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Design, synthesis, and biological evaluation of 4-phenylpyrrole derivatives as novel androgen receptor antagonists

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ABSTRACT

A series of 4-phenylpyrrole derivatives **D** were designed, synthesized, and evaluated for their potential as novel orally available androgen receptor antagonists therapeutically effective against castration-resistant prostate cancers. 4-Phenylpyrrole compound **1** exhibited androgen receptor (AR) antagonistic activity against T877A and W741C mutant-type ARs as well as wild-type AR. An arylmethyl group incorporated into compound **1** contributed to enhancement of antagonistic activity. Compound **4n**, 1-{[6-chloro-5-(hydroxymethyl)pyridin-3-yl]methyl}-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carbonitrile exhibited inhibitory effects on tumor cell growth against the bicalutamide-resistant LNCaP-cxD2 cell line as well as the androgen receptor-dependent JDCaP cell line in a mouse xenograft model. These results demonstrate that this series of pyrrole compounds are novel androgen receptor antagonists with efficacy against prostate cancer cells, including castration-resistant prostate cancers such as bicalutamide-resistant prostate cancer.

1. Introduction

Prostate cancer (PC) is the most common cancer in American males and the second-leading cause of cancer deaths after lung cancer.[1] Since PC growth is primarily stimulated by androgen, blockade of the androgen signal is very important and effective to treat PC. In addition to surgical or chemical castration by gonadotropin-releasing hormone (GnRH) analogues, androgen receptor (AR) antagonists, such as cyproterone acetate,[2] flutamide,[3,4] nilutamide[5,6] and bicalutamide[7–12] have been used to block the androgen signal (Fig. 1). AR antagonists are used as a single agent (monotherapy) or in combination with castration. The latter use, referred to as 'combined androgen blockade (CAB) therapy', shows significant effects by blocking adrenal androgen signals as well as suppressing transient testosterone increase induced by GnRH analogues.[13–18]

However, AR antagonist therapy ultimately results in castration-resistance such as antiandrogen withdrawal syndrome.[19] AR gene mutation such as T877A and W741C/L is an important mechanism for castration-resistance.[20–25] The T877A mutant is activated by hydroxyflutamide, an active metabolite of flutamide.[26] Bicalutamide is known to act as an agonist through the W741C/L mutation of the AR.[24,25] The W741C mutant AR was detected in bicalutamide-resistant PC patient tissue.[27,28]

Here, we report the design, synthesis, and biological evaluation of novel orally available AR antagonists effective against mutant ARs as well as wild-type (WT) AR.

In the course of exploring novel AR antagonists, we screened our compound library and found that pyrrole derivative **1** showed AR antagonistic activity (Table 1). Compound **1** possessed a unique structure, distinct from that of known AR antagonists, containing an anilide structure, as shown in Figure 1. In addition, compound **1** exhibited antagonistic activity against T877A and W741C mutant-type ARs as well as wild-type (WT) AR. We anticipated that a series of compounds derived from compound **1** may comprise a novel class of AR antagonists effective against PCs, including castration-resistant PC. Thus, we selected compound **1** as a lead for the design of novel AR antagonists with improved efficacy. Our design was initially based on the hypothesis that the 4-nitrophenyl moiety of compound **1** corresponded to the 4-cyano-3-(trifluoromethyl)phenyl moiety of bicalutamide (Fig. 2(1)) or the 4-nitro-3-(trifluoromethyl)phenyl moiety of flutamide, fulfilling

Abbreviations: PC, prostate cancer; AR, androgen receptor; GnRH, gonadotropin-releasing hormone; CAB, combined androgen blockade; PSA, prostate-specific antigen; WT, wild-type; DHT, dihydrotestosterone; SAR, structure–activity relationship; DIBAL, diisobutylaluminium hydride; TosMIC, tosylmethyl isocyanide; TFA, trifluoroacetic acid; AIBN, azobisisobutyronitrile.

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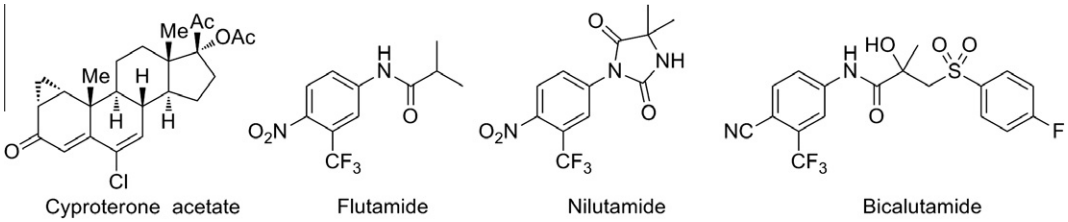


Figure 1. Known androgen receptor antagonists.

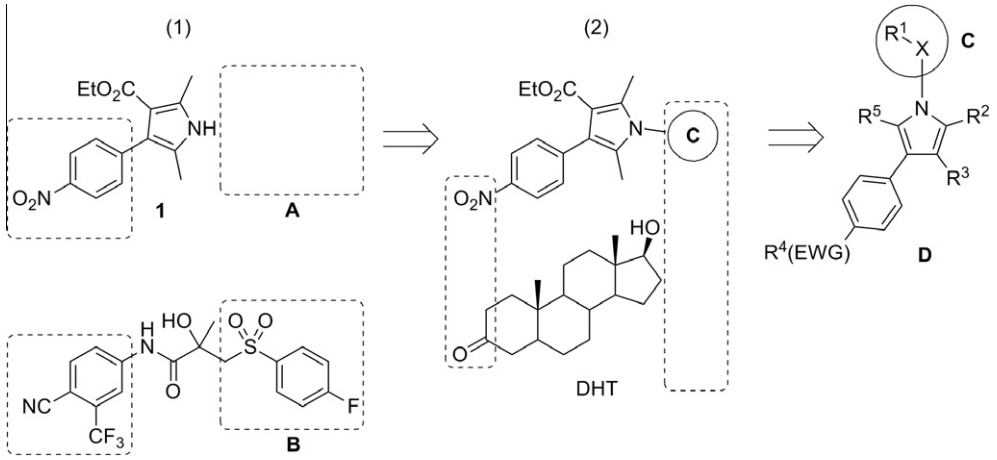


Figure 2. Design of 1-substituted-4-phenylpyrrole AR antagonists.

the requirement of electron-deficient aromatic rings for antagonistic activity.[8] On the basis of this hypothesis, we focused on region **A**, which corresponds to region **B** occupied by the arylsulfonyl moiety of bicalutamide. We supposed that a substituent filling region **A** could be incorporated into compound **1** by attachment at the nitrogen atom of the pyrrole skeleton (group **C**). If the nitro group of compound **1** mimics the carbonyl group of the natural ligand dihydrotestosterone (DHT), schematic superimposition of these two compounds suggested that DHT was missing a moiety corresponding to group **C** (Fig. 2(2)). Taking into account the agonistic activity of DHT and the antagonistic activity of bicalutamide, we considered that introduction of group **C** would contribute to increase the antagonistic activity. On the basis of these hypotheses, we designed scaffold **D**, possessing an electron-deficient aromatic ring and group **C**. For this scaffold, we studied modifications at **C** (linker X plus terminal moiety R^1), substituents on the phenyl ring at the 4-position (R^4), and substituents at the 2-, 3-, and 5-positions of the pyrrole skeleton (R^2, R^3, and R^5).

2. Chemistry

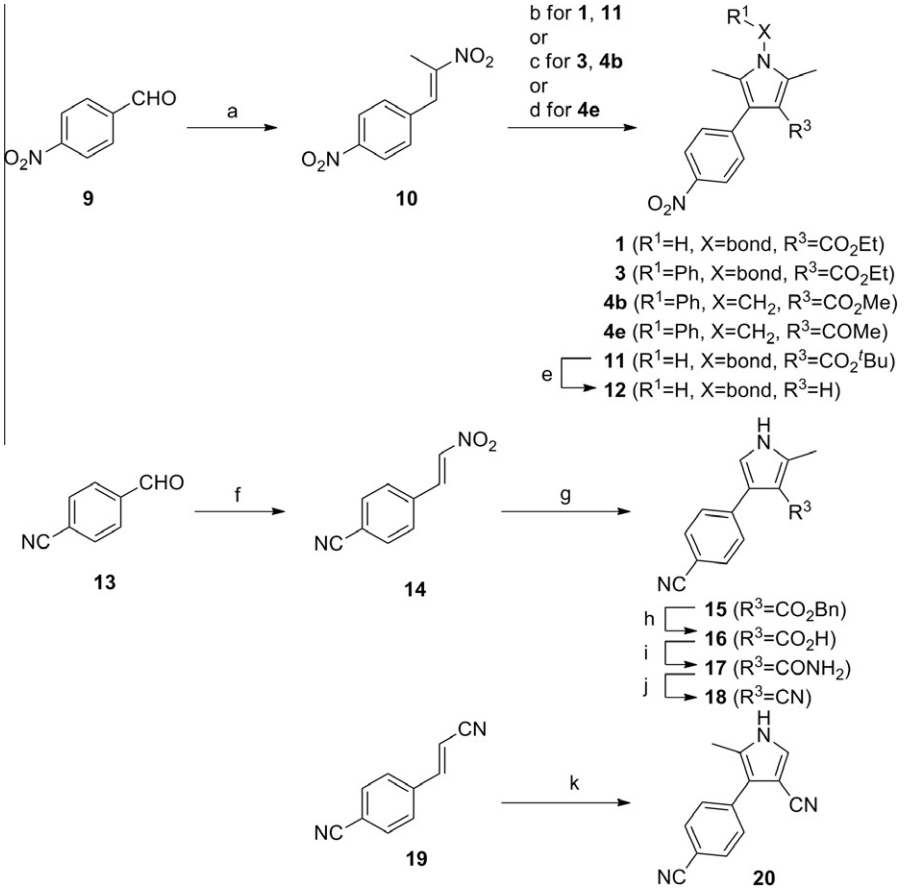
The synthesis of compounds **1**, **3**, **4b**, **4e**, **12**, **18**, and **20** is shown in Scheme 1. Nitroethane or nitromethane was treated with benzaldehyde **9** or **13** in the presence of *n*-butylamine[29] to afford (*E*)-nitroalkenes **10** and **14**. Cyclization of nitroalkenes **10** and **14** with acetoacetates[30] gave 1-unsubstituted pyrroles **1**, **11**, and **15**. On the other hand, cyclization of **10** with acetoacetate or 2,4-pentanedione, and aniline or benzylamine afforded 1-substituted pyrroles **3**, **4b**, and **4e**. Treatment of *tert*-butyl ester **11** with trifluoroacetic acid (TFA) generated decarboxylated compound **12**. Carboxylic acid **16** was obtained by catalytic hydrogenation of benzyl ester **15**. Amidation of carboxylic acid **16** followed by dehydration gave pyrrole carbonitrile **18**. Synthesis of the 2-unsubstituted pyrrole compound **20** was achieved by reacting cyanoalkene **19**[31] with tosylmethyl isocyanide (TosMIC).[32]

