Eur J Med Chem (1996) 31, 675–681
© Elsevier, Paris

Synthesis and testosterone 5α-reductase inhibitory activity of 11-substituted 4-aza-5α-androstane compounds

K Ishibashi[1], H Kurata[1], T Hamada[2], H Horikoshi[2], K Kojima[1]*

[1]*Medicinal Chemistry Research Laboratories, Sankyo Co Ltd;*
[2]*Pharmacology and Molecular Biology Research Laboratories, Sankyo Co Ltd, 1-2-58 Hiromachi, Sinagawa-ku, Tokyo, 140, Japan*

(Received 16 November 1995; accepted 11 March 1996)

Summary — 11α-Acetoxy-, 11α-hydroxy-, 11β-hydroxy-, and 11-oxo-4-aza-5α-androstane compounds with an *N*-diphenylmethylcarbamoyl moiety at the C-17 position were synthesized and their inhibitory activities against rat and human testosterone 5α-reductase were tested. Introduction of the 11α-acetoxy, 11α-hydroxy, 11β-hydroxy and 11-oxo groups into 4-aza-5α-androstane compounds reduced the inhibitory activity against rat and human 5α-reductase. The 11α-acetoxy-4-aza-5α-androstane compound in particular lost almost all its activity. However, several compounds with an 11β-hydroxy or 11-oxo group showed inhibitory activities comparable to MK-906. The 4-methyl-11β-hydroxy-4-aza-5α-androstane derivative showed the most potent inhibitory activity against rat and human enzyme, and was more active than MK-906.

testosterone 5α-reductase inhibitor/ synthesis/ 4-aza-5α-androstane/ steroid/ prostatic hypertrophy

Introduction

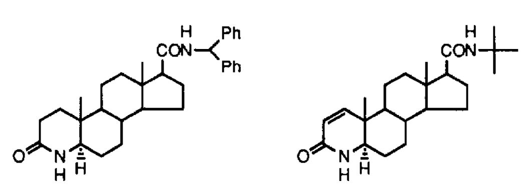
Benign prostatic hypertrophy is known to be caused by excessive accumulation of dihydrotestosterone, which is formed from testosterone by testosterone 5α-reductase. In a previous paper we reported the synthesis and biological activities of new testosterone 5α-reductase inhibitors, 4-aza-5α-androstane compounds modified in the B-ring or C-17 carbamoyl group [1]. In that study, we found that 4-aza-5α-androstane compounds with two aromatic moieties in the C-17 carbamoyl group showed potent inhibitory activities against rat and human 5α-reductase. In the course of our studies on steroidal 5α-reductase inhibitors we became interested in introducing a hydroxy function onto the C-11 position of 4-aza-5α-androstane **1** (fig 1) with an *N*-diphenylmethylcarbamoyl group at the C-17 position. The importance of the 11β-hydoxy group for steroidal activity is well established [2]. In this paper, we describe a new chemical synthesis of 11-oxygenated 4-aza-5α-androstane derivatives including 11β-hydroxy-4-aza-5α-androstane compound **12** and their 5α-reductase inhibitory activities [3]. The structure–activity relationships are also discussed.

*Present address: Neuroscience Research Laboratories, Sankyo Co Ltd.

Chemistry

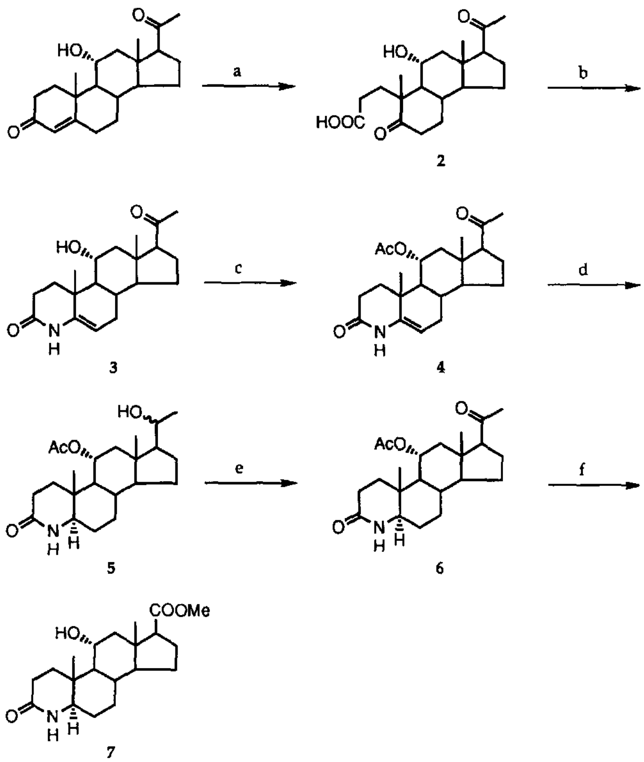
First, a key intermediate, 11α-acetoxy-4-aza-5α-pregnane-3,20-dione **6**, was synthesized from 11α-hydroxyprogesterone according to a method similar to that described by Rasmusson et al (scheme 1) [4, 5]. Oxidative cleavage of the A ring of 11α-hydroxyprogesterone gave a carboxylic acid **2** (86% yield). Heating of **2** with ammonia, followed by acetylation with acetic anhydride afforded 3-oxo-4-aza-5-ene derivative **4** (53% yield). Hydrogenation of **4** in the presence of a platinum catalyst and then Jones oxidation gave key intermediate **6** (58% yield).

