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Design, synthesis, and biological evaluation of 4-arylmethyl-1-phenylpyrazole and 4-aryloxy-1-phenylpyrazole derivatives as novel androgen receptor antagonists

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ABSTRACT

A series of 4-arylmethyl-1-phenylpyrazole and 4-aryloxy-1-phenylpyrazole compounds **B** were designed, synthesized, and evaluated for their potential as new-generation androgen receptor (AR) antagonists therapeutically effective against castration-resistant prostate cancer (CRPC). Introduction of a bulky amide substituent (R^2) to the terminal aryl ring of the 4-arylmethyl group favored the reduction of agonistic activity and improved the pharmacokinetic (PK) properties. Similarly, introduction of a bulky substituent in the 4-aryloxy derivatives also resulted in improved PK properties. Compounds **28h** and **44b** exhibited potent antitumor effects against a CRPC model of LNCaP-hr cell line in a mouse xenograft model. On the contrary, bicalutamide showed only partial suppression of tumor growth. These results suggest that the novel pyrazole derivatives are new-generation AR antagonists, different from the 'first-generation' antagonists such as bicalutamide in a CRPC treatment model.

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] Treatment of PCs can be of various types such as surgery, radiation therapy, and endocrine therapy. These therapies are provided alone or in combination depending on the stage of the PCs, age of the patients, and so on. In endocrine therapy, progression of PCs is suppressed by inhibiting the action and/or secretion of androgens, which play a pivotal role in the proliferation of PC cells.[2] Secretion of testosterone (95% from the testis) is prevented by surgical or chemical castration using gonadotropin-releasing hormone (GnRH) analogs. Androgen receptor (AR) antagonists are used in the treatment of PCs; AR antagonists suppress the action of androgens by inhibiting their binding to the ARs. Currently, AR antagonists such as cyproterone acetate,[3] flutamide,[4,5] nilutamide,[6,7] and bicalutamide[8–17] are used for clinical purposes (Fig. 1). When a combination of AR antagonists and chemical castration is used, AR antagonists such as bicalutamide show significant synergistic effects by blocking adrenal androgen signals as well as by suppressing transient increase in testosterone levels induced by GnRH analogs. This 'combined androgen blockade (CAB)' therapy is at least initially effective for PC treatment.[15] However, in spite of the initial success of the PC treatment, there is a considerable population of patients who develop castration-resistant prostate cancer (CRPC) after the prolonged use of an AR antagonist.[18–20] In such cases, discontinuation of CAB therapy or change to another AR antagonist should be considered.[21] Despite its risk of severe adverse effects, docetaxel, a microtubule-stabilizing agent, is the only approved chemotherapeutic agent against CRPC.[22] Thus, it is necessary to develop effective new-generation AR antagonists against CRPC. In recent years, intensive clinical and nonclinical studies have been carried out.[23–28] An AR antagonist MDV-3100 is reported to be effective against CRPC, and it is currently under phase 3 clinical trials.[29,30] Because of its unique pharmaceutical properties, this agent is categorized as a 'second-generation' AR antagonist.

We discovered novel 1-arylmethyl-4-(4-cyanophenyl)pyrrole derivatives **1** as orally available AR antagonists (Fig. 2).[31] Among these antagonists, compound **2** showed antitumor effects against bicalutamide-resistant LNCaP-cxD2 cell line as well as androgen dependent JDCaP cell line in a mouse xenograft model upon oral administration.[31] In optimizing pyrroles **1**, we found that cyanophenyl moiety **a**, which might correspond to the A ring of dihydrotestosterone (DHT), was favorable for the antagonistic activity.

Abbreviations: AR, androgen receptor; CRPC, castration-resistant prostate cancer; CAB, combined androgen blockade; DHT, dihydrotestosterone; SAR, structure–activity relationship; EDC, *N*-[3-(dimethylamino)propyl]-*N* ′-ethylcarbodiimide hydrochloride; DMT-MM, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholin-4-ium chloride.

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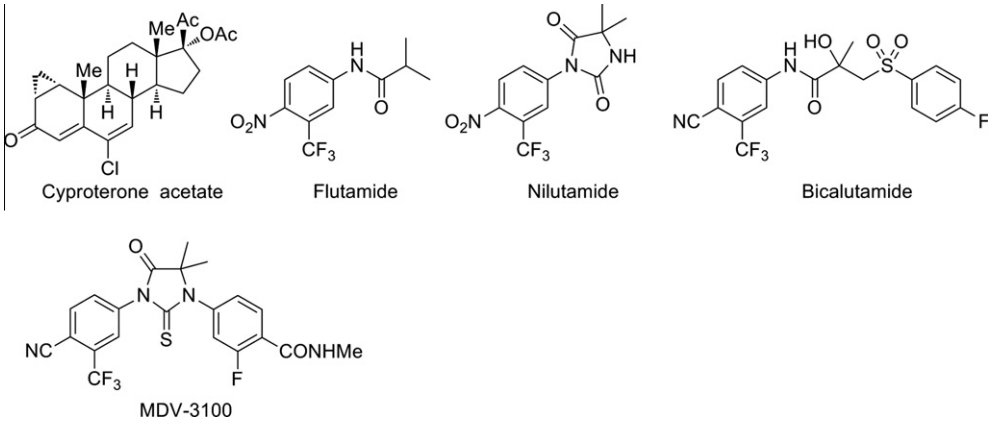


Figure 1. Known androgen receptor antagonists.

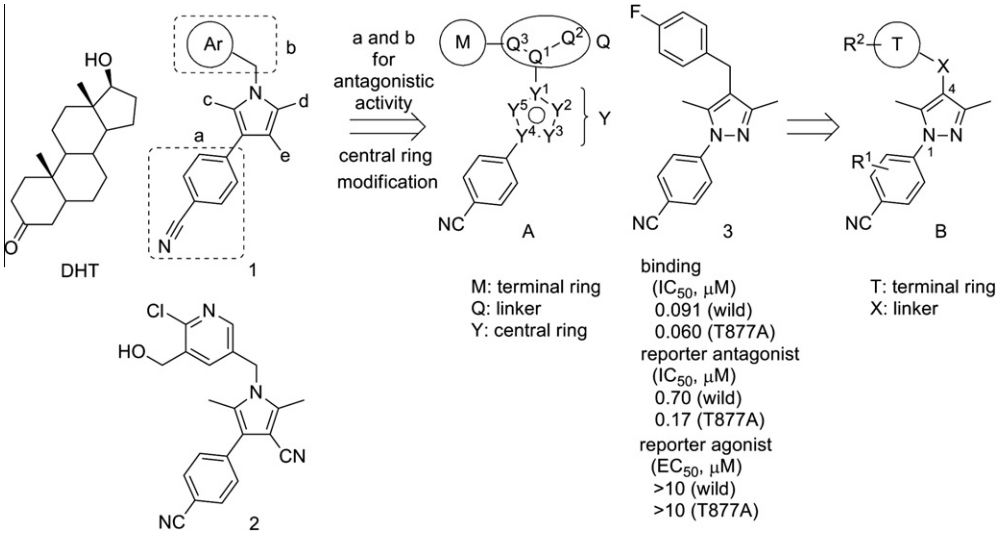


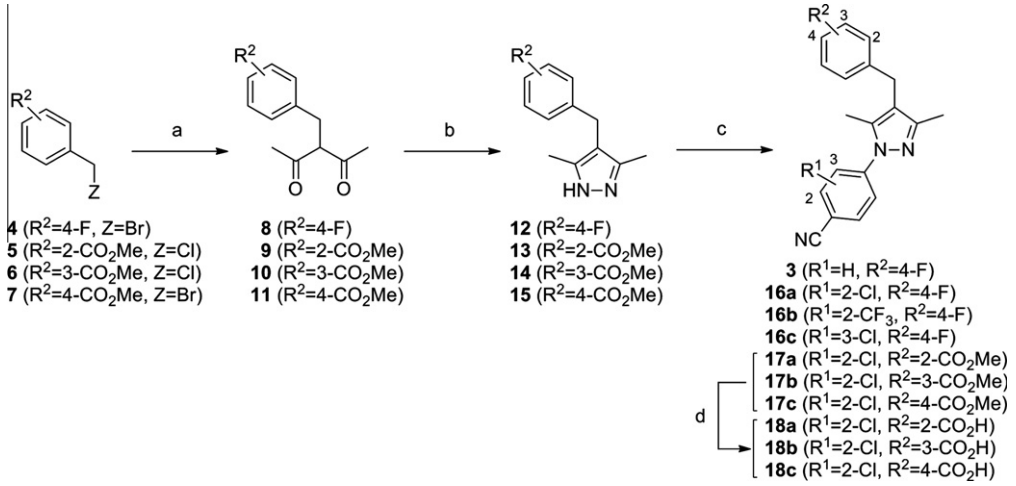
Figure 2. Design of 1-(4-cyanophenyl)-4-substitutedpyrazole AR antagonists.

