Synthesis and aromatase inhibition by potential metabolites of exemestane (6-methylenandrosta-1,4-diene-3,17-dione)

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Exemestane (6-methylenandrosta-1,4-diene-3,17-dione; FCE 24304) is an orally active irreversible aromatase inhibitor which is in phase II clinical evaluation for the potential therapy of postmenopausal breast cancer. A series of exemestane analogs, with modifications at the 6-methylene group and with additional reduction at the 17-keto group, were synthesized as potential metabolites and tested in vitro for their effect on human placental aromatase. All these new analogs were found to be less potent in inhibiting aromatase than exemestane. The most effective compound was the 17β-hydroxy-derivative (compound 2), which is 2.6-fold less potent than exemestane [50% inhibitory concentration (IC_{50}) 69 and 27 nM, respectively]. The various C-6 modified derivatives of the 17-oxo series were found to inhibit the aromatase enzyme in the following descending order: 6-methylene (exemestane) > 6-spirooxirane (6) > 6β-hydroxymethyl (11) > 6-hydroxymethyl (7) > 6β-carboxy (13), showing IC_{50} values of 27, 206, 295, 2,300, and 7,200 nM, respectively. The 17β-hydroxy analogs of some of the above mentioned compounds were also synthesized (3, 4, 12) and found to be 3–8-fold less potent than the corresponding 17-keto analogs. (Steroids **58:**527–532, 1993)

Keywords: steroids; aromatase inhibitors; exemestane; 6-methylenandrosta-1,4-diene-3,17-dione; potential metabolites.

Introduction

Exemestane (6-methylenandrosta-1,4-diene-3,17-dione; FCE 24304) is an orally active irreversible aromatase inhibitor[1–5] which is currently in phase II clinical evaluation for the potential therapy of postmenopausal breast cancer. The compound was shown to inactivate the human placental aromatase with K_i of 26 nM and $t_{1/2}$ of 13.9 min.[1,2] Given orally in pregnant mare's serum gonadotropin-primed rats, exemestane was found very potent in inhibiting ovarian aromatase activity, both subcutaneously and orally (ED_{50} 1.8 and 3.7 mg/kg, respectively).[1,2] In a first phase I trial in healthy postmenopausal volunteers, a single oral dose of 25 mg exemestane was found very effective in causing a long-lasting (for at least 5 days) reduction of plasma and urinary estrogens.[4,5]

Since preliminary studies in animals have shown that the compound is extensively metabolized (G. Cocchiara et al., unpublished results), in the present paper we report on the synthesis of a number of potential metabolites of exemestane and on their inhibitory effect on human placental aromatase. Since the 6-methylene group is likely the most reactive position with respect to the P_{450}-catalyzed oxidation a number of compounds modified at this position were synthesized (compounds **6, 7, 8, 11,** and **13**). In addition the corresponding 17β-alcohols, which are formed via the reversible 17β-hydroxysteroidoxidoreductase, were also prepared (**3, 4, 5,** and **12**). Other compounds structurally related to exemestane with modifications at C-6 (compounds **14, 18,** and **20**) were also synthesized.

Experimental

The synthetic routes are depicted in Schemes 1–3. Melting points (mp) were determined in open capillaries and are uncorrected. ^1H NMR spectra were run on a Varian VXR-200 spectrometer using $CDCl_3$ solutions. Chemical shifts are in δ ppm relative to TMS internal standard. Elemental analyses for all compounds were within 0.4% of the theoretical value. Mass spectra (MS m/z) were recorded with a Varian CH7 instrument. The homogeneity of the compounds was checked by TLC on silica gel-G.

17β-hydroxy-6-methylenandrosta-1,4-dien-3-one (2)

A mixture of paraformaldehyde (3.30 g, 0.11 mol) and dimethylamine hydrochloride (10.53 g, 0.13 mol) in isoamyl alcohol (150