Scheme 2 shows the synthesis of pyrrole derivatives by Suzuki coupling. Compounds **24** and **4g** were obtained by coupling of phenylboronic acids and bromopyrroles **22** and **23**, respectively. Deprotection of **24** by boron tribromide generated carboxylic acid **25**. Amidation of **25** afforded carbamoylpyrrole **26**, which was dehydrated to afford cyanopyrrole **27**. Treatment of **23** with *n*BuLi and borate gave pyrroleboronic acid **28**. Aniline **29** was reacted with nitrite and copper (II) bromide to generate bromobenzene **30**. Coupling of **28** and **30** afforded 4-phenylpyrrole **4i**.

Phenylpyrroles **2**, **4a**, **4c**, **4d**, **4f**, **4h**, **4j**–**4p**, and **5**–**8** were synthesized as shown in Scheme 3. Cyanoalkene **34**[33] was treated with $POCl_3$ in DMF so that ring formation proceeded to give methylpyridine **35**, which was brominated to afford (bromomethyl)pyridine **36**. Alkylation, acylation, or sulfonylation of 1-unsubstituted pyrroles **1**, **12**, **18**, **20**, **24**, and **27** afforded compounds **2**, **4a**, **4f**, **4h**, **4j**–**4l**, **5**–**8**, and **37**–**40**. Acidic hydrolysis of ester **4a** generated carboxylic acid **4c**. Hydroxymethyl derivative **4d** was synthesized by reduction of **4a** using diisobutylaluminium hydride (DIBAL). (Hydroxymethyl)pyridines **4m**–**4p** were prepared from corresponding ester derivatives **37**–**40** by reduction using a cocktail of sodium borohydride and calcium chloride.

3. Results and discussion

The synthesized compounds were initially evaluated using an AR binding inhibitory test and an AR reporter gene assay. The AR binding inhibitory test was conducted to examine each compound's binding affinity to AR, while the AR reporter gene assay was used to determine antagonistic and agonistic activities of the compounds. In these tests, wild-type AR (used in the binding inhibitory test and reporter gene assay), T877A mutant-type AR



Scheme 1. Synthesis of compounds **1**, **3**, **4b**, **4e**, **12**, **18**, and **20**. Reagents and conditions: (a) $EtNO_2$, $nBuNH_2$, 100 °C, 46%; (b) $CH_3COCH_2R^3$, KOH, THF, 0 °C to room temperature, 41% (**1**) and 10% (**11**); (c) $CH_3COCH_2CO_2Et$ or $CH_3COCH_2CO_2Me$, R^1NH_2, EtOH, room temperature to 70 °C, 9% (**3**) and 32% (**4b**); (d) 2,4-pentanedione, $BnNH_2$, DMF, room temperature to 80 °C, 17%; (e) TFA, room temperature, 90%; (f) $MeNO_2$, $nBuNH_2$, 100 °C, 32%; (g) $CH_3COCH_2CO_2Bn$, MeONa, 20%NH_3–MeOH, MeOH, room temperature, 32%; (h) Pd/C, H_2, MeOH, THF, room temperature, 97%; (i) (1) $SOCl_2$, THF, room temperature, (2) NH_4OH, THF, room temperature, 68% for 2 steps; (j) $(COCl)_2$, pyridine, DMF, 0 °C, 46%; (k) TosMIC, NaH, THF, 0 °C, 30%.

(used in the binding inhibitory test and reporter gene assay), and W741C mutant-type AR (used in the reporter gene assay) were utilized to estimate the efficacy against PCs, including flutamide-resistant and/or bicalutamide-resistant PC, two types of castration-resistant PCs. In these studies, we sought to discover novel AR antagonists that possess strong binding affinity and antagonistic activity without any significant agonistic activity, which was deemed important for efficacy against PCs including castration-resistant PCs. Results are shown in Tables 1 and 2.

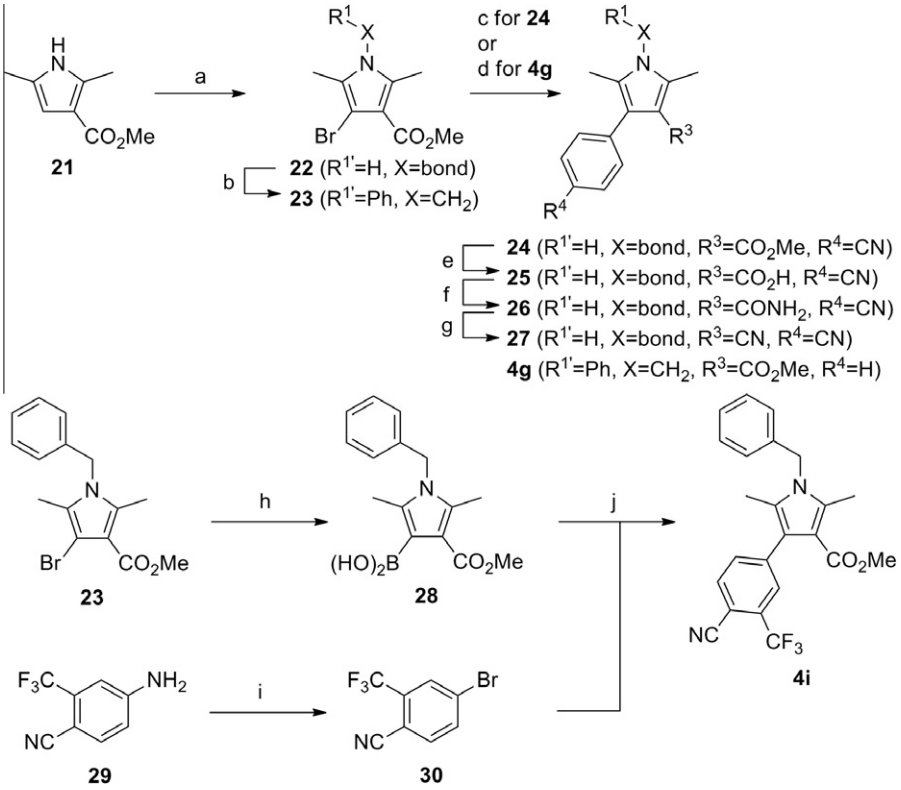
First, SAR trends for group **C** (a linker X plus a terminal moiety R^1) were investigated. As shown in Table 1, the presence of a methyl group did not increase binding affinity (**2** vs **1**). Introduction of a phenyl group diminished activity (**3** vs **1**). However, a benzyl group resulted in notably increased binding affinity (**4a** vs **1**). Along with strong binding affinity, compound **4a** showed antagonistic activity against the wild-type, T877A mutant-type, and W741C mutant-type ARs without any significant agonistic activity. In compound **4a**, replacement of the benzene ring with a cyclohexane ring diminished the activity (**5**). For linker X, ethylene (**6**), carbonyl (**7**), and sulfonyl (**8**) exhibited less potent activity than methylene (**4a**). These results indicated that a terminal aromatic ring R^1 and a methylene linker X were favorable for group **C**. Therefore, we selected 1-arylmethyl-4-phenylpyrrole compounds **4** for further study, and investigated R^1–R^5 as shown in Table 2.

We investigated the requirement for an electron-withdrawing group on the aromatic ring at R^4, and found that a cyano group was tolerable for antagonistic activity (**4b** vs **4h**). Removal of the nitro or cyano group decreased activity (**4g**). It has been reported that a nitro or cyano group is primarily required for binding to the AR ligand-binding domain.[35] The importance of these groups was also observed in our study. Introduction of a trifluoromethyl group into the 3-position of 4-cyanophenyl moiety resulted in agonistic activity against the T877A mutant-type AR (**4i**).

In assessing the substituent of R^3, exchange of the ester moiety with a cyano group resulted in a significant increase in binding affinity and antagonistic activity (**4h** vs **4j**). However, a carboxy group (**4c**), a hydroxymethyl group (**4d**), or an acetyl group (**4e**) decreased the activity. Compound **4f**, lacking a substituent at the 3-position of pyrrole ring, showed reduced activity against the W741C mutant-type AR.

Replacement of the R^1 benzene ring with other aromatic heterocycles was studied in order to enhance solubility. Compound **4j** exhibited strong binding affinity and antagonistic activity, but possessed low solubility (0.35 μg/mL in pH 6.8 solution). Improvement of solubility was achieved by replacement of the benzene ring with pyridine (**4k**: 5.1 μg/mL in pH 6.8 solution). Compound **4k** showed strong binding affinity and antagonistic activity against the wild-type, T877A mutant-type, and W741C mutant-type ARs. However, weak agonistic activity was also observed.

