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The Synthesis of Novel 7α, 19-Bifunctional Androstenediones as Aromatase Inhibitors

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Abstract

Both 7α and 19-substituted androstenediones exhibit good inhibitory activities against aromatase. The combination effects of those two functional groups were examined by comparing the activity of19-methylthiomethoxy-7α-phenylthioandrost-4-en-3,17-dione (IC_{50}591 nM) with 19-methylthiomethoxy-androst-4,6-dien-3,17-dione (IC_{50}389 nM). The addition of a 7α-phenylthio functional group leads to a slight loss of inhibitory activity for this 7α, 19-bifunctional androstenedione. These results indicate the relative location of the pharmacophores on C-19 and in the active center. The 7α-position may not be compatible for the enhancement of inhibitory activity.

Keywords: Aromatase, Aromatase Inhibition, Aromatase Inhibitor

Introduction

Aromatase is the enzyme responsible for catalyzing the conversion of androgens to estrogens in the last step of estrogen biosynthesis. It is also vital in estrogen metabolic and reproductive processes. The inhibition of aromatase is a specific route to control estrogen levels and estrogen-dependent diseases. (1, 2) Since the discovery of steroidal substrate analogues as effective aromatase inhibitors, (2, 3, 4), many substituted C-19 steroids have been synthesized and evaluated on this basis.

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The important pharmacophores explored on the C-19 steroid nucleus include 1) keto functionalities at C-3 and C-17, 2) hetero atom substituents at C-4 and C-19, and 3) hydrophobic groups at C-7α. (5-9) These aromatase inhibitors have potential use as therapeutic agents for estrogen-dependent diseases. They can also serve as biochemical tools, "molecular probes", for deducing the types of drug-enzyme interactions. The steric location of pharmacophores in the enzyme active center was based on the hypothesis that these inhibitors take the same orientation as the substrate. The incorporation of more pharmacophores on the C-19 steroid nucleus should enhance the inhibitory activity against aromatase. (2, 7, 10) In our recent study on N-aryl androsterone pyrazoles, we discovered that this hypothesis may not hold true in this case. (11) In order to further explore these combination effects and provide more information for the molecular modeling of the aromatase active center, (12) we have synthesized methylthiomethoxy-7α-phenylthioandrost-4-en-3,17-dione by using 6,19-oxyandrost-4-en-3,17-dione as a starting material. However, a slight loss of activity was observed for this 7α,19-bifunctional androstenone in comparison with the mono-functional 19-methylthiomethoxy-androst-4,6-dien-3,17-dione in human placental aromatase bioassays. However, the inhibitory activity of both synthesized compounds have similar activity to a clinically used drug, 4-hydroxyandrost-4-en-3,17-dione (4-OHA). These results indicate the relative location of the pharmacophores on the steroid nucleus at positions C-19 and 7α- may have an affinity foractivitywith the receptor.

Experimental Procedures

3β-Acetoxyandrost-5-en-17-one was obtained from the Sigma Chemical Co. (St. Louis, MO). The synthesis of 6,19-oxyandrost-4-en-3,17-dione has been described. (13) Procedures for recording melting points (mp), infrared (IR), ^{1}H-NMR, ^{13}C-NMR, and mass spectra (MS) together with details concerning column and thin layer chromatography (TLC) have been described. (14, 15) Microsomes were prepared from term human placentas and stored at –70°C until used 1-3 days later in the aromatase assays. Time-dependent aromatase assays were performed as described previously. (16)

Preparation of 19-acetoxyandrost-4,6-dien-3,17-dione, 2

6,19-oxyandrost-4-en-3,17-dione (3.0 g, 10 mmol) was dissolved in chloroform (50 ml). Zinc chloride (20 mg) and acetyl bromide (2.5 g, 20 mmol) were added. The reaction mixture was stirred at 0°C for 30 min, warmed to room temperature for 1 h, and then heated at reflux for 2 h. The solution was poured into a cold saturated sodium bicarbonate solution (200 mL) and extracted with chloroform. The chloroform extracts were washed with water and brine and dried over sodium sulfate.

