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6β,19-Bridged androstenedione analogs as aromatase inhibitors

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ABSTRACT

Inhibition of aromatase is an efficient approach for the prevention and treatment of breast cancer. New 6β,19-bridged steroid analogs of androstenedione, 6β,19-epithio- and 6β,19-methano compounds **11** and **17**, were synthesized starting from 19-hydroxyandrostenedione (**6**) and 19-formylandrost-5-ene-3β,17β-yl diacetate (**12**), respectively, as aromatase inhibitors. All of the compounds including known steroids 6β,19-epoxyandrostenedione (**4**) and 6β,19-cycloandrostenedione (**5**) tested were weak to poor competitive inhibitors of aromatase and, among them, 6β,19-epoxy steroid **4** provided only moderate inhibition (K_i: 2.2 μM). These results show that the 6β,19-bridged groups of the inhibitors interfere with binding in active site of aromatase.

1. Introduction

Aromatase is the cytochrome P-450–enzyme complex responsible for estrogen biosynthesis [1–3]. The aromatase reaction is thought to proceed through three sequential oxygenations at C-19 of the androgen androst-4-ene-3,17-dione (androstenedione) and testosterone [4–11] (Fig. 1). The first two oxygenations occur at C-19 position to produce 19-hydroxy and 19,19-dihydroxy intermediates. In the third step, C-19 and the 1β,2β-hydrogens are eliminated as formic acid and water, respectively. There appear to be a major clinical role for methods of controlling estrogen-dependent breast cancer. The inhibitors of aromatase represent one route to such control [12–15].

A number of potent aromatase inhibitors, analogs of the natural substrate androstenedione, have been the subjects of clinical trials. Previously, we have reported a series of 6β-alkylandrostenediones as aromatase inhibitors of which 6β-ethyl compound is extremely powerful inhibitor with K_i of 1.4 nM [16–19]. The 6β-hydroxy derivative, a polar steroid, has also high activity to aromatase [20,21]. On the other hand, l9-substituted (hydroxy, methyl and sulfur) androstenedione analogs have been reported as good to powerful competitive inhibitors of the enzyme [22–27]. Considering the facts obtained by the 6β- and 19-substituted steroids, we aimed at compounds with 6β,19-bridged androstenediones as aromatase inhibitors. Previously, 6β,19-bridged steroids, 6β,19-epoxy- [28,29], 6β,19-epithio- [30], 6β,19-methano- [31], and 6β,19-cyclo- [32] progesterones and its 21-hydroxy analogs, have been shown to possess new biological activities of steroid hormones (Fig. 2). However, effect of 6β,19-bridged structures on the aromatase activity have not been explored so far. In this paper, we describe the synthesis of 6β,19-bridged steroids, epoxy-, cyclo-, epithio-, and methano-androstenediones (**4**, **5**, **11**, and **17**), and their inhibitory activity of human placental aromatase.

2. Experimental

2.1. Materials and general methods

6β,19-Epoxyandrost-4-ene-3,17-dione (**4**) [33] and 6β,19-cycloandrost-4-ene-3,17-dione (**5**) [34] were synthesized by the previous reported methods. [1β-^{3}H] androstenedione (specific activity, 27.5 Ci/mmol; ^{3}H-distribution 74–79%) was obtained from New England Nuclear (Boston, MA, USA). NADPH was purchased from Sigma–Aldrich (St. Louise, MO, USA).

Melting points were measured on a Yanagimoto melting point apparatus (Kyoto, Japan) and are uncorrected. Infra red (IR) spectra were recorded on a PerkinElmer FT-IR 1725X spectrophotometer in a KBr pellet or Nujor form, and ultra-violet (UV) spectra were determined in 95% ethanol on a Hitachi 150-20 spectrophotometer (Tokyo, Japan). Nuclear magnetic resonance (NMR) spectra were obtained in $CDCl_3$ solution with JEOL LA 400 (400 MHz for ^{1}H) and JEOL LA 600 (600 MHz for ^{1}H and 150 MHz for ^{13}C) spectrometer (Tokyo, Japan) using tetramethylsilane as an internal standard, and mass (MS) spectra (electron impact mode) and high resolution