As described above, minimising agonistic activity is important for efficacy against PCs, including castration-resistant PCs. We found that introduction of substituents into the pyridine ring of **4k** reduced the observed agonistic activity (**4l**–**4n**). Significantly, compound **4n**, which possessed a chloro and a hydroxymethyl



Scheme 2. Synthesis of compounds **4g**, **4i**, and **24**–**27**. Reagents and conditions: (a) pyridinium bromide perbromide, Et_3N, CH_2Cl_2, 0 °C, 92%; (b) $PhCH_2Br$, NaH, THF, 0 °C, 54%; (c) 4-cyanophenylboronic acid, $Pd(Ph_3P)_4$, Na_2CO_3, DMF, H_2O, 130 °C, 32%; (d) $PhB(OH)_2$, $Pd(Ph_3P)_4$, Na_2CO_3, DME, H_2O, 130 °C, 75%; (e) BBr_3, CH_2Cl_2, 0 °C, 71%; (f) (1) $SOCl_2$, THF, room temperature, (2) NH_4OH, THF, room temperature, 53% for two steps; (g) $(COCl)_2$, pyridine, DMF, 0 °C, 82%; (h) *n*BuLi, $B(OCH_3)_3$, THF, -78 °C, 84%; (i) *t*BuONO, $CuBr_2$, MeCN, 0 °C to room temperature, 82%; (j) $Pd(Ph_3P)_4$, Na_2CO_3, DMF, H_2O, 130 °C, 64%.

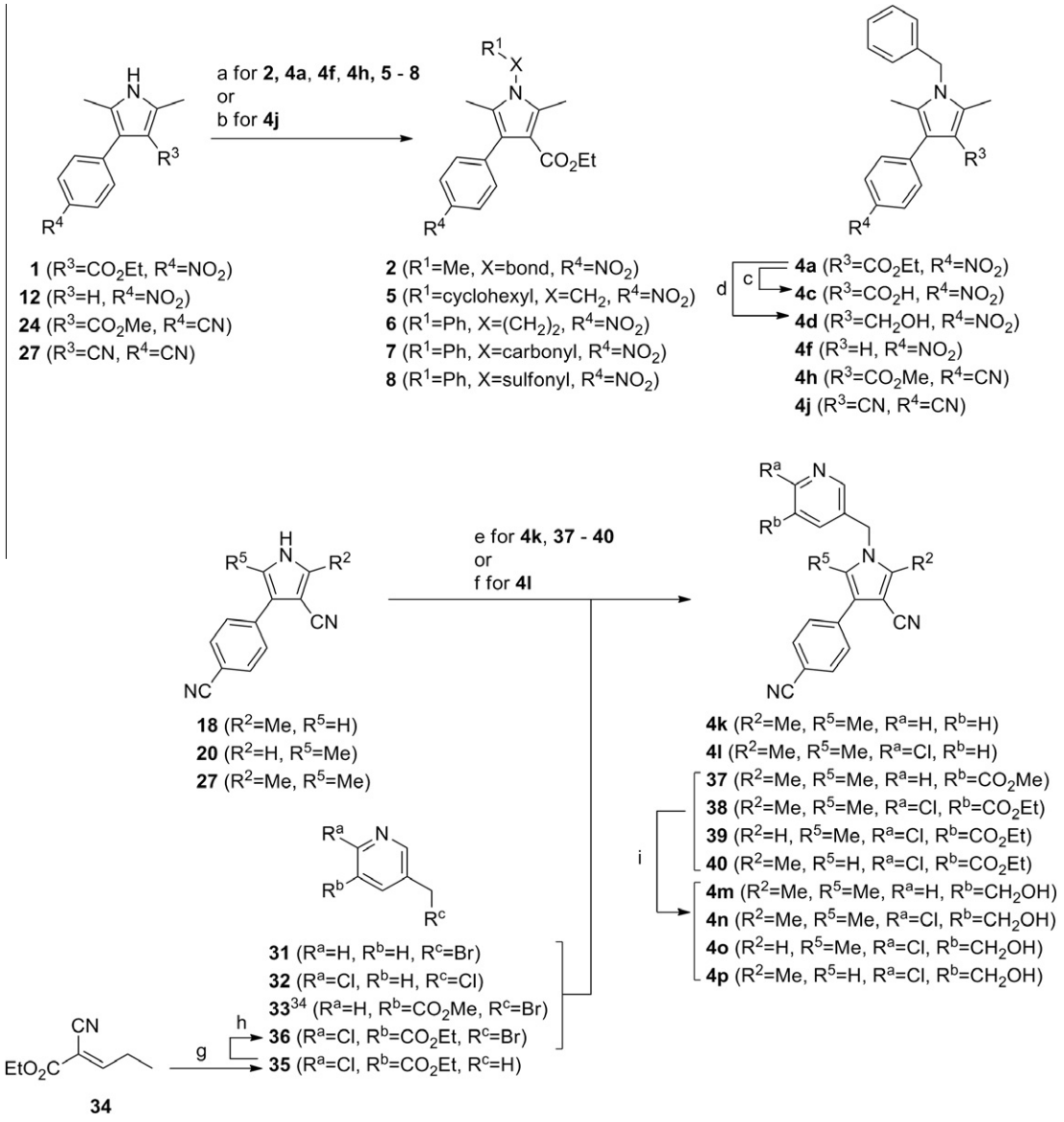
group on the pyridine ring, showed strong pan-antagonistic activity and an additional increase in solubility (15 μg/mL in pH 6.8 solution). The increase in solubility was not observed for chloropyridine **4l** and (hydroxymethyl)pyridine **4m** (0.83 and 3.5 μg/mL in pH 6.8 solution, respectively). Thus, a combination of a chloro and a hydroxymethyl group on the pyridine ring is favorable for both biological and physical properties.

Finally, we evaluated R^2 and R^5 on the pyrrole skeleton. Removal of the methyl group on either R^2 or R^5 in **4n**, particularly on R^2, decreased the antagonistic activity (**4o** and **4p**), indicating that both R^2 and R^5 methyl groups are favorable for the antagonistic activity. On the basis of these biological and physical profiles, we selected compound **4n** for further evaluation. Compound **4n** was orally available in mice (AUC = 19.2 μg h/mL, F = 104%, MRT = 4.85 h at a dose of 5 mg/kg, po (n = 3)).

Compound **4n** was evaluated for its reducing effects on the weight of ventral prostate in intact mice in comparison with bicalutamide. This test mimics monotherapy for hormone-dependent PCs.[36] As shown in Table 3, compound **4n** showed comparable weight-reducing effects to bicalutamide. Compound **4n** exhibited an approximately 74% weight reduction at an oral dose of 25 mg/kg, bid, while bicalutamide exhibited an approximately 40% reduction at an oral dose of 100 mg/kg, qd. Thus, **4n** is anticipated to be effective in monotherapy for hormone-dependent PC. Next, **4n** was evaluated for its antitumor effects in mouse xenograft models in comparison with bicalutamide (Tables 4 and 5). In these tests, two cell lines, a JDCaP cell line[37] harboring a wild-type AR cell line, and LNCaP-cxD2, a cell line harboring a mutated AR (T877A + W741C), were used to estimate the therapeutic potential against PCs, including bicalutamide-resistant PC. The JDCaP cell line exhibited strict androgen-dependency on its in vivo growth. The LNCaP-cxD2 cell line has been established in vitro as a model of bicalutamide-resistant PC[25] by culturing the human PC cell line LNCaP-FGC, harboring the T877A mutant AR, in androgen-depleted medium in the continuous presence of bicalutamide to mimic CAB therapy. During the first 6–13 weeks of culture, cell growth was kept suppressed, after which, cells resumed growth. We identified W741C/L mutation in these re-growing cells. Compounds were orally administered daily for 4 weeks. Tumor volume was measured once a week during the treatment period. As shown in Table 4, **4n** exhibited potent antitumor effects in a JDCaP xenograft model. After 4-week administration of **4n** at a dose of 3.125 mg/kg, bid, the tumor regressed to half of the original volume. Bicalutamide also exhibited comparable antitumor effects in this model. In an LNCaP-cxD2 xenograft, bicalutamide stimulated tumor growth to a T/C (volume change in a test compound/volume change in control) value of 138% at a dose of 20 mg/kg, qd and failed to decrease serum levels of prostate-specific antigen (PSA), a PC progression biomarker (Table 5). Body weight was decreased by approximately 20% during the treatment period. Similar body weight loss was observed in the castration group, which was considered the control in this study. In contrast, compound **4n** potently inhibited tumor growth to a T/C value of 16% at a dose of 25 mg/kg, bid, and significantly reduced serum PSA levels. Compound **4n** also inhibited body weight loss caused by tumor burden, suggesting low toxicity at this dose level. These results indicated that **4n** was potentially effective against both hormone-dependent and bicalutamide-resistant PCs.

4. Conclusion

In exploring novel orally available AR antagonists effective against castration-resistant PC as well as hormone-dependent PC, we designed, synthesized, and evaluated 4-phenylpyrrole



Scheme 3. Synthesis of compounds **2**, **4a**, **4c**, **4d**, **4f**, **4h**, **4j**–**4p**, and **5**–**8**. Reagents and conditions: (a) MeI, $PhCH_2Br$, cyclohexylCH_2Br, $Ph(CH_2)_2Br$, PhCOCl or $PhSO_2Cl$, NaH, THF, 0 °C, 8–89%; (b) $PhCH_2Br$, NaH, DMF, room temperature, 75%; (c) H_2SO_4, room temperature, quant.; (d) DIBAL, toluene, 0 °C to room temperature, 51%; (e) NaH, DMF, room temperature, 20–64%; (f) NaH, THF, 0 °C, 60%; (g) $POCl_3$, DMF, 0 °C to 80 °C, 54%; (h) NBS, AIBN, CCl_4, 90 °C, 51%; (i) $NaBH_4$, $CaCl_2$, THF, EtOH, room temperature, 49%–78%. (See above mentioned references for further information).