Next, the crucial transformation of the C-17 acetyl group of **6** to a methoxy carbonyl moiety was per-



1   MK-906

Fig 1. Compounds **1** and MK-906.



Scheme 1. (a) $KMnO_4$, $NaIO_4$, Na_2CO_3, *t*BuOH-H_2O, reflux; (b) liquid NH_3, ethylene glycol, 0→170 °C; (c) Ac_2O, pyridine, rt; (d) H_2/Pt, AcOH, 60 °C; (e) Jones reagent, acetone, rt; (f) 1.1 eq I_2, pyridine, 50→110 °C then MeONa, MeOH, reflux.

formed using a modified method to that described by Rasmusson et al (scheme 1) [4]. Treatment of **6** with 1.1 equiv of I_2 in pyridine while the reaction temperature was raised gradually from 50 to 110 °C over 10.5 h, was followed by treatment with NaOMe to give **7** in 35% yield.

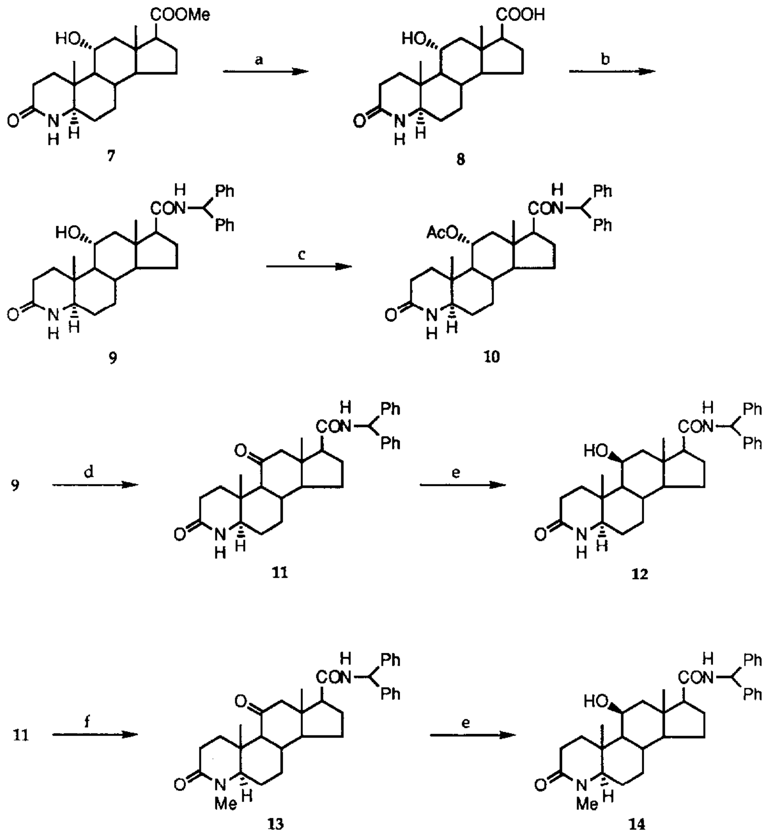
Finally, ester **7** was led to the desired products by the following reactions. Hydrolysis of **7** afforded carboxylic acid **8** (61% yield), which was converted to 11-oxygenated derivatives **9**–**14** (scheme 2). Condensation reaction of **8** with diphenylmethylamine and diethyl phosphorocyanidate [1] afforded the 11α-hydroxy compound **9** (92% yield). Compound **9** was oxidized to afford the 11-oxo compound **11** (84% yield). Reduction of **11** with $NaBH_4$ selectively gave the 11β-hydroxy compound **12** (75% yield). This selectivity due to steric hindrance on the upper side by the 18- and 19-methyl groups is well known in steroid chemistry. Acetylation of the 11α-hydroxy compound **9** gave the 11α-acetoxy compound **10** (84% yield). *N*-Methylation of **11** gave the 4-methyl-11-oxo compound **13** (61% yield). Compound **13** was reduced to afford the 4-methyl-11β-hydroxy compound **14** (96% yield).

The synthesis of Δ^1-11-oxo derivatives **18** and **19** is shown in scheme 3. Dehydrogenation of **7** with bistrimethylsilyltrifluoroacetamide and DDQ gave the Δ^1-ester **15** (67% yield) [6]. Oxidation of **15** with a Jones reagent followed by hydrolysis afforded the Δ^1-11-oxo carboxylic acid **17** (71% yield). Amidation of **17** with diphenylmethylamine and diethyl phosphorocyanidate gave the Δ^1-11-oxo derivative **18** (68% yield). *N*-Methylation of **18** with potassium *t*-butoxide and iodomethane gave the Δ^1-4-methyl-11- oxo compound **19** (87% yield).

Results and discussion

The testosterone 5α-reductase inhibitory activities of the synthesized compounds are listed in table I. The 11α-acetoxy derivative **10**, 11-oxo derivative **11**, 11α-hydroxy derivative **9** and 11β-hydroxy derivative **12** showed relatively low inhibitory rates in rat 5α-reductase tests compared with the original 11-unsubstituted compound **1** [1]. The activities in descending order are **12**, **9**, **11** and **10**. The 11α-acetoxy compound **10** had almost no activity. The 11β-hydroxy compound **12** showed potent inhibitory activity comparable to MK906 (finasteride, a drug for benign prostatic hypertrophy) [1, 5] against rat and human 5α-reductase. Further modification of **11** and **12** afforded 4-methyl compounds **13** and **14**, which showed increased inhibitory activities more active than MK-906 against rat and human enzyme. Introduction of a double bond at the C-1 position in **11** and **13** gave derivatives **18** and **19**. Compounds **18** and **19** showed more potent inhibitory activities than MK-906 against rat enzyme. However, compound **18** was very weakly active against human 5α-reductase. In contrast, compound **19** retained its inhibitory activity against human enzyme.