Our structure–activity relationship (SAR) also suggested that the arylmethyl moiety **b**, which did not mimic any moiety of DHT, was an important contributor to the strong antagonistic activity. To date some studies on other AR antagonists possessing a cyanophenyl moiety and a second aryl ring have been reported.[27,28,32–34]

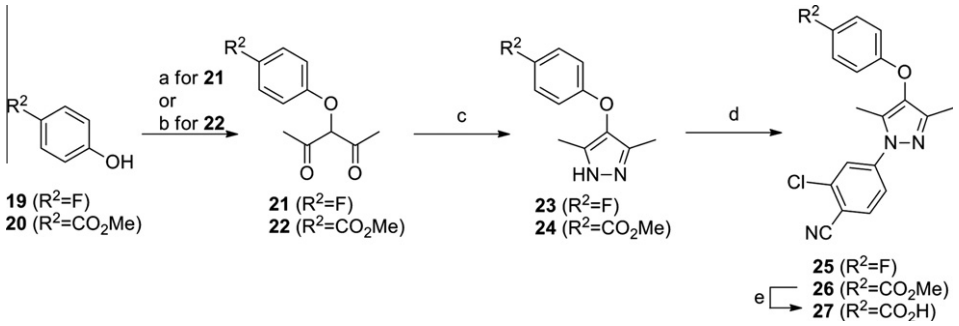
In addition to optimizing pyrroles **1**, we investigated a new series of compounds for novel AR antagonists by modifying the central pyrrole ring. A basic scaffold **A** was designed based on the importance of **a** and **b** for antagonistic activity (Fig. 2). This scaffold contains a cyanophenyl group, a central ring Y, a linker Q, and a terminal ring M. In contrast to our previous pyrrole studies (Y^1 = N), the Y^1 atom of ring Y was fixed at carbon so that a hetero atom or carbon could be introduced at Q^1 or Q^2. We set out to explore novel AR antagonists based on scaffold **A**, and found that pyrazole derivative **3** showed AR antagonistic activity. It is noteworthy that compound **3** possessed an activity profile that was deemed appropriate for efficacy against CRPC: strong AR antagonistic activity without any significant agonistic activity. Thus we selected **3** as the lead for a new series of AR antagonists, and studied substituent modifications at R^1 on the cyanophenyl group, linker X, and terminal ring T (compound **B**) to obtain compounds with good efficacy and pharmacokinetic (PK) properties. In this paper, we describe the design, synthesis, and biological evaluation of pyrazole compounds as novel AR antagonists.

2. Chemistry

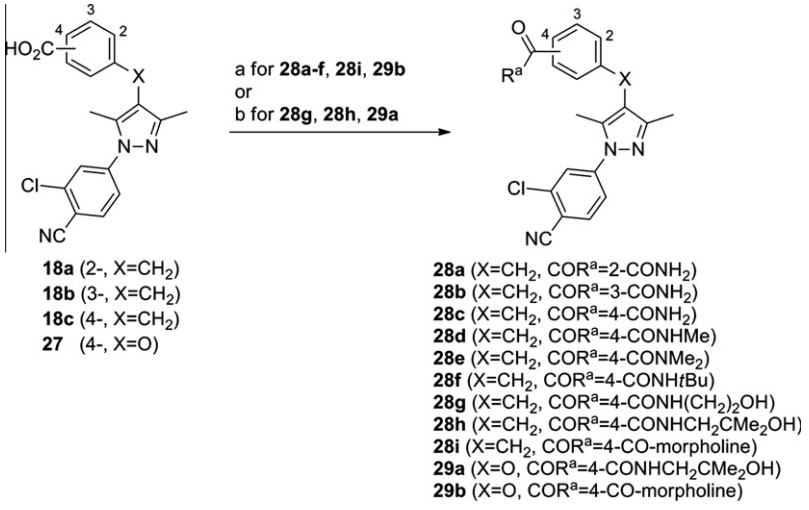
4-Benzyl-1-phenylpyrazoles **3**, **16a**–**c**, and **18a**–**c** were prepared as shown in Scheme 1. Reaction of benzyl halides **4**–**7** with 2,4-pentanedione gave diketones **8**–**11**. Cyclization of **8**–**11** with hydrazine hydrate gave 4-benzylpyrazoles **12**–**15**, which were arylated with 4-fluorobenzonitriles to afford 4-benzyl-1-phenylpyrazoles **3**, **16a**–**c**, and **17a**–**c**. Basic hydrolysis of esters **17a**–**c** gave carboxylic acids **18a**–**c**. Scheme 2 illustrates the synthesis of 1-phenyl-4-phenyloxypyrazoles **25** and **27**. The reaction of phenols **19** and **20** with 3-chloropentan-2,4-dione afforded phenyl ether **21** and **22**, which were treated with hydrazine hydrate to give 4-phenoxypyrazoles **23** and **24**. Coupling of **23** and **24** with 2-chloro-4-fluorobenzonitrile afforded **25** and **26**, respectively. Ester **26** was subjected to basic hydrolysis to afford carboxylic acid **27**. 4-Benzyl-1-phenylpyrazoles **28a**–**i** and 1-phenyl-4-phenyloxypyrazoles **29a**,**b**, which possessed an amide moiety on the terminal benzene ring, were synthesized as



Scheme 1. Synthesis of compounds **3**, **16a**–**c**, and **18a**–**c**. Reagents and conditions: (a) 2,4-Pentanedione, EtONa, EtOH, reflux; (b) hydrazine hydrate, EtOH, reflux, 17–72% for two steps; (c) 4-fluorobenzonitrile (**3**), 2-chloro-4-fluorobenzonitrile (**16a** and **17a**–**c**), 4-fluoro-2-(trifluoromethyl)benzonitrile (**16b**) or 3-chloro-4-fluorobenzonitrile (**16c**), NaH, DMF, 0 °C to room temp, 44%–quant.; (d) 1 N NaOH, MeOH, THF, 50 °C, 66%–quant.

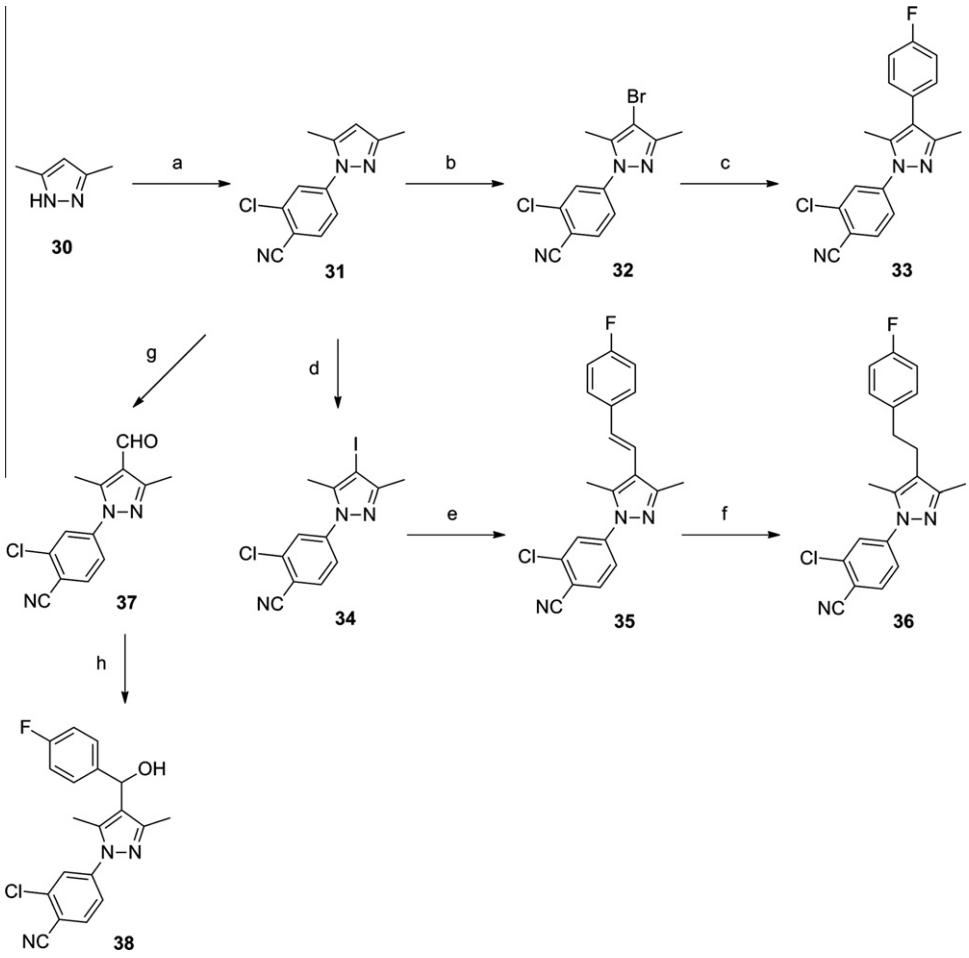


Scheme 2. Synthesis of compounds **25** and **27**. Reagents and conditions: (a) 3-Chloropentan-2,4-dione, Cs_2CO_3, acetone, reflux; (b) 3-chloropentan-2,4-dione, K_2CO_3, DMF, 80 °C; (c) hydrazine hydrate, AcOH, room temp, 29% (**23**) and 52% (**24**) for two steps; (d) NaH, 2-chloro-4-fluorobenzonitrile, DMF, 0 °C, 53% (**25**); (e) 1 N NaOH, MeOH, THF, 40 °C, 72% from **24** (two steps).



