Synthesis and *in Vitro* Evaluation of 4-Substituted *N*-(1,1-Dimethylethyl)-3-oxo-4-androstene-17β-carboxamides as 5α-Reductase Inhibitors and Antiandrogens

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4-Substituted *N*-(1,1-dimethylethyl)-3-oxo-4-androstene-17β-carboxamides with the hydroxy (OH) **3d**, mercapto (SH) **3e**, chloro (Cl) **3f**, and bromo (Br) **3g** substituents at the 4-position were prepared in a two-step sequence with overall yields of 21%, 27%, 41%, and 37%, respectively. Compounds **3d**–**g** showed weak inhibitory activity on human type I 5α-reductase ($IC_{50} \geq 700$ nM) while they had intermediate inhibitory activity on human type II 5α-reductase at IC_{50}s of 172, 437, 192, and 387 nM, respectively. In androgen-sensitive Shionogi cells, the inhibition of dihydrotestosterone (DHT) stimulatory action on the proliferation of the androgen-sensitive cancer cells by all four compounds was high at IC_{50}s of 170–279 nM compared with 117 nM for hydroxyflutamide. The present data show compounds having both moderate inhibition of human type II 5α-reductase activity and relatively potent antiandrogenic action, two beneficial characteristics in the therapy of androgenic-sensitive diseases.

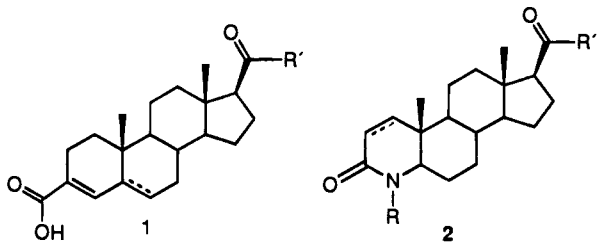
Introduction

Study of the mechanisms and inhibition of steroid 5α-reductase (Δ^4-3-oxosteroid 5α-oxidoreductase; EC 1.3.99.5)[1] is a topic of major current biological and pharmaceutical interest, since this enzyme catalyzes the irreversible reduction of the C-4–C-5 double bond of 3-oxo-4-ene steroids to their corresponding 5α-3-oxo-steroid derivatives,[2] using a nicotinamide adenine dinucleotide hydrogen phosphate (NADPH) as co-factor.[3] The main interest in 5α-reductase arises from its role in the conversion of testosterone (T) to dihydrotestosterone (DHT), the most active androgen known to be essential for male differentiation,[4] growth and function of androgen-sensitive sex organs and in the pathophysiology of many diseases, especially benign prostatic hyperplasia (BPH), prostate cancer,[5] acne,[6] hirsutism,[7] and androgenic alopecia (male-pattern baldness).[8]

Recently, the isolation and characterization of human types I and II 5α-reductase genes have been described.[9] The human type II 5α-reductase is presumed to be involved in the prostatic androgen-related diseases mentioned above. However, human type I 5α-reductase encodes for the isozyme presumed to be expressed in the skin. A rationale treatment of these androgen-sensitive conditions would be the selective inhibition of either one or both enzymes with specific inhibitors.

Although several steroidal and nonsteoridal compounds[10] have been reported as inhibitors of 5α-reductase during the last decade, steroidal compounds have attracted more attention. It has been observed that for a compound to be a good to excellent inhibitor, a conjugated system (sp^2–sp^2–sp^2) should be present at the C-3, -4, and -5 positions of the A ring of steroids together with a lipophilic group at the C-17 position.[11,12] On the basis of these observations, remarkable progress has been made in the design and synthesis of steroidal inhibitors, by selectively modifying the 3-, 4-, 5-, and 17-positions of steroids: the two best known series of compounds are 3-carboxy steroids 1,[11] and 4-azasteroids **2** (Chart 1).[12] The *in vitro* evaluation of compounds **1** on inhibitory activity of human and rat type II prostatic steroid 5α-reductases has shown that 17β-carbamoyl-3-androstene-3-carboxylic acids are highly potent inhibitors of the enzyme. The inhibitory activity is enhanced in analogues possessing an additional C-5–C-6 double bond while it is decreased in analogues

Chart 1

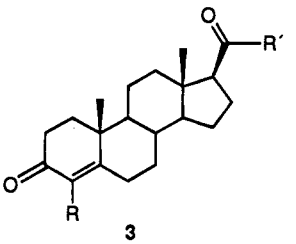


1. Δ^2, Δ^3, $\Delta^{2,4}$, or $\Delta^{3,5}$
R′ = N-monoalkyl, N,N-dialkyl

1a. $\Delta^{3,5}$; R′ = N-C(CH$_3$)$_3$

2. R = H, CH$_3$
R′ = N-monoalkyl, N,N-dialkyl

2a. R = H; R′ = N-C(CH$_3$)$_3$



3a. R = CN, R′ = N,N-diethyl or methyl
3b. R = NH_2, R′ = N-monoalkyl or N,N-dialkyl
3c. R = N_3, R′ = N-monoalkyl or N,N-dialkyl
3d. R = OH, R′ = N-(1,1-dimethylethyl)
3e. R = SH, R′ = N-(1,1-dimethylethyl)
3f. R = Cl, R′ = N-(1,1-dimethylethyl)
3g. R = Br, R′ = N-(1,1-dimethylethyl)