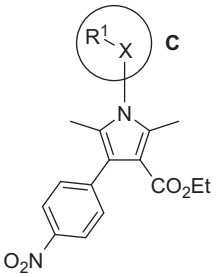
compounds **D**. SAR analyses indicated that an arylmethyl group at 1-position of the pyrrole skeleton was important for antagonistic activity, resulting in compound **4j**. Replacement of the R^1 benzene ring of **4j** with pyridine improved solubility to generate compound **4k**. Compound **4n**, discovered by further modification of **4k**, showed potent oral antitumor effects against the androgen-dependent JDCaP cell line. In addition, oral administration of **4n** exhibited significant effects against the bicalutamide-resistant LNCaP-cxD2 cell line in a mouse xenograft model. Our data suggest that this series of pyrrole compounds, such as **4n**, are novel androgen receptor antagonists effective against prostate cancer cells in clinics, including castration-resistant PCs such as bicalutamide-resistant PC.

5. Experimental section

5.1. Chemistry

Melting points were determined with a Yanagimoto melting point apparatus or a Büchi melting point apparatus B-545 and are uncorrected. ^{1}H NMR spectra were obtained at 200 or 300 MHz on a Varian Gemini-200, a Varian Ultra-300, or a Bruker DPX-300 spectrometer. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard. Peak multiplicities are expressed as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; br, broad; br s, broad singlet; m, multiplet. Elemental analyses were carried out by Takeda Analytical Laboratories Ltd. Reactions were followed by TLC on Silica Gel 60 F 254 precoated TLC plates (E. Merck) or NH TLC plates (Fuji Silysia Chemical Ltd). Chromatographic separations were carried out on Silica Gel 60 (0.063–0.200 or 0.040–0.063 mm, E. Merck) or basic silica gel (Chromatorex® NH, 100–200 mesh, Fuji Silysia Chemical Ltd) using the indicated eluents. Yields are unoptimized. The HPLC analyses were performed using a Shimadzu UFLC instrument. Elution was done with a gradient of 5–90% solvent B in solvent A (solvent A was 0.1% TFA in water, and solvent B was 0.1% TFA in acetonitrile) through a L-column 2 ODS (3.0 × 50 mm, 2 μm) column at 1.2 mL min^{-1}. Area% purity was measured at 254 nm. Microwave reactions were performed using Initiator Sixty system (Biotage, Inc.).

Table 1
Binding inhibitory and antagonistic activities of 1-substituted-4-phenylpyrrole derivatives



Compd	R^1	X	Binding[a]		Reporter[a]					
					Antagonist			Agonist		
			IC_{50}[b] (μM)		IC_{50}[b] (μM)			EC_{50}[b] (μM)		
			Wild	T877A	Wild	T877A	W741C	Wild	T877A	W741C
1	H	Bond	0.022	0.034	0.24	0.34	4.2	>10	>10	>10
2	Me	Bond	0.047	0.030	0.50	0.21	6.2	>10	>10	>10
3	Phenyl	Bond	0.55	0.22	4.3	2.1	>10	>10	>10	>10
4a	Phenyl	CH_2	0.0055	0.0052	0.34	0.16	3.2	>10	>10	>10
5	Cyclo-hexyl	CH_2	0.31	0.35	3.8	3.7	7.2	>10	>10	>10
6	Phenyl	$(CH_2)_2$	0.15	0.052	4.1	0.97	>10	>10	>10	>10
7	Phenyl	C=O	0.15	0.085	2.4	1.5	7.7	>10	>10	>10
8	Phenyl	SO_2	0.13	0.062	4.7	4.7	>10	>10	>10	>10
Bicalu-tamide			0.054	0.12	0.33	0.47	>10	>10	>10	0.18
DHT			0.0021	0.0026	>10	>10	>10	0.0062	0.015	0.012

[a] Human AR was utilized.
[b] IC_{50} and EC_{50} values shown are the mean values of duplicate measurements.

5.1.1. 1-Nitro-4-[(1*E*)-2-nitro-1-propenyl]benzene (10)

To a mixture of 4-nitrobenzaldehyde (23.9 g, 158 mmol) and nitroethane (30.0 g, 410 mmol) was added *n*-butylamine (1.20 g, 17.0 mmol). After stirring at 100 °C for 18 h, the mixture was concentrated in vacuo, and the residue was diluted with EtOH. The precipitated compound was collected and washed with EtOH and diisopropyl ether to give **10** (15.6 g, 46%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.46 (3H, s), 7.60 (2H, d, J = 8.7 Hz), 8.09 (1H, s), 8.33 (2H, d, J = 8.7 Hz).

5.1.2. Ethyl 2,5-dimethyl-4-(4-nitrophenyl)-1*H*-pyrrole-3-carboxylate (1)

To a suspension of KOH (0.15 g, 2.67 mmol) in THF (5.0 mL) was added ethyl acetoacetate (1.0 mL, 7.85 mmol) dropwise at 0 °C. After stirring at 0 °C for 15 min, **10** (0.56 g, 2.69 mmol) was added. The mixture was stirred at room temperature for 3 h, poured into H_2O, and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. To the residue were added MeOH (16 mL), H_2O (1.2 mL) and hydrochloric acid (0.2 mL), and the mixture was refluxed for 2 h. The mixture was concentrated in vacuo, diluted with a saturated aqueous solution of sodium bicarbonate, and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc). The product was recrystallized from EtOAc–Et_2O to give **1** (0.32 g, 41%) as yellow crystals, mp 164–165 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.11 (3H, t, J = 7.2 Hz), 2.14 (3H, s), 2.52 (3H, s), 4.12 (2H, q, J = 7.2 Hz), 7.41 (2H, d, J = 8.7 Hz), 8.20 (2H, d, J = 8.7 Hz), 8.23 (1H, br s). Anal. Calcd for $C_{15}H_{16}N_2O_4$: C, 62.49; H, 5.59; N, 9.72. Found: C, 62.40; H, 5.48; N, 9.80.

5.1.3. Ethyl 1,2,5-trimethyl-4-(4-nitrophenyl)-1*H*-pyrrole-3-carboxylate (2)

To a suspension of NaH (60% in mineral oil, 0.03 g, 0.80 mmol) in THF (1 mL) was added a solution of **1** (0.23 g, 0.80 mmol) in THF (1 mL) dropwise at 0 °C. After stirring at 0 °C for 1 h, methyl iodide (0.05 mL, 0.80 mmol) was added. The mixture was stirred at 0 °C for 3 h, poured into brine, and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc). The product was recrystallized from hexane–EtOAc to give **2** (0.09 g, 69%) as yellow crystals, mp 115–116 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (3H, t, J = 7.2 Hz), 2.11 (3H, s), 2.56 (3H, s), 3.49 (3H, s), 4.07 (2H, q, J = 7.2 Hz), 7.37 (2H, d, J = 8.7 Hz), 8.19 (2H, d, J = 8.7 Hz). Analytical HPLC showed 99.6% purity.

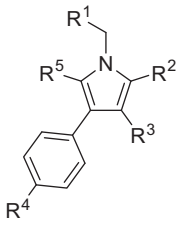
5.1.4. Ethyl 2,5-dimethyl-4-(4-nitrophenyl)-1-phenyl-1*H*-pyrrole-3-carboxylate (3)

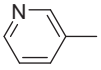
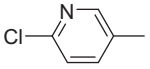
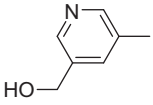
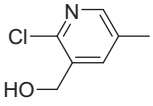
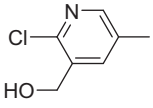
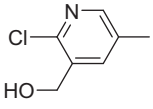
A mixture of **10** (0.50 g, 2.40 mmol), aniline (0.26 g, 2.76 mmol), ethyl acetoacetate (0.66 g, 5.04 mmol), and EtOH (10 mL) was stirred at room temperature for 18 h. The mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography (hexane–EtOAc). The product was washed with diisopropyl ether at room temperature to give **3** (0.075 g, 9%) as a pale yellow powder, mp 136–137 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.08 (3H, t, J = 7.2 Hz), 1.89 (3H, s), 2.32 (3H, s), 4.12 (2H, q, J = 7.2 Hz), 7.23–7.26 (2H, m), 7.44–7.57 (5H, m), 8.22 (2H, d, J = 9.0 Hz). Anal. Calcd for $C_{21}H_{20}N_2O_4$: C, 69.22; H, 5.53; N, 7.69. Found: C, 69.18; H, 5.62; N, 7.66.

5.1.5. Ethyl 1-(cyclohexylmethyl)-2,5-dimethyl-4-(4-nitrophenyl)-1*H*-pyrrole-3-carboxylate (5)

Compound **5** was prepared in a manner similar to that described for **2** in 64% yield as yellow crystals, mp 149–153 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.94–1.25 (5H, m), 0.96 (3H, t, J = 7.2 Hz), 1.50–1.75 (6H, m), 2.09 (3H, s), 2.48 (3H, s), 3.74 (2H, d, J = 7.2 Hz), 3.96 (2H, q, J = 7.2 Hz), 7.41 (2H, d, J = 8.4 Hz), 8.17 (2H, d, J = 8.4 Hz). Anal. Calcd for $C_{22}H_{28}N_2O_4 \cdot 0.25H_2O$: C, 67.93; H, 7.37; N, 7.20. Found: C, 67.80; H, 7.34; N, 7.06.

