Discovery of an Orally-Active Nonsteroidal Androgen Receptor Pure Antagonist and the Structure–Activity Relationships of Its Derivatives

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The 3-(4-cyano-3-trifluoromethylphenyl)-5,5-dimethylthiohydantoin derivatives which have carboxy-terminal side chains were synthesized and their agonistic/antagonistic activities against androgen receptor (AR) measured. Among them, compound 13b showed antagonistic activity (IC_{50}=130 nM) with no agonistic activity even at 10000 nM. This compound exhibited significant metabolic stability and oral antiandrogenic activity (ED_{50}= 7 mg/kg).

Key words androgen receptor; pure antagonist; prostate cancer

Prostate cancer is the most common cancer amongst men and the second most common malignant cause of male death after lung cancer in the U.S.[1] Since the growth of prostate cancer is dependent on androgen, androgen receptor (AR) antagonists such as flutamide (**1**), a precursor of its active form hydroxyflutamide (**2**), and bicalutamide (**3**) are currently used as hormone therapy (Chart 1).[2] These antiandrogens exhibit good efficacy in many cases and comprise an important part of effective therapeutics.[3—6] However, a considerable problem with these antiandrogens is that recurrence occurs after a short period of response.[7] They have partial agonistic activities at high concentration *in vitro*,[8] which may attribute to recurrence. Therefore, the search for new antiandrogenic agents that exhibit no agonistic activities, so-called "AR pure antagonists," has been conducted.[9,10]

We previously reported that some steroidal compounds that have a side chain at the position 7α such as compound **4**, exhibited AR pure antagonistic activities in reporter gene assay (RGA) (Chart 1).[11,12] Since the steroidal core structures have intrinsic agonistic activities, the side chains are critical for pure antagonistic activities. Drawing on the analogy between the estrogen receptor (ER), for which the molecular mechanisms of the expression of antagonistic activities have been investigated,[13] and AR, the side chain should inhibit the folding of helix 12 of the ligand binding domain (LBD), which is necessary for agonistic activity. Thus, we considered the introduction of a side chain to an AR affinity ligand to be a useful strategy for the discovery of AR pure antagonists. However, our compounds didn't show antiandrogenic activity *in vivo*, probably due to the lack of metabolic stability of the steroidal core structures.[12]

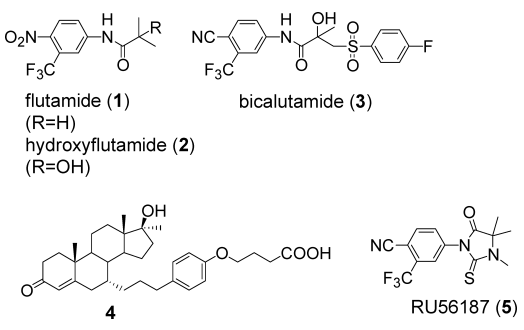

Chart 1. Structures of AR Antagonists

Among a number of nonsteroidal compounds that have been reported to have binding affinities for AR, thiohydantoin derivative RU56187 (**5**) has a high affinity for AR and oral antiandrogenic activity *in vivo* (Chart 1).[14] But this compound also showed agonistic activities in RGA.[15,16] Coupled with our previous results, we speculated that these agonistic activities are caused by the lack of a side chain to inhibit the folding of helix 12. The nitrogen atom on position 1 in thiohydantoin seemed appropriate for the introduction of a side chain and we therefore hypothesized that our strategy could be applied to thiohydantoin to discover an orally active nonsteroidal AR pure antagonist.

In our previous report, terminal-carboxylic acid side chains were introduced to the steroidal core structures because the carboxylic acid was expected to increase the water-solubility and oral absorption of the compounds.[12,17] Unfortunately, as mentioned above, the compounds did not show *in vivo* antiandrogenic activity, probably due to the lack of metabolic stability of the steroidal core structure. However, some compounds exhibited AR pure antagonistic activities in RGA, suggesting that the terminal-carboxylic acid side chains are valuable for the discovery of AR pure antagonists. Furthermore, it was found that the length or the structure of the linker part of the side chain significantly affects the pure antagonistic activities. Therefore, we decided to introduce terminal-carboxylic acid side chains with various types of linkers to the thiohydantoin core structure in order to clarify the structure–activity relationships and discover orally active AR pure antagonists.

We here report the synthesis of novel thiohydantoin derivatives and their structure–activity relationships for AR pure antagonistic activities. Moreover, we report that one of the derivatives showed a high metabolic stability *in vitro* and antiandrogenic activity from oral administration *in vivo*.

Results and Discussion

The target compounds **13a**—**h** and **18a**—**d** were prepared according to the synthetic sequences outlined in Charts 2—4.

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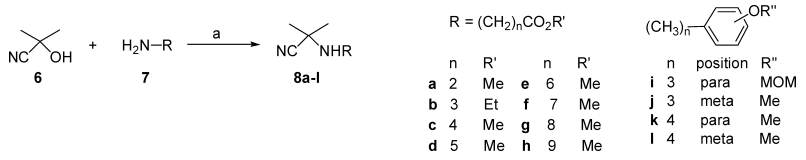
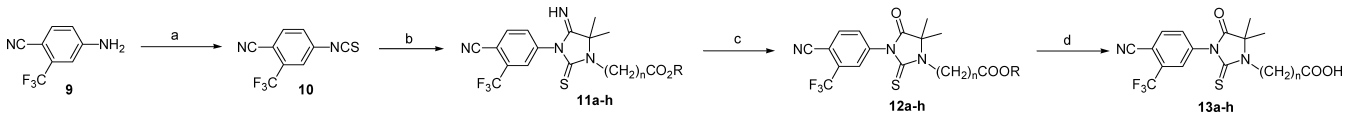