Evaporating the solvent yielded a brownish oil, which was subjected to silica gel column chromatography. 19-Acetoxyandrost-4,6-dien-3,17-dione, **2,** (2.0 g, 60% yield) and 6β-acetoxy-19-bromoandrost-4-en-3,17-dione, **3**, (1.3 g, 30% yield) were isolated.

2: mp: 163-166°C; MS (EI): 342.20 (M, 100), 343.20 (M+1, 23.6), 300.20 (M-acetate, 17.2); ^{1}H-NMR: 0.957 (s, 3H, H-18), 4.245 (d, J=11.9 Hz, 1H, H-19), 4.339 (d, J=11.9 Hz, 1H, H-19), 2.049 (s, 3H, acetate), 5.822 (s, 1H, H-4), 6.227 (m, 2H, H-6,7). HR-MS (EI): $C_{21}H_{26}O_4$theoretical 342.1832, measured 342.1846. ^{13}C-NMR: 218.6, 198.3, 170.0, 156.8, 138.4, 128.3, 127.4, 125.9, 63.6, 50.1, 48.7, 47.8, 39.3, 36.9, 35.1, 33.6, 31.4, 29.3, 20.9, 20.6, 13.2. IR: 2945, 2882, 1737, 1723, 1661, 1621, 1579, 1474, 1387, 1033 cm^{-1}.

3: light yellow oil; MS (CI, NH_3); 359.1 (M+NH_3-HBr, 100.0), 341.1 (M-Br, 87.8); ^{1}H-NMR: 0.841 (s, 3H, H-18), 3.459 (d, J=8.1 Hz, 1H, H-19), 4.153 (d, J=8.1 Hz, 1H, H-19), 2.046 (s, 3H, acetate), 5.748 (s, 1H, H-4), 4.674 (d, J=4.5Hz, 1H, H-6). $C_{21}H_{27}O_4Br$ theoretical C, 59.58%; H, 6.43%, measured C, 59.31%; H, 6.67%. IR: 2939, 2885, 1736, 1672, 1454, 1419, 1032 cm^{-1}.

Preparation of 19-hydroxyandrost-4,6-dien-3,17-dione, 4

19-Acetoxyandrost-4,6-dien-3,17-dione (2.0 g, 5.8 mmol) was dissolved in 100 mL methanol and sodium bicarbonate (1.0 g, 1.2 mmol) was added. The solution was heated at reflux for 4.0 h. After evaporating the solvent, the residue was extracted with 3 portions of 30 mL ethyl acetate. The combined ethyl acetate extracts were washed with sodium bicarbonate, water, a saturated NaCl solution, and dried with anhydrous sodium sulfate. Evaporating the solvent yielded a light yellow solid. This crude product was purified by silica gel column chromatography. 19-Hydroxyandrost-4,6-dien-3,17-dione (1.6 g, 92% yield) was isolated as a white solid.

4: mp: 197-198°C (lit.15 198-199°C); MS (EI): 300.20 (M, 4.5), 270.20 (M-CH_2O, 100); HR-MS (EI): $C_{19}H_{24}O_3$theoretical 300.1726, measured 300.1738. ^{1}H-NMR: 0.982 (s, 3H, H-18), 3.260 (s, br, OH), 3.772 (d, J=11.5 Hz, 1H, H-19), 3.898 (d, J=11.5 Hz, 1H, H-19), 5.783 (s, 1H, H-4), 6.222 (m, 2H, H-6,7). IR: 3433, 2951, 1736, 1655, 1614, 1583, 1458, 1362, 1271, 1069 cm^{-1}.

Preparation of 19-methylthiomethoxyandrost-4,6-dien-3,17-dione, 5

A suspension of 19-hydroxyandrost-4,6-dien-3,17-dione (0.16 g, 0.50 mmol) and chloromethyl methyl sulfide (50 μL, 0.60 mmol) in dry benzene (1.0 mL) was added to a stirred mixture of silver nitrate (94 mg, 0.60 mmol), triethylamine (84 μL, 0.60 mmol), and dry benzene (1.0 mL). Then, the mixture was stirred at room temperature overnight. The resulting reaction mixture was subjected to silica gel column chromatography using a mixture of ethyl ether and toluene as the eluting solvent. 19-Methylthiomethoxyandrost-4,6-dien-3,17-dione (94 mg, 55% yield) was isolated as a colorless oil.