In conclusion, 11α-acetoxy-, 11α-hydroxy-, 11β-hydroxy-, and 11-oxo-4-aza-5α-androstane compounds with an *N*-diphenylmethylcarbamoyl moiety at the C-17 position were synthesized and their inhibitory activities against rat and human testosterone 5α-reductase tested. Introduction of 11α-acetoxy, 11α-hydroxy, 11β-hydroxy, and 11-oxo groups into compound **1** reduced the inhibitory activity against rat and human 5α-reductase. The 11α-acetoxy compound **10** lost almost all its activity. However, compounds **12**–**14**, **18**, and **19** showed potent inhibitory activities comparable to MK-906. 4-Methyl-11β-hydroxy-4-aza-5α-androstane derivative **14** showed the most



Scheme 2. (a) KOH, $MeOH/H_2O$, reflux; (b) Ph_2CHNH_2, DEPC, Et_3N, CH_2Cl_2, rt; (c) Ac_2O, pyridine, rt; (d) Jones reagent, acetone, rt; (e) $NaBH_4$, $EtOH/H_2O$, rt; (g) *t*-BuOK, MeI, *t*-BuOH, rt.

potent inhibitory activity against rat and human enzyme; it was more active than MK-906 and as active as compound **1**.

Experimental protocols

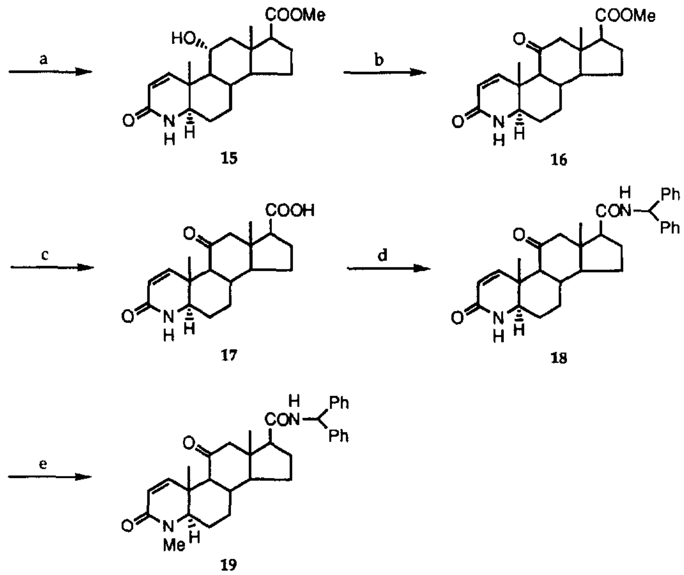
Chemistry

Melting points were determined with a Yanagimoto micro melting point apparatus MP-500D and are uncorrected. ^{1}H-NMR spectra were recorded on a Jeol JNM-GX270 or Jeol JNM-EX270 spectrometer (270 MHz). Chemical shifts are given in ppm (δ) using tetramethylsilane as an internal standard. IR spectra were measured with a Nic.5SXC, Jasco A-302, Jasco FTIR8300, Jasco FTIR8900, or Jasco A-102 spectrometer. Mass spectra were measured with a Jeol JMS-D300, JMS-AX505H, or JMS-AX505W spectrometer. Thin-layer chromatography (TLC) was run on silica gel-coated plates (E Merck, Silica gel 60 F_{254} precoated) of thickness 0.25 mm. Preparative thin-layer chromatography was carried out on similar plates with a thickness of 2 mm. Silica gel 60 (E Merck, 70–230 mesh) was used for column chromatography.

5,20-Dioxo-11α-hydroxy-A-nor-3,5-secopregnan-3-oic acid **2**

A solution of 11α-hydroxy-3,20-dioxopregn-4-ene (10.5 g, 31.8 mmol) and Na_2CO_3 (4.40 g, 41.5 mmol) in a mixture of 2-methyl-2-propanol (150 mL) and water (20 mL) was brought to reflux. A solution of $KMnO_4$ (0.34 g, 2.15 mmol) and $NaIO_4$ (41.0 g, 192 mmol) in hot water (200 mL) was added dropwise over 30 min and the mixture refluxed for 1 h. The reaction mixture was cooled to room temperature and the formed solid was filtered and washed with water. After evaporation of 2-methyl-2-propanol from the filtrate, the remaining water solution was acidified with 1 N HCl. The precipitate was filtered, washed with water, and dried under air to give **2** (9.54 g, 86%), mp 101–104 °C. ^{1}H-NMR (pyridine-d_5) δ: 0.73 (3H, s), 1.12–1.30 (3H, m), 1.36 (3H, s), 1.52–1.82 (6H, m), 2.09 (3H, s), 2.37 (1H, m), 2.45–2.63 (4H, m), 2.77 (1H, m), 2.92–3.05 (2H, m), 3.12 (1H, m), 4.27 (1H, dt, J = 5, 10 Hz). IR (KBr): 3483, 2976, 2951, 2889, 1726, 1688 cm^{-1}. HR-MS *m/z*: calc for $C_{20}H_{30}O_5$ (M^+): 350.2094; found: 350.2100.