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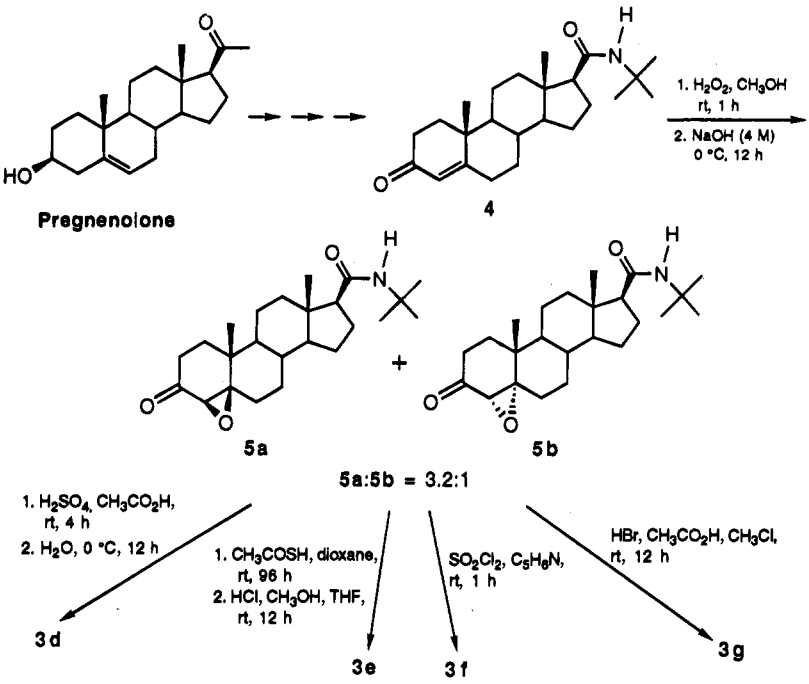
Scheme 1



lacking the C-3–C-4 double bond.[11d] Epristeride (**1a**) of this series, which is under clinical trials, reduces the serum DHT levels by 25–54% at 0.4-160 mg doses after 8 days.[13] It is proposed that these unsaturated steroidal acid functions mimic the putative enolate intermediate of the enzymatic process.[11a,b] However, 3-carboxyaryl isomers of **1** showed significantly reduced activity with the rodent enzyme relative to the human 5α-reductase.[11c]

Another series of mechanism-based steroidal inhibitors are the 4-azasteroids, which were prepared by structural modifications of the A ring and of moieties attached at the C-17 position of the steroid nucleus. High *in vitro* activity was seen with 4-methyl-4-aza derivatives on both human and rat type II prostatic 5α-reductases. However, 4-unsubstituted 4-aza derivatives are usually less active than 4-methyl derivatives.[12a] The activity-enhancing features of a 4-methyl group and a semipolar side chain at the 17-position indicate that the enzyme has steric and lipophilic preferences at these positions.[12c] One of the well-known compounds in this series is Finasteride (**2a**) (PROSCAR) which has the above-indicated substituents in the A and D rings. Potent *in vitro* inhibition was verified for both human and rat type II enzymes. Clinical trials in patients with BPH, using Finasteride, have demonstrated its therapeutic efficacy in BPH with an 80% reduction in circulating DHT levels and a 28% reduction in prostate size.[12b–d] It is believed that these compounds behave as "intermediate state" inhibitors and the A-ring lactam of 3-oxo-4-azasteroids mimics the conformation of the intermediate enol form.[14] Recently, another series of azasteroids were prepared and evaluated for their inhibitory activities. The 6-azasteroids were found to be active on both types I and II 5α-reductases.[15]

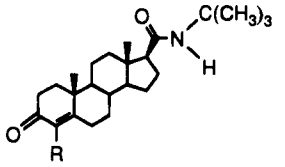
4-Substituted 3-oxo-4-androstenes having an *N*-(1,1-dimethylethyl)-17β-carboxamide group were prepared and tested *in vitro* as human type II 5α-reductase inhibitors.[16] The 4-cyano analogue **3a** showed high *in vitro* potency (IC_{50} = 2.9 nM). However, when a 4-amino or 4-azido group was present, a decrease in activity was observed (IC_{50} = 54 nM for **3b**).[14b,c] It thus appears that the steroids having a 4-substituted 3-oxo group in the A ring and a 17β-carboxamide substituent in the D ring have good to excellent 5α-reductase inhibitory activity. The 4-fluoro and 4-cyano derivatives of androstenedione and progesterone were also evaluated on the type II enzyme.[17] The fluoro compounds exhibited weak *in vitro* activity and were androgen agonists in *in vivo*, while the cyano compounds were potent inhibitors.

