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Vinyl Fluoride as a Mimic of the 'Intermediate' Enol Form in the 5α-Reductase Transformation: Synthesis and In Vitro Activity of (*N*-1′,1′-Dimethylethyl)-3-haloandrost-3,5-diene-17β-carboxamides

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Abstract—(*N*-1′,1′-Dimethylethyl)-3-haloandrost-3,5-diene-17β-carboxamides (**9–11**) and the methyl ester **8** were prepared from 3-chloro/bromoandrost-3,5-diene-17β-carboxylic chloride/bromide (**6/7**), which were obtained from pregnenolone. In comparison with finasteride and 4-MA, compounds **8–11** showed very weak inhibitory activity (≤10% inhibition) on human type I 5α-reductase (transfected 293 cells) at 100 and 1000 nM concentrations. Against the type II enzyme, chloro compounds **8** and **9**, and bromo **10** had no effect at 100 nM concentration, however, they were weak inhibitors of the type II (6.0% < inhibition < 30%) at a higher concentration. The best activity (IC_{50} = 480 nM) was observed with the 3-vinyl fluoride analogue **11**.

Introduction

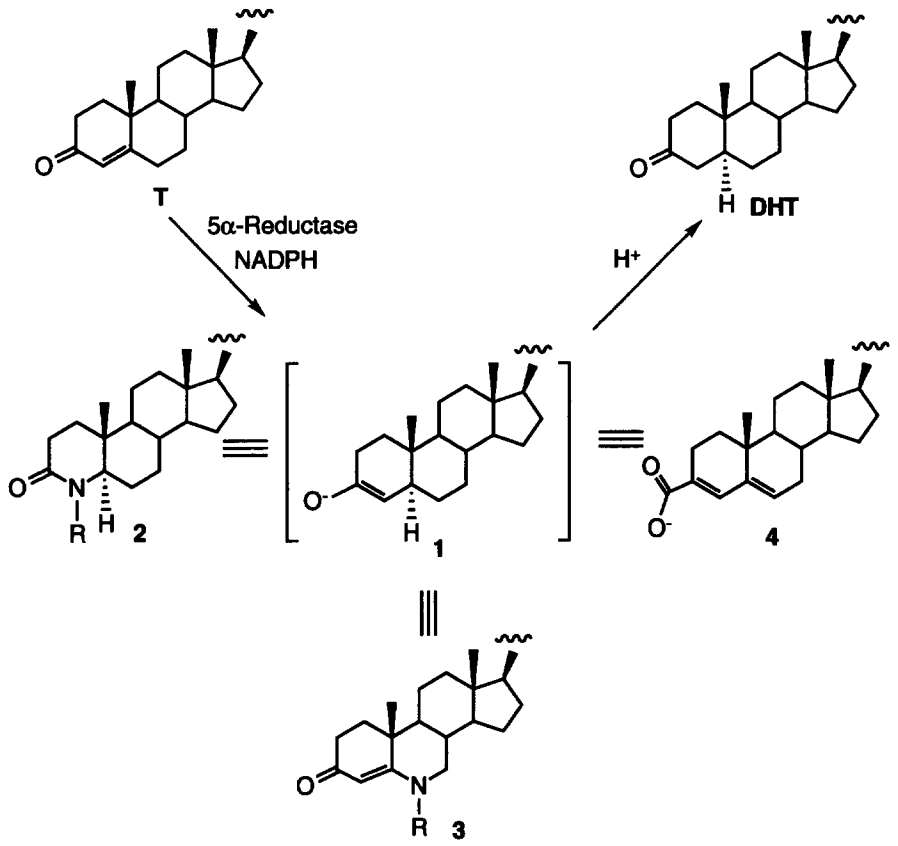
Steroid 5α-reductase irreversibly converts testosterone (T) into the more potent intracellular androgenic 5α-reduced metabolite, dehydrotestosterone (DHT),[1,2] which plays a predominant role in the development of prostate cancer,[3–5] benign prostatic hyperplasia (BPH),[6,7] and other disorders such as acne,[8] female hirsutism[9,10] and male pattern baldness.[11] Two types of human 5α-reductase, chronologically identified as type I[12,13] and type II[14,15] have been isolated and characterized from human prostatic cDNA libraries. Earlier reports suggest that the type I is expressed in skin[11] while type II is responsible for male pseudohypermaphroditism and is the main type expressed in the human prostate.[15] During the catalytic transformation, reduction of the 4,5-double bond of testosterone occurs via the formation of an 'intermediate' 3-enol form **1** to give DHT (Scheme 1). A variety of 5α-reductase inhibitors based on the 3-enol form have been developed. 4-Azasteroids **2**, which mimic the enol form, were prepared and tested as powerful inhibitors of both isozymes.[16,17] Finasteride of this series, which is a selective inhibitor of human type II 5α-reductase, is currently used for the treatment of BPH. 6-Azasteroids (**3**) are another good example of the enol based inhibitors, which inhibit the transformation via the formation of the putative 3,5-diene-3-ol intermediate.[18,19] This class of compounds acts as a dual inhibitor of both 5α-reductases. Vinyl carboxylic acids **4** which also mimic the enol form were reported as potent inhibitors of human type II 5α-reductase.[20,21] Epristeride is a good example of this series.