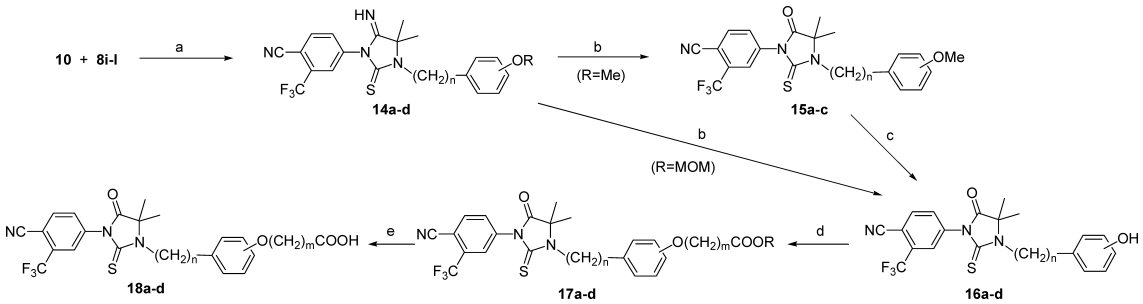
Reagents and conditions: (a) NEt_3 or MeOH, r.t.

Chart 2



Reagents and conditions: (a) thiophosgene, THF–H_2O; (b) compounds **8a**—**h**, NEt_3, THF, reflux; (c) 2 N-HCl, EtOH, reflux; (d) 2 N-NaOH, EtOH, r.t.

Chart 3



Reagents and conditions: (a) NEt_3, THF, reflux; (b) 6 N-HCl, dioxane, 80 °C; (c) BBr_3, CH_2Cl_2, −78 to 0 °C; (d) $Br(CH_2)_mCOOR$, NaH, DMF, 0 °C to r.t.; (e) 2 N-NaOH, MeOH, r.t.

Chart 4

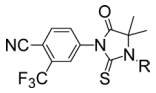
In the preparation of aminonitriles **8a**—**l** by the coupling of corresponding amines **7** with acetone cyanohydrin **6**, NEt_3 was used when the amine was HCl salt (Chart 2). Isothiocyanate **10**, which was derived from **9** and thiophosgene, was coupled with compounds **8a**—**l** in THF in the presence of NEt_3 under reflux to give 5-imino-2-thioxoimidazolidines **11a**—**h** and **14a**—**d**. The imino groups and terminal esters of compounds **11a**—**h** were hydrolyzed successively to give methylene linker compounds **13a**—**h** (Chart 3). Compound **14a**, in which the protecting group of phenolic OH was the methoxymethyl group, was converted to phenol-terminal thiohydantoin **16a** directly by heating in 6 N-HCl and dioxane. Compounds **14b**—**d**, in which the hydroxyl groups were protected by the methyl group, were converted to compounds **15a**—**c** under the same conditions followed by deprotection of the methyl group by BBr_3. Alkylation of the hydroxyl groups of **16a**—**d** followed by hydrolysis of the terminal esters gave phenyl-inserted derivatives **18a**—**d** (Chart 4).

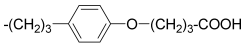
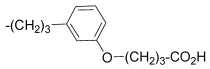
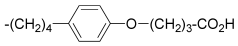
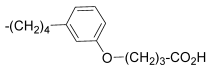
The synthesized compounds were evaluated for their *in vitro* binding affinities and agonist/antagonist activities for AR using the same procedures as reported previously.[11] The binding affinity for AR was determined by displacement of [^{3}H]-mibolerone with the test compound utilizing CHO-K1/hAR cells. The agonistic and antagonistic activities of the compounds for AR were determined by RGA using hAR-transfected Hela cells. Antagonistic activity was determined by the IC_{50} value, the concentration of a compound that inhibits the transcriptional activity of 0.1 nM of DHT by 50%. To determine agonistic activity, we calculated the value of EC_5, the concentration of a compound-treated group in which the transcriptional activity is 5% of the transcriptional activity of 0.1 nM of DHT. The maximum transcriptional activity of each compound indicated as its efficacy (% of 0.1 nM of DHT). A "pure antagonist" was defined as having an EC_5 value greater than 10000 nM.

First, we evaluated RU56187 (**5**) for its binding and transcriptional activities for AR in our assay systems to estimate the validity of the compound as a template (Table 1, Fig. 1). As shown in Fig. 1, it exhibited remarkable agonistic activity at concentration as low as 1 nM. Although it tended to show antagonistic activities at lower concentrations, its own agonistic activities diminished the effect at higher concentrations, thus strongly suggesting that this compound is an appropriate template for the introduction of a side chain to verify our hypothesis.

The agonistic/antagonistic activities of the compounds with a terminal-carboxylic acid side chain are shown in Table 1. The activities of the compounds with a simple alkyl side chain (**13a**—**h**) were found to be influenced by the length of the alkyl chain. Compound **13a**, with two methylene groups, did not show any agonistic or antagonistic activities, possibly because of an electric repulsion of a carboxyl group in close proximity to the hydrophobic ligand binding pocket in the LBD of AR. Compounds with three to five methylene groups (**13b**—**d**) exhibiting antagonistic activities of IC_{50}s of 130—800 nM, with no agonistic activities even at 10000 nM, were best suited to be pure antagonists. Among them, **13b** showed the highest antagonistic activity (IC_{50}=130 nM). Compounds with more than 5 methylene groups in the side chain tended to show partial agonistic activities (**13e**—**h**). The difference

Table 1. Binding and Agonistic/Antagonistic Activities[a] of Thiohydantoin Derivatives