7



Scheme 3. (a) DDQ, *N,O*-bistrimethylsilyltrifluoracetamide, dioxane, rt to reflux; (b) Jones reagent, acetone, rt; (c) KOH, dioxane/H_2O, reflux; (d) Ph_2CHNH_2, DEPC; Et_3N, CH_2Cl_2, rt; (e) *t*-BuOK, MeI, *t*-BuOH, rt.

3,20-Dioxo-11α-hydroxy-4-azapregn-5-ene 3

Liquid NH_3 (20.0 mL) was added to a suspension of **2** (9.50 g, 27.1 mmol) in ethylene glycol (65 mL) at 0 °C. The whole was gradually heated to 170 °C over 1 h and stirred for 45 min at the same temperature. The reaction mixture was cooled to room temperature, acidified with 1 N HCl and extracted with CH_2Cl_2. The combined organic layer was washed with brine. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on a silica-gel column. The fraction eluted with CH_2Cl_2/acetone (3:1 to 2:3) was evaporated and the residue was triturated with Et_2O to give **3** (7.16 g, 80%), mp 189–193 °C. ^{1}H-NMR (pyridine-d_5) δ: 0.72 (3H, s), 1.08–1.84 (9H, m), 1.35 (3H, m), 1.98–2.15 (1H, m), 2.09 (3H, s), 2.36 (1H, m), 2.52–2.82 (4H, m), 3.41 (1H, m), 4.28 (1H, m), 5.23 (1H, dd, *J* = 2, 5 Hz), 5.84 (1H, d, *J* = 6 Hz), 10.31 (1H, s). IR (KBr): 3400, 2941, 2920, 1703, 1690, 1659 cm^{-1}. HR-MS *m/z*: calc for $C_{20}H_{29}NO_3$ (M^+): 331.2148; found: 331.2131.

11α-Acetoxy-3,20-dioxo-4-azapregn-5-ene 4

A solution of **3** (2.28 g, 6.88 mmol) in a mixture of Ac_2O (10.0 mL) and pyridine (20.0 mL) was stirred at room temperature for 3 h. After addition of ice, the reaction mixture was extracted with CH_2Cl_2. The combined organic layer was washed with 1 N HCl and brine. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on a silica-gel column. The fraction eluted with CH_2Cl_2/acetone (4:1 to 2:3) was evaporated and the residue was triturated with Et_2O to give **4** (1.70 g, 66%), mp 199–204 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.74 (3H, s), 1.15–2.63 (16H, m), 1.18 (3H, s), 2.06 (3H, s), 2.12 (3H, s), 4.90 (1H, dd, *J* = 2, 5 Hz), 5.29 (1H, dt, *J* = 5, 10 Hz), 7.32 (1H, br). IR (KBr): 3182, 3065, 2980, 2922, 2886, 1735, 1703, 1664 cm^{-1}. HR-MS *m/z*: calc for $C_{22}H_{31}NO_4$ (M^+): 373.2253; found: 373.2244.

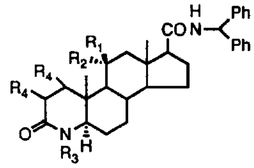
11α-Acetoxy-20-hydroxy-3-oxo-4-aza-5α-pregnane 5

A suspension of **4** (904 mg, 2.42 mmol) and platinum dioxide (300 mg) in acetic acid (20 mL) was stirred under hydrogen atmosphere at 60 °C for 2 h. The platinum catalyst was filtered off and washed with MeOH. The filtrate was evaporated and the residue was chromatographed on a silica-gel column. The fraction eluted with CH_2Cl_2/acetone (3:2 to 3:7) was evaporated to give **5** (697 mg, 76%) as a foam. ^{1}H-NMR ($CDCl_3$) δ: 0.70 and 0.82 (total 3H, each s), 0.99 and 1.00 (total 3H, each s), 1.07–1.88 (18H, m), 1.99 and 2.03 (total 3H, each s), 2.31–2.58 (3H, m), 3.13 and 3.17 (total 1H, each m), 3.71 (1H, m), 5.19 (1H, m), 5.95 and 6.09 (total 1H, each br). IR (KBr): 3210, 2967, 2943, 2871, 1731, 1707, 1666 cm^{-1}. HR-MS *m/z*: calc for $C_{22}H_{36}NO_4$ ($(M+1)^+$): 378.2644; found: 378.2667.

11α-Acetoxy-3,20-dioxo-4-aza-5α-pregnane 6

Jones reagent (4.00 mL) was added to a solution of **5** (4.60 g, 12.2 mmol) in acetone (50 mL) at room temperature and the mixture was stirred for 30 min. Additional Jones reagent (2.00 mL) was added and the mixture stirred for a further 1 h. After addition of 2-propanol, the reaction mixture was diluted with brine and extracted with CH_2Cl_2. The combined organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was chromatographed on a silica-gel column. The fraction eluted with CH_2Cl_2/acetone (3:1 to 1:1) was evaporated and the residue was triturated with Et_2O to give **6** (3.50 g, 76%), mp 178–183 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.70 (3H, s), 0.82–2.65 (18H, m), 0.99 (3H, s), 2.04 (3H, s), 2.11 (3H, s), 3.14 (1H, dd, *J* = 4, 12 Hz), 5.19 (1H, dt, *J* = 5, 11 Hz), 6.19 (1H, br). IR ($CHCl_3$): 2960, 2870, 1725, 1700, 1655 cm^{-1}. HR-MS *m/z*: calc for $C_{22}H_{33}NO_4$ (M^+): 375.2409; found: 375.2385.

Table I. Inhibitory activities of 11-substituted 4-aza-5α-androstane compounds.



