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7-SUBSTITUTED 1,4,6-ANDROSTATRIENE-3,17-DIONES AS ENZYME-ACTIVATED IRREVERSIBLE INHIBITORS OF AROMATASE*

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Summary—7-Phenyl-1,4,6-androstatriene-3,17-dione (**4**), 7-benzyl-1,4,6-androstatriene-3,17-dione (**5**) and 7-phenethyl-1,4,6-androstatriene-3,17-dione (**6**) were synthesized and evaluated *in vitro* in human placental microsomes as enzyme-activated irreversible inhibitors of aromatase. The compounds were synthesized from appropriate 7-substituted 4,6-androstadiene-3,17-diones by reaction with DDQ under neutral conditions. All the compounds produced a first order inactivation of aromatase in the presence of NADPH but not in the absence of NADPH. Substrate 4-androstene-3,17-dione protected the enzyme from inactivation by the inhibitors. Furthermore, cysteine failed to protect aromatase from inactivation by compounds **5** and **6**. In contrast, cysteine partially protected aromatase from inactivation by compound **4**. Irreversibility studies illustrated the covalent nature of the inactivation by **4**, **5** and **6**. The above experimental evidence demonstrated that compounds **5** and **6** are effective enzyme-activated irreversible inhibitors of aromatase.

INTRODUCTION

Aromatase is the cytochrome *P*-450 enzyme complex responsible for the conversion of androgens to estrogens. Inhibitors of aromatase have proven to be effective in causing regression of hormone-dependent breast tumors in rats [1–3] and humans [4, 5]. Recently, research efforts have focused on enzyme-activated irreversible inhibitors of aromatase. The irreversible nature of the inhibition involves the conversion of the inhibitor to a chemically reactive species by the enzyme, and subsequently a covalent bond is formed between the enzyme and the inhibitor. Such agents are expected to be more effective agents *in vivo* than reversible inhibitors. Several enzyme-activated inhibitors of aromatase have been developed [6–12].

Several 7α-thiosubstituted androstenediones result in inhibitors with high affinity to the enzyme aromatase [13–15]. 7α-(4′-Amino)phenylthio-4-androstene-3,17-dione (7α-APTA) was the most potent competitive inhibitor among the compounds synthesized [13]. Androstenedione derivatives with extended linear conjugation in ring A and/or ring B have demonstrated good inhibition [16, 17]. Additionally, several 4,6-androstene-3,17-diones containing 7-substituents with carbon–carbon linkages were recently synthesized and proven to be effective inhibitors of aromatase [18]. The introduction of a C_1–C_2 double bond into 7α-APTA yielded 7α-(4′-amino)phenylthio-1,4-androstadiene-3,17-dione (7α-APTADD) [19], an enzyme-activated inhibitor of aromatase. Addition of a C_1–C_2 double bond to the 7-substituted 4,6-androstatriene-3,17-diones would result in 7-substituted 1,4,6-androstatriene-3,17-diones, which may be effective enzyme-activated inhibitors of aromatase. The synthesis and biochemical evaluation of these new enzyme-activated inhibitors of aromatase are described in this report.

EXPERIMENTAL

General

Steroids were purchased from Searle Laboratories (Skokie, Ill.) or Steraloids (Wilton, N.H.) and checked for purity by thin-layer chromatography or melting point. The 7-substituted 4,6-androstadiene-3,17-diones were prepared as previously described [16]. Chemicals were purchased from Aldrich Chemical Co. (Milwaukee, Wis.). Dioxane was dried with calcium hydride and distilled from sodium. Aluminum oxide (basic) was obtained from Fischer Scientific (Fair Lawn, N.J.). TLC plates were purchased from Analtech Inc. (Newark, N.J.). Biochemicals were obtained from Sigma Chemical Co. [1β-^{3}H]4-Androstene-3,17-dione was purchased from New England Nuclear (Boston, Mass). Melting points were obtained on a Thomas–Hoover capillary

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melting point apparatus and were uncorrected. i.r. spectral data was recorded on a Beckman i.r. 4230 spectrophotometer. NMR spectra were obtained with either a Bruker HX-90E NMR spectrometer (90 MHz), a Bruker WP-80DS NMR spectrometer (80 MHz) or an IBM AF/250 spectrometer in the pulse mode. Mass spectra were obtained at The Ohio State University Chemical Instrumentation Center using a Kratos MS-30 mass spectrometer. Elemental analyses were performed by Galbraith Labs Inc. (Knoxville, Tenn.). Centrifugation was performed on a Sorvall RC2-B centrifuge and a Beckman L5-50B ultracentrifuge was used for ultracentrifugation. Radioactive samples were detected with a Beckman LS 6800 scintillation counter using Formula 963 (New England Nuclear) as the counting solution.

