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Structure–activity relationships of bioisosteric replacement of the carboxylic acid in novel androgen receptor pure antagonists

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ABSTRACT

A series of 5,5-dimethylthiohydantoin derivatives were synthesized and evaluated for androgen receptor pure antagonistic activities for the treatment of hormone refractory prostate cancer. **CH4933468** (**32d**) with a sulfonamide side chain not only exhibited antagonistic activity with no agonistic activity in the reporter gene assay but also inhibited the growth of bicalutamide-resistant cell lines. This compound also inhibited tumor growth of the LNCaP xenograft in mice dose-dependently.

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**) (a precursor of its active form hydroxyflutamide (**2**)) and bicalutamide (**3**) (Chart 1) are clinically used to treat prostate cancer.[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] Hydroxyflutamide and bicalutamide have partial agonistic activities at high concentrations in vitro,[8] which may contribute to recurrence. Therefore, research into new antiandrogenic agents that exhibit no agonistic activities, so-called 'AR pure antagonists', has been conducted.[9–12]

Estrogen pure antagonists have been reported to have a side chain which inhibits the folding of helix 12 of the estrogen receptor (ER) ligand binding domain (LBD).[13,14] Since AR and ER belong to the nuclear receptor superfamily and the tertiary structure of the AR LBD is similar to that of ER LBD, we thought that the same strategy would be effective.[9,10] Therefore, we introduced side chains to a nonsteroidal known androgen ligand, RU56187 (**4**), which have been reported to have binding affinity for AR. As expected, we recently reported a thiohydantoin derivative (**5**) with a carboxyl terminal side chain that showed pure antagonistic activities in vitro and oral AR antagonistic activity in vivo.[15]

In further experiments, however, the carboxylic acid derivatives exhibited weak cell growth inhibition in T877A mutation AR cell line LNCaP, a common androgen-dependent human prostate cancer cell line. Moreover, compound **5** also showed weak antagonistic activity in LNCaP-BC2, a bicalutamide-resistant and androgen-hypersensitive prostate cell line established in our

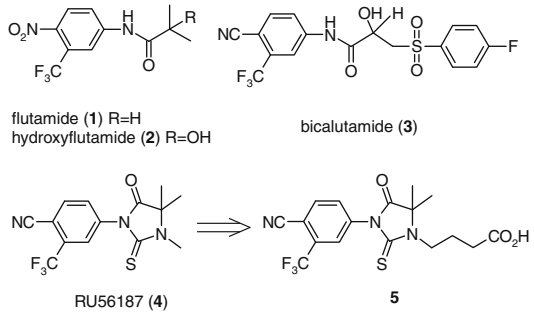

Chart 1. Structure of AR antagonists.

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group.[16] Because these cell lines reflect clinical hormone refractory prostate cancer, it is necessary to identify potent and orally active AR pure antagonists not only in wild type RGA but also in AR mutant cell lines. In this paper, we describe two strategies to increase antiandrogenic activities. One is bioisosteric replacement of the carboxylic acid side chain to find functional groups that would effectively inhibit the folding of helix 12 of the AR LBD. The other is optimization of the substituent groups on the phenyl ring to increase AR affinity.

2. Chemistry

Some of the requisite analogs were prepared according to the synthetic sequence outlined in Scheme 1. Carboxylic acid **5** was treated with $ClCO_2Et$ in the presence of Et_3N followed by ammonia to give the corresponding amide **6**. Multistep reactions of compound **7** afforded amine **8**. Compound **8** was treated with TMS isocyanate to afford urea **9**. Compound **8** was also treated with chlorosulfonyl isocyanate and *tert*-BuOH followed by TFA to afford sulfamide **10**. Tetrazole **13** was synthesized through multistep reactions starting from material **11**. Protection of tetrazole with *p*-methoxybenzyl (PMB) group gave regioisomers of **12a** and **12b** as a mixture. This mixture was treated with 4-CN-3-CF_3-PhNCS followed by deprotection of PMB group to give tetrazole **13**. Sulfonic acid derivative **15** was synthesized from compound **14**.

Sulfonamide derivatives were synthesized according to the synthetic sequence shown in Scheme 2. In the case of compounds **16a** and **16b**, the sulfonamide group of compound **17** was protected with *N*,*N*-(dimethylimino)methylene group. After cyclization with 4-CN-3-CF_3-PhNCS, deprotection of sulfonamide group with 6 N HCl afforded corresponding sulfonamide **18**. In the case of compound **21**, PMB group was used to protect the sulfonamide group. Monomethyl sulfonamide **24** was protected with the Boc group, but dimethyl sulfonamide **27** was directly given from treatment with isothiocyanate.

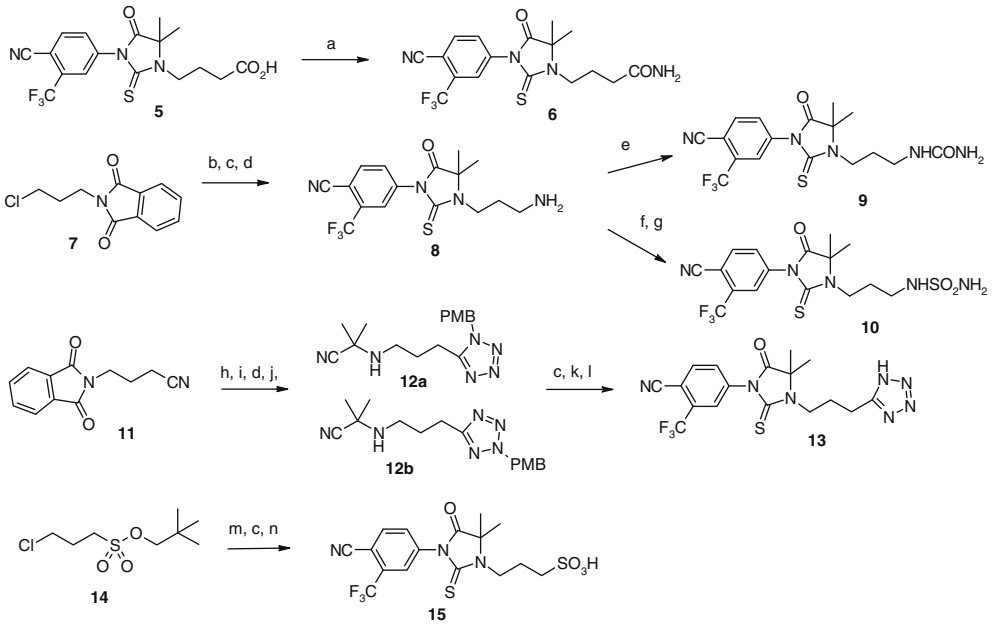
Further optimization of the substituent groups on the phenyl rings of sulfonamide derivatives is shown in Scheme 3. The introduction of the methyl group of compound **28** by treatment with LDA and MeI gave **29a** and **29b** at a ratio of 2 to 1. Further replacement of the chloride group by nitrile followed by treatment with 6 N HCl afforded **30a** and **30b**, respectively. Isothiocyanates were synthesized by treatment of the corresponding anilines with thiophosgene. Target compounds **32a–f** were prepared by coupling the isothiocyanates with amine **17a** followed by acidic hydrolysis of the *N*,*N*-dimethylimino group.