Scheme 3. Synthesis of compounds **28a**–**i** and **29a**,**b**. Reagents and conditions: (a) EDC, HOBt, amines (R^aH), DMF, room temp, 20–93%; (b) DMT-MM, amines (R^aH), THF, isopropanol, room temp, 60–89%.

shown in Scheme 3. Condensation of acids **18a**–**c** and **27** with amines was carried out using *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC) or 4-(4,6-dimethoxy-1,3,-5-triazin-2-yl)-4-methylmorpholin-4-ium chloride (DMT-MM). DMT-MM was used for the condensation of amines possessing an alcoholic hydroxyl group, so that acylation of the amino group proceeded selectively over esterification of the hydroxyl group.



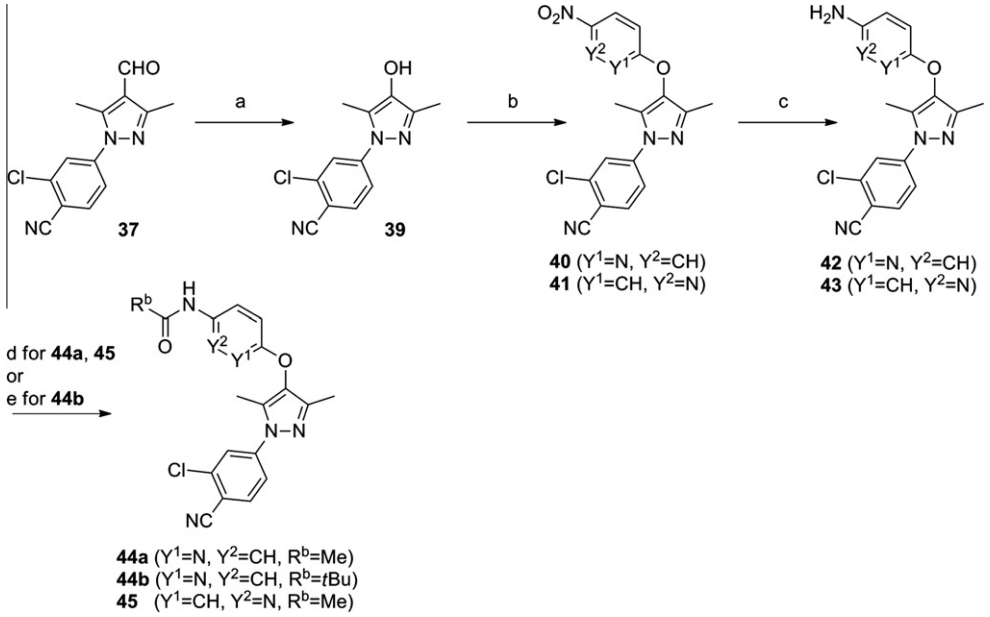
Scheme 4. Synthesis of compounds **33**, **36**, and **38**. Reagents and conditions: (a) NaH, 2-chloro-4-fluorobenzonitrile, DMF, 0 °C, 58%; (b) *N*-bromosuccinimide, AcOH, room temp, 57%; (c) 4-fluorophenylboronic acid, PS-Ph_3P-Pd, Na_2CO_3, DMF, H_2O, 150–170 °C (microwave), 20%; (d) *N*-iodosuccinimide, MeCN, room temp, 77%; (e) 4-fluorophenylstyrene, $Pd(OAc)_2$, $NaHCO_3$, tetrabutylammonium chloride, DMF, 120–130 °C (microwave), 67%; (f) H_2, Pd-C, EtOAc, room temp, 34%; (g) $POCl_3$, DMF, 0–80 °C, 56%; (h) 4-fluorophenylmagnesium bromide, THF, 0 °C, 80%.

1-Phenyl-4-phenylpyrazole compound **33**, 1-phenyl-4-(2-phenylethyl)pyrazole derivative **36**, and 4-(α-hydroxybenzyl)-1-phenylpyrazole derivative **38** were prepared as shown in Scheme 4. Compounds **33** and **36** were synthesized by a palladium-catalyzed coupling reaction. 3,5-Dimethylpyrazole **30** was deprotonated with sodium hydride and then treated with 2-chloro-4-fluorobenzonitrile to give 1-phenylpyrazole **31**. Pyrazole **31** was halogenated at the 4-position by *N*-halogenosuccinimide to give 4-halogenopyrazoles **32** and **34**. Suzuki coupling of 4-bromopyrazole **32** with 4-fluorophenylboronic acid afforded 4-phenylpyrazole **33**. 4-Iodopyrazole **34** was allowed to react with 4-fluorostyrene under modified Heck reaction conditions[35] to give olefin **35**. 4-(2-Phenylethyl)pyrazole **36** was obtained by the catalytic hydrogenation of **35**. 4-(α-Hydroxybenzyl)-1-phenylpyrazole **38** was also prepared from **31**. Formylation of the pyrazole skeleton of **31** by Vilsmeier reaction afforded 4-formyl-1-phenylpyrazole **37**. Reaction of **37** with 4-fluorophenylmagnesium bromide yielded compound **38**.

1-Phenyl-4-pyridyloxypyrazole derivatives **44a**,**b** and **45** were synthesized from **37** as described in Scheme 5. Baeyer-Villiger oxidation of **37** and subsequent solvolysis afforded 4-hydroxy-1-phenylpyrazole **39**. Coupling of **39** with 2-bromo-5-nitropyridine and 5-bromo-2-nitropyridine gave 1-phenyl-4-pyridyloxypyrazoles **40** and **41**, respectively. Reduction of the nitro group of **40** and **41** using zinc powder afforded the corresponding aminopyridines **42** and **43**. Acylation of **42** and **43** afforded compounds **44a**,**b** and **45**.

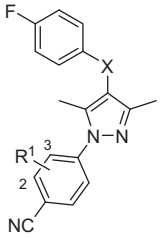
3. Results and discussion

The synthesized compounds were evaluated using in vitro and in vivo biological tests as follows. The binding affinities of the compounds to the ARs were measured by the AR binding inhibitory test (Tables 1–4). The antagonistic and agonistic activities of the compounds toward the ARs were determined by using the AR reporter assay system (Tables 1–4). In these assays, we used the wild-type AR and the T877A mutant-type AR, and estimated their efficacy against PCs, including flutamide-resistant PC. To evaluate their potential against CRPC, we established LNCaP-hr cell line as a model of CRPC.[36] This cell line expresses high levels of AR with a single mutation (T877A, inherited from the parent LNCaP-FGC cells). The cells are highly responsive to androgens and exhibit continuous growth in androgen-depleted medium. LNCaP-hr cells produce high levels of prostate specific antigen (PSA) in vitro in the absence of any androgens, indicating the constitutive activity of AR. We performed an in vitro assay using LNCaP-hr cells; the amount of PSA secreted by LNCaP-hr cells was measured after the addition of the test compound (Tables 2–4). The PK profile of the compounds was analyzed by a mouse cassette dosing test (Tables 2–4). The in vivo therapeutic efficacy of the compounds against CRPC



Scheme 5. Synthesis of compounds **44a**,**b** and **45**. Reagents and conditions: (a) (1) *m*CPBA, MeCN, EtOAc, room temp, (2) Et_3N, MeOH, room temp, 69% for two steps; (b) 2-bromo-5-nitropyridine (**40**) or 5-bromo-2-nitropyridine (**41**), Cs_2CO_3, DMF, 100 °C, 56% (**40**) and 80% (**41**); (c) zinc powder, AcOH, THF, room temp, 72% (**42**) and 83% (**43**); (d) acetic anhydride, pyridine, 0 °C to room temp, 70% (**44a**) and 63% (**45**); (e) 2,2-dimethylpropanoyl chloride, Et_3N, THF, 0 °C, 92%.

Table 1
In vitro activities of compounds **3**, **16a–c**, **25**, **33**, **36**, and **38**



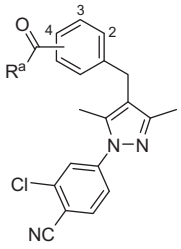
Compd	X	R^1	Binding IC_{50}[a] (μM)		Reporter			
					Antagonist IC_{50}[a] (μM)		Agonist EC_{50}[a] (μM)	
			Wild	T877A	Wild	T877A	Wild	T877A
3	CH_2	H	0.091	0.060	0.70	0.17	>10	>10
16a	CH_2	2-Cl	0.00047	0.00070	0.075	>10	1.1	0.062
16b	CH_2	2-CF_3	0.0014	0.00095	0.11	>10	>10	0.062
16c	CH_2	3-Cl	1.5	0.35	>10	0.82	>10	>10
25	O	2-Cl	0.11	0.0063	0.67	0.11	>10	>10
33	Bond	2-Cl	0.18	0.033	6.2	0.27	>10	>10
36	$(CH_2)_2$	2-Cl	0.053	0.018	1.1	0.14	>10	>10
38	CH(OH)	2-Cl	0.026	0.0083	0.048	0.18	>10	1.6
Bicalutamide			0.054	0.12	0.33	0.47	>10	>10

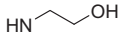
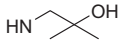
[a] IC_{50} and EC_{50} values shown are the mean values of duplicate measurements.

was evaluated by performing animal experiments in which LNCaP-hr cells were subcutaneously injected into a mouse xenograft model (Figs. 4 and 5). In these studies, we sought to discover novel AR antagonists that primarily possess strong binding affinity and antagonistic activity without significant agonistic activity, which was deemed important for efficacy against CRPC.

