Synthesis of Androst-5-en-7-ones and Androsta-3,5-dien-7-ones and Their Related 7-Deoxy Analogs as Conformational and Catalytic Probes for the Active Site of Aromatase

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Received January 19, 1994*

A series of androst-5-en-7-ones and androsta-3,5-dien-7-ones and their 7-deoxy derivatives, respectively, were synthesized and tested for their abilities to inhibit aromatase in human placental microsomes. All the steroids inhibited the enzyme in a competitive manner with $K_i$'s ranging from 0.058 to 45 $\mu$M. The inhibitory activities of 17-oxo compounds were much more potent than those of the corresponding 17$\beta$-alcohols in each series. Steroids having an oxygen function (hydroxy or carbonyl) at C-19 were less potent inhibitors than the corresponding parent compounds having a 19-methyl group. 3,5-Dien-7-one 24 and its 19-hydroxy and 19-oxo derivatives (12 and 13) as well as 19-oxo-5-en-7-one 3 caused a time-dependent inactivation of aromatase only in the presence of NADPH in which the $k_{inact}$ values of 19-als 3 and 13 (0.143 and 0.189 min$^{-1}$, respectively) were larger than those of the corresponding 19-methyl (23 and 24) and 19-hydroxy (1 and 12) steroids, respectively. 19-Nor-5-en-7-one 4 but not its 3,5-diene derivative 14 also inactivated the enzyme in a time-dependent manner. In contrast, 7-deoxy steroids 21 and 27, having a 19-methyl group, did not cause it. The inactivations were prevented by the substrate androstenedione, and no significant effects of L-cysteine on the inactivations were observed in each case. The results suggest that oxygenation at C-19 would be at least in part involved in the inactivations caused by the inhibitors 23 and 24. The conjugated enone structures should play a critical role in the inactivation sequences.

The conversion of 4-en-3-one androgens to the phenolic estrogens represents the last step in the multienzyme transformations of cholesterol to the female sex steroids. Aromatase is a unique cytochrome P-450 enzyme complex which is responsible for this conversion. Aromatization of the androgens appears to involve three oxygenation steps, eventual loss of the angular methyl group at C-19, and the elimination of the 19- and 23-hydrogens, resulting in the aromatization of the A-ring of the androgen molecule to form estrogen. Two of these oxygenations appear to occur at the C-19 position, while it is presently unknown whether the third oxygenation takes place at this position or at a separate site, such as the C-22 position. In the absence of a 19-methyl group, the oxygenative requirements for the aromatization of estr-4-ene-3,17-dione (19-norandrostenedione, 29) by placental aromatase must be different from the above. However, a single enzyme system seems to aromatize both androgen and 19-norandrogen in the stallion testis, and the aromatization of 19-noradrenogen involves also stereospecific loss of the 18$\alpha$ and 22$\beta$-hydrogens.

Inhibitors of aromatase may be valuable as therapeutic agents in the treatment of estrogen-sensitive breast tumors and as possible antifertility agents. Present knowledge of the mechanism of aromatization had led to the successful design and development of a wide variety of suicide substrates of aromatase. The known suicide substrates primarily have made use of the oxygenation of the 19-angular methyl of a 4-en-3-one steroid in the inactivation process. We have previously reported that a C$_{19}$ steroid having a unique $\alpha\beta$-unsaturated ketone, a 4-en-6-one, instead of the 4-en-3-one, or a 4-one system efficiently blocks the aromatase activity in a reversible manner, even though there is no oxygen function at the C-3 position.

The 4-ene steroids are the most potent competitive inhibitors among those reported so far. On the other hand, 5-en-7-one steroid 23, another $\alpha\beta$-unsaturated keto steroid, and its 19-hydroxy derivative 1 inactivate the enzyme in a mechanism-based manner.

To gain further insight regarding the structure-activity relationships of aromatase inhibitors having a 5-en-7-one structure, we synthesized a series of 5-en-7-one and 3,5-dien-7-one steroids, with or without a 7-carbonyl function, including 19-oxygenated and 19-nor analogs. All the steroids examined inhibited human placental aromatase in a competitive manner in which a 17-carbonyl group is necessary for a tight binding to the active site of aromatase. Moreover, 3,5-dien-7-one derivatives 12, 13, and 24 as well as 5-en-7-oxo steroids 3 and 4 were proved to be mechanism-based inactivators of the enzyme. The 7-oxo function is essential for the irreversible inactivations by the 5-ene and 3,5-diene steroids.

