Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 174–185
Bioorganic & Medicinal Chemistry

Discovery of 7α-substituted dihydrotestosterones as androgen receptor pure antagonists and their structure–activity relationships

Kazutaka Tachibana,* Ikuhiro Imaoka, Hitoshi Yoshino, Takashi Emura, Hirohumi Kodama, Yoshiyuki Furuta, Nobuaki Kato, Mitsuaki Nakamura, Masateru Ohta, Kenji Taniguchi, Nobuyuki Ishikura, Masahiro Nagamuta, Etsuro Onuma and Haruhiko Sato

Fuji Gotemba Research Laboratories, Chugai Pharmaceutical Co., Ltd, 1-135 Komakado, Gotemba, Shizuoka 412-8513, Japan

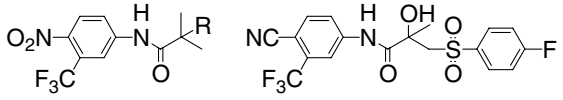
Received 16 August 2006; revised 28 September 2006; accepted 30 September 2006
Available online 4 October 2006

Abstract—A series of 7α-substituted dihydrotestosterone derivatives were synthesized and evaluated for androgen receptor (AR) pure antagonistic activity. From reporter gene assay (RGA), the compound with a side chain containing *N*-*n*-butyl-*N*-methyl amide (**19a**) showed pure antagonistic activity (IC_{50} = 340 nM, FI_5 > 10,000 nM), whereas known AR antagonists showed partial agonistic activities. The optimization of **19a** led to compound **23** (**CH4892280**), which showed more potent pure antagonistic activity (IC_{50} = 190 nM, FI_5 > 10,000 nM). The SARs of tested compounds suggested that the length of the side chain and the substituents on the amide nitrogen are important for pure antagonistic activities.

1. Introduction

Prostate cancer is the most common cancer amongst men in the USA and the second most common malignant cause of male death worldwide after lung cancer.[1] Since the growth of prostate cancer is dependent on androgen, androgen receptor (AR) antagonists such as flutamide (**1**) and bicalutamide (**3**) are currently used as hormone therapy.[2] These antiandrogens exhibit good efficacy in many cases and comprise an important part of effective therapeutics.[3–6] However, the most considerable problem with these antiandrogens is that recurrence occurs after a short period of response.[7] Since they have partial agonistic activities at high concentration in vitro,[8] this may be attributed to recurrence. Therefore, new antiandrogenic agents that exhibit no agonistic activities, so-called 'AR pure antagonists', are expected.

It has been reported that 7α-substituted estradiols such as fulvestrant (**4**)[9] and ICI 164,384 (**5**)[10] show pure antagonistic activities against estrogen receptor (ER). Fulvestrant showed efficacy in tamoxifen-resistant breast cancer[11,12] and was launched in 2002. Moreover, it was found in the X-ray crystallography of the complex of **5** and ligand-binding domain (LBD) of ERβ that the side chain of **5** inhibits the folding of helix 12 of ERβ LBD,[13] presumed to be the important mechanism of expression of pure antagonistic activity. As AR pure antagonists, Hashimoto et al. discovered isoxazolone derivatives designed on the basis of 'the helix-folding



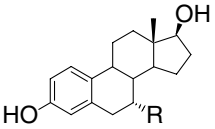


Chart 1. Structures of AR and ER antagonists.

Keywords: Androgen receptor; Pure antagonist; Dihydrotestosterone; Prostate cancer.
* Corresponding author. E-mail: tachibanakzt@chugai-pharm.co.jp

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.09.072

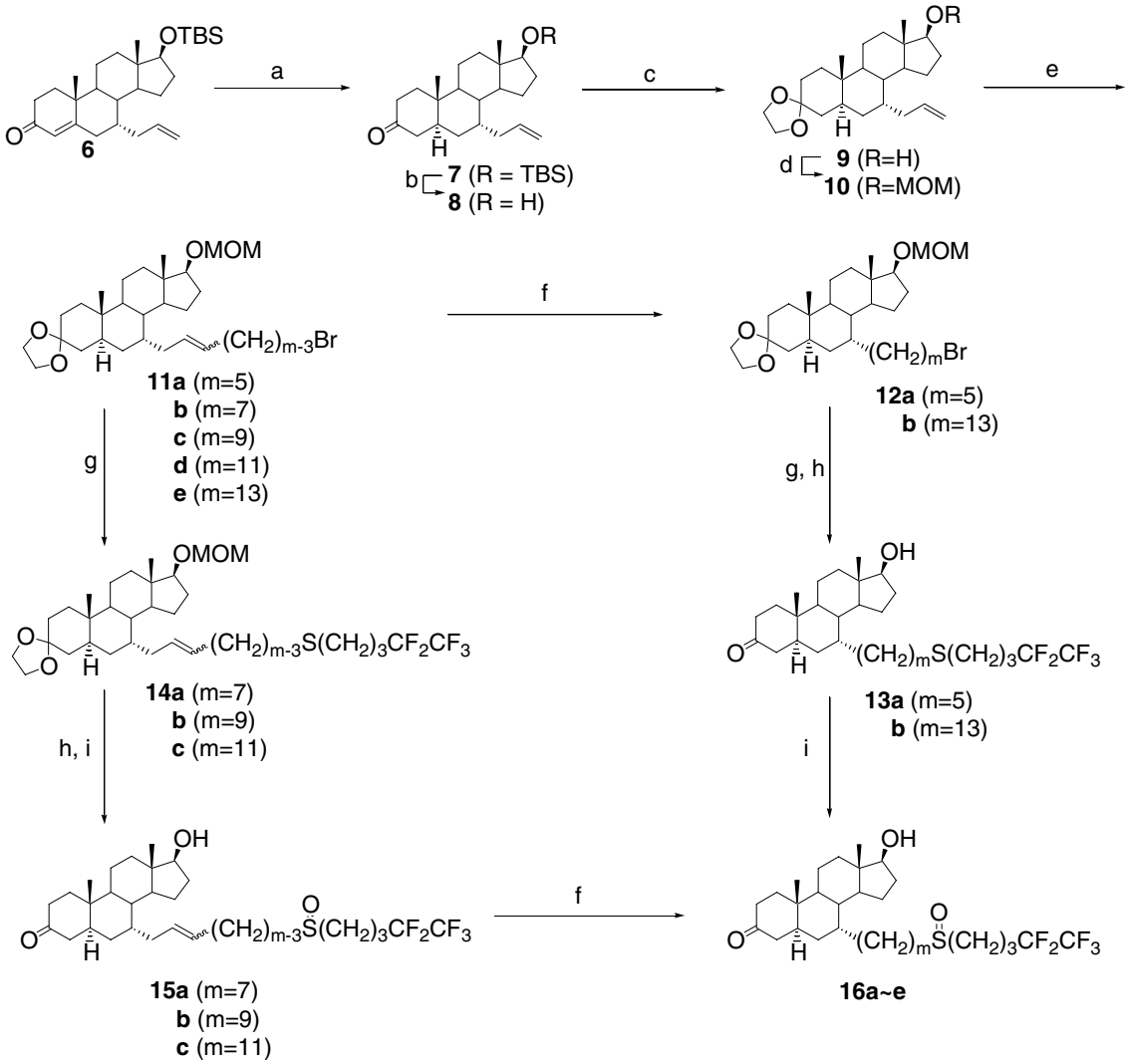
inhibition hypothesis'.[14,15] Since AR and ER belong to the nuclear receptor superfamily and the tertiary structure of AR LBD is similar to that of ER LBD,[16,17] we hypothesized that dihydrotestosterone (DHT) derivatives which have side chain in position 7α similar to ER pure antagonists could be AR pure antagonists (see Chart 1).

In this paper, we report the design and synthesis of new DHT derivatives with the side chain in position 7α and their AR pure antagonistic activity in vitro. Moreover, we report the structure–activity relationships determined from the process of the investigation.