MS (EI): 360.00 (M, 0.99), 361.00 (M+1, 0.25), 284.00 (M-CH_3SCH_2O, 2.17); HR-MS (EI): $C_{21}H_{28}O_3S$ theoretical 360.1760, measured 360.1781. ^{1}H-NMR: 0.985 (s, 3H, H-18), 2.137 (s, 3H, CH_3-MTM group), 4.614 (dd, 2H, CH_2-MTM group), 3.605 (d, J=9.9 Hz, 1H, H-19), 3.725 (d, J=9.9 Hz, 1H, H-19), 5.797 (s, 1H, H-4), 6.209 (m, 2H, H-6,7). ^{13}C-NMR: 219.4, 199.4, 158.6, 138.7, 129.0, 125.9, 76.1, 68.7, 50.7, 49.1, 48.3, 40.3, 37.4, 35.6, 34.0, 31.8, 29.9, 21.4, 21.2, 14.5, 13.6. IR: 2922, 1738, 1663, 1618, 1453, 1268 cm^{-1}.

Preparation of 19-hydroxy-7α-phenylthioandrost-4-en-3,17-dione, 6

19-Hydroxyandrost-4,6-dien-3,17-dione (0.60 g, 2.0 mmol) was dissolved in thiophenol (15 mL). Sodium (5 mg, 0.2 mmol) was added and the reaction mixture was stirred at 60°C under dry nitrogen for 24 h. After cooling to room temperature, ice water (100 mL) was added. The mixture was extracted with chloroform and the chloroform extracts were washed with water and brine and dried over magnesium sulfate. The residue was subjected to column chromatography. 19-Hydroxy-7α-phenylthioandrost-4-en-3,17-dione (0.74 g, 90%) was isolated as a light yellow solid.

mp: 172-173°C; MS (EI): 410.2 (M, 1.0), 300.2 (M-HSPh, 27.4), 270.2 (M-HSPh-CH_2O, 100); HR-MS (EI): $C_{25}H_{30}O_3S$ theoretical 410.1919, measured 410.1934. ^{1}H-NMR ($CDCl_3$) δ 0.841 (s, 3H, H-18), 3.824 (d, J=5.7 Hz, 1H, H-19), 3.920 (d, J=5.7 Hz, 1H, H-19), 5.744 (s, 1H, H-4), 3.513 (m, 1H, H-7β), 7.252 (m, 5H, aromatic). ^{13}C-NMR ($CDCl_3$) δ 220.0, 199.5, 164.3, 134.0, 132.9, 129.05, 129.00, 127.4, 66.0, 49.5, 47.6, 47.4, 43.1, 39.8, 39.4, 35.4, 34.4, 33.2, 31.1, 20.9, 20.5, 13.6. IR: 3441, 2943, 1734, 1662, 1473, 1439, 1053 cm^{-1}.

Preparation of 19-methylthiomethoxy-7α-phenylthioandrost-4-en-3,17-dione, 7

A suspension of 19-hydroxy-7α-phenylthioandrost-4-en-3,17-dione (0.10 g, 0.25 mmol) and chloromethyl methylsulfide (25 μL, 0.30 mmol) in dry benzene (0.5 mL) was added to a stirred mixture of silver nitrate (47 mg, 0.28 mmol), triethylamine (42 μL, 0.30 mmol), and dry benzene (0.5 mL). The mixture was then heated at 60°C overnight. The resulting reaction mixture was subjected to silica gel column chromatography and eluted with a solvent gradient of ethyl ether and toluene. 19-Methylthiomethoxy-7α-phenylthioandrost-4-en-3,17-dione (58 mg, 50% yield) was isolated as a white solid.

mp: 115-117°C; MS (EI): 470.00 (M, 0.18), 471.00 (M+1, 0.04), 361.00 (M-SPh, 6.86); HR-MS (EI): $C_{27}H_{34}O_3S_2$theoretical 470.1953, measured 470.1932. IR: 2945, 1738, 1668, 1621, 1439, 1021 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.952 (s, 3H, H-18), 2.074 (s, 3H, CH_3-MTM group), 4.589 (dd, 2H, CH_2-MTM group), 3.725 (d, J=7.4 Hz, 1H, H-19), 3.847 (d, J=7.4 Hz, 1H, H-19), 5.828 (s, 1H, H-4), 3.610 (m, 1H, H-7β), 7.340 (m, 5H, aromatic). ^{13}C-NMR ($CDCl_3$) δ 219.6, 199.0, 162.9, 134.0, 133.3 (2C), 129.2 (3C), 127.6, 71.7, 49.5, 47.7, 47.5, 42.1, 40.0, 39.1, 35.6, 34.6, 33.7, 31.3, 21.1, 20.9, 14.5, 13.8.