Results

Chemistry. We initially focused on preparation of 5-en-7-oxo steroids (Scheme 1). Treatment of 19-hydroxyandrostan-5-ene-7,17-dione (1), which was previously synthesized, with a limited amount of NaBH$_4$ in chilled MeOH yielded the 17$\beta$-reduced product 2 (60%) whereas oxidation of it with pyridinium dichromate (PDC) gave 19-oxo steroid 3 (20%). Reaction of the 19-al 3 with KOH in aqueous MeOH afforded the 19-nor derivative 4 (21%). The conformation of a proton at C-10 was determined to be $\beta$ based on the previous findings reported for the similar reaction with 19-oxo-4-en-3-one steroids. Treatment of 3,17$\beta$-dihydroxy-5-en-7-one 5 with p-toluenesulfonyl (tosyl) chloride in pyridine gave 3-tosylate 6 as well as its 17$\beta$-isomer 7 and 3,17$\beta$-ditosylate 8. Reductive
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Scheme 1*

\[
\begin{align*}
1 & \quad \text{i} \quad \text{ii} \\
2 & \\
3 & \\
4 & \\
5: R_1 = R_2 = H \\
6: R_1 = Ts, R_2 = H \\
7: R_1 = H, R_2 = TS \\
8: R_1 = H, R_2 = TS \\
9 & \\
10 & \\
11 & \\
12 & \\
13 & \\
14 & \\
15 & \\
16 & \\
17 & \\
18 & \\
19 & \\
20 & \\
21 & \\
22 & \\
\end{align*}
\]

* Reagents: (i) NaBH₄, MeOH; (ii) pyridinium dichromate, CH₂Cl₂; (iii) KOH, aqueous MeOH; (iv) Ts-Cl, pyridine; (v) Zn powder, NaI.

Scheme 2*

\[
\begin{align*}
10 & \quad \text{i} \\
11 & \quad \text{ii} \\
12 & \quad \text{iii} \\
13 & \quad \text{iv} \\
14 & \\
\end{align*}
\]

* Reagents: (i) N-methylpyrrolidone, 80 °C; (ii) (n-Bu)₃NF, THF; (iii) pyridinium dichromate, CH₂Cl₂; (iv) KOH, aqueous MeOH.

deoxygenation of compound 6 with Zn powder in the presence of NaI produced 3-deoxy compound 9 in good yield.

A series of androsta-3,5-diene-7,17-dione (24) derivatives were synthesized as shown in Scheme 2. Elimination reaction of the known 3β,19-dihydroxy-5-ene-7,17-dione derivative 10, which has a tosyl group at C-3 and a tert-

Butyldimethylsilyl (TBDMS) group at C-19, in basic media at elevated temperature gave 19-hydroxy-3,5-diene-7,17-dione 12 (20%) as well as its 19-TBDMS derivative 11 (65%), deprotection of which was achieved with (n-Bu)₃NF to also produce compound 12. The 3,5-dien-7-one structure was confirmed by the UV (λₘₐₓ 280 nm) and ¹H NMR spectra [conjugated olefinic protons at δ 5.84 (1H, s) and 6.19 (2H, m)]. The 19-ol 12 was converted into 19-al 13 (30%) by the PDC oxidation. Treatment of steroid 13 with KOH in aqueous MeOH similarly gave 19-nor-3,5-dien-7-one 14 as described in the synthesis of compound 4.

The known steroid 3β,19-di hydroxy-5-en-17-one 19-TBDMS ether 15 was converted into 3-tolylation 16 and the subsequent reductive elimination reaction with Zn powder and NaI yielded 3-deoxy steroid 17 (Scheme 3), similarly as described in the synthesis of compound 9. The 19-silyl ether 17 was hydrolyzed to afford 19-01 18 on treatment with the fluoride reagent as described above. Reduction of the 17-one 18 with NaBH₄ in MeOH at 0 °C gave 17β,19-diol 19 in fair yield whereas oxidation of it with PDC yielded 19-al 20 (47%). Bromination of 17-one 21 with cupric bromide in MeOH under reflux afforded 16α-bromo ketone 22 (29%).