Compound	R	Binding IC_{50} (nM)	RGA	
			EC_5 (nM) efficacy (%)	IC_{50} (nM)
RU56187 (**5**)	Me	15	<1 (135)	ND[b]
13a	$-(CH_2)_2CO_2H$	>10000	>10000 (0.5)	>10000
13b	$-(CH_2)_3CO_2H$	3900	>10000 (2.0)	130
13c	$-(CH_2)_4CO_2H$	3800	>10000 (3.3)	330
13d	$-(CH_2)_5CO_2H$	>10000	>10000 (3.1)	800
13e	$-(CH_2)_6CO_2H$	6000	2700 (9.6)	720
13f	$-(CH_2)_7CO_2H$	460	3500 (7.9)	380
13g	$-(CH_2)_8CO_2H$	180	4500 (6.6)	200
13h	$-(CH_2)_9CO_2H$	150	3000 (8.8)	89
18a		180	370 (28)	160
18b		59	110 (26)	240
18c		100	11 (33)	60
18d		35	54 (16)	45

a) All data are mean values of duplicate experiments. *b*) ND: Not determined.

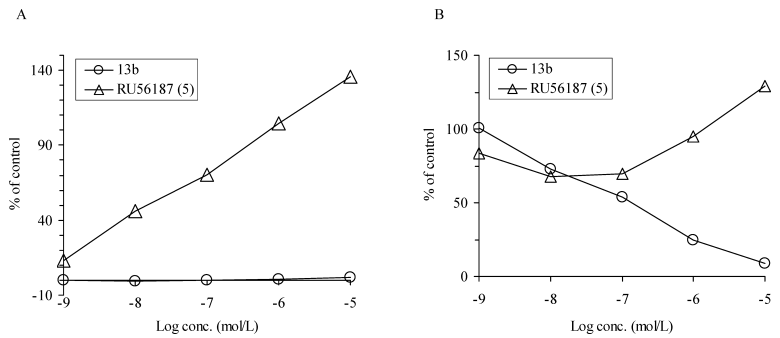


Fig. 1. Agonistic and Antagonistic Activities of RU56187 (**5**) and Compound **13b** in Reporter Gene Assay with hAR-Transfected Hela Cells

(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.

in pure antagonistic activities between **13b** and **13e** was investigated using the docking model illustrated in Fig. 2. In this model, the side-chain carboxyl group of **13b** locates at a region in which the hydrophobic helix 12 would be located in the agonistic form, leading to the inhibition of the folding of helix 12. In the case of **13e**, the interaction of the terminal carboxyl group with His874 would lead the side chain in a direction allowing the folding of helix 12.

We also investigated the effects of a side chain with a phenyl ring in the center. Previously, we reported that the side chain with a 4-(3-carboxypropoxy)phenylpropyl group and a 3-(3-carboxypropoxy)phenylbutyl group exhibited pure antagonistic activities when introduced at position 7α of 17α-methyltestosterone.[12] However, compounds **18a** and **18d**, which have these groups as a side chain, showed relatively high agonistic activities with EC_5s of 370 nM and 54 nM, respectively. We also investigated the activities of compounds **18b** and **18c**, in which the substituted position of the terminal carboxypropyloxy groups were changed. However, these compounds also exhibited only partial agonistic activities. The differences in the pure antagonistic activities between **4** and **18a** were also investigated by a docking model (Fig. 3). The side-chain carboxyl group of **4** locates at a region in which hydrophobic helix 12 would be found in the agonistic form, resulting in pure antagonistic activity. In the case of **18a**, the interaction of the terminal carboxyl group with the imidazole of His874 and the NH group of the main chain of Leu741 would lead the side chain in a direction which allows the folding of helix 12.

The metabolic stability of the two thiohydantoin deriva-

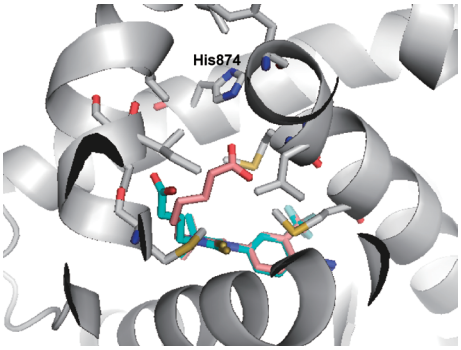


Fig. 2. Docking Model of Compounds **13b** (Blue) and **13e** (Magenta) to AR (Gray)

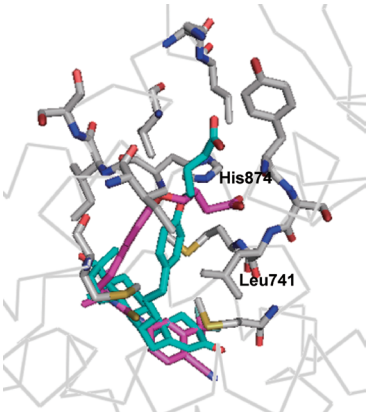


Fig. 3. Docking Model of Compounds **4** (Blue) and **18a** (Magenta) to AR (Gray)

Table 2. Metabolic Stabilities of Compounds in Mouse-Liver Microsomes

Compound	CL_{int} (μl/min/mg protein)
13b	11.5
18a	10.3
4	219

tives was examined in mouse liver microsomes (Table 2). Compound **13b**, the most potent AR pure antagonist in this study, showed significantly high metabolic stability. Furthermore, compound **18a** also exhibited high metabolic stability compared to that of compound **4**, which is a steroidal derivative with a side chain identical to that of **18a**. These results suggest that the thiohydantoin core structure compared to the steroidal core structure contributes significantly to the metabolic stability.

Last, the antiandrogenic activities of **13b** were evaluated on seminal vesicle (SV) wet weights in castrated mice (Fig. 4). Compound **13b** inhibited seminal vesicle wet-weight gain by 10 μg/body subcutaneously (s.c.) of testosterone propionate (TP) dose dependently (ED_{50}=7 mg/kg). Furthermore, at a dose of 50 mg/kg, the activity reached almost the same level as with castration.

