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Design and synthesis of an androgen receptor pure antagonist (CH5137291) for the treatment of castration-resistant prostate cancer

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ABSTRACT

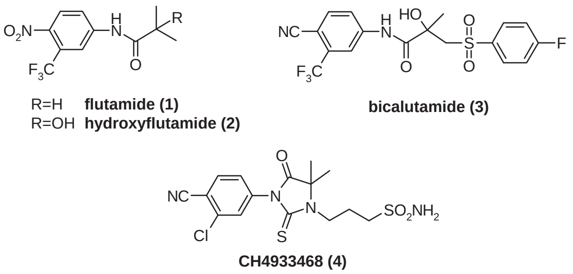
A series of 5,5-dimethylthiohydantoin derivatives were synthesized and evaluated for androgen receptor pure antagonistic activities for the treatment of castration-resistant prostate cancer. Since CH4933468, which we reported previously, had a problem with agonist metabolites, novel thiohydantoin derivatives were identified by applying two strategies. One was the replacement of the alkylsulfonamide moiety by a phenylsulfonamide to avoid the production of agonist metabolites. The other was the replacement of the phenyl ring with a pyridine ring to improve in vivo potency and reduce hERG affinity. Pharmacological assays indicated that CH5137291 (**17b**) was a potent AR pure antagonist which did not produce the agonist metabolite. Moreover, CH5137291 completely inhibited in vivo tumor growth of LNCaP-BC2, a castration-resistant prostate cancer model.

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.[1] Since the growth of prostate cancer is dependent on androgen, androgen receptor (AR) antagonists such as flutamide (**1**) (a precursor of the active form hydroxyflutamide (**2**)) and bicalutamide (**3**) are clinically used to treat prostate cancer[2] (Chart 1). These AR antagonists exhibit good efficacy in many cases and comprise an important part of effective therapeutics.[3–6] However, after a short period of response, the tumor progresses through treatment to a castration-resistant prostate cancer (CRPC) stage.[7] Therefore, treatment of CRPC would benefit from novel AR antagonists. We previously reported that hydroxyflutamide and bicalutamide have partial agonistic activities at high concentrations in vitro.[8] Therefore, we hypothesized that the AR antagonists exhibiting no agonist activities, so-called AR pure antagonists[9,10] would be efficacious against CRPC.

We recently reported that a thiohydantoin derivative, CH4933468 (**4**),[11] with a sulfonamide terminal side chain, not only showed AR pure antagonistic activities in the reporter gene assay (RGA) but also inhibited the growth of LNCaP-BC2,[12] a bicalutamide-resistant cell line modeling CRPC. Furthermore, CH4933468 also inhibited tumor growth of the LNCaP xenograft dose-dependently in mice.

However, CH4933468 did not show antitumor activity against a mouse LNCaP-BC2 xenograft model,[13] an AR hypersensitive CRPC model, because of the existence of a small amount of 3-dealkylated thiohydantoin metabolite (**5**) with strong AR agonistic activity. Therefore, the amount of **5** was evaluated in vitro and in vivo to predict the production of **5** in human (Table 1). In in vitro liver microsome experiments with various animal species, the concentration of the metabolite was determined to be less than 0.1%. However, from in vivo pharmacokinetic studies after oral administration of CH4933468, the AUC of metabolite **5** was found to be in the range of 0.005–8.6% in various animals. Especially, a high concentration of **5** was detected in rats. In parallel, we evaluated the



R=H **flutamide (1)**
R=OH **hydroxyflutamide (2)**

bicalutamide (3)

CH4933468 (4)

Chart 1. Structure of AR antagonists.

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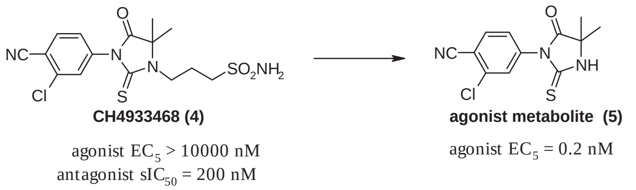
Table 1
Agonist metabolite (**5**) of CH4933468 and its amount in vitro and in vivo in several animal species



	In vitro liver microsome concd @ 30 min[a] (% of intact CH4933468)	In vivo PK[b] AUC% of intact
Human	0.022%	
Mouse	0.038%	0.7%[c]
Rat	0.075%	8.6%[c]
Dog	<0.001%	0.005%[d]
Monkey	0.027%	0.15%[c]

[a] Lower limit of quantification: 0.1 nM, substrate concn: 10 mM, enzyme concn: 1 mg/protein/mL. The values were determined by a single experiment run in duplicate.
[b] Lower limit of quantification: 0.1 nM, $n = 3$.
[c] Dose: 100 mg/kg multiple.
[d] Dose: 100 mg/kg single.

pharmacological effects of the agonist metabolite in vitro. The addition of metabolite **5** at a concentration of only 0.1% to CH4933468 affected the pure AR antagonistic activities of CH4933468 (data not shown). These results showed that it was difficult to develop CH4933468 as an AR pure antagonist.

Based on the above results, we applied a novel modification strategy to obtain AR pure antagonists without the propensity to produce agonist metabolites. Our strategy was to mimic the structure of the alkyl sulfonamide by incorporating an aryl sulfonamide (Chart 2).

2. Chemistry

The agonist metabolite **5** and 3-phenylthiohydantoin analogs were prepared according to the synthetic sequence outlined in Scheme 1. Isothiocyanate **6a** and aminoisobutyric acid ester were treated with Et$_3$N to give compound **5**. Isothiocyanate **6b** was treated with 2-methyl-2-phenylaminopropanenitrile and Et$_3$N, followed by hydrolysis with 6 N HCl to give 3-phenyl thiohydantoin **7**. For the synthesis of phenyl sulfonamides **11a** and **11b**, bromide derivatives **9a** and **9b** were used as intermediates. Alkylation of 3-amino-*N*,*N*-bis(phenylmethyl)benzenesulfonamide[14] with **9a** and **9b** and NaH, followed by thiohydantoin ring formation with ClCSOPh and NaH, gave compounds **10a** and **10b**, respectively. Deprotection of the benzyl group on sulfonamide by concentrated H$_2$SO$_4$[15] afforded target compounds **11a** and **11b**.