3. Biological assays

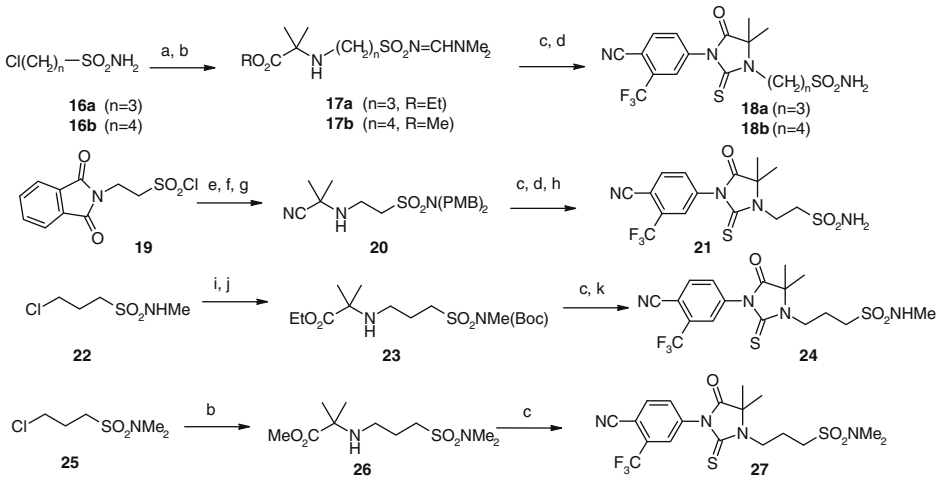
The synthesized compounds were evaluated for their in vitro binding affinities and agonist/antagonist activities to AR using the same procedures as previously reported.[15] The binding affinity to AR was determined by displacement of [^{3}H]-mibolerone with the test compound utilizing CHO-K1/hAR cells. The reporter gene assay initially used a transient assay system until a stable assay system using hAR-transfected HeLa cells was utilized. The 0.1 nM DHT-induced transcriptional activity was set at 100%. A 'pure antagonist' was defined as having a 5% effective concentration (EC_5) value greater than 10,000 nM. To estimate the potential antagonistic activity, the transcriptional activity value determined by the agonistic assay was subtracted from that of the antagonistic activity. The resulting value was termed the subtracted antagonistic activity (sIC_{50}). The sIC_{50} value was determined from the dose–response curve of the subtracted antagonistic activity. Cell growth was determined in LNCaP cells and bicalutamide-resistant LNCaP-BC2 cells. Agonistic activity was expressed as + or − according to the dose–response curve of growth. A 'pure antagonist' was expressed as −. Bicalutamide showed pure antagonistic activity in LNCaP cells but clearly exhibited agonistic activity in LNCaP-BC2 cells.

4. Results and discussion

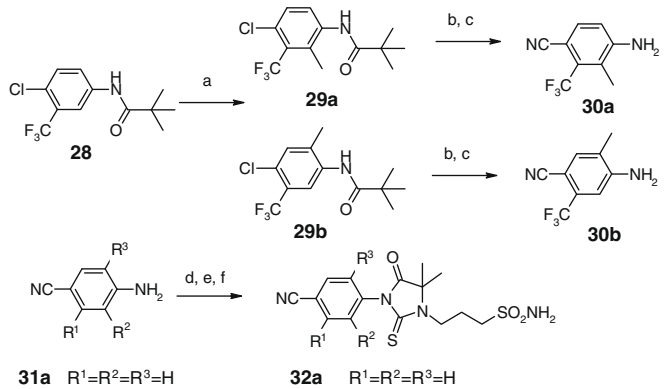
In vitro activities of the thiohydantoin derivatives with various terminal side chains are shown in Table 1. Although compound **5**



Scheme 1. Reagents and conditions: (a) $ClCO_2Et$, Et_3N, THF, 0 °C then NH_4OH, rt; (b) aminoisobutyric acid methyl ester, K_2CO_3, NaI, DMF, 80 °C; (c) 4-CN-3-CF_3-PhNCS, Et_3N, THF, rt; (d) NH_2NH_2/H_2O, CH_2Cl_2, EtOH, rt; (e) trimethylsilyl isocyanate, CH_2Cl_2, rt; (f) chlorosulphonyl isocyanate, *tert*-BuOH, CH_2Cl_2; (g) TFA, CH_2Cl_2, rt; (h) NaN_3, $AlCl_3$, 1,4-dioxane, reflux; (i) *p*-methoxybenzylalcohol, DEAD, PPh_3, CH_2Cl_2, rt; (j) acetone cyanohydrin, Et_3N, MeOH, 50 °C; (k) 2 N HCl, MeOH, 60 °C; (l) TFA, 80 °C; (m) aminoisobutyric acid methyl ester, K_2CO_3, *n*-Bu_4NI, DMF, MeCN, reflux; (n) Me_4NCl, DMF, reflux.



Scheme 2. Reagents and conditions: (a) DMF dimethylacetal, DMF, rt; (b) 2-aminoisobutyric acid methyl or ethyl ester, K_2CO_3, NaI, DMF, 80 °C; (c) 4-CN-3-CF_3-PhNCS, Et_3N, THF, rt; (d) 6 N HCl, 1,4-dioxane, reflux; (e) PMB_2NH, Et_3N, CH_2Cl_2, rt; (f) NH_2NH_2/H_2O, EtOH, rt; (g) acetone cyanohydrin, Et_3N, MeOH, 50 °C; (h) TFA, anisole, reflux; (i) Boc_2O, DMAP, CH_3CN, rt; (j) 2-aminoisobutyric acid ethyl ester, K_2CO_3, NaI, DMF, MeCN, 80 °C; (k) TFA, CH_2Cl_2, rt.



31a R^1=R^2=R^3=H
31b R^1=Me,R^2=R^3=H
31c R^1=OMe,R^2=R^3=H
31d R^1=Cl,R^2=R^3=H
30a R^1=CF_3,R^2=Me,R^3=H
30b R^1=CF_3,R^2=H,R^3=Me

32a R^1=R^2=R^3=H
32b R^1=Me,R^2=R^3=H
32c R^1=OMe,R^2=R^3=H
32d R^1=Cl,R^2=R^3=H
32e R^1=CF_3,R^2=Me,R^3=H
32f R^1=CF_3,R^2=H,R^3=Me

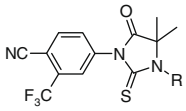
Scheme 3. Reagents and conditions: (a) MeI, *n*-BuLi, THF, −30 °C; (b) CuCN, NMP, 170 °C; (c) concd HCl, EtOH, reflux; (d) thiophosgene, THF, 0 °C; (e) **17a**, DMAP, THF, reflux; (f) 6 N HCl, 1,4-dioxane, 80 °C.

with a carboxylic acid showed strong pure antagonistic activity in transient RGA, it showed not only weak binding affinity to AR but also weak antagonistic activity in stable RGA and cell growth inhibition assays. Overall, antagonistic activities in stable RGA tended to be weaker than those in transient RGA. Although amide **6** and urea **9** exhibited improved binding affinity, they showed agonistic activities in transient RGA. On the other hand, tetrazole **13** and sulfonic acid **15**, which are generally known as carboxylic acid bioisosteres, did not show AR binding activity. Sulfamide **10** and sulfonamide **18a** showed the desired androgen pure antagonistic activities in all the cell lines assayed. Particularly, the cell growth inhibition of **18a** was more than 10 times more potent than that of **5**. Because we found that sulfonamide had the desired potency, we expanded the research to further derivatizations of the sulfonamide side chains. Although *N*-methyl sulfonamide **24** showed potent antagonistic activities in RGA, agonistic activity was observed in LNCaP-BC2 cell growth. In the case of *N*,*N*-dimethyl sulfonamide **27**, more potent agonistic activities were observed in transient RGA and LNCaP. These results indicated that an increase in lipophilicity leads to increased agonistic activities. Next, we evaluated the effect of the length of the alkyl chain. Even though all of the compounds which had between two to four methylene groups in the sulfonamide side chain showed pure antagonistic activities, those with three methylene groups showed the most potent activities. The results indicate the same tendencies as the carboxylic acid side chains,[15] but the sulfonamide derivatives bound more strongly to AR than the carboxylic acid derivative. The difference between the binding affinities of compound **18a** and **5** might come from the different charge states of the compound. Based on the calculated pK_a values, compound **18a** is neutrally charged and **5** is negatively charged. The binding of neutral compound **18a** is stronger than that of the negatively charged compound. Therefore, the sulfonamide side chain has the desired lipophilicity and pK_a value for AR antagonistic activity. For the next step, we turned our attention to the optimization of the phenyl group with a sulfonamide terminal side chain (Table 2). According to Refs. 17,18 an electron-withdrawing group such as a nitrile group is essential for AR binding, so we mainly optimized the trifluoromethyl group to other substituent groups. With compound **32a**, the replacement of the trifluoromethyl group with hydrogen resulted in a drastic loss of activity. Methyl-substituted compound **32b** had less potency than trifluoromethyl in the RGA assay but was as active as **18a** in the cell growth assays. Although that result indicates the growth inhibition of **32b** had some causes other than AR signal, the reason cannot be clearly explained.