To better understand structure–activity relationships in connection with both types of human 5α-reductases, we have synthesized and assayed the *in vitro* activity of 4-substituted (OH, SH, Cl and Br) 3-oxo-4-androstene-17β-carboxamides as inhibitors of both types of human 5α-reductases (types I and II). These compounds were also evaluated for their inhibitory activities of the androgen-induced proliferation of Shionogi cells.

Results and Discussion

Chemistry. The 4-substituted 3-oxo-4-ene-steroid-17β-carboxamides were prepared from the key intermediate, 3-oxo-4-ene-steroid-17β-carboxamide **4** while compound **4** was prepared from commercially available pregnenolone (Scheme 1).[12c] Thus, *N*-(1,1-dimethylethyl)-3-oxo-4-androstene-17β-carboxamide (**4**) was obtained in 89% yield from a one-pot reaction, in which the 17β-carboxylic acid was first converted to 3-oxo-4-androstene-17β-carboxylic chloride *in situ* by stirring with oxalyl chloride in pyridine at −10 °C and then with *tert*-butylamine in toluene.[12c,18] Treatment of the amide 4 with 30% hydrogen peroxide in methanol at room temperature for 1 h followed by 4 M sodium hydroxide at 0 °C for 12 h provided an isomeric mixture of 4,5-epoxides **5a/5b** (β:α = 3.2:1).[19a] The isomeric ratio was determined by ^1H NMR spectroscopy. Reaction of epoxides with a mixture of concentrated sulfuric acid[19] and acetic acid[19a] gave *in situ* 4-oxyacetate which, after subsequent hydrolysis, yielded 4-hydroxy-3-oxo-4-androstene-17β-carboxamide (**3d**) at a 40% yield.[19a,d] The

Table 1. *In Vitro* Activity of 4-Substituted *N*-(1,1-Dimethylethyl)-3-oxo-4-androstene-17β-carboxamides



entry	R	*in vitro* bioactivity[a] (IC_{50}, nM) human type I 5α-R (transfected 293 cells)	human type II 5α-R (transfected SW-13 cells)	DHT[d]-stimulated Shionogi cells
finasteride (**2a**)[b]		218	8.47	NA
Flu-OH[c]		NA	NA	117
Δ^4-dione[e]		2700	83.9	NA
3d	OH	≫1000	172	242
3e	SH	709	437	279
3f	Cl	≫1000	192	170
3g	Br	981	387	184

[a] The results of the inhibition of 5α-reductase (types I and II) and DHT-stimulated proliferation of Shionogi cells were obtained by following standard procedures described in the Experimental Section. The concentration of the compounds required to inhibit 5α-reductase activity or 5α-dihydrotestosterone-induced stimulation of Shionogi cells proliferation by 50% is represented as IC_{50} values. [b] Finasteride[12c,d] was used as a standard reference for the inhibition of 5α-reductase. [c] Hydroxyflutamide (Flu-OH) was added as a standard control for the antiandrogen test. [d] 5α-Dihydrotestosterone (DHT, 0.3 nM) was used as a standard substrate for the growth assay. [e] [^{3}H]-Δ^4-Androstenedione ([^{3}H]-Δ^4-dione, 2.5 nM) was added as the substrate for 5α-reductases.

6α-proton of compound **3d** resonated at 3.0 ppm, which is a characteristic signal for the 4-substituted 3-oxo-4-ene system. In a similar fashion, 4-mercapto-3-oxo-4-androstene-17β-carboxamide (**3e**) was also obtained in 50% yield, after stirring 4,5-epoxides **5a/5b** with thioacetic acid.[19a-c] Both ^{1}H and ^{13}C NMR spectra were in agreement with the assigned structure. When the epoxides **5a/5b** were treated with a mild chlorinating reagent, such as sulfuryl chloride (SO_2Cl_2), in dry pyridine at room temperature, 4-chloro-3-oxo-4-androstene-17β-carboxamide (**3f**) was formed in 77% yield.[20a,c] However, the preparation of compound **3f** from **3d** or **3e**[20b] under acidic conditions gave more side products. 4-Bromo-3-oxo-4-androstene-17β-carboxamide (**3g**) was prepared in 69% yield from **5a/5b** and hydrogen bromide in a mixture of acetic acid and chloroform at room temperature.[20b]

Inhibition of Human 5α-Reductase (Types I and II). Finasteride (**2a**) was used as the standard reference in both *in vitro* protocols. In comparision with finasteride (IC_{50} = 218 nM), 4-substituted 3-oxo-4-androstene-17β-carboxamides showed little or no inhibitory effect on human type I 5α-reductase. In fact, the IC_{50} value of each compound was at approximately or above 700 nM (Table 1). However, intermediate inhibitory activity was seen on human type II 5α-reductase (Figure 1). Thus, the halogenated compounds **3d** (IC_{50} = 172 nM) and **3f** (IC_{50} = 192 nM) were 2-fold more potent than the thiol **3e** (IC_{50} = 437 nM) and the hydroxy compound **3g** (IC_{50} = 387 nM). However, all compounds were 20 times or above less potent than finasteride (IC_{50} = 8.47 nM).