Vinyl fluoride analogues as pharmacophores of the enol form have been used successfully in the design and synthesis of enzyme inhibitors.[22–24] Because vinyl fluoride is chemically stable, the polarity of the C—F bond is similar to the C—O bond and the van der Waals radius of the fluorine atom is comparable to the oxygen atom (Table 1).[22] Based on available information, we decided to prepare 3-fluorovinyl/halosteroids and test their compatibility as inhibitors, and thus, herein, we describe synthesis and in vitro activity of (*N*-1′,1′-dimethylethyl)-3-fluoro/chloro/bromoandrost-3,5-diene-17β-carboxamides as inhibitors of 5α-reductase.

Results and Discussion

Chemistry

(*N*-1′,1′-Dimethylethyl)-3-haloandrost-3,5-diene-17β-carboxamides (**8–11**) were prepared from the commercially available pregnenolone. Thus, 3-oxo-4-androsten-17β-carboxylic acid (**5**) was prepared following the method of Rasmusson et al.[16,17] Acid **5** was treated with oxalyl chloride/bromide in the presence of oxalic acid in dry benzene to give the crude 3-chloro/bromoandrost-3,5-diene-17β-carboxylic chloride/bromide (**6/7**),[25] which was not purified and used in the next step (Scheme 2). Reaction of 3-chloro-3,5-diene (**6**) with methanol under the basic catalytic condition afforded a 17β-methyl ester (**8**) in 61% yield, while the reaction with *t*-butylamine in the presence of a base (K_2CO_3) gave 3-chloroandrost-



Scheme 1.

3,5-diene-17β-carboxamide (**9**) in 60% yield. Similar reaction with 3-bromo compound **7** gave 3-bromo-androst-3,5-diene-17β-carboxamide (**10**) in 65% yield. Treatment of 3-bromo-3,5-diene (**10**) with *t*-butyl lithium in dry ether at −78 °C, followed the electrophilic capture of anion with *N*-fluorodibenzene-sulfonylamide $(C_6H_5SO_2)_2)NF$) gave the fluoro compound **11** in 50% yield.

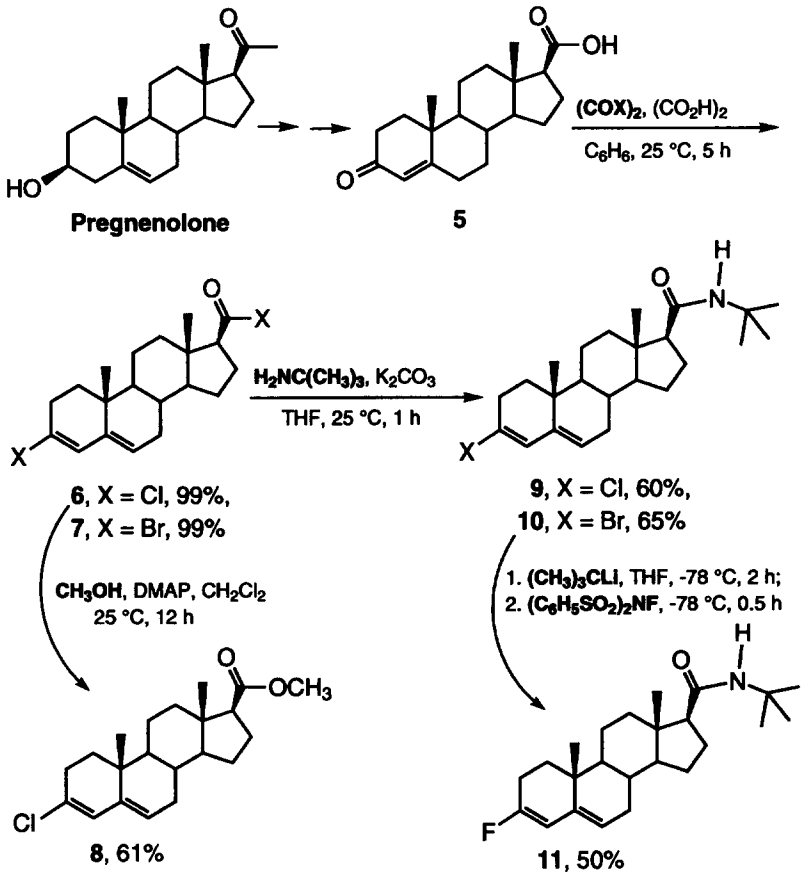
Inhibition of human type I and type II 5α-reductases (transfected 293 cells)[14]