The SAR data obtained from the initial synthetic study of compounds **B** are shown in Table 1. In the substituents on the cyanophenyl moiety, introduction of a chloro group or a trifluoromethyl group at the 2-position greatly increased the binding affinity, although agonistic activity was also observed (**16a**, **16b**). Shifting of the chloro group to the 3-position decreased the binding affinity (**16c**). For compound **16a**, we analyzed the linker X. The AR binding affinity was found to be highly dependent on the linker length. Removal (**33**, X = bond) or elongation (**36**, X = $(CH_2)_2$) of the linker diminished the binding affinities, indicating that an atomic length of 1 unit was favorable. Interestingly, conversion of the methylene linker (**16a**, X = CH_2) to an ether linker (**25**, X = O) resulted in reduction of the agonistic activity in addition to enhancement of the antagonistic activity against T877A mutant-type AR. In contrast, when a hydroxyl group was introduced in the methylene linker (**38**, X = CH(OH)), weak agonistic activity was observed. Thus we find that the linker X is very sensitive to determining the balance between agonistic and/or antagonistic activity. On the basis of these results, we focused on the strong binder **16a**, and studied reduction of the observed agonistic activity via an

Table 2
In vitro activities and PK profiles (mouse cassette dosing) of compounds **28a–i**



Cmpd	Posi-tion	R[a]	Binding IC_{50}[a] (μM)		Reporter[b] antagonist IC_{50}[a] (μM)		LNCaP-hr PSA secretion % of control[c]		Mouse cassette dosing[d] 10 mg/kg po	
			Wild	T877A	Wild	T877A	1 μM	10 μM[e]	AUC (μg h/mL)	MRT (h)
28a	2	NH_2	4.2	1.5	>10	>10	NT[f]	NT	12.3	2.2
28b	3	NH_2	0.26	0.20	0.70	1.9	21	35	0.6	2.0
28c	4	NH_2	0.016	0.010	0.42	0.14	76	NT	5.0	3.2
28d	4	NHMe	0.027	0.013	1.3	0.11	NT	NT	NT	NT
28e	4	NMe_2	0.92	0.46	4.0	6.3	21	NT	NT	NT
28f	4	NH*t*Bu	0.11	0.10	0.97	0.77	33	57	14.1	4.0
28g	4		0.16	0.099	2.7	2.1	33	NT	30.5	3.3
28h	4		0.34	0.16	0.59	1.1	27	31	38.9	3.9
28i	4		0.51	0.70	2.4	>10	35	NT	6.2	2.4

[a] IC_{50} and EC_{50} values shown are the mean values of duplicate measurements.
[b] Agonistic activities of **28a–i** were EC_{50} >10 μM for wild-type or T877A mutant-type AR.
[c] $n = 3$.
[d] $n = 3$.
[e] Bicalutamide exhibited more than 100% secretion of control at 10 μM.
[f] Not tested.

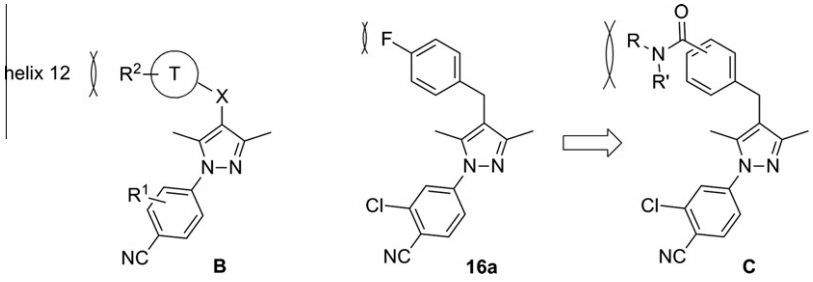
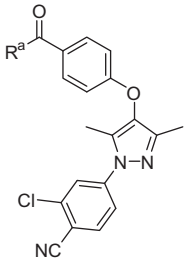
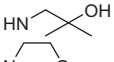


Figure 3. Introduction of a Bulky Amide Moiety in Arylmethyl Group for Inhibition of Folding of Helix 12.

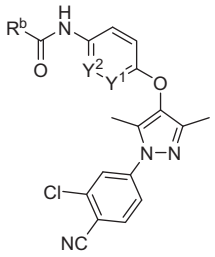
Table 3
In vitro activities and PK profiles (mouse cassette dosing) of compounds **29a** and **29b**



Compd	R[a]	Binding IC_{50}[a] (μM)		Reporter[b] antagonist IC_{50}[a] (μM)		LNCaP-hr PSA secretion % of control[c]		Mouse cassette dosing[d] 10 mg/kg, po	
		Wild	T877A	Wild	T877A	1 μM	10 μM	AUC (μg h/mL)	MRT (h)
29a		1.1	0.055	1.8	0.18	33	>100	24.0	4.8
29b		1.3	0.18	3.0	0.72	22	22	13.9	3.0

[a] IC_{50} and EC_{50} values shown are the mean values of duplicate measurements.
[b] Agonistic activities of **29a** and **29b** were EC_{50} >10 μM for wild-type or T877A mutant-type AR.
[c] $n = 3$.
[d] $n = 3$.

Table 4
In vitro activities and PK profiles (mouse cassette dosing) of compounds **44a**,**b** and **45**



Compd	Y^1	Y^2	R^b	Binding IC_{50}[a] (μM)		Reporter[b] antagonist IC_{50}[a] (μM)		LNCaP-hr PSA secretion % of control[c]		Mouse cassette dosing[d] 10 mg/kg, po	
				Wild	T877A	Wild	T877A	1 μM	10 μM	AUC (μg h/mL)	MRT (h)
44a	N	CH	Me	5.4	0.27	>10	1.6	8	14	18.2	2.8
44b	N	CH	*t*Bu	4.3	0.34	4.0	0.19	8	5	29.5	4.2
45	CH	N	Me	1.2	0.083	7.1	0.35	11	39	0.13	1.9

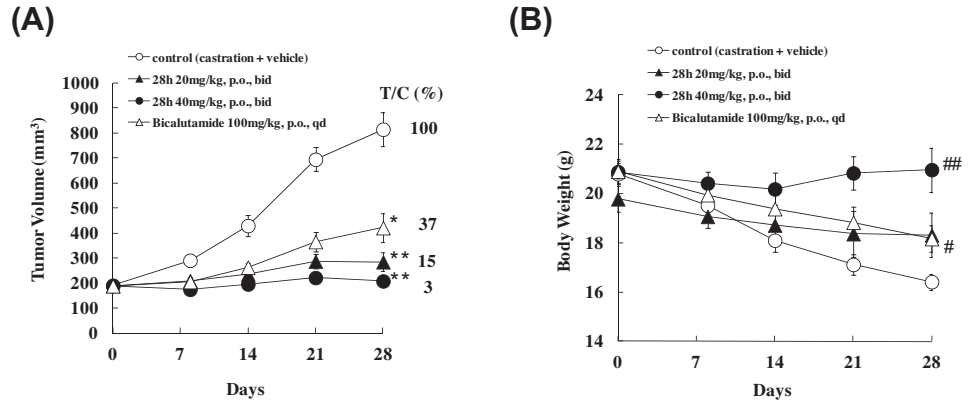
[a] IC_{50} and EC_{50} values shown are the mean values of duplicate measurements.
[b] Agonistic activities of **44a**,**b** and **45** were EC_{50} >10 μM for wild-type or T877A mutant-type AR.
[c] $n = 3$.
[d] $n = 3$.

alternative approach to linker X modification. Apart from **16a**, we selected compound **25**, which showed strong antagonistic activity free from agonistic activity, for further investigation.

To analyze the reduction of the agonistic activity of **16a**, we focused on the folding of helix 12 of the AR ligand binding domain (LBD).[37–40] Folding of the helix is an important factor in the expression of agonistic activity. Therefore, inhibition of folding is an effective method for reducing agonistic activity. We hypothesized that the arylmethyl group (X–T–R^2 in **B**) inhibited folding, as shown in Figure 3, and that the size of the 4-fluorobenzyl group of **16a**, which possessed AR agonistic activity as well as antagonistic activity, was not sufficient for the inhibition. On the basis of this hypothesis, we investigated whether introduction of a bulky group into the benzyl moiety could result in steric repulsion to reduce the agonistic activity. With the goal of obtaining compounds with good PK properties, we set out to prepare compounds with relatively polar bulky groups, such as amide compounds (**C** in Figure 3). The results are shown in Table 2. Notably, none of the evaluated compounds exhibited agonistic activity (EC_{50} >10 μM) in a reporter assay of wild-type or mutated AR. Among the 3 regioisomers of carboxamides (**28a**–**c**) used in the reporter antagonist assay, the 4-carboxamide compound **28c** was the most potent. The secondary amide exhibited higher antagonistic activity than that of the tertiary amide (**28d** vs **28e**). Carboxamides of polar amines had better PK properties than that of simple alkyl amides (**28f** vs **28g** or **28h**), presumably because of their increased hydrophilicity. In particular, 2-hydroxy-2-methylpropylamide derivative **28h** showed moderate PK profile and inhibited LNCaP-hr PSA secretion, for which bicalutamide exhibited no inhibition (more than 100% secretion of control at 10 μM). It was noteworthy that **28h** showed significantly different response in CRPC in vitro model from bicalutamide. We thus selected **28h** for further studies.