Inhibition of the Proliferation of Androgen-Sensitive Shionogi Cancinoma Cells (Clone SEM-107). The antiandrogenic activity was determined by inhibiting DHT action on the proliferation of the androgen-sensitive Shionogi cells. Hydroxyflutamide was used as the standard reference with an IC_{50} value of 117 nM. The inhibitory ability of the 4-substituted 3-oxo-4-androstene-17β-carboxamides was dependent on the nature of the substituent at 4-position of the A ring (Table 1). The 4-halogenated compounds **3f** and **3g** have high inhibitory activity (IC_{50}s = 170 and 184 nM, respectively) on the proliferation of DHT-stimulated Shionogi cells. However, the 4-hydroxy compound (**3d**) and the thiol (**3e**) have some what lower potency (IC_{50}s = 242 and 297 nM, respectively) (Figure 2). This study reveals that 4-substituted compounds are androgen antagonists and are less active compared to hydroxyflutamide.

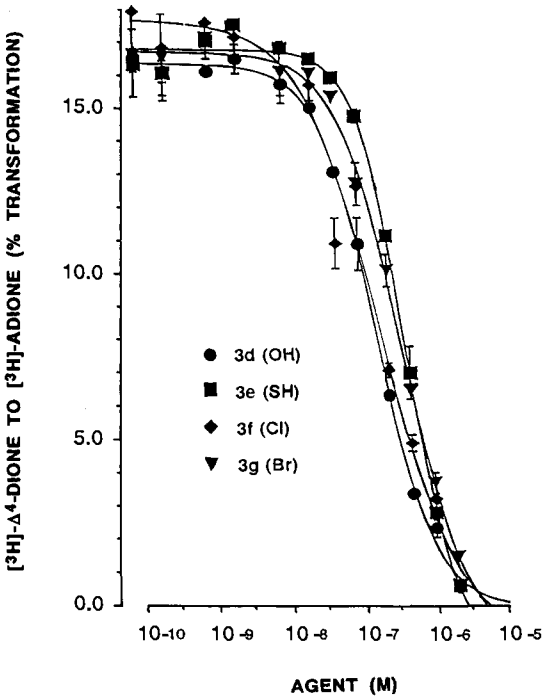


Figure 1. Inhibition of human type II 5α-reductase by 4-substituted 3-oxo-4-androstene-17β-carboxamides.

Conclusion

Compounds **3d-g** are intermediate inhibitors of human type II 5α-reductase. However, they are very weak inhibitors of human type I 5α-reductase. In addition, these compounds show relatively good inhibitory activity on the proliferation of androgen-sensitive cells, thus

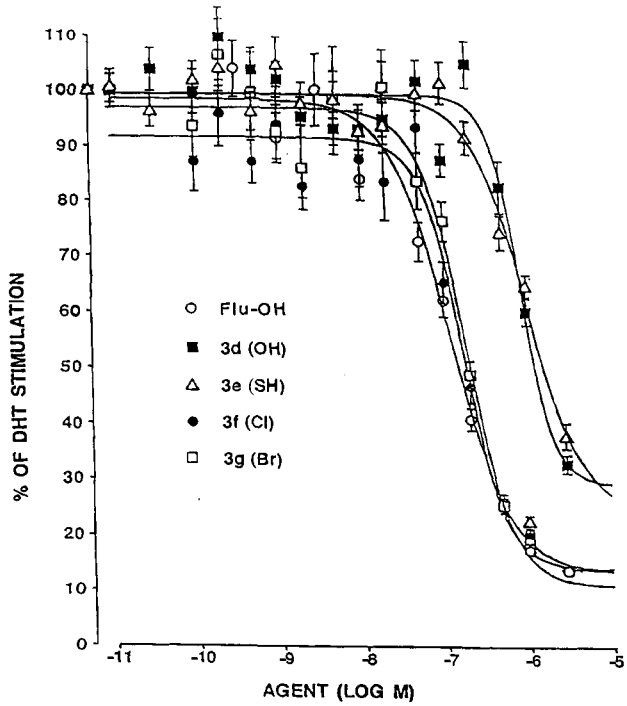


Figure 2. Inhibition of DHT-stimulated proliferation of androgen-sensitive Shionogi cells (clone SEM 107) by 4-substituted 3-oxo-4-androstene-17β-carboxamides.

adding to the positive characteristics of these compounds. These observations should lead to design of more potent and selective inhibitors of androgenic formation and action.