Chemistry

7-Phenyl-1,4,6-androstatriene-3,17-dione (4). To a solution of 7-phenyl-4,6-androstadiene-3,17-dione **1** (0.4 g, 1.11 mmol) in benzene (20 ml) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 0.302 g, 1.33 mmol). The mixture was heated under reflux for 12 h. The solution was then cooled to room temperature and solvent evaporated under reduced pressure to leave a dark green residue. This material was dissolved in $CHCl_3$ and applied to a basic alumina column (5 g). Elution with $CHCl_3$ afforded **4** which was recrystallized from acetone/hexane to give pure **4** (0.21 g, 52.8%). m.p. 118–120°C i.r. (KBr) 3050, 2960, 2940, 2870, 1738, 1655, 1600, 1380, 800 cm^{-1}; [^{1}H]NMR δ 1.05 (s, 3H, C_{18}), 1.29 (s, 3H, C_{19}), 6.10 (t, 1H, C_4), 6.27–6.33 (m, 2H, C_2 and C_6), 7.09 (d, 1H, C_1), 7.25–7.39 (m, 5H, aromatic); MS *m/e* (rel. intensity) 358 (M^+, 1.00), 219 (0.152), 210 (0.467), 149 (0.127), 91 (0.100). Anal. C, H for $C_{25}H_{26}O_2$.

7-Benzyl-1,4,6-androstatriene-3,17-dione (5). To compound **2** (1 g, 2.67 mmol) in benzene (30 ml) was added DDQ (1.2 g, 5.28 mmol). The mixture was treated in a similar manner as in **4**. Compound **5** was isolated as yellowish crystals (0.49 g, 49%). m.p. 87–89°C. i.r. (KBr) 3020, 2920, 2870, 1730, 1640, 1595, 1370, 690 cm^{-1}; [^{1}H]NMR δ 0.95 (s, 3H, C_{18}), 1.16 (s, 3H, C_{19}), 3.64 (s, 2H, benzyl), 5.93 (s, 1H, C_4), 6.04 (Br s, 1H, C_6), 6.23–6.28 (d of d, 1H, C_2), 7.03 (d, 1H, C_1), 7.17–7.37 (m, 5H, aromatic); MS *m/e* (rel. intensity) 372 (M^+, 0.150), 281 (0.224), 253 (0.500), 219 (0.120), 171 (0.148), 131 (0.132), 106 (0.370), 91 (1.00). Anal. C, H for $C_{26}C_{28}O_2$.

7-phenethyl-1,4,6-androstatriene-3,17-dione (6). Compound **3** (1 g, 2.59 mmol) in benzene (30 ml) was added DDQ (1.2 g, 5.28 mmol). The mixture was treated with similar manner as in **4**. Compound **6** was isolated as yellowish crystals (0.56 g, 56%). m.p. 174.5–176°C i.r. (KBr) 3020, 2980, 2930, 2870, 1735, 1650, 1600, 1370, 800 cm^{-1}; [^{1}H]NMR δ 1.02 (s, 3H, C_{18}), 1.04 (s, 3H, C_{19}), 5.98 (s, 1H, C_4), 6.23–6.27 (m, 2H, C_2 and C_6), 7.04 (d, 1H, C_1), 7.18–7.38 (m, 5H, aromatic); MS *m/e* (rel. intensity) 386 (M^+, 0.015), 185 (0.014), 171 (0.016), 147 (0.020), 115 (0.018), 105 (0.042), 91 (0.024). Anal. C, H for $C_{27}H_{30}O_2$.