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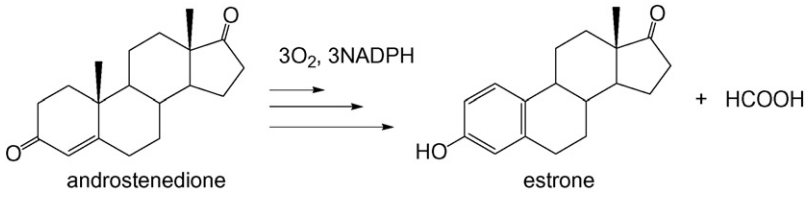

Fig. 1. Aromatase reaction of androstenedione by human placental aromatase.

(HR)-MS with a JEOL JMS-700 spectrometer. Thin-layer chromatography (TLC) was performed on E. Merck precoated plates (silica gel 60F-254, Darmstadt, Germany). Column chromatography was conducted with silica gel 60, 70–230 mesh (E. Merck).

2.2. 6β,19-Cycloandrost-4-ene-3,17-dione (**5**)

p-Toluenesulfonyl chloride (1.13 g, 5.93 mmol) was added to a solution of 19-hydroxyandrostenedione (**6**) (556 mg, 1.84 mmol) in pyridine (27.8 mL) at 0 °C [34]. The reaction mixture was stand for 30 min and allowed to warm to room temperature. After 116 h, the mixture was poured to ice water, acidified by 10% HCl, extracted with EtOAc. The organic layer was washed with 5% $NaHCO_3$ solution and water and dried with Na_2SO_4. The evaporation of the organic solvent afforded 19-tosylate (802 mg, 96%). 28% $NaOCH_3$ in CH_3OH solution (0.48 mL) was added to the 19-tosylate (402 mg, 0.88 mmol) in CH_3OH (12.4 mL) under nitrogen. The mixture was heated under reflux for 5.5 h, diluted with EtOAc, washed with sat. $NaHCO_3$ solution and water, and dried with Na_2SO_4. Evaporation of the solvent gave an oil which was purified by column chromatography (hexane–EtOAc, 5:1, v/v) followed by preparative TLC (hexane–EtOAc, 1:1, v/v). A solid material was recrystallized from acetone to give 6β,19-cycloandrostenedione (**5**) (110 mg, 44%), mp 128–132 °C (reported, 132 °C [34]). ^{1}H NMR δ: 0.97 (3H, s, 18-CH_3), 3.24 (1H, t, J = 5.6 Hz, 6-H), 5.61 (1H, s, 4-H). ^{13}C NMR δ: 14.43 (C-18), 21.28 (C-11), 24.39 (C-15), 30.67 (C-12), 31.69 (C-2), 32.35 (C-1), 33.72 (C-8), 33.95 (C-19), 35.73 (C-16), 43.24 (C-6), 49.02 (C-13), 49.48 (C-9), 49.79 (C-14), 49.99 (C-10), 111.06 (C-4), 180.19 (C-5), 198.88 (C-3), 219.46 (C-17). IR (KBr) cm^{-1}: 1732 and 1655 (C=O). UV λ_{max} nm (ε): 242 (13,900). MS m/z: 284 (M^+, 100%). Analysis calculated for $C_{19}H_{24}O_2$: C, 80.24; H, 8.51. Found: C, 80.15; H, 8.56.

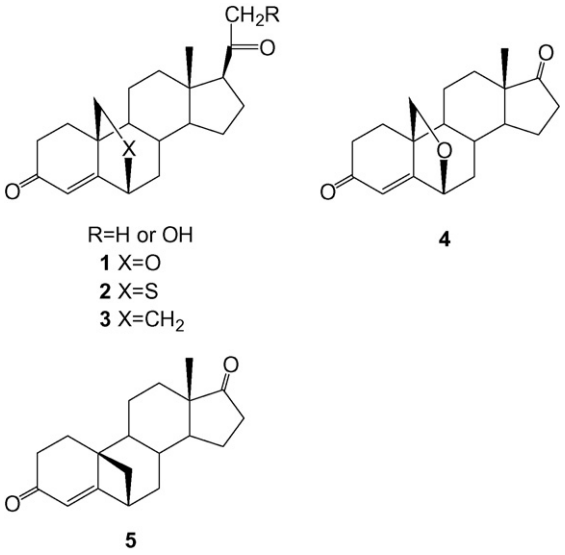

Fig. 2. Structures of 6,19-bridged progesterone analogs and androstenediones.