Pyridine sulfonamide derivatives were synthesized according to the synthetic sequence shown in Scheme 2. Dibromopyridines **12a** and **12b** were treated with *i*PrMgCl and SO$_2$Cl$_2$,[16] followed by amidation with dibenzylamine to give corresponding bromo sulfonamides **13a** and **13b**. An amino group on the pyridine ring was installed by treatment with *p*-methoxybenzylamine (PMBNH$_2$), followed by deprotection of the PMB group with TFA. Alkylation of **14a** and **14b** with 2-bromo-2-methylpropionic acid, followed by esterification gave **15a** and **15b**, respectively. Cyclization of the thiohydantoin ring with corresponding isothiocyanates in the presence of DMAP followed by deprotection of the benzyl group gave target molecules **17a**, **17b**, **17c**, and **17d**.

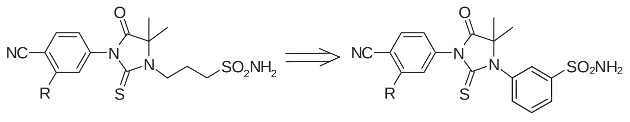
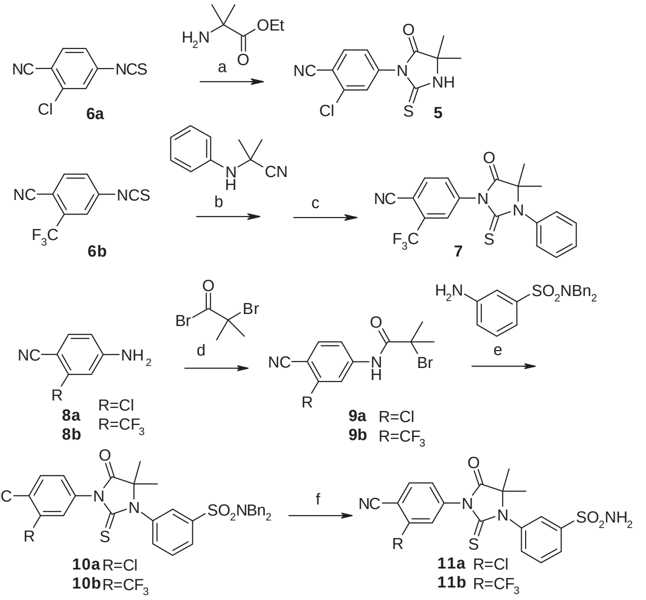


Chart 2. Modification strategy to avoid agonist metabolite formation.



Scheme 1. Reagents and conditions: (a) Et$_3$N, toluene, 100 °C; (b) Et$_3$N, THF, reflux; (c) 6 N HCl, 1,4-dioxane, 80 °C; (d) *i*Pr$_2$NEt, CH$_2$Cl$_2$, rt; (e) (i) NaH, THF, 45 °C; (ii) NaH, ClCSOPh, THF, rt; (f) concd H$_2$SO$_4$, rt.

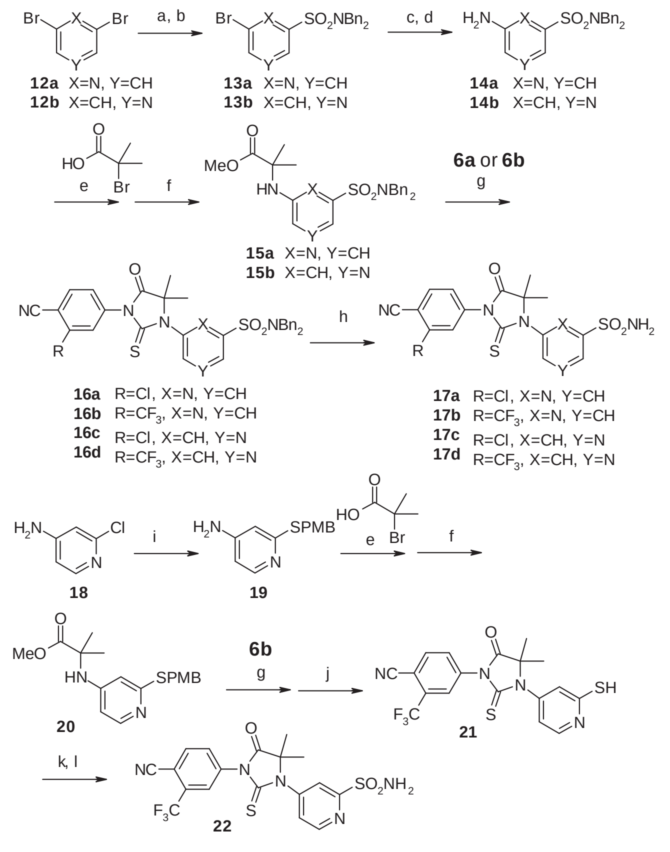
Compound **22** was prepared by a different route due to the difficulty of the synthesis of 4-aminopyridine-2-sulfonamide. Specifically, 2-chloropyridin-4-ylamine (**18**) was treated with PMBSH in the presence of *t*BuOK to give **19**. Alkylation of **19** with 2-bromo-2-methylpropionic acid, followed by esterification gave **20**. After cyclization of the thiohydantoin ring, deprotection of PMB group with TFA gave thiol **21**. Treatment of **21** with KNO$_3$ and SO$_2$Cl$_2$, followed by ammonia, afforded target compound **22**.

3. Results and discussion

The synthesized compounds were evaluated for their in vitro agonist/antagonist activities to AR using the same procedures as previously reported.[11] In RGA, a ‘pure antagonist’ was defined to have a EC$_5$ value greater than 10,000 nM. Cell growth was also determined in LNCaP-BC2 cells as previously reported.[11] Agonistic activity was expressed as + or − according to the dose–response curve of growth. A ‘pure antagonist’ was expressed as −.

Phenyl sulfonamide compounds **11a** and **11b** showed almost the same activity as CH4933468 in the in vitro RGA and cell growth inhibition assays. Since 3-phenyl thiohydantoin (**7**) showed partial agonist activities, these results were attributed to the sulfonamide moiety. In addition, as expected, compound **11a** did not produce the agonist metabolite **5** either in vitro or in vivo (data not shown). So, we succeeded in converting an alkyl linker to an aryl linker with the activities maintained without the production of the agonist metabolite.