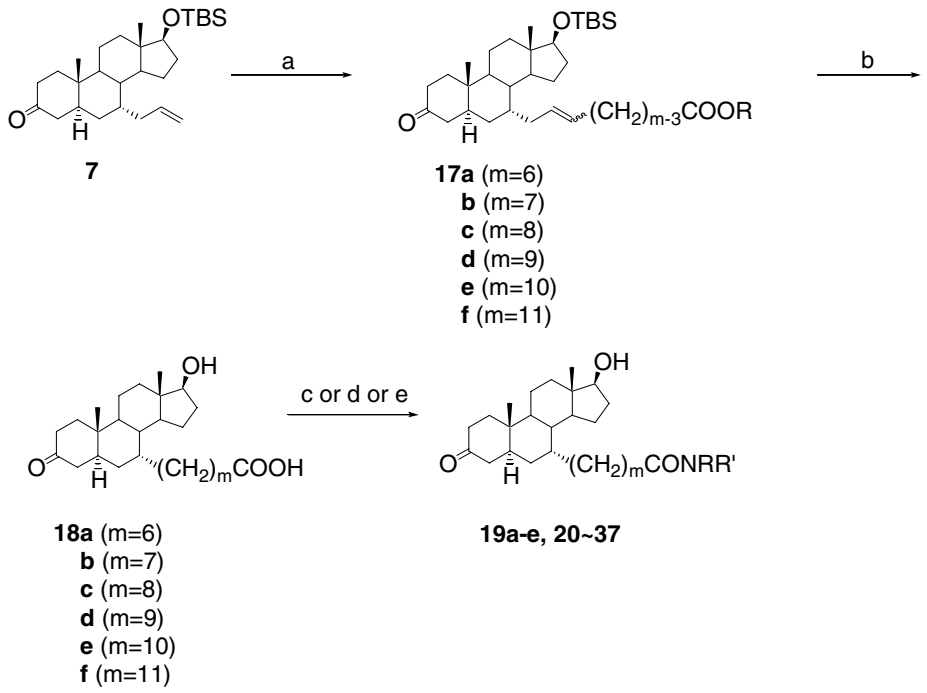
2. Chemistry

The target compounds with a sulfoxide side chain (**16a–e**) were prepared according to the synthetic sequence outlined in Scheme 1. Starting material **6**, synthesized from testosterone according to the reported procedures,[18–20] was converted to the key intermediate **10** in four steps. For the next step, we selected olefin cross-metathesis[21] as the key reaction to effectively introduce side chains of various lengths. The reaction of **10** and two equivalents of bromoalkenes in the presence of a catalytic amount of $Cl_2(Cy_3P)_2Ru{=}CHPh$ gave **11a–e** in yields of 69–98%. Further derivatizations to the target compounds were carried out by two routes as described in Scheme 1. For both routes, bromide **11** was converted to 4,4,5,5,5-pentafluoropentylsulfides **13** or **14** followed by oxidation to a sulfoxide using OXONE®.[22]

The synthetic route of the amide side chain derivatives (**19a–e, 20–37**) is shown in Scheme 2. In this scheme also, olefin cross-metathesis was adopted as the key reaction to form alkenyl esters **17a–f**. After the reduction of the olefins by Pd-catalyzed hydrogenation, removal of the *tert*-butyldimethylsilyl group and hydrolysis of the ester were performed simultaneously by 1 N HCl to give carboxylic acids **18a–f**. Finally, these compounds were converted to target compounds



Scheme 1. Reagents and conditions: (a) Li, liquid NH_3, −78 °C, 20 min; (b) 2 N HCl, acetone, rt, 4 h; (c) ethylene glycol, *p*-TsOH, benzene, reflux, 2 h; (d) MOMCl, $^{i}Pr_2NEt$, CH_2Cl_2, rt, 14 h; (e) $CH_2{=}CH(CH_2)_{m-3}Br$, $Cl_2(Cy_3P)_2Ru{=}CHPh$ (cat.), CH_2Cl_2, reflux; (f) H_2, 10% Pd/C (cat.), AcOEt, rt; (g) $AcS(CH_2)_3CF_2CF_3$, 1 M MeONa in MeOH, THF–MeOH, rt; (h) 2 N HCl, acetone, 60 °C; (i) OXONE®, THF–H_2O, 0 °C, 30 min.



Scheme 2. Reagents and conditions: (a) $CH_2{=}CH(CH_2)_{m-3}CO_2R$, $Cl_2(Cy_3P)_2Ru{=}CHPh$ (cat.), CH_2Cl_2, reflux; (b) H_2, 10% Pd/C (cat.), AcOEt, rt, then 1 N HCl, acetone, reflux; (c) HNRR′, EDC, HOBt, THF, rt; (d) HNRR′, HATU, $^{i}Pr_2NEt$, THF, rt; (e) $ClCO_2Et$, NEt_3, THF, 0 °C, then NH_3 gas.

19a–e and **20–37** by amidation using corresponding amines.

3. Biological assay

Affinities of tested compounds against AR were determined by competitive receptor-binding assay using [^{3}H]mibolerone and CHO-K1/hAR cells.[8,23] Agonistic and antagonistic activities of the compounds for AR were determined by reporter gene assay (RGA) using hAR-transfected HeLa cells.[24,25] Antagonistic activity was described as IC_{50} value, which is the concentration of a compound to inhibit the transcriptional activity of 0.1 nM of DHT by 50%. To describe agonistic activity, we calculated the value of 'FI_5', the concentration of a compound-treated group in which the transcriptional activity is five times the transcriptional activity of the case without the addition of a compound. A 'pure antagonist' was defined to have a FI_5 value greater than 10,000 nM.

4. Results

The characterization of hydroxyflutamide (**2**), an active metabolite of flutamide, and bicalutamide (**3**) in RGA (Table 1) showed antagonistic activities of IC_{50}s of 31 and 140 nM, and FI_5 values of 1000 and 770 nM, respectively, and thus clarified that these known AR antagonists have partial agonistic activities.

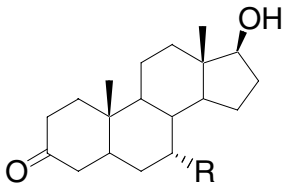
As the first step in our investigation, we designed and synthesized the compounds (**16a–e** and **19a–e**) shown in Table 1. They have various lengths of side chains which have the 4,4,5,5,5-pentafluoropentylsulfoxide group (**16a–e**) or the *N*-*n*-butyl-*N*-methylamide group (**19a–e**). Most of the sulfoxide derivatives exhibited no antagonistic activities, only **16c** showed weak antagonistic activity (IC_{50} = 2900 nM), and all of them exhibited agonistic activities. These results were quite different from those from ER antagonists.

In the case of *N*-*n*-butyl-*N*-methylamide derivatives, their profiles were dependent on the length of the side chain. Specifically, **19b** (m = 8), **19d** (m = 10), and **19e** (m = 11) have agonistic activities, but **19a** (m = 7) and **19c** (m = 9) showed no agonistic activities even at 10,000 nM. Therefore, by our definition, **19a** and **c** are considered to be the pure antagonists.

In the next step, we attempted the optimization of the N-substituents of **19a** (Table 2).

The same activities as **19a** were retained by the substitution of an *n*-butyl group with smaller groups such as with isopropyl (**21**), ethyl (**22**), or methyl (**23**). The antagonistic activity was decreased by the substitution of an *n*-butyl group to a larger group such as a benzyl group (**25**). Moreover, the RGA activity and binding affinity disappeared when both groups on the nitrogen atom were substituted for larger groups (**26**). On the other hand, although agonistic activity resulted from removal of substituents from the nitrogen atom (**30**), the introduction of a methyl group also led to the disappearance of agonistic activity (**31**). The larger the methyl group of **31** was, the lower the antagonistic activities became (**32–35**). The compounds with cyclic

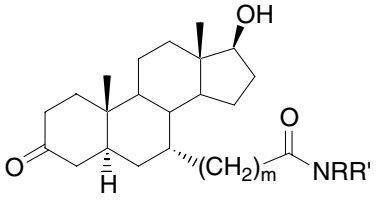
Table 1. Binding and agonistic/antagonistic activities of 7α-substituted DHT derivatives[a]



Compound	R	*m*	Binding affinity IC_{50} (nM)	RGA	
				FI_5 (nM)	IC_{50} (nM)
16a	$(CH_2)_m$–SO–$(CH_2)_3C_2F_5$	5	6,800	16	>10,000
16b	$(CH_2)_m$–SO–$(CH_2)_3C_2F_5$	7	960	200	>10,000
16c	$(CH_2)_m$–SO–$(CH_2)_3C_2F_5$	9	110	490	2,900
16d	$(CH_2)_m$–SO–$(CH_2)_3C_2F_5$	11	>10,000	120	>10,000
16e	$(CH_2)_m$–SO–$(CH_2)_3C_2F_5$	13	>10,000	310	>10,000
19a	$(CH_2)_m$–CON(Me)^{n}Bu	7	280	>10,000	340
19b	$(CH_2)_m$–CON(Me)^{n}Bu	8	550	41	3,400
19c	$(CH_2)_m$–CON(Me)^{n}Bu	9	570	>10,000	1,800
19d	$(CH_2)_m$–CON(Me)^{n}Bu	10	1,900	31	1,300
19e	$(CH_2)_m$–CON(Me)^{n}Bu	11	9,900	410	3,500
2 (hydroxyflutamide)	—	—	200	1,000	31
3 (bicalutamide)	—	—	200	770	140

[a] All data are mean values of duplicate experiments.

Table 2. Binding and agonistic/antagonistic activities of **19a** derivatives[a]



Compound	*m*	NRR′	Binding affinity IC_{50} (nM)	RGA	
				FI_5 (nM)	IC_{50} (nM)
19a	7	N(Me)^{n}Bu	280	>10,000	340
20	7	N(Me)Pr	300	>10,000	1,100
21	7	N(Me)^{i}Pr	430	>10,000	420
22	7	N(Me)Et	660	>10,000	420
23	7	NMe_2	260	>10,000	190
24	7	NEt_2	120	>10,000	300
25	7	N(Me)Bn	820	>10,000	1,600
26	7	N(*n*-hexyl)$_2$	>10,000	>10,000	>10,000
27	7		1,300	>10,000	730
28	7		350	>10,000	300
29	7		120	>10,000	490
30	7	NH_2	260	2,400	450
31	7	NHMe	710	>10,000	480
32	7	NHEt	1,500	>10,000	940
33	7	NH^{n}Pr	1,600	>10,000	1,800
34	7	NH^{n}Bu	NT[b]	1,600	6,300
35	7	NH*n*-hexyl	2,100	>10,000	6,600
36	6	NMe_2	110	490	200
37	6	NEt_2	330	1,700	530

[a] All data are mean values of duplicate experiments unless otherwise noted.
[b] Not tested.