Next, we studied the modification of 4-phenyloxypyrazole compound **25**, which showed AR antagonistic activity without significant agonistic activity. The data for the PK profile of this compound was insufficient for further evaluation (mouse cassette dosing, 10 mg/kg, po AUC = 1.00 μg·h/mL, MRT_{po} = 2.2 h). As described above, in the case of the 4-benzylpyrazole derivatives, better PK profiles and biological activities were obtained by the introduction of a bulky amide substituent at the 4-position of the benzyl moiety. To identify 4-aryloxypyrazole compounds which had improved PK profiles and activities, we applied this SAR information to 4-aryloxylpyrazole derivatives. As shown in Table 3, similar to the 4-benzylpyrazole compounds, 2-hydroxy-2-methylpropylamide derivative **29a** exhibited moderate PK profile and activities in binding and reporter gene assays. However, unlike 4-benzylpyrazole compounds, the **29a** exhibited reduced inhibitory activity against LNCaP-hr PSA secretion at high concentrations. On the other hand, morpholine derivative **29b** exhibited increased inhibitory activity in an LNCaP-hr PSA assay. The PK profile of **29b** was not as good as that of **29a**. Considering the good PK profile of the compounds, we also studied replacement of the phenyloxy moiety with a heteroaryloxy group.[31] The results for compounds **44a**, **44b**, and **45** are shown in Table 4. None of these compounds showed agonistic activity (EC_{50} >10 μM) in a reporter assay of wild-type or mutated AR. Pyridin-2-yloxy compounds **44a** and **44b** exhibited moderate PK profiles. Compound **44b** had moderate binding affinity and antagonistic activity. It was noteworthy that **44a** and **44b** showed strong inhibitory activity against LNCaP-hr PSA secretion. Pyridin-3-yloxy compound **45** showed higher binding affinities and antagonistic activities than did **44a**. On the other hand, the PK profile of pyridin-2-yloxy compounds was better than that of pyridin-3-yloxy compounds. The AUC value of **44a** in mouse cassette dosing was approximately 100-fold as large as that of **45**. On the basis of these results, **44b** was selected for further evaluations.

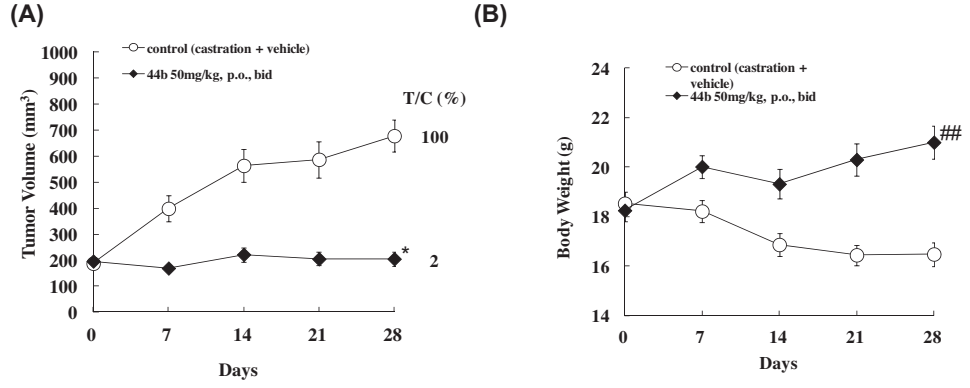
We evaluated the antitumor effects of **28h**, **44b**, and bicalutamide in mouse xenograft model using LNCaP-hr cells. Bicalutamide is reported to show long duration of plasma concentration in men (mean $t_{1/2}$ of *R*-isomer and *S*-isomer is 4.2 days and 19 h, respectively[41]). Long duration was also observed in mice (data not shown). On the basis of this data for bicalutamide and the PK data for **28h** and **44b** (Tables 2 and 4), these compounds were orally administered twice (**28h**, **44b**) or once (bicalutamide) a day for 4 weeks. Tumor volume and plasma PSA concentration were measured after the treatment period. As shown in Figs. 4 and 5, compounds **28h** and **44b** showed extremely potent tumor growth inhibition by *T*/*C* values (volume change in a test compound/volume change in control) of 3% and 2% at doses of 40 and 50 mg/kg, bid, respectively. The plasma PSA levels in these treatment groups were also markedly reduced to 23% and 17% of those in the vehicle treatment groups, respectively. In addition, these compounds resulted in almost no loss of body weight. On the other hand, bicalutamide showed only partial suppression of tumor growth (*T*/*C* value of 37%) and had almost no effect on plasma PSA level even at a dose of 100 mg/kg, qd. These results, as well



(C)

Compound	Dose (mg/kg, *p.o.*)	Mean ± SE (ng / mL) Day 28	T / C (%)
Control (castration + vehicle)	–	31.4 ± 8.2	100
28h	20, bid	27.6 ± 11.1	88
28h	40, bid	7.2 ± 3.9	23
Bicalutamide	100, qd	44.0 ± 12.4	142

Figure 4. Antitumor effects of **28h** and bicalutamide against LNCaP-hr cell line in mouse xenograft model ($n = 6$ animals per group). (A) Tumor volume (mm^3). (B) Body weight (g). (C) Plasma PSA (ng / mL). *T*/*C* indicates increase in a test compound group during the treatment period/increase in a control group during the treatment period × 100. $^{*}P < 0.05$, Steel test versus control. $^{**}P < 0.025$, Shirley–Williams test versus control. $^{\#}P < 0.05$ Student's *t*-test versus control. $^{\#\#}P < 0.025$ Williams test versus control.



(C)

Compound	Dose (mg/kg, *p.o.*)	Mean ± SE (ng / mL) Day 28	T / C (%)
Control (castration + vehicle)	–	17.8 ± 3.1	100
44b	50, bid	3.0 ± 1.2$^{\#}$	17

Figure 5. Antitumor effects of **44b** against LNCaP-hr cell line in mouse xenograft model ($n = 6$ animals per group). (A) Tumor volume (mm^3). (B) Body weight (g). (C) Plasma PSA (ng/mL). *T*/*C* indicates increase in a test compound group during the treatment period/increase in a control group during the treatment period × 100. $^{*}P < 0.01$, Steel test versus control. $^{\#}P < 0.01$, Student's *t*-test versus control. $^{\#\#}P < 0.001$, Student's *t*-test versus control.

as the significantly different activity observed in CRPC in vitro model between compounds **28h** and **44b** in contrast to bicalutamide, suggested that **28h** and **44b** represent a class of new-generation AR antagonists effective against CRPC model and that their properties are different from those of 'first-generation' antagonists such as bicalutamide.

4. Conclusion

In the course of investigating new-generation AR antagonists therapeutically effective against CRPC, we designed, synthesized, and evaluated the activities of 4-arylmethyl-1-phenylpyrazole and 4-aryloxy-1-phenylpyrazole compounds **B**. In 4-arylmethyl derivatives, the introduction of a bulky amide group at the 4-position of the benzyl group afforded **28h** with significantly reduced agonistic activity and improved pharmacokinetics. In 4-aryloxy derivatives, the introduction of a bulky substituent at the 4-position of the aryloxy group and replacement of the phenyloxy moiety with a pyridyloxy group improved the pharmacokinetics and gave **44b**. Oral administration of **28h** and **44b** induced extremely potent antitumor effects against LNCaP-hr cell line, a CRPC model, in a mouse xenograft. On the other hand, administration of bicalutamide caused only partial suppression of the growth of LNCaP-hr. It was indicated that the 4-arylmethyl-1-phenylpyrazole and 4-aryloxy-1-phenylpyrazole compounds represent a class of new-generation AR antagonists effective against CRPC model and that their properties are different from those of 'first-generation' AR antagonists such as bicalutamide.

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 300 MHz on 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. 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. 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 mm, Merck KGaA), basic silica gel (Chromatorex® NH, 100–200 mesh, Fuji Silysia Chemical Ltd) or Purif-Pack (Si or NH, Moritex Corporation) using the indicated eluents. Yields are unoptimized. Preparative HPLC separations were performed by Unipoint system (Gilson, Inc.) outfitted with YMC ODS column (30 × 75 mm or 50 × 20 mm, YMC Co., Ltd). Samples were eluted with a gradient of 5–100% water in acetonitrile containing 0.1% TFA, and detected by the UV absorbance at 220 nm and 254 nm. Microwave reactions were performed using Optimizer system (Personal Chemistry, Inc.) or Initiator Sixty system (Biotage, Inc.). The polymer-bound reagents were pre-conditioned prior to use.

5.1.1. Methyl 4-[(3,5-dimethyl-1*H*-pyrazol-4-yl)methyl]-benzoate (15)

To a mixture of 2,4-pentanedione (6.72 mL, 65.4 mmol), sodium ethoxide (20%w/w in EtOH, 8.55 mL, 21.8 mmol), and EtOH (46.0 mL) warmed at 50 °C was added a solution of methyl 4-(bromomethyl)benzoate **7** (5.00 g, 21.8 mmol) in EtOH (40.0 mL) dropwise over 30 min. The mixture was refluxed for 2 h, cooled to room temperature, and concentrated in vacuo. The residue was mixed with 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 methyl 4-(2-acetyl-3-oxobutyl)benzoate **11** (4.56 g) as a colorless gum. This product **11** (4.56 g, 18.4 mmol) was mixed with EtOH (90 mL) and hydrazine hydrate (0.983 mL, 20.2 mmol), and the mixture was refluxed for 2 h. After cooling, the mixture was concentrated in vacuo, and the residue was recrystallized from toluene to give **15** (3.86 g, 72% from **7**) as colorless tabular crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.14 (6H, s), 3.79 (2H, s), 3.90 (3H, s), 7.17 (2H, d, J = 8.7 Hz), 7.93 (2H, d, J = 8.7 Hz), 9.47 (1 H, br s).