Compound	R_1	R_2	R_3	R_4	*Rat 5α-reductase % inhibition at 10^{-8} M*	*Human 5α-reductase relative inhibitory potency*[a] *to MK-906 (MK-906 = 1)*
9	H	OH	H	H	37	<0.1
11	O		H	H	17	0.30
12	OH	H	H	H	50	0.55
10	H	OAc	H	H	6.8	<0.1
13	O		Me	H	74	1.6
14	OH	H	Me	H	74	2.9
18	O		H	–[b]	39	<0.1
19	O		Me	–[b]	33	1.0
1	H	H	H	H	89	5.6
MK-906					28	1.0

[a]The relative inhibitory potency was calculated from the inhibition curve of MK-906 (0.25×10^{-8} M to 8×10^{-8} M) and the % inhibition of compounds at 10^{-7} M or 10^{-8} M. The % inhibition of MK-906 at 10^{-8} M varied from 31 to 65% for human 5α-reductase from BPH patients. [b]– means a single bond combined with both R_4.

Methyl 11α-hydroxy-3-oxo-4-aza-5α-androstane-17β-carboxylate ***7***

A solution of a mixture of **6** (10.2 g, 27.2 mmol) and I_2 (7.90 g, 31.1 mmol) in pyridine (200 mL) was heated from 50 to 110 °C over 10.5 h and stirred at the same temperature for 6 h under N_2 atmosphere. The reaction mixture was cooled to room temperature and absolute MeOH (250 mL) and 28% MeONa in MeOH solution (110 ml, 540 mmol) were added. The mixture was stirred at 65 °C for 1 h. 28% MeONa in MeOH solution (100 mL, 490 mmol) was added and the mixture stirred for an additional 1 h. The reaction mixture was neutralized with 1 N HCl at 0 °C and the MeOH evaporated. The residue was diluted with 1 N HCl and extracted with $CHCl_3$. The combined organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on a silica-gel column. The fraction eluted with 2-propanol/toluene/acetone (3:37:60 to 5:35:60) was evaporated and the residue was triturated with Et_2O to give **7** (3.34 g, 35%), mp 248–251 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.69 (3H, s), 0.88–2.48 (17H, m), 1.02 (3H, s), 2.68 (1H, m), 3.14 (1H, dd, J = 4, 12 Hz), 3.69 (3H, s), 3.96 (1H, dt, J = 5, 10 Hz), 6.19 (1H, br). IR (KBr): 3396, 2940, 2876, 1733, 1657 cm^{-1}. HR-MS *m/z*: calc for $C_{20}H_{31}NO_4$ (M^+): 349.2253; found: 349.2239.

11α-Hydroxy-3-oxo-4-aza-5α-androstane-17β-carboxylic acid ***8***

A solution of **7** (102 mg, 0.29 mmol) in a mixture of MeOH (2.0 mL) and 10% KOH aqueous solution (1.0 mL) was refluxed under N_2 atmosphere for 4 h. After evaporation of MeOH, the residue was acidified with 1 N HCl. The precipitate was filtered and washed with water to give **8** (60 mg, 61%), mp 245 °C (dec). ^{1}H-NMR (pyridine-d_5) δ: 0.85–2.02 (12H, m), 0.97 (3H, s), 1.11 (3H, s), 2.40–2.70 (4H, m), 2.97 (1H, dd, J = 4, 12 Hz), 3.13–3.29 (2H, m), 4.21 (1H, br), 5.56 (1H, br). IR (KBr): 3420, 3269, 3177, 2966, 2935, 1699, 1632 cm^{-1}. HR-MS *m/z*: calc for $C_{19}H_{29}NO_4$ (M^+): 335.2096; found: 335.2107.

N-Diphenylmethyl-11α-hydroxy-3-oxo-4-aza-5α-androstan-17β-carboxamide ***9***

A solution of **8** (40 mg, 0.12 mmol), diphenylmethylamine (80 μL, 0.46 mmol), diethyl phosphorocyanidate (70 μL, 0.46 mmol), and triethylamine (55 μL, 0.40 mmol) in CH_2Cl_2 (2.0 mL) was stirred at room temperature for 24 h. The reaction mixture was diluted with 1 N HCl and extracted with CH_2Cl_2. The combined organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on a silica-gel column. The fraction eluted with 2-propanol/CH_2Cl_2/acetone (3:37:60 to 1:3:6) was evaporated and the residue was crystallized from EtOAc to give **9** (55 mg, 92%), mp 160–163 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.70 (3H, s), 0.84–1.95 (14H, m), 1.01 (3H, s), 2.15–2.30 (2H, m), 2.44 (1H, m), 2.68 (1H, m), 3.15 (1H, dd, J = 5, 12 Hz), 3.98 (1H, dt, J = 4, 10 Hz), 5.88 (1H, d, J = 8 Hz), 6.28 (1H, d, J = 8 Hz), 6.48 (1H, br), 7.19–7.38 (10H, m). IR (KBr): 3304, 2931, 2873, 1656 cm^{-1}. HR-MS *m/z*: calc for $C_{32}H_{40}N_2O_3$ (M^+): 500.3039; found: 500.3068. Anal calc for $C_{32}H_{40}N_2O_3$·3/4H_2O: C, 74.75; H, 8.14; N, 5.45; found: C, 74.47; H, 7.91; N, 5.31.