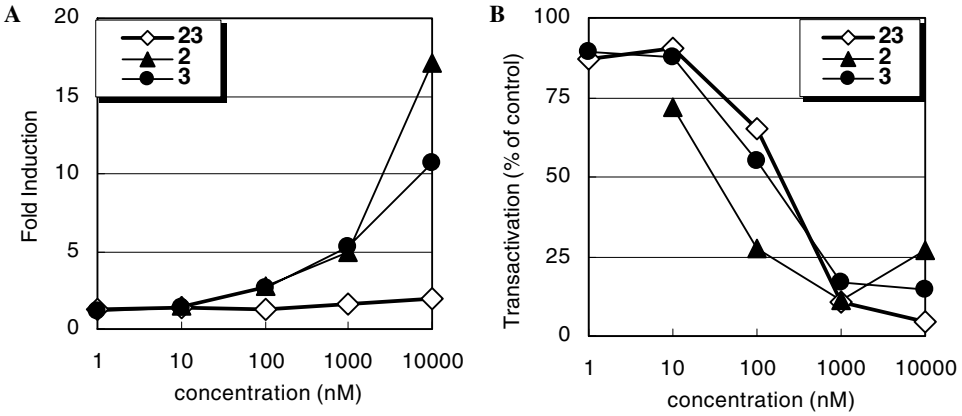


Figure 1. Agonistic and antagonistic activities of **CH4892280** (**23**), hydroxyflutamide (**2**), and bicalutamide (**3**) in reporter gene assay with hAR-transfected HeLa cells. (A) Dose-dependent agonistic activities of compounds without DHT. (B) Dose-dependent antagonistic activities of compounds in the presence of 0.1 nM of DHT. All data are mean values of duplicate experiments.

groups (**27–29**) as well as **19a** exhibited pure antagonistic activities.

With the aim of optimization of the length of side chain, two compounds with side chains shorter by one carbon than the others were synthesized (**36** and **37**). However, both of them showed only partial agonistic activities.

Compound **23** (**CH4892280**) exhibited the highest antagonistic activity of the pure antagonists.

The dose-dependent agonistic and antagonistic activities of **CH4892280** (**23**), hydroxyflutamide (**2**), and bicalutamide (**3**) in RGA are shown in Figure 1. **CH4892280** (**23**) showed no agonistic activity even at 10,000 nM, but hydroxyflutamide (**2**) and bicalutamide (**3**) exhibited obvious agonistic activities at 100 nM which increased dose dependently (Fig. 1A). On the other hand, **CH4892280**, but not **2** or **3**, inhibited transcriptional activity of 0.1 nM DHT completely at 10,000 nM (Fig. 1B).

5. Discussion

The value 'FI_5' was established as the criterion for agonistic activity of a compound. Hydroxyflutamide (**2**) exhibited an FI_5 of 1000 nM in this study and the agonistic activity was comparable to the concentration as reported in the literature.[23] Plasma levels of **2** in prostate cancer patients have been reported to reach 78 ng/ml (8 μM).[23] Therefore, we considered our criterion to be valid.

Our strategy for the discovery of AR pure antagonists was based on the fact that estradiol derivatives having a side chain in position 7α exhibit pure antagonistic activities against ER. This suggests that the folding of helix 12 of ER LBD is inhibited by the side chain of the antagonist sticking out of the pocket.[13] It has also been reported that the optimal length of the side chain is 16–18 atoms.[10] Since the optimal length of the side chain has not been clarified for AR pure antagonists, we synthesized compounds with sulfoxide side chains over the wide range of 11–19 atoms in length. However, contrary to our expectations, these compounds exhibited full agonistic activities with FI_5 values of 16–486 nM, with the exception of **16c**, which showed weak antagonistic activity. That the side chains of these compounds may not prevent the folding of helix 12 to the agonistic form can be explained by the following two reasons: (1) the side chain might turn in a direction in which it cannot inhibit the folding of helix 12 to an agonistic form and (2) compounds such as **16d** and **16e** might have exhibited agonistic activities by the allosteric effect because they showed no binding affinities to AR, even though it is extraordinary that there are such differences in the binding affinities of the compounds in which there are only slight differences in the length of side chain (e.g., **16c** vs **16d**).

For *N*-methyl-*N*-*n*-butyl amide derivatives, contrary to the sulfoxide derivatives, the agonistic activities disappeared alternately in the range of seven to 10 methylene groups (**19a** and **19c**). Therefore, compounds with a sulfoxide side chain which have an even number of methylene groups might exhibit pure antagonistic activity.

In the optimization of **19a**, it was found that the antagonistic activities are influenced by substituents on the nitrogen atom of the amide moiety. In the case of N,N-disubstituted derivatives, the same level of antagonistic activities was expressed in the *N*,*N*-dimethyl derivative (**23**) through the *N*-*n*-butyl-*N*-methyl derivative (**19a**). On the contrary, with N-monosubstituted derivatives, antagonistic activities decreased as the substituent became larger. These distinct results were probably the result of the difference in the conformation of the substituents; that is, in N,N-disubstituted amides, the substituents may have turned to a direction different than the substituent in N-monosubstituted amides because of the steric hindrance of the geminal substituent.

We also studied the effect of **CH4892280** (**23**) on seminal vesicle wet weight in mice to investigate the in vivo antiandrogenic activity of the compound. However, **CH4892280** showed no activities from either by oral or subcutaneous administration (data not shown). These

results might be attributed to the metabolic instability of the compound because it was significantly instable in rat liver microsome (data not shown). Improvement of metabolic stability would be important for the clarification of the in vivo activities of AR pure antagonists.

6. Conclusion

Some of the compounds synthesized in this study including **CH4892280** (**23**) exhibited AR antagonistic activities of IC_{50}s of 100 nM order and no agonistic activities even at 10,000 nM and thus meet the criteria for AR pure antagonists in vitro. Although the structure–activity relationships (e.g., optimum length or optimum structure of side chain) of the compounds differ, our hypothesis that AR pure antagonists could be obtained by the introduction of a side chain to the natural ligand, similar to ER pure antagonists, have been established.

7. Experimental

7.1. Chemistry: instruments

Column chromatography was carried out on Merck Silicagel 60 (230–400 mesh) if not otherwise specified. R_f was determined with Merck Silicagel 60 F^{254} plates. ^{1}H NMR spectra were recorded on JEOL EX-270. Mass spectra (MS) were measured with VG Analytical VG70-250SEQ (FAB) or Thermo Electron LCQ Classic (ESI). High resonance mass spectra (HRMS) were recorded by a Micromass Q-Tof Ultima API mass spectrometer.

7.1.1. 7α-Allyl-17β-(*tert*-butyldimethylsilyl)oxy-5α-androstan-3-one (7). Li (220 mg, 31.7 mmol) was added to liquid ammonia (150 ml) at −78 °C. After stirring for 5 min, 7α-allyl-17β-[(*tert*-butyldimethylsilyl)oxy]androst-4-en-3-one (**6**) (1.261 g, 2.85 mmol) and *tert*-butanol (0.41 ml) in THF (20 ml) were added and the mixture was stirred for 20 min. 1,2-Dibromoethane (3 ml) and NH_4Cl (30 g) were added, and the mixture was stirred at 25 °C for 30 min. Water was added to the mixture and extracted with AcOEt. The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (AcOEt/hexane 1:10) to give **7** (810.7 mg, 64%) as white solid. Mp 130–132 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.01 (6H, s), 0.73 (3H, s), 0.88 (9H, s), 1.04 (3H, s), 0.92–2.45 (23H, m), 3.55 (1H, t, J = 8.3 Hz), 4.93 (1H, d, J = 3.8 Hz), 4.99 (1H, s), 5.58–5.72 (1H, m); R_f 0.54 (AcOEt/hexane 1:10).

7.1.2. 7α-Allyl-17β-hydroxy-5α-androstan-3-one (8). Compound **7** (1.00 g, 2.26 mmol) was dissolved in acetone (20 ml) and then 2 N HCl (2 ml) was added dropwise. After stirring for 17 h at room temperature, water was added to the reaction mixture and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (0–33% AcOEt/hexane) to give **8** (655.7 mg, 88%) as white solid. Mp 197–198 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.77 (3H, s), 1.04 (3H, s), 0.96–2.44 (23H, m), 3.60–3.70 (1H, m), 4.94 (1H, d, J = 3.5 Hz), 5.00 (1H, s), 5.58–5.72 (1H, m); R_f 0.54 (AcOEt/hexane 1:1). Anal. Calcd for $C_{22}H_{34}O_2$: C, 79.95; H, 10.37. Found: C, 79.73; H, 10.39.