5.1.2. 4-(4-Fluorobenzyl)-3,5-dimethyl-1*H*-pyrazole (12)

Compound **12** was prepared in a manner similar to that described for **15** in 55% yield as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.98 (3H, s), 2.08 (3H, s), 3.63 (2H, s), 6.74–7.29 (4H, m), 11.98 (1H, br s).

5.1.3. 4-[4-(4-Fluorobenzyl)-3,5-dimethyl-1*H*-pyrazol-1-yl]-benzonitrile (3)

To an ice-cooled solution of **12** (0.200 g, 0.979 mmol) in DMF (2.0 mL) was added NaH (60% in mineral oil, 0.047 g, 1.18 mmol), and the mixture was stirred at 0 °C for 30 min. To the mixture was added 4-fluorobenzonitrile (0.237 g, 1.96 mmol), and the mixture was stirred at 0 °C for 2 h and room temperature for 18 h. The mixture was acidified with saturated aqueous solution of NH_4Cl and extracted twice with EtOAc. The organic layers were combined, 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 **3** (0.131 g, 44%) as white crystals, mp 100–101 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.19 (3H, s), 2.30 (3H, s), 3.76 (2H, s), 6.91–7.03 (2H, m), 7.05–7.16 (2H, m), 7.55–7.64 (2H, m), 7.70–7.78 (2H, m). Anal. Calcd for $C_{19}H_{16}FN_3$: C, 74.74; H, 5.28; N, 13.76. Found: C, 74.68; H, 5.23; N, 13.74.

5.1.4. Methyl 2-[(3,5-dimethyl-1*H*-pyrazol-4-yl)methyl]-benzoate (13)

Compound **13** was prepared in a manner similar to that described for **15** in 26% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.08 (6H, s), 3.90 (3H, s), 4.12 (2H, s), 7.21–7.28 (2H, m), 7.32–7.40 (1H, m), 7.88 (1H, dd, J = 7.9 and 1.5 Hz).

5.1.5. Methyl 3-[(3,5-dimethyl-1*H*-pyrazol-4-yl)methyl]-benzoate (14)

Compound **14** was prepared in a manner similar to that described for **15** in 17% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.15 (6H, s), 3.78 (2H, s), 3.90 (3H, s), 7.27–7.38 (2H, m), 7.79–7.93 (2H, m).

5.1.6. 2-Chloro-4-[4-(4-fluorobenzyl)-3,5-dimethyl-1*H*-pyrazol-1-yl]benzonitrile (16a)

Compound **16a** was prepared in a manner similar to that described for **3** in quantitative yield as colorless gum. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.18 (3H, s), 2.32 (3H, s), 3.76 (2H, s), 6.97 (2H, t, J = 8.7 Hz), 7.05–7.13 (2H, m), 7.51 (1H, dd, J = 8.5 and 2.1 Hz), 7.70–7.77 (2H, m). Anal. Calcd for $C_{19}H_{15}ClFN_3$: C, 67.16; H, 4.45; N, 12.37. Found: C, 67.02; H, 4.57; N, 12.20.

5.1.7. 4-[4-(4-Fluorobenzyl)-3,5-dimethyl-1*H*-pyrazol-1-yl]-2-(trifluoromethyl)benzonitrile (16b)

Compound **16b** was prepared in a manner similar to that described for **3** in 87% yield as a white solid, mp 111–112 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.19 (3H, s), 2.35 (3H, s), 3.77 (2H, s), 6.91–7.03 (2H, m), 7.04–7.13 (2H, m), 7.75–7.82 (1H, m), 7.88–7.96 (1H, m), 8.02 (1H, d, J = 1.9 Hz). Anal. Calcd for $C_{20}H_{15}F_4N_3$: C, 64.34; H, 4.05; N, 11.25. Found: C, 64.22; H, 4.02; N, 11.36.

5.1.8. 3-Chloro-4-[4-(4-fluorobenzyl)-3,5-dimethyl-1*H*-pyrazol-1-yl]benzonitrile (16c)

Compound **16c** was prepared in a manner similar to that described for **3** in 89% yield as a white solid, mp 109–110 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.04 (3H, s), 2.17 (3H, s), 3.77 (2H, s), 6.98 (2H, t, J = 8.7 Hz), 7.05–7.14 (2H, m), 7.55 (1H, d, J = 8.3 Hz), 7.66–7.73 (1H, m), 7.84 (1H, s). Anal. Calcd for $C_{19}H_{15}ClFN_3$: C, 67.16; H, 4.45; N, 12.37. Found: C, 67.20; H, 4.43; N, 12.40.

5.1.9. Methyl 2-{[1-(3-chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]methyl}benzoate (17a)

Compound **17a** was prepared in a manner similar to that described for **3** in 60% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.12 (3H, s), 2.26 (3H, s), 3.91 (3H, s), 4.20 (2H, s), 7.01 (1H, d, J = 7.9 Hz), 7.30 (1H, d, J = 6.8 Hz), 7.36–7.44 (1H, m), 7.53 (1H, dd, J = 8.5 and 2.1 Hz), 7.70–7.80 (2H, m), 7.90–7.96 (1H, m).

5.1.10. Methyl 3-{[1-(3-chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]methyl}benzoate (17b)

Compound **17b** was prepared in a manner similar to that described for **3** in 53% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.18 (3H, s), 2.34 (3H, s), 3.84 (2H, s), 3.91 (3H, s), 7.28–7.41 (2H, m), 7.52 (1H, dd, J = 8.5 and 2.1 Hz), 7.70–7.77 (2H, m), 7.84 (1H, s), 7.89 (1H, d, J = 7.5 Hz).

5.1.11. Methyl 4-{[1-(3-chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]methyl}benzoate (17c)

Compound **17c** was prepared in a manner similar to that described for **3** in 70% yield as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.09 (3H, s), 2.39 (3H, s), 3.83 (3H, s), 3.87 (2H, s), 7.32 (2H, d, J = 7.9 Hz), 7.71–7.78 (1H, m), 7.85–7.97 (3H, m), 8.08 (1H, d, J = 8.3 Hz).

5.1.12. 4-{[1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]methyl}benzoic acid (18c)

To a suspension of **17c** (0.500 g, 1.32 mmol) in THF (5.0 mL) and MeOH (5.0 mL) at 50 °C was added 1 N NaOH (5.0 mL, 5.0 mmol) dropwise. The mixture was stirred at 50 °C for 1 h to give a colorless solution. The solution was cooled to room temperature, neutralized with 2 N HCl (2.5 mL), and extracted twice with EtOAc. The organic extracts were combined, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give **18c** (0.455 g, 94%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.10 (3H, s), 2.40 (3H, s), 3.86 (2H, s), 7.29 (2H, d, J = 8.3 Hz), 7.75 (1H, dd, J = 8.5 and 2.1 Hz), 7.86 (2H, d, J = 8.7 Hz), 7.95 (1H, d, J = 1.9 Hz), 8.08 (1H, d, J = 8.7 Hz), 12.83 (1H, br s).

5.1.13. 2-{[1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]methyl}benzoic acid (18a)

Compound **18a** was prepared in a manner similar to that described for **18c** in 66% yield as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.00 (3H, s), 2.30 (3H, s), 4.15 (2H, s), 7.06 (1H, d, J = 7.5 Hz), 7.31 (1H, t, J = 7.5 Hz), 7.40–7.48 (1H, m), 7.74 (1H, dd, J = 8.5 and 2.1 Hz), 7.78–7.85 (1H, m), 7.94 (1H, d, J = 2.3 Hz), 8.08 (1H, d, J = 8.3 Hz), 13.01 (1H, br s).

5.1.14. 3-{[1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]methyl}benzoic acid (18b)

Compound **18b** was prepared in a manner similar to that described for **18c** in quantitative yield as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.10 (3H, s), 2.40 (3H, s), 3.87 (2H, s), 7.39–7.46 (2H, m), 7.72–7.82 (3H, m), 7.96 (1H, d, J = 2.3 Hz), 8.08 (1H, d, J = 8.7 Hz), 12.94 (1H, br s).

5.1.15. 4-(4-Fluorophenoxy)-3,5-dimethyl-1*H*-pyrazole (23)

A mixture of 4-fluorophenol **19** (1.00 g, 8.92 mmol), 3-chloropentan-2,4-dione (1.17 mL, 9.81 mmol), Cs_2CO_3 (3.20 g, 9.81 mmol), and acetone (20 mL) was refluxed for 5 h. After cooling, white solid was removed by filtration and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give 3-(4-fluorophenoxy)pentane-2,4-dione **21** as a pale yellow gum (1.30 g). This product **21** (1.20 g, 5.71 mmol) was dissolved in acetic acid (12 mL), and hydrazine hydrate (0.333 mL, 6.85 mmol) was added to the solution. The mixture was stirred at room temperature for 1 h, concentrated in vacuo, and neutralized with saturated aqueous solution of $NaHCO_3$. The mixture was extracted twice with EtOAc. The organic layers were combined, 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 **23** (0.499 g, 29% from **19**) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.11 (6H, s), 6.78–6.87 (2H, m), 6.91–7.00 (2H, m).