Although, methoxy-substituted compound **32c** showed almost the same potency in the RGA assay, agonistic activities were observed in the cell growth inhibition assays. Among them, chloro-substituted derivative **32d** showed the most potent pure antagonistic activities not only in RGA but also in all cell growth inhibition. With respect to the substituent R^1, compounds with an electron-withdrawing group at R^1 showed stronger affinity than those with an electron-donating group. Our docking model suggests that substituent R^1 interacts with the main chain carbonyl group Met745 of AR (Fig. 1). In the case of compounds **32d** (R^1 = Cl) and **18a** (R^1 = CF_3), $\delta-$ of R and $\delta+$ of the carbon atom of Met745 makes contact at an appropriate distance, 3.4 and 3.0 Å, respectively. In the case of compound **32c** (R^1 = OMe), the distance between OMe and the carbon atom of Met745 was slightly longer than that of **32d** and **18a** (3.8 Å). This difference in distance might be the reason for the weaker binding of compound **32c**. In the case of compound **32b** (R^1 = Me), there is no $\delta-$ in the substituent R^1.

We introduced a methyl group to compound **18a** to improve AR binding. However, compound **32e** with a methyl group introduced at position R^2 showed enhanced agonistic activity, but the

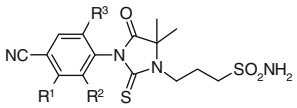
Table 1
In vitro activities of thiohydantoin derivatives



No.	R	Binding	RGA transient		RGA stable		LNCaP		LNCaP-BC2		clog *P*
		IC_{50} (nM)	EC_5 (nM)	sIC_{50} (nM)	EC_5 (nM)	sIC_{50} (nM)	Agonist +/−	Antagonist IC_{50} (nM)	Agonist +/−	Antagonist IC_{50} (nM)	
3		200	200	100	25	190			+	550	
4	Me	15	<1	ND	0.08	1	+	ND	NT	NT	
5	$(CH_2)_3CO_2H$	3900	>10,000	130	>10,000	2000	–	2000	–	400	3.27
6	$(CH_2)_3CONH_2$	480	3600	290	>10,000	560	–	400	–	55	2.49
9	$(CH_2)_3NHCONH_2$	800	650	490	350	490	–	1600	–	200	2.33
10	$(CH_2)_3NHSO_2NH_2$	>1000	>10,000	260	>10,000	1000	–	780	–	89	2.33
13	$(CH_2)_3$-5-tetrazolyl	>1000	>10,000	>10,000	NT	NT	–	ND	NT	NT	2.91
15	$(CH_2)_3SO_3H$	>1000	>10,000	>10,000	NT	NT	+	ND	NT	NT	1.37
18a	$(CH_2)_3SO_2NH_2$	400	>10,000	64	>10,000	320	–	160	–	18	2.16
24	$(CH_2)_3SO_2NHMe$	170	>10,000	64	1400	350	–	190	+	30	2.81
27	$(CH_2)_3SO_2NMe_2$	80	110	21	NT	NT	+	3700	NT	NT	3.14
21	$(CH_2)_2SO_2NH_2$	770	>10,000	160	7200	550	–	750	–	79	1.93
18b	$(CH_2)_4SO_2NH_2$	>1000	>10,000	230	>10,000	1300	–	1400	–	110	2.14

NT: Not tested, ND: Not determined.

Table 2
In vitro activities of sulfonamide derivatives



No.	R^1	R^2	R^3	Binding	RGA stable		LNCaP		LNCaP-BC2		Metabolic stability (human liver MS)
				IC_{50} (nM)	EC_5 (nM)	sIC_{50} (nM)	Agonist +/−	Antagonist IC_{50} (nM)	Agonist +/−	Antagonist IC_{50} (nM)	Residue (%) after 15 min
5				3900	>10,000	2000	–	2000	–	400	97
18a	CF_3	H	H	400	>10,000	320	–	160	–	18	95
32a	H	H	H	>5000	>10,000	>10,000	NT	NT	NT	NT	92
32b	Me	H	H	>1000	>10,000	1900	–	300	–	51	94
32c	OMe	H	H	1900	>10,000	330	+	1900	+	ND	100
32d	Cl	H	H	440	>10,000	190	–	410	–	12	100
32e	CF_3	Me	H	260	2000	110	+	1100	+	ND	89
32f	CF_3	H	Me	>5000	>10,000	2300	NT	NT	NT	NT	91

NT: Not tested, ND: Not determined.

introduction of a methyl group at R^3 (**32f**) decreased the AR binding affinity. Change in the location of the side chain sulfonamide group would result in a slight change in the phenyl ring.

Metabolic stability of the sulfonamide derivatives was examined in human liver microsomes. All of the compounds tested were metabolically stable. Among them, compound **32d** showed significantly high metabolic stability as well as potent pure antagonistic activities. To confirm the in vivo activities of **32d**, the antiandrogenic activities were evaluated by seminal vesicle (SV) wet weights in castrated mice (data not shown).[15] Compound **32d** inhibited dose-dependent SV wet weight gain by 10 mg/body from sc administration of testosterone propionate (ED_{50} = 10 mg/kg). In addition, we confirmed the in vivo efficacy of **32d** against prostate cancer. We used LNCaP xenograft models of castrated SCID mice (Fig. 2). As shown in the growth inhibition of LNCaP cells in vitro, **32d** inhibited tumor growth of LNCaP xenograft in mice dose-dependently at the same level as bicalutamide. Hence, we believe **32d** (**CH4933468**) is a promising candidate for development as an orally available antiandrogen for the treatment of bicalutamide-resistant prostate cancer.

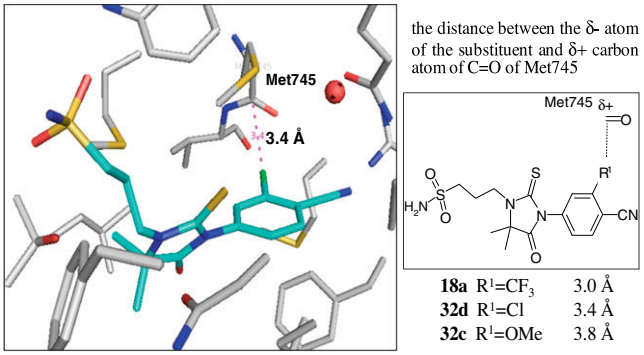


Figure 1. Docking model of compound **32d** to AR.

5. Conclusion

We have discovered novel thiohydantoin derivatives which have a sulfonamide side chain. The compounds showed pure

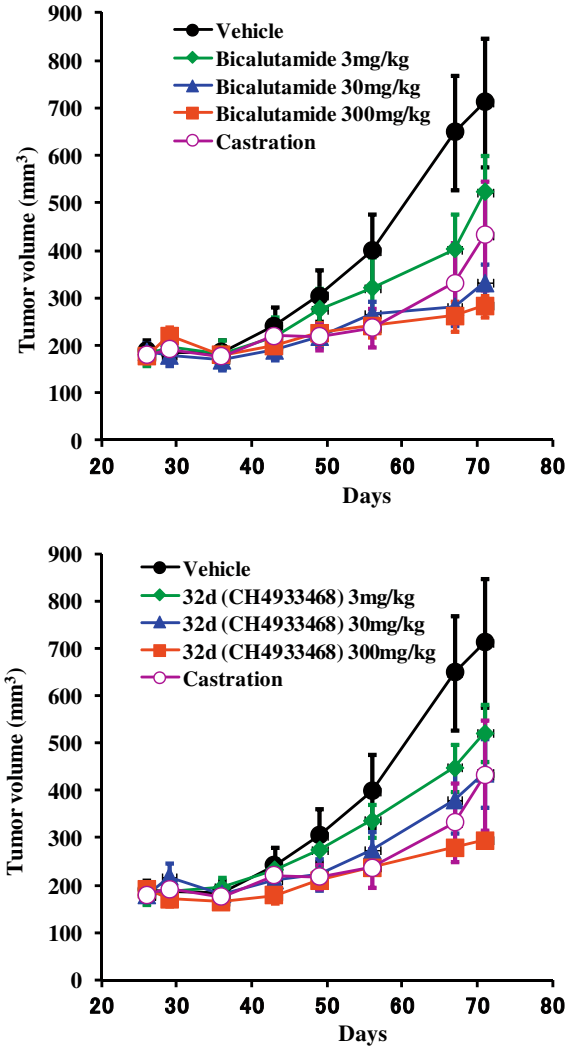


Figure 2. Antitumor activities on LNCaP xenograft in mice. LNCaP was subcutaneously inoculated into 6-week-old male SCID mice. When the tumor size reached 90–400 mm^3, the mice were randomized into groups and orally administered either a test compound or a vehicle (5% gum arabic) in seven cycles of 5 days on, 2 days off (n = 6 animals per group).

antagonistic activities not only in RGA assays but also in cell growth assays in LNCaP and LNCaP-BC2, a bicalutamide-resistant prostate cancer cell line. Among the compounds, **CH4933468** exhibited antiandrogenic activities. Furthermore, **CH4933468** inhibited tumor growth of the LNCaP xenograft in mice dose-dependently at a level similar to bicalutamide. This compound is promising as a candidate for the development of novel agents for bicalutamide refractory prostate cancer.