7.1.3. 7α-Allyl-3,3-ethylenedioxy-17β-hydroxy-5α-androstane (9). To a solution of **8** (126 mg, 0.381 mmol) in benzene (5 ml) were added ethylene glycol (2 ml) and *p*-TsOH (13.2 mg, 0.0767 mmol), and the mixture was refluxed for 2 h using Dean-Stark trap. After cooling to room temperature, satd $NaHCO_3$ aq was added under cooling with ice and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$, filtered, and concentrated in vacuo to give **9** (141 mg, 99%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.74 (3H, s), 0.85 (3H, s), 0.92–2.20 (23H, m), 3.56–3.70 (1H, m), 3.93 (4H, s), 4.92–5.04 (2H, m), 5.62–5.80 (1H, m); R_f 0.60 (AcOEt/hexane 1:1). This material was used in the next step without purification.

7.1.4. 7α-Allyl-3,3-ethylenedioxy-17β-methoxymethoxy-5α-androstane (10). To a solution of **9** (141 mg, 0.376 mmol) in CH_2Cl_2 (4 ml) were added ^{i}Pr$_2$NEt (0.227 ml, 1.33 mmol) and MOMCl (0.087 ml, 1.16 mmol) dropwise under cooling with ice. After stirring for 14 h at 25 °C, satd $NaHCO_3$ aq was added to the reaction mixture and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (AcOEt/hexane 1:4) to give **10** (131 mg, 83%) as white solid. Mp 112–114 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.77 (3H, s), 0.84 (3H, s), 0.92–2.20 (23H, m), 3.35 (3H, s), 3.53 (1H, t, J = 8.3 Hz), 3.92 (4H, s), 4.62 (2H, d, J = 1.8 Hz), 4.92–5.04 (2H, m), 5.62–5.80 (1H, m); R_f 0.50 (AcOEt/hexane 1:4). Anal. Calcd for $C_{26}H_{42}O_4$: C, 74.60; H, 10.11. Found: C, 74.40; H, 10.13.

7.1.5. 7α-(13-Bromo-2-tridecen-1-yl)-3,3-ethylenedioxy-17β-methoxymethoxy-5α-androstane (11e). The mixture of **10** (42.6 mg, 0.102 mmol), 12-bromododecene (50.4 mg, 0.204 mmol), and $Cl_2(Cy_3P)_2Ru{=}CHPh$ (8.4 mg, 0.0102 mmol) in CH_2Cl_2 (1.5 ml) was heated under reflux for 5 h under Ar atmosphere. After cooling, purification by column chromatography (AcOEt/hexane 1:10) gave **11e** (56.0 mg, 86%) as brown oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.83 (3H, s), 0.94–2.14 (41H, m), 3.34 (3H, s), 3.41 (2H, t, J = 6.9 Hz), 3.52 (1H, t, J = 8.3 Hz), 3.92 (4H, s), 4.62 (2H, d, J = 1.8 Hz), 5.22–5.46 (2H, m); R_f 0.56 (AcOEt/hexane 1:4).

7.1.6. 7α-(5-Bromo-2-penten-1-yl)-3,3-ethylenedioxy-17β-methoxymethoxy-5α-androstane (11a). This compound was prepared using a procedure similar to that described for **11e**. Yield: 79%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.83 (3H, s), 0.90–2.38 (23H, m), 2.50–2.68 (2H, m), 3.34 (3H, s), 3.37 (2H, t, J = 7.3 Hz), 3.52 (1H, t, J = 8.3 Hz), 3.92 (4H, s), 4.62 (2H, d, J = 1.8 Hz), 5.30–5.46 (2H, m); R_f 0.50 (AcOEt/hexane 1:4).

7.1.7. 7α-(7-Bromo-2-hepten-1-yl)-3,3-ethylenedioxy-17β-methoxymethoxy-5α-androstane (11b). This compound was prepared using a procedure similar to that described for **11e**. Yield: 74%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.77 (3H, s), 0.84 (3H, s), 0.93–2.32 (29H, m), 3.35 (3H, s), 3.40 (2H, t, J = 6.8 Hz), 3.52 (1H, t, J = 8.3 Hz), 3.93 (4H, s), 4.62 (2H, d, J = 1.5 Hz), 5.25–5.46 (2H, m); R_f 0.53 (AcOEt/hexane 1:4); HRMS calcd for $C_{30}H_{50}O_4Br$ 553.2892. Found 553.2868.

7.1.8. 7α-(9-Bromo-2-nonen-1-yl)-3,3-ethylenedioxy-17β-methoxymethoxy-5α-androstane (11c). This compound was prepared using a procedure similar to that described for **11e**. Yield: 69%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.77 (3H, s), 0.83 (3H, s), 0.92–2.30 (33H, m), 3.34 (3H, s), 3.40 (2H, t, J = 6.8 Hz), 3.52 (1H, t, J = 8.2 Hz), 3.92 (4H, s), 4.62 (2H, d, J = 1.6 Hz), 5.18–5.43 (2H, m); HRMS calcd for $C_{32}H_{54}O_4Br$ 581.3205. Found 581.3227.

7.1.9. 7α-(11-Bromo-2-undecen-1-yl)-3,3-ethylenedioxy-17β-methoxymethoxy-5α-androstane (11d). This compound was prepared using a procedure similar to that described for **11e**. Yield: 98%; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.77 (3H, s), 0.84 (3H, s), 0.95–2.32 (37H, m), 3.35 (3H, s), 3.41 (2H, t, J = 6.9 Hz), 3.52 (1H, t, J = 8.2 Hz), 3.92 (4H, s), 4.62 (2H, d, J = 1.7 Hz), 5.20–5.45 (2H, m).

7.1.10. 7α-(13-Bromotridecyl)-3,3-ethylenedioxy-17β-methoxymethoxy-5α-androstane (12b). To a solution of **11e** (55.3 mg, 0.0867 mmol) in AcOEt (2 ml) was added 10% Pd/C (10 mg), and the mixture was stirred for 13 h at room temperature in a hydrogen atmosphere. After filtering the reaction mixture, the solvent was distilled off at reduced pressure to give **12b** (47.4 mg, 86%) as colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.84 (3H, s), 0.94–2.10 (45H, m), 3.34 (3H, s), 3.41 (2H, t, J = 6.9 Hz), 3.53 (1H, t, J = 8.3 Hz), 3.93 (4H, s), 4.62 (2H, d, J = 1.8 Hz); R_f 0.56 (AcOEt/hexane 1:4).

7.1.11. 17β-Hydroxy-7α-{13-(4,4,5,5,5-pentafluoropentylsulfanyl)tridecyl}-5α-androstan-3-one (13b). 4,4,5,5,5-Pentafluoropentanethioacetate (35.0 mg, 0.148 mmol) was dissolved in MeOH (1 ml) and then 1 M MeONa in MeOH (0.12 ml) was added dropwise. After stirring for 30 min, a solution of **12b** (47.4 mg, 0.0742 mmol) in THF (1 ml) was added to the reaction mixture. After stirring for 18 h, water was added to the reaction mixture and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. After filtering, the solvent was distilled off at reduced pressure. The obtained residue was dissolved in acetone (2 ml), and then 2 N HCl (0.5 ml) was added. The mixture was heated under reflux for 3 h at 60 °C. After cooling to 0 °C, water was added and extracted with $CHCl_3$. The organic layer was washed with brine and dried with $MgSO_4$. After filtering, the solvent was distilled off at reduced pressure. Purification by silica gel column chromatography (AcOEt/hexane 1:4) gave **13b** (40.2 mg, 82%) as colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 1.04 (3H, s), 0.88–2.40 (49H, m), 2.50 (2H, t, J = 7.3 Hz), 2.59 (2H, t, J = 7.9 Hz), 3.58–3.70 (1H, m); R_f 0.32 (AcOEt/hexane 1:4).

7.1.12. 17β-Hydroxy-7α-{13-(4,4,5,5,5-pentafluoropentylsulfinyl)tridecyl}-5α-androstan-3-one (16e). To a solution of **13b** (26.0 mg, 0.0391 mmol) in THF (1 ml) were added OXONE® (14.4 mg, 0.0234 mmol) and water (0.2 ml) at 0 °C. After stirring for 30 min, satd $NaHCO_3$ aq was added to the reaction mixture and extracted with AcOEt. The organic layer was washed with brine and dried with $MgSO_4$. After filtering, the solvent was distilled off at reduced pressure. Purification by silica gel column chromatography (AcOEt/hexane 2:1) gave **16e** (19.5 mg, 73%) as colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 1.04 (3H, s), 0.98–2.40 (50H, m), 2.58–2.82 (4H, m), 3.60–3.70 (1H, m); MS (FAB) m/z 681 [(M+H)$^+$]; HRMS calcd for $C_{37}H_{62}F_5O_3S$ 681.4340. Found 681.4355; R_f 0.10 (AcOEt/hexane 1:4).

7.1.13. 17β-Hydroxy-7α-{5-(4,4,5,5,5-pentafluoropentylsulfinyl)pentyl}-5α-androstan-3-one (16a). This compound was prepared from **11a** using a procedure similar to that described for **16e**. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 1.04 (3H, s), 0.98–2.40 (34H, m), 2.58–2.82 (4H, m), 3.60–3.70 (1H, m); MS (FAB) m/z 569 [(M+H)$^+$]; HRMS calcd for $C_{29}H_{46}O_3F_5S$ 569.3088. Found 569.3116.