2.3. 19-Acetylthioandrost-4-ene-3,17-dione (**8**)

A solution of 19-hydroxyandrostenedione (**6**) (1.08 g, 3.57 mmol) in cold pyridine (10.8 mL) was added dropwise to a stirred solution of trifluoromethanesulfonic anhydride (1.34 mL, 7.96 mmol) in cold pyridine (7.4 mL) under nitrogen [30]. The mixture was allowed to warm to room temperature for 1.5 h and diluted with EtOAc. After acidification with 5% HCl, the organic layer was washed with sat. $NaHCO_3$ solution and water and dried with Na_2SO_4. A yellow solid, a crude 19-trifilate (1.42 g), obtained by evaporation of the solvent, was used without further purification in the following step.

A solution of the 19-triflate **7** (1.42 g) and potassium thioacetate (1.42 g, 12.45 mmol) in acetone (73 mL) was stirred at room temperature for 24 h under nitrogen. The reaction mixture was then diluted with EtOAc, washed with sat. $NaHCO_3$ solution and water, and dried with Na_2SO_4. Evaporation of the solvent yielded an oil which was purified by column chromatography (hexane–EtOAc, 3:1, v/v) to afford 19-thioacetate **8** as pale yellow oil (558 mg, 43%). ^{1}H NMR δ: 0.95 (3H, s, 18-CH_3), 2.34 (3H, s, $SCOCH_3$), 3.20 and 3.52 (1H each, d, J = 14.0 Hz, 19-H_2), 5.90 (1H, s, 4-H). IR (Nujor) cm^{-1}: 3586 (OH), 1733, 1683, and 1652 (C=O). UV λ_{max} nm (ε): 238 (16,600). MS m/z: 360 (M^+, 31%), 317 (58), 284 (18), 272 (100). HR-MS calculated for $C_{21}H_{28}O_3S$: 360.1759. Found: 360.1766.

2.4. 3,17-Diethylenedioxy-19-acetylthioandrost-5-ene (**9**)

A mixture of the 19-thioacetate **8** (502 mg, 1.39 mmol) and ethylene glycol (3.5 mL) in benzene (16 mL) was distilled for 15 min to remove traces water. *p*-Toluenesulfonic acid monohydrate (15 mg) was added to the reaction mixture and the mixture was heated under reflux for 4 h, diluted with EtOAc, and added sat. $NaHCO_3$ solution. The organic layer was washed with water, dried with Na_2SO_4, and evaporated to give a semi-solid which was purified by column chromatography (hexane–EtOAc, 20:1, v/v) to afford ketal **9** (432 mg, 69%) as an oil. ^{1}H NMR δ: 0.87 (3H, s, 18-CH_3), 2.31 (3H, s, $SCOCH_3$), 3.05 and 3.43 (1H each, d, J = 14.2 Hz, 19-H_2), 3.85 (8H, m, 3,17-OCH_2CH_2O–), 5.46 (1H, m, 6-H). IR (Nujor) cm^{-1}: 1689 (C=O). UV λ_{max} nm (ε): 235 (5200). MS m/z: 448 (M^+, 21%), 405 (17), 359 (32), 297 (69), 99 (100). HR-MS calculated for $C_{25}H_{36}O_5S$: 448.2284. Found: 448.2292.