In summary, we have discovered novel thiohydantoin derivatives which have a carboxy-terminal side chain similar to AR pure antagonists. The structure of a side chain was found

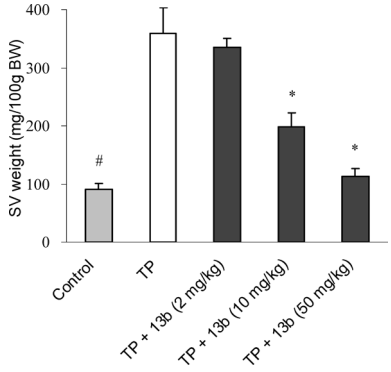


Fig. 4. Antiandrogenic Activities on Seminal Vesicle (SV) Wet Weights in Mice

Each bar represents the mean±S.E.M. (n=4—5 animals per group). $*p<0.05$ compared with the TP group by Dunnett's multiple-comparison test. $\#p<0.05$ compared with the TP group by Student's t test.

to affect pure antagonistic activity. Compound **13b**, one of the pure antagonists found in this investigation, exhibited substantially high *in vitro* metabolic stabilities compared to a steroidal compound. In addition, **13b** showed obvious antiandrogenic activities with an ED_{50} of 7 mg/kg and a level of efficacy similar to castration.

Experimental

Column chromatography was carried out on Merck Silicagel 60 (230—400 mesh) if not otherwise specified. *Rf* was determined using Merck Silicagel 60 F^{254} plates. ^{1}H-NMR spectra were recorded on JEOL EX-270 or JEOL ECP-400. Mass spectra (MS) were measured by Thermo Electron LCQ Classic (ESI) or Shimadzu GCMS-QP5050A (EI). High resonance mass spectra (HR-MS) were recorded by a Micromass Q-Tof Ultima API mass spectrometer or Applied Biosystems QSTAR XL MS/MS system.

Typical Procedures for Synthesis of Carboxyalkylthiohydantoins (13a—h). 4-[(Cyanodimethylmethyl)amino]butyric Acid Ethyl Ester (8b) The mixture of acetone cyanohydrin (**6**) (1.0 g, 11.8 mmol) and 4-aminobutyric acid ethyl ester HCl salt (**7b**) (1.97 g, 11.8 mmol) in NEt_3 (6 ml, 43.0 mmol) was stirred at room temperature for 1 h. Water was added and the mixture was extracted with ether. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under vacuum to give crude compound **8b** (1.91 g, 82%) as an oil. This compound was used in the next step without purification. *Rf* 0.38 (AcOEt/Hexane=1 : 2); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.26 (3H, t, J=7.1 Hz), 1.45 (6H, s), 1.78—1.88 (2H, m), 2.40 (2H, t, J=7.3 Hz), 2.76 (2H, t, J=6.5 Hz), 4.14 (2H, q, J=7.1 Hz).

4-[3-(4-Cyano-3-trifluoromethylphenyl)-4-imino-5,5-dimethyl-2-thioxoimidazolidin-1-yl]butyric Acid Ethyl Ester (11b) To a solution of 4-isothiocyanato-2-trifluoromethylbenzonitrile (**10**) (1.2 g, 5.26 mmol) and compound **8b** (1.1 g, 5.37 mmol) in THF (20 ml) was added NEt_3 (0.2 ml, 1.43 mmol) and the mixture was stirred at reflux for 50 min. After cooling to room temperature, the mixture was concentrated under vacuum and purified by silica gel column chromatography (AcOEt/Hexane=1 : 1) to give compound **11b** (938 mg, 42%) as an oil. *Rf* 0.28 (AcOEt/hexane=2 : 1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.29 (3H, t, J=7.2 Hz), 1.59 (6H, s), 2.05—2.21 (2H, m), 2.44 (2H, t, J=6.8 Hz), 3.69—3.75 (2H, m), 4.17 (2H, q, J=7.2 Hz), 7.44 (1H, s), 7.64—8.03 (3H, m).

4-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]butyric Acid Ethyl Ester (12b) To a solution of compound **11b** (938 mg, 2.2 mmol) in EtOH (10 ml) was added 2 N-HCl (10 ml) and the mixture was stirred at reflux for 1 h. After cooling to room temperature, the mixture was extracted with $CHCl_3$. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated under vacuum. The purification by silica gel column chromatography (MeOH/$CHCl_3$=0 : 1—1 : 30) gave compound **12b** (765 mg, 81%) as an oil. *Rf* 0.70 (AcOEt/hexane=2 : 1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.29 (3H, t, J=7.1 Hz), 1.62 (6H, s), 2.11—2.20 (2H, m), 2.45 (2H, t, J=6.7 Hz), 3.73—3.79 (2H, m), 4.17 (2H, q, J=7.1 Hz), 7.76 (1H, d, J=8.3 Hz), 7.89 (1H, s), 7.95 (1H, d, J=8.3 Hz); MS (ESI) m/z: 428 [$(M+H)^+$].

4-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]butyric Acid (13b) To a solution of compound **12b**

(765 mg, 1.8 mmol) in EtOH (8 ml) was added 2 N-NaOH (8 ml) and the mixture was stirred at room temperature for 1.5 h. After addition of 2 N-HCl the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under vacuum. The purification by silica gel column chromatography (MeOH/$CHCl_3$=1 : 100) gave compound **13b** (445 mg, 64%) as a white solid. mp 161—162 °C (AcOEt–hexane); *Rf* 0.13 (AcOEt/Hexane=1 : 1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.61 (6H, s), 2.14—2.20 (2H, m), 2.54 (2H, t, *J*=6.8 Hz), 3.75—3.81 (2H, m), 7.77 (1H, dd, *J*=1.7, 8.1 Hz), 7.89 (1H, d, *J*=1.7 Hz), 7.95 (1H, d, *J*=8.1 Hz); MS (ESI) *m/z*: 400 [(M+H)$^+$]; *Anal.* Calcd for $C_{17}H_{16}F_3N_3O_3S$: C, 51.12; H, 4.04; N, 10.52. Found: C, 51.34; H, 4.16; N, 10.64.