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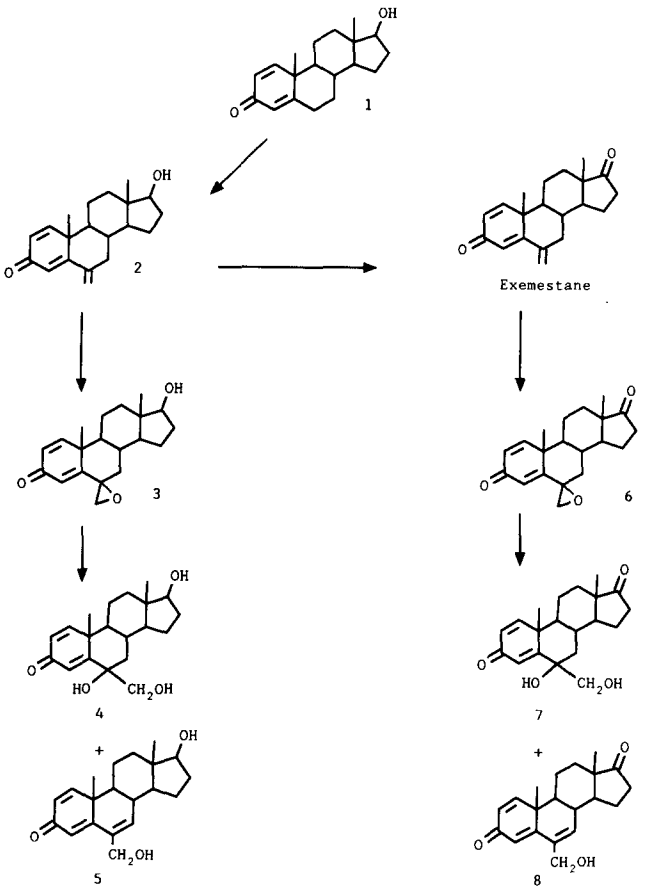
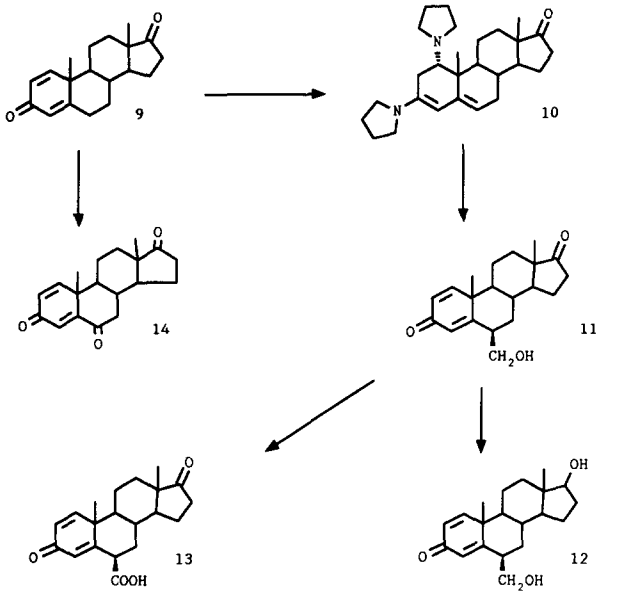
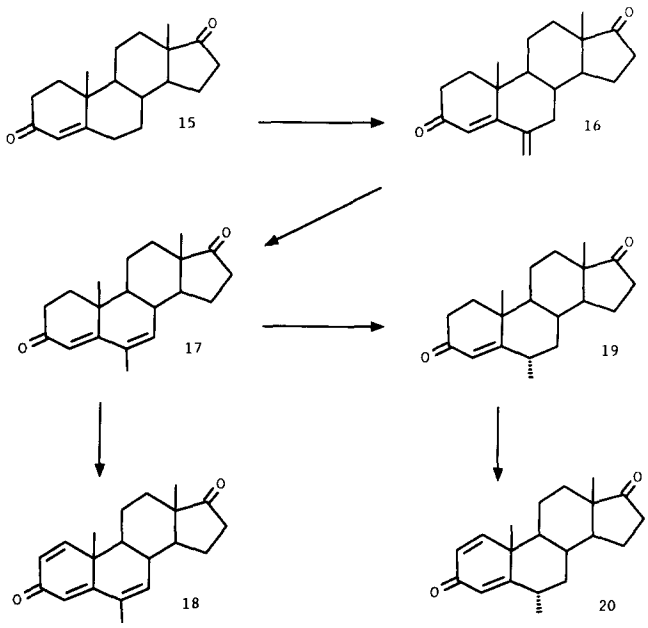