Biochemical Properties. Reversible inhibition of aromatase activity by the 5-ene and 3,5-diene steroids with or without a 7-oxo function were initially tested in vitro by enzyme kinetics studies using human placental microsomes. The inhibition by 19-norandrostenedione (29, Chart 1) was also examined in our hands. 5-En-7,17-dione 23 and its 19-hydroxy derivative 1 and androst-4-en-17-one (30) are listed for comparison. The results are
Table 1. Aromatase Inhibition by Various Androst-5-ene Derivatives

<table>
<thead>
<tr>
<th>compound</th>
<th>IC₅₀ μM</th>
<th>Kᵢ μM</th>
<th>inhibition¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-one 23</td>
<td>1.8</td>
<td>0.25</td>
<td>competitive</td>
</tr>
<tr>
<td>17β-ol 9</td>
<td>42</td>
<td>5.5</td>
<td>competitive</td>
</tr>
<tr>
<td>19-hydroxy-17-one 1</td>
<td>56</td>
<td>11</td>
<td>competitive</td>
</tr>
<tr>
<td>17,19-diol 2</td>
<td>140</td>
<td>45</td>
<td>competitive</td>
</tr>
<tr>
<td>17C,19-diol 3</td>
<td>60</td>
<td>13</td>
<td>competitive</td>
</tr>
<tr>
<td>19-nor-17-one 4</td>
<td>28</td>
<td>2.4</td>
<td>competitive</td>
</tr>
<tr>
<td>3,5-Dien-7-one Steroid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-one 24</td>
<td>1.8</td>
<td>0.22</td>
<td>competitive</td>
</tr>
<tr>
<td>17β-ol 25</td>
<td>42</td>
<td>5.3</td>
<td>competitive</td>
</tr>
<tr>
<td>19-hydroxy-17-one 12</td>
<td>60</td>
<td>15</td>
<td>competitive</td>
</tr>
<tr>
<td>17,19-diol 13</td>
<td>11</td>
<td>1.8</td>
<td>competitive</td>
</tr>
<tr>
<td>19-nor-17-one 14</td>
<td>33</td>
<td>3.0</td>
<td>competitive</td>
</tr>
<tr>
<td>5-En Steroid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-one 21</td>
<td>0.66</td>
<td>0.12</td>
<td>competitive</td>
</tr>
<tr>
<td>17β-ol 26</td>
<td>30</td>
<td>3.0</td>
<td>competitive</td>
</tr>
<tr>
<td>19-hydroxy-17-one 18</td>
<td>6.9</td>
<td>1.0</td>
<td>competitive</td>
</tr>
<tr>
<td>17β,19-diol 19</td>
<td>62</td>
<td>9.8</td>
<td>competitive</td>
</tr>
<tr>
<td>17,19-diene 20</td>
<td>9.0</td>
<td>1.4</td>
<td>competitive</td>
</tr>
<tr>
<td>16a-bromo-17-one 22</td>
<td>55</td>
<td>11</td>
<td>competitive</td>
</tr>
<tr>
<td>3,5-Diene Steroid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17-one 27</td>
<td>0.34</td>
<td>0.068</td>
<td>competitive</td>
</tr>
<tr>
<td>17β-ol 28</td>
<td>18</td>
<td>2.0</td>
<td>competitive</td>
</tr>
</tbody>
</table>

For Comparison

<table>
<thead>
<tr>
<th>compound</th>
<th>IC₅₀ μM</th>
<th>Kᵢ μM</th>
<th>inhibition¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>androstenedione</td>
<td>0.30</td>
<td>0.020</td>
<td>(Kᵢ)</td>
</tr>
<tr>
<td>19-norandrostenedione (29)</td>
<td>1.2</td>
<td>0.14</td>
<td>competitive</td>
</tr>
<tr>
<td>androst-4-en-17-one (30)</td>
<td>0.11</td>
<td>0.013</td>
<td>competitive</td>
</tr>
</tbody>
</table>

IC₅₀, μM = IC₅₀ concentration (μM) for half-maximal inhibition of aromatase activity.
Kᵢ, μM = inhibition constant (μM) for half-maximal inhibition of aromatase activity.