To confirm the in vivo activities of the compounds, antiandrogenic activities were evaluated on the basis of seminal vesicle (SV) wet weight in castrated mice.[17] However, phenyl sulfonamide derivatives **11a** and **11b** tended to show weaker activities in vivo than CH4933468. Since these compounds were metabolically stable in human liver microsome and showed acceptable permeability in a parallel artificial membrane permeation assay (PAMPA), weak in vivo antiandrogenic activities might be mainly attributed to low solubility.



Scheme 2. Reagents and conditions: (a) *i*PrMgCl, hexane, rt, then SO_2Cl_2, 0 °C; (b) Bn_2NH, Et_3N, CH_2Cl_2, rt; (c) $PMBNH_2$, *i*Pr_2NEt, 1,3-dimethly-2-imidazolidinone (DMI), microwave, 100 °C; (d) TFA, microwave, 100 °C; (e) *i*Pr_2NEt, 1,4-dioxane, 60 °C; (f) trimethylsilyl diazomethane, MeOH, toluene, rt; (g) DMAP, toluene, 100 °C; (h) concd H_2SO_4, rt; (i) PMBSH, *t*BuOK, DMI, microwave, 120 °C; (j) TFA, microwave, 120 °C; (k) KNO_3, SO_2Cl_2, 0 °C; (l) NH_3 aq, rt.

In addition, **11a** and **11b** strongly inhibited the human *ether-a-go-go* related gene (hERG) potassium channel. Therefore, further investigation was necessary to address issues of weak in vivo potency and potent hERG inhibitory activity. According to Zhao et al., both solubility and hERG affinity are highly related to the lipophilicity of the molecule.[18] On the other hand, our previous research showed that the thiohydantoin ring and left side phenyl ring were important for AR binding.[11] Therefore, we focused our efforts on the conversion of the right side phenyl ring. Specifically, we replaced the phenyl ring with pyridine rings to reduce lipophilicity.

All pyridine derivatives showed AR pure antagonistic activity in both RGA and LNCaP-BC2 (Table 2). Although there were some differences in potency between RGA and LNCaP-BC2, all compounds in which the phenyl ring had been replaced by a pyridine ring showed acceptable biological activity.

Concerning the in vitro ADME profile, some improvements were observed after replacing the phenyl ring with a pyridine ring. Although the metabolic stability of the pyridine derivatives was slightly lower than that of the phenyl derivatives, the solubility of the pyridine derivatives was improved. In the mouse PK screening, all of the pyridine derivatives had higher AUC values than **11a** and **11b**. Among them, the AUCs for 2-pyridine derivatives **17a** and **17b** were tenfold higher than that of **11b**. On the other hand, 4-pyridine compound **22** showed only a slight increase in AUC value. The in vivo SV wet weights in mice for **17a** and **17b** showed potent antiandrogenic activities, indicating that increased oral AUC values might lead to improved in vivo activity. As expected, the pyridine derivatives showed a reduction in hERG inhibitory activity. Especially, 4-pyridine compound **22** drastically reduced hERG binding. On the other hand, 5-pyridine compounds **17c** and **17d** resulted in only slightly reduced hERG binding. The results indicated that the position of the nitrogen on the pyridine ring significantly affects hERG channel binding. The replacement of the phenyl ring by a pyridine ring contributed to solve both issues at the same time.

Among the compounds, **17b** (CH5137291) attracted the most attention as an AR pure antagonist. First, the existence of the 3-dearylated thiohydantoin metabolite (**23**)[19] was examined (Table 3). Since **23** also showed strong agonistic activity in RGA like compound **5**, the amount of **23** produced after oral administration of CH5137291 was a significant issue. In the in vitro liver microsome stability experiments with CH5137291, the production of **23** was below the lower limit of quantification (0.001%) in any animal species, including human. After oral administration of CH5137291, less than 0.1% of metabolite **23** was determined in rat and was not determined in other species. In addition, other metabolites which showed AR agonist activity were not detected in any species. These results encouraged us to believe that the amount of the agonist metabolite **23** produced from CH5137291 would be acceptable in clinical development.

Since CH5137291 showed weak hERG inhibition in vitro, potential cardiovascular effects of CH5137291 were evaluated using anesthetized dogs. Fortunately, no significant adverse changes of electrocardiographic parameters including QT prolongation were observed (data not shown).

To confirm the in vivo bicalutamide-resistant antitumor effect of CH5137291, we examined the LNCaP-BC2 mouse xenograft model. Treatment with CH5137291 (10 and 100 mg/kg) inhibited tumor growth in this model (Fig. 1a). On the other hand, bicalutamide (10 and 100 mg/kg) did not inhibit tumor growth. This tendency was clarified by the relative PSA (prostate specific antigen; the transcriptional product of AR) concentration results. In the case of CH4933468, the relative PSA increased significantly.[13] Treatment with CH5137291 completely inhibited production of PSA (Fig. 1b). In contrast, bicalutamide did not completely inhibit production of PSA. These results were corroborated by the in vitro agonist activities of bicalutamide.

4. Conclusion

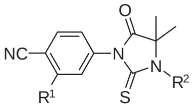
Starting from AR pure antagonist CH4933468, which had the problem of forming an agonist metabolite, novel thiohydantoin derivatives were identified through two strategies: the replacement of the alkylsulfonamide by a phenylsulfonamide to reduce the production of the agonist metabolites and the replacement of the phenyl ring by a pyridine ring to improve in vivo potency and reduce hERG affinity. Pharmacological assays indicated that CH5137291 (**17b**) was a potent AR pure antagonist which would not produce the agonist metabolite **23**. Moreover, CH5137291 completely inhibited in vivo tumor growth and PSA production in the LNCaP-BC2 xenograft model, a bicalutamide-resistant model. In addition to that, the results of early safety studies including a 2-week mouse safety study indicated that CH5137291 was well-tolerated. These results indicate that CH5137291 is expected to contribute to novel therapeutic approaches against CRPC.