Scheme 1



Scheme 2

ml) was heated to reflux for 2 hours. Then 17β-hydroxyandrosta-1,4-dien-3-one (compound **1**, 2.86 g, 0.01 mol) was added and the refluxing was continued for another 15 hours. During both these operations the distilled off water was collected by a Dean-



Scheme 3

Stark trap. The reaction mixture was cooled, sodium hydroxide 1N was added and the mixture stirred for 1/2 hour. The layers were separated and the organic layer was evaporated under vacuum. The resulting raw product (1.04 g, 35% yield) can be employed without further purification for the next step. A sample was purified by column chromatography on silica gel for characterization purposes. Compound **2**: mp 130 C; NMR 0.81 (s, 3H, 18-CH_3), 1.14 (s, 3H, 19-CH_3), 2.07 (m, 2H, 16α-CH), 2.50 (m, 1H, 7β-CH), 3.66 (t, J = 8.5 Hz, 1H, 17-CH), 4.94 (t, J = 1.9 Hz, 1H, $=CH_2$), 5.01 (t, J = 1.9 Hz, 1H, $=CH_2$), 6.14 (d, J = 1.8 Hz, 1H, 4-CH), 6.24 (dd, J = 1.8, 10.0 Hz, 1H, 2-CH), 7.09 (d, J = 10.0 Hz, 1H, 1-CH); MS 298.

17β-Hydroxy-6α/β-spirooxiranandrosta-1,4-dien-3-one (3)

To a solution of compound **2** (2.98 g, 0.010 mol) in dichloromethane (100 ml) was added under cooling m-chloroperbenzoic acid 50% (3.80 g, 0.011 mol) and the mixture was stirred for 48 hours at room temperature. The excess of peracid was destroyed with sodium metabisulfite solution, the organic layer washed with bicarbonate and saline solution and dried over sodium sulfate, and the solvent removed under vacuum. The crude product was chromatographed on silica gel using dichloromethane/ethanol 2% as eluant to yield pure compound **3** (2.51 g, 80%); mp 140 C; MS 314; NMR 0.82 (s, 3H, 18-CH_3), 0.83 (s, 3H, 18-CH_3), 1.24 (s, 3H, 19-CH_3), 1.33 (s, 3H, 19-CH_3), 2.65 (dd, J = 1.6, 5.7 Hz, 1H, -CH_2-O-), 2.96 (d, J = 5.7 Hz, 1H, -CH_2-O-), 2.73, 3.08 (2 d, J = 4.2 Hz, 2H, -CH_2-O-), 3.65 (m, 1H, 17-CH), 6.1-6.3 (m, 2H, 2-CH, 4-CH), 7.05 (d, J = 10.1 Hz, 1H, 1-CH), 7.08 (d, J = 10.1 Hz, 1H, 1-CH).

6α/β,17β-Dihydroxy-6α/β-hydroxymethylandrosta-1,4-dien-3-one (4) and 17β-hydroxy-6-hydroxymethylandrosta-1,4,6-trien-3-one (5)

To a solution of compound **3** (3.14 g, 0.010 mol) in THF (150 ml) was added under cooling perchloric acid 20% (30 ml) and the mixture was stirred for 3 hours at room temperature. Then

the reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was separated, washed with bicarbonate and saline solution, dried over sodium sulfate, and the solvent removed under vacuum. The crude product was chromatographed on silica gel using dichloromethane/ethanol 4% as eluant to give pure compounds **4** (1.495 g, 45%) and **5** (1.10 g, 35%). Compound **4**: MS 332, NMR 0.80 (s, 3H, 18-CH_3), 0.83 (s, 3H, 18-CH_3), 1.23 (s, 3H, 19-CH_3), 1.45 (s, 3H, 19-CH_3), 2.4 (bs, 1H, CH_2OH), 2.8 (bs, 1H, 6-OH), 3.4-4.0 (m, 3H, CH_2OH, 17-CH), 6.1-6.3 (m, 2H, 2-CH, 4-CH), 7.04 (d, J = 10.2 Hz, 1H, 1-CH), 7.08 (d, J = 10.1 Hz, 1H, 1-CH). Compound **5**: mp 160 C; MS 314; NMR 0.85 (s, 3H, 18-CH_3), 1.17 (s, 3H, 19-CH_3), 2.31 (ddd, J = 1.7, 11.5, 11.5 Hz, 1H, 8-CH), 3.65 (m, 1H, 17-CH), 4.32, 4.37 (2 d, J = 13.1 Hz, 2H, CH_2OH), 6.10 (s, 1H, 7-CH), 6.20 (d, J = 1.8 Hz, 1H, 4-CH), 6.25 (dd, J = 1.8, 10.0 Hz, 1H, 2-CH), 7.08 (d, J = 10.0 Hz, 1H, 1-CH).

