid product obtained was recrystallization from MeOH to give **9** (114 mg, 68%) as colorless needles: mp 63.5–64.5 °C; ^{1}H NMR (400 MHz) δ 0.00 and 0.01 (3 H each, s, 17-OSiMe_2), 0.72 (3 H, s, 18-Me), 0.88 (9 H, s, 17-OSi(Me$_2$)CMe_3), 2.05 (3 H, s, 19-OCOMe), 3.53 (1 H, t, J = 8.3 Hz, 17α-H), 4.12 and 4.43 (1 H each, d, J = 11.2 Hz, 19-H), 5.50 (1 H, br s, 4-H); IR (KBr) 1740 (C=O) cm^{-1}. Anal. ($C_{27}H_{46}O_3Si$) C, H.

19-Acetoxyandrost-4-en-17β-ol (10). To a solution of compound **9** (105 mg, 0.291 mmol) in propan-2-ol (2.2 mL) and THF (2.2 mL) was added 3 M HCl (0.72 mL) and the mixture was stirred at room temperature for 1 day. After this time, the reaction mixture was neutralized with $NaHCO_3$ and then the solvent was removed under reduced pressure below 50 °C and the residue was diluted with AcOEt (100 mL). The organic layer was washed with water, dried (Na_2SO_4), and evaporated to give an oil, which was purified by preparative TLC (hexane–AcOEt, 3:1) to yield **10** (53 mg, 68%) as an oil: ^{1}H NMR (400 MHz) δ 0.76 (3 H, s, 18-Me), 2.05 (3 H, s, 19-OCOMe), 3.62 (1 H, dd, J = 8.3 and 8.8 Hz, 17α-H), 4.12 and 4.42 (1 H each, d, J = 11.2 Hz, 19-H), 5.50 (1 H, br s, 4-H); exact mass found 272.2145, calcd for $C_{19}H_{28}O$ (M^+ – 60) 272.2140.

19-(*tert*-Butyldimethylsiloxy)-17β-acetoxyandrost-4-ene (11). Compound **7** (25 mg, 0.062 mmol) was acetylated with acetic anhydride (0.15 mL)–pyridine (0.3 mL) as above. Recrystallization of the crude product from acetone–water afforded **11** (15 mg, 55%) as colorless needles: mp 90–90.5 °C; ^{1}H NMR (400 MHz) δ 0.00 and 0.01 (3 H each, s, 19-OSiMe_2), 0.79 (3 H, s, 18-Me), 0.85 (9 H, s, 19-OSi(Me$_2$)CMe_3), 2.00 (3 H, s, 17-OCOMe), 3.73 and 3.77 (1 H each, d, J = 10.3 Hz, 19-H), 4.54 (1 H, dd, J = 9.2 and 8.0 Hz, 17α-H), 5.37 (1 H, br s, 4-H); IR (KBr) 1735 (C=O) cm^{-1}. Anal. ($C_{27}H_{46}O_3Si$) C, H.

17β-Acetoxyandrost-4-en-19-ol (12). Protecting group at C-19 of compound **11** (45 mg, 0.10 mmol) was hydrolyzed with 3 M HCl in a similar fashion as described for the synthesis of **10**. The product was purified by preparative TLC (hexane–AcOEt, 1:1) and recrystallization from acetone to give **12** (15 mg, 45%) as colorless needles: mp 130–131 °C; ^{1}H NMR (400 MHz) δ 0.80 (3 H, s, 18-Me), 2.03 (3 H, s, 17-OCOMe), 3.53 and 3.94 (1 H each, d, J = 10.3 Hz, 19-H), 4.57 (1 H, dd, J = 7.8 and 9.8 Hz, 17α-H), 5.72 (1 H, br s, 4-H); IR (KBr) 3375 (OH), 1735 (C=O) cm^{-1}. Anal. ($C_{21}H_{32}O_3$) C, H.