7.1.14. 7α-[7-(4,4,5,5,5-Pentafluoropentylsulfanyl)hept-2-enyl]-3,3-ethylenedioxy-17β-methoxymethoxy-5α-androstane (14a). 4,4,5,5,5-Pentafluoropentanethioacetate (29.0 mg, 0.123 mmol) was dissolved in MeOH (1 ml) and then 1 M MeONa in MeOH (0.115 ml) was added dropwise. After stirring for 15 min, a solution of **11b** (45.5 mg, 0.0822 mmol) in THF (0.5 ml) was added to the reaction mixture. After stirring for 13 h, water was added to the reaction mixture and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. After filtering, the solvent was distilled off at reduced pressure. Purification by silica gel column chromatography (AcOEt/hexane 1:10) gave **14a** (49.8 mg, 90%) as colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.77 (3H, s), 0.83 (3H, s), 0.93–2.27 (33H, m), 2.48–2.61 (4H, m), 3.35 (3H, s), 3.52 (1H, t, J = 8.1 Hz), 3.93 (4 H, s), 4.62 (2H, d, J = 1.7 Hz), 5.23–5.44 (2H, m); HRMS calcd for $C_{35}H_{59}NO_4F_5S$ 684.4085. Found 684.4108 [(M+NH$_4$)$^+$].

7.1.15. 17β-Hydroxy-7α-{7-(4,4,5,5,5-pentafluoropentylsulfinyl)hept-2-enyl}-5α-androstan-3-one (15a). To a solution of **14a** (24.5 mg, 0.0367 mmol) in acetone (2.0 ml) was added 2 N HCl (0.5 ml) and the mixture was stirred at 60 °C for 2 h. After cooling to room temperature, water was added to the reaction mixture and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over $MgSO_4$. After filtering, the solvent was distilled off at reduced pressure. The resulting residue was dissolved to THF (1 ml) and then OXONE® (16.6 mg, 0.0271 mmol) and water (0.2 ml) were added at 0 °C. After stirring for 40 min, satd $NaHCO_3$ aq was added to the reaction mixture and extracted with AcOEt. The organic layer was washed with brine and dried with $MgSO_4$. After filtering, the solvent was

distilled off at reduced pressure. Purification by silica gel column chromatography (AcOEt) gave **15a** (17.6 mg, 66%) as colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.95–2.45 (34H, m), 1.04 (3H, s), 2.60–2.82 (4H, m), 3.63 (1H, brs), 5.20–5.43 (2H, m); R_f 0.31 (AcOEt); HRMS calcd for $C_{31}H_{48}O_3F_5S$ 595.3244. Found 595.3244.

7.1.16. 17β-Hydroxy-7α-{7-(4,4,5,5,5-pentafluoropentylsulfinyl)heptyl}-5α-androstan-3-one (16b). To a solution of **15a** (15.4 mg, 0.0259 mmol) in AcOEt (2 ml) was added 10% Pd/C (5.0 mg), and the mixture was stirred for 17 h at room temperature in a hydrogen atmosphere. After filtering the reaction mixture, the solvent was distilled off at reduced pressure. Purification by silica gel column chromatography (AcOEt) gave **16b** (13.7 mg, 89%) as colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 1.04 (3H, s), 0.98–2.40 (38H, m), 2.60–2.82 (4H, m), 3.60–3.70 (1H, m); MS (FAB) *m/z* 597 [$(M+H)^+$]; HRMS calcd for $C_{31}H_{50}O_3F_5S$ 597.3401. Found 597.3402.

7.1.17. 17β-Hydroxy-7α-{9-(4,4,5,5,5-pentafluoropentylsulfinyl)nonyl}-5α-androstan-3-one (16c). This compound was prepared from **11c** using a procedure similar to that described for **16b**. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 1.04 (3H, s), 0.98–2.40 (42H, m), 2.60–2.82 (4H, m), 3.60–3.70 (1H, m); MS (FAB) *m/z* 625 [$(M+H)^+$]; HRMS calcd for $C_{33}H_{54}O_3F_5S$ 625.3714. Found 625.3708.

7.1.18. 17β-Hydroxy-7α-{11-(4,4,5,5,5-pentafluoropentylsulfinyl)undecyl}-5α-androstan-3-one (16d). This compound was prepared from **11d** using a procedure similar to that described for **16b**. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 1.04 (3H, s), 0.98–2.40 (42H, m), 2.60–2.82 (4H, m), 3.60–3.70 (1H, m); MS (FAB) *m/z* 653 [$(M+H)^+$]; HRMS calcd for $C_{35}H_{58}O_3F_5S$ 653.4027. Found 653.4036.

7.1.19. 8-(17β-*tert*-Butyldimethylsilyloxy-5α-androstan-3-one-7α-yl)oct-6-enoic acid methyl ester (17b). To a solution of **7** (596.1 mg, 1.34 mmol) and methyl 6-heptenoate (384.4 mg, 2.70 mmol) in CH_2Cl_2 (5 ml) was added benzylidenebis(tricyclohexylphosphine)dichlororuthenium (57.0 mg, 0.0693 mmol), and the mixture was heated under reflux for 5 h under Ar. After cooling to room temperature, the reaction mixture was purified directly by silica gel column chromatography (AcOEt/hexane 1:10) to give **17b** (527.6 mg, 70%) as brown oil; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.01 (6H, s), 0.71 (3H, s), 0.88 (9H, s), 1.03 (3H, s), 0.90–2.10 (26H, m), 2.18–2.43 (5H, m), 3.51 (1H, t, J = 8.4 Hz), 3.67 (3H, s), 5.18–5.40 (2H, m); R_f 0.43 (AcOEt/hexane 1:4).

7.1.20. 7-(17β-*tert*-Butyldimethylsilyloxy-5α-androstan-3-one-7α-yl)hept-5-enoic acid *tert*-butyl ester (17a). This compound was prepared from **7** and *tert*-butyl 5-hexenoate using a procedure similar to that described for **17b**. Yield: 85%. Brown oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.01 (6H, s), 0.71 (3H, s), 0.87 (9H, s), 1.02 (3H, s), 0.90–2.12 (24H, m), 2.18–2.44 (5H, m), 3.54 (1H, t, J = 8.4 Hz), 3.66 (3H, s), 5.20–5.45 (2H, m); R_f 0.42 (AcOEt/hexane 1:15).

7.1.21. 9-(17β-*tert*-Butyldimethylsilyloxy-5α-androstan-3-one-7α-yl)non-7-enoic acid *tert*-butyl ester (17c). This compound was prepared from **7** and *tert*-butyl 7-octenoate using a procedure similar to that described for **17b**. Yield: 74%. Brown oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.01 (6H, s), 0.71 (3H, s), 0.88 (9H, s), 1.03 (3H, s), 0.90–2.08 (37H, m), 2.15–2.42 (5H, m), 3.52 (1H, t, J = 8.4 Hz), 5.15–5.40 (2H, m); R_f 0.56 (AcOEt/hexane 1:4).

7.1.22. 10-(17β-*tert*-Butyldimethylsilyloxy-5α-androstan-3-one-7α-yl)dec-8-enoic acid methyl ester (17d). This compound was prepared from **7** and methyl 8-nonenoate using a procedure similar to that described for **17b**. Yield: 61%. Brown oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.01 (6H, s), 0.71 (3H, s), 0.87 (9H, s), 1.02 (3H, s), 0.90–2.08 (30H, m), 2.18–2.43 (5H, m), 3.54 (1H, t, J = 8.3 Hz), 3.66 (3H, s), 5.17–5.41 (2H, m); R_f 0.33 (ether/hexane 1:2).

7.1.23. 11-(17β-*tert*-Butyldimethylsilyloxy-5α-androstan-3-one-7α-yl)undec-9-enoic acid methyl ester (17e). This compound was prepared from **7** and methyl 9-decenoate using a procedure similar to that described for **17b**. Yield: 51%. Brown oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.01 (6H, s), 0.71 (3H, s), 0.87 (9H, s), 1.02 (3H, s), 0.90–2.08 (32H, m), 2.17–2.43 (5H, m), 3.53 (1H, t, J = 8.2 Hz), 3.65 (3H, s), 5.17–5.38 (2H, m); R_f 0.21 (AcOEt/hexane 1:15).

7.1.24. 12-(17β-*tert*-Butyldimethylsilyloxy-5α-androstan-3-one-7α-yl)dodec-10-enoic acid methyl ester (17f). This compound was prepared from **7** and methyl 10-undecenoate using a procedure similar to that described for **17b**. Yield: 67%. Brown oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.01 (6H, s), 0.72 (3H, s), 0.88 (9H, s), 1.03 (3H, s), 0.90–2.08 (34H, m), 2.18–2.43 (5H, m), 3.54 (1H, t, J = 8.1 Hz), 3.66 (3H, s), 5.17–5.40 (2H, m); R_f 0.42 (AcOEt/hexane 1:4).