References:
2. Lineweaver-Burk plot
3. Competition type

Figure 1. Lineweaver-Burk plot of inhibition of human placental aromatase by 3,5-dien-17-one 27 with androstenedione as a substrate. Each point represents the mean of two determinations which varied by less than 10% of the mean. The inhibition experiments with all the other steroids examined gave essentially similar plots to Figure 1 (data not shown).

A series of the 5-en-7-ones (3 and 4) and the 3,5-dien-7-ones (12-14, and 24) were then tested for their abilities to cause a time-dependent inactivation of aromatase. All the inhibitors examined, except 19-nor-3,5-dien-7-one 14, showed the time-dependent inactivation when they were incubated in the presence of NADPH, whereas the 19-nor steroid 14, at concentrations employed (1.5, 3.0, and 4.5 μM), did not cause it. Pseudo-first-order kinetics were obtained during the first 12 min of the incubation of the inhibitors when the kinetic data were analyzed according to the method of Kitz and Wilson (1988) (Figures 2 and 3).

Double-reciprocal plots of kₐ and kᵢ, versus inhibitor concentration gave kᵢ, and kᵢ, respectively, for the inhibitors (Table 2). 19-Nor-4-en-3-one 29, which is a regioisomer of compound 4, inhibited the aromatase activity in a competitive manner but did not cause the time-dependent inactivation (data not shown).

NADPH was essential for the time-dependent activity loss by the irreversible inhibitors (Figure 4A). The substrate androstenedione blocked the inactivation while a nucleophile, L-cysteine, had no significant effect on it in every case (Figure 4B, C).

On the other hand, the 5-ene steroids 18, 20, and 21 and the 3,5-diene steroid 27, without a carbonyl function at C-7, at concentrations employed (0.7, 1.4, and 2.8 μM for 18 and 20, 0.12 and 0.24 μM for 21, and 0.06, 0.12, and 0.24 μM for 27), did not cause a time-dependent inactivation of aromatase (data not shown).
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Table 2. Kinetic Analysis of Time-Dependent Inactivation of Aromatase Caused by Androst-5-ene-7,17-dione Derivatives

<table>
<thead>
<tr>
<th>compound</th>
<th>$K_i$, $\mu M$</th>
<th>$k_{inact}$, min$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-En-7-one Steroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-methyl steroid 23</td>
<td>0.15</td>
<td>0.069$^a$</td>
</tr>
<tr>
<td>19-ox 1</td>
<td>11</td>
<td>0.058$^a$</td>
</tr>
<tr>
<td>19-al 3</td>
<td>20</td>
<td>0.143</td>
</tr>
<tr>
<td>19-nor steroid 4</td>
<td>2.6</td>
<td>0.068</td>
</tr>
<tr>
<td>3,5-Dien-7-one Steroid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19-methyl steroid 24</td>
<td>0.19</td>
<td>0.119</td>
</tr>
<tr>
<td>19-ol 12</td>
<td>7.0</td>
<td>0.088</td>
</tr>
<tr>
<td>19-al 13</td>
<td>2.9</td>
<td>0.189</td>
</tr>
<tr>
<td>19-nor steroid 14</td>
<td>NT$^c$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Apparent $K_i$ and $k_{inact}$ were obtained by Kitz-Wilson plot.
$^b$ Reference 10. $^c$ NT: the time-dependent inactivation was not observed.

27 is similar to that of the natural substrate androstenedione ($K_m = 0.020 \mu M$). Conformational change of the A,B-ring system and/or stereoelectronic effect may be involved in the increased affinity, although there is no evidence of that at present. The inhibitory activities of the 17-alcohols are, respectively, weaker than those of the corresponding 17-oxo analogs ($K_i, \mu M$: 0.12 vs 0.058 (21 vs 27), 0.15 vs 0.025 (2 vs 25), 5.5 vs 2.0 (9 vs 26), 9.8 vs 0.53 (19 vs 14), 5.3 vs 2.0 (23 vs 27), 11 vs 1.0 (1 vs 18), 45 vs 9.8 (2 vs 19), and 13 vs 2.0 (23 vs 27)). These results indicate that a conjugated carbonyl group is not necessary for tight bindings of the 5-ene and 3,5-diene steroids to the active site.