2.5. 6β,19-Epithioandrost-4-ene-3,17-dione (**11**)

A solution of the ketal **9** (287 mg, 0.64 mmol) in dry CH_3OH (8 mL) was deoxygenated by bubbling dry nitrogen for 15 min. Then a solution of potassium hydroxide (58 mg, 1.04 mmol) in CH_3OH (0.4 mL) was added and the mixture was stirred at room temperature for 30 min. The reaction mixture was neutralized with 5% HCl and diluted EtOAc. The organic layer was washed with sat. $NaHCO_3$ solution and water, and dried with Na_2SO_4, and evaporated to afford the crude 19-thiol **10** (176 mg). Iodine (234 mg, 0.93 mmol) and triethylamine (61 mL, 0.43 mmol) in dry CH_2Cl_2 (115 mL) were added to a solution of the crude 19-thiol **10** (176 mg) in CH_2Cl_2 (72 mL) at 0 °C, then the mixture was stirred and allowed to warm to room temperature. After 2 h, the reaction mixture was diluted with EtOAc and washed with sat. $Na_2S_2O_3$ solution and water, and dried with

Na_2SO_4. Evaporation of the solvent gave a solid which was purified by column chromatography (hexane–EtOAc, 3:1, v/v) to yield a crude 6,19-epithioandrostenedione **11** (33 mg, 16%). Recrystallization of the crude compound from acetone afforded compound **11**, mp 251–256 °C. ^{1}H NMR δ: 0.99 (3H, s, 18-CH_3), 2.61 and 3.07 (1H each, d, J = 10.3 Hz, 19-H_2), 3.95 (1H, m, 6-H), 5.81 (1H, s, 4-H). IR (KBr) cm^{-1}: 1738 and 1667 (C=O). UV λ_{max} nm (ε): 241 (14,000). MS m/z: 316 (M^+, 100%), 288 (10), 260 (18), 242 (15), 152 (34). Analysis calculated for $C_{19}H_{24}O_2S$: C, 72.11; H, 7.64; S, 10.13. Found: C, 72.35; H, 7.30; S, 10.20.

2.6. 5α-Chloro-19a-hydroxy-6β,19-methanoandrostane-3β,17β-yl diacetate (**13**)

A solution of 19-formylandrost-5-ene-3β,17β-yl diacetate (**12**) (608 mg, 1.51 mmol) in dry CH_2Cl_2 (6 mL) was added to a solution of titanium chloride (3 mL) in CH_2Cl_2 (10 mL) at −70 °C under a nitrogen atmosphere [31]. The reaction mixture was stirred at −30 °C for 2 h, and C_2H_5OH–water (1:3, 12 mL) was added. The resulting solution was further stirred at 0 °C for 15 min. The mixture was diluted with EtOAc, washed with sat. $NaHCO_3$ solution and water, and dried with Na_2SO_4. The solvent was evaporated to give a solid which was purified by column chromatography (hexane–EtOAc, 8:1, v/v) and recrystallization from acetone to afford 5α-chloro-19a-hydroxy-6β,19-methanoandrostane-3β,17β-yl diacetate (**13**) (266 mg, 40%), mp 158–162 °C. ^{1}H NMR δ: 0.79 (3H, s, 18-CH_3), 2.72 (1H, t, J = 12.8 Hz, 6α-H), 4.27 (1H, d, J = 7.7 Hz, 19a-CH), 4.60 (1H, t, J = 8.4 Hz, 17-H), 5.15 (1H, m, 3-H). ^{13}C NMR δ: 12.49 (C-18), 21.14 and 21.36 (3β- or 17β-OCO<u>C</u>H$_3$), 21.67 (C-11), 22.96 (C-15), 25.89 (C-1), 26.71 (C-2), 27.58 (C-16), 29.86 (C-7), 33.87 (C-8), 36.54 (C-19), 36.70 (C-12), 41.45 (C-4), 43.31 (C-10), 47.50 (C-14), 48.03 (C-13), 49.15 (C-9), 54.35 (C-6), 70.30 (C-3), 73.31 (C-19a), 82.08 (C-5), 82.41 (C-17), 170.45 and 171.10 (3β- or 17β-O<u>C</u>OCH$_3$). IR (KBr) cm^{-1}: 3465 (OH) and 1723 (C=O). MS m/z: 438 (M^+, 0.5%), 378 (11), 334 (100), 298 (85). Analysis calculated for $C_{24}H_{35}ClO_5$: C, 65.66; H, 8.04. Found: C, 65.87; H, 8.25.