6-Methylenandrosta-1,4-diene-3,17-dione (exemestane)

To a solution of compound **2** (2.98 g, 0.010 mol) in acetone (60 ml) was added dropwise Jones reagent (6 ml) under cooling at −20C and the stirring was continued for 1/4 hours at −20 C. Then the reaction was quenched with isopropanol (6 ml) and the mixture extracted with ethyl acetate after dilution with water. The organic phase was washed with bicarbonate and saline solution, dried and evaporated under vacuum. The residue was submitted to flash chromatography on silica gel using *n*-hexane/ethyl acetate (7 : 3) as eluant to give pure exemestane (2.52 g, 85%); mp 195 C; MS 296; NMR 0.95 (s, 3H, 18-CH_3), 1.17 (s, 3H, 19-CH_3), 5.03 (m, 2H, $=CH_2$), 6.24 (m, 2H, 2-CH, 4-CH), 7.07 (d, 1H, 1-CH).

6α/β-Spirooxiranandrosta-1,4-diene-3, 17-dione (6)

Exemestane (2.96 g, 0.010 mol) was epoxidized with *m*-chloroperbenzoic acid as described above to give pure compound **6** (2.50 g, 80%); mp 210 C; MS 312; NMR 0.95 (s, 3H, 18-CH_3), 1.36 (s, 3H, 19-CH_3), 2.78, 3.11 (2 d, J = 4.2 Hz, 2H, -CH_2-O-), 6.14 (d, J = 1.9 Hz, 1H, 4-CH), 6.24 (dd, J = 1.9, 10.2 Hz, 1H, 2-CH), 7.07 (d, J = 10.2 Hz, 1H, 1-CH).

6α/β-Hydroxy-6α/β-hydroxymethylandrosta-1,4-diene-3,17-dione (7) and 6-hydroxymethylandrosta-1,4,6-triene-3,17-dione (8)

Compound **6** was hydrolyzed with perchloric acid 20% and worked up as described above to afford compound **7** (42% yield) and **8** (38% yield). Compound **7**: amorphous solid; MS 330; NMR 0.93 (s, 3H, 18-CH_3), 0.96 (s, 3H, 18-CH_3), 1.25 (s, 3H, 19-CH_3), 1.47 (s, 3H, 19-CH_3), 2.86 (bs, 1H, 6-OH), 2.92 (bs, 1H, 6-OH), 3.5-4.1 (m, 2H, CH_2OH), 6.1-6.3 (m, 2H, 2-CH, 4-CH), 6.69 (d, J = 1.9 Hz, 1H, 4-CH), 7.04 (d, J = 10.1 Hz, 1H, 1-CH), 7.07 (d, J = 10.2 Hz, 1H, 1-CH). Compound **8**: mp 210–212 C; MS 312; NMR 0.98 (s, 3H, 18-CH_3), 1.19 (s, 3H, 19-CH_3), 4.38 (bs, 2H, CH_2OH), 6.21 (m, 2H, 4-CH, 7-CH), 6.26 (dd, J = 1.8, 10.1 Hz, 1H, 2-CH), 7.07 (d, J = 10.1 Hz, 1H, 1-CH).

6β-Hydroxymethylandrosta-1,4-diene-3,17-dione (11)

Androsta-1,4-diene-3,17-dione (**9**) was reacted with pyrrolidine at reflux temperature as described by Wagner and Ponsold[6] to give 1α,3-dipyrrolidinylandrosta-3,5-dien-17-one (**10**). Treatment of the latter with formaldehyde 30% in ethanol-benzene solution at room temperature according to Wagner and Ponsold[6] led to the formation of compound **11** in 90% yield. mp 193 C; MS 314; NMR 0.93 (s, 3H, 18-CH_3), 1.24 (s, 3H, 19-CH_3), 2.8 (m, 1H, 6-CH), 3.95 (m, 2H, CH_2OH), 6.2 (m, 2H, 2-CH, 4-CH), 6.99 (d, J = 10.4 Hz, 1H, 1-CH).