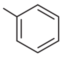
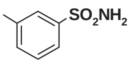
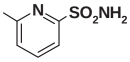
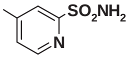
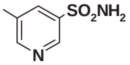
5. Experimental

5.1. Chemistry: instruments

Column chromatography was carried out on Merck Silicagel 60 (230–400 mesh) if not otherwise specified. Reverse phase column chromatography was carried out on Fuji Silysia Chromatorex Pro. No. DM2035MT. NH silica gel column chromatography was carried

Table 2
Activities of thiohydantoin derivatives



No.	R^1	R^2	RGA[a]		LNCaP-BC2[b]		Human liver MS stability[c] Residue (%)	PBS[d] (μg/mL)	PAMPA (cm/s)	In vivo SV[e] ED$_{50}$ (mg/kg)	In vivo PK[f] AUC (mg/mL h)	hERG[g] Inhibition% at 10 μM
			EC$_5$ (nM)	sIC$_{50}$ (nM)	Agonist +/–	Antagonist IC$_{50}$ (nM)						
3			30	200	+	600	99	<3	2.5E–05	3	NT	7.4
4	Cl	$(CH_2)_3SO_2NH_2$	>10,000	200	–	10	100	13	2.5E–05	10	21[h]	17
7	CF_3		3000	200	+	100	80	NT	6.9E–07	NT	NT	NT
11a	Cl		>10,000	400	–	100	100	<3	1.6E–06	84	75[h]	58
11b	CF_3		>10,000	300	–	100	100	11	8.1E–06	63	39[h]	41
17a	Cl		>10,000	600	–	100	100	5	1.3E–06	6	669[i]	26
17b	CF_3		>10,000	700	–	100	94	38	1.4E–05	7	684[h]	10
22	CF_3		>10,000	1000	–	80	97	29	1.2E–05	NT	101[i]	0
17c	Cl		>10,000	2000	–	50	95	76	1.3E–05	NT	163[i]	52
17d	CF_3		>10,000	1000	–	30	95	60	1.4E–05	6	167[h]	33

NT: not tested.

[a] EC$_5$ and IC$_{50}$ values were determined by a single experiment run in duplicate.

[b] IC$_{50}$ values were determined by a single experiment run in triplicate, A 'pure antagonist' was expressed as –.

[c] NADPH added and residue (%) were determined after 15 min..

[d] Phosphate buffer (pH 7.4).

[e] In vivo antiandrogenic activities on seminal vesicle (SV) wet weight in mice (n = 4 or 5).

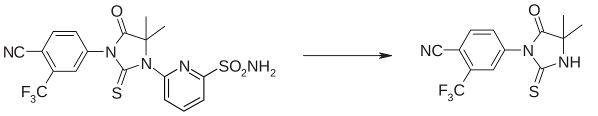
[f] Oral administration in mice (n = 2), dose 10 mg/kg.

[g] Using whole cell patch-clamp technique, the values were determined by a single experiment run in duplicated.

[h] Vehicle 5% arabic gum.

[i] Vehicle 5% DMSO and 20% Cremophor EL.

Table 3
Agonist metabolite (**23**) of CH5137291 and its amount in vitro and in vivo in several animal species



CH5137291 (17b)

agonist EC$_5$ > 10000 nM
antagonist sIC$_{50}$ = 700 nM

agonist metabolite (23)

agonist EC$_5$ = 1.0 nM

	In vitro liver microsome concn @ 30 min[a] (% of intact CH5137291)	In vivo SDPK[b] AUC% of intact
Human	<0.001%	
Mouse	<0.001%	ND
Rat	<0.001%	0.023%
Dog	<0.001%	NT
Monkey	<0.001%	ND

ND: not detected, NT: not tested.

[a] Lower limit of quantification: 0.1 nM, substrate conc: 10 mM, enzyme concn: 1 mg/protein/mL. The values were determined by a single experiment run in duplicate.

[b] Lower limit of quantification: 1 nM, dose: 500 mg/kg single (mouse, rat), 10 mg/kg single (monkey). Administration vehicle: 0.5% CMC suspension (mouse, rat), 5% DMSO/20% Cremophore (monkey), n = 3.

out on Fuji Silysia NH-DM1020. R_f was determined with Merck Silicagel 60 F^{254} plates. ^1H NMR spectra were recorded on 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.

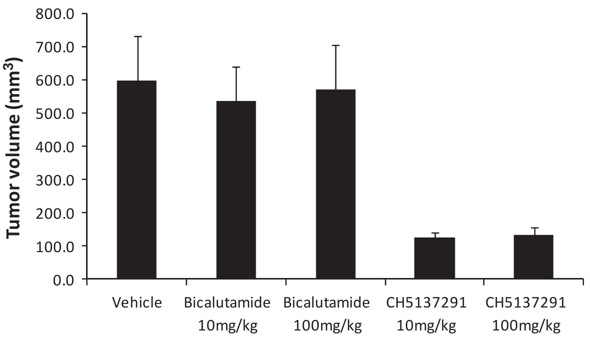
5.1.1. 2-Chloro-4-(4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl)benzonitrile (5)

To a solution of 2-chloro-4-isothiocyanatobenzonitrile (**6a**) (15.0 g, 77.1 mmol) and 2-aminoisobutyric acid ethyl ester hydrochloride (19.4 g, 116 mmol) in toluene (500 mL) was added Et_3N (26.9 mL, 193 mmol) at 0 °C. The mixture was then stirred at 100 °C for 30 min. After cooling to room temperature, water was added to the reaction mixture and extracted with AcOEt. The organic layer was washed with 2 N HCl, saturated aq $NaHCO_3$ and brine and then dried with $MgSO_4$. After filtering, the solvent was distilled off at reduced pressure. The residue was recrystallized from AcOEt/hexane (1:1) to give **5** (18.6 g, 86%) as a colorless solid. R_f 0.34 (AcOEt/hexane = 1:1); mp: 228–229 °C, ^1H NMR (400 MHz, $CDCl_3$) δ: 7.47 (1H, dd, J = 1.8, 8.3 Hz), 7.48 (1H, br s), 7.62 (1H, d, J = 1.8 Hz), 7.80 (1H, d, J = 8.3 Hz); HRMS calcd for $C_{12}H_{11}ClN_3OS$ 280.0306. Found 280.0305.