N-Diphenylmethyl-11α-acetoxy-3-oxo-4-aza-5α-androstane-17β-carboxamide **10**

A solution of **9** (52 mg, 0.10 mmol) in a mixture of pyridine (1.0 mL) and Ac_2O (0.5 mL) was stirred at room temperature for 12 h. After ice was added to the reaction mixture, the solution was diluted with 1 N HCl and extracted with CH_2Cl_2. The combined organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on a silica-gel column. The fraction eluted with CH_2Cl_2/acetone (7:3 to 4:6) was evaporated and the residue was crystallized from a mixture of CH_2Cl_2/EtOAc to give **10** (47 mg, 84%), mp 258–261 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.72 (3H, s), 0.96–1.90 (14H, m), 0.98 (3H, s), 2.00 (3H, s), 2.15–2.43 (4H, m), 3.13 (1H, dd, *J* = 4, 12 Hz), 5.16 (1H, dt, *J* = 5, 10 Hz), 5.64 (1H, br), 5.84 (1H, d, *J* = 8 Hz), 6.27 (1H, d, *J* = 8 Hz), 7.19–7.39 (10H, m). IR (KBr): 3306, 2969, 2936, 1731, 1664 cm^{-1}. HR-MS *m/z*: calc for $C_{34}H_{42}N_2O_4$ (M^+): 542.3144; found: 542.3167. Anal calc for $C_{34}H_{42}N_2O_4$•2/3H_2O: C, 73.62; H, 7.87; N, 5.05. Found: C, 73.57; H, 8.00; N, 4.97.

N-Diphenylmethyl-3,11-dioxo-4-aza-5α-androstane-17β-carboxamide **11**

Jones reagent (0.20 mL) was added to a solution of **9** (208 mg, 0.42 mmol) in acetone (15 mL) at room temperature and the mixture was stirred for 1 h. After addition of 2-propanol, the reaction mixture was made basic with 1 N NaOH and diluted with CH_2Cl_2. The solution was passed through celite and the filtrate extracted with CH_2Cl_2. The combined organic layer was washed with brine. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on a silica-gel column. The fraction eluted with 2-propanol/CH_2Cl_2/acetone (0:1:1 to 2:38:60) was evaporated and the residue crystallized from a mixture of CH_2Cl_2/EtOAc to give **11** (171 mg, 83%), mp 279 °C (dec). ^{1}H-NMR ($CDCl_3$) δ: 0.65 (3H, s), 1.09 (3H, s), 1.17–2.03 (10H, m), 2.17–2.65 (8H, m), 3.02 (1H, dd, *J* = 4, 11 Hz), 5.72 (1H, br), 5.85 (1H, d, *J* = 8 Hz), 6.25 (1H, d, *J* = 8 Hz), 7.19–7.39 (10H, m). IR (KBr): 3305, 2943, 2874, 1706, 1657 cm^{-1}. HR-MS *m/z*: calc for $C_{32}H_{38}N_2O_3$ (M^+): 498.2883; found: 498.2876. Anal calc for $C_{32}H_{38}N_2O_3$•3/4H_2O: C, 75.04; H, 7.77; N, 5.47. Found: C, 74.93; H, 7.86; N, 5.43.

N-Diphenylmethyl-11β-hydroxy-3-oxo-4-aza-5α-androstane-17β-carboxamide **12**

$NaBH_4$ (77 mg, 2.00 mmol) was added to a solution of **11** (102 mg, 0.20 mmol) in a mixture of EtOH (6.0 mL) and water (0.50 mL) and the mixture was stirred at room temperature for 24 h. The reaction mixture was cooled to 0 °C and AcOH was added. The solution was diluted with water and extracted with CH_2Cl_2. The combined organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on a silica-gel column. The fraction eluted with CH_2Cl_2/acetone (1:1 to 4:6) was evaporated and the residue was triturated with Et_2O to give **12** (76 mg, 75%), mp 153–156 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.77–2.22 (16H, m), 0.91 (3H, s), 1.12 (3H, s), 2.40–2.49 (2H, m), 3.03 (1H, dd, *J* = 4, 11 Hz), 4.30 (1H, d, *J* = 2 Hz), 5.58 (1H, br), 5.89 (1H, d, *J* = 8 Hz), 6.28 (1H, d, *J* = 8 Hz), 7.21–7.38 (10H, m). IR (KBr): 3309, 2931, 2871, 1652 cm^{-1}. HR-MS *m/z*: calc for $C_{32}H_{40}N_2O_3$ (M^+): 500.3039; found: 500.3063. Anal calc for $C_{32}H_{40}N_2O_3$•3/4H_2O: C, 74.75; H, 8.14; N, 5.45. Found: C, 74.79; H, 8.34; N, 5.21.

N-Diphenylmethyl-3,11-dioxo-4-methyl-4-aza-5α-androstan-17β-carboxamide **13**

t-BuOK (34 mg, 0.30 mmol) was added to a solution of **11** (60 mg, 0.12 mol) in 2-methyl-2-propanol (4.0 mL) and the mixture was stirred at room temperature for 1 h under N_2 atmosphere. Methyl iodide (0.12 mL, 1.93 mmol) was then added to the reaction mixture and stirring continued for 6 h. The reaction mixture was diluted with 1 N HCl and extracted with CH_2Cl_2. The combined organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by preparative thin-layer chromatography developed with CH_2Cl_2/acetone (1:1). A part showing an R_f-value of 0.5 was extracted with acetone. After evaporation of the acetone, the residue was crystallized from a mixture of CH_2Cl_2/EtOAc to give **13** (38 mg, 61%), mp 142–145 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.65 (3H, s), 1.07–2.65 (18H, m), 1.08 (3H, s), 2.92 (3H, s), 3.00 (1H, dd, *J* = 3, 12 Hz), 5.84 (1H, d, *J* = 8 Hz), 6.25 (1H, d, *J* = 8 Hz), 7.20–7.39 (10H, m). IR (KBr): 3308, 2959, 2876, 1706, 1645 cm^{-1}. HR-MS *m/z*: calc for $C_{33}H_{40}N_2O_3$ (M^+): 542.3039; found: 512.3011. Anal calc for $C_{33}H_{40}N_2O_3$•3/4H_2O: C, 75.32; H, 7.95; N, 5.32. Found: C, 75.40; H, 8.21; N, 5.30.