17β-hydroxy-6β-hydroxymethylandrosta-1,4-dien-3-one (12)

To a stirred solution of compound **11** (3.14 g, 0.010 mol) in methanol (100 ml) was added sodium borohydride (0.570 g, 0.015 mol) over a period of 20 minutes at 0 C and stirring was continued for 1 hour at 0 C. After addition of few drops of acetic acid the mixture was concentrated under vacuum, diluted with water, and then extracted with ethyl acetate. The combined organic phases were washed with saline solution, dried and then evaporated in vacuum. The residue was submitted to column chromatography on silica gel. Gradient elution with benzene-ether mixtures afforded pure compound **12** in 65% yield (2.055 g); mp 186–190 C; MS 316; NMR 0.81 (s, 3H, 18-CH_3), 1.23 (s, 3H, 19-CH_3), 2.05 (m, 1H, 16α-CH), 2.75 (m, 1H, 6-CH), 3.64 (t, J = 8.5 Hz, 1H, 17-CH), 3.8 (m, 2H, CH_2OH), 6.16 (d, J = 1.9 Hz, 1H, 4-CH), 6.19 (dd, J = 1.9, 10.2 Hz, 1H, 2-CH), 7.02 (d, J = 10.2 Hz, 1H, 1-CH).

6β-Carboxyandrosta-1,4-diene-3,17-dione (13)

To a solution of compound **11** (3.14 g, 0.010 mol) in acetone (60 ml) was added dropwise Jones reagent (6 ml) under cooling at 0 C and the oxidation was continued for another 1/2 hour at this temperature. After quenching with isopropanol (6 ml) and dilution with water the mixture was extracted with ethyl acetate. The organic layer was separated and extracted with bicarbonate solution. The aqueous phase was separated and acidified with 2N HCl under cooling to precipitate the crude acid. The latter was submitted to column chromatography on silica gel using gradient elution with dichloromethane/ethanol 2-8% to give pure compound **13** in 65% yield (2.13 g): mp 150 C dec. MS 328; NMR 0.93 (s, 3H, 18-CH_3), 1.22 (s, 3H, 19-CH_3), 3.51 (d, J = 4.6 Hz, 1H, 6-CH), 6.24 (m, 2H, 2-CH, 4-CH), 7.02 (d, J = 9.8 Hz, 1H, 1-CH).

Androsta-1,4-diene-3,6,17-trione (14)

To a solution of compound **9** (2.84 g, 0.01 mol) in *t*-butanol (80 ml) was added portionwise potassium *t*-butoxide (2.80 g, 0.025 mol) and then dry air was bubbled through the mixture for 8 hours at room temperature. After dilution with water and neutralization with acetic acid and the raw product was extracted with ethyl acetate and the separated organic phase washed, dried, and evaporated under vacuum. The residue was chromatographed on silica gel using gradient elution with *n*-hexane/ethyl acetate 20-40% to give pure compound **14**[7] in 15% yield. mp 205–207 C; MS 298; NMR 0.95 (s, 3H, 18-CH_3), 1.22 (s, 3H, 19-CH_3), 6.30 (dd, J = 1.9, 10.2 Hz, 1H, 2-CH), 6.38 (d, J = 1.9 Hz, 1H, 4-CH), 7.06 (d, J = 10.2 Hz, 1H, 1-CH).

6-Methylenandrost-4-ene-3,17-dione (16)

Androst-4-ene-3,17-dione (**15**) was transformed into compound **16** according to Annen et al.[8] in 60% yield; mp 167 C; MS 298; NMR 0.91 (s, 3H, 18-CH_3), 1.12 (s, 3H, 19-CH_3), 4.99 (bt, 1H, $=CH_2$), 5.10 (t, 1H, $=CH_2$), 5.93 (s, 1H, 4-CH).