The affinities of 19-ols to the active site of aromatase are significantly lower than those of the parent 19-methyl steroids in each series ($K_i, \mu M$: 0.15 vs 0.025 (21 vs 27), 5.5 vs 3.0 (9 vs 26), 11 vs 1.0 (1 vs 18), 45 vs 9.8 (2 vs 19), and 13 vs 2.0 (23 vs 27)). The similar structure-activity relationships are also observed in the 3,5-diene steroid series ($K_i, \mu M$: 0.22 vs 0.058 (24 vs 27) and 5.3 vs 2.0 (25 vs 28)). These results indicate that a conjugated carbonyl group is not necessary for tight bindings of the 5-ene and 3,5-diene steroids to the active site.

The affinities of 19-ols to the active site of aromatase are significantly lower than those of the parent 19-methyl steroids in each series ($K_i, \mu M$: 0.15 vs 0.025 (21 vs 27), 5.5 vs 3.0 (9 vs 26), 11 vs 1.0 (1 vs 18), 45 vs 9.8 (2 vs 19), and 13 vs 2.0 (23 vs 27)). The similar results were also obtained for 19-oxo steroids 3, 13, and 20 with $K_i$'s ranging from 1.4 to 13 $\mu M$. It has been reported that in the series of androstenedione and its 16a-hydroxy$^{1,21}$ and 6-oxo$^{22}$ derivatives, which are substrates for aromatase, their 19-ols and 19-als have lower affinities for the enzyme compared to the corresponding parent steroids.

We$^10$ have previously reported that the 5-ene-7-one 23 as well as its 19-hydroxy derivative 1 inactive aromatase in a suicidal manner. In this study, not only 19-oxo and 19-nor derivatives (3 and 4) of inhibitor 23 but also 3,5-dien-7-one steroids and 19-methyl (24), 19-hydroxy (12), and 19-oxo (13) compounds inactivated the aromatization of androstenedione in a time-dependent, pseudo-first-order manner in the presence of NADPH in air; on the other hand, 19-nor-3,5-dien-7-one 14 did not. A double-reciprocal plot$^{18}$ 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 (Table 2). This indicates formation of a dissociable enzyme-inhibitor complex followed by unimolecular inactivation. The apparent $K_i$'s are similar to the apparent $K_i$'s obtained from the competitive experiments described in Table 1, suggesting that the initial binding of the inhibitors to the enzyme is not rate-limiting in the inactivation process.

Discussion

The 5-en-17-one steroid 21 and the 3,5-diene-7,17-dione steroid 24 are good competitive inhibitors of human placental aromatase, of which $K_i$ values (0.12 $\mu M$ for 21 and 0.22 $\mu M$ for 24) are comparable to that of the 5-ene-7,17-dione 23 ($K_i = 0.25 \mu M$) reported previously.$^{19}$ An introduction of a double bond at C-3 of compound 21 markedly enhanced affinity to the active site of aromatase in which $K_i$ value (0.058 $\mu M$) of the 3,5-diene derivative

Figure 2. Time-dependent inactivation (A) and concentration-dependent inactivation (B) by 3,5-dien-7-one 24 in the presence of NADPH in air. Concentrations of the inhibitor: control (0 $\mu M$), $\bullet$: 0.25 $\mu M$, $\times$: 0.50 $\mu M$, $\triangle$: 0.75 $\mu M$. Each point represents the mean of two determinations which varied by less than 10% of the mean.

Figure 3. Time-dependent inactivation (A) and concentration-dependent inactivation (B) of human placental aromatase by 19-nor-5-en-7-one 4 in the presence of NADPH in air. Concentrations of the inhibitor: control (0 $\mu M$), $\bullet$: 1.25 $\mu M$, $\times$: 2.5 $\mu M$, $\triangle$: 5.0 $\mu M$. Each point represents the mean of two determinations which varied by less than 10% of the mean. The time-dependent inactivation experiments with compounds 3, 12, and 13 gave essentially similar plots to Figures 2 and 3 (data not shown).
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.