2.7. 5α-Chloro-6β,19-methanoandrostane-3β,17β-yl diacetate (**14**)

A mixture of DBU (110 μL) and CS_2 (348 μL) was added to a solution of the 19a-hydroxy-6β,19-methano steroid **13** (91 mg, 0.21 mmol) in dimethylformamide (1.25 mL) under nitrogen atmosphere. The reaction mixture was stirred for 1 h, then CH_3I (2.5 mL) was added [35]. After 2 h, the mixture was diluted with EtOAc which was washed successively with sat. $KHSO_4$ solution, sat. $NaHCO_3$ solution, and water. The organic layer was dried with Na_2SO_4. Evaporation of the solvent gave the residue which was dissolved in dry toluene (2 mL). Bu_3SnH (73 μL) was added to the solution under nitrogen and the mixture was heated under reflux for 17 h and another Bu_3SnH (73 μL) was added to the mixture which was heated under reflux for 6.5 h. Then the reaction mixture was diluted with EtOAc and insoluble materials were filtered off. Evaporation of the solvent yielded a solid which was purified by column chromatography (hexane–EtOAc, 10:1, v/v) followed by recrystallization from CH_3OH to give 5α-chloro-6β,19-methanoandrostane-3β,17β-yl diacetate **14** (47 mg, 54%), mp 110–114 °C. ^{1}H NMR δ: 0.82 (3H, s, 18-CH_3), 4.60 (1H, t, J = 8.3 Hz, 17-H), 5.15 (1H, m, 3-H). IR (KBr) cm^{-1}: 1733 (C=O). MS m/z: 422 (M^+, 6%), 362 (45), 326 (76), 119 (100). Analysis calculated for $C_{24}H_{35}ClO_4$: C, 68.15; H, 8.34. Found: C, 68.33; H, 8.51.

2.8. 6β,19-Methanoandrost-4-ene-3,17-dione (**17**)

Potassium hydroxide (23.5 mg, 0.42 mmol) in CH_3OH (0.71 mL) was added to a solution of the diacetate **14** (50 mg, 0.12 mmol) in CH_3OH (1.5 mL) under nitrogen and warmed at 50 °C. After 1.5 h, the reaction mixture was diluted with EtOAc and neutralized with 5% HCl. The organic layer was washed with sat. $NaHCO_3$ solution and water, and dried with Na_2SO_4. Evaporation of the solvent gave a crude 5α-chloro-6β,19-methanoandrostane-3β,17β-diol (**15**) (43 mg, 99%).

Pyridinium chlorochromate (113 mg, 0.53 mmol) and $BaCO_3$ (66 mg, 0.34 mmol) were added to a solution of the crude diol **15** (43 mg, 0.13 mmol) in CH_2Cl_2 (1.5 mL) under nitrogen atmosphere. The reaction mixture was stirred for 1.5 h and passed through silica gel column (5 g) to remove a dark brawn solid. Elution with CH_2Cl_2 and evaporation of the solvent gave a crude 5α-chloro-6β,19-methanoandrostane-3,17-dione (**16**) (31 mg, 72%).

A solution of the crude 5α-androstane steroid **16** (29 mg, 0.09 mmol) in CH_2Cl_2 (1 mL) was stirred for 6 h in the presence of Al_2O_3 (192 mg, 1.88 mmol). Al_2O_3 was removed by filtration and the solvent was evaporated to yield a solid. The solid was recrystallized from acetone to afford 6β,19-methanoandrost-4-ene-3,17-dione (**17**) (10 mg, 42%), mp 196–199 °C. ^{1}H NMR δ: 0.95 (3H, s, 18-CH_3), 2.85 (1H, br. s, 6-H), 5.77 (1H, s, 4-H). IR (KBr) cm^{-1}: 2927 (SR), 1738 and 1670 (C=O). UV λ_{max} nm (ε): 243 (14,400). MS m/z: 298 (M^+, 100%), 256 (84), 148 (44), 135 (82), 91 (39). Analysis calculated for $C_{20}H_{26}O_2$: C, 80.50; H, 8.78. Found: C, 80.28; H, 8.90.