6-Methylandrosta-1,4,6-triene-3,17-dione (18)

Compound **16** was isomerized to 6-methylandrosta-4,6-diene-3,17-dione (**17**) in 90% yield with palladium charcoal 5% in re-

fluxing ethanol under benzyl alcohol addition as described for an analogous isomerization by Burn et al.[9] mp 160–161 C. A mixture of compound **17** (0.500 g, 1.67 mmol) and dichlorodicyanobenzoquinone (DDQ) (0.570 g, 2.51 mmol) in anhydrous dioxane (20 ml) was refluxed for about 15 hours. To remove the DDQ the suspension was filtered through alumina. After evaporation of the solvent the residue was dissolved in ethyl acetate, the organic layer washed with water and dried over sodium sulfate, and the solvent removed under vacuum. The crude product was chromatographed on silica gel using hexane/ethyl acetate 40% to give pure compound **18** in 50% yield. mp 222 C; MS 296; NMR 0.95 (s, 3H, 18-CH_3), 1.15 (s, 3H, 19-CH_3), 2.10 (bs, 3H, 6-CH_3), 5.80 (bs, 1H, 7-CH), 6.25 (m, 2H, 2-CH, 4-CH), 7.05 (d, 1H, 1-CH).

6α-Methylandrosta-1,4-diene-3,17-dione (20)

Compound **17** was submitted to transfer hydrogenation over palladium-charcoal using cyclohexene as hydrogen donor in refluxing ethanol as reported by Burn et al.[9] in an analogous reaction. Thus 6α-methyl-androst-4-ene-3,17-dione (**19**)[10] was obtained in 60% yield (mp 167 C). Compound **19** was dehydrogenated in 50% yield with DDQ as described by Burn et al.[11] to give compound **20**: mp 219–220 C; MS 298; NMR 0.91 (d, 3H, 6-CH_3), 0.94 (s, 3H, 18-CH_3), 1.16 (s, 3H, 19-CH_3), 6.24 (m, 2H, 2-CH, 4-CH), 7.08 (d, 1H, 1-CH).

Aromatase inhibition

Microsomes from human placenta were used as the enzyme source. Aromatase activity was tested by measuring the release of ^3H_2O from [1β-^{3}H]androstenedione (specific activity 15–30 Ci/mmol, supplied by New England Nuclear, Boston, MA), according to Thompson and Siiteri.[12] All incubations were performed in a Dubnoff shaking incubator at 37 C in air in phosphate buffer (10 mM potassium phosphate buffer pH 7.5, containing 100 mM KCl, 1 mM EDTA, and 1 mM dithiothreitol). The assay was carried out in duplicate in 1 ml final incubation volume containing 50 nM [1β-^{3}H]androstenedione, various concentrations of the tested compounds, 100 μM NADPH, and an aliquot of the microsomal preparation. After 15 minutes incubation the enzymatic reaction was terminated by the addition of 4 ml $CHCl_3$. The extraction procedure was repeated twice. Radioactivity in the water phase was determined by liquid scintillation counting in Ria Luma^R (cumene, supplied by Lumac LSC). The concentration of each compound required to reduce control aromatase activity by 50% (IC_{50}) was calculated by linear regression analysis. Each compound was tested in at least two separate assays. The aromatase inhibitor formestane (4-hydroxyandrost-4-ene-3,17-dione) was used as reference standard. In comparison with the generally used standard procedure,[12] we have omitted the further elimination of traces of labeled substrate in the water phase with dextran-coated charcoal (DCC), since we have found no difference in the determination of the IC_{50} values (for exemestane and formestane) obtained with and without the DCC step.