Experimental Section

General. Unless otherwise indicated, materials obtained from commercial suppliers were used without further purification. Diethyl ether and tetrahydrofuran (THF) were distilled from sodium/benzophenone immediately prior to use. All reactions except those involving water as a reagent were conducted under argon atmosphere. Melting points were measured on a Gallenkamp capillary melting point apparatus and are uncorrected. IR spectra were determined with a Perkin-Elmer 1600 Series FT infrared spectrometer. ^{1}H NMR spectra were determined on a Bruker Aspect-3000 (300 MHz). ^{13}C NMR spectra were measured at 75.14 MHz with a Bruker Aspect-3000. Low-resolution mass spectra were obtained with a Varian Model 3700 gas chromatography/micromass 16F mass spectrometer. High-resolution mass spectra were measured at the Department of Chemistry, University of Montreal, Montreal, Quebec. Combustion analyses (C, H, N, S, Cl, Br) were performed by Galbraith Laboratories (Knoxville, TN). All the final products were at least 99.5% pure, and the purity was determinated by a high-performance liquid chromatography (HPLC) of Waters Model 600E (Millipore).

***N*-(1,1-Dimethylethyl)-3-oxo-4-androstene-17β-carboxamide (4)** was prepared by the method of Rasmusson et al.[12c,18]

***N*-(1,1-Dimethylethyl)-3-oxo-4,5-epoxyandrostane-17β-carboxamides (5a/5b).** In a 1-L flask equipped with a magnetic stir bar, a septum and an argon inlet was placed *N-tert*-butyl-3-oxo-4-androstene-17β-carboxamide (14.0 g, 37.7 mmol) in a 1:10 mixture of methylene chloride/methanol. Hydrogen peroxide (30% aqueous solution, 19.8 mL, 174.3 mmol) was added dropwise over a period of 30 min to the above mixture. After 30 min of stirring at room temperature, the flask was cooled to 0 °C and 4 M aqueous sodium hydroxide (6.8 mL) was added dropwise. The reaction mixture was left for 12 h at 0–4 °C; after that, it was neutralized with 1 N aqueous HCl. The reaction mixture was concentrated on a rotary evaporator to a volume of about 50 mL. Ice–water (500 mL) was added, and the mixture was stirred for 15 min to give precipitate which was filtered, washed with water, and dried under vacuum to afford the product (8.2 g, 53% yield). The analysis of the NMR spectrum gave a 3.2:1 mixture of β:α-isomer: mp 94–96 °C; IR (KBr, cm^{-1}) 3447 (sh), 3398, 2942, 2847, 1711, 1673, 1513, 1451, 1390, 1383, 1251, 1218, 862; ^{1}H NMR ($CDCl_3$) δ 0.67 (s, 2.28 H, 18-CH_3), 1.04 (s, 0.72 H, 18-CH_3), 1.06 (s, 0.72 H, 19-CH_3), 1.13 (s, 2.28 H, 19-CH_3), 0.8–1.17 (m, 2H), 1.18–1.27 (m, 3H), 1.31 (s, 9 H, 3 CH_3), 1.40–1.58 (m, 4 H), 1.67 (dd, J = 8.2, 13.0 Hz, 2H), 1.74–2.09 (m, 4 H), 2.11–2.42 (m, 5 H), 2.95 (s, 0.76 H, 4α-H), 3.01 (s, 0.24 H, 4β-H), 5.10 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$) (β/α) δ 206.8, 171.6, 70.3/70.1, 62.8/62.7, 57.6/57.5, 55.6/55.4, 51.1/50.8, 46.5, 43.7, 38.5/38.3, 37.2/37.1, 35.4/35.0, 33.1/32.5, 30.3/29.7, 29.7/29.6, 29.0 (3C), 26.2, 24.4, 23.2, 21.5/21.4, 18.9, 16.5, 13.1; EI-MS m/s (relative intensity) 387 (M^+, 100), 372 (10), 332 (18), 215 (16), 141 (25), 128 (86), 115 (44), 95 (29), 72 (57); HRMS calcd for $C_{24}H_{37}O_3N_1$ 387.2773, found 387.2759. Anal. ($C_{24}H_{36}O_3N_1$) C, H, N.