Preparation of 19-mesylateandrost-4,6-dien-3,17-dione, 8

19-Hydroxyandrost-4,6-dien-3,17-dione (0.60 g, 2.0 mmol) was dissolved in pyridine (15 mL). Mesyl chloride (0.46 g, 4.0 mmol) was added dropwise at 0°C. The reaction mixture was gradually warmed to room temperature and stirred for another 2 h. Ice water (200 mL) was added and the resulting white solid was filtered and washed with water. The crude product was purified by recrystallization with acetone-water. 19-Hydroxyandrost-4,6-dien-3,17-dione (0.70 g, 92% yield) was obtained after recrystallization.

mp: 83-86°C; MS (EI): 378.00 (M, 12.17), 379.00 (M+1, 2.50); HR-MS (EI), $C_{20}H_{26}O_5S$ theoretical 378.1502, measured 378.1514. ^{1}H-NMR ($CDCl_3$) δ 0.776 (s, 3H, H-18), 4.269 (d, J=10.2 Hz, 1H, H-19), 4.401 (d, J=10.2 Hz, 1H, H-19), 3.014 (s, 3H, mesyl-CH_3), 5.824 (s, 1H, H-4) 6.180 (m, 2H, H-6,7). IR: 2941, 1734, 1658, 1536, 1429, 1032 cm^{-1}.

Preparation of 19-hydroxy-7α-thiophenolandrost 4-en-3,17-dione 19-methanesulfonic ester,9

19-Hydroxy-7α-phenylthioandrost-4-en-3,17-dione (0.41 g, 1.0 mmol) was dissolved in pyridine (10 mL). Mesyl chloride (0.23 g, 2.0 mmol) was added dropwise at 0°C. The reaction mixture was gradually warmed to room temperature and stirred for another 2 h. Ice water (200 mL) was added and the resulting white solid was filtered and washed with water. The crude product was purified by recrystallization using acetone-water. 19-Hydroxy-7α-thiophenolandrost 4-en-3,17-dione 19-methanesulfonic ester (0.44 g, 91% yield) was obtained after recrystallization.

mp: 87-90°C; MS (EI): m/z, 488.20 (M, 100.0), 394.20 (M-Ms, 89.0); HR-MS (EI), $C_{26}H_{32}O_5S_2$theoretical 488.1693, measured 488.1674. ^{1}H-NMR ($CDCl_3$) δ 0.949 (s, 3H, H-18), 4.384 (d, J= 9.8 Hz, 1H, H-19), 4.584 (d, J=9.8 Hz, 1H, H-19), 5.905 (s, 1H, H-4), 3.634 (m, 1H, H-7β), 7.352 (m, 5H, aromatic). ^{13}C-NMR ($CDCl_3$) δ 219.4, 197.7, 159.7, 133.5, 133.1, 130.5, 129.3, 127.8, 70.9, 49.1, 47.5, 47.4, 41.5, 39.8, 38.6, 37.6, 35.4, 34.1, 33.1, 31.1, 20.9, 20.7, 13.7. IR: 2948, 1734, 1653, 1617, 1522, 1419, 929 cm^{-1}.

Preparation of 19-bis(19-hydroxyandrost-4,6-dien-3,17-dione) sulfonic ester, 10

Thionyl choloride (0.20mL, 2.0 mmol) was added dropwise at 0°C to a solution of 19-hydroxyandrost-4,6-dien-3,17-dione (0.30 g, 1.0 mmol) in pyridine (dried over solid potassium hydroxide, 10 ml). The mixture was stirred for 45 min at 0°C. It was then poured onto water (100 mL) and the resulting precipitate was collected by filtration and washed with water. The crude product was purified by column chromatography to give bis (19-hydroxyandrost-4,6-dien-3,17-dione) sulfonic ester (0.3 g, 90% yield) as a white solid.