Compounds **13a** and **13c**—**h** were prepared by a procedure similar to that described for **13b**.

3-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propionic Acid (13a) White solid. mp 180—181 °C (AcOEt–hexane); *Rf* 0.10 (AcOEt/Hexane=1 : 1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.61 (6H, s), 3.01 (2H, t, *J*=7.4 Hz), 4.02 (2H, t, *J*=7.4 Hz), 7.77 (1H, dd, *J*=1.7, 8.3 Hz), 7.88 (1H, d, *J*=1.7 Hz), 7.96 (1H, d, *J*=8.3 Hz); MS (ESI) *m/z*: 386 [(M+H)$^+$]; *Anal.* Calcd for $C_{16}H_{14}F_3N_3O_3S$: C, 49.87; H, 3.66; N, 10.90. Found: C, 50.10; H, 3.78; N, 11.13.

5-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]pentanoic Acid (13c) White solid. mp 117—118 °C (AcOEt–hexane); *Rf* 0.10 (AcOEt/hexane=1 : 1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.59 (6H, s), 1.71—1.98 (4H, m), 2.47 (2H, t, *J*=7.1 Hz), 3.71 (2H, t, *J*=8.1 Hz), 7.76 (1H, dd, *J*=1.6, 8.1 Hz), 7.88 (1H, d, *J*=1.6 Hz), 7.95 (1H, d, *J*=8.1 Hz); MS (ESI) *m/z*: 414 [(M+H)$^+$]; *Anal.* Calcd for $C_{18}H_{18}F_3N_3O_3S$: C, 52.29; H, 4.39; N, 10.16. Found: C, 52.32; H, 4.45; N, 10.29.

6-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]hexanoic Acid (13d) Colorless oil. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.40—1.55 (2H, m), 1.58 (6H, s), 1.65—1.94 (4H, m), 2.41 (2H, t, *J*=7.1 Hz), 3.66—3.72 (2H, m), 7.77 (1H, dd, *J*=1.7, 8.3 Hz), 7.89 (1H, d, *J*=1.7 Hz), 7.95 (1H, d, *J*=8.3 Hz); MS (ESI) *m/z*: 428 [(M+H)$^+$]; HR-MS Calcd for $C_{19}H_{21}N_3O_3F_3S$ 428.1250. Found 428.1289.

7-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]heptanoic Acid (13e) Colorless oil. *Rf* 0.17 (AcOEt/hexane=1 : 1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.40—1.93 (14H, m), 2.39 (2H, t, *J*=7.1 Hz), 3.62—3.71 (2H, m), 7.76 (1H, d, *J*=8.1 Hz), 7.88 (1H, s), 7.95 (1H, d, *J*=8.1 Hz); MS (ESI) *m/z*: 442 [(M+H)$^+$]; HR-MS Calcd for $C_{20}H_{23}N_3O_3F_3S$ 442.1406. Found 442.1428.

8-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]octanoic Acid (13f) Colorless oil. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.30—1.45 (6H, m), 1.58 (6H, s), 1.58—1.90 (4H, m), 2.37 (2H, t, *J*=7.3 Hz), 3.64—3.70 (2H, m), 7.77 (1H, dd, *J*=1.5, 8.2 Hz), 7.89 (1H, d, *J*=1.5 Hz), 7.94 (1H, d, *J*=8.2 Hz); MS (ESI) *m/z*: 456 [(M+H)$^+$]; HR-MS Calcd for $C_{21}H_{24}F_3N_3O_3S$ 455.1490. Found 455.1486.

9-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]nonanoic Acid (13g) Colorless oil. *Rf* 0.27 (AcOEt/hexane=1 : 1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.30—1.90 (18H, m), 2.36 (2H, t, *J*=7.3 Hz), 3.63—3.69 (2H, m), 7.76 (1H, d, *J*=8.2 Hz), 7.88 (1H, s), 7.94 (1H, d, *J*=8.2 Hz); MS (ESI) *m/z*: 470 [(M+H)$^+$]; HR-MS Calcd for $C_{22}H_{26}N_3O_3F_3NaS$ ([M+Na]$^+$) 492.1539. Found 492.1560.

10-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]decanoic Acid (13h) Colorless oil. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.25—1.42 (10H, m), 1.55—1.73 (2H, m), 1.58 (6H, s), 1.75—1.80 (2H, m), 2.35 (2H, t, *J*=7.4 Hz), 3.63—3.71 (2H, m), 7.76 (1H, dd, *J*=1.6, 8.1 Hz), 7.89 (1H, d, *J*=1.6 Hz), 7.95 (1H, d, *J*=8.1 Hz); MS (ESI) *m/z*: 484 [(M+H)$^+$]; HR-MS Calcd for $C_{23}H_{28}F_3N_3O_3S$ 483.1803. Found 483.1788.