***N*-(1,1-Dimethylethyl)-4-hydroxy-3-oxo-4-androstene-17β-carboxamide (3d).** Epoxides **5a/5b** (1.0 g, 2.59 mmol) was added in small portions to a well-stirred mixture of concentrated sulfuric acid (0.16 mL) and glacial acetic acid (7.8 mL). The mixture, which turned progressively orange, was then stirred for 4 h at room temperature under argon. The mixture was poured into a beaker containing 250 g of ice and allowed to stand at 4 °C for 12 h. The aqueous solution was extracted with methylene chloride (4 × 120 mL). The combined organic phase was washed with brine (100 ML), dried, and concentrated to oil. The crude product was purified by silica gel flash column chromatography (C_6H_6:EtOAc:CH_3COCH_3, 80:15:5) to give the product **3d** (0.397 g, 40% yield): mp 163–165 °C; IR (KBr, cm^{-1}) 3403, 2961, 2842, 1664, 1635 (sh), 1509, 1451, 1378, 1249, 1202; ^{1}H NMR ($CDCl_3$) δ 0.70 (s, 3 H, 18-CH_3), 0.38–1.12 (m, 4 H), 1.16 (s, 3 H, 19-CH_3), 1.25 (ddd, J = 4.7, 11.9, 12.0 Hz, 1 H), 1.32 (s, 9 H, 3 CH_3), 1.43 (ddd, J = 3.4, 13.3, 13.1 Hz, 1 H), 1.56 (ddd, J = 3.5, 6.9, 10.8 Hz, 1 H), 1.61–1.76 (m, 4 H), 1.82–2.02 (m, 5 H), 2.10–2.20 (m, 1H), 2.22–2.52 (m, 2 H), 3.0 (dq, J = 2.2, 4.3, 14.9 Hz, 1 H, 6α-H), 5.09 (br s, 1 H, NH), 6.08 (s, 1 H, 4-OH); ^{13}C NMR ($CDCl_3$) δ 193.5, 171.7, 141.2, 139.8, 57.5, 55.7, 54.2, 51.0, 43.5, 38.5, 37.8, 35.2, 34.7, 31.8, 30.9, 29.0 (3 C), 24.4 23.3, 22.9, 20.9, 17.1, 13.1; EI-MS m/s (relative intensity) 387 (M^+, 100), 372 (7), 314 (5), 286 (8), 250 (10), 230 (6), 147 (44), 128 (14), 93 (15), 72 (19); HRMS calcd for $C_{24}H_{37}O_3N_1$ 387.2773, found 387.2744. Anal. ($C_{24}H_{37}O_3N_1$) C, H, N.

***N*-(1,1-Dimethylethyl)-4-mercapto-3-oxo-4-androstene-17β-carboxamide (3e).** Thioacetic acid (0.25 mL) in dioxane (0.8 mL) was added to an ice-cold solution of epoxides **5a/5b** (1.0 g, 2.59 mmol) in dioxane (8.3 mL). After 4 days of stirring at room temperature under argon, another batch of thioacetic acid (0.25 mL) was added to the dark mixture and stirred for 24 h at room temperature. The mixture was concentrated on a rotary evaporator (equipped with a vacuum pump) to give a crude 4-acetylthio compound, which was dissolved in a 1:1 mixture of methanol/tetrahydrofuran (50 mL). Concentrated HCl (0.34 mL) was added, and the mixture was stirred for 12 h at room temperature. Concentrated HCl (0.34 mL) was again added, and the mixture was stirred for 24 h at room temperature. The mixture was then poured into crushed ice–water (80 mL). The aq solution was extracted with methylene chloride (4 × 120 mL). The combined organic phase was washed with brine (100 ML), dried and concentrated to oil. The crude product was purified by silica gel flash column chromatography (C_6H_6:EtOAc:CH_3COCH_3; 80:15:5) to give the produce **3e** (0.521 g, 50% yield): mp 152–154 °C; IR (KBr, cm^{-1}) 3389, 2985, 2854, 1675, 1653 (sh), 1572, 1514, 1452, 1387, 1367, 1294, 1253, 1221, 1098, 938, 892; ^{1}H NMR ($CDCl_3$) δ 0.72 (s, 3H, 18-CH_3), 0.93–1.10 (m, 4 H), 1.18 (s, 3 H, 19-CH_3), 1.27 (ddd, J = 4.5, 11.6, 11.6 Hz, 1 H), 1.34 (s, 9 H, 3 CH_3), 1.43 (dd, J = 2.8, 3.6 Hz, 1 H), 1.51–1.71 (m, 5 H), 1.84–2.04 (m, 5 H), 2.30 (ddd, J = 5.2, 14.2, 14.3 Hz, 1 H), 2.45–2.62 (m, 2 H), 2.74 (dq, J = 2.0, 4.1, 14.7 Hz, 1 H, 6α-H), 4.73 (s, 1 H, 4-SH),[16a] 5.09 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$) δ 192.2, 171.6, 161.1, 126.6, 57.5, 55.6, 54.0, 51.1, 43.6, 40.2, 38.5, 35.2, 34.6, 33.4, 31.5, 31.2, 29.1 (3C), 24.3, 23.3, 21.1, 17.4, 13.1; EI-MS m/s (relative intensity) 403 (M^+, 100), 388 (4), 329 (4),

304 (15), 276 (16), 220 (8), 147 (17), 128 (12), 91 (15), 72 (18); HRMS calcd for $C_{24}H_{37}O_2N_1S_1$ 403.2545, found 403.2541. Anal. ($C_{24}H_{37}O_2N_1S_1$) C, H, N, S.