Bromination of Androst-4-en-17-one (13) with $CuBr_2$. A solution containing **13** (1.1 g, 4.04 mmol) and $CuBr_2$ (2.7 g, 12.1 mmol) in MeOH (40 mL) was heated under reflux for 9 h and then poured into ice–water (500 mL) and extracted with AcOEt (500 mL × 2). The organic layer was washed with 5% $NaHCO_3$ solution and water, dried (Na_2SO_4), and evaporated to give a solid, which an ^{1}H NMR spectrum showed to be a ca. 7:3:2 mixture of **13**, 16α-bromide **14**, and its 16β-isomer **15** [δ 0.90, 0.93, and 1.10 (18-Me)]. Thus, the mixture was subjected to column chromatography (silica gel, 80 g). Elution with hexane–AcOEt (95:5) gave three fractions: 16α-bromide-rich, ca. 1:1 mixture, and 16β-bromide-rich. Repeated recrystallization of the first or last fraction from MeOH gave compound **14** or **15** in a pure state, respectively.

16α-Bromoandrost-4-en-17-one (14): yield 7% (97 mg); mp 170–172 °C; ^{1}H NMR (400 MHz) δ 0.93 (3 H, s, 18-Me), 1.04 (3 H, s, 19-Me), 4.52 (1 H, m, 16β-H), 5.34 (1 H, br s, 4-H); IR (KBr) 1745 (C=O) cm^{-1}; EI-MS m/z 352 (M^+). Anal. ($C_{19}H_{27}OBr$) C, H.

16β-Bromoandrost-4-en-17-one (15): yield 5% (75 mg); mp 162–165 °C; ^{1}H NMR (400 MHz) δ 1.04 (3 H, s, 19-Me), 1.10 (3 H, s, 18-Me), 4.09 (1 H, m, 16α-H), 5.33 (1 H, br s, 4-H); IR (KBr) 1760 cm^{-1}; EI-MS m/z 352 (M^+). Anal. ($C_{19}H_{27}OBr$) C, H.

3,3-(Ethylenedithio)-19,19-difluoroandrost-4-en-17-one (17). A solution of 19,19-difluoroandrost-4-ene-3,17-dione (**16**)[13] (218 mg, 0.678 mmol), ethane-1,2-dithiol (66 mg, 0.70 mmol), and *p*-toluenesulfonic acid monohydrate (62 mg, 0.324 mmol) in 6 mL of acetic acid was allowed to stand at room temperature for 1.5 h. After this time, the mixture was poured into 100 mL of chilled water and then extracted with AcOEt (100 mL × 3). The organic layer was washed with 5% $NaHCO_3$ solution and water, dried (Na_2SO_4), and evaporated to afford a solid product. Purification of the product with silica gel column chromatography (silica gel, 20 g; hexane–AcOEt, 5:1) and recrystallization from AcOEt gave **17** (118 mg, 41%) as colorless needles: mp 154–155 °C; ^{1}H NMR

(60 MHz) δ 0.93 (3 H, s, 18-Me), 3.35 (4 H, m, 3,3-SCH_2CH_2S-), 5.90 (1 H, s, 4-H), 5.98 (1 H, t, J = 56 Hz, 19-HF_2); IR (KBr) 1728 (C=O) cm^{-1}. Anal. ($C_{21}H_{28}OS_2F_2$) C, H.

19,19-Difluoroandrost-4-en-17-one (18). Compound 17 (110 mg, 0.276 mmol) was desulfurized with sodium metal–liquid NH_3 similarly as described for the synthesis of compounds 2 and 3 [THF, 2 mL; sodium metal, 510 mg (22.2 mmol); 18 mL of liquid NH_3; reaction time, 1 h]. After essentially same workup as above, the oily product was subjected to column chromatography (silica gel, 20 g). Elution with hexane–AcOEt (4:1) gave an oil 18 along with the more polar solid 19. Product 18 (17 mg, 22%) was obtained in a pure state after further purification with preparative TLC (hexane–AcOEt, 2:1): ^1H NMR (400 MHz) δ 0.92 (3 H, s, 18-Me), 5.67 (1 H, s, 4-H), 5.96 (1 H, t, J = 56.2 Hz, 19-CHF_2); IR ($CHCl_3$) 1730 (C=O) cm^{-1}; exact mass found 308.2020, calcd for $C_{19}H_{26}OF_2$ (M^+) 308.1952.