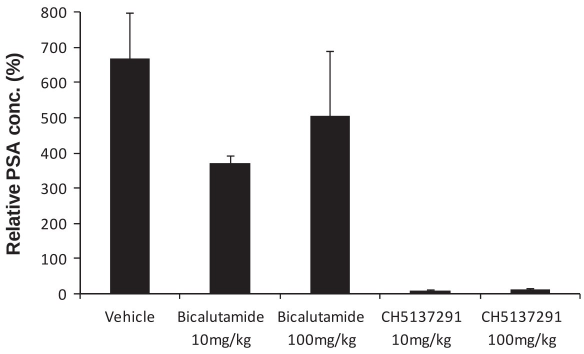
5.1.2. 4-(3-Phenyl-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl)-2-trifluoromethylbenzonitrile (7)

To a solution of 4-isothiocyanato-2-trifluoromethylbenzonitrile (**6b**) (678 mg, 2.97 mmol) and 2-methyl-2-phenylaminopropanenitrile (356 mg, 2.22 mmol) in THF (7 mL) was added Et_3N (1.07 mL, 7.77 mmol), the mixture was then refluxed for 5 h. After cooling to room temperature, solvent was distilled off at reduced pressure. The residue was dissolved in 1,4-dioxane (5 mL) and 6 N HCl (5 mL) and the mixture was refluxed for 1 h. After cooling to room temperature, water was added to the reaction mixture and extracted with CH_2Cl_2. The organic layer was washed with brine,

a Tumor volume



b Relative PSA concentration*



*: Relative PSA concentration (day 17 of administration) / PSA concentration (day 0 of administration)

Figure 1. Antitumor activity in LNCaP-BC2 mouse xenografts. LNCaP-BC2 was subcutaneously inoculated into castrated 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. ($n = 5$ animals per group). After 17 days, the tumor size and plasma PSA level were measured. *Relative PSA concentration (day 17 of administration)/PSA concentration (day 0 of administration).

then 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 **7** (56 mg, 6.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.60 (6H, s), 7.29–7.33 (2H, m), 7.52–7.59 (3H, m), 7.83–7.88 (1H, m), 7.96–8.00 (2H, m); HRMS calcd for $C_{19}H_{15}F_3N_3OS$ 390.0882. Found 390.0882.

5.1.3. 2-Bromo-*N*-(4-cyano-3-trifluoromethylphenyl)-2-methylpropionamide (9b)

To a solution of 4-amino-2-trifluoromethylbenzonitrile (**8b**) (10.0 g, 53.7 mmol) in CH_2Cl_2 (110 mL) were added *i*Pr_2NEt (10.3 mL, 59.1 mmol) and 2-bromobutyryl bromide (7.24 mL, 59.1 mmol) at 0 °C and the mixture was stirred at room temperature. After stirring for 2 h, 2 N HCl was added at 0 °C and the reaction mixture was 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. The residue was recrystallized from AcOEt/hexane (1:3) to give **9b** (18.0 g, quant). R_f 0.55 (AcOEt/hexane = 1:2); mp: 126–127 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.82 (1H, d, J = 8.4 Hz), 7.92 (1H, d, J = 2.4 Hz), 8.06 (1H, s), 8.76 (1H, br s).

5.1.4. 2-Bromo-*N*-(3-chloro-4-cyanophenyl)-2-methylpropionamide (9a)

This compound was prepared from 4-amino-2-chlorobenzonitrile (**8a**) using a procedure similar to that described for **9b**. Yield 73%. R_f 0.43 (AcOEt/hexane = 1:3); mp: 135–136 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.51 (1H, dd, J = 1.8, 8.8 Hz), 7.48 (1H, br s), 7.64 (1H, d, J = 8.8 Hz), 7.92 (1H, d, J = 1.8 Hz), 8.60 (1H, br s).

5.1.5. *N,N*-Dibenzyl-3-[3-(4-cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]benzenesulfonamide (10b)

To a solution of **9b** (11.4 g, 34.0 mmol) and 3-amino-*N,N*-bis(phenylmethyl) benzenesulfonamide (12.0 g, 34.0 mmol) in THF (100 mL) was added NaH (1.63 g, 40.8 mmol) at room temperature, the mixture was heated at 45 °C for 1 h. After cooling to 0 °C, NaH (8.16 g, 204 mmol) and phenyl chlorothionoformate (17.6 g, 102 mmol) were added then stirred for 5 min. After further stirring at room temperature for 1 h, the mixture was diluted with *t*BuOMe. To saturated aq NH_4Cl was slowly added the reaction mixture at 0 °C and extracted with *t*BuOMe. 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 = 2:3) and NH silica gel chromatography (AcOEt/hexane = 2:3) gave **10b** (12.4 g, 56%) as a brown amorphous solid. R_f 0.32 (AcOEt/hexane = 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.60 (6H, s), 4.37 (4H, s) 7.00–7.10 (4H, m), 7.18–7.30 (6H, m), 7.51–7,55 (1H, m), 7.67 (1H, t, J = 8.0 Hz), 7.82–7.86 (2H, m), 7.92–8.01 (3H, m); MS (ESI) *m/z* 649 ($[M+H]^+$).

5.1.6. *N,N*-Dibenzyl-3-[3-(3-chloro-4-cyanophenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]benzenesulfonamide (10a)

This compound was prepared from **9a** using a procedure similar to that described for **10b**. R_f 0.30 (AcOEt/hexane = 1:2); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.58 (6H, s), 4.37 (4H, s) 7.02–7.10 (4H, m), 7.20–7.30 (6H, m), 7.51–7,55 (1H, m), 7.64–7.70 (2H, m), 7.81–7.86 (2H, m), 7.93 (1H, d, J = 8.1 Hz); MS (EI) *m/z* 615 ($[M]^+$).

5.1.7. 3-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]benzenesulfonamide (11b)

Compound **10b** (12.4 g, 19.1 mmol) was dissolved in concd H_2SO_4 at 0 °C, the mixture was stirred at room temperature for 15 min. After cooling to 0 °C, iced water was added and extracted with AcOEt. The organic layer was washed with saturated aq $NaHCO_3$ and brine, and then 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 **11a** (7.53 g, 84%) as a colorless solid. R_f 0.34 (AcOEt/hexane = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.60 (6H, s), 4.94 (2H, s), 7.53–7.57 (1H, m), 7.74 (1H, t, J = 8.0 Hz), 7.83 (1H, dd, J = 1.8, 8.0 Hz), 7.90–7.93 (1H, m), 7.96 (1H, d, J = 1.8 Hz), 8.00 (1H, d, J = 8.0 Hz), 8.06–8.10 (1H, m); HRMS calcd for $C_{19}H_{16}F_3N_4O_3S_2$ 469.0610. Found 469.0608.