Biochemical methods

Placental microsome preparation. Human placenta were obtained immediately upon delivery from the Ohio State University Hospital and stored on ice during transportation to the laboratory. The preparation of microsomes was performed according to the method of Ryan[20]. All procedures were carried out at 0–4°C. The placenta was cut free of connective tissue and large blood vessels with scissors. The tissue was then homogenized in a cold Waring blender with two parts of tissues to one part of homogenization buffer. The buffer, pH 7, contained 0.05 M sodium phosphate, 0.25 M sucrose and 0.04 M nicotinamide. The homogenate was centrifuged at 10,000 ***g*** for 30 min. The debris was discarded and the supernatant centrifuged at 105,000 ***g*** for 1 h. The microsomal pellet obtained was resuspended in 0.1 M sodium phosphate buffer, pH 7, and centrifuged at 105,000 ***g*** for 1 h. The procedure was repeated once again and the resulting pellet was stored at −70°C until needed.

Aromatase activity in human placental microsomes was assayed by radiometric method developed by Siiteri and Thompson[21] in which the tritium from [1β-^{3}H]4-androstene-3,17-dione was released as ^3H_2O and used as an index of estrogen formation.

Competitive inhibition studies. The procedure is similar to that of Brueggemeier *et al.*[13]. [1β-^{3}H]4-Androstene-3,17-dione (300,000 dpm), various concentration of 4-androstene-3,17-dione (60–500 nM) and a single concentrtion of inhibitor (**4**, **5**, or **6**) were preincubated with propylene glycol, (100 μl), NADP (1.8 mM), glucose-6-phosphate (2.85 mM) and glucose-6-phosphate dehydrogenase (5 units) at 37°C for 5 min. Placental microsomes (0.07–0.1 mg), diluted to 3.5 ml with 0.1 M sodium phosphate buffer, pH 7, were added to the preincubated mixture in shaking water bath and the solution was incubated at 37°C for 15 min. The reaction was stopped by addition of $CHCl_3$ (5 ml), followed by vortexing the samples for 20 s. The samples were then centrifuged for 10 min (1000 ***g***). Aliquots of water (200 μl) were mixed with scintillation cocktail (5 ml) and counted for radioactivity. Assays were run in duplicate and control samples containing no inhibitor were run simultaneously. Blank samples were obtained by incubating boiled microsomes. Kinetic data was analyzed by programs of Cleland[22]. Protein concentrations were determined by the method of Lowry *et al.*[23].

Time-dependent inactivation studies. All incubations were carried out in 0.1 M sodium phosphate buffer, pH 7. Incubations at 37°C contained microsomal protein (0.2–0.3 mg/ml), propylene glycol (100 μl), and NADPH (0.2 mM) in 0.1 M sodium phosphate buffer, pH 7, to a total volume of 10 ml. Various concentrations of inhibitor (0.5–15 μM) were added to the incubations. Aliquots (1.5 ml) were removed at different time periods (0–20 min) and

immediately diluted 1:10 with cold sodium phosphate buffer (0.1 M). Aromatase activity was assayed by adding the microsomal suspension (3 ml) to a mixture of [1β-^{3}H]4-androstene-3,17-dione (0.5 μM, 300,000 dpm), propylene glycol (100 μl), NADP (1.8 mM), glucose-6-phosphate (2.8 mM) and glucose-6-phosphate dehydrogenase (5 units) to a total of 3.6 ml and incubated for 30 min at 37°C. The reaction was stopped by addition of $CHCl_3$ (5 ml) followed by vortexing the samples for 20 s. The samples were then centrifuged for 10 min (1000 *g*). Aliquots of water (1 ml) were mixed with scintillation cocktail (4 ml) and counted for radioactivity. Controls were run simultaneously with the absence of inhibitor. Inactivation studies in the absence of NADPH were performed in the same manner but without NADPH in the initial incubation. Protection studies with substrate were carried out analogous to the inactivation studies with unlabeled 4-androstene-3,17-dione (0.5–0.8 μM) and inhibitor (1.5 μM) included in the initial incubation. Cysteine protection studies were carried out analogous to the inactivation studies with cysteine (0.5 mM) and inhibitor included in the incubation.