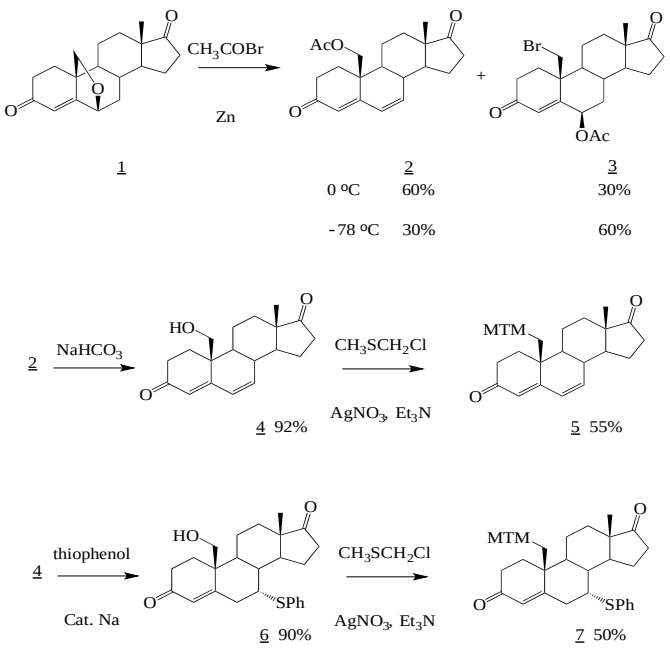
mp: 140-142°C; MS (EI): 646.00 (M, 2.75), 299.00 (M-$C_{19}H_{24}O_4S$, 4.58), 284.00 (M- $C_{19}H_{24}O_4S$-CH_3, 15.9), 269.0 (M-$C_{19}H_{24}O_4S$-CH_2O, 46.1); HR-MS (EI), $C_{38}H_{46}O_7S$theoretical 646.2791, measured 646.2777. ^{1}H-NMR ($CDCl_3$) δ 0.971 (d, 6H, H-18), 3.842 (d, J= 5.7 Hz, 2H, H-19), 4.205 (d, J=5.7 Hz, 2H, H-19), 5.748 (s, 2H, H-4), 6.228 (m, 4H, H-6,7). IR: 2961, 2864, 1738, 1662, 1618, 1454, 1375, 1267, 1053 cm^{-1}.

Results and Discussion

The chemical synthesis utilized 6,19-oxyandrost-4-en-3,17-dione, **1**, which was prepared from 3β-acetoxyandrost-5-en-17-one according to literature methods. (13)