4-{5-Imino-3-[3-(4-methoxymethoxyphenyl)propyl]-4,4-dimethyl-2-thioxoimidazolidin-1-yl}-2-trifluoromethylbenzonitrile (14a) To a solution of 5-amino-2-cyanobenzotrifluoride (**9**) (180 mg, 0.967 mmol) in THF/H_2O (1 : 4, 5 ml) was added thiophosgene (88.5 μl, 1.16 mmol) and the mixture was stirred at room temperature for 1 h. The mixture was extracted with AcOEt and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under vacuum to give a crude product of 3-trifluoromethyl-4-cyanoisothiocyanate (**10**). Acetone cyanohydrin (90%, 149 mg, 1.57 mmol) and 3-(4-methoxymethoxyphenyl)propylamine (280 mg, 1.43 mmol) was dissolved in MeOH (2 ml) and the mixture was stirred at room temperature for 15 h. Water was added and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under vacuum to give a crude product of 2-[3-(4-methoxymethoxyphenyl)propylamino]-2-methylpropionitrile (**8i**). These two crude products were dissolved in THF (2 ml) and NEt_3 (207 μl, 1.5 mmol) was added and the mixture was stirred at reflux for 1 h. After cooling to room temperature, water was added and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under vacuum. The purification by silica gel column chromatography (AcOEt/hexane=1 : 1) gave compound **14a** (442 mg, 43%) as a colorless oil. *Rf* 0.33 (AcOEt/Hexane=1 : 1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.57 (6H, s), 2.03—2.19 (2H, m), 2.67 (2H, t, *J*=7.6 Hz), 3.48 (3H, s), 3.62—3.68 (2H, m), 5.16 (2H, s), 6.98 (2H, d, *J*=8.6 Hz), 7.14 (2H, d, *J*=8.6 Hz), 7.62—8.01 (3H, m).

4-{5-Imino-3-[3-(3-methoxyphenyl)propyl]-4,4-dimethyl-2-thioxoimidazolidin-1-yl}-2-trifluoromethylbenzonitrile (14b) This compound was prepared by a procedure similar to that described for **14a**. Colorless oil. *Rf* 0.23 (AcOEt/hexane=1 : 1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.58 (6H, s), 2.11—2.22 (2H, m), 2.71 (2H, t, *J*=7.5 Hz), 3.63—3.69 (2H, m), 3.81 (3H, s), 6.75—6.83 (3H, m), 7.23 (1H, t, *J*=9.1 Hz), 7.61—8.02 (3H, m).

4-{3-[3-(3-Methoxyphenyl)propyl]-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl}-2-trifluoromethylbenzonitrile (15a) Compound **14b** (328 mg, 0.712 mmol) was dissolved in 1,4-dioxane (1 ml) and 6 N-HCl (3 ml) and the mixture was stirred at 80 °C for 1 h. After cooling to room temperature, water was added and the mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under vacuum. The purification by silica gel column chromatography (AcOEt/hexane=1:2) gave compound **15a** (98 mg, 30%) as colorless oil. *Rf* 0.70 (AcOEt/hexane=1 : 1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.52 (6H, s), 2.15—2.21 (2H, m), 2.71 (2H, t, *J*=7.4 Hz), 3.66—3.72 (2H, m), 3.81 (3H, s), 6.78—6.83 (3H, m), 7.23 (1H, t, *J*=8.6 Hz), 7.76 (1H, dd, *J*=1.7, 8.3 Hz), 7.88 (1H, d, *J*=1.7 Hz), 7.94 (1H, d, *J*=8.3 Hz); MS (EI) *m/z*: 461 [M$^+$].

4-{3-[3-(3-Hydroxyphenyl)propyl]-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl}-2-trifluoromethylbenzonitrile (16b) To a solution of compound **15a** (91 mg, 0.197 mmol) in CH_2Cl_2 (2 ml) was added BBr_3 (1.0 M in CH_2Cl_2, 591 μl, 0.591 mmol) at −78 °C. The mixture was stirred at −78 °C for 10 min and at 0 °C for 40 min. After workup with sat. $NaHCO_3$aq., the mixture was extracted with $CHCl_3$. Organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under vacuum. The purification by silica gel column chromatography (AcOEt/hexane=1 : 2) gave compound **16b** (79.8 mg, 91%) as a colorless oil. *Rf* 0.50 (AcOEt/hexane=1 : 1); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.53 (6H, s), 2.13—2.20 (2H, m), 2.69 (2H, t, *J*=7.6 Hz), 3.68—3.70 (2H, m), 4.68 (1H, s), 6.68—6.71 (2H, m), 6.80 (1H, d, *J*=7.3 Hz), 7.18 (1H, t, *J*=7.8 Hz), 7.76 (1H, dd, *J*=2.0, 8.3 Hz), 7.89 (1H, d, *J*=2.0 Hz), 7.95 (1H, d, *J*=8.3 Hz); MS (EI) *m/z*: 447 [M$^+$].

Compounds **16c** and **16d** were prepared by a procedure similar to that described for **16b**.

4-{3-[4-(4-Hydroxyphenyl)butyl]-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl}-2-trifluoromethylbenzonitrile (16c) Colorless oil. *Rf* 0.27 (AcOEt/hexane=1 : 4); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.54 (6H, s), 1.60—1.75 (2H, m), 1.75—1.92 (2H, m), 2.63 (2H, t, *J*=7.3 Hz), 3.65—3.71 (2H, m), 4.71 (1H, s), 6.76 (2H, d, *J*=8.4 Hz), 7.06 (2H, d, *J*=8.4 Hz), 7.76 (1H, dd, *J*=1.6, 8.3 Hz), 7.88 (1H, d, *J*=1.6 Hz), 7.94 (1H, d, *J*=8.3 Hz); MS (ESI) *m/z*: 462 [M+H$^+$].