Table 2
Binding inhibitory and antagonistic activities of 1-arylmethyl-4-phenylpyrrole derivatives **4**



Compd	R^1	R^2	R^3	R^4	R^5	Binding[a]		Reporter[a]					
						IC_{50}[b] (μM)		Antagonist IC_{50}[b] (μM)			Agonist EC_{50}[b] (μM)		
						Wild	T877A	Wild	T877A	W741C	Wild	T877A	W741C
4a		Me	CO_2Et	NO_2	Me	0.0055	0.0052	0.34	0.16	3.2	>10	>10	>10
4b		Me	CO_2Me	NO_2	Me	0.0050	0.0038	0.30	0.15	0.74	>10	>10	>10
4c		Me	CO_2H	NO_2	Me	0.62	0.19	8.7	6.5	>10	>10	>10	>10
4d		Me	CH_2OH	NO_2	Me	0.30	0.092	3.8	0.66	>10	>10	>10	>10
4e		Me	COMe	NO_2	Me	0.39	0.13	4.6	0.90	6.5	>10	>10	>10
4f		Me	H	NO_2	Me	0.0067	0.0022	0.49	0.21	3.1	>10	>10	>10
4g		Me	CO_2Me	H	Me	0.13	0.065	2.6	1.3	3.7	>10	>10	>10
4h		Me	CO_2Me	CN	Me	0.027	0.023	0.27	0.12	0.71	>10	>10	>10
4i		Me	CO_2Me	[c]	Me	0.0039	0.0047	0.32	0.69	3.3	>10	5.4	>10
4j		Me	CN	CN	Me	0.0014	0.0030	0.040	0.041	0.38	>10	>10	>10
4k		Me	CN	CN	Me	0.0034	0.023	0.065	0.19	0.18	2.2	>10	3.6
4l		Me	CN	CN	Me	0.0090	0.024	0.045	0.10	0.91	>10	>10	>10
4m		Me	CN	CN	Me	0.014	0.15	0.10	0.44	0.60	>10	>10	>10
4n		Me	CN	CN	Me	0.037	0.10	0.025	0.11	0.28	>10	>10	>10
4o		H	CN	CN	Me	0.49	1.1	0.32	0.81	1.4	>10	>10	>10
4p		Me	CN	CN	H	0.078	0.16	0.17	0.91	0.76	>10	>10	>10
Bicalutamide						0.054	0.12	0.33	0.47	>10	>10	>10	0.18
DHT						0.0021	0.0026	>10	>10	>10	0.0062	0.015	0.012

[a] Human AR was utilized.
[b] IC_{50} and EC_{50} values shown are the mean values of duplicate measurements.
[c] 4-CN-3-CF_3.

5.1.6. Ethyl 2,5-dimethyl-4-(4-nitrophenyl)-1-(2-phenylethyl)-1*H*-pyrrole-3-carboxylate (6)

Compound **6** was prepared in a manner similar to that described for **2** in 8% yield as yellow crystals, mp 134–136 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.04 (3H, t, J = 7.2 Hz), 1.92 (3H, s), 2.51 (3H, s), 2.94 (2H, t, J = 7.5 Hz), 4.04–4.11 (4H, m), 7.07–7.12 (2H, m), 7.25–7.37 (5H, m), 8.17–8.21 (2H, m). Anal. Calcd for $C_{23}H_{24}N_2O_4 \cdot 0.25H_2O$: C, 69.59; H, 6.22; N, 7.06. Found: C, 69.38; H, 6.12; N, 6.67.

Table 3
Effects of **4n** and bicalutamide on ventral prostate weight in mice[a]

Compound	Dose (mg/kg, po)	Ventral prostate weight Day 7	
		Mean ± SE (g)	Weight reduction[b] (%)
Castration	—	6.9 ± 1.2	—
Control[c]	—	14.6 ± 1.6	—
4n	2.5, bid	13.4 ± 2.1	16
4n	7.5, bid	12.2 ± 1.9**	31
4n	25, bid	8.9 ± 2.1**	74
Bicalutamide	100, qd	11.5 ± 3.1*	40

[a] $n = 8$ animals per group.
[b] Decrease in ventral prostate weight in a test compound group during the treatment period/decrease in ventral prostate weight in a castration group during the treatment period × 100.
[c] Vehicle.
* $P < 0.05$, unpaired Student's test versus control.
** $P < 0.025$, Williams test versus control.

Table 4
Antitumor effects of **4n** and bicalutamide against JDCaP cell line in a mouse xenograft model[a]

Compound	Dose (mg/kg, po)	Tumor volume (mm^3)		
		Mean ± SE		T/C[b]
		Day 0	Day 28	(%)
Castration[c]	—	265.7 ± 38.7	48.0 ± 8.9***	−52.8
Control[d]	—	269.3 ± 42.1	681.8 ± 75.0	100
4n[e]	3.125, bid	272.7 ± 43.2	88.4 ± 15.8**	−44.7
4n[e]	6.25, bid	279.7 ± 42.3	94.8 ± 14.4**	−44.8
Castration[c]	—	862.2 ± 137.4	134.2 ± 23.6*	−76.0
Control[d]	—	779.5 ± 133.7	1736.6 ± 486.2	100
Bicalutamide[e]	20, qd	884.4 ± 143.4	212.2 ± 28.0*	−70.2

[a] $n = 7$ animals per group.
[b] Increase in tumor volume in a test compound group during the treatment period/increase in tumor volume in a control group during the treatment period × 100.
[c] Castration + vehicle + vehicle during the treatment period.
[d] Castration + vehicle + testosterone propionate 0.2 mg/kg, qd during the treatment period.
[e] Castration + test compound + testosterone propionate 0.2 mg/kg, qd during the treatment period.
* $P < 0.01$, Steel test versus control.
** $P < 0.025$, Shirley–Williams test versus control.
*** $P < 0.001$, Welch test versus control.

5.1.7. Ethyl 2,5-dimethyl-4-(4-nitrophenyl)-1-(phenylcarbonyl)-1*H*-pyrrole-3-carboxylate (7)

Compound **7** was prepared in a manner similar to that described for **2** in 67% yield as yellow crystals, mp 210–212 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.98 (3H, t, J = 7.2 Hz), 1.90 (3H, s), 2.32 (3H, s), 4.03 (2H, q, J = 7.2 Hz), 7.54 (2H, d, J = 8.7 Hz), 7.63–7.68 (2H, m), 7.78–7.84 (3H, m), 8.23 (2H, d, J = 8.7 Hz). Anal. Calcd for $C_{22}H_{20}N_2O_5$: C, 67.34; H, 5.14; N, 7.14. Found: C, 67.14; H, 5.13; N, 6.94.

5.1.8. Ethyl 2,5-dimethyl-4-(4-nitrophenyl)-1-(phenylsulfonyl)-1*H*-pyrrole-3-carboxylate (8)

Compound **8** was prepared in a manner similar to that described for **2** in 53% yield as yellow crystals, mp 146–148 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.85 (3H, t, J = 7.2 Hz), 2.26 (3H, s), 2.67 (3H, s), 3.94 (2H, q, J = 7.2 Hz), 7.46 (2H, d, J = 9.0 Hz), 7.71–7.76 (2H, m), 7.82–7.87 (1H, m), 7.94–7.97 (2H, m), 8.22 (2H, d, J = 8.7 Hz). Anal. Calcd for $C_{21}H_{20}N_2O_6S$: C, 58.87; H, 4.70; N, 6.54. Found: C, 58.65; H, 4.60; N, 6.31.

5.1.9. *tert*-Butyl 2,5-dimethyl-4-(4-nitrophenyl)-1*H*-pyrrole-3-carboxylate (11)

Compound **11** was prepared in a manner similar to that described for **1** in 10% yield as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.32 (9H, s), 2.13 (3H, s), 2.51 (3H, s), 7.39 (2H, d, J = 8.7 Hz), 7.99 (1H, br s), 8.21 (2H, d, J = 8.7 Hz).

5.1.10. 2,5-Dimethyl-3-(4-nitrophenyl)-1*H*-pyrrole (12)

A mixture of **11** (0.80 g, 2.53 mmol) and TFA (7.0 mL) was stirred at room temperature for 7 h. The mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography (hexane–EtOAc). The product was recrystallized from hexane–EtOAc to give **12** (0.49 g, 90%) as light brown crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.27 (3H, s), 2.43 (3H, s), 6.07–6.08 (1H, m), 7.46–7.53 (2H, m), 7.87 (1H, br), 8.16–8.23 (2H, m).

5.1.11. 4-[(1*E*)-2-Nitro-1-ethenyl]benzonitrile (14)

Compound **14** was prepared in a manner similar to that described for **10** in 32% as a brown solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.61 (1H, d, J = 13.6 Hz), 7.66 (2H, d, J = 8.4 Hz), 7.76 (2H, d, J = 8.4 Hz), 7.99 (1H, d, J = 13.6 Hz).

5.1.12. Benzyl 4-(4-cyanophenyl)-2-methyl-1*H*-pyrrole-3-carboxylate (15)

To a mixture of **14** (26.0 g, 149 mmol), benzyl acetoacetate (29.4 g, 153 mmol) and MeOH (35 mL) was added sodium methoxide (2.03 g, 37.6 mmol) at room temperature. After stirring at room temperature for 1 h, was added 20% NH_3 in MeOH. The mixture was stirred at room temperature for 14 h and partitioned between EtOAc and H_2O. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **15** (15.2 g, 32%) as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.56 (3H, s), 5.16 (2H, s), 6.62 (1H, d, J = 2.6 Hz), 7.14–7.50 (9H, m), 8.29 (1H, br).

Table 5
Antitumor effects of **4n** and bicalutamide against LNCaP-cxD2 cell line in a mouse xenograft model[a]

Compd	Dose (mg/kg, po)	Tumor volume (mm^3)			Plasma PSA (ng/mL)	Body weight (g)	
		Mean ± SE		T/C[b]	Mean ± SE	Mean ± SE	
		Day 0	Day 28	(%)	Day 28	Day 0	Day 28
Castration[c]	—	242.9 ± 10.7	402.0 ± 47.7	100	35.8 ± 10.7	21.4 ± 0.6	17.6 ± 0.4
4n[d]	25, bid	244.1 ± 11.4	269.3 ± 42.7	15.8	1.0 ± 0.4	22.8 ± 0.9	22.9 ± 1.0
Bicalutamide[d]	20, qd	242.1 ± 10.4	461.5 ± 40.7	137.9	38.5 ± 10.0	21.8 ± 0.4	17.9 ± 0.6

[a] $n = 7$ animals per group.
[b] Increase in tumor volume in a test compound group during the treatment period/increase in tumor volume in a castration group during the treatment period × 100.
[c] Castration + vehicle during the treatment period.
[d] Castration + test compound during the treatment period.