5.1.8. 3-[3-(3-Chloro-4-cyanophenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]benzenesulfonamide (11a)

This compound was prepared from **10a** using a procedure similar to that described for **11b**. Yield 79%. R_f 0.44 (AcOEt/hexane = 2:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.60 (6H, s), 4.95 (2H, s), 7.49–7.56 (2H, m), 7.68 (1H, d, J = 1.8 Hz), 7.73 (1H, t, J = 8.0 Hz), 7.82 (1H, d, J = 8.4 Hz), 7.89–7.92 (1H, m), 8.07 (1H, d, 8.0 Hz); HRMS calcd for $C_{18}H_{16}ClN_4O_3S_2$ 435.0347. Found 435.0348.

5.1.9. 6-Bromopyridine-2-sulfonic acid dibenzylamide (13a)

1 M *i*PrMgCl in THF (15 mL, 15.0 mmol) was added dropwise to 2,6-dibromopyridine (**12a**) (2.36 g, 9.96 mmol) and the mixture was stirred at room temperature for 90 min, To a solution of SO_2Cl_2 (1.60 mL, 19.9 mmol) in hexane (75 mL) was slowly added the above mixture at 0 °C. After stirring for 10 min, the reaction mixture was distilled off at reduced pressure and the residue was di-

luted with heptane. The mixture was distilled off at reduced pressure and the residue was dissolved in CH_2Cl_2 (35 mL). To the above solution were added dibenzylamine (1.90 mL, 9.92 mmol) and Et_3N (2.5 mL, 17.9 mmol) at 0 °C. After stirring at room temperature for 1 h, water was added 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 (AcOEt/hexane = 1:4) gave **13a** (2.17 g, 52%) as a brown oil. R_f 0.31 (AcOEt/hexane = 1:4); ^1H NMR (400 MHz, $CDCl_3$) δ: 4.50 (4H, s), 7.18–7.26 (10H, m), 7.58 (1H, d, *J* = 8.1 Hz), 7.65 (1H, t, *J* = 7.3 Hz), 7.85 (1H, d, *J* = 7.3 Hz); MS (ESI) *m/z* 417, 419 ($[M+H]^+$).

5.1.10. 5-Bromopyridine-3-sulfonic acid dibenzylamide (13b)

This compound was prepared from 3,5-dibromopyridine (**12b**) using a procedure similar to that described for **13a**. Yield 43%. ^1H NMR (400 MHz, $CDCl_3$) δ: 4.41 (4H, s), 7.10–7.36 (10H, m), 7.97 (1H, s), 8.78 (1H, d, *J* = 1.8 Hz), 8.86 (1H, d, *J* = 1.8 Hz); MS (ESI) *m/z* 417, 419 ($[M+H]^+$).

5.1.11. 6-Aminopyridine-2-sulfonic acid dibenzylamide (14a)

To a solution of **13a** (1.05 g, 2.53 mmol) in DMI (5 mL) were added *p*-methoxybenzylamine (0.490 mL, 3.79 mmol) and *i*Pr_2NEt (0.878 mL, 5.04 mmol) and the mixture was rapidly heated by microwave irradiation at 100 °C for 30 min. After cooling to room temperature, 0.5 N HCl was added 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. The residue was dissolved in TFA (5 mL) and the mixture was heated by microwave irradiation at 100 °C for 10 min. After cooling to room temperature, the solvent was distilled off at reduced pressure. To the residue was added saturated aq $NaHCO_3$ 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 (AcOEt/hexane = 1:2) gave **14a** (551 mg, 62%). R_f 0.26 (AcOEt/hexane = 1:2); ^1H NMR (400 MHz, $CDCl_3$) δ: 4.44 (4H, s), 4.65 (2H, br s), 6.62 (1H, d, *J* = 8.1 Hz), 7.13–7.15 (4H, m), 7.20–7.24 (6H, m), 7.33 (1H, d, *J* = 7.3 Hz), 7.56 (1H, t, *J* = 7.9 Hz); MS (ESI) *m/z* 354 ($[M+H]^+$).

5.1.12. 5-Aminopyridine-3-sulfonic acid dibenzylamide (14b)

This compound was prepared from **13b** using a procedure similar to that described for **14a**. Yield 34%. ^1H NMR (400 MHz, $CDCl_3$) δ: 4.35 (4H, s), 7.08–7.30 (11H, m), 8.21 (1H, d, *J* = 1.8 Hz), 8.40 (1H, d, *J* = 1.8 Hz); MS (ESI) *m/z* 354 ($[M+H]^+$).

5.1.13. 2-(6-Dibenzylsulfamoylpyridin-2-ylamino)-2-methylpropionic acid methyl ester (15a)

To a crude solution of **14a** (550 mg, 1.56 mmol) and *i*Pr_2NEt (5.40 mL, 31.0 mmol) in 1,4-dioxane (5 mL) was added a solution of 2-bromo-2-methylpropionic acid (2.60 g, 15.6 mmol) in 1,4-dioxane (1 mL) and the mixture was stirred at 90 °C for 13 h. After cooling to 0 °C, 2 N NaOH (10 mL) and MeOH (20 mL) were added and the mixture was heated at 70 °C for 30 min. After cooling to room temperature, the solvent was distilled off at reduced pressure. The residue was acidified (pH 5) with 5 N HCl and the mixture was 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 and the residue was dissolved in toluene (2 mL) and MeOH (2 mL). To the above solution was added a 2 M solution of (trimethylsilyl)diazomethane in hexane (1.0 mL, 2.0 mmol) and the mixture was stirred at room temperature for 30 min. After distilling off the solvent, the residue was purified by silica gel column chromatography (AcOEt/hexane = 1:2) to give **15a** (328 mg, 46%) as a colorless solid. R_f 0.26 (AcOEt/hexane = 1:2); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.51 (6H, s), 3.64 (3H, s), 4.45 (4H, s), 5.00 (1H, s), 6.54 (1H, d, *J* = 8.4 Hz), 7.07–7.10 (4H, m), 7.18–7.21 (6H, m), 7.33 (1H, d, *J* = 7.0 Hz), 7.52 (1H, t, *J* = 7.9 Hz); MS (ESI) *m/z* 476 ($[M+Na]^+$).