19,19-Difluoroandrost-4-en-17β-ol (19). Crude steroid 19 obtained above was recrystallized from hexane–ether to yield 19 (46 mg, 56%) as colorless needles: mp 102–104 °C; ^1H NMR (400 MHz) δ 0.79 (3 H, s, 18-Me), 3.62 (1 H, t, J = 5.6 Hz, 17α-H), 5.64 (1 H, s, 4-H), 5.98 (1 H, t, J = 56.6 Hz, 19-HF_2); IR (KBr) 3420 (OH) cm^{-1}; exact mass found 310.2152, calcd for $C_{19}H_{28}OF_2$ (M^+) 310.2108. Anal. ($C_{19}H_{28}OF_2$) C, H.

Compound 19 (30 mg, 0.097 mmol) in acetone (4 mL) at 0 °C was stirred during dropwise addition of Jones reagent until there was a permanent orange color and the mixture was allowed to stand at 0 °C for 5 min. After this time, the mixture was poured into chilled water (50 mL) saturated with NaCl, extracted with AcOEt (50 mL × 2), washed with 5% $NaHCO_3$ solution and water, and dried (Na_2SO_4). Evaporation of the solvent gave an oil, which was purified by preparative TLC (hexane–AcOEt, 2:1) to give 18 (18 mg, 60%) as an oily substance. Compound 18 obtained by this method was identical with that obtained above in every respect.

19-(*tert*-Butyldimethylsiloxy)-3,3-(ethylenedithio)-androst-4-en-17-one (20). To a solution of 19-alcohol 1 (1.24 g, 3.29 mmol) in DMF (31 mL) were added TBDMS-Cl (2.39 g, 15.86 mmol) and imidazole (1.03 g, 15.13 mmol), and the reaction mixture was stirred at room temperature for 17 h. After the same workup as described for the silylation of compound 3, the crude product obtained was purified by column chromatography (silica gel, 180 g; hexane–AcOEt, 95:5) and recrystallization from acetone to afford 20 (1.55 g, 95%) as colorless plates: mp 122–124 °C; ^1H NMR (60 MHz) δ 0.00 (6 H, s, 19-OSiMe_2), 0.90 (12 H, s, 18-Me and 19-OSi(Me_2)CMe_3), 3.37 (4 H, br s, 3,3-SCH_2CH_2S-), 4.09 and 4.53 (1 H each, d, J = 11 Hz, 19-H), 5.77 (1 H, br s, 4-H); IR (KBr) 1737 (C=O) cm^{-1}. Anal. ($C_{27}H_{44}O_2S_2Si$) C, H.

19-(*tert*-Butyldimethylsiloxy)androst-4-en-17-one (21). Thioacetal 20 (1.75 g, 3.56 mmol) was treated with sodium metal–liquid NH_3 essentially as described for the desulfurization of compound 1 (THF, 300 mL; liquid NH_3, 60 mL; Na metal, 5 g; reaction time, 1 h). The product obtained was purified by column chromatography (silica gel, 110 g; hexane–AcOEt, 9:1) to yield 17β-ol 7 (850 mg, 59%) as the major product along with the desired 17-ketone 21 (180 mg, 12%). Thus, 7 (800 mg, 1.96 mmol) was converted to 21 by oxidation with Jones reagent (200 mL of acetone, 1 mL of Jones reagent, 0 °C, 5 min). After usual workup, the product was recrystallized from MeOH to give 21 (570 g, 71%) as colorless needles: mp 67–68 °C; ^1H NMR (400 MHz) δ 0.00 and 0.01 (3 H each, s, 19-OSiMe_2), 0.85 (9 H, s, 19-OSi(Me_2)CMe_3), 0.86 (3 H, s, 18-Me), 3.65 and 3.78 (1 H each, d, J = 10.3 Hz, 19-H), 5.39 (1 H, br s, 4-H); IR (KBr) 1735 (C=O) cm^{-1}. Anal. ($C_{25}H_{42}O_2Si$) C, H.