5.1.13. 4-(4-Cyanophenyl)-2-methyl-1*H*-pyrrole-3-carboxylic acid (16)

A mixture of **15** (15.1 g, 47.7 mmol) and 10% Pd/C (50% wet, 3.0 g), MeOH (200 mL), and THF (100 mL) was stirred at room temperature under H_2 atmosphere (1 atm) for 3 h, followed by filtration through a pad of Celite. The filtrate was concentrated in vacuo to afford **16** (10.5 g, 97%) as a colorless powder. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.36 (3H, s), 6.81 (1H, d, J = 2.2 Hz), 7.48 (2H, d, J = 8.4 Hz), 7.66 (2H, d, J = 8.4 Hz), 11.39 (1H, br).

5.1.14. 4-(4-Cyanophenyl)-2-methyl-1*H*-pyrrole-3-carboxamide (17)

To a solution of **16** (10.4 g, 46.0 mmol) in THF (100 mL) was added thionyl chloride (8.4 mL, 115 mmol) dropwise at room temperature. The mixture was stirred at room temperature for 1 h, and concentrated in vacuo. The residue was dissolved in THF (100 mL), and the resulting solution was added dropwise to 25% NH_4OH (200 mL) at room temperature. After stirring at room temperature for 1 h, the mixture was poured into brine and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **17** (7.01 g, 68%) as a colorless powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.55 (3H, s), 5.10–5.40 (2H, m), 6.68 (1H, d, J = 2.6 Hz), 7.53 (2H, d, J = 8.8 Hz), 7.65 (2H, d, J = 8.8 Hz), 8.28 (1H, br).

5.1.15. 4-(4-Cyanophenyl)-2-methyl-1*H*-pyrrole-3-carbonitrile (18)

To a solution of **17** (7.00 g, 31.1 mmol) and pyridine (3.9 mL, 48.1 mmol) in DMF (150 mL) was added oxalyl chloride (3.3 mL, 38.9 mmol) dropwise at 0 °C. After stirring at 0 °C for 0.5 h, the mixture was poured into brine and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **18** (2.97 g, 46%) as a colorless powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.50 (3H, s), 6.94 (1H, d, J = 2.4 Hz), 7.66 (2H, d, J = 8.8 Hz), 7.74 (2H, d, J = 8.8 Hz), 8.46 (1H, br).

5.1.16. 4-(4-Cyanophenyl)-5-methyl-1*H*-pyrrole-3-carbonitrile (20)

To a suspension of NaH (60% in mineral oil, 0.48 g, 12.0 mmol) in THF (20 mL) was added a mixture of 4-[(*E*)-2-cyanoethenyl]benzonitrile **19** (1.54 g, 10.0 mmol) and TosMIC (2.09 g, 10.7 mmol) in THF (50 mL) dropwise at 0 °C. After stirring at 0 °C for 2 h, the mixture was poured into brine and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **20** (0.60 g, 30%) as crystals. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.29 (3H, s), 7.59 (2H, d, J = 8.4 Hz), 7.66 (1H, s), 7.91 (2H, d, J = 8.4 Hz), 11.92 (1H, br s).

5.1.17. Methyl 4-bromo-2,5-dimethyl-1*H*-pyrrole-3-carboxylate (22)

To a mixture of methyl 2,5-dimethyl-1*H*-pyrrole-3-carboxylate **21** (6.85 g, 45.0 mmol), triethylamine (8.7 mL, 63.0 mmol) and CH_2Cl_2 (270 mL) was added pyridinium bromide perbromide (15.7 g, 15.7 mmol) portionwise at 0 °C. After stirring at 0 °C for 2 h, the mixture was poured into brine and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc). The product was recrystallized from hexane–EtOAc to give **22** (7.59 g, 92%) as yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.19 (3H, s), 2.47 (3H, s), 3.82 (3H, s), 8.20 (1H, s).

5.1.18. Methyl 1-benzyl-4-bromo-2,5-dimethyl-1*H*-pyrrole-3-carboxylate (23)

Compound **23** was prepared in a manner similar to that described for **2** in 54% yield as a colorless powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.15 (3H, s), 2.45 (3H, s), 3.84 (3H, s), 5.08 (2H, s), 6.80–7.00 (2H, m), 7.20–7.40 (3H, m).

5.1.19. Methyl 4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylate (24)

A mixture of **22** (0.23 g, 1.00 mmol), 4-cyanophenylboronic acid (0.16 g, 1.09 mmol), tetrakis(triphenylphosphine)palladium(0) (0.06 g, 0.052 mmol), anhydrous Na_2CO_3 (0.32 g, 3.02 mmol), DMF (8 mL), and H_2O (2 mL) was stirred at 130 °C for 2 h under argon atmosphere. After cooling to room temperature, the mixture was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc). The product was recrystallized from hexane–EtOAc to give **24** (0.082 g, 32%) as pale yellow crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.13 (3H, s), 2.52 (3H, s), 3.63 (3H, s), 7.35 (2H, d, J = 8.4 Hz), 7.62 (2H, d, J = 8.4 Hz), 8.14 (1H, s).

5.1.20. 4-(4-Cyanophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylic acid (25)

To a solution of **24** (1.01 g, 3.97 mmol) in CH_2Cl_2 (8.0 mL) was added 1 M boron tribromide in CH_2Cl_2 (15.9 mL, 15.9 mmol) dropwise at 0 °C. After stirring at 0 °C for 1 h, the mixture was poured into brine and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **25** (0.68 g, 71%) as a yellow powder. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 2.05 (3H, s), 2.39 (3H, s), 7.35 (2H, d, J = 8.4 Hz), 7.71 (2H, d, J = 8.4 Hz).

5.1.21. 4-(4-Cyanophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxamide (26)

Compound **26** was prepared in a manner similar to that described for **17** in 53% yield as colorless crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.15 (3H, s), 2.52 (3H, s), 4.99 (1H, br s), 5.25 (1H, br s), 7.43 (2H, d, J = 8.0 Hz), 7.68 (2H, d, J = 8.0 Hz), 8.27 (1H, s).

5.1.22. 4-(4-Cyanophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carbonitrile (27)

Compound **27** was prepared in a manner similar to that described for **18** in 82% yield as colorless crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.32 (3H, s), 2.44 (3H, s), 7.52 (2H, d, J = 8.4 Hz), 7.70 (2H, d, J = 8.4 Hz), 8.26 (1H, s).

5.1.23. [1-Benzyl-4-(methoxycarbonyl)-2,5-dimethyl-1*H*-pyrrol-3-yl]boronic acid (28)

To a solution of **23** (0.55 g, 1.71 mmol) in THF (8.5 mL) was added 1.6 M butyllithium in hexane (1.1 mL, 1.71 mmol) dropwise at −78 °C under argon atmosphere. After stirring at −78 °C for 1 h, was added a solution of trimethyl borate (1.94 mL, 17.1 mmol) in THF (34 mL). The mixture was stirred at −78 °C for 1 h, followed by addition of H_2O (5 mL) and MeOH (5 mL). After warming to room temperature, the mixture was poured into brine and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **28** (0.41 g, 84%) as a colorless amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.41 (3H, s), 2.47 (3H, s), 3.86 (3H, s), 5.11 (2H, s), 6.78–6.94 (2H, m), 7.18–7.37 (3H, m), 7.45 (2H, s).

5.1.24. 4-Bromo-2-(trifluoromethyl)benzonitrile (30)

To a solution of 4-amino-2-(trifluoromethyl)benzonitrile **29** (1.02 g, 5.48 mmol) in acetonitrile (30 mL) was added *tert*-butyl nitrite (0.95 mL, 7.19 mmol) dropwise at 0 °C. After stirring at 0 °C for 0.5 h, copper (II) bromide (1.39 g, 6.22 mmol) was added and the mixture was warmed to room temperature. The mixture was stirred at room temperature for 14 h, poured into brine, and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **30** (1.13 g, 82%) as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.60–8.10 (3H, m).

5.1.25. Ethyl 2-chloro-5-methylpyridine-3-carboxylate (35)

To a mixture of ethyl (2*E*)-2-cyanopent-2-enoate **34** (30.0 g, 196 mmol) and DMF (76 mL, 982 mmol) was added phosphoryl chloride (36 mL, 386 mmol) dropwise at 0 °C. After stirring at 80 °C for 2 h, the mixture was poured into ice water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **35** (21.1 g, 54%) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.42 (3H, t, J = 7.2 Hz), 2.37 (3H, s), 4.42 (2H, q, J = 7.2 Hz), 7.96 (1H, d, J = 2.6 Hz), 8.32 (1H, d, J = 2.6 Hz).

5.1.26. Ethyl 5-(bromomethyl)-2-chloropyridine-3-carboxylate (36)

A mixture of **35** (14.7 g, 73.6 mmol), AIBN (1.31 g, 7.98 mmol), *N*-bromosuccinimide (17.1 g, 96.1 mmol), and CCl_4 (200 mL) was stirred at 90 °C for 5 h. The mixture was partitioned between EtOAc and brine. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **36** (10.5 g, 51%) as an oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.39–1.45 (3H, m), 4.30–4.55 (4H, m), 7.97 (1H, d, J = 2.6 Hz), 8.19 (1H, d, J = 2.6 Hz).

5.1.27. Ethyl 1-benzyl-2,5-dimethyl-4-(4-nitrophenyl)-1*H*-pyrrole-3-carboxylate (4a)

Compound **4a** was prepared in a manner similar to that described for **2** in 89% yield as yellow crystals, mp 122.5 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.98 (3H, t, J = 7.2 Hz), 2.03 (3H, s), 2.43 (3H, s), 3.99 (2H, q, J = 7.2 Hz), 5.25 (2H, s), 7.00 (2H, d, J = 8.4 Hz), 7.26–7.31 (1H, m), 7.35–7.40 (2H, m), 7.46 (2H, d, J = 8.7 Hz), 8.19 (2H, d, J = 8.7 Hz). Anal. Calcd for $C_{22}H_{22}N_2O_4$: C, 69.83; H, 5.86; N, 7.40. Found: C, 69.68; H, 5.87; N, 7.41.