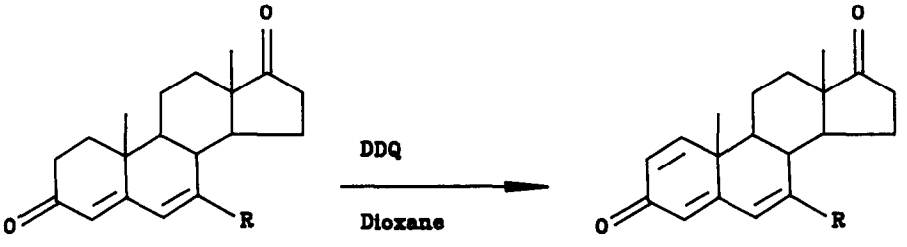
Irreversibility experiments. Inhibitors at various concentrations (4–15 μM) were incubated at 37°C with placental microsomes (2–3 mg) in the presence of 1 mM NADPH, 10 mM glucose-6-phosphate, 5 mM $MgCl_2$, and 0.2 mg of glucose-6-phosphate dehydrogenase in 0.1 M sodium phosphate buffer, pH 7, to a total volume of 3.5 ml. After 1 h, the incubations were diluted with cold buffer, and the microsomes were precipitated by ultracentrifugation. The pellets were resuspended in buffer, diluted, recentrifuged, and resuspended a second time. The amount of aromatase activity was then assayed as described in time-dependent inactivation studies. Identical procedures were performed with inhibitor and microsomes in the abence of NADPH. The control incubation lacked both inhibitor and NADPH.

RESULTS

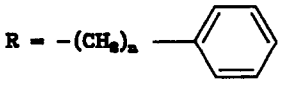
The preparation of 7-substituted 4,6-androstadiene-3,17-diones have been described previously [16]. The synthetic goal in obtaining the potential enzyme-activated inhibitors is the incorporation of C_1–C_2 double bond into 7-substituted 4,6-androstadiene-3,17-diones. The introduction of a double bond preferentially at C_1–C_2 was achieved by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) under neutral condition (Scheme 1). Product isolation was easily achieved by chromatography using basic alumina.

Inhibition studies were conducted on compounds **4**, **5**, and **6** under initial velocity conditions. All the inhibitors demonstrated competitive inhibition under these experimental conditions with apparent K_is ranged from 73.9 to 2256.0 nM (Fig. 1, Table 1). These values are similar to the apparent K_is of 61 nM to 1.42 μM for the 7-substituted 4,6-androstadiene-3,17-diones. Thus, the introduction of C_1–C_2 double bond into the 7-substituted 4,6-androstadiene-3,17-diones does not alter the inhibitory activity to any significant extent.

Compounds **4**, **5**, and **6** were also examined in inactivation studies. All three compounds exhibited time-dependent, first order inactivation of placental aromatase when incubated at various concentrations in the presence of NADPH, as shown for compound **5** in Fig. 2. In the absence of NADPH, no inactivation was observed (Fig. 3). Thus, catalytic activity of aromatase is necessary for inactivation. In order to determine if inactivation occurs at the active site,



1, 2, 3 **4, 5, 6**



1, 4 n=0

2, 5 n=1

3, 6 n=2

Scheme 1

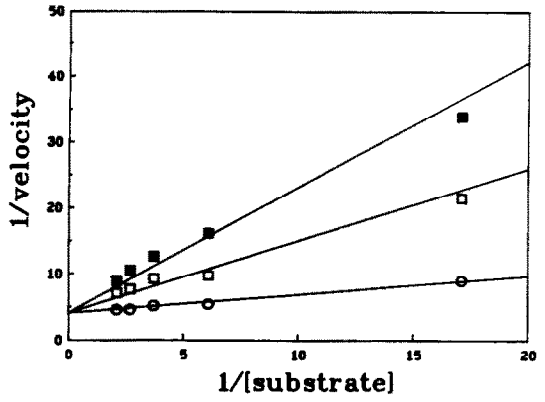
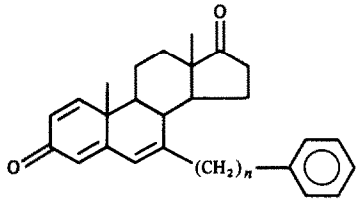