The in vitro study of human type I and II 5α-reductases is summarized in Table 2. The purpose of this study is to see the compatibility of halovinyl analogues as the metaphore of intermediate enol form, to define the molecular changes which influence their activity in each of these assays and thus, to provide information on the structure–activity relationships of these compounds. The inhibitory activity of each compound was measured at low (100 nM) and high (1000 nM) concentrations on both isozymes. The inhibition of androstenedione is expressed as a percentage and an IC_{50} value. Finasteride and 4-MA were used as the standard references. Finasteride had IC_{50} values of 650 and 1.2 nM for type I and II isozymes, respectively; while, the IC_{50} values of 4-MA were 40.2 and 6.6 nM against type I and II isozymes, respectively. Compounds **8–11** showed very weak inhibitory activity ($\leq$10% inhibition) on human type I 5α-reductase at 100 and 1000 nM. However, against the type II enzyme, chloro compounds **8** and **9**, and bromo **10** had no effect at 100 nM and were weak inhibitors (6.0% < inhibition < 30%) at a higher concentration (1000 nM). Compound **9** having a 17β-carboxamide substituent was more active than the 17β-methyl ester **8**. 3-Fluorovinyl analogue **11** showed good inhibitory activity (IC_{50} = 480 nM) against type II enzyme. In general, compounds **8–11** showed better activity on the type II 5α-reductase than the type I enzyme at 1000 nM.

Table 1. Comparison of vinyl fluoride and vinyl alcohol using MNDO[22]

	OH	F
$r_{C\text{-}C}$ (Å)	1.35	1.35
$r_{C\text{-}X}$ (Å)	1.36	1.325
E_{HOMO} (e-V)	-9.3	-10.2
μ (D)	1.72	1.702

In conclusion, although the semi-empirical MO calculations provide similarities between vinyl fluoride and the enol form, the practical implications of this, thus far, have failed in this study. This may be due to



Scheme 2.

the fact that in neutral inhibitors like **2** and 3-nitrovinylsteroid,[27] introduction of the 5,6-double bond reduces the potency of the compound compared to the parent one. This observation may be true for the compound **11**, which also has the 5,6-double bond and may be less active than 3-fluorovinylsteroid. Synthesis and comparison of potency of 3-fluorovinylsteroids will be a subject of future discussion.

Experimental

General procedures

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

Table 2. In vitro percentage (%) inhibition of androstenedione by 3-halo-3,5,diene-steroids[a]

Entry	% Inhibition and IC_{50} (nM)					
	Human type I 5α-R (transfected 293 cells)			Human type II 5α-R (transfected 293 cells)		
	100 nM[b]	1000 nM[b]	IC_{50}	100 nM	1000 nM	IC_{50}
Finasteride	32	77	650	98	99	1.2
4-MA	88	100	40.2	100	100	6.6
8	0.0	6	-	0.0	6	-
9	0.0	8	-	0.0	30	-
10	9	10	-	0.0	18	-
11	3	4	1300	15	59	480

[a]Bio-assays were carried out as described in the Experimental.
[b]Concentration of inhibitors.

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 AC/F 300 (300 MHz). ^{13}C NMR spectra were measured at 75.14 MHz with a Bruker AC/F 300. 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, Québec. All the final products were at least 97% pure and the purity was determinated by a high performance liquid chromatography (HPLC) of Waters Model 600E (Millipore).