7.1.25. 8-(17β-*tert*-Butyldimethylsilyloxy-5α-androstan-3-one-7α-yl)octanoic acid (18b). To a solution of **17b** (505.5 mg, 0.904 mmol) in AcOEt (30 ml) was added 10% Pd/C (148 mg), and the mixture was stirred at room temperature for 4 h in a hydrogen atmosphere. The reaction mixture was filtered and the solvent was distilled off at reduced pressure. The resulting residue was dissolved in acetone (10 ml) and, after adding 1 N HCl (1 ml), the mixture was heated under reflux for 26 h. After cooling to room temperature, water was added and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated at reduced pressure. Purification by silica gel column chromatography (AcOEt/hexane 1:2–1:1) gave **18b** (362.6 mg, 93%) as yellow oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 1.04 (3H, s), 1.00–1.82 (27H, m), 1.98–2.15 (3H, m), 2.23–2.48 (5H, m), 3.65 (1H, t, J = 8.7 Hz); MS (FAB) *m/z* 433 [$(M+H)^+$]; R_f 0.27 (AcOEt/hexane 1:1).

7.1.26. *N*,*N*-Dimethyl-8-(17β-hydroxy-5α-androstan-3-one-7α-yl)octanamide (23). To a solution of **18b** (9.9 mg, 0.0229 mmol) in THF (0.5 ml) were added EDC (13.0 mg, 0.0678 mmol), HOBt (10.5 mg, 0.0686 mmol), and 2.0 M dimethylamine in THF (68.7 μl, 0.137 mmol). The mixture was stirred at room temperature for 15 h. After adding AcOEt (2.0 ml), the mixture was washed with 1 N HCl, satd $NaHCO_3$ aq, and brine. After being dried over $MgSO_4$, the mixture was filtered through NH silica gel (DM1020, Fuji Silicia Chemical Co., Ltd) and the solvent was distilled off at reduced pressure to give **23** (10.5 mg, 99.7%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 1.04 (3H, s), 1.00–1.83 (28H, m), 1.95–2.16 (3H, m), 2.23–2.47 (5H, m), 2.94 (3H, s), 3.01 (3H, s), 3.65 (1H, t, J = 8.7 Hz); MS (ESI) m/z 460 [(M+H)$^+$]; R_f 0.28 (MeOH/$CHCl_3$ 1:10); HRMS calcd for $C_{29}H_{50}NO_3$ 460.3791. Found 460.3794.

7.1.27. *N*-*n*-Butyl-*N*-methyl-8-(17β-hydroxy-5α-androstan-3-one-7α-yl)octanamide (19a). This compound was prepared from **18b** using a procedure similar to that described for **23**. Yield: 91%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.92 (3/2H, t, J = 7.4 Hz), 0.95 (3/2H, t, J = 7.4 Hz), 1.04 (3H, s), 1.18–2.45 (40H, m), 2.91 (3/2H, s), 2.97 (3/2H, s), 3.25 (2/2H, t, J = 7.6 Hz), 3.35 (2/2H, t, J = 7.6 Hz), 3.64 (1H, t, J = 8.2 Hz); MS (ESI) m/z 502 [(M+H)$^+$]; HRMS calcd for $C_{32}H_{56}NO_3$ 502.4260. Found 502.4239.

7.1.28. *N*-*n*-Butyl-*N*-methyl-9-(17β-hydroxy-5α-androstan-3-one-7α-yl)nonanamide (19b). This compound was prepared from **17c** using a procedure similar to that described for **23**. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.92 (3/2H, t, J = 7.4 Hz), 0.95 (3/2H, t, J = 7.4 Hz), 1.04 (3H, s), 1.18–2.45 (42H, m), 2.91 (3/2H, s), 2.97 (3/2H, s), 3.25 (2/2H, t, J = 7.6 Hz), 3.35 (2/2H, t, J = 7.6 Hz), 3.63 (1H, t, J = 8.2 Hz); MS (ESI) m/z 538 [(M+Na)$^+$]; HRMS calcd for $C_{33}H_{58}NO_3$ 516.4417. Found 516.4391.

7.1.29. *N*-*n*-Butyl-*N*-methyl-10-(17β-hydroxy-5α-androstan-3-one-7α-yl)decanamide (19c). This compound was prepared from **17d** using a procedure similar to that described for **23**. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.92 (3/2H, t, J = 7.4 Hz), 0.95 (3/2H, t, J = 7.4 Hz), 1.04 (3H, s), 1.18–2.45 (44H, m), 2.91 (3/2H, s), 2.97 (3/2H, s), 3.25 (2/2H, t, J = 7.4 Hz), 3.36 (2/2H, t, J = 7.4 Hz), 3.62–3.70 (1H, m); MS (ESI) m/z 530 [(M+H)$^+$]; HRMS calcd for $C_{34}H_{60}NO_3$ 530.4573. Found 530.4575.

7.1.30. *N*-*n*-Butyl-*N*-methyl-11-(17β-hydroxy-5α-androstan- 3-one-7α-yl)undecanamide (19d). This compound was prepared from **17e** using a procedure similar to that described for **23**. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.92 (3/2H, t, J = 7.4 Hz), 0.95 (3/2H, t, J = 7.4 Hz), 1.04 (3H, s), 1.12–2.45 (46H, m), 2.91 (3/2H, s), 2.96 (3/2H, s), 3.25 (2/2H, t, J = 7.4 Hz), 3.36 (2/2H, t, J = 7.4 Hz), 3.64 (1H, br); MS (ESI) m/z 544 [(M+H)$^+$]; HRMS calcd for $C_{35}H_{62}NO_3$ 544.4730. Found 544.4728.

7.1.31. *N*-*n*-Butyl-*N*-methyl-12-(17β-hydroxy-5α-androstan-3-one-7α-yl)dodecanamide (19e). This compound was prepared from **17f** using a procedure similar to that described for **23**. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.92 (3/2H, t, J = 7.4 Hz), 0.95 (3/2H, t, J = 7.4 Hz), 1.04 (3H, s), 1.12–2.45 (46H, m), 2.90 (3/2H, s), 2.96 (3/2H, s), 3.25 (2/2H, t, J = 7.4 Hz), 3.35 (2/2H, t, J = 7.4 Hz), 3.62 (1H, br); MS (ESI) m/z 558 [(M+H)$^+$]; HRMS calcd for $C_{36}H_{64}NO_3$ 558.4886. Found 558.4893.

7.1.32. *N*-Methyl-*N*-propyl-8-(17β-hydroxy-5α-androstan-3-one-7α-yl)octanamide (20). This compound was prepared from **18b** using a procedure similar to that described for **23**. Yield: 58%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.88 (3/2H, t, J = 7.4 Hz), 0.92 (3/2H, t, J = 7.4 Hz), 1.01–1.90 (34H, m), 1.04 (3H, s), 1.95–2.45 (8H, m), 2.91 (3/2H, s), 2.97 (3/2H, s), 3.23 (2/2H, t, J = 7.7 Hz), 3.33 (2/2H, t, J = 7.7 Hz), 3.62–3.69 (1H, m); MS (ESI) m/z 488 [(M+H)$^+$]; HRMS calcd for $C_{31}H_{54}NO_3$ 488.4104. Found 488.4085.

7.1.33. *N*-Isopropyl-*N*-methyl-8-(17β-hydroxy-5α-androstan-3-one-7α-yl)octanamide (21). This compound was prepared from **18b** using a procedure similar to that described for **23**. Yield: 80%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.90–1.82 (28H, m), 1.04 (3H, s), 1.07 (6× 3/5H, d, J = 6.8 Hz), 1.18 (6× 2/5H, d, J = 6.6 Hz), 1.95–2.18 (3H, m), 2.20–2.43 (5H, m), 2.77 (3× 2/5H, 2), 2.80 (3× 3/5H, s), 3.65 (1H, t, J = 8.8 Hz), 4.02–4.15 (1× 2/5H, m), 4.82–4.95 (1× 3/5H, m); MS (FAB) m/z 488 [(M+H)$^+$]; HRMS calcd for $C_{31}H_{54}NO_3$ 488.4104. Found 488.4099.

7.1.34. *N*-Methyl-*N*-ethyl-8-(17β-hydroxy-5α-androstan-3-one-7α-yl)octanamide (22). This compound was prepared from **18b** using a procedure similar to that described for **23**. Yield: 72%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.98–1.90 (32H, m), 1.04 (3H, s), 1.09 (3/2H, t, J = 7.1 Hz), 1.17 (3/2H, t, J = 7.1 Hz), 1.95–2.45 (8H, m), 2.91 (3/2H, s), 2.97 (3/2H, s), 3.34 (2/2H, t, J = 7.1 Hz), 3.41 (2/2H, t, J = 7.7 Hz), 3.62–3.69 (1H, m); MS (ESI) m/z 474 [(M+H)$^+$]; HRMS calcd for $C_{30}H_{52}NO_3$ 474.3947. Found 474.3948.

7.1.35. *N*,*N*-Diethyl-8-(17β-hydroxy-5α-androstan-3-one-7α-yl)octanamide (24). This compound was prepared from **18b** using a procedure similar to that described for **23**. Yield: 95%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 1.04 (3H, s), 1.00–1.83 (34H, m), 1.95–2.16 (3H, m), 2.22–2.47 (5H, m), 3.30 (2H, q, J = 7.1 Hz), 3.37 (2H, q, J = 7.1 Hz), 3.65 (1H, t, J = 9.0 Hz); MS (ESI) m/z 488 [(M+H)$^+$]; HRMS calcd for $C_{31}H_{54}NO_3$ 488.4104. Found 488.4088.