***N*-(1,1-Dimethylethyl)-4-chloro-3-oxo-4-androstene-17β-carboxamide (3f).** *N,N*-(1,1-Dimethylethyl)-3-oxo-4-androstene-17β-carboxamide (0.6 g, 1.62 mmol) was dissolved in dry pyridine (10 mL). Freshly distilled sulfuryl chloride (0.56 g, 4.04 mmol) was added dropwise to the above agitating mixture at 20–25 °C over a period of 5 min. After 1 h, the reaction mixture was poured into water and extracted with diethyl ether (3 × 50 mL). The organic layer was washed with 10% aqueous HCl, 5% aqueous Na_2CO_3, and brine. Drying and removal of the solvent gave the crude product which was purified by silica gel flash column chromatography (CH_2Cl_2:C_6H_6:EtOAc; 50:50:2 to 60:40:3) to give the product **3f** (0.51 g, 77% yield): mp 153–155 °C, IR (KBr, cm^{-1}) 3390 (sh), 2966, 2852, 1693, 1668, 1583, 1525, 1451, 1390, 1362, 1292, 1252, 1224, 1185, 1115, 952, 812; ^1H NMR ($CDCl_3$) δ 0.73 (s, 3 H, 18-CH_3), 0.88 (t, J = 6.9, 7.0 Hz, 1 H), 0.94–1.13 (m, 3 H), 1.23 (s, 3 H, 19-CH_3), 1.24–1.25 (m, 1 H), 1.35 (s, 9 H, 3 CH_3), 1.43 (ddd, J = 3.5, 14.7, 14.6 Hz, 1 H), 1.52–1.63 (m, 3 H), 1.66–1.79 (m, 3 H), 1.90–2.06 (m, 4 H), 2.17 (ddd, J = 5.1, 14.5, 14.5 Hz, 2 H), 2.57 (dd, J = 4.5, 7.1 Hz, 2 H), 2.74 (dt, J = 3.3, 3.4, 14.6 Hz, 1 H, 6α-H), 5.08 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$) δ 190.6, 171.6, 164.5, 127.4, 57.5, 55.5, 54.0, 51.1, 43.5, 41.4, 38.4, 35.2, 34.5, 34.1, 34.0, 31.1, 29.1 (3C), 24.3, 23.3, 21.1, 17.8, 13.1; EI-MS m/s (relative intensity) 405 (M^+, 100), 390 (16), 369 (25), 350 (11), 269 (22), 252 (12), 227 (11), 141 (34), 128 (60), 115 (38), 93 (41), 81 (36), 72 (64); HRMS calcd for $C_{24}H_{36}O_2N_1Cl_1$ 405.2434, found 405.2405. Anal. ($C_{24}H_{36}O_2N_1Cl_1$) C, H, N, Cl.

***N*-(1,1-Dimethylethyl)-4-bromo-3-oxo-4-androstene-17β-carboxamide (3g).** Aqueous 40% HBr (10 mL) was added to a solution of *N,N*-(1,1-dimethylethyl)-3-oxo-4,5-epoxyandrostane-17β-carboxamide (1.0 g, 2.59 mmol) in chloroform (100 mL) and glacial acetic acid (10 mL). After stirring for 16 h at room temperature under argon, the mixture was diluted with water (120 mL) and extracted with chloroform (3 × 100 mL). Drying and removal of the solvent gave an oil which was purified by silica gel flash column chromatography (CH_2Cl_2:C_6H_6:EtOAc; 50:50:2.5 to 60:40:2.5) to give the product **3g** (0.81 g, 69% yield): mp 167–169 °C; IR (KBr, cm^{-1}) 3438 (sh), 3390, 2966, 2846, 1687, 1670, 1572, 1504, 1451, 1392, 1364, 1293, 1253, 1227, 1105, 948, 810; ^1H NMR ($CDCl_3$) δ 0.73 (s, 3 H, 18-CH_3), 0.96–1.121 (m, 4 H), 1.24 (s, 3 H, 19-CH_3), 1.29 (dd, J = 4.8, 4.9 Hz, 1 H), 1.35 (s, 9 H, 3 CH_3), 1.44 (ddd, J = 3.7, 13.0, 12.9 Hz, 1 H), 1.54–1.62 (m, 3 H), 1.66–1.80 (m, 3 H), 1.89–2.07 (m, 4 H), 2.17 (dd, J = 10.4, 10.7 Hz, 1 H), 2.26 (ddd, J = 5.2, 14.4, 14.5 Hz, 1 H), 2.56 (ddd, J = 4.6, 14.8, 14.8 Hz, 1 H), 2.62 (ddd, J = 3.9, 5.3, 11.1 Hz, 1 H), 3.27 (dt, J = 3.3, 3.6, 14.5 Hz 1 H, 6α-H),[17c] 5.08 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$) δ 190.6 171.6, 167.8, 121.9, 57.5, 55.5, 54.0, 51.1, 43.5, 42.5, 38.4, 35.3, 34.6, 34.0, 32.8, 31.2, 29.1 (3C), 24.3, 23.3, 21.2, 17.9, 13.1; EI-MS m/s (relative intensity) 451 (M^+ + 2, 67), 449 (M^+, 66), 436 (7), 434 (8), 395 (7), 397 (7), 370 (100), 297 (22), 269 (32), 256 (11), 173 (23), 147 (32), 128 (37), 91 (47), 81 (35), 79 (35), 72 (53), HRMS calcd for $C_{24}H_{36}O_2N_1Br_1$ 449.1930, found 449.1905. Anal. ($C_{24}H_{36}O_2N_1Br_1$) C, H, N, Br.