3-Oxo-4-androsten-17β-carboxylic acid (5). This was prepared following the method of Rasmusson et al.[16,17]: mp 243–246 °C, (lit.[26] 244–248 °C). IR (KBr, cm^{-1}) 3465 (br), 2937, 2853, 1730, 1659, 1207, 1187, 1162. ^{1}H NMR ($CDCl_3$) δ 0.77 (s, 3 H, 18-CH_3), 0.92–1.15 (m, 3 H), 1.18 (s, 3 H, 19-CH_3), 1.19–1.36 (m, 2 H), 1.40 (dd, J=3.8, 12.8 Hz, 1 H), 1.47–1.99 (m, 6 H), 2.0–2.14 (m, 3 H), 2.24–2.47 (m, 5 H), 2.88 (t, J=9.3 Hz, 1 H), 5.38 (dd, J=2.8 Hz, 1 H, 6-H), 6.05 (d, J=2.1 Hz, 1 H, 4-H) 9.8–10.6 (br s, 1 H, COOH). ^{13}C NMR ($CDCl_3$) δ 174.6, 140.5, 130.4, 126.8, 123.5, 67.1, 56.0, 47.7, 45.1, 37.9, 34.7, 34.4, 31.8, 31.4, 30.6, 25.2, 24.2, 21.0, 18.8, 13.2.

3-Chloroandrost-3,5-diene-17β-carboxylic chloride (6). Acid **5** (1.0 g, 3.17 mmol) was dissolved in dry benzene (30 mL) and stirred with oxalyl chloride (7.79 g, 60.0 mmol) and oxalic acid (0.09 g, 0.95 mmol) for 4 h. The organic solvent was evaporated under reduced pressure. The residue was taken into Et_2O (80 mL) and washed with satd $NaHCO_3$ (80 mL), and brine (50 mL). The solvent was dried and evaporated to give the product **6** (1.17 g, 99%) which was further dried under high vacuum for 12 h and used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 0.83 (s, 3 H, 18-CH_3), 0.97 (s, 3 H, 19-CH_3), 1.05–1.24 (m, 2 H), 1.28–1.48 (m, 4 H), 1.65–1.76 (m, 4 H), 1.83–1.98 (m, 3 H), 2.0–2.36 (m, 4 H), 2.42–2.61 (m, 1 H), 5.36 (dd, J=2.1, 5.6 Hz, 1 H, 6-H), 5.96 (d, J=1.9 Hz, 1 H, 4-H). ^{13}C NMR ($CDCl_3$) δ 199.4, 171.6, 171.0, 123.9, 57.5, 55.6, 51.0, 43.5, 38.6, 38.4, 35.7, 35.6, 33.9, 32.8, 31.9, 24.4, 23.2, 21.0, 17.4, 13.1.

3-Bromoandrost-3,5-diene-17β-carboxylic bromide (7). Acid **5** (1.0 g, 3.17 mmol) was dissolved in dry benzene (30 mL) and stirred with oxalyl bromide (6.83 g, 31.60 mmol) and oxalic acid (0.09 g, 0.95 mmol) for 4 h. The reaction was worked up as described in the preparation for compound **6** to give compound **7** (1.44 g, 99%): ^{1}H NMR ($CDCl_3$) δ 0.85 (s, 3 H, 18-CH_3), 0.96 (s, 3 H, 19-CH_3), 1.05–1.46 (m, 6 H), 1.64–1.82 (m, 4 H), 1.84–2.18 (m, 4 H), 2.17–2.50 (m, 3 H), 3.01 (t, J=9.3 Hz, 1 H), 5.37 (d, J=2.9 Hz, 1 H, 6-H), 6.28 (d, J=1.9 Hz, 1 H, 4-H). ^{13}C NMR ($CDCl_3$) δ 170.6, 141.0, 130.8, 123.7, 121.0, 71.5, 55.9, 47.6, 45.1, 37.9, 35.4, 34.3, 32.9, 31.7, 31.4, 26.0, 24.0, 20.9, 18.8, 13.4.