Compound 21 (565 mg, 1.41 mmol) was treated with 3 M HCl in a similar fashion as described for the synthesis of 10 to afford compound 2 (309 mg, 76%), mp 155–156 °C (from acetone–hexane), which was identical with the authentic sample in every respect.

17,17-(Ethylenedioxy)androst-4-en-19-ol (22). A solution of 17-ketone 2 (640 mg, 2.22 mmol) in 7 mL of ethylene glycol and 40 mL of toluene containing *p*-toluenesulfonic acid monohydrate (35 mg, 0.18 mmol) was heated under reflux for 2 h with a water separator. Saturated $NaHCO_3$ solution was added to the cooled mixture and the toluene layer was separated. The organic layer was washed with water, dried (Na_2SO_4), and evaporated. The residue was recrystallized from acetone–hexane to give 22 (570 mg, 77%) as colorless leaflets: mp 111–113 °C; ^1H NMR (400 MHz) δ 0.85 (3 H, s, 18-Me), 3.83–3.95 (6 H, m, 17,17-OCH_2CH_2O- and 19-H_2), 5.72 (1 H, m, 4-H); IR (KBr) 3520 (OH) cm^{-1}. Anal. ($C_{21}H_{32}O_3$) C, H.

17,17-(Ethylenedioxy)androst-4-en-19-al (23). To a stirred solution of 19-alcohol 22 (627 mg, 1.89 mmol) in CH_2Cl_2 (21 mL) was added pyridinium dichromate (1.04 g, 2.76 mmol) and the mixture was stirred at room temperature for 8 h. After this time, the reaction mixture was passed through silica gel column (silica gel, 50 g) and eluted with hexane–AcOEt (7:1) to give the crude product, which was recrystallized from acetone–water to give 23 (455 mg, 73%) as colorless needles: mp 120–121 °C; ^1H NMR (400 MHz) δ 0.85 (3 H, s, 18-Me), 3.87 (4 H, m, 17,17-OCH_2CH_2O-), 5.68 (1 H, m, 4-H), 9.82 (1 H, s, 19-H); IR (KBr) 1710 (C=O) cm^{-1}. Anal. ($C_{21}H_{30}O_3$) C, H.

(19*R*/*S*)-19-Ethynyl-17,17-(ethylenedioxy)androst-4-en-19-ol (24). Acetylene (passed through a −70 °C trap and H_2SO_4) was bubbled at room temperature into THF (51 mL, freshly distilled from $LiAlH_4$) for 10 min before and during the dropwise addition of 3 M ethylmagnesium bromide in ether (5 mL, 15 mmol), all at room temperature. Acetylene was bubbled into the clear solution for another 60 min. N_2 was passed over the ethynylmagnesium bromide solution, and aldehyde 23 (335 mg, 1.02 mmol) in dry THF (5 mL) was added dropwise, followed by rinse with THF (3 mL). After vigorous stirring for 40 min, the reaction mixture was poured into 300 mL each of AcOEt and saturated NH_4Cl solution, acidified with 10% HCl, shaken, and separated. The organic layer was washed with 5% $NaHCO_3$ solution and saturated NaCl solution, dried (Na_2SO_4), and evaporated to give a solid. The product was recrystallized from acetone to yield 24 (265 mg, 73%) as colorless needles: mp 181–183 °C; ^1H NMR (400 MHz) δ 0.90 (3 H, s, 18-Me), 2.48 (1 H, d, J = 2.2 Hz, 19-C≡CH), 3.88 (4 H, m, 17,17-OCH_2CH_2O-), 4.80 and 4.82 (0.6 and 0.4 H, d, J = 2.2 Hz, 19-CHOH), 5.55 (1 H, m, 4-H); IR (KBr) 3287 (OH), 2362 and 2344 (C≡C) cm^{-1}; $[\alpha]^{23}_D$ = +47.9° (c = 1.99). Anal. ($C_{23}H_{32}O_3$) C, H.