4-{3-[4-(3-Hydroxyphenyl)butyl]-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl}-2-trifluoromethylbenzonitrile (16d) Colorless oil. *Rf* 0.43 (AcOEt/hexane=1 : 1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.54 (6H, s), 1.69—1.89 (4H, m), 2.65 (2H, t, *J*=7.4 Hz), 3.65—3.71 (2H, m), 4.77 (1H, s), 6.64—6.79 (3H, m), 7.16 (1H, t, *J*=7.6 Hz), 7.77 (1H, dd, *J*=2.0, 8.3 Hz), 7.89 (1H, d, *J*=2.0 Hz), 7.94 (1H, d, *J*=8.3 Hz); MS (ESI) *m/z*: 462 [M+H$^+$].

4-{3-[3-(4-Hydroxyphenyl)propyl]-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl}-2-trifluoromethylbenzonitrile (16a) To a solution of compound **14a** (440 mg, 0.897 mmol) in 1,4-dioxane (2 ml) was added 6 N-HCl (4 ml) and the mixture was stirred at 80 °C for 2 h. After cooled to room temperature, the reaction mixture was extracted with $CHCl_3$. Organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under vacuum. The purification by silica gel column chromatography (AcOEt/hexane=1 : 3) gave compound **16a** (164 mg, 41%) as a colorless oil. *Rf* 0.37 (AcOEt/hexane=1 : 1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.52 (6H, s), 2.07—2.19 (2H, m), 2.66 (2H, t, *J*=7.6 Hz), 3.64—3.70 (2H, m), 4.65 (1H, s), 6.78 (2H, d, *J*=8.4 Hz), 7.09 (2H, d, *J*=8.4 Hz), 7.76 (1H, dd, *J*=2.0, 8.2 Hz), 7.89 (1H, d, *J*=2.0 Hz), 7.95 (1H, d, *J*=8.2 Hz); MS (EI) *m/z*: 447 [M$^+$].

4-(4-{3-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propyl}phenoxy)butyric Acid Ethyl Ester (17a) To a solution of compound **16a** (57.8 mg, 0.129 mmol) in DMF (1 ml) were

added NaH (60% in oil, 6.2 mg, 0.155 mmol) and ethyl 4-bromobutyrate (22.4 μl, 0.155 mmol) at 0 °C under N_2. After stirring at room temperature for 30 min, sat.NH_4Claq. was added and the mixture was extracted with AcOEt. Organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated under vacuum. The purification by silica gel column chromatography (AcOEt/hexane=1 : 4) gave compound **17a** (15.6 mg, 21%) as colorless oil. *Rf* 0.63 ($CHCl_3$); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.26 (3H, t, *J*=7.2 Hz), 1.52 (6H, s), 2.08—2.16 (4H, m), 2.51 (2H, t, *J*=7.3 Hz), 2.67 (2H, t, *J*=7.4 Hz), 3.64—3.70 (2H, m), 3.99 (2H, t, *J*=6.1 Hz), 4.15 (2H, q, *J*=7.2 Hz), 6.83 (2H, d, *J*=8.6 Hz), 7.12 (2H, d, *J*=8.6 Hz), 7.76 (1H, dd, *J*=1.7, 8.3 Hz), 7.88 (1H, d, *J*=1.7 Hz), 7.94 (1H, d, *J*=8.3 Hz) ; MS (EI) *m/z*: 561 [M^+].

4-(4-{3-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propyl}phenoxy)butyric Acid (18a) To a solution of compound **17a** (10.6 mg, 0.0189 mmol) in MeOH (1 ml) was added 2 N-NaOH (0.5 ml) and the mixture was stirred at room temperature 15 h. The reaction mixture was acidified by 2 N-HCl and extracted with AcOEt. Organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under vacuum. The purification by preparative TLC (AcOEt/hexane=1 : 1) gave compound **18a** (8.4 mg, 83%) as a colorless oil. *Rf* 0.13 (AcOEt/hexane=1 : 1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.52 (6H, s), 2.02—2.20 (4H, m), 2.56—2.70 (4H, m), 3.64—3.70 (2H, m), 4.01 (2H, t, *J*=6.0 Hz), 6.83 (2H, d, *J*=8.4 Hz), 7.12 (2H, d, *J*=8.4 Hz), 7.76 (1H, dd, *J*=1.8, 8.2 Hz), 7.88 (1H, d, *J*=1.8 Hz), 7.94 (1H, d, *J*=8.2 Hz); HR-MS Calcd for $C_{26}H_{26}N_3O_4F_3NaS$ ([M+Na]$^+$) 556.1488. Found 556.1452.

Compounds **18b**—**d** were prepared by a procedure similar to that described for **18a**.

4-(3-{3-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propyl}phenoxy)butyric acid (18b) Colorless oil. *Rf* 0.20 (AcOEt/hexane=1 : 1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.53 (6H, s), 2.07—2.25 (4H, m), 2.59 (2H, t, *J*=7.3 Hz), 2.70 (2H, t, *J*=7.3 Hz), 3.66—3.72 (2H, m), 4.02 (2H, t, *J*=5.9 Hz), 6.74—6.82 (3H, m), 7.21 (1H, t, *J*=8.6 Hz), 7.76 (1H, dd, *J*=1.7, 8.4 Hz), 7.88 (1H, d, *J*=1.7 Hz), 7.94 (1H, d, *J*=8.4 Hz); MS (EI) *m/z*: 533 [M^+]; HR-MS Calcd for $C_{26}H_{26}N_3O_4F_3NaS$ ([M+Na]$^+$) 556.1488. Found 556.1505.

4-(4-{4-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]butyl}phenoxy)butyric acid (18c) Colorless oil. *Rf* 0.33 (MeOH/$CHCl_3$=1:20); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.54 (6H, s), 1.65—1.73 (2H, m), 1.81—1.85 (2H, m), 2.08—2.15 (2H, m), 2.59 (2H, t, *J*=7.3 Hz), 2.63 (2H, t, *J*=7.3 Hz), 3.66—3.70 (2H, m), 4.00 (2H, t, *J*=6.4 Hz), 6.82 (2H, d, *J*=8.6Hz), 7.10 (2H, d, *J*=8.6Hz), 7.77 (1H, dd, *J*=2.0, 8.3 Hz), 7.89 (1H, d, *J*=2.0 Hz), 7.94 (1H, d, J=8.3 Hz); HR-MS Calcd for $C_{27}H_{28}N_3O_4F_3NaS$ ([M+Na]$^+$) 570.1644. Found 570.1666.