5.1.28. Methyl 1-benzyl-2,5-dimethyl-4-(4-nitrophenyl)-1*H*-pyrrole-3-carboxylate (4b)

Compound **4b** was prepared in a manner similar to that described for **3** in 32% yield as yellow crystals, mp 158–159 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.05 (3H, s), 2.51 (3H, s), 3.62 (3H, s), 5.13 (2H, s), 6.94–6.96 (2H, m), 7.29–7.43 (5H, m), 8.22 (2H, d, J = 8.7 Hz). Anal. Calcd for $C_{21}H_{20}N_2O_4$: C, 69.22; H, 5.53; N, 7.69. Found: C, 69.41; H, 5.49; N, 7.45.

5.1.29. 1-Benzyl-2,5-dimethyl-4-(4-nitrophenyl)-1*H*-pyrrole-3-carboxylic acid (4c)

Compound **4a** (0.086 g, 0.23 mmol) was added to sulfuric acid (1.0 mL). After stirring at room temperature for 0.5 h, the mixture was poured into ice water and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was recrystallized from EtOAc to give **4c** (0.079 g, quant.) as yellow crystals, mp 148–149 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.02 (3H, s), 2.43 (3H, s), 5.24 (2H, s), 7.00 (2H, d, J = 7.5 Hz), 7.26–7.42 (3H, m), 7.47 (2H, d, J = 8.7 Hz), 8.17 (2H, d, J = 8.7 Hz). Anal. Calcd for $C_{20}H_{18}N_2O_4$·EtOAc: C, 65.74; H, 5.97; N, 6.38. Found: C, 65.51; H, 6.00; N, 6.55.

5.1.30. [1-Benzyl-2,5-dimethyl-4-(4-nitrophenyl)-1*H*-pyrrol-3-yl]methanol (4d)

To a solution of **4a** (0.30 g, 0.79 mmol) in toluene (6.3 mL) was added 1 M diisobutylaluminium hydride in toluene (1.6 mL, 1.60 mmol) dropwise at 0 °C. After stirring at room temperature for 1 h, the mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **4d** (0.14 g, 51%) as a yellow amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.20 (3H, s), 2.24 (3H, s), 4.51 (2H, s), 5.11 (2H, s), 6.94–6.97 (2H, m), 7.24–7.36 (3H, m), 7.57–7.62 (2H, m), 8.22–8.77 (2H, m). Analytical HPLC showed 99.8% purity.

5.1.31. 1-[1-Benzyl-2,5-dimethyl-4-(4-nitrophenyl)-1*H*-pyrrol-3-yl]ethanone (4e)

A mixture of **10** (0.42 g, 2.02 mmol), 2,4-pentanedione (0.41 mL, 3.99 mmol), benzylamine (0.24 mL, 2.20 mmol), and DMF (10 mL) was stirred at room temperature for 18 h and at 80 °C for 24 h. The mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc). The product was recrystallized from hexane–EtOAc to give **4e** (0.12 g, 17%) as yellow crystals, mp 121–122 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.98 (3H, s), 2.04 (3H, s), 2.46 (3H, s), 5.13 (2H, s), 6.97 (2H, d, J = 7.2 Hz), 7.30–7.45 (5H, m), 8.26 (2H, d, J = 9.0 Hz). Anal. Calcd for $C_{21}H_{20}N_2O_3$: C, 72.40; H, 5.79; N, 8.04. Found: C, 72.45; H, 5.69; N, 8.26.

5.1.32. 1-Benzyl-2,5-dimethyl-3-(4-nitrophenyl)-1*H*-pyrrole (4f)

Compound **4f** was prepared in a manner similar to that described for **2** in 18% yield as yellow crystals, mp 106 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.21 (3H, s), 2.31 (3H, s), 5.09 (2H, s), 6.17 (1H, s), 6.94 (2H, d, J = 7.2 Hz), 7.24–7.36 (3H, m), 7.49–7.54 (2H, m), 8.19–8.23 (2H, m). Anal. Calcd for $C_{19}H_{18}N_2O_2$: C, 66.66; H, 4.71; N, 8.18. Found: C, 66.58; H, 4.62; N, 8.11.

5.1.33. Methyl 1-benzyl-2,5-dimethyl-4-phenyl-1*H*-pyrrole-3-carboxylate (4g)

A mixture of **23** (0.40 g, 1.24 mmol), phenylboronic acid (0.20 g, 1.61 mmol), sodium carbonate (0.26 g, 2.48 mmol), tetrakis(triphenylphosphine)palladium(0) (0.070 g, 0.06 mmol), DME (3 mL) and water (1 mL) was heated at 130 °C for 1 h under microwave irradiation. The reaction was quenched with water and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **4g** (0.295 g, 75%) as colorless crystals, mp 100–102 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.04 (3H, s), 2.49 (3H, s), 3.59 (3H, s), 5.11 (2H, s), 6.80–7.07 (2H, m), 7.17–7.49 (8H, m). Analytical HPLC showed 99.0% purity.

5.1.34. Methyl 1-benzyl-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carboxylate (4h)

Compound **4h** was prepared in a manner similar to that described for **2** in 60% yield as colorless crystals, mp 121–122 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.01 (3H, s), 2.42 (3H, s), 3.50 (3H, s), 5.24 (2H, s), 6.99 (2H, d, J = 7.5 Hz), 7.26–7.30 (1H, m), 7.34–7.39 (4H, m), 7.77 (2H, d, J = 8.4 Hz). Anal. Calcd for $C_{22}H_{20}N_2O_2$: C, 76.72; H, 5.85; N, 8.13. Found: C, 76.57; H, 5.79; N, 8.06.

5.1.35. Methyl 1-benzyl-4-[4-cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-1*H*-pyrrole-3-carboxylate (4i)

A mixture of **28** (0.15 g, 0.522 mmol), **30** (0.13 g, 0.520 mmol), tetrakis(triphenylphosphine)palladium(0) (0.03 g, 0.026 mmol), anhydrous Na_2CO_3 (0.17 g, 1.56 mmol), DMF (4 mL), and H_2O (1 mL) was stirred at 130 °C for 2 h under argon atmosphere. After cooling to room temperature, the mixture was partitioned between EtOAc and H_2O. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **4i** (0.14 g, 64%) as a colorless amorphous. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.05 (3H, s), 2.51 (3H, s), 3.62 (3H, s), 5.14 (2H, s), 6.92–6.97 (2H, m), 7.28–7.38 (3H, m), 7.53–7.57 (1H, m), 7.68 (1H, s), 7.80 (1H, d, J = 8.1 Hz). Analytical HPLC showed 99.3% purity.

5.1.36. 1-Benzyl-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carbonitrile (4j)

To a solution of **27** (0.10 g, 0.452 mmol) in DMF (1.2 mL) was added NaH (60% in mineral oil, 0.03 g, 0.678 mmol) portionwise at room temperature. After stirring at room temperature for 15 minutes, benzyl bromide (0.093 g, 0.542 mmol) was added. The mixture was stirred at room temperature for 1.5 h, poured into H_2O, and extracted with EtOAc. The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **4j** (0.105 g, 75%) as a colorless powder, mp 125–126 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.20 (3H, s), 2.38 (3H, s), 5.12 (2H, s), 6.92–6.94 (2H, m), 7.28–7.40 (3H, m), 7.53 (2H, d, J = 8.4 Hz), 7.70 (2H, d, J = 8.4 Hz). Anal. Calcd for $C_{21}H_{17}N_3$: C, 81.00; H, 5.50; N, 13.49. Found: C, 80.83; H, 5.45; N, 13.47.

5.1.37. Methyl 5-{[3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl]methyl}pyridine-3-carboxylate (37)

Compound **37** was prepared in a manner similar to that described for **4j** in 20% yield as a colorless powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.20 (3H, s), 2.36 (3H, s), 3.88 (3H, s), 5.45 (2H, s), 7.59 (2H, d, J = 8.1 Hz), 7.91–7.94 (3H, m), 8.52 (1H, d, J = 2.1 Hz), 9.02 (1H, d, J = 2.1 Hz).

5.1.38. Ethyl 2-chloro-5-{[3-cyano-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrol-1-yl]methyl}pyridine-3-carboxylate (38)

Compound **38** was prepared in a manner similar to that described for **4j** in 64% yield as crystals. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.31 (3H, t, J = 7.2 Hz), 2.20 (3H, s), 2.37 (3H, s), 4.34 (2H, q, J = 7.2 Hz), 5.40 (2H, s), 7.59 (2H, d, J = 8.1 Hz), 7.91–7.94 (3H, m), 8.24 (1H, d, J = 2.7 Hz).

5.1.39. Ethyl 2-chloro-5-{[3-cyano-4-(4-cyanophenyl)-5-methyl-1*H*-pyrrol-1-yl]methyl}pyridine-3-carboxylate (39)

Compound **39** was prepared in a manner similar to that described for **4j** in 50% yield as colorless crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.42 (3H, t, J = 7.2 Hz), 2.31 (3H, s), 4.44 (2H, q, J = 7.2 Hz), 5.30 (2H, s), 7.27 (1H, s), 7.49 (2H, d, J = 8.7 Hz), 7.74 (2H, d, J = 8.7 Hz), 7.84 (1H, d, J = 2.6 Hz), 8.21 (1H, d, J = 2.6 Hz).

5.1.40. Ethyl 2-chloro-5-{[3-cyano-4-(4-cyanophenyl)-2-methyl-1*H*-pyrrol-1-yl]methyl}pyridine-3-carboxylate (40)

Compound **40** was prepared in a manner similar to that described for **4j** in 36% yield as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.40 (3H, t, J = 7.2 Hz), 2.41 (3H, s), 4.42 (2H, q, J = 7.2 Hz), 5.13 (2H, s), 6.87 (1H, s), 7.60–7.77 (4H, m), 7.80–7.87 (1H, m), 8.30 (1H, d, J = 2.6 Hz).