Results and discussion

Synthesis

In the preceding we describe the synthesis of some potential metabolites (and related analogs) of exemestane. Their design was based on the hypothesis that the metabolic attack occurs mainly at the exocyclic double bond besides the reduction of the 17-keto group. Scheme 1 shows the synthetic route for the compounds **2–8**. Starting from 17β-hydroxyandrosta-1,4-dien-3-one (**1**) Mannich reaction with paraformaldehyde and dimethylamine hydrochloride affords 17β-hydroxy-6-methylenandrosta-1,4-dien-3-one (**2**). Epoxidation of compound **2** with m-chloroperbenzoic acid led to the formation of an epimeric mixture of 17β-hydroxy-6α/β-spirooxiranandrosta-1,4-dien-3-one (**3**), which could not be separated by column chromatography. Subsequent hydrolysis with aqueous perchloric acid gave an unseparated epimeric mixture of 6α/β,17β-dihydroxy-6α/β-hydroxymethylandrosta-1,4-dien-3-one (**4**) besides 17β-hydroxy-6-hydroxymethylandrosta-1,4,6-trien-3-one (**5**). It is clear that compound **5** derives from compound **4** by dehydration and that its yield increases under more vigorous conditions. Jones oxidation of compound **2** affords exemestane. Epoxidation of the latter with *m*-chloroperbenzoic acid afforded an epimeric mixture of 6α/β-spirooxiranandrosta-1,4-diene-3,17-dione (**6**), which submitted to hydrolysis gave an epimeric mixture of 6α/β-hydroxy-6α/β-hydroxymethylandrosta-1,4-diene-3,17-dione (**7**) besides 6-hydroxymethylandrosta-1,4,6-triene-3,17-dione (**8**). The epoxidation and hydrolysis was carried out as described above for the 17β-hydroxy series.

Scheme 2 reports on the syntheses of compounds **11–14**, of which compounds **12** and **13** were unknown. Reaction of androsta-1,4-diene-3,17-dione (**9**) with pyrrolidine afforded 1α,3-dipyrrolidinylandrosta-3,5-dien-17-one (**10**),[6] which when treated with formaldehyde 30% led to the formation 6β-hydroxymethylandrosta-1,4-diene-3,17-dione (**11**).[6] Selective reduction of the latter with sodium borohydride yielded 17β-hydroxy-6β-hydroxymethyl-androsta-1,4-dien-3-one (**12**). On the other hand oxidation of compound **11** afforded 6β-carboxyandrosta-1,4-diene-3,17-dione (**13**). Finally alkaline autooxidation of compound **9** provided androsta-1,4-diene-3,6,17-trione (**14**).[7]

According to the literature[6] compound **11** has a 6β-configuration and we agree with this assignment as it is in accordance with the NMR data found for the epimeric analogs 6α- and 6β-hydroxy-methylandrost-4-ene-3,17-dione.[13] As we did not observe a coupling constant $J_{4,6}$ of about 1.5 Hz as would be required for a 6α-epimer, we concluded that compound **11** is the 6β-epimer. For the same reason also compound **12** has a 6β-configuration. As regards compound **13**, we postulate a 6β-configuration because of the relatively low coupling constant ($J_{6,7}$ = 4.6 Hz). If compound **13** had a 6α-configuration, the 6βH-7αH diaxial coupling would have given a higher coupling constant $J_{6,7}$ of about 10 Hz.

Scheme 3 depicts the synthetic pathway for compounds **18** and **20**. Reaction of androst-4-ene-3,17-dione (**15**) with formaldehyde diethyl acetal and phosphoryl chloride according to Annen et al.[8] afforded 6-methylenandrost-4-ene-3,17-dione (**16**), which was isomerized by treatment with palladium-charcoal as described by Burn et al.[9] in an analogous reaction to give 6-methylandrosta-4,6-diene-3,17-dione (**17**). Subsequent dehydrogenation with DDQ afforded 6-methylandrosta-1,4,6-triene-3,17-dione (**18**). Otherwise transfer hydrogenation of compound **17** with cyclohexene over Pd/C yielded 6α-methylandrost-4-ene-3,17-dione (**19**),[10] which was dehydrogenated with DDQ to give 6α-methylandrosta-1,4-diene-3,17-dione (**20**).[11]

Table 1. Inhibition of human placental aromatase[a]