5.1.14. 2-(5-Dibenzylsulfamoylpyridin-3-ylamino)-2-methylpropionic acid methyl ester (15b)

This compound was prepared from **14b** using a procedure similar to that described for **15a**. Yield 14%. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.58 (6H, s), 3.69 (3H, s), 4.33 (4H, s), 7.00–7.30 (11H, m), 8.11 (1H, d, *J* = 1.8 Hz), 8.39 (1H, d, *J* = 1.8 Hz); MS (ESI) *m/z* 454 ($[M+H]^+$).

5.1.15. 6-[3-(3-Chloro-4-cyanophenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]pyridine-2-sulfonic acid dibenzylamide (16a)

To a solution of **15a** (63.0 mg, 0.139 mmol) and **6a** (135 mg, 0.691 mmol) in toluene (0.3 mL) was added 4-dimethylaminopyridine (DMAP) (43.0 mg, 0.352 mmol) and the mixture was stirred at 100 °C for 16 h. After cooling to room temperature, the solvent was distilled off at reduced pressure. Purification by silica gel column chromatography (AcOEt/hexane = 1:2) gave **16a** (22.5 mg, 26%). ^1H NMR (400 MHz, $CDCl_3$) δ: 1.70 (6H, s), 4.51 (4H, s), 7.06–7.14 (4H, m), 7.22–7.30 (6H, m), 7.49 (1H, dd, *J* = 1.8, 8.1 Hz), 7.66 (1H, d, *J* = 1.8 Hz), 7.85 (1H, d, *J* = 8.1 Hz), 7.91–8.00 (2H, m), 8.09 (1H, d, *J* = 6.6 Hz); MS (ESI) *m/z*: 616 ($[M+H]^+$).

5.1.16. 6-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]pyridine-2-sulfonic acid dibenzylamide (16b)

This compound was prepared from **15a** and **6b** using a procedure similar to that described for **16a**. Yield 21%. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.71 (6H, s), 4.51 (4H, s), 7.07–7.13 (4H, m), 7.20–7.30 (6H, m), 7.81 (1H, dd, *J* = 1.8, 8.1 Hz), 7.93–8.07 (4H, m), 8.08 (1H, d, *J* = 7.6 Hz); MS (ESI) *m/z*: 650 ($[M+H]^+$).

5.1.17. 5-[3-(3-Chloro-4-cyanophenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]pyridine-3-sulfonic acid dibenzylamide (16c)

This compound was prepared from **15b** and **6a** using a procedure similar to that described for **16a**. Yield 40%. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.58 (6H, s), 4.41 (4H, s), 7.07–7.13 (4H, m), 7.20–7.30 (6H, m), 7.51 (1H, dd, *J* = 1.8, 8.3 Hz), 7.68 (1H, d, *J* = 1.8 Hz), 7.84 (1H, d, *J* = 8.3 Hz), 8.05 (1H, t, *J* = 2.2 Hz), 8.72 (1H, d, *J* = 2.2 Hz), 9.03 (1H, d, *J* = 2.2 Hz); MS (ESI) *m/z*: 616 ($[M+H]^+$).

5.1.18. 5-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]pyridine-3-sulfonic acid dibenzylamide (16d)

This compound was prepared from **15b** and **6b** using a procedure similar to that described for **16a**. Yield 73%. ^1H NMR (400 MHz, $CDCl_3$) δ: 1.60 (6H, s), 4.42 (4H, s), 7.07–7.13 (4H, m), 7.20–7.30 (6H, m), 7.83 (1H, dd, *J* = 1.8, 8.4 Hz), 7.96 (1H, d, *J* = 1.8 Hz), 8.01 (1H, d, *J* = 8.4 Hz), 8.06 (1H, t, *J* = 2.2 Hz), 8.73 (1H, d, *J* = 2.2 Hz), 9.04 (1H, d, *J* = 2.2 Hz); MS (ESI) *m/z*: 650 ($[M+H]^+$).

5.1.19. 6-[3-(3-Chloro-4-cyanophenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]pyridine-2-sulfonic acid amide (17a)

This compound was prepared from **16a** using a procedure similar to that described for **11a**. Yield 65%. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.69 (6H, s), 7.62 (2H, br s), 7.77 (1H, dd, *J* = 1.5, 8.4 Hz), 7.97 (1H, d, *J* = 7.7 Hz), 8.08 (1H, d, *J* = 1.5 Hz), 8.11 (1H, d, *J* = 8.0 Hz), 8.21 (1H, d, *J* = 8.4 Hz), 8.26 (1H, dd, *J* = 7.7, 8.0 Hz); HRMS calcd for $C_{17}H_{15}ClN_5O_3S_2$ 436.0299. Found 436.0300.

5.1.20. 6-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]pyridine-2-sulfonic acid amide (17b)

This compound was prepared from **16b** using a procedure similar to that described for **11a**. Yield 36%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.72 (6H, s), 7.64 (2H, br s), 7.99 (1H, d, J = 7.7 Hz), 8.10–8.18 (2H, m), 8.27 (1H, dd, J = 7.7, 8.0 Hz), 8.37 (1H, s), 8.43 (1H, d, J = 8.4 Hz); HRMS calcd for $C_{18}H_{15}F_3N_5O_3S_2$ 470.0563. Found 470.0562.

5.1.21. 5-[3-(3-Chloro-4-cyanophenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]pyridine-3-sulfonic acid amide (17c)

This compound was prepared from **16c** using a procedure similar to that described for **11a**. Yield 24%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.62 (6H, s), 5.15 (2H, br s), 7.52 (1H, d, J = 8.4 Hz), 7.67 (1H, d, J = 1.8 Hz), 7.83 (1H, d, J = 8.4 Hz), 8.19 (1H, d, J = 1.8 Hz), 8.76 (1H, d, J = 2.2 Hz), 9.24 (1H, d, J = 1.8 Hz); HRMS calcd for $C_{17}H_{15}ClN_5O_3S_2$ 436.0299. Found 436.0298.

5.1.22. 5-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]pyridine-3-sulfonic acid amide (17d)

This compound was prepared from **16d** using a procedure similar to that described for **11a**. Yield 52%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.64 (6H, s), 5.19 (2H, br s), 7.82 (1H, d, J = 8.4 Hz), 7.95 (1H, br s), 8.01 (1H, d, J = 8.4 Hz), 8.20 (1H, t, J = 1.8 Hz), 8.78 (1H, d, J = 1.8 Hz), 9.25 (1H, d, J = 1.8 Hz); HRMS calcd for $C_{18}H_{15}F_3N_5O_3S_2$ 470.0563. Found 470.0556.