N-Diphenylmethyl-11β-hydroxy-4-methyl-3-oxo-4-aza-5α-androstane-17β-carboxamide **14**

Compound **14** (cryst, CH_2Cl_2/EtOAc) was synthesized in 96% yield from **13** using a method similar to that described for the preparation of **12**. Mp 164–167 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.78–2.23 (16H, m), 0.91 (3H, s), 1.10 (3H, s), 2.50–2.58 (2H, m), 2.94 (3H, s), 2.98 (1H, dd, *J* = 3, 12 Hz), 4.31 (1H, d, *J* = 3 Hz), 5.87 (1H, d, *J* = 8 Hz), 6.29 (1H, d, *J* = 8 Hz), 7.20–7.38 (10H, m). IR (KBr): 3441, 3320, 2943, 2874, 1623 cm^{-1}. HR-MS *m/z*: calc for $C_{33}H_{42}N_2O_3$ (M^+): 514.3196; found: 514.3176. Anal calc for $C_{33}H_{42}N_2O_3$•1/2H_2O: C, 75.68; H, 8.28; N, 5.35. Found: C, 75.83; H, 8.04; N, 5.26.

Methyl 11α-hydroxy-3-oxo-4-aza-5α-androst-1-ene-17β-carboxylate **15**

Dichlorodicyanohydroquinone (240 mg, 1.06 mmol) and *N,O*-bistrimethylsilyltrifluoroacetamide (1.45 mL, 5.50 mmol) were added to a solution of **7** (316 mg, 0.90 mmol) in dry dioxane (10 mL). The mixture was stirred at room temperature for 5 h under N_2 atmosphere and refluxed for 18 h. The reaction mixture was cooled to room temperature, and then CH_2Cl_2 (8.0 mL) and 1% aqueous $NaHSO_3$ solution (2.0 mL) were added. The precipitated solid was filtered off and washed with CH_2Cl_2. The filtrate was diluted with 10% aqueous HCl solution and extracted with $CHCl_3$. The combined organic layer was washed with 1 N HCl and then brine. The organic layer was dried over Na_2SO_4 and evaporated. The residue was chromatographed on a silica-gel column. The fraction eluted with 2-propanol/CH_2Cl_2/acetone (0:1:1 to 1:39:60) was evaporated and the residue was triturated with Et_2O to give **15** (210 mg, 67%), mp 260 °C (dec). ^{1}H-NMR ($CDCl_3$) δ: 0.70 (3H, s), 1.05–1.94 (H, m), 2.16 (1H, m), 2.31 (1H, dd, *J* = 4, 12 Hz), 2.41 (1H, t, *J* = 10 Hz), 3.38 (1H, t, *J* = 8 Hz), 3.69 (3H, s), 4.05 (1H, dt, *J* = 5, 10 Hz), 5.34 (1H, br), 5.74 (1H, dd, *J* = 2, 10 Hz), 7.95 (1H, d, *J* = 10 Hz). IR ($CHCl_3$): 3405, 3340, 2970, 2935, 1718, 1660 cm^{-1}. HR-MS *m/z*: calc for $C_{20}H_{29}NO_4$ (M^+): 347.2097; found: 347.2092.

Methyl 3,11-dioxo-4-aza-5α-androst-1-ene-17β-carboxylate **16**

Compound **16** was synthesized in 75% yield from **15** using a method similar to that described for the preparation of **11**. Mp 284–287 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.63 (3H, s), 1.18–2.03 (10H, m), 1.19 (3H, s), 2.18–2.42 (2H, m), 2.57–2.72 (2H, m), 3.30 (1H, dd, *J* = 7, 9 Hz), 3.69 (3H, s), 5.40 (1H, br), 5.80 (1H, d, *J* = 10 Hz), 7.18 (1H, d, *J* = 10 Hz). IR (KBr): 3177, 3102, 2933, 1717, 1703, 1679, 1603 cm^{-1}. HR-MS *m/z*: calc for $C_{20}H_{27}NO_4$ (M^+): 345.1940; found: 345.1944.

3,11-Dioxo-4-aza-5α-androst-1-ene-17β-carboxylic acid ***17***
A solution of **16** (65 mg, 0.19 mmol) in a mixture of dioxane (3.0 mL) and 10% KOH aqueous solution (1.5 mL) was refluxed for 3 h under N_2 atmosphere. After evaporation of the dioxane, the residue was acidified with 1 N HCl. The precipitate was filtered and washed with water to give **17** (58 mg, 94%), mp >300 °C. ^{1}H-NMR ($CDCl_3$ + CD_3OD) δ: 0.69 (3H, s), 1.17–2.77 (14H, m), 1.18 (3H, s), 3.29 (1H, t, J = 8 Hz), 5.77 (1H, d, J = 10 Hz), 7.20 (1H, d, J = 10 Hz). IR (KBr): 3309, 2951, 2867, 2525, 1702, 1648, 1593 cm^{-1}. HR-MS *m/z*: calc for $C_{19}H_{25}NO_4$ (M^+): 331.1783; found: 331.1788.