7.1.36. *N*-Benzyl-*N*-methyl-8-(17β-hydroxy-5α-androstan-3-one-7α-yl)octanamide (25). This compound was prepared from **18b** using a procedure similar to that described for **23**. Yield: 85%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.97–1.82 (28H, m), 1.04 (3H, s), 1.95–2.15 (3H, m), 2.22–2.43 (5H, m),

2.92 (3× 4/7H, s), 2.94 (3× 3/7H, s), 3.60–3.70 (1H, m), 4.54 (2× 3/7H, s), 4.59 (2× 4/7H, s), 7.15–7.39 (5H, m); MS (ESI) *m/z* 536 [(M+H)$^+$]; HRMS calcd for $C_{35}H_{54}NO_3$ 536.4104. Found 536.4103.

7.1.37. 8-(17β-Hydroxy-5α-androstan-3-one-7α-yl)-1-(morpholin-4-yl)octan-1-one (27). This compound was prepared from **18b** using a procedure similar to that described for **23**. Yield: 96%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.97–1.82 (28H, m), 1.04 (3H, s), 1.95–2.15 (3H, m), 2.22–2.43 (5H, m), 3.45–3.48 (2H, m), 3.60–3.69 (7H, m); MS (ESI) *m/z* 502 [(M+H)$^+$]; HRMS calcd for $C_{31}H_{52}NO_4$ 502.3896. Found 502.3900.

7.1.38. 8-(17β-Hydroxy-5α-androstan-3-one-7α-yl)-1-(pyrrolidin-1-yl)octan-1-one (28). This compound was prepared from **18b** using a procedure similar to that described for **23**. Yield: 97%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.97–2.10 (35H, m), 1.04 (3H, s), 2.22–2.43 (5H, m), 3.39–3.48 (4H, m), 3.61–3.70 (1H, m); MS (ESI) *m/z* 486 [(M+H)$^+$]; HRMS calcd for $C_{31}H_{52}NO_3$ 486.3947. Found 486.3956.

7.1.39. 8-(17β-Hydroxy-5α-androstan-3-one-7α-yl)-1-(piperidin-1-yl)octan-1-one (29). This compound was prepared from **18b** using a procedure similar to that described for **23**. Yield: 88%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 1.04 (3H, s), 1.00–1.83 (34H, m), 1.95–2.16 (3H, m), 2.21–2.47 (5H, m), 3.39 (2H, t, J = 5.3 Hz), 3.54 (2 H, t, J = 5.3 Hz), 3.65 (1H, t, J = 9.0 Hz); MS (ESI) *m/z* 500 [(M+H)$^+$]; HRMS calcd for $C_{32}H_{54}NO_3$ 500.4104. Found 500.4104.

7.1.40. *N*-Methyl-8-(17β-hydroxy-5α-androstan-3-one-7α-yl)octanamide (31). This compound was prepared from **18b** using a procedure similar to that described for **23**. Yield: 72%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.97–1.85 (28H, m), 1.04 (3H, s), 1.98–2.42 (8H, m), 2.81 (3H, d, J = 4.9 Hz), 3.61–3.70 (1H, m), 5.48 (1H, br); MS (ESI) *m/z* 446 [(M+H)$^+$]; HRMS calcd for $C_{28}H_{48}NO_3$ 446.3634. Found 446.3655.

7.1.41. *N*-Ethyl-8-(17β-hydroxy-5α-androstan-3-one-7α-yl)octanamide (32). This compound was prepared from **18b** using a procedure similar to that described for **23**. Yield: 64%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.97–1.85 (28H, m), 1.04 (3H, s), 1.14 (3H, t, J = 7.3 Hz), 1.98–2.42 (8H, m), 3.24–3.35 (2H, m), 3.65 (1H, t, J = 8.2 Hz), 5.43 (1H, br); MS (FAB) *m/z* 460 [(M+H)$^+$]; HRMS calcd for $C_{29}H_{50}NO_3$ 460.3791. Found 460.3811.

7.1.42. *N*-Butyl-8-(17β-hydroxy-5α-androstan-3-one-7α-yl)octanamide (34). This compound was prepared from **18b** using a procedure similar to that described for **23**. Yield: 92%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.92 (3H, t, J = 7.3 Hz), 0.95–1.82 (32H, m), 1.04 (3H, s), 1.93–2.45 (8H, m), 3.25 (2H, dt, J = 6.1, 7.1 Hz), 3.65 (1H, t, J = 9.0 Hz), 5.44 (1H, br); MS (ESI) *m/z* 488 [(M+H)$^+$]; HRMS calcd for $C_{31}H_{54}NO_3$ 488.4104. Found 488.4099.

7.1.43. *N*,*N*-Dimethyl-7-(17β-hydroxy-5α-androstan-3-one-7α-yl)heptanamide (36). This compound was prepared from **7** using a procedure similar to that described for **23**. Yield: 76%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.85–1.83 (26H, m), 1.04 (3H, s), 1.95–2.15 (3H, m), 2.22–2.45 (5H, m), 2.94 (3H, s), 3.01 (3H, s), 3.65 (1H, t, J = 9.0 Hz); MS (FAB) *m/z* 446 [(M+H)$^+$]; HRMS calcd for $C_{28}H_{48}NO_3$ 446.3634. Found 446.3631.

7.1.44. *N*,*N*-Diethyl-7-(17β-hydroxy-5α-androstan-3-one-7α-yl)heptanamide (37). This compound was prepared from **7** using a procedure similar to that described for **23**. Yield: 7.1%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.82–1.90 (26H, m), 1.04 (3H, s), 1.11 (3H, t, J = 7.2 Hz), 1.17 (3H, t, J = 7.2 Hz), 1.95–2.15 (3H, m), 2.20–2.45 (5H, m), 3.30 (2H, q, J = 7.1 Hz), 3.37 (2H, q, J = 7.1 Hz), 3.65 (1H, t, J = 9.0 Hz); MS (FAB) *m/z* 474 [(M+H)$^+$]; HRMS calcd for $C_{30}H_{52}NO_3$ 474.3947. Found 474.3954.

7.1.45. *N*,*N*-Dihexyl-8-(17β-hydroxy-5α-androstan-3-one-7α-yl)octanamide (26). To a solution of **18b** (10.3 mg, 0.0238 mmol) and *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluromium hexafluorophosphate (30 mg, 0.0789 mmol) in THF (1 ml) were added ^{i}Pr$_2$NEt (25 μl, 0.0167 mmol) and di-*n*-hexylamine (17.2 μl, 0.0735 mmol), and the mixture was stirred at room temperature for 2 h. After adding AcOEt, the reaction mixture was washed with satd $NaHCO_3$ aq, 1 N HCl, and brine. To the organic layer, NH silica gel (Pro. No. DM1020; Fuji Silicia Chemical Co., Ltd) was added and the mixture was stirred for 5 min. After filtering, the solvent was distilled off at reduced pressure. The resulting residue was purified by silica gel column chromatography (MeOH/$CHCl_3$ 1:10) to give **26** (9.5 mg, 67%) as colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.88 (3H, t, J = 6.9 Hz), 0.90 (3H, t, J = 6.9 Hz), 1.04 (3H, s), 1.00–1.83 (44H, m), 1.95–2.16 (3H, m), 2.21–2.47 (5H, m), 3.19 (2H, t, J = 8.5 Hz), 3.28 (2H, t, J = 8.5 Hz), 3.65 (1 H, t, J = 9.0 Hz); MS (ESI) *m/z* 600 [(M+H)$^+$]; HRMS calcd for $C_{39}H_{70}NO_3$ 600.5356. Found 600.5332.

7.1.46. *N*-Propyl-8-(17β-hydroxy-5α-androstan-3-one-7α-yl)octanamide (33). This compound was prepared from **18b** using a procedure similar to that described for **26**. Yield: 99%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.92 (3H, t, J = 7.6 Hz), 0.95–1.82 (30 H, m), 1.04 (3H, s), 1.93–2.45 (8H, m), 3.21 (2H, dt, J = 6.3, 6.8 Hz), 3.65 (1H, t, J = 9.0 Hz), 5.48 (1H, br); MS (FAB) *m/z* 474 [(M+H)$^+$]; HRMS calcd for $C_{30}H_{52}NO_3$ 474.3947. Found 474.3938.

7.1.47. *N*-Hexyl-8-(17β-hydroxy-5α-androstan-3-one-7α-yl)octanamide (35). This compound was prepared from **18b** using a procedure similar to that described for **26**. Yield: 99%. Colorless oil. ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.88 (3H, t, J = 6.9 Hz), 1.04 (3H, s), 1.10–1.82 (36H, m), 1.93–2.45 (8H, m), 3.24 (2H, dt, J = 6.1, 6.9 Hz), 3.65 (1H, t, J = 9.0 Hz), 5.45 (1H, br); MS (ESI) *m/z* 516 [(M+H)$^+$]; HRMS calcd for $C_{33}H_{58}NO_3$ 516.4417. Found 516.4399.