5.1.23. 2-(4-Methoxybenzylsulfanyl)pyridin-4-ylamine (19)

To a solution of 2-chloropyridin-4-ylamine (**18**) (1.28 g, 9.96 mmol) in DMI (8 mL) were added 4-methoxy-α-toluenethiol (1.67 mL, 12.0 mmol) and *t*BuOK (2.46 g, 21.9 mmol) and the mixture was heated by microwave irradiation at 120 °C for 2 h. After cooling to room temperature, water was added and extracted with *t*BuOMe. The organic layer was washed with brine and dried with $MgSO_4$. After filtering, the solvent was distilled off at reduced pressure. Purification by reverse phase chromatography (H_2O/MeCN/0.1% TFA = 90:10:1–50:50:1) gave **19** (583 mg, 24%). MS (ESI) *m/z* 247 ($[M+H]^+$).

5.1.24. 2-[2-(4-Methoxybenzylsulfanyl)pyridin-4-ylamino]-2-methylpropionic acid methyl ester (20)

To a solution of **19** (103 mg, 0.415 mmol) and 2-bromo-2-methylpropionic acid (76.3 mg, 0.457 mmol) in 1,4-dioxane (2 mL) was added *i*Pr_2NEt (0.0796 mL, 0.457 mmol), the mixture was stirred at 60 °C for 5 h. After further addition of *i*Pr_2NEt (0.159 mL, 0.914 mmol) and 2-bromo-2-methylpropionic acid (153 mg, 0.914 mmol), the mixture was stirred at 80 °C for 2 h. To the reaction mixture was added 2 N NaOH in MeOH (3 mL) and the mixture was heated at 80 °C for 15 min. After cooling to room temperature, the solvent was distilled off at reduced pressure and the residue was dissolved in THF (2 mL). To the above solution was added diazomethane in Et_2O solution (prepared from *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and 6 N NaOH) until the yellow color did not disappear. To the mixture was added acetic acid until the yellow solution disappeared. The solvent was distilled off at reduced pressure. Purification by silica gel column chromatography (AcOEt/hexane = 2:3) gave **20** (96.6 mg, 67%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.55 (6H, s), 3.71 (3H, s), 3.78 (3H, s), 4.32 (2H, s), 4.53 (1H, s), 6.14 (1H, dd, J = 2.4, 5.8 Hz), 6.24 (1H, d, J = 2.4 Hz), 6.82 (2H, d, J = 8.7 Hz), 7.30 (2H, d, J = 8.7 Hz), 8.05 (1H, d, J = 5.8 Hz).

5.1.25. 4-[3-(2-Mercaptopyridin-4-yl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl]-2-trifluoromethylbenzonitrile (21)

To a solution of **20** (96.6 mg, 0.279 mmol) and 4-isothiocyanato-2-trifluoromethylbenzonitrile (127 mg, 0.558 mmol) in toluene (1 mL) was added DMAP (34.0 mg, 0.279 mmol) and the mixture stirred at 80 °C for 1 day. After further addition of 4-isothiocyanato-2-trifluoromethylbenzonitrile (127 mg, 0.558 mmol), the mixture was stirred at 80 °C for 1 day. After cooling to room temperature, purification by silica gel column chromatography (AcOEt/hexane = 3:2) gave 4-{3-[2-(4-methoxybenzylsulfanyl)pyridin-4-yl]-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl}-2-trifluoromethylbenzonitrile (127 mg, 50%). The above obtained compound (127 mg, 0.235 mmol) was dissolved in TFA (2.0 mL) and the mixture heated by microwave irradiation at 120 °C for 30 min. After cooling to room temperature, the solvent was distilled off at reduced pressure. Purification by reverse phase chromatography (H_2O/MeCN/0.1% TFA = 90:10:1–50:50:1) gave **21** (48.1 mg, 49%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.67 (6H, s), 5.30 (1H, s), 6.80 (1H, dd, J = 2.0, 6.9 Hz), 7.49 (1H, d, J = 2.0 Hz), 7.65 (1H, d, J = 6.9 Hz), 7.80 (1H, dd, J = 2.1, 8.4 Hz), 7.92 (1H, d, J = 2.1 Hz), 8.00 (1H, d, J = 8.4 Hz); MS (ESI) *m/z*: 423 ($[M+H]^+$).

5.1.26. 4-[3-(4-Cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]pyridine-2-sulfonic acid amide (22)

To a solution of **21** (31.0 mg, 0.0734 mmol) and KNO_3 (14.8 mg, 0.147 mmol) in MeCN (1.0 mL) was added SO_2Cl_2 (0.0118 mL, 0.147 mmol) at 0 °C, After stirring at 0 °C for 30 min, concd NH_3 was added. After stirring at room temperature for 30 min, water was added 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 reverse phase chromatography (H_2O/MeCN = 80:20–55:45) gave **22** (6.1 mg, 18%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.64 (6H, s), 5.30 (2H, br s), 7.52 (1H, dd, J = 2.0, 5.2 Hz), 7.80 (1H, dd, J = 2.1, 8.2 Hz), 7.92 (1H, d, J = 2.1 Hz), 7.99 (1H, d, J = 8.2 Hz), 8.03 (1H, d, J = 2.0 Hz), 8.88 (1H, d, J = 5.2 Hz); HRMS calcd for $C_{18}H_{15}F_3N_5O_3S_2$ 470.0563. Found 470.0558.

5.2. Biology

5.2.1. 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 onto 96-well plates at 1×10^4 cells/well in phenol red-free DMEM containing 3% DCC-FCS. 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.

5.2.2. In vitro LNCaP-BC2 cell growth assay

Pre-starved 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 0.01 nM R1881 for 6 days. Cell proliferation was determined using a CellTiter 96® AQueous One Solution Cell Proliferation Assay kit (Promega).

5.2.3. In vivo antitumor activities on LNCaP-BC2 xenograft in mice

LNCaP-BC2 (2×10^6 cells) was subcutaneously inoculated into castrated 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 and plasma PSA levels were measured at appropriate time points. ELISA (Eiken Chemical) was used to measure the PSA levels.

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