Fig. 1. Double reciprocal plots of aromatase inhibition by inhibitor **5**. Varying concentrations and androstenedione were incubated with aromatase at single inhibitor concentrations of 0 μM (○), 0.3 μM (□), or 0.6 μM (■). Each point represents the average of two determinations with variation of less than 7%.

protection studies were performed by addition of substrate 4-androstene-3,17-dione to the incubation mixture of enzyme, inhibitor and NADPH. The enzyme half-life was lengthened when the substrate concentration was increased from 0–0.8 μM, indicating that inhibitors are inactivating aromatase at the active site (Fig. 4).

Enzyme-activated irreversible inhibition requires that once the inhibitor is converted to a reactive intermediate, it immediately forms a covalent bond with the enzyme at the active site without first diffusing out to the incubation medium. Nucleophiles such as cysteine present in the incubation medium react with the electrophilic intermediate only after it diffuses out of the active site. This would result in a decrease in the rate of inactivation. Inactivation studies were conducted in which cysteine (0.5 mM) was added to the incubation mixture with the inhibitor, enzyme and NADPH. Cysteine failed to protect aromatase from inactivation by inhibitors **5** and **6** after correcting for its general stabilizing effect on the microsomal enzyme preparation (Fig. 5). For inhibitor **4**, cysteine partially protected the enzyme from inactivation (Fig. 6). At the inhibitor concentration of 15 μM, the half-time of inactivation increased from 8.38 to 32.65 min with the presence of cysteine (0.5 mM).

Table 1. Aromatase inhibition by various 7-substituted 1,4,6-androstatriene-3,17-diones



Compound	n	K_i (nM)
4	0	2256 ± 520
5	1	73.9 ± 15.6
6	2	172 ± 41

K_m of androstenedione = 54 nM (SE = 8 nM).

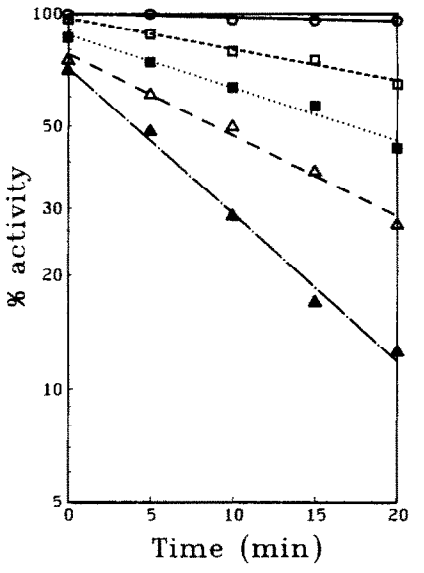


Fig. 2. Inactivation of aromatase by inhibitor **5** in the presence of NADPH. A time-dependent, first-order inactivation of aromatase activity is produced at inhibitor concentration of 0.5 μM (□), 1 μM (■), 1.5 μM (△) and 3 μM (▲). Control samples contained no inhibitor (○). Each point represents the average of two determinations with variation of less than 7%. The value for 100% aromatase activity is 0.022 ± 0.0009 nmol mg^{-1} min^{-1}.

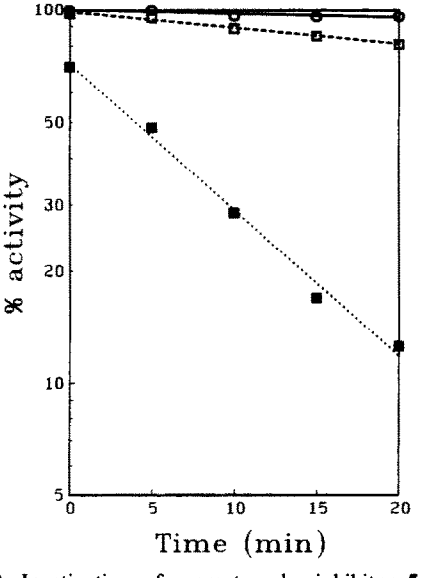


Fig. 3. Inactivation of aromatase by inhibitor **5** in the absence of NADPH. In the absence of NADPH, the inhibitor at concentration of 4 μM (□) failed to produce an inactivation of aromatase while in the presence of NADPH, a first-order inactivation was observed (■). Control samples contained no inhibitor (○). Each point represents the average of two determinations with variation of less than 7%. The value for 100% aromatase activity is 0.019 ± 0.0007 nmol mg^{-1} min^{-1}.