(19*R*/*S*)-19-Ethynyl-19-hydroxyandrost-4-en-17-one (25). To a solution of acetal 24 (40 mg, 0.16 mmol) in acetone (2 mL) was added 10% H_2SO_4 (0.1 mL). The mixture was stirred at room temperature for 1 h and then diluted with AcOEt (50 mL), washed with 5% $NaHCO_3$ solution and water, dried (Na_2SO_4), and evaporated to give an oil. The product was purified by preparative TLC (hexane–AcOEt, 4:1), and recrystallization from acetone–hexane to yield 25 (24 mg, 69%) as colorless prisms: mp 143–144 °C; ^1H NMR (400 MHz) δ 0.93 (3 H, s, 18-Me), 2.51 (1 H, d, J = 2.2 Hz, C≡C–H), 4.80 and 4.81 (0.6 and 0.4 H, d, J = 2.2 Hz, 19-H), 5.60 (1 H, m, 4-H); IR (KBr) 3267 (OH), 2343 and 2361 (C≡C), 1720 (C=O); $[\alpha]^{25}_D$ = +148.3 (c = 0.30). Anal. ($C_{21}H_{30}O_2$) C, H.

Biochemical Studies. Chemicals. [1β-^{3}H]Androstenedione (25.4 Ci/mmol) (^{3}H distribution: β/α = 74.2/25.8) was purchased from New England Nuclear Corp. (Boston, MA) and NADPH from Kohjin Co., Ltd. (Tokyo, Japan).

Enzyme Preparation. Human term placental microsomes (particles sedimenting at 105000*g* for 60 min) were obtained as described by Ryan.[28] They were washed twice with 0.5 mM dithiothreitol solution, lyophilized, and stored at −20 °C. No loss of activity occurred over the period of the study.

Screening Assay Procedure. Aromatase activity was measured according to the original procedure of Thompson and Siiteri.[17] All enzymatic studies were carried out in 67 mM phosphate buffer, pH 7.5, at a final incubation of volume of 0.5 mL. The incubation mixture contained 180 μM NADPH, 1 μM [1β-^{3}H]-androstenedione (3×10^5 dpm), 40 μg of protein of the lyophilized microsomes, various concentration of inhibitors, and 25 μL of MeOH. Incubation were performed at 37 °C for 20 min in air and terminated by addition of 3 mL of $CHCl_3$, followed by vortexing for 40 s. After centrifugation at 700*g* for 10 min, aliquots (0.3 mL) were removed from the water phase and added to scintillation mixture for determination of ^3H_2O production.

Time-Dependent Inactivation Procedure. Various concentrations of inhibitors 4, 14, 18, and 25 were incubated with

(28) Ryan, K. J. Biological Aromatization of Steroids. *J. Biol. Chem.* **1959**, *234*, 268–272.

or without androstenedione and L-cysteine at 37 °C with the placental microsomes (1 mg of protein), 600 μM NADPH, and MeOH (50 μL) in 67 mM phosphate buffer, pH 7.5, in a total volume of 1 mL in air or N_2 atmosphere. Aliquots (50 μL), in duplicate, were removed at various time periods (0–12 min) and added to a solution of [1β-^{3}H]androstenedione (1 μM, 3.0×10^5 dpm), NADPH (180 μM) in 67 mM phosphate buffer, pH 7.5 (total volume, 0.5 mL), and the mixture was incubated at 37 °C for 20 min. ^3H_2O release was determined as described above.