2.9. Enzyme preparation

Human placental microsomes (sedimented after 60 min at 105,000 × g) were obtained as described by Ryan [36]. They were washed once with 0.05 mM dithiothreitol, lyophilized and stored at −80 °C. No significant loss of activity occurred during the period (6 months) of this study. The preparation of human placental microsomes was conducted under the approval of the ethical review committee of Tohoku Pharmaceutical University in accordance with the standard of the Helsinki Declaration.

2.10. Aromatase assay procedure

Aromatase activity was measured essentially according to the original procedure of Siiteri and Thompson [37]. The screening assay for determination of IC_{50} value and the kinetic assay were carried out essentially according to the assay methods described in our previous work [21]. Briefly, 20 μg of protein from the lyophilized microsomes and 20-min incubation period were used for the screening assay, and 20 μg of protein from the microsomes and a 5-min period were used for the kinetic assay. The assays were carried out at 37 °C in 67 mM phosphate buffer, pH 7.4, in the presence of NADPH under air.

Apparent K_i values were calculated using non-linear regression analysis with GraFit software [38].

3. Results and discussion

3.1. Chemistry

6β,19-Epoxyandrostenedione (**4**) [33] and 6β,19-cycloandrostenedione (**5**) [34] were prepared according to the reported methods. The structure of the 6β,19-cyclo compound **5**, obtained by reaction of 19-tosylate of 19-hydroxyandrostenedione (**6**) with sodium methoxide, was reconfirmed by two-dimensional NMR spectroscopy.

Burton et al. [30,31] have reported synthesis of series of 6β,19-epithio- and 6β,19-methano-progesterone analogs to expect new biological activities as steroid hormones. In the course of our studies, we were of interest in 6β,19-bridged androstenedione analogs as the aromatase inhibitors. We initially tried

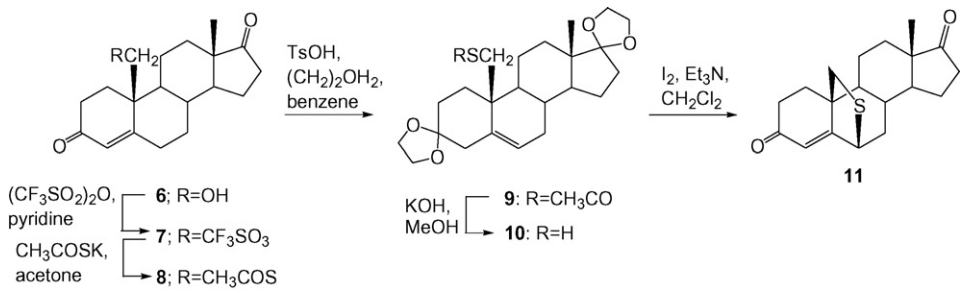

Fig. 3. Synthesis of 6,19-epithioandrostenedione (**11**).

synthesis of 6β,19-epithioandrostenedione (**11**) starting from 19-hydroxyandrostenedione (**6**) (Fig. 3). The 19-hydroxy group of compound **6** was converted to triflate **7** by treatment with trifluoromethanesulfonic anhydride in pyridine and displaced with potassium thioacetate in acetone to give 19-thioacetyl derivative **8**. The ^{1}H NMR spectrum of this compound showed a singlet peak of an acetylthio group (3H, 2.34 ppm) and two doublet peaks of 19-CH_2 (3.20 and 3.52 ppm, J = 14.0 Hz). Compound **8** was converted to ethyleneketal **9** in which the double bond migrated to the 5,6-position (^{1}H NMR: multiplet signal at 5.46 ppm of C-6) as required for the 6β,19-cyclization [30], and the 19-thioacetate was hydrolyzed with potassium hydroxide in methanol to give the free thiol **10**. Treatment of the thiol **10** by triethylamine and iodine resulted in the one-pot conversion of compound **10** into 6β,19-epithio compound **11** based upon the ^{1}H NMR spectrum which showed 2.61 and 3.07 ppm (1H each, d, J = 10.3 Hz) for 19-H, and 3.95 ppm (1H, m) for 6-H.