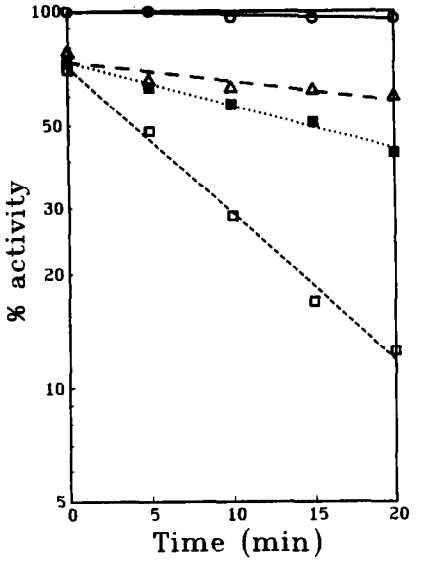


Fig. 4. Protection of inhibitor **5** inactivation of aromatase by substrate. Androstenedione at concentration of 0 μM (□), 0.5 μM (■), 0.8 μM (△) was incubated with microsomal aromatase, inhibitor (1.5 μM), and NADPH and protected the enzyme from inactivation. Control samples were run simultaneously and contained no inhibitor (○). Each point represents the average of two determinations with variation of less than 7%. The value for 100% aromatase activity is 0.026 ± 0.0008 nmol mg^{-1} min^{-1}.

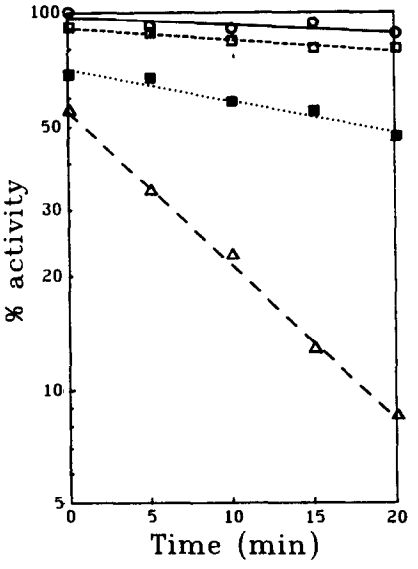


Fig. 6. Inactivation of aromatase by inhibitor **4** in the presence of a nucleophilic trapping agent. A first order inactivation of aromatase by inhibitor **4** (15 μM) was observed in the presence (■) or absence (△) of 0.5 mM cysteine. Control samples with (○) and without (□) cysteine contained no inhibitor. Each point represents the average of three determinations with variation of less than 7%. The value for 100% aromatase activity is 0.023 ± nmol mg^{-1} min^{-1}.

The irreversible nature of the enzyme-inhibitor interaction was further supported by irreversibility studies. In these studies, placental microsomal protein was incubated with inhibitors (5–15 μM) in the presence and absence of 1 mM NADPH. The control incubation lacked both inhibitor and NADPH. After 1 h, the microsomal pellet was precipitated by ultracentrifugation. The placental microsomes incubated with both inhibitor **5** and NADPH exhibited significantly lower aromatase activity after exhaustive washing to remove the inhibitor (Fig. 7). On the other hand, microsomes incubated with only inhibitor **5** exhibited aromatase activity to the same level as control samples after exhaustive washing to remove the inhibitor.