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Syntheses of Tolrestat Analogues Containing Additional Substituents in the Ring and Their Evaluation as Aldose Reductase Inhibitors. Identification of Potent, Orally Active 2-Fluoro Derivatives

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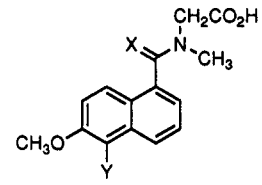
A series of aldose reductase inhibitors were prepared which were analogues of the potent, orally active inhibitor tolrestat (1). These compounds (**5**, **7**, **9**, and **10**) have an extra substituent on one of the unoccupied positions on the naphthalene ring of **1**. Primary amide prodrugs of several members from the series **5** and **7**, namely **6** and **8**, respectively, were also prepared. These compounds were evaluated in two in vitro systems: an isolated enzyme preparation from bovine lens to assess their intrinsic inhibitory activity and an isolated sciatic nerve assay to determine their ability to penetrate membranes of nerve tissue. These compounds were also evaluated in vivo as inhibitors of galactitol accumulation in the lens, sciatic nerve, and diaphragm of galactose-fed rats. In general, compounds in series **5**, **7**, **9**, and **10** were potent inhibitors of bovine lens aldose reductase. 2-Halo-substituted analogues from the series **5**, **7**, and **9** exhibited high activity in the nerve of the 4-day-galactose-fed rat, and in several instances, the primary amide prodrug **8** enhanced the in vivo potency of the respective carboxylic acid **7**. Two 2-fluoro-derivatives, **8a** and **9a**, had especially high activity in vivo and were chosen for additional studies. These compounds were found to be approximately equipotent to tolrestat in the sciatic nerve of the galactose-fed rat and the STZ rat, as judged by their ED_{50}'s in these assays. Although primary amide analogue **8a** did not have intrinsic inhibitory activity toward aldose reductase, it was metabolized to an active form in vivo and also in vitro within the sciatic nerve.

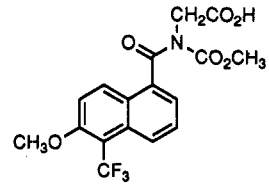
Introduction

Tolrestat[1] (1) is an orally effective aldose reductase inhibitor which is currently marketed under the tradename Alredase for the treatment of diabetic complications.[2,3] The naphthoylamide analogue of tolrestat, **2**,[3a] and the 5-bromo bioisostere, **3**,[3a] were also shown to be potent aldose reductase inhibitors, although both compounds were somewhat less active in vivo than tolrestat. In addition, the *N*-carbomethoxy derivative **4**,[4] was recently shown to have similar potency to **2**. Tolrestat and its analogues belong to the carboxylic acid class of aldose reductase inhibitors. The phthalazine acetic acids, ponalrestat[3d] and zopalrestat,[3e] are other members in this class reported to show good activity in vivo. Sorbinil[2] is the prototypical example for the other major structural class, the five-membered ring cyclic imides.

Our earlier studies demonstrated that the intrinsic and oral activities of analogues of **1** were strongly influenced by the nature and position of the substituents on the naphthalene ring. Optimal activity in vivo was associated with 6-methoxy-5-(trifluoromethyl) substitution. As one aspect of our program to identify new aldose reductase inhibitors, we further explored the scope of these substituent effects. In this regard, we synthesized derivatives of **1–4**, represented by **5**, **7**, **9**, and **10**, in which an extra substituent was added to one of the unoccupied positions on the naphthalene ring.

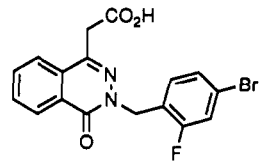
We had also previously shown that the carboxylic acid moiety in **1–4** was a key pharmacophore.[3a,4] Alteration of this group by, for instance, esterification or amidation resulted in intrinsically inactive compounds. However,

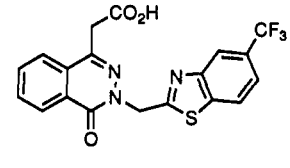




1, X = S, Y = CF_3, Tolrestat
2, X = O, Y = CF_3, "Oxo-tolrestat"
3, X = S, Y = Br

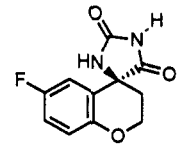
4





Ponalrestat

Zopalrestat



Sorbinil

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