Next, 6β,19-methanoandrostenedione (**17**) was synthesized from 19-formylandrost-5-ene-3β,17β-yl diacetate (**12**) through six steps (Fig. 4). The key step in the synthesis was the cyclization of an additional carbon atom at C-19a [31]. Titanium tetrachloride has been the Lewis acid of choice for the Prins reaction to give bridged chloroalcohol [39]. Treatment of compound **12** with the titanium salt in CH_2Cl_2 at −50 °C gave the Prins reaction product, 5α-chloro-6β,19-methano steroid **13**, with a hydroxyl group at the C-19a position. The ^{1}H NMR spectrum showed a methine proton at 4.27 ppm (J = 7.7 Hz) for the 19a-position and 2.72 ppm (J = 12.8 Hz) for the 6α-position. Thus the formation of the 6β,19-methano ring proceeded through a stereo-controlled way; the stereochemistry of the 19a-hydroxy group was not established by the NMR spectrometries. This hydroxyl group was eliminated by Barton deoxygenation procedure [40]. The 19a-alcohol **13** was converted into the 19a-xanthate derivative by reaction with CS_2 and methyl iodide and this was treated with tributyltin hydride in toluene to give the deoxygenated compound **14**. The ^{1}H NMR spectrum of this compound showed an absence of a hydroxymethine resonance at 3.5–4.5 ppm. The 3β,17β-acetoxy moieties of compound **14** was eliminated by the alkaline hydrolysis to yield 3β,17β-dihydroxy compound **15**. Pyridinium chlorochromate oxidation of compound **15** followed by treatment with Al_2O_3 afforded the other desired compound, 6β,19-methano compound **17**.

The structures of the compounds synthesized were confirmed by the spectrometric analysis, HR-MS, and elemental analysis.

3.2. Biochemical properties

The inhibitory activities of human placental aromatase by the 6β,19-bridged steroids **4**, **5**, **11** and **17** were tested. Aromatization activity in placental microsomes was determined using a radiometric assay in which tritiated water released from [1β-^{3}H] androstenedione into the incubation medium during aromatization was measured [37]. To characterize the nature of inhibitor binding to the active site of aromatase, aromatization was measured at several concentrations and substrate concentrations. The results of these studies were plotted on a typical Lineweaver–Burk plot in all cases. The results are given in Table 1. In these studies, apparent K_m and V_{max} values for androstenedione were 35 nM and 110 pmol/min/mg protein, respectively. All of the steroids showed clear-cut competitive inhibition (Fig. 5). The apparent K_i values were obtained by Dixon plots. The bridged steroids **4**, **5**, **11** and **17** were weak to poor competitive inhibitors to aromatase in human placental microsomes in which the 6β,19-epoxy steroid **4** was the

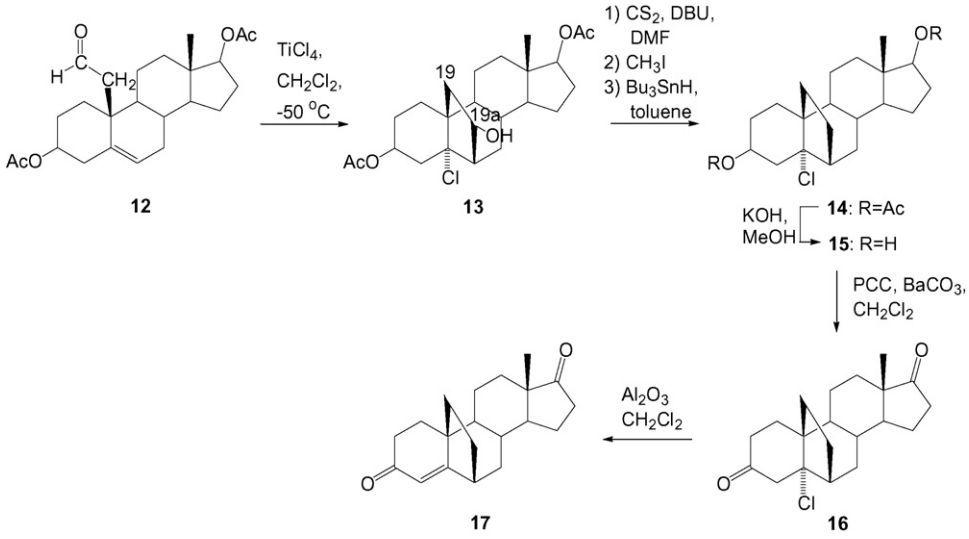

Fig. 4. Synthesis of 6,19-methanoandrostenedione (**17**).