7.1.48. 8-(17β-Hydroxy-5α-androstan-3-one-7α-yl)octanamide (30). To a solution of **18b** (12.6 mg, 0.0291 mmol) in THF (0.5 ml) were added NEt_3 (8.1 μl, 0.0584 mmol) and ClCOOEt (4.2 μl, 0.0441 mmol) dropwise at 0 °C. After stirring for 5 min, ammonia gas was bubbled into the solution for 30 s. After stirring for 30 min, water was added to the reaction mixture and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated at reduced pressure. Purification by silica gel column chromatography (CH_2Cl_2/MeOH 20:1) gave **30** (11.6 mg, 92%) as colorless oil. ^1H NMR (270 MHz, $CDCl_3$) δ 0.76 (3H, s), 0.98–1.83 (28H, m), 1.04 (3H, s), 1.95–2.16 (3H, m), 2.22–2.47 (5H, m), 3.65 (1H, t, $J = 8.3$ Hz), 5.41 (2H, br); MS (FAB) *m/z* 432 [$(M+H)^+$]; HRMS calcd for $C_{27}H_{46}NO_3$ 432.3478. Found 432.3498.

7.2. Biological assay

7.2.1. Competitive binding assay. CHO-K1/hAR cells (5×10^4/well) were plated in 24-well plates and cultured for 2 days. Adhered cells were washed with PBS(−) and replaced with phenol red-free DMEM containing 0.34 nmol/L [^{3}H]mibolerone in the presence or absence of test compound. Nonspecific binding of [^{3}H]mibolerone was determined separately by adding 200-fold excess of cold mibolerone. Following 2 h incubation at 37 °C, cells were washed with PBS(−) and solubilized in 10 mmol/L Tris–HCl, pH 6.8, containing 2% SDS and 10% glycerol. Radioactivity was counted using a scintillation counter.

The binding affinity was described as an IC_{50} value (the concentration of a compound required to inhibit 50% of [^{3}H]mibolerone).

7.2.2. Reporter gene assay. Twenty-four hours before transfection, 1.0×10^5 HeLa cells were cultured in phenol red-free DMEM/5% DCC-FBS on 12-well microplates. Five hundred nanograms/well of MMTV-Luc vector, 100 ng/well pSG5-hAR, and 5 ng/well of *Renilla* Luc vector were transfected into the HeLa cells. The transfection was performed in a liquid culture of the phenol red-free DMEM using 3 ml/well of Lipofectamine. Nine hours after the transfection, the liquid culture was replaced by phenol red-free DMEM/3% DCC-FBS containing 1, 10, 100, 1000, or 10,000 nmol/L of test compound. The transcriptional activity value was measured 48 h after the replacement of the liquid culture. Transcriptional activity was measured with a dual luciferase reporter assay system. The transcriptional activity value was calculated as the value for firefly luciferase divided by the value for sea pansy luciferase. The agonist activity was computed by the following formula and the determined agonist activity was used to compute the FI_5 value (the concentration of a compound-treated group that shows a transcriptional activity five times the transcriptional activity of the group without a test compound).

$$\text{Agonist activity} = \text{Compound-treated transcriptional activity} / \text{Non-treated transcriptional activity}.$$

Twenty-four hours before transfection, 1.0×10^5 HeLa cells were cultured in phenol red-free DMEM/5% DCC-FBS on 12-well microplates. Five hundred nanograms/well of MMTV-Luc vector, 100 ng/well of pSG5-hAR, and 5 ng/well of *Renilla* Luc vector were transfected into the HeLa cells. The transfection was performed in a liquid culture of the phenol red-free DMEM using 3 ml/well of Lipofectamine. Nine hours after the transfection, the liquid culture was replaced by phenol red-free DMEM/3% DCC-FBS containing 0.1 nmol/L of DHT and 1, 10, 100, 1000, or 10,000 nmol/L of test compound. The transcriptional activity value was measured 48 h after the replacement of the liquid culture. Transcriptional activity was measured with a dual luciferase reporter assay system. The transcriptional activity value was calculated as the value for firefly luciferase divided by the value for sea pansy luciferase. The antagonist activity was computed by the following formula and the determined antagonist activity was used to compute the IC_{50} value (the concentration for a compound-treated group at which it shows a 50% decrease in the transcriptional activity of DHT 0.1 nmol/L when the compound was not added).

$$\text{Antagonist activity}\,(\%) = (\text{Compound-treated transcriptional activity} / \text{Non-treated transcriptional activity}) \times 100.$$

Acknowledgments

The authors thank Mr. Kenichiro Kotake and coworkers for HRMS measurements of the compounds, and Ms. Frances Ford of Chugai Pharmaceutical Co., Ltd for assistance with English usage.

References and notes

1. Landis, S. H.; Murray, T.; Bolden, S.; Wingo, P. A. *CA Cancer J. Clin.* **1999**, *49*, 8.
2. Stewart, A. B.; Lwaleed, B. A.; Douglas, D. A.; Birch, B. R. *Curr. Med. Chem. Anti-canc. Agents* **2005**, *5*, 603.
3. Kennealey, G. T.; Furr, B. J. *Urol. Clin. North Am.* **1991**, *18*, 99.
4. Blackledge, G.; Kolvenbag, G.; Nash, A. *Anticancer Drugs* **1996**, *7*, 27.
5. Tyrrell, C. J.; Altwein, J. E.; Klippel, F.; Varenhorst, E.; Lunglmayr, G.; Boccardo, F.; Holdaway, I. M.; Haefliger, J. M.; Jordaan, J. P. *J. Urol.* **1991**, *146*, 1321.
6. Eisenberger, M. A.; Blumenstein, B. A.; Crawfold, E. D.; Miller, G.; McLeod, D. G.; Leohrer, P. J.; Wilding, G.; Sears, K.; Culkin, D. J.; Thompson, I. M., Jr.; Bueschen, A. J.; Lowe, B. A. *N. Engl. J. Med.* **1998**, *339*, 1036.
7. Wirth, M. P.; Froschermaier, S. E. *Urol. Res.* **1997**, *25*(Suppl. 2), S67.
8. Kemppainen, J. A.; Wilson, E. M. *Urology* **1996**, *48*, 157.
9. Brunner, N.; Frandsen, T. L.; Holst-Hansen, C.; Bei, M.; Thompson, E. W.; Wakeling, A. E.; Lippman, M. E.; Clarke, R. *Cancer Res.* **1993**, *53*, 3229.
10. Bowler, J.; Lilley, T. J.; Pittam, J. D.; Wakeling, A. E. *Steroids* **1989**, *54*, 71.

11. Osborne, C. K.; Pippen, J.; Jones, S. E.; Parker, L. M.; Ellis, M.; Come, S.; Gertler, S. Z.; May, J. T.; Burton, G.; Dimery, I.; Webster, A.; Morris, C.; Elledge, R.; Buzdar, A. *J. Clin. Oncol.* **2002**, *20*, 3386.
12. Howell, A.; Robertson, J. F. R.; Quaresma Albano, J.; Aschermannova, A.; Mauriac, L.; Kleeberg, U. R.; Vergote, I.; Erikstein, B.; Webster, A.; Morris, C. *J. Clin. Oncol.* **2002**, *20*, 3396.
13. Pike, A. C. W.; Brzozowski, A. M.; Walton, J.; Hubbard, R. E.; Thorsell, A. G.; Li, Y. L.; Gustafsson, J. Å.; Carlquist, M. *Structure* **2001**, *9*, 145.
14. Ishioka, T.; Tanatani, A.; Nagasawa, K.; Hashimoto, Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2655.
15. Hashimoto, Y.; Miyachi, H. *Bioorg. Med. Chem.* **2005**, *13*, 5080.
16. Matias, P. M.; Donner, P.; Coelho, R.; Thomaz, M.; Peixoto, C.; Macedo, S.; Otto, N.; Joschko, S.; Scholz, P.; Wegg, A.; Bäsler, S.; Schäfer, M.; Egner, U.; Carrondo, M. A. *J. Biol. Chem.* **2000**, *275*, 26164.
17. Brzozowski, A. M.; Pike, A. C. W.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engström, O.; Öhman, L.; Greene, G. L.; Gustafsson, J. Å.; Carlquist, M. *Nature* **1997**, *389*, 753.
18. He, W.; Huang, F.-C.; Morytko, M.; Jariwala, N.; Yu, K.-T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2825.
19. O'Reilly, J. M.; Li, N.; Duax, W. L.; Brueggemeier, R. W. *J. Med. Chem.* **1995**, *38*, 2842.
20. Nickisch, K.; Laurent, H. *Tetrahedron Lett.* **1988**, *29*, 1533.
21. Brümmer, O.; Rückert, A.; Blechert, S. *Chem. Eur. J.* **1997**, *3*, 441.
22. Kanbe, Y.; Kim, M.-H.; Nishimoto, M.; Ohtake, Y.; Kato, N.; Tsunenari, T.; Taniguchi, K.; Ohizumi, I.; Kaiho, S.; Morikawa, K.; Jo, J.-C.; Lim, H.-S.; Kim, H.-Y. *Bioorg. Med. Chem.* **2006**, *14*, 4803.
23. Wong, C.-I.; Kelce, W. R.; Sar, M.; Wilson, E. M. *J. Biol. Chem.* **1995**, *270*, 19998.
24. Hollenberg, S. M.; Evans, R. M. *Cell* **1988**, *55*, 899.
25. Simental, J. A.; Sar, M.; Lane, M. V.; French, F. S.; Wilson, E. M. *J. Biol. Chem.* **1991**, *266*, 510.