5.1.16. Methyl 4-[(3,5-dimethyl-1*H*-pyrazol-4-yl)oxy]benzoate (24)

To a solution of methyl 4-hydroxybenzoate **20** (5.00 g, 32.9 mmol) in DMF (125 mL) were added K_2CO_3 (9.10 g, 65.8 mmol) and 3-chloropentane-2,4-dione (7.84 mL, 65.8 mmol) at 0 °C. The mixture was stirred at 80 °C for 30 min, cooled to room temperature, and filtrated to remove an insoluble solid. The filtrate was diluted with EtOAc, washed with saturated aqueous solution of $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give methyl 4-(1-acetyl-2-oxopropoxy)benzoate **22** (11.8 g). A mixture of **22** (11.8 g), hydrazine hydrate (1.94 mL, 39.5 mmol), and acetic acid (40 mL) was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo, neutralized with saturated aqueous solution of $NaHCO_3$ at 0 °C, and extracted twice with EtOAc. The organic layers were combined, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo to give a yellow solid. The residue was purified by silica gel column chromatography (hexane–EtOAc) to afford **24** (4.20 g, 52% from **20**) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.11 (6H, s), 3.89 (3H, s), 6.91 (2H, d, J = 7.7 Hz), 7.98 (2H, d, J = 7.7 Hz).

5.1.17. 2-Chloro-4-[4-(4-fluorophenoxy)-3,5-dimethyl-1*H*-pyrazol-1-yl]benzonitrile (25)

To an ice-cooled mixture of **23** (0.100 g, 0.485 mmol) and DMF (1.0 mL) was added NaH (60% in mineral oil, 0.023 g, 0.582 mmol), and the mixture was stirred at 0 °C for 30 min. To the mixture was added 2-chloro-4-fluorobenzonitrile (0.151 g, 0.970 mmol), and the mixture was stirred at 0 °C for 1 h. The mixture was mixed with saturated aqueous solution of $NaHCO_3$ and extracted twice with EtOAc. The organic layers were combined, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to afford **25** (0.088 g, 53%) as a white solid, mp 118–119 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.13 (3H, s), 2.32 (3H, s), 6.82–6.91 (2H, m), 6.96–7.05 (2H, m), 7.55 (1 H, dd, J = 8.7 and 1.9 Hz), 7.72–7.81 (2H, m). Anal. Calcd for $C_{18}H_{13}ClFN_3O$: C, 63.26; H, 3.83; N, 12.29. Found: C, 63.22; H, 3.78; N, 12.35.

5.1.18. 4-{[1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]oxy}benzoic acid (27)

To a solution of **24** (7.18 g, 29.2 mmol) in DMF (75 mL) was added NaH (60% in mineral oil, 1.40 g, 35.0 mmol) at 0 °C. After stirring at 0 °C for 30 min, 2-chloro-4-fluorobenzonitrile (9.07 g, 58.3 mmol) was added. The mixture was stirred at 0 °C for 2 h, neutralized with saturated aqueous solution of NH_4Cl, and

extracted twice with EtOAc. The organic layers were combined, 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 methyl 4-{[1-(3-chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]oxy}benzoate **26** (14.5 g) as a white solid. To a warmed solution of **26** (14.5 g) in THF (220 mL) and methanol (220 mL) was added 1 N NaOH (110 mL) at 40 °C. The resulting colorless solution was stirred at the same temperature for 2 h, cooled on ice, and acidified with 1 N HCl. The mixture was concentrated in vacuo, and the residue was extracted twice with EtOAc. The organic layers were combined, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to afford **27** (7.68 g, 72% from **24**) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.06 (3H, s), 2.30 (3H, s), 7.06 (2H, d, J = 8.3 Hz), 7.81 (1H, d, J = 8.5 Hz), 7.94 (2H, d, J = 8.1 Hz), 8.01 (1H, s), 8.13 (1H, d, J = 8.7 Hz), 12.77 (1 H, br s).

5.1.19. *N*-*tert*-Butyl-4-{[1-(3-chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]methyl}benzamide (28f)

To a solution of **18c** (2.00 g, 5.47 mmol), *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (1.57 g, 8.21 mmol), and 1-hydroxybenzotriazole (1.11 g, 8.21 mmol) in DMF (20 mL) was added *tert*butylamine (0.862 mL, 8.21 mmol). After stirring at room temperature for 13 h, the mixture was mixed with 10% aqueous solution of $NaHSO_4$ and extracted twice with EtOAc. The organic layers were combined, washed with 1 N HCl, saturated aqueous solution of $NaHCO_3$ 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 **28f** (1.81 g, 79%) as a white solid, mp 135–136 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.46 (9H, s), 2.17 (3H, s), 2.32 (3H, s), 3.82 (2H, s), 5.89 (1H, br s), 7.17 (2H, d, J = 8.3 Hz), 7.51 (1 H, dd, J = 8.6 and 2.0 Hz), 7.62–7.67 (2H, m), 7.74 (2H, dd, J = 5.2 and 3.1 Hz). Anal. Calcd for $C_{24}H_{25}ClN_4O$: C, 68.48; H, 5.99; N, 13.31. Found: C, 68.49; H, 5.97; N, 13.34.

5.1.20. 2-{[1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]methyl}benzamide (28a)

Compound **28a** was prepared in a manner similar to that described for **28f** in 88% yield as a white solid, mp 208–209 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.03 (3H, s), 2.32 (3H, s), 3.94 (2H, s), 7.02 (1H, d, J = 6.8 Hz), 7.17–7.46 (4H, m), 7.73 (1H, dd, J = 8.5 and 2.1 Hz), 7.80 (1H, s), 7.93 (1H, d, J = 1.9 Hz), 8.08 (1H, d, J = 8.7 Hz). Anal. Calcd for $C_{20}H_{17}ClN_4O{\cdot}0.2$AcOEt: C, 65.32; H, 4.90; N, 14.65. Found: C, 65.36; H, 4.82; N, 14.50.

5.1.21. 3-{[1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]methyl}benzamide (28b)

Compound **28b** was prepared in a manner similar to that described for **28f** in 73% yield as a white solid, mp 200–201 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.10 (3H, s), 2.40 (3H, s), 3.83 (2H, s), 7.26–7.42 (3H, m), 7.65–7.80 (3H, m), 7.95 (2H, d, J = 2.3 Hz), 8.08 (1H, d, J = 8.7 Hz). Anal. Calcd for $C_{20}H_{17}ClN_4O$: C, 65.84; H, 4.70; N, 15.36. Found: C, 65.60; H, 4.77; N, 15.03.

5.1.22. 4-{[1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]methyl}benzamide (28c)

Compound **28c** was prepared in a manner similar to that described for **28f** in 64% yield as a white solid, mp 200–201 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.10 (3H, s), 2.40 (3H, s), 3.83 (2H, s), 7.24 (2H, d, J = 8.3 Hz), 7.29 (1H, br s), 7.71–7.82 (3H, m), 7.89 (1H, br s), 7.95 (1H, d, J = 2.3 Hz), 8.08 (1H, d, J = 8.7 Hz). Anal. Calcd for $C_{20}H_{17}ClN_4O$: C, 65.84; H, 4.70; N, 15.36. Found: C, 65.70; H, 4.69; N, 15.29.

5.1.23. 4-{[1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]methyl}-*N*-methylbenzamide (28d)

Compound **28d** was prepared in a manner similar to that described for **28f** in 22% yield as a white solid, mp 173–174 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.17 (3H, s), 2.32 (3H, s), 3.01 (3H, d, J = 4.9 Hz), 3.83 (2H, s), 6.12 (1H, br s), 7.19 (2H, d, J = 8.3 Hz), 7.52 (1H, d, J = 8.3 Hz), 7.65–7.82 (4H, m). Anal. Calcd for $C_{21}H_{19}ClN_4O{\cdot}0.5H_2O$: C, 65.03; H, 5.20; N, 14.45. Found: C, 65.37; H, 5.01; N, 14.48.

5.1.24. 4-{[1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]methyl}-*N*,*N*-dimethylbenzamide (28e)

Compound **28e** was prepared in a manner similar to that described for **28f** in 20% yield as a white solid, mp 131–132 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.21 (3H, s), 2.34 (3H, s), 3.00 (3H, s), 3.12 (3H, s), 3.83 (2H, s), 7.17 (2H, d, J = 8.3 Hz), 7.37 (2H, d, J = 8.3 Hz), 7.53 (1H, dd, J = 8.5 and 2.1 Hz), 7.72–7.80 (2H, m). Anal. Calcd for $C_{22}H_{21}ClN_4O$: C, 66.49; H, 5.45; N, 14.10. Found: C, 66.46; H, 5.36; N, 14.15.