N-Diphenylmethyl-3,11-dioxo-4-aza-5α-androst-1-ene-17β-carboxamide ***18***
Compound **18** (cryst, CH_2Cl_2/EtOAc) was synthesized in 69% yield from **17** by a similar method to that described for the preparation of **9**. Mp 209–212 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.65 (3H, s), 1.17–2.03 (10H, m), 1.18 (3H, s), 2.18–2.50 (3H, m), 2.61 (1H, d, J = 12 Hz), 3.29 (1H, t, J = 8 Hz), 5.36 (1H, br), 5.79 (1H, d, J = 10 Hz), 5.86 (1H, d, J = 8 Hz), 6.25 (1H, d, J = 8 Hz), 7.16 (1H, d, J = 10 Hz), 7.20–7.39 (10H, m). IR (KBr): 3310, 2940, 1706, 1675, 1600 cm^{-1}. HR-MS *m/z*: calc for $C_{32}H_{36}N_2O_3$ (M^+): 496.2726; found: 496.2743. Anal calc for $C_{32}H_{36}N_2O_3$•5/4H_2O: C, 74.03; H, 7.47; N, 5.40. Found: C, 73.86; H, 7.23; N, 5.47.

N-Diphenylmethyl-3,11-dioxo-4-methyl-4-aza-5α-androst-1-ene-17β-carboxamide ***19***
Compound **19** (cryst, CH_2Cl_2/EtOAc) was synthesized in 87% yield from **18** using a method similar to that described for the preparation of **13**. Mp 262–265 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.65 (3H, s), 1.12–2.08 (10H, m), 1.14 (3H, s), 2.17–2.50 (3H, m), 2.61 (1H, d, J = 12 Hz), 2.95 (3H, s), 3.30 (1H, dd, J = 4, 12 Hz), 5.82 (1H, d, J = 10 Hz), 5.85 (1H, d, J = 7 Hz), 6.25 (1H, d, J = 7 Hz), 6.98 (1H, d, J = 10 Hz), 7.20–7.65 (10H, m). IR (KBr): 3312, 2946, 1706, 1664, 1602 cm^{-1}. HR-MS *m/z*: calc for $C_{33}H_{38}N_2O_3$ (M^+): 510.2883; found: 510.2891. Anal calc for $C_{33}H_{38}N_2O_3$•6/5H_2O: C, 74.46; H, 7.65; N, 5.26. Found: C, 74.43; H, 7.41; N, 4.98.

Biological activity

Preparation of 5α-reductase from human and rat prostates
Human and rat prostates were minced into small pieces. The minced tissue was homogenized in approximately three tissue volumes of buffer A (20 mM potassium phosphate, pH 6.5, containing 0.32 M sucrose, 1 mM dithiothreitol, 50 μM NADPH and 0.001% PMSF), first with a Polytron (Kinematica GmbH) and then with a Teflon/glass homogenizer. The homogenate was centrifuged at 140 000 *g* for 60 min and then the pellets were washed with approximately three tissue volumes of buffer A. The washed pellets were used as the 5α-reductase.

Assay of 5α-reductase inhibitory activity
The reaction solutions contained 1 μM [^{14}C]testosterone, 1 mM dithiothreitol, 40 mM buffer (potassium phosphate, pH 6.5, for the rat enzyme; Tris-citrate, pH 5.5, for the human enzyme), prostatic particulate (0.2–1 mg protein) and 0.5 mM NADPH in a final volume of 0.5 mL. The samples were added to 5 μL DMSO and the control tubes received the same volume of DMSO. The reactions were carried out for 10–30 min and were stopped with 2 mL ethyl acetate containing testosterone, 5α-dihydrotestosterone and androstenedione (10 μg each). After centrifugation at 1000 *g* for 5 min, the ethyl acetate phase (upper) was transferred to a tube and then evaporated under nitrogen to dryness. The steroids were taken up in 30 μL ethyl acetate and the solutions were applied to Whatman LK5DF or LK6DF silica plates, and the plates were developed in ethyl acetate/cyclohexane (1:1) at room temperature. The plates were air-dried, and the chromatography was repeated. The radioactivity profiles were determined by bioimage analyzer (Fuji Film Co, Ltd).

The testosterone 5α-reductase inhibitory activities against human enzyme seem to reflect approximately the inhibition of the type 2 enzyme [7]. On the other hand, isozyme type is uncertain for rat enzyme [8].

References

1 Ishibashi K, Kurata H, Kojima K, Horikoshi H (1994) *Bioorg Med Chem Lett* 4, 729–732

2 Hogg JA (1992) *Steroids* 57, 593–616

3 Ishibashi K, Kojima K, Hamada T, Horikoshi H (1993) Jpn Patent 5-170789; *Chem Abstr* 120, 107465d

4 Rasmusson GH, Reynolds GF, Utne T et al (1984) *J Med Chem* 27, 1690–1701

5 Rasmusson GH, Reynolds GF, Steinberg NG et al (1986) *J Med Chem* 29, 2298–2315

6 Bhattacharya A, DiMichele LM, Dolling UH, Douglas AW, Grabowski EJJ (1988) *J Am Chem Soc* 110, 3318–3319

7 Anderson S, Berman DM, Jenkins EP, Russell DW (1991) *Nature* 354, 159–161

8 Normington K, Russell DW (1992) *J Biol Chem* 267, 19548–19554