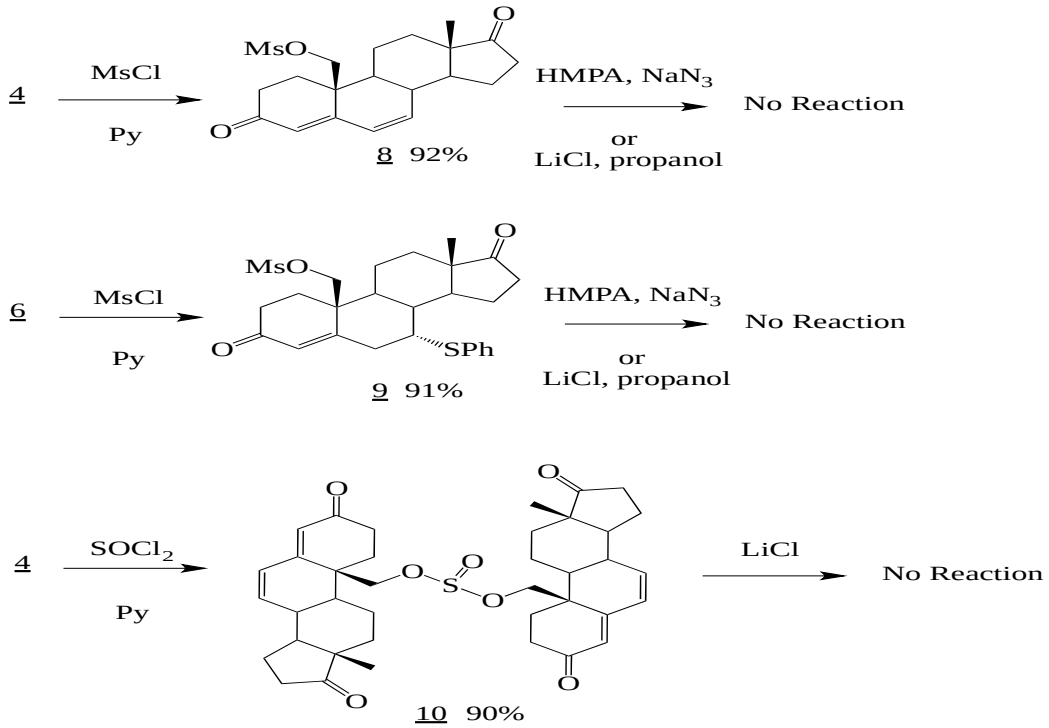
The cleavage of the 6,19-ether of **1** with acetyl bromide and zinc chloride resulted in 19-acetoxyandrost-4,6-dien-3,17-dione, **2,** and 19-bromo-6-acetoxyandrost-4-en-3,17-dione, **3** (Scheme 1). (17) The ratio of products was dependent on the reaction temperature. If the reaction was conducted at 0°C, 19-acetoxy-4,6-dien-3,17-dione was the major product in 60% yield, plus 30% of 19-bromo-6-acetoxyandrost-4-en-3,17-dione. At -78°C, the ratio of those two products was reversed. Mechanistically, 19-bromo-6-acetoxyandrost-4-en-3,17-dione was produced via an S_N2-type mechanism and 19-acetoxyandrost-4,6-dien-3,17-dione was produced as the E1 elimination product. Raising the reaction temperature favored the elimination product. The hydrolysis of compound **2** with sodium carbonate in methanol produced 19-hydroxyandrost-4,6-dien-3,17-dione, **4,** in 92% yield. The 19-methylthiomethoxide derivative, **5,** was prepared in 55% yield by treating compound **4** with chloromethyl methylsulfide and silver nitrate in a refluxing benzene solution. (18) The 1,6-conjugate addition yielded 90% product when thiophenol was added to **4**resulting in 19-hydroxy-7α-phenylthioandrost-4-en-3,17-dione, **6**. (7) The 19-methylthiomethoxide derivative of 7α-phenylthioandrost-4-en-3,17-dione, **7**, was prepared using the same procedure as compound **5** in 50% yield.

Scheme1. The synthesis of 19-substituted and 19, 7α-disubstituted androstenones.



Efforts have also been directed towards the synthesis of 19-chloro and 19-azide analogues via 19-mesylandrost-4,6-dien-3,17-dione, **8,** and 19-hydroxy-7α-thiophenolandrost 4-en-3,17-dione 19-methanesulfonic ester, **9,** (Scheme 2). It was found that these 19-mesylates could not be displaced by sodium azide and lithium chloride under refluxing conditions. These similar circumstances were encountered with 19-mesylandrost-4-en-3,17-dione. (7) Usually the nucleophilic displacement of primary mesylates should proceed via an S_N2 mechanism. However, this backside attack was blocked by the ring system in these cases. The nucleophilic displacement of the 19-mesylate in the 5-ene system was considered to proceed via an S_N1 mechanism. (19) The primary C-19 cation was stabilized by the π-electrons of the C-5,6 double bond. Possibly, this stabilization effect is not available in Δ^4 and $\Delta^{4,6}$-3-ketone systems during the conjugation with the electron withdrawing 3-ketone. The reaction of 19-hydroxyandrost-4,6-dien-3,17-dione, **4,** with thionyl chloride in pyridine formed a sulfonic ester, **10**, instead of the normal chlorination product. The formed sulfonic ester was very stable and could not be displaced by chloride in refluxing propanol, containing lithium chloride. This novel linkage between two steroid nuclei could be a useful template in the design of potential aromatase inhibitors with this type of novel structure. (20)

Scheme 2. The attempted synthesis of 19-chloro and 19-azido androstenones.



Compounds **5** and **7** were subjected to a time-dependent aromatase assay using the methods described previously. (16) Microsomes were prepared from term human placentas and stored at –70°C until used 1-3 days later in the aromatase assays. The following IC_{50} values were obtained from a 50% reduction of enzymatic activity: **5,** 389 nM and **7,** 591 nM.