5.1.41. 4-(4-Cyanophenyl)-2,5-dimethyl-1-(pyridin-3-ylmethyl)-1*H*-pyrrole-3-carbonitrile (4k)

Compound **4k** was prepared in a manner similar to that described for **4j** in 55% yield as colorless crystals, mp 160–164 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.20 (3H, s), 2.36 (3H, s), 5.34 (2H, s), 7.39–7.40 (2H, m), 7.59 (2H, d, J = 8.1 Hz), 7.92 (2H, d, J = 8.1 Hz), 8.37 (1H, br s), 8.52 (1H, t, J = 3.0 Hz). Anal. Calcd for $C_{20}H_{16}N_4$: C, 76.90; H, 5.16; N, 17.94. Found: C, 76.68; H, 5.15; N, 17.82.

5.1.42. 1-[(6-Chloropyridin-3-yl)methyl]-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carbonitrile (4l)

Compound **4l** was prepared in a manner similar to that described for **2** in 60% yield as colorless crystals, mp 209–210 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.01 (3H, s), 2.40 (3H, s), 5.13 (2H, s), 7.16 (1H, dd, J = 2.2, 8.0 Hz), 7.35 (1H, d, J = 8.0 Hz), 7.51 (2H, d, J = 8.4 Hz), 7.72 (2H, d, J = 8.4 Hz), 8.13 (1H, d, J = 2.2 Hz). Analytical HPLC showed 98.3% purity.

5.1.43. 1-{[6-Chloro-5-(hydroxymethyl)pyridin-3-yl]methyl}-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carbonitrile (4n)

A mixture of sodium borohydride (0.50 g, 13.2 mmol), calcium chloride (1.00 g, 9.01 mmol), THF (40 mL), and EtOH (20 mL) was stirred at room temperature for 0.5 h. To the mixture was added **38** (1.80 g, 4.30 mmol), and the mixture was stirred at room temperature for 14 h. The mixture was poured into a saturated aqueous solution of citric acid and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc). The product was recrystallized from EtOH to give **4n** (1.26 g, 78%) as colorless crystals, mp 147–148 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.21 (3H, s), 2.37 (3H, s), 4.52 (2H, d, J = 5.1 Hz), 5.37 (2H, s), 5.61 (1H, t, J = 5.1 Hz), 7.57–7.60 (3H, m), 7.92 (2H, d, J = 8.4 Hz), 8.06 (1H, d, J = 2.4 Hz). Anal. Calcd for $C_{21}H_{17}ClN_4O$: C, 66.93; H, 4.55; N, 14.87. Found: C, 66.74; H, 4.46; N, 14.68.

5.1.44. 1-{[5-(Hydroxymethyl)pyridin-3-yl]methyl}-4-(4-cyanophenyl)-2,5-dimethyl-1*H*-pyrrole-3-carbonitrile (4m)

Compound **4m** was prepared in a manner similar to that described for **4n** in 49% yield as colorless powder, mp 214–215 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.21 (3H, s), 2.37 (3H, s), 4.52 (2H, d, J = 5.4 Hz), 5.32–5.37 (3H, m), 7.36 (1H, br s), 7.59 (2H, d, J = 8.1 Hz), 7.92 (2H, d, J = 8.1 Hz), 8.23 (1H, d, J = 2.4 Hz), 8.45 (1H, d, J = 1.5 Hz). Analytical HPLC showed 99.2% purity.

5.1.45. 1-{[6-Chloro-5-(hydroxymethyl)pyridin-3-yl]methyl}-4-(4-cyanophenyl)-5-methyl-1*H*-pyrrole-3-carbonitrile (4o)

Compound **4o** was prepared in a manner similar to that described for **4n** in 74% yield as a colorless amorphous powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.21 (3H, s), 4.53 (2H, d, J = 5.7 Hz), 5.35 (2H, s), 5.64 (1H, t, J = 5.7 Hz), 7.57 (2H, d, J = 8.7 Hz), 7.79 (1H, d, J = 2.4 Hz), 7.92 (2H, d, J = 8.7 Hz), 7.94 (1H, s), 8.24 (1H, d, J = 2.4 Hz). Analytical HPLC showed 100% purity.

5.1.46. 1-{[6-Chloro-5-(hydroxymethyl)pyridin-3-yl]methyl}-4-(4-cyanophenyl)-2-methyl-1*H*-pyrrole-3-carbonitrile (4p)

Compound **4p** was prepared in a manner similar to that described for **4n** in 55% yield as a colorless powder, mp 181–182 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.15 (1H, br s), 2.41 (3H, s), 4.79 (2H, s), 5.10 (2H, s), 6.88 (1H, s), 7.59–7.79 (5H, m), 8.13 (1H, d, J = 2.3 Hz). Analytical HPLC showed 100% purity.

5.2. Solubility determination

Small volumes of the compound DMSO solutions were added to the aqueous buffer solution (pH 6.8). After incubation, precipitates were separated by filtration. The solubility was determined by HPLC analysis of each filtrate.

5.3. Biology

5.3.1. AR binding inhibitory assay (wild-type and T877A mutant-type AR)

After FreeStyle293F (Invitrogen) cells were transfected with pcDNA3.1 containing an androgen receptor (AR) gene (wild-type AR or T877A mutant-type AR) by using 293fectin transfection reagent (Invitrogen), these cells were seeded into an Erlenmeyer flask (Corning, 1L, 430518) at 1.1×10^6 cells/mL in FreeStyle293 Expression Medium (Invitrogen). After 48 h shaking incubation (125 rpm) at 37 °C in a 8% CO_2 atmosphere, these cells were washed with TEG Buffer (10 mM Tris–HCl (pH 7.2), 50 mM EDTA, 10% Glycerol), and suspended with TEGM Buffer (10 mM Tris–HCl (pH 7.2), 1 mM EDTA, 10% glycerol, 10 mM Na_2MoO_4, 1 mM DTT, 1 mM 2-ME, 1 × Complete protease inhibitor tablet (Roche)). After freezing and thawing to lyse cells, lysate was centrifuged at 228,000*g* at 4 °C for 20 min. The supernatant was stored at −80 °C as AR cell lysate. To cell lysate solution containing an AR or a T877A mutant-type AR were added [17-α-methyl-^{3}H] mibolerone (final 3 nM, PerkinElmer NET-919) and a compound, and the mixture was incubated at 4 °C for 3 h. B (Bound)/F (Free) were separated by the dextran/charcoal method.[38] The label count of B was measured, and the inhibitory rate of the compound was calculated.

5.3.2. AR reporter gene assay (wild-type, T877A mutant-type, and W741C mutant-type AR)

Cos-7 (5×10^6 cells) were sown in a 150 cm^2 flask (Corning), and cultured in culture medium (DMEM medium containing 10% Dextran Charcoal (DCC)-Fetal Bovine Serum (FBS), 2 mM glutamine) for 24 h. pcDNA3.1 (Invitrogen) containing AR genes (wild-type, T877A mutant-type or W741C mutant-type), and pGL3-MMTV-luc vector containing luciferase gene bound at the downstream of an AR promoter derived from Mouse Mammary Tumor Virus (MMTV) were co-transfected by using SuperFect transfection reagent (QIAGEN). After culturing at 37 °C in a 5% CO_2 atmosphere for 4 h, these cells were harvested and plated in a 96 well plate (10,000 cells/well) and cultured for 2 h. Dihydrotestosterone (DHT, final 0.1 μM) and a compound were added, and the cells were further cultured for 24 h, after which the luciferase activity was measured. The inhibitory rate by the compound was calculated with the luciferase activity induced by the addition of 0.1 μM DHT.

5.3.3. Effects on ventral prostate weight in mice

A test compound was orally administered to 8-week-old male ICR mice for seven days ($n = 8$). On the next day of the final administration, prostate weight was measured.

5.3.4. Antitumor effects against JDCaP cell line in mouse xenograft model

Androgen-dependent JDCaP tumor maintained in nude mice was cut into small pieces and the tumor piece was inserted to the flank region of a 5-week-old male BALB/c nude mouse with a trocar. When the steady tumor growth was observed, tumor-bearing mice were allocated to four groups ($n = 7$ for each group) to give the similar average tumor volume. On the next day of the grouping, the nude mice were castrated. Testosterone propionate (TP) at a dose of 0.2 mg/kg or vehicle was subcutaneously injected to the castrated mice once daily for 29 days. This injection gives the human castration level of serum testosterone in a nude mouse. Compound **4n** at doses of 3.125 and 6.25 mg/kg, twice daily, bicalutamide at a dose of 20 mg/kg, once daily, or vehicle (0.5% methylcellulose), twice daily was orally administered to TP-treated, castrated mice for the same period. Tumor size was measured with a caliper. Antitumor effects were expressed as T/C% (increase in tumor volume in a test compound group during the treatment period/increase in tumor volume in a vehicle group during the treatment period × 100).

5.3.5. Antitumor effects against LNCaP-cxD2 cell line in mouse xenograft model

100 μL of LNCaP-cxD2 cell suspension in PBS/Matrigel (1:1) at a cell density of 5×10^7 was inoculated into the flask region of a 5-week-old male BALB/c nude mouse. When the average tumor volume reached to approximately 200 mm^3, tumor-bearing mice were allocated to four groups ($n = 7$ for each group) to give the similar average tumor volume. On the next day of the grouping, the nude mice were castrated. Compound **4n** at a dose of 25 mg/kg, twice daily, bicalutamide at a dose of 20 mg/kg, once daily, or vehicle (0.5% methylcellulose), twice daily was orally administered to the mice for 29 days from the day of castration. Measurement of tumor size with a caliper and body weight was conducted once weekly. Antitumor effects were expressed as T/C% (increase in tumor volume in a test compound group during the treatment period/increase in tumor volume in a vehicle group during the treatment period × 100). At the end of experiments, blood samples were obtained to measure the plasma PSA levels.

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