6. Experimental

6.1. Chemistry: instruments

Column chromatography was carried out on Merck Silica Gel 60 (230–400 mesh) if not otherwise specified. NH silica gel column chromatography was carried out on Fuji Silysia NH-DM1020. R_f was determined with Merck Silica Gel 60 F^{254} plates. ^1H NMR spectra were recorded on JEOL EX-270, Bruker ARX 300, Varian Mercury300 or JEOL ECP-400. Mass spectra (MS) were measured by a Thermo Electron LCQ Classic (ESI) or a Shimadzu GCMS-QP5050A (EI). High resonance mass spectra (HRMS) were recorded by a Micromass Q-Tof Ultima API mass spectrometer.

6.1.1. 4-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]butyramide (6)

To a solution of 4-[3-(4-cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]butyric acid[15] (**5**) (2.30 g, 6.80 mmol) in THF (50 mL) were added Et_3N (2.8 mL, 20.4 mmol) and $ClCO_2Et$ (1.00 mL, 10.2 mmol) at 0 °C. After stirring for 3 min, ammonia hydroxide (10 mL) was added to the reaction mixture. After stirring for 15 min at room temperature, water 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 = 10:1) gave **6** (868 mg, 55%) as a colorless amorphous solid. R_f 0.22 (AcOEt); ^1H NMR (300 MHz, $CDCl_3$) δ: 1.62 (6H, s), 2.15–2.24 (2H, m), 2.41 (2H, t, J = 6.7 Hz), 3.78–3.84 (2H, m), 5.38 (1H, br s), 5.59 (1H, br s), 7.74 (1H, dd, J = 1.8, 8.3 Hz), 7.92 (1H, d, J = 1.8 Hz), 7.97 (1H, d, J = 8.3 Hz); MS (EI) m/z 398 ($[M]^+$); HRMS Calcd for $C_{17}H_{18}F_3N_4O_2S$ 399.1097. Found 399.1090.

6.1.2. 4-[3-(3-Aminopropyl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl]-2-trifluoromethylbenzonitrile (8)

To a solution of 2-(3-chloropropyl)isoindole-1,3-dione (**7**) (2.00 g, 8.94 mmol) in DMF (40 mL) were added 2-aminoisobutyric acid methyl ester HCl salt (2.75 g, 17.9 mmol), K_2CO_3 (4.94 g, 35.8 mmol) and NaI (1.34 g, 8.94 mmol); the mixture was then stirred at 80 °C for 12 h. After cooling to room temperature, water 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 = 1:1) gave 2-[3-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)propylamino]-2-methylpropionic acid methyl ester (2.21 g, 81%).

To a solution of the above obtained compound (400 mg, 1 mmol) in THF (7 mL) were added 3-trifluoromethyl-4-cyanoisothiocyanate[15] (330 mg, 1.45 mmol) and Et_3N (0.036 mL, 0.26 mmol); the mixture was stirred at room temperature for 15 h; water was then 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 = 1:2) gave 4-{3-[3-(1,3-dioxo-1,3-dihydroisoindol-2-yl)propyl]-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl}-2-trifluoromethylbenzonitrile (630 mg, 96%).

To a solution of the above obtained compound (5.30 g, 10.6 mmol) in CH_2Cl_2 (40 mL) and EtOH (100 mL) was added NH_2NH_2/H_2O (1.65 mL, 53.0 mmol), the mixture was stirred at room temperature. After stirring for 2 days, the solvent was distilled off at reduced pressure. To the residue was added CH_2Cl_2, insoluble material was filtered. Distillation of the filtrate at reduced pressure gave a crude product of **8** (3.80 g, 62%). This compound was used in the next step without purification. R_f 0.38 (NH silica gel, CH_2Cl_2/MeOH = 20:1); ^1H NMR (300 MHz, $CDCl_3$) δ: 1.60 (6H, s), 1.92–2.03 (2H, m), 2.84 (2H, t, J = 6.7 Hz), 3.79–3.85 (2H, m), 7.77 (1H, dd, J = 1.5, 8.7 Hz), 7.90 (1H, d, J = 1.5 Hz), 7.95 (1H, d, J = 8.7 Hz).

6.1.3. 3-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propylurea (9)

To a solution of **8** (100 mg, 0.270 mmol) in CH_2Cl_2 (3 mL) was added trimethylsilyl isocyanate (0.073 mL, 0.540 mmol) and the mixture was stirred at room temperature for 12 h, water was then added to the reaction mixture and extracted with CH_2Cl_2. 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 (CH_2Cl_2/MeOH = 20:1) gave **9** (58 mg, 52%) as a colorless amorphous solid. R_f 0.26 (NH

silica gel, CH_2Cl_2/MeOH = 20:1); ^1H NMR (300 MHz, $CDCl_3$) δ: 1.60 (6H, s), 2.00–2.06 (2H, m), 3.28–3.35 (2H, m), 3.80–3.86 (2H, m), 4.41 (2H, br s), 5.09 (1H, t, J = 5.7 Hz), 7.76 (1H, dd, J = 1.9, 8.0 Hz), 7.89 (1H, d, J = 1.9 Hz), 7.96 (1H, d, J = 8.0 Hz); MS (ESI) m/z 414 ($[M+H]^+$) HRMS Calcd for $C_{17}H_{19}F_3N_5O_2S$ 414.1206. Found 414.1233.

6.1.4. 3-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propylsulfamide (10)

N-Chlorosulfonyl-*tert*-butylcarbamate was prepared by the addition of *tert*-BuOH (0.031 mL, 0.32 mmol) to a solution of chlorosulfonyl isocyanate (0.028 mL, 0.32 mmol) in CH_2Cl_2 (1 mL) at 0 °C.[19] To a solution of **8** (120 mg, 0.324 mmol) and Et_3N (0.050 mL, 0.36 mmol) in CH_2Cl_2 (1.1 mL) was slowly added prepared *N*-chlorosulfonyl-*tert*-butylcarbamate at 0 °C, the mixture was stirred for 1 h. After further stirring for 2 h at room temperature, water was added to the reaction mixture and extracted with CH_2Cl_2. The organic layer was washed with brine and dried with $MgSO_4$. After filtering, the solvent was distilled off at reduced pressure to give a crude product of *N*-*tert*-butyloxycarbonyl-*N*′-(3-(4-cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]-propyl)sulfamide (111 mg, 62%).

To a solution of the above obtained compound (105 mg, 0.19 mmol) in CH_2Cl_2 (2 mL) was added TFA (0.147 mL) and the mixture was stirred at room temperature for 12 h. To the reaction mixture was added water and then extracted with CH_2Cl_2. 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 = 3:2) gave **10** (65 mg, 82%) as a colorless amorphous solid. R_f 0.22 (AcOEt/hexane = 1:1); ^1H NMR (300 MHz, $CDCl_3$) δ: 1.61 (6H, s), 2.07–2.17 (2H, m), 3.25–3.32 (2H, m), 3.85–3.90 (2H, m), 4.58 (2H, br s), 4.91 (1H, t, J = 6.8 Hz), 7.76 (1H, dd, J = 1.8, 8.3 Hz), 7.89 (1H, d, J = 1.8 Hz), 7.96 (1H, d, J = 8.3 Hz); HRMS Calcd for $C_{16}H_{19}F_3N_5O_3S_2$ 450.0875. Found 450.0896.