Methyl 3-chloroandrost-3,5-diene-17β-carboxylate (8). To a stirring solution of compound **7** (1.13 g, 3.06 mmol) in CH_2Cl_2 (40 mL), DMAP (0.04 g, 0.1 mmol) and CH_3OH (0.98 g, 30.62 mmol) were added and the mixture was stirred at room temperature for 12 h. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with 5% aq HCl (100 mL) followed by brine (100 mL). Evaporation gave the crude product, which was purified by flash column chromatography (n-C_6H_{14}:EtOAc, 98:2) to give compound **8** (0.65 g, 61%): mp 143–145 °C. IR (KBr, cm^{-1}) 2940, 2851, 1727, 1620, 1443, 1378, 1232, 1156. ^{1}H NMR ($CDCl_3$) δ 0.69 (s, 3 H, 18-CH_3), 0.94 (s, 3 H, 19-CH_3), 1.01–1.19 (m, 2 H), 1.25–1.35 (m, 4 H), 1.36–1.78 (m, 4 H), 1.75–1.87 (m, 2 H), 2.02 (dt, J=3.4, 11.9 Hz, 1 H), 2.12–2.39 (m, 4 H), 2.40–2.58 (m, 1 H), 3.67, (s, 3H, $COOCH_3$), 5.38 (dd, J=2.7, 5.9 Hz, 1 H, 6-H), 6.04 (d, J=2.1 Hz, 1 H, 4-H). ^{13}C NMR ($CDCl_3$) δ 174.5, 140.6, 130.3, 126.9, 123.9, 56.2, 55.2, 51.2, 47.9, 44.0, 38.1, 34.8, 34.5, 31.8, 31.7, 30.7, 24.5, 23.7, 21.0, 18.9, 13.4. EI-MS *m/s* (relative intensity) 348 (M^+, 100), 313 (24), 206 (22), 147 (56), 133 (38), 105 (26), 91 (38). HRMS calcd for $C_2H_{29}O_2Cl_1$, 348.1861; found 348.1865.

(*N*-1′,1′-Dimethylethyl)-3-chloroandrost-3,5-diene-17β-carboxamide (9). To a solution of compound **6** (1.17 g, 3.17 mmol) in THF (40 mL), K_2CO_3 (4.36 g, 31.65 mmol) and t-butylamine (1.16 g, 15.8 mmol) were added, and the mixture was stirred at room temperature for 1 h. The organic layer was evaporated under reduced pressure and the residue was dissolved in CH_2Cl_2 (100 mL), and washed successively with 5% HCl (2×100 mL), and brine (100 mL). The solvent was dried and evaporated to crude product, which was purified by flash column chromatography (n-C_6H_{14}:EtOAc, 95:5) to give compound **9** (0.74 g, 60%): mp 152–154 °C. IR (KBr, cm^{-1}) 3458, 3421 , 2965, 2910, 2872, 2846, 1664, 1620, 1509, 1449, 1364, 1253, 1223. ^{1}H NMR ($CDCl_3$) δ 0.70 (s, 3 H, 18-CH_3), 0.95 (s, 3 H, 19-CH_3), 1.01–1.22 (m, 3 H), 1.23–1.31 (m, 3 H), 1.34 (s, 9 H, $C(CH_3)_3$), 1.44 (dd, J=3.9, 12.6 Hz, 1 H), 1.58–1.78 (m, 5-H), 1.84 (dd, J=4.4, 12.7 Hz, 1 H), 1.90–2.04 (m, 2 H), 2.05–2.19 (m, 2 H), 2.26 (dd, J=5.2, 13.4 Hz, 1 H), 5.08 (s, 1 H, NH), 5.37 (dd, J=2.9, 5.3 Hz, 1 H, 6-H), 6.04 (d, J=3.0 Hz, 1 H, 4-H). ^{13}C NMR ($CDCl_3$) δ 171.8, 140.5, 130.1, 127.0, 124.0, 57.6, 56.5, 51.0, 48.0, 43.6, 38.5, 34.8, 34.5, 31.7, 31.4, 30.6, 29.1 (3C), 24.4, 23.3, 21.1, 18.9, 13.0. EI-MS *m/z* (relative intensity) 389 (M^+, 100), 374 (62), 354 (3), 301 (18), 249 (12), 192 (42), 91 (31), 72 (32). HRMS calcd for $C_{24}H_{36}O_1N_1Cl_1$, 389.2485; found 389.2482.

(*N*-1′,1′-Dimethylethyl)-3-bromoandrost-3,5-diene-17β-carboxamide (10). Compound **10** (0.90 g, 65%) was yielded from 3-bromo-17β-carboxylic bromide **7** (1.45 g, 3.17 mmol) following the procedure for compound **9**: mp 165–167 °C. IR (KBr, cm^{-1}) 3422, 2962, 2854, 1664, 1510, 1449, 1365, 1224. ^{1}H NMR ($CDCl_3$) δ 0.65