Cpd	Name	IC_{50} (nM)	Ratio[b]
	Exemestane (6-methylenandrosta-1,4-diene-3,17-dione)	27	1.0
	Formestane (4-hydroxyandrost-4-ene-3,17-dione)	67	2.5
2	17β-Hydroxy-6-methylenandrosta-1,4-dien-3-one	69	2.6
6	6α/β-Spirooxiranandrosta-1,4-diene-3,17-dione	206	7.6
3	17β-Hydroxy-6α/β-spirooxiranandrosta-1,4-dien-3-one	552	20.4
7	6α/β-Hydroxy-6α/β-hydroxymethylandrosta-1,4-diene-3,17-dione	2,300	85.2
4	6α/β,17β-Dihydroxy-6α/β-hydroxymethylandrosta-1,4-dien-3-one	11,100	411.1
8	6-hydroxymethylandrosta-1,4,6-triene-3,17-dione	560	20.7
5	17β-Hydroxy-6-hydroxymethylandrosta-1,4,6-trien-3-one	4,400	163.0
11	6β-Hydroxymethylandrosta-1,4-diene-3,17-dione	295	10.9
12	17β-Hydroxy-6β-hydroxymethylandrosta-1,4-dien-3-one	1,157	42.9
13	6β-Carboxyandrosta-1,4-diene-3,17-dione	7,200	266.7
14	Androsta-1,4-diene-3,6,17-trione	268	9.9
18	6-Methylandrosta-1,4,6-triene-3,17-dione	91	3.4
20	6α-Methylandrosta-1,4-diene-3,17-dione	99	3.7

[a] The compounds were incubated for 15 minutes with 50 nM [1β-^{3}H]androstenedione. The results are the mean of at least two separate assays.
[b] IC_{50} of tested compound/IC_{50} of exemestane.

Compound **19** was assigned with a 6α-configuration on the basis of its melting point (167 C). In fact, Ackroyd et al.,[10] who synthesized the 6α- and the 6β-methylandrost-4-ene-3,17-dione in a different way using a sterically controlled multistep procedure, have shown that the 6-epimers have very different melting points (167 C and 212 C, respectively). Furthermore compound **20** had the same melting point as that reported for the 6α-epimer.[11]

Aromatase inhibition

Table 1 shows the IC_{50} values for inhibition of human placental aromatase obtained with a number of analogs of exemestane. The parent compound was confirmed to be a very potent aromatase inhibitor with an IC_{50} value of 27 nM. In comparison with formestane (IC_{50} 67 nM), exemestane resulted 2.5-fold more potent.

None of the new analogs of exemestane here described (some of which were synthesized as potential metabolites) had aromatase inhibitory potency higher than the parent compound. The most potent was the exemestane derivative with a reduced 17-keto group (compound **2**, IC_{50} 69 nM), which however is 2.6-fold less potent than exemestane.

Among the C-6 modified analogs of exemestane, the following results have been obtained: the epoxidation of the exocyclic double bond (compound **6**, IC_{50} 206 nM) caused a 7.6-fold decrease in activity; the cleavage of the epoxide giving the diol **7** (IC_{50} 2,300 nM) and subsequent dehydration to yield the 6-hydroxymethyl-Δ^6-steroid (compound **8**, IC_{50} 560 nM) caused a further decrease in activity; the reductive cleavage of the epoxide affords a compound (**11**, IC_{50} 295 nM) which resulted 11-fold less potent than exemestane. The 17β-ol derivatives obtained by reduction at C-17 (compounds **3, 4, 5,** and **12**) appeared all approximately 3- to 8-fold less potent than the corresponding 17-keto derivatives (compounds **6, 7, 8,** and **11**). In addition, the 6β-carboxy derivative (compound **13**, IC_{50} 7,200 nM) resulted 267-fold less potent than exemestane, whereas the super-oxidation of the exocyclic double bond to give the 6-oxo steroid (compound **14**, IC_{50} 268 nM) caused a 10-fold decrease in activity. Isomerization or reduction of exocyclic double bond (compounds **18** and **20**) produced a 3-fold decrease in activity (IC_{50} 91 and 99 nM, respectively).

Exemestane has been characterized in vivo as a long-lasting inhibitor of aromatase. Although the duration of its effects on estrogen synthesis has been attributed to the irreversible nature of aromatase inhibition[4,5] rather than to the pharmacokinetic property of the compound, the possible contribution of some active metabolites cannot be excluded. Among the potential metabolites of exemestane studied in the present report, the 17β-hydroxy derivative (compound **2**) has already been identified both in animal (G. Cocchiara et al., unpublished results) and human[5] studies. However the plasma levels of this metabolite which were found, e.g., after single oral doses of exemestane in postmenopausal women, were always less than 1/10th of the unchanged drug,[5] and we now report that this metabolite is 2.6-fold less potent than exemestane in inhibiting human placental aromatase. The identification of other exemestane metabolites is still in progress. However, none of the potential metabolites at C-6 or at C-17 here described were found to be more potent than the unchanged drug.

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