6.1.5. 2-{3-[1-(4-Methoxybenzyl)-1*H*-tetrazol-5-yl] propylamino}-2-methylpropionitrile (12a) and 2-{3-[2-(4-methoxybenzyl)-2*H*-tetrazol-5-yl]propylamino}-2-methylpropionitrile (12b)

To a solution of 4-(1,3-dioxo-1,3-dihydroisoindol-2-yl)butyronitrile (**11**) (2.30 g, 10.8 mmol) in 1,4-dioxane (10 mL) were added NaN_3 (2.11 g, 32.4 mmol) and $AlCl_3$ (1.31 g, 9.80 mmol), the mixture was heated at 130 °C for 36 h. After cooling to room temperature, AcOEt and 3 N HCl were added. After stirring for 30 min, water was added to the reaction mixture and then extracted with AcOEt. The organic layer was washed with brine and dried with Na_2SO_4. After filtering, the solvent was distilled off at reduced pressure. Purification by trituration (AcOEt/hexane = 2:1) gave 2-[3-(1*H*-tetrazol-5-yl)propyl]isoindole-1,3-dione (1.72 g, 62%).

To a solution of the above obtained compound (1.70 g, 6.60 mmol) in CH_2Cl_2 (85 mL) were added *p*-methoxybenzyl alcohol (855 mg, 6.67 mmol), PPh_3 (1.75 g, 6.67 mmol) and diethyl azodicarboxylate (DEAD) (1.15 g, 6.60 mmol) and the mixture was stirred at room temperature. After stirring for 12 h, the solvent was distilled away at reduced pressure. To the residue were added CH_2Cl_2 (50 mL), EtOH (10 mL) and NH_2NH_2/H_2O (11 g), the mixture was stirred at room temperature for 12 h. After filtering, purification by silica gel column chromatography (CH_2Cl_2/MeOH = 5:1) gave a mixture of 3-[1-(4-methoxybenzyl)-1*H*-tetrazol-5-yl]propylamine and 3-[2-(4-methoxybenzyl)-2*H*-tetrazol-5-yl]propylamine (980 mg, 60%).

To a solution of the above obtained mixture (980 mg, 3.96 mmol) in MeOH (20 mL) was added acetone cyanohydrin (1.00 mL), the mixture was stirred at 50 °C for 4 h. Distillation of the solvent at reduced pressure gave crude products of **12a** and **12b** (1.20 g, 96%) as a mixture. R_f 0.50 (AcOEt/hexane = 2:1); ^1H NMR (300 MHz, CD_3OD) δ: 1.41 and 1.42 (6H, s), 1.86–2.02 (2H, m), 2.71–2.85 (2H, m), 2.83 and 2.97 (2H, t, J = 7.5 Hz), 5.45 and 5.64 (2H, s), 6.89 (2H, d, J = 8.8 Hz), 7.16 and 7.32 (2H, d, J = 8.8 Hz).

6.1.6. 4-{4,4-Dimethyl-5-oxo-3-[3-(1*H*-tetrazol-5-yl)propyl]-2-thioxoimidazolidin-1-yl}-2-trifluoromethylbenzonitrile (13)

To a crude solution of **12a** and **12b** (620 mg, 2.00 mmol) in THF (7 mL) were added 3-trifluoromethyl-4-cyanoisothiocyanate[15] (450 mg, 2.00 mmol) and Et_3N (0.450 mL, 3.20 mmol). The mixture was stirred at room temperature for 15 h, and water was then 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 a crude product of 4-(5-imino-3-{3-[2-(4-methoxybenzyl)-2*H*-tetrazol-5-yl]propyl}-4,4-dimethyl-2-thioxoimidazolidin-1-yl)-2-trifluoromethylbenzonitrile (280 mg, 26%).

To a solution of the above obtained compound (280 mg, 0.516 mmol) in MeOH (15 mL) was added 2 N HCl (6 mL), 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 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 4-(3-{3-[2-(4-methoxybenzyl)-1*H*-tetrazol-5-yl]-propyl}-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl)-2-trifluoromethylbenzonitrile (275 mg, 98%).

The above obtained compound (150 mg, 0.277 mmol) was dissolved in TFA (10 mL) and the mixture was stirred at 80 °C for 2 h. After cooling to room temperature, 2 N HCl (6 mL) was added and the mixture was stirred at 60 °C for 2 h. After cooling to room temperature, the solvent was distilled off at reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 9:1) to give **13** (92.0 mg, 78%) as a colorless amorphous solid. R_f 0.28 (AcOEt/hexane = 2:1); ^1H NMR (300 MHz, CD_3OD) δ: 1.51 (6H, s), 2.19–2.30 (2H, m), 2.98 (2H, t, J = 7.6 Hz), 3.75–3.81 (2H, m), 3.80 (3H, s), 5.66 (2H, s), 6.89 (2H, d, J = 8.5 Hz), 7.34 (1H, d, J = 8.5 Hz), 7.76 (1H, dd, J = 1.5, 8.3 Hz), 7.87 (1H, d, J = 1.5 Hz), 7.94 (1H, d, J = 8.3 Hz); HRMS Calcd for $C_{17}H_{17}F_3N_7OS$ 424.1161. Found 424.1134.

6.1.7. 3-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propane-1-sulfonic acid (15)

To a solution of 3-chloropropane-1-sulfonic acid 2,2-dimethylpropyl ester (**14**)[20] (405 mg, 1.77 mmol) in MeCN (10 mL) and DMF (2 mL) were added 2-amino-isobutyric acid methyl ester HCl salt (816 mg, 5.31 mmol), K_2CO_3 (1.54 g, 11.2 mmol) and *n*-tetrabutylammonium iodide (654 mg, 1.77 mmol), the mixture was stirred at 80 °C for 2 days. After cooling to room temperature, water 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 = 1:1) gave 2-[3-(2,2-dimethylpropoxysulfonylpropylamino]-2-methylpropionic acid methyl ester (126 mg, 23%).

The above compound was treated with a procedure similar to that described for **8** to obtain 3-[3-(4-cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propane-1-sulfonic acid 2,2-dimethylpropyl ester (yield 78%).

To a solution of the above obtained compound (118 mg, 0.233 mmol) in DMF (6 mL) was added tetramethylammonium chloride (128 mg, 1.17 mmol) and the mixture was heated under reflux for 6 h. After cooling, water was added and the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with water and brine, and then dried over $MgSO_4$. After filtration

and evaporation of the solvent under reduced pressure, the resulting residue was purified by silica gel column chromatography (AcOEt/MeOH = 10:1) to give **15** (85 mg, 84%) as a colorless amorphous solid. ^{1}H NMR (300 MHz, CD_3OD) δ: 1.80 (6H, s), 2.48–2.54 (2H, m), 3.12 (2H, t, J = 3.1 Hz), 4.10–4.16 (2H, m), 8.11 (1H, dd, J = 1.5, 8.4 Hz), 8.28 (1H, d, J = 1.5 Hz), 8.31 (1H, d, J = 8.4 Hz); MS (ESI) m/z: 436 $[M+H]^+$; HRMS Calcd for $C_{16}H_{15}F_3N_3O_4S_2$ ($[M-H]^-$) 434.0461. Found 434.0476.

6.1.8. 2-(3-{[1-Dimethylaminomethylidene]sulfamoyl}propylamino)-2-methylpropionic acid ethyl ester (17a)

To a solution of compound 3-chloropropane-1-sulfonic acid amide (**16a**) (4.00 g, 25.4 mmol) in DMF (20 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (3.70 mL, 43.3 mmol), stirred at room temperature for 1 h. After the addition of AcOEt, the organic layer was washed with water and dried over $MgSO_4$. After filtration, the solvent was distilled off under reduced pressure to give 3-chloropropane-1-sulfonic acid 1-dimethylaminomethylideneamide (3.05 g, 57%).

2-Aminoisobutyric acid ethyl ester hydrochloride (4.33 g, 28.2 mmol) and K_2CO_3 (7.80 g, 56.4 mmol) were dissolved in DMF (30 mL) and stirred at room temperature for 30 min. To this solution, a solution of the above obtained compound (3.00 g, 10.2 mmol) in DMF (20 mL) and NaI (2.11 g, 14.1 mmol) were added and stirred at 80 °C for 15 h. After cooling to room temperature, water was added and the reaction mixture was extracted with AcOEt. The organic layer was washed with water and dried over $MgSO_4$. After filtration, the solvent was distilled off under reduced pressure. The resulting residue was purified by NH silica gel column chromatography (AcOEt/hexane = 4:1) to give **17a** (1.81 g, 44%) as a colorless oil. R_f 0.37 (AcOEt/MeOH = 4:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.29 (6H, s), 1.89–1.94 (2H, m), 2.57 (2H, t, J = 6.8 Hz), 3.04 (3H, s), 3.07–3.11 (2H, m), 3.13 (3H, s), 3.70 (3H, s), 8.03 (1H, s); MS (ESI) m/z 330 ($[M+Na]^+$).