For comparison, the clinical-used drug, 4-hydroxyandrost-4-en-3,17-dione (4-OHA), was also tested in the same assay with an of IC_{50} of 370 nM. (4) However, the bifunctional androstenone, **7**, showed a slightly lower inhibitory activity than the monofunctional androstenone, **5**. The inhibitory activity of both synthesized compounds are in the same range as 4-OHA. The percentage of conversion of labeled substrates in the presence of compounds **5** and **7** are presented in Table 1 and in Figures 1 and 2.

Table 1. The normalized percentage of conversion of substrate in the presence of compound **5**.

Concentration of **5** (nM)	Percentage of conversion	Error
0	100.0	3.9
10	82.4	3.9
50	82.8	2.1
100	76.3	0.5
200	69.9	0.1
400	48.8	3.2
800	41.9	1.3
1000	45.5	0.9

Table 2. The normalized percentage of conversion of substrate in the presence of compound **7**.

Concentration of **7** (nM)	Percentage of conversion	Error
0	100.0	3.9
10	93.9	0.5
50	90.7	0.6
100	86.1	3.9
200	62.6	12.2
400	54.8	0.9
800	39.7	3.8
1000	41.1	1.4

Figure 1. Percentage of labeled substrate conversion vs concentration of compound 5.

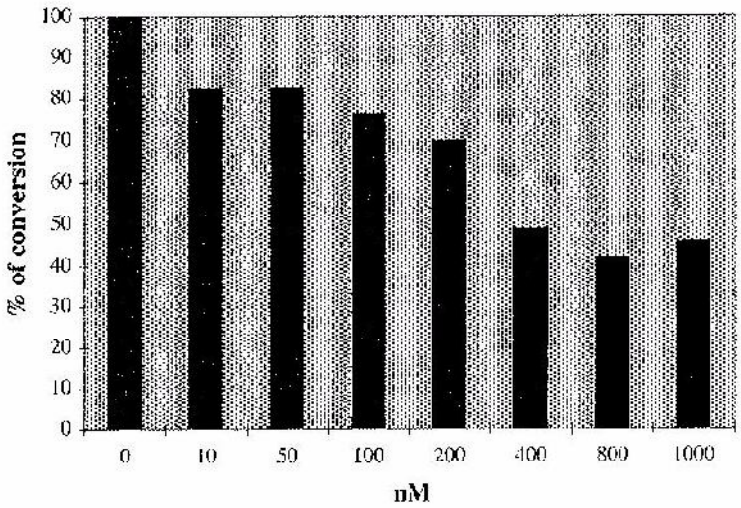
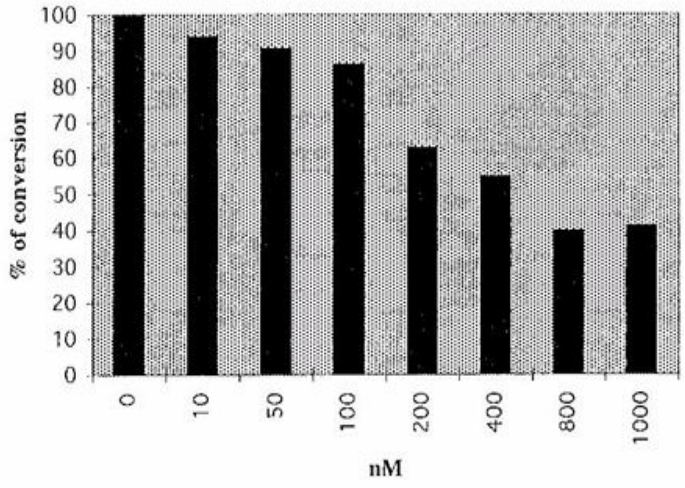


Figure 2. Percentage of labeled substrate conversion vs concentration of compound 7.



In summary, we have described the chemical synthesis of a new type 7α,19-bifunctional androstenone and have found this compound to be an effective inhibitor of human placental aromatase. However, a slight loss of activity was observed in comparison with the 19-monofunctional analogue. In addition, we have described the preparation of a novel dimeric steroidal sulfonic ester.

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