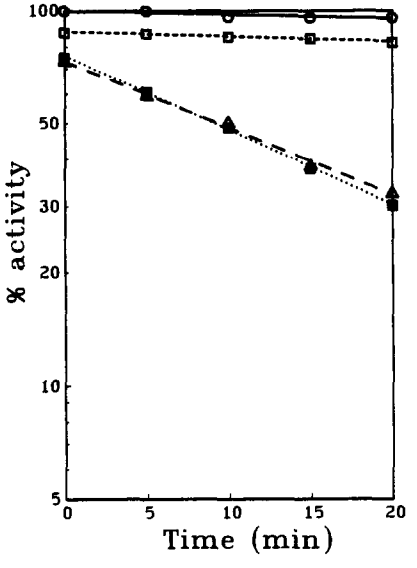


Fig. 5. Inactivation of aromatase by inhibitor **5** in the presence of a nucleophilic trapping agent. A first order inactivation of aromatase by inhibitor **5** (1.5 μM) was observed in the presence (△) or absence (■) of 0.5 mM cysteine. Control samples with (○) and without (□) cysteine contained no inhibitor. Each point represents the average of three determinations with variation of less than 7%. The value for 100% aromatase activity is 0.017 ± 0.0005 nmol mg^{-1} min^{-1}.

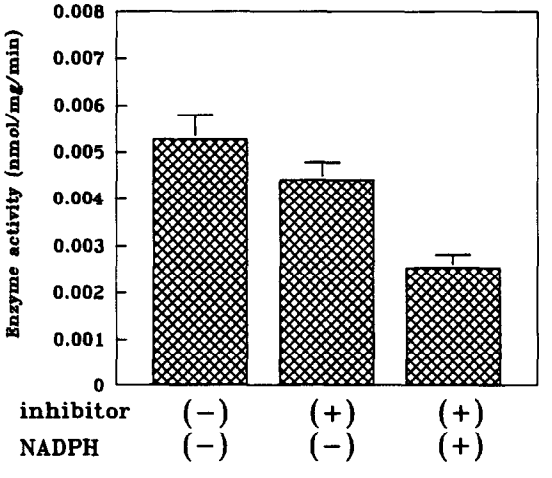


Fig. 7. Irreversibility studies of inhibitor **5**. Placental microsomes were incubated for 1 h at 37°C with inhibitor **5** (5 μM) in the presence and absence of 1 mM NADPH. The control incubation lacked both inhibitor and NADPH.

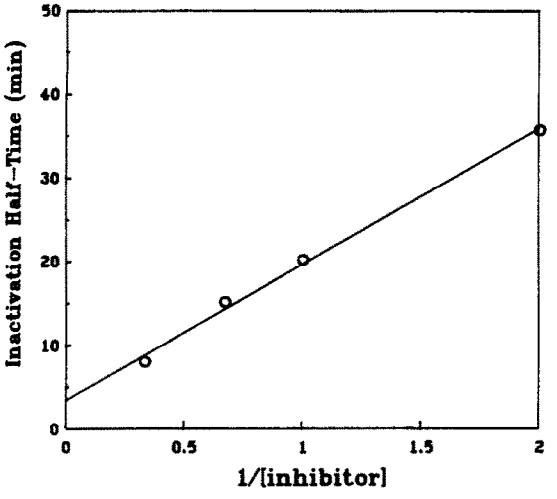


Fig. 8. Plot of the inactivation half-time (min) vs 1/[I] (μM^{-1}) for inhibitor **5**. The apparent K_{inact} for inhibitor **5** is 4.69 μM and the apparent rate of inactivation, k_{app}, is 3.35×10^{-3} sec^{-1}. The inactivation half-time of aromatase at infinite inhibitor concentration is 3.45 min.

The kinetics of the inactivation process were evaluated by plotting the half-time of inactivation vs the reciprocal of inhibitor concentration. Linear plots were obtained exhibiting saturation kinetics (Fig. 8). The apparent K_{inact} and minimum half-time of inactivation of the inhibitors is shown in Table 2. The minimum half-time of inactivation at saturating concentrations of inhibitors **4**, **5** and **6** are 4.62, 3.45 and 5.65 min, respectively.

DISCUSSION

Androstenedione derivatives with additional C_1–C_2 double bond inactivate aromatase through enzyme-activated mechanism [12, 17]. Both ATD and 7α-APTADD are efficient inactivators of aromatase. 7-Substituted 1,4,6-androstatriene-3,17-dione were synthesized as potential enzyme-activated inhibitors of aromatase.