6.1.9. 2-(4-{[1-Dimethylaminomethylidene]sulfamoyl}-butylamino)-2-methylpropionic acid methyl ester (17b)

This compound was prepared from **16b** using a procedure similar to that described for **17a**. Colorless oil. R_f 0.08 (AcOEt/MeOH = 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.29 (6H, s), 1.56–1.62 (2H, m), 1.78–1.92 (2H, m), 2.47 (2H, t, J = 7.2 Hz), 2.98–3.03 (2H, m), 3.04 (3H, s), 3.13 (3H, s), 3.70 (3H, s), 8.03 (1H, s).

6.1.10. 3-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propane-1-sulfonamide (18a)

To a solution of **17a** (2.20 g, 7.50 mmol) in THF (34 mL) were added Et_3N (0.21 mL, 1.51 mmol) and 4-cyano-3-trifluoromethylphenylisothiocyanate[15] (1.71 g, 7.49 mmol), the mixture was stirred at room temperature for 2 h. The reaction solution was concentrated and the resulting residue was purified by NH silica gel column chromatography (AcOEt/hexane = 4:1) to give 3-[3-(4-cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propane-1-sulfonic acid 1-dimethylaminomethylideneamide (2.60 g, 71%)

To a solution of the above obtained compound (2.60 g, 5.31 mmol) in 1,4-dioxane (25 mL) was added 6 N HCl (25 mL), the resulting mixture was heated under reflux for 1 h. After cooling to room temperature, water was added and the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with water and dried over $MgSO_4$. After filtration and concentration under reduced pressure, the resulting residue was purified by silica gel column chromatography (AcOEt/hexane = 4:1) to give **18a** (1.62 g, 70%) as a colorless amorphous solid. R_f 0.18 (AcOEt/hexane = 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.62 (6H, s), 2.36–2.46 (2H, m), 3.28 (2H, t, J = 7.1 Hz), 3.90–3.95 (2H, m), 4.85 (2H, s), 7.77 (1H, dd, J = 2.3, 8.4 Hz), 7.90 (1H, d, J = 2.3 Hz), 7.97 (1H, d, J = 8.4 Hz); MS (ESI) m/z 433 ($[M-H]^-$); HRMS Calcd for $C_{16}H_{18}F_3N_4O_3S_2$ 435.0766. Found 435.0797.

6.1.11. 4-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]butane-1-sulfonamide (18b)

This compound was prepared from **17b** using a procedure similar to that described for **18a**. Colorless amorphous solid. R_f 0.18 (AcOEt/hexane = 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.60 (6H, s), 1.97–2.04 (4H, m), 3.21–3.26 (2H, m), 3.72–3.77 (2H, m), 4.66 (2H, s), 7.77 (1H, dd, J = 1.9, 8.5 Hz), 7.89 (1H, d, J = 1.9 Hz), 7.96 (1H, d, J = 8.5 Hz). MS (ESI) m/z 447 ($[M-H]^-$); HRMS Calcd for $C_{17}H_{20}F_3N_4O_3S_2$ 449.0923. Found 449.0901.

6.1.12. 2-[(Cyanodimethylmethyl)amino]ethanesulfonic acid bis(4-methoxybenzyl)amide (20)

Bis(4-methoxybenzyl) amine (900 mg, 3.50 mmol) was dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C. To this solution, Et_3N (1.02 mL) was added and then 2-(1,3-dioxo-1,3-dihydroisoindol-2-yl)ethanesulfonyl chloride (**19**) (1.05 g, 3.84 mmol) was added in small portions, followed by stirring at room temperature for 3 h. After the addition of water, the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over $MgSO_4$. After filtration and evaporation of the solvent under reduced pressure, the resulting residue was purified by silica gel column chromatography (AcOEt) to give 2-phthalimidoethanesulfonic acid bis(4-methoxybenzyl) amide (1.40 g, 81%).

To the suspension of the above obtained compound (1.40 g, 2.83 mmol) in EtOH (15 mL) was added NH_2NH_2/H_2O (0.151 mL), the mixture was stirred at room temperature for 12 h. The reaction solution was filtered and the filtrate was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 20:1) to give 2-aminoethanesulfonic acid bis(4-methoxybenzyl)amide (460 mg, 45%).

To a solution of the above obtained compound (450 mg, 1.23 mmol) in MeOH (5 mL) was added acetone cyanohydrin (0.136 mL) and stirred at room temperature for 12 h. After further addition of acetone cyanohydrin (0.226 mL), the mixture was heated and maintained at 40–50 °C for 3 h. The reaction solution was concentrated under reduced pressure and purified by silica gel chromatography (CH_2Cl_2/MeOH = 50:1) to give **20** (330 mg, 62%). R_f 0.67 (CH_2Cl_2/MeOH = 20:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.44 (6H, s), 1.95 (1H, br s), 3.00–3.16 (4H, m), 3.82 (6H, s), 4.30 (4H, s), 6.89 (4H, d, J = 8.7 Hz), 7.23 (4H, d, J = 8.7 Hz).

6.1.13. 2-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]ethanesulfonamide (21)

To a solution of **20** (220 mg, 0.510 mmol) in THF (4.5 mL) were added Et_3N (0.014 mL, 0.100 mmol)) and 4-cyano-3-trifluoromethylphenyl isothiocyanate[15] (116 mg, 0.508 mmol); the mixture was stirred at room temperature for 3 h. The reaction solution was concentrated under reduced pressure and purified by silica gel column chromatography (CH_2Cl_2/MeOH = 40:1) to give 2-[3-(4-cyano-3-trifluoromethylphenyl)-4-imino-5,5-dimethyl-2-thioxoimidazolidin-1-yl]ethanesulfonic acid bis(4-methoxybenzyl)amide (259 mg, 77%).

To a solution of the above obtained compound (259 mg, 0.392 mmol) in 1,4-dioxane (2.5 mL) was added 6 N HCl (2.5 mL), the mixture was heated under reflux for 1 h. After cooling to room temperature, water was added and the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over $MgSO_4$. After filtration and concentration under reduced pressure, the resulting residue was purified by silica gel column chromatography (AcOEt/hexane = 1:1) to give 2-[3-(4-cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]ethanesulfonic acid bis-(4-methoxybenzyl) amide (144 mg, 56%).

The above obtained compound (140 mg, 0.211 mmol), TFA (1 mL) and anisole (0.02 mL) were mixed and stirred at room temperature for 2 h, followed by heating under reflux for 1 h. After cooling to room temperature, water was added and the reaction mixture was extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. After filtration and concentration under reduced pressure, the resulting residue was purified by silica gel column chromatography (AcOEt/hexane = 4:1) to give **21** (64 mg, 72%) as a colorless amorphous solid. R_f 0.21 (AcOEt/hexane = 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.64 (6H, s), 3.67–3.72 (2H, m), 4.17–4.22 (2H, m), 4.88 (2H, br s), 7.76 (1H, dd, J = 1.8, 8.5 Hz), 7.88 (1H, d, J = 1.8 Hz), 7.97 (1H, d, J = 8.5 Hz); HRMS Calcd for $C_{15}H_{16}F_3N_4O_3S_2$ 421.061. Found 421.0642.

6.1.14. 2-(4-{*N*-methyl-*N*-(*tert*-butoxycarbonyl)sulfamoyl}-butylamino)-2-methylpropionic acid ethyl ester (23)

To a solution of 3-chloropropane-1-sulfonic acid methylamide (**22**)[21] (1.08 g, 6.29 mmol) were added Boc_2O (2.06 g, 9.43 mmol) and 4-dimethylaminopyridine (DMAP) (77 mg, 0.630 mmol) in CH_3CN (12.6 mL); the mixture was stirred at room temperature for 17 h. After the addition of water, the reaction mixture was extracted with CH_2Cl_2 and the organic layer was dried over $MgSO_4$. After filtration, the solvent was distilled off under reduced pressure to give *N*-methyl-*N*-(*tert*-butyloxycarbonyl)-3-chloropropanesulfonamide (1.65 g, 96%).