The inactivation rates of the 19-als 3 and 13 are faster than those of the others in each series and the 19-ols 1 and 12 are also suicide substrates of aromatase. On the basis of these facts along with the relative affinities to the active site of the enzyme of the 19-oxygenated steroids and their parent 19-methyl steroids, it is suggested that the inactivation may proceed through 19-oxygenation in each series, although there is no direct evidence of this. Robinson’s group and our group have demonstrated that the 19-methyl group of 3-deoxy-4-ene steroids having a double bond at C-2 or difluoro and acetylenic alcohol functions at C-19 are oxygenated by aromatase. In contrast, all the 7-deoxy steroids examined did not cause a significant time-dependent inactivation of aromatase, so a conjugated carbonyl structure, 5-en-7-one or 3,5-dien-7-one, should be essential for the formation of the reactive electrophile.

19-Nor steroid 4, having a 5-en-7-one structure, unexpectedly, inactivated aromatase in a time-dependent manner only in the presence of NADPH in air whereas another 19-nor steroid 14 having a 3,5-dien-7-one structure or 19-norandrostenedione (29) (K_i = 0.14 μM) having a 4-en-3-one structure did not. 19-Norandrogens are aromatized far more slowly than androgens. Thompson and Sliteri have postulated that the lack of the C-19 methyl group would result in an unfavorable orientation of the substrate with regard to the heme of aromatase, which would lead to a slowing down of the rate of oxygenation. The aromatization mechanism of 19-norandrogens is currently unknown. Compound 29 is converted to its 1β-hydroxy derivative by human placental aromatase, although it is not clear whether the 1β-hydroxylation is involved in the aromatization mechanism. Based on these previous findings, the following mechanism is plausible for the inactivation by the 19-nor-4-en-7-one 4. The 1β-hydroxy derivative of inhibitor 4 is initially produced by aromatase reaction, followed by its conversion to 1(10),5-dien-7-one through further oxygenation by the enzyme or nonenzymatic dehydration. The 1(10),5-diene intermediate covalently binds to the enzyme in a 1,6-addition manner to result in the irreversible inactivation of the enzyme. The 3,5-dien-7-one 14, which is not an inactivator of the enzyme, would not be hydroxylated at C-1 principally because of the conformational changes of the A,B-ring system caused by introduction of a double bond at C-3 of compound 4. We have currently carried out the synthesis of the 1,6-acceptor in order to clarify the inactivation mechanism.

In conclusion, 5-en-7-one and 3,5-diene steroids with or without a carbonyl function at C-7 are good to poor competitive inhibitors of human placental aromatase in which a C-17 carbonyl function is essential for tight binding to the active site of aromatase in each series. The 7-oxo derivatives including the 19-nor steroid 4 inactivate aromatase in a suicidal manner. The present results add new aspects to structure–activity relationships of aromatase inhibitors, especially suicide substrates of aromatase. Furthermore, the inhibitors examined are promising to play an important role for understanding not only catalytic functions of aromatase as well as the mechanism.

Figure 4. Inactivation of human placental aromatase by 3,5-dien-7-one 24 under various conditions. (A) In the absence of NADPH, the inhibitor at concentrations of 0.25 μM (X), 0.50 μM (△), and 0.75 μM (●) failed to produce the inactivation. Control sample contained no inhibitor (○). (B) Androstenedione at concentration of 0.3 μM (X) protected aromatase from inactivation caused by the inhibitor (0.25 μM) (●) in the presence of NADPH. Control sample with (△) or without (○) androstenedione contained no inhibitor. (C) In the presence (X) or absence (△) of L-cysteine (0.5 mM), a pseudo-first-order inactivation of aromatase by the inhibitor (0.25 μM) was observed. Control sample with (△) or without (○) L-cysteine contained no inhibitor. Each point represents the mean of two determinations which varied by less than 10% of the mean. The inactivation experiments with compounds 3, 4, 12, and 13 in the absence of NADPH and in the presence of androstenedione or L-cysteine gave essentially similar results to Figure 4 (data not shown).

The rate of inactivation decreased when the substrate androstenedione was included in the incubation mixture. In the nucleophile protection experiment, L-cysteine failed to protect aromatase from inactivation by the inactivators.
of the aromatase reaction but also conformational features of the active site.