Compound **4**, **5** and **6** have similar binding affinity to the enzyme when compared with their respective 7-substituted 4,6-androstadiene-3,17-dione analogs (Table 2) [16]. The 7 benzyl- and 7-phenethyl-1,4,6-androstatriene-3,17-diones, **5** and **6**, are effective competitive inhibitors of aromatase under initial velocity conditions with apparent K_is of 73.9 and 172.0 nM, respectively. In contrast, 7-phenyl-1,4,6-androstatriene-3,17-dione, **4**, is a poor competitive inhibitor with apparent K_i of 2256.0 nM. Previous investigations on aromatase inhibitors have shown that the enzyme has considerable tolerance for androstenedione and testosterone derivatives with bulky 7α-substituents. The biochemical results of the 7-substituted 1,4,6-androstatriene-3,17-diones suggest that the orientation of the substituents can affect the affinity of the inhibitors to the enzyme complex. The 7-phenyl analog can only adopt a pseudo β position. The 7-benzyl- and 7-phenethyl-groups of 1,4,6-androstatriene-3,17-diones orient themselves in a way that the phenyl rings can protrude into the 7α pocket.

Table 2. Comparison of inactivation kinetics of irreversible aromatase inhibitors

Inhibitor	K_i (nM)	K_{inact} (μM)	$T_{1/2}$ (min)	k_{app} (s^{-1})
4	2220	6.61	4.62	2.50×10^{-3}
5	60.9	4.69	3.45	3.35×10^{-3}
6	172	0.92	5.65	2.04×10^{-3}
7α-APTADD	9.9	0.16	1.40	8.4×10^{-3}
PED	4.5	NR	11.2	1.0×10^{-3}
4-OH-A	10.2	NR	2.9	1.0×10^{-3}
ATD	110	NR	29.8	3.9×10^{-3}

The kinetic parameters of 7-APTADD was reported in Ref. 17 and kinetic parameters of PED, 4-OHA and ATD were taken in part from Ref. 24.
NR = not reported.

All the inhibitors produce a first-order inactivation of aromatase in the presence but not in the absence of NADPH. The rate of inactivation decreased when the substrate androstenedione was included in the incubation mixture. In cysteine protection experiments, cysteine failed to protect aromatase from inactivation by inhibitors **5** and **6**. Thus, covalent bond formation between the enzyme and the reactive inhibitor intermediate appears to occur rapidly at the active site, therefore preventing diffusion of the activated inhibitor into the surrounding media. For inhibitor **4**, cysteine partially protected the enzyme from inactivation. This is conceivable because of the low affinity and high apparent K_i (2.256 μM) of this inhibitor for aromatase. Once the inhibitor is activated, it may slowly diffuse out into the incubation medium where it reacts with cysteine. Thus, lower concentrations of activated inhibitor are available for formation of a covalent bond at the active site. Irreversibility studies involving ultracentrifugation and extensive washing of the microsomal fractions incubated with inhibitors confirm that these 7-substituted 1,4,6-androstatriene-3,17-diones are interacting in an irreversible manner at the active site of the enzyme.

The inactivations of aromatase produced by these agents are more rapid than the inactivations produced by 10-propargyl-4-estrene-3,17-dione (PED; MDL 18,962) and 1,4,6-androstatriene-3,17-dione (ATD) [24]. However, the values are less rapid than either 7α-APTADD and 4-OHA [19, 24]. The mechanism of inactivation of aromatase by all of these enzyme-activated irreversible inhibitors remains to be elucidated. Further studies with enzyme-activated inhibitors and purified aromatase preparations may provide additional information on the mechanism(s) of inactivation.

Thus, three 7-substituted 1,4,6-androstatriene-3,17-diones were synthesized and these steroids demonstrated effective enzyme-activated irreversible inhibition of aromatase. The most effective inhibitors were those with flexibility at the 7-substitution i.e. the benzyl or phenethyl moiety. The 7-substituted 1,4,6-androstatriene-3,17-diones may offer an advantage *in*

vivo over the 7α-thiosubstituted analog (7α-APTADD) by providing greater metabolic stability. Further evaluation of these new analogs in cell culture systems and *in vivo* will provide additional information on the efficacy of these new androstadienediones as potential aromatase inhibitors for the treatment of estrogen-dependent cancers.

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