Table 1
In vitro aromatase inhibition by 6β,19-bridged ADs (**4**, **5**, **11** and **17**).

Compound	IC_{50} (μM[a])	Apparent K_i (μM[b])
6,19-epoxy, **4**	44 ± 2.0	2.2 ± 0.17
6,19-cyclo, **5**	>100	18 ± 1.0
6,19-epithio, **11**	>100	18 ± 1.4
6,19-methano, **17**	>100	16 ± 1.0

[a] 300 nM of [1β-^{3}H]AD 20 μg protein from human placental microsomes and 20-min incubation time were used.

[b] All of the inhibitors examined showed a competitive type inhibition on the basis of the Lineweaver–Burk plot and apparent inhibition constant (K_i) was obtained by Dixon plot. Twenty micrograms protein from the placental microsomes and 5-min incubation time were used.

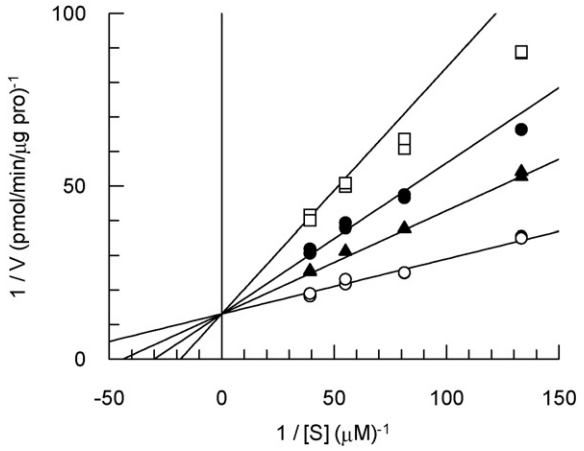

Fig. 5. Lineweaver–Burk plots of aromatase inhibition by 6β,19-oxidoandrostenedione (**4**). Concentrations of the inhibitor: control (0 μM) (○); 1.92 μM (▲); 3.83 μM (●); 7.66 μM (□).

most potent one among the steroids examined (K_i value: 2.2 μM of compound **4** and 16–18 μM of compound **5**, **11**, and **17**, respectively).

The affinity of the polar steroid, 19-hydroxyandrostenedione (**6**), to aromatase is known to be about one-third of androstenedione [27] and that of the other polar steroid, 6β-hydroxyandrostenedione, is relatively high to be 130 nM of the K_i value [20,21]. The previous reports have shown that the affinity of 19-methyl-substituted androstenedione for aromatase is comparable to that of androstenedione [22] and the 6β-methyl compound [16] has very high affinity for the active site (K_i: 11 nM). It has shown that 19-thio- and 19-methylthio-androstenediones act as very strong inhibitors of aromatase [23,24]. However, it was demonstrated that the affinities of the 6β,19-bridged compounds **4**, **5**, **11** and **17** were extremely low, compared to the above reported ones, and the K_i values were more than 2.2 μM. In the bridged compounds, the 19-substituents cannot rotate freely around the $C_{10\beta}$–C_{19} bond. This may cause the low affinities. On the other hand, in the 4-en-3-one steroids, the 6β,19-bridged structure bends the steroid skeleton at the A/B ring junction stabilizing the quasi-cis conformation with an inverted ring A 1β-half chair [28]. The inverted ring A conformation caused by the 6β,19-bridged moiety may also prevent the access of the active site of aromatase through the steric reason.

4. Conclusion

6β,19-Bridged androstenediones (**4**, **5**, **11**, and **17**) were synthesized and tested the inhibitory activities of the placental aromatase. The synthesis of the compounds **11** and **17** were achieved starting from the 19-hydroxy steroid **6** and the 19-formyl steroid **12**, respectively. The aromatase inhibitory activities of the 6β,19-brigded compounds were disappointingly low, however, their new biological properties such as androgenic/antiandrogenic activities are of interest and studies of them will be carried in our laboratory.

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