To a solution of 2-aminoisobutyric acid ethyl ester hydrochloride (592 mg, 3.53 mmol) in CH_3CN (5 mL) and DMF (1 mL) was added K_2CO_3 (1.02 g, 7.38 mmol), the mixture was stirred at room temperature for 1 h. To a solution of the mixture were added the above obtained compound (800 mg, 2.94 mmol) and NaI (441 mg, 2.94 mmol), the reaction mixture was maintained at 80 °C for 22 h. After cooling to room temperature, water was added and the reaction mixture was extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. After filtration and evaporation of the solvent under reduced pressure, the resulting residue was purified by silica gel column chromatography (AcOEt/hexane = 1:1) to give **23** (813 mg, 75%). R_f 0.32 (AcOEt/hexane = 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.27 (3H, t, J = 7.1 Hz), 1.28 (6H, s), 1.54 (9H, s), 1.87–1.92 (2H, m), 2.59 (2H, t, J = 6.5 Hz), 3.19 (3H, s), 3.54–3.59 (2H, m), 4.16 (2H, q, J = 7.1 Hz).

6.1.15. 3-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propane-1-sulfonic acid methylamide (24)

Compound **23** was treated with a procedure similar to that described for **18a** to obtain *tert*-butyl 3-[3-(4-cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propane-*N*-methylsulfonylcarbamate (yield 100%).

To a solution of the above obtained compound (300 mg, 0.547 mmol) in CH_2Cl_2 (2.7 mL) was slowly added TFA (0.421 mL) at 0 °C, and the mixture stirred at room temperature for 5.5 h. The reaction solution was concentrated under reduced pressure and purified by silica gel column chromatography (AcOEt/hexane/CH_2Cl_2 = 1:1:1) to give **24** (235 mg, 96%) as a colorless amorphous solid. R_f 0.18 (AcOEt/hexane = 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.62 (6H, s), 2.33–2.39 (2H, m), 2.84 (3H, d, J = 5.2 Hz), 3.16 (2H, t, J = 7.1 Hz), 3.89–3.94 (2H, m), 4.35 (1H, q, J = 5.2 Hz), 7.77 (1H, dd, J = 1.7, 8.4 Hz), 7.90 (1H, d, J = 1.7 Hz), 7.96 (1H, d, J = 8.4 Hz); MS (ESI) m/z 447 ($[M-H]^-$); HRMS Calcd for $C_{17}H_{20}F_3N_4O_3S_2$ 449.0923. Found 449.0941.

6.1.16. 2-(3-Dimethylsulfamoylpropylamino)-2-methylpropionic acid methyl ester (26)

This compound was prepared from 3-chloropropane-1-sulfonic acid dimethylamide (**25**) using a procedure similar to that described for **17a**. Yield 29%; R_f 0.43 (AcOEt/MeOH = 10:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.23 (6H, s), 1.82–1.92 (2H, m), 2.59 (2H, t, J = 6.7 Hz), 2.81 (6H, s), 2.95–3.00 (2H, m), 3.64 (3H, s).

6.1.17. 3-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propane-1-sulfonic acid dimethylamide (27)

This compound was prepared from **26** using a procedure similar to that described for **18a**. Yield 56%; R_f 0.77 (CH_2Cl_2/acetone = 20:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.62 (6H, s), 2.32–2.43 (2H, m), 2.91 (6H, s), 3.04 (2H, t, J = 6.9 Hz), 3.89–3.95 (2H, m), 7.78 (1H, dd, J = 1.8, 8.3 Hz), 7.90 (1H, d, J = 1.8 Hz), 7.96 (1H, d, J = 8.3 Hz); MS (ESI) m/z 485 ($[M+Na]^+$); HRMS Calcd for $C_{18}H_{22}F_3N_4O_3S_2$ 463.1079. Found 463.1092.

6.1.18. *N*-(4-Chloro-2-methyl-3-trifluoromethylphenyl)-2,2-dimethylpropionamide (29a) and *N*-(4-chloro-2-methyl-5-trifluoromethylphenyl)-2,2-dimethylpropionamide (29b)

To a solution of *N*-(4-chloro-3-trifluoromethylphenyl)-2,2-dimethylpropionamide (**28**) (11.2 g, 40.0 mmol) in THF was slowly added *n*-BuLi (1.6 M in hexane, 60 mL) at −30 °C, the mixture was stirred at the same temperature for 45 min. To the reaction mixture was added MeI (5.1 mL, 55.0 mmol) at −30 °C, the mixture was stirred at the same temperature for 30 min. After the addition of water, the reaction mixture was extracted with AcOEt. The organic layer was washed with water and dried over $MgSO_4$. After filtration, the reaction solution was concentrated under reduced pressure and purified by silica gel column chromatography (CH_2Cl_2) to give **29a** (8.63 g, 74%) and **29b** (2.12 g, 18%).

Compound **29a**: R_f 0.79 (CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.35 (9H, s), 2.35–2.37 (3H, m), 7.21 (1H, br s), 7.34 (1H, d, J = 8.7 Hz), 7.77 (1H, d, J = 8.7 Hz); MS (ESI) m/z 294 ($[M+H]^+$).

Compound **29b**: R_f 0.88 (CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.35 (9H, s), 2.28 (3H, s), 7.32 (1H, s), 8.31 (1H, s); MS (ESI) m/z 294 ($[M+H]^+$).

6.1.19. 4-Amino-3-methyl-2-trifluoromethylbenzonitrile (30a)

Under nitrogen atmosphere, CuCN (177 mg, 1.98 mmol) was added to a solution of compound **29a** (340 mg, 1.16 mmol) in NMP (3.5 mL). The mixture was heated and maintained at 200 °C for 4 days. After cooling to room temperature, water was added. The precipitate was filtered, washed with water and dried. To a solution of the resulting solid in EtOH (2.7 mL) was added concd HCl (2.7 mL) and the mixture was refluxed for 2 h. After cooling to 0 °C, 2 N NaOH was added to neutralize the reaction mixture followed by extraction with CH_2Cl_2. The organic layer was washed with brine and dried over $MgSO_4$. After filtration and evaporation of the solvent under reduced pressure, the resulting residue was purified by NH silica gel column chromatography (CH_2Cl_2) to give **30a** (200 mg, 86%). R_f: 0.43 (CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.56 (9H, s), 4.38 (2H, br s), 6.82 (1H, d, J = 8.4 Hz), 7.46 (1H, d, J = 8.4 Hz); MS (EI) m/z 200 ($[M]^+$).

6.1.20. 4-Amino-5-methyl-2-trifluoromethylbenzonitrile (30b)

This compound was prepared from **29b** using a procedure similar to that described for **30a**. Yield 20%; R_f: 0.48 (CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.20 (9H, s), 4.30 (2H, br s), 6.94 (1H, s), 7.45 (1H, s); MS (EI) m/z 200 ($[M]^+$).

6.1.21. 3-[3-(3-Chloro-4-cyanophenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propane-1-sulfonamide (32d)

Under nitrogen atmosphere, thiophosgene (27.5 mL, 361 mmol) was added to a solution of 4-amino-2-chloro-benzonitrile (**31d**) (50.0 g, 327 mmol) in THF (1500 mL) at 0 °C. After stirring at 5 °C for 1 h, water was added and the reaction mixture was extracted with Et_2O. The organic layer was washed with brine and dried over $MgSO_4$. After filtration, the solvent was distilled off under reduced

pressure. The resulting residue was recrystallized from acetone/hexane to give 2-chloro-4-isothiocyanatobenzonitrile (44.5 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.19 (1H, dd, J = 2.1, 8.7 Hz), 7.35 (1H, d, J = 2.1 Hz), 7.65 (1H, d, J = 8.7 Hz).

To a solution of **17a** (100 mg, 0.330 mmol) in THF (0.7 mL) were added the above obtained compound (63.3 mg, 0.330 mmol) and DMAP (60.4 mg, 0.494 mmol) and the mixture was heated at 60 °C under nitrogen atmosphere. After stirring for 45 min, water 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 (CH_2Cl_2/MeOH = 20:1) gave 3-[3-(3-chloro-4-cyanophenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propane-1-sulfonic acid 1-dimethylaminomethylideneamide (136 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.60 (6H, s), 2.32–2.38 (2H, m), 3.06 (3H, s), 3.13 (2H, t, J = 6.6 Hz), 3.16 (3H, s), 3.93–3.95 (2H, m), 7.44 (1H, dd, J = 1.5, 8.4 Hz), 7.60 (1H, d, J = 1.5 Hz), 7.78 (1H, d, J = 8.4 Hz), 8.07 (1H, s); MS (ESI) m/z 460 ([M+H]$^+$).