Experimental Section

Chemistry. Materials and General Methods. Melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 1725X spectrophotometer and UV spectra in 95% EtOH solutions on a Hitachi 150-20 spectrophotometer. "H NMR spectra were obtained in CDC13 solutions with JEOL GSX 400 (400 MHz) and JOEL EX 270 (270 MHz) spectrometers using tetramethylsilane (δ = 0.00) or CHCl3 (δ = 7.26, for TBDMS derivatives) as an internal standard, and mass spectra (MS) with a JEOL JMS-DX 303 spectrometer. Thin-layer chromatography (TLC) was performed on E. Merck precoated silica gel plates (Kieselgel 60 F254).

17-Bis[(-Tolylsulfonyl)]oxy]-38-hydroxyandrost-5-en-3-one (7) was obtained from the more polar fraction than that of 5 (35%): "H NMR (270 MHz) δ 0.80 (3H, s, 18-Me), 1.15 (3H, s, 19-Me), 1.45 (3H, s, 20-Me), 3.85 (1H, br m, 3α-H), 4.99 (1H, br d, 17α-H), 5.78 and 5.80 (2H each, δ = 8.1 Hz, aromatic protons); exact MS found 458.2090, calcd for C37H30O3S 458.2127.

17b-[Toluenesulfonyloxy]-androst-5-en-3-one (6): yield 25%; "H NMR (270 MHz) δ 0.75 (3H, s, 18-Me), 1.18 (3H, s, 19-Me), 2.46 (3H, s, Ph-Me), 3.65 (1H, t, J = 7.8 Hz, 17α-H), 4.38 (1H, br m, 3α-H), 5.61 (1H, d, J = 2.0 Hz, 6-H), 7.38 and 7.50 (2H each, δ = 8.1 Hz, aromatic protons); exact MS found 458.2127, calcd for C37H30O3S 458.2127.

17b-[Toluenesulfonyloxy]-androst-5-en-3-one (6): yield 25%; "H NMR (270 MHz) δ 0.75 (3H, s, 18-Me), 1.18 (3H, s, 19-Me), 2.46 (3H, s, Ph-Me), 3.65 (1H, t, J = 7.8 Hz, 17α-H), 4.38 (1H, br m, 3α-H), 5.61 (1H, d, J = 2.0 Hz, 6-H), 7.38 and 7.50 (2H each, δ = 8.1 Hz, aromatic protons); exact MS found 458.2127, calcd for C37H30O3S 458.2127.

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to silica gel column chromatography (hexane–AcOEt, 6/1) in which the product that eluted was recrystallized from benzene–ether to afford 14 (18 mg, 38%); mp 159–160 °C; UV λ max (ε) (278 nm) = 300 (800); IR (KBr) v max (cm−1) = 3590, 3380, 1720, and 1600 cm−1 (C=O). A 32 mg (11.2 μmol) of this tosylated product was treated with Zn powder (2.0 g, 30.6 mmol) and NaI (2.1 g, 14 mmol) similarly to the synthesis of 8 (ethylene glycol dimethyl ether, 45 mL; water, 15 mL; pH 7); the reaction mixture was stirred for 22 h. Silicagel column chromatography (hexane–AcOEt, 8/1) of the product and a subsequent recrystallization from acetone yielded 16 (11.2 g, 76%): mp 139–141 °C; IR (KBr) v max (cm−1) = 3230, 1740 (C=O), 1351 (S=O), 1277 (pTolylsulfonyl) cm−1; 1H NMR (270 MHz) δ 0.93 (3H, s, 18-Me), 5.74 (1H, s, 6-H), 6.21 (1H, dd, J = 10.9 Hz, 19-Ha), 5.68 (1H, t, J = 8.0 Hz, aromatic protons). Anal. (C32H40O5S) C, H.

The synthesis of 8 (ethylene glycol dimethyl ether, 45 mL; water, 15 mL; pH 7); the reaction mixture was stirred for 22 h. Silicagel column chromatography (hexane–AcOEt, 8/1) of the product and a subsequent recrystallization from acetone yielded 16 (1.12 g, 76%): mp 139–141 °C; IR (KBr) v max (cm−1) = 3230, 1740 (C=O), 1351 (S=O), 1277 (pTolylsulfonyl) cm−1; 1H NMR (270 MHz) δ 0.93 (3H, s, 18-Me), 5.74 (1H, s, 6-H), 6.21 (1H, dd, J = 10.9 Hz, 19-Ha), 5.68 (1H, t, J = 8.0 Hz, aromatic protons). Anal. (C32H40O5S) C, H.

Reference