(s, 3 H, 18-CH_3), 0.91 (s, 3 H, 19-CH_3), 0.93–1.18 (m, 3 H), 1.20–1.41 (m, 3 H), 1.29 (s, 9 H, $C(CH_3)_3$), 1.42–1.68 (m, 5 H), 1.75 (dd, J=4.2, 12.3 Hz, 1 H), 1.88 (dt, J=3.2, 11.6 Hz, 1 H), 1.98 (t, J=8.6 Hz, 1 H), 2.07–2.14 (m, 2 H), 2.40 (dd, J=5.2, 12.9 Hz, 1 H), 2.44–2.63 (m, 1 H), 5.12 (s, 1 H, NH), 5.33 (d, J=3.0 Hz, 1 H, 6-H), 6.20 (d, J=1.8 Hz, 1 H, 4-H). ^{13}C NMR ($CDCl_3$) δ 171.6, 141.0, 130.9, 124.2, 120.6, 57.3, 53.6, 50.9, 47.8, 43.5, 38.3, 35.4, 34.3, 32.9, 31.7, 31.5, 28.9 (3C), 24.2, 23.1, 20.9, 18.7, 12.9. EI-MS *m/z* (relative intensity) 436 (M^++2, 98), 434 (M^+, 100), 418 (34), 420 (32), 354 (34), 236 (15), 238 (27), 143 (10), 91 (12). HRMS Calcd for $C_{24}H_{36}O_1N_1Br_1$, 434.1980; found 434.2001.

(*N*-1′,1′-Dimethylethyl)-3-fluoroandrost-3,5-diene-17β-carboxamide (11). To a stirring solution of compound **10** (0.20 g, 0.46 mmol) in Et_2O (45 mL) at −78 °C, *t*-BuLi (0.20 g, 3.11 mmol, 1.6 M in hexanes) was added dropwise and the mixture was stirred at −78 °C for 2 h. *N*-Fluorodibenzenesulfonamide (0.11 g, 1.40 mmol) in THF (5 mL) was added to the above solution and the mixture was stirred at −78 °C for 30 min then at room temperature for 4 h. The reaction mixture was quenched with aq NH_4Cl (100 mL) and the mixture was extracted with EtOAc (3 × 40 mL). The organic layer was washed with brine and evaporated to give compound **11**, which was purified by flash column chromatography (*n*-C_6H_{14}:EtOAc, 95:5): yield (0.09 g, 50%): mp 121–123 °C. IR (KBr, cm^{-1}) 3424, 2969, 2934, 2881, 1666, 1504, 1451, 1381, 1255, 1225, 1135. ^{1}H NMR ($CDCl_3$) δ 0.71 (s, 3 H, 18-CH_3), 0.97 (s, 3 H, 19-CH_3), 0.89–1.09 (m, 4 H), 1.25–1.66 (m, 6 H), 1.35 (s, 9 H, $C(CH_3)_3$), 1.67–2.05 (m, 4 H), 2.16–2.26 (m, 3 H), 2.31–2.44 (m, 1 H), 5.08 (s, 1 H, NH), 5.32 (d, J=3.1 Hz, 1 H, 6-H), 5.57 (d, J=2.1, 15.4 Hz, 1 H, 4-H). ^{13}C NMR ($CDCl_3$) d 171.8, 138.8, 130.7, 122.1, 106.6, 57.6, 56.6, 51.0, 48.0, 43.6, 38.6, 35.0, 33.7, 31.8, 31.7, 29.1 (3C), 24.4, 23.6, 23.3, 21.3, 18.8, 13.0. EI-MS *m/z* (relative intensity) 373 (M^+, 100), 358 (50), 285 (12), 176 (30), 57 (24), 50 (44). HRMS calcd for $C_{24}H_{36}O_1N_1F_1$, 373.2781; found 373.2783.

Type I 5α-reductase. 293 Cells[14] (ATCC CRL 1573) were transfected with the human type I 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 μM 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, 100 nM {^{3}H}-androstenedione, 500 μM NADPH and the cell homogenate were added to the sample tubes to a final volume of 1 mL. Following the 60-minute incubation at 37 °C, the media were extracted twice with ether after the addition of 25 μg each of non-radioactive steroid carriers (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. 293 Cells (ATCC CRL 1573) were transfected with the human 5α-reductase type II cDNA 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 μM and 0.1 μM. Compounds showing 50% or more inhibition at the 1 μM concentration were subsequently tested at twelve concentrations ranging from 0.1 to 1000 nM for the measurement of the IC_{50} value. The indicated compound, 100 nM {^{3}H}-androstenedione, 500 μM NADPH and the cell homogenate were added to the sample tubes to a final volume of 1 mL. Following the 60-minute incubation at 37 °C, the media were extracted twice with ether after the addition of 25 μg each of non-radioactive steroid carriers (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.

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