Evaluation of the Inhibition of Human 5α-Reductase (Types I and II). The measurements of *in vitro* inhibitory activity of compounds **3d–g** on human 5α-reductase (types I and II) were carried out according to the following procedures.

Type I 5α-Reductase.[9b] A total of 293 cells (ATCC CRL 1573) were transfected with the human type 1 5α-reductase cDNA and were used as the source of type 1 5α-reductase. After the transfection, cells were homogenized for the *in vitro* assay. Compounds to be tested were dissolved in ethanol and diluted with 50 mM Tris-HCl buffer containing 20% glycerol and 1 mM EDTA at pH 7.5. Inhibitors were first screened at two concentrations for 5α-reductase inhibitory activity: 1 and 0.1 μM. Compounds showing 50% or more inhibition at the 1 μM concentration were subsequently tested at 12 concentrations ranging from 0.1 to 1000 nM for the measurement of the IC_{50} value. The indicated compound, 1 nM [^3H]androstenedione, 500 μM NADPH, and the cell homogenate were added to the sample tubes to a final volume of 1 mL. Following the 60-min incubation at 37 °C, the media were extracted twice with ether after the addition of 25 μg each of nonradioactive steroid cariers (androstenedione and androstanedione). Steroids were separated by TLC, and the radioactivity was counted. Results are expressed as the amount of androstanedione produced as a percentage of control values.

Type II 5α-Reductase.[9a,b] SW-13 cells (ATCC HTB81) were transfected with the human 5α-reductase type II cDNA[9c] and were used as the source of type II 5α-reductase. After transfection, cells were homogenized for the *in vitro* assay. Compounds to be tested were dissolved in ethanol and diluted with 50 mM Tris-HCl buffer containing 20% glycerol and 1 mM EDTA at pH 7.5. Inhibitors were first screened at two concentrations for 5α-reductase inhibitory activity: 1 and 0.1 μM. Compounds showing 50% or more inhibition at the 1 μM concentration were subsequently tested at 12 concentration ranging from 0.1 to 1000 nM for the measurement of the IC_{50} value. The indicated compound, 5 nM [^3H]androstenedione, 500 μM NADPH, and the cell homogenate were added to the sample tubes to a final volume of 1 mL. Following a 60-min incubation at 37 °C, the media were extracted twice with ether after the addition of 25 μg each of nonradioactive steroid carriers (androstanedione and androstenedione). Steroid were separated by TLC, and the radioactivity was counted. Results are expressed as the amount of androstanedione produced as a percentage of control values.

Inhibition of the Proliferation of Androgen-Sensitive Shionogi Cells. An androgen-sensitive cell line (clone SEM-107) derived from Shionogi mouse mammary carcinoma cells[21] was used at passage 23. Cells were routinely grown as described previously.[22] For the measurement of cell growth and sensitivity to anti-steroids, cells were plated at a density of 17400 cells/mL in minimal essential medium (MEM) supplemented with 2% dextran-coated charcoal-treated fetal calf serum, 1% nonessential amino acids, 10 IU/mL penicillin and 50 μg/mL streptomycin. Steroids and anti-steroids were dissolved in ethanol, and stock solutions were prepared to yield a final ethanol concentration of less than 0.01% in the culture medium. Twenty-four hours after plating, medium was changed and the indicated concentrations of anti-steroids and DHT were added to triplicate dishes. Cells were grown for 13 days with medium changes every 3–4 days. Cells were than fixed in methanol, and their number was assessed by measurement of DNA content by a modification[23] of the method of Fiszer-Szafarz.[24] Dose–response curves and IC_{50} values were calculated using a weighted iterative nonlinear least squares regression.[25] Results are presented as means ± SEM of triplicate measurements.

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