4-(3-{4-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]butyl}phenoxy)butyric acid (18d) Colorless oil. *Rf* 0.23 (AcOEt/hexane=1 : 1); ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.54 (6H, s), 1.65—1.75 (2H, m), 1.81—1.88 (2H, m), 2.09—2.15 (2H, m), 2.59 (2H, t, *J*=6.8 Hz), 2.67 (2H, t, *J*=7.6 Hz), 3.67—3.71 (2H, m), 4.02 (2H, t, *J*=6.1 Hz), 6.71—6.74 (2H, m), 6.79 (1H, d, *J*=7.8 Hz), 7.20 (1H, t, *J*=8.1 Hz), 7.77 (1H, dd, *J*=2.0, 8.3 Hz), 7.89 (1H, d, *J*=2.0 Hz), 7.96 (1H, d, *J*=8.3 Hz); HR-MS Calcd for $C_{27}H_{29}N_3O_4F_3S$ ([M+H]$^+$) 548.1825. Found 548.1852.

Competitive Binding Assay CHO-K1/hAR cells (5×10^4/well) were placed in 24-well plates and cultured for 2 d. 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).

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 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 lipofectoamine. Nine hours after the transfection, the liquid culture was replaced by phenol red-free DMEM/3% DCC-FBS containing 1, 10, 100, 1000, or 10000 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 "EC_5" value (the concentration of a compound-treated group in which the transcriptional activity is 5% of the transcriptional activity of 0.1 nM of DHT) was computed to evaluate the agonist 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 lipofectoamine. 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 10000 nmol/l of test compound. The transcriptional activity was measured 48 h after the replacement of the liquid culture. Transcriptional activity was measured using a dual luciferase reporter assay system. The transcriptional activity was calculated by dividing the value for firefly luciferase by the value for sea pansy luciferase. The antagonist activity was computed using the following formula and the antagonist activity determined was used to compute the IC_{50} value (the concentration of compound that shows a 50% decrease in the transcriptional activity of DHT 0.1 nmol/l without compound).

antagonist activity (%)=(compound-treated transcriptional activity/non-treated transcriptional activity)×100

***In Vitro* Metabolic Stability in Mouse Liver Microsome** To a 0.1 M potassium phosphate buffer (pH 7.4) containing 1 mM NADPH and 0.5 mg/ml mouse liver microsome, a test compound (final conc. 1 μM) was added to start the reaction. After incubation for 0, 5, 15, or 30 min at 37 °C, CH_3CN was added to stop the reaction. The concentration of the test compound at each time point was measured by LC/MS/MS (QTRAP, Applied Biosystems). Hepatic intrinsic clearance (CL_{int}) was determined by the temporal changes in the concentration of the test compound.

***In Vivo* Antiandrogenic Activities on Seminal Vesicle (SV) Wet Weights in Mice** Immature male ICR mice (7—8 weeks old) were castrated on day 0. After 1 d, animals were divided into groups and dosed on days 1—4 and 7—10 with one of following: (1) control vehicle (5% gum arabic, orally); (2) TP (10 μg/body/d, subcutaneously) plus control vehicle; (3) TP plus compound **13b** (2, 10, or 50 mg/kg/d in 5% gum arabic, orally). Animals were sacrificed on day 11 and SVs were collected and weighed after formalin fixation. To compare data from different experiments, organ weights were first standardized as mg/100 g of body weight. Reduction ratios were calculated by the following equation;

reduction ratio (%)=$100\times(B-A)/(B-C)$

A: SV weights of groups under condition (3)

B: SV weights of a group under condition (2)

C: SV weights of a group under condition (1)

Molecular Modeling. Docking Model of Compounds 13b and 13e to AR This model was built based on the X-ray crystal structure of human AR in complex with the ligand R1881 (PDB ID: le3g).[18] 3D structures of compounds **13b** and **13e** were modeled independently using software SYBYL[19] with a Tripos force field.[20] Compounds **13b** and **13e** were manually docked into AR such that (i) the binding mode of the cyanophenyl moiety of compounds **13b** and **13e** is similar to that of Casodex (PDB ID: 1z95)[21] and (ii) the thiohydantoin ring is modeled so that the side chain attached to the thiohydantoin ring of **13b** and **13e** is directed to helix 12 of AR. After checking the bumps between the compound and AR, energy minimization of the compound/AR complex was performed using a molecular mechanics method with the Tripos force field on condition that the coordinates of AR are fixed. Conformations obtained for **13b** and **13e** are local minimum energy conformations.

Docking Model of Compounds 4 and 18a to AR 3D structures of compounds **4** and **18a** were modeled independently using software SYBYL with a Tripos force field. Compound **4** was manually docked into AR in a way that (i) the oxygen atom of the carbonyl group attached at position 3 of the steroid interacts with the side chain of Arg752 of AR and (ii) the hydroxy group attached at position 17 of the steroid interacts with the hydroxy group of the side chain of Thr877 of AR and (iii) the side chain attached to position 7α of the steroid core of **4** is directed to helix 12 of AR. The binding mode of **18a** was determined by manual docking in the same way as for **13b** and **13e**. After checking the bumps between the compound and AR, energy minimization of the compound/AR complex was performed using a

molecular mechanics method with the Tripos force field on condition that the coordinates of AR are fixed.

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