To a solution of the above obtained compound (136 mg, 0.299 mmol) in 1,4-dioxane (2.4 mL) was added 6 N HCl (1.2 mL); the resulting mixture was heated under reflux for 1 h. After cooling to room temperature, water was added and the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with water and dried over $MgSO_4$. After filtration and concentration under reduced pressure, the resulting residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 30:1) to give **32d** (104 mg, 86%) as a colorless amorphous solid. R_f: 0.23 (CH_2Cl_2/MeOH = 20:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.53 (6H, s), 2.12–2.22 (2H, m), 3.10 (2H, t, J = 7.3 Hz), 3.81 (2H, t, J = 7.3 Hz), 6.86 (2H, s), 7.65 (1H, d, J = 8.1 Hz), 7.96 (1H, s), 8.15 (1H, d, J = 8.1 Hz); MS (ESI) m/z 401 ([M+H]$^+$; HRMS Calcd for $C_{15}H_{18}ClN_4O_3S_2$ 401.0503. Found 401.0517.

Compounds **32a–c**, **e** and **f** were prepared using a procedure similar to that described for **32d**.

6.1.22. 3-[3-(4-Cyanophenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propane-1-sulfonamide (32a)

R_f 0.30 (AcOEt/hexane = 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.60 (6H, s), 2.35–2.48 (2H, m), 3.28 (2H, t, J = 7.0 Hz), 3.88–3.98 (2H, m), 4.70 (2H, br s), 7.51 (2H, d, J = 8.4 Hz), 7.79 (2H, d, J = 8.4 Hz); MS (ESI) m/z 367 ([M+H]$^+$; HRMS Calcd for $C_{15}H_{19}N_4O_3S_2$ 367.0893. Found 367.0899.

6.1.23. 3-[3-(4-Cyano-3-methylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propane-1-sulfonamide (32b)

^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.58 (6H, s), 2.37–2.42 (2H, m), 2.59 (3H, s), 3.25 (2H, t, J = 6.9 Hz), 3.15 (3H, s), 3.88–3.94 (2H, m), 5.00 (2H, br s), 7.26–7.32 (2H, m), 7.72 (1H, d, J = 8.2 Hz), 8.07 (1H, s); MS (ESI) m/z 379 ([M−H]$^-$; HRMS Calcd for $C_{16}H_{21}N_4O_3S_2$ 381.1049. Found 381.1049.

6.1.24. 3-[3-(4-Cyano-3-methoxyphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propane-1-sulfonamide (32c)

R_f 0.20 (CH_2Cl_2/MeOH = 20:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.54 (6H, s), 2.12–2.25 (2H, m), 3.08–3.17 (2H, m), 3.82 (2H, t, J = 7.7 Hz), 3.91 (3H, s), 6.87 (2H, s), 7.14 (1H, d, J = 8.1 Hz), 7.36 (1H, s), 7.87 (1H, d, J = 8.1 Hz); MS (ESI) m/z 397 ([M+H]$^+$; HRMS Calcd for $C_{16}H_{21}N_4O_4S_2$ 397.0998. Found 397.1031.

6.1.25. 3-[3-(4-Cyano-2-methyl-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propane-1-sulfonamide (32e)

R_f 0.20 (CH_2Cl_2/MeOH = 20:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.56 (3H, s), 1.58 (3H, s), 2.15–2.20 (2H, m), 2.23 (3H, s), 3.10 (2H, t, J = 8.1 Hz), 3.78–3.90 (2H, m), 6.87 (2H, s), 8.00 (1H, d, J = 8.1 Hz), 8.18 (1H, d, J = 8.1 Hz); MS (ESI) m/z 449 ([M+H]$^+$); HRMS Calcd for $C_{17}H_{20}F_3N_4O_3S_2$ 449.0923. Found 449.0911.

6.1.26. 3-[3-(4-Cyano-2-methyl-5-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]propane-1-sulfonamide (32f)

R_f 0.33 (CH_2Cl_2/MeOH = 20:1); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.54 (3H, s), 1.58 (3H, s), 2.12–2.25 (2H, m), 2.22 (3H, s), 3.05–3.15 (2H, m), 3.75–3.90 (2H, m), 6.87 (2H, s), 8.22 (1H, s), 8.30 (1H, s); HRMS Calcd for $C_{17}H_{20}F_3N_4O_3S_2$ 449.0923. Found 449.0937.

6.2. Competitive androgen receptor binding assay

CHO-K1/hAR cells (5×10^4/well) were plated onto 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 a 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.

6.3. Reporter gene assay

6.3.1. Transient reporter gene assay

Twenty four hours before transfection, the Hela cells were plated at 1×10^4 cells/well in phenol red-free DMEM containing 3% DCC-FCS onto 96-well plates. The cells were co-transfected with GM-LUC (50 ng/well) and pSG5-hAR (10 ng/well) using a FuGENE™ 6 Transfection Reagent (Roche). six hours after the transfection, the cells were treated with 1, 10, 100, 1000 or 10,000 nmol/L of test compound in the absence or presence of DHT (0.1 nmol/L) for 48 h. The luciferase activity of each sample was measured using a Bright-Glo™ Luciferase Assay System (Promega).

6.3.2. Stable reporter gene assay

Hela cells were co-transfected with MMTV-Luc-Hyg and pSG5-hAR-neo using a FuGENE™ 6 Transfection Reagent. The transfected cells were selected in DMEM containing 500 μg/mL neomycin, 300 μg/mL hygromycin and 10% FBS and the cloned 11A11B2 cells were maintained in DMEM containing 400 μg/mL neomycin, 200 μg/mL hygromycin and 10% FBS. Pre-starved 11A11B2 cells were plated at 1×10^4 cells/well in phenol red-free DMEM containing 3% DCC-FCS onto 96-well plates. Following overnight attachment, the cells were treated with 1, 10, 100, 1000 or 10,000 nmol/L of test compound in the absence or presence of DHT (0.1 nmol/L) for 48 h. The luciferase activity of each sample was measured using a Bright-Glo™ Luciferase Assay System.

6.4. In vitro cell growth assay LNCaP and LNCaP-BC2

Pre-starved LNCaP or LNCaP-BC2 cells were plated onto 96-well plates in phenol red-free RPMI 1640 containing 5% DCC-FBS. Following overnight attachment, the cells were treated with the test compound in the absence or presence of R1881 (LNCaP: 0.1 nM, LNCaP-BC2: 0.01 nM) for 6 days. Cell proliferation was determined by CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega).

6.5. 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, test compound

(final concn 1 mM) was added to start the reaction. After incubation for 15 min at 37 °C, CH_3CN was added to stop the reaction. The concentration of the test compound was measured by LC/MS/MS (QTRAP, Applied Biosystems).

6.6. Molecular modeling. Docking model of compound 32d to AR

This model was built based on the X-ray crystal structure of human AR in complex with the ligand R1881 (PDB ID: 1e3g).[22] 3D structures of compound **32d** were modeled using software SYBYL[23] with a Tripos force field.[24] Compound **32d** was manually docked into AR such that (i) the binding mode of the cyanophenyl moiety of compound **32d** was similar to that of bicalutamide (PDB ID: 1z95)[17] and (ii) the thiohydantoin ring was modeled so that the side chain attached to the thiohydantoin ring of **32d** was 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 the condition that the coordinates of AR are fixed. Conformations obtained for **32d** are local minimum energy conformations.

6.7. In vivo antitumor activities on LNCaP Xenograft in mice

LNCaP (2×10^6 cells) was subcutaneously inoculated into noncastrated 6-week-old male SCID mice (CLEA Japan). When the tumor size reached 90–400 mm^3, the animals were randomized and orally administered an agent or the agent vehicle (5% gum arabic) at 10 mL/kg body weight. The agents were administered once a day in seven cycles of 5 days on, 2 days off; the tumor size was measured at time points designated to be appropriate.

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