5.1.25. 4-{[1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]methyl}-*N*-(2-hydroxy-2-methylpropyl)benzamide (28h)

A suspension of **26c** (2.00 g, 5.47 mmol), 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholin-4-ium chloride (2.27 g, 8.21 mmol) and 1-amino-2-methylpropan-2-ol (0.585 g, 6.56 mmol) in THF (25 mL) and 2-propanol (25 mL) was stirred at room temperature for 19 h. The mixture was concentrated in vacuo, acidified with 1 N HCl, and extracted twice with EtOAc. The organic layers were combined, washed with saturated aqueous solution of NH_4Cl and brine, dried over anhydrous Na_2SO_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 **28h** (2.06 g, 89%) as a white solid, mp 171–172 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.09 (6H, s), 2.11 (3H, s), 2.40 (3H, s), 3.24 (2H, d, J = 6.0 Hz), 3.84 (2H, s), 4.54 (1H, s), 7.26 (2H, d, J = 8.1 Hz), 7.73–7.80 (3H, m), 7.94 (1H, d, J = 1.8 Hz), 8.08 (1H, d, J = 8.7 Hz), 8.15 (1H, t, J = 6.0 Hz). Anal. Calcd for $C_{24}H_{25}ClN_4O_2$: C, 65.97; H, 5.77; N, 12.82. Found: C, 66.12; H, 5.71; N, 12.87.

5.1.26. 4-{[1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]methyl}-*N*-(2-hydroxyethyl)benzamide (28g)

Compound **28g** was prepared in a manner similar to that described for **28h** in 60% yield as a white solid, mp 146–147 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.17 (3H, s), 2.32 (3H, s), 2.56 (1H, br s), 3.58–3.67 (2H, m), 3.84 (2H, s), 3.95 (2H, d, J = 9.4 Hz), 6.58 (1H, br s), 7.20 (2H, d, J = 7.9 Hz), 7.51 (1H, d, J = 7.9 Hz), 7.67–7.78 (4H, m). Analytical HPLC showed 100% purity.

5.1.27. 2-Chloro-4-{3,5-dimethyl-4-[4-(morpholin-4-ylcarbonyl)benzyl]-1*H*-pyrazol-1-yl}benzonitrile (28i)

Compound **28i** was prepared in a manner similar to that described for **28f** in 82% yield as a white solid, mp 141–142 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.19 (3H, s), 2.34 (3H, s), 3.35–3.94 (8H, m), 3.82 (2H, s), 7.18 (2H, d, J = 7.9 Hz), 7.34 (2H, d, J = 8.3 Hz), 7.52 (1H, dd, J = 8.5 and 2.1 Hz), 7.71–7.78 (2H, m). Anal. Calcd for $C_{24}H_{23}ClN_4O_2{\cdot}0.2H_2O$: C, 65.73; H, 5.38; N, 12.78. Found: C, 65.72; H, 5.36; N, 12.86.

5.1.28. 4-{[1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]oxy}-*N*-(2-hydroxy-2-methylpropyl)benzamide (29a)

Compound **29a** was prepared in a manner similar to that described for **28h** in 78% yield as a white solid, mp 194–195 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.30 (6H, s), 2.14 (3H, s), 2.19 (1H, s), 2.32 (3H, s), 3.48 (2H, d, J = 6.0 Hz), 6.47–6.54 (1H, m), 6.94–7.00 (2H, m), 7.56 (1H, dd, J = 8.6 and 2.2 Hz), 7.74–7.82 (4H, m). Anal.

Calcd for $C_{23}H_{23}ClN_4O_3$: C, 62.94; H, 5.28; N, 12.77. Found: C, 62.91; H, 5.31; N, 12.80.

5.1.29. 2-Chloro-4-{3,5-dimethyl-4-[4-(morpholin-4-ylcarbonyl)phenoxy]-1*H*-pyrazol-1-yl}benzonitrile (29b)

Compound **29b** was prepared in a manner similar to that described for **28f** in 93% yield as a white solid, mp 156–157 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.15 (3H, s), 2.31 (3H, s), 3.70 (8H, br s), 6.92–6.98 (2H, m), 7.37–7.43 (2H, m), 7.56 (1H, dd, J = 8.5 and 2.1 Hz), 7.76 (1H, d, J = 8.5 Hz), 7.79 (1H, d, J = 1.9 Hz). Anal. Calcd for $C_{23}H_{21}ClN_4O_3$: C, 63.23; H, 4.84; N, 12.82. Found: C, 63.28; H, 4.76; N, 12.78.

5.1.30. 2-Chloro-4-(3,5-dimethyl-1*H*-pyrazol-1-yl)benzonitrile (31)

To a cooled suspension of NaH (60% in mineral oil, 6.24 g, 156 mmol) in DMF (100 mL) at 5 °C was added 3,5-dimethylpyrazole **30** (15.0 g, 156 mmol) dropwise. After stirring at 5 °C for 30 min, 2-chloro-4-fluorobenzonitrile (24.3 g, 156 mmol) was added dropwise, and the whole was stirred at 0 °C for 1 h. The mixture was diluted with water. The resulting precipitates were collected by filtration, washed with water and hexane, and dried in air. The resulting solid was purified by silica gel column chromatography (NH silica gel, hexane–EtOAc). The product was recrystallized from hexane–EtOAc to afford **31** (21.0 g, 58%) as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.29 (3H, s), 2.42 (3H, s), 6.07 (1H, s), 7.51 (1H, dd, J = 8.5 and 2.1 Hz), 7.70–7.76 (2H, m).

5.1.31. 4-(4-Bromo-3,5-dimethyl-1*H*-pyrazol-1-yl)-2-chlorobenzonitrile (32)

To a solution of **31** (7.30 g, 31.5 mmol) in AcOH (50 mL) was added bromine (5.54 g, 34.7 mmol) dropwise. After stirring at room temperature for 30 min, the mixture was diluted with water. The resulting precipitates were collected by filtration, washed with water, and dried in air. Recrystallization from EtOAc–hexane gave **32** (5.60 g, 57%) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.30 (3H, s), 2.42 (3H, s), 7.49 (1H, dd, J = 8.5 and 2.0 Hz), 7.71 (1H, d, J = 2.0 Hz), 7.76 (1H, d, J = 8.5 Hz).

5.1.32. 2-Chloro-4-[4-(4-fluorophenyl)-3,5-dimethyl-1*H*-pyrazol-1-yl]benzonitrile (33)

A mixture of polystyrene-bound triphenylphosphine palladium (0) (PS-PPh_3-Pd, 0.1 mmol/g, 0.025 g, 0.0025 mmol), **32** (0.100 M in 1,1-dimethoxyethane, 2.0 mL, 0.200 mmol), 4-fluorophenylboronic acid (0.111 M in 1,1-dimethoxyethane, 2.0 mL, 0.222 mmol), and 0.5 M aqueous solution of Na_2CO_3 (0.60 mL, 0.300 mmol) was heated at 150 °C for 4 min and at 170 °C for 6 min under microwave irradiation. The resin reagent was removed by filtration and the filtrate was concentrated in vacuo. The residue was purified by preparative HPLC to give **33** (0.013 g, 20%) as a solid, mp 114–115 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.23 (3H, s), 2.38 (3H, s), 7.28–7.42 (4H, m), 7.79 (1H, dd, J = 8.7 and 2.1 Hz), 7.99 (1H, d, J = 1.9 Hz), 8.13 (1H, d, J = 8.5 Hz). Analytical HPLC showed 100% purity.

5.1.33. 2-Chloro-4-(4-iodo-3,5-dimethyl-1H-pyrazol-1-yl)benzonitrile (34)

A mixture of **31** (5.00 g, 21.6 mmol), N-iodosuccinimide (4.86 g, 710 21.6 mmol), and acetonitrile (100 mL) was stirred at room temperature for 6 days. The mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography (hexane–EtOAc) to give a white solid. This solid was washed with diisopropyl ether to give **34** as a white solid (5.05 g, 65%). The diisopropyl ether extract was concentrated and recrystallized from hexane–EtOAc to give **34** (0.95 g, 12%) as a white solid. 1H NMR (300 MHz, $CDCl_3$) d: 2.31 (3H, s), 2.45 (3H, s), 7.48 (1H, dd, J = 8.5 and 2.1 Hz), 7.70 (1H, d, J = 1.9 Hz), 7.76 (1H, d, J = 8.3 Hz).

5.1.34. 2-Chloro-4-{4-[(*E*)-2-(4-fluorophenyl)ethenyl]-3,5-dimethyl-1*H*-pyrazol-1-yl}benzonitrile (35)

A mixture of **34** (0.100 g, 0.280 mmol), 4-fluorostyrene (0.05 mL, 0.42 mmol), $Pd(OAc)_2$ (0.0031 g, 0.014 mmol), $NaHCO_3$ (0.059 g, 0.700 mmol), Bu_4NCl (0.078 g, 0.280 mmol), and DMF (1.0 mL) was heated at 120 °C for 15 min and 130 °C for 15 min under microwave irradiation. The mixture was cooled to room temperature and extracted twice with EtOAc. The organic layers were combined, washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) followed by preparative HPLC separation to afford **35** (0.066 g, 67%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.45 (3H, s), 2.50 (3H, s), 6.74 (1H, d, J = 16.2 Hz), 6.84 (1H, d, J = 16.2 Hz), 7.06 (2H, t, J = 8.7 Hz), 7.40–7.55 (3H, m), 7.72–7.79 (2H, m).

5.1.35. 2-Chloro-4-{4-[2-(4-fluorophenyl)ethyl]-3,5-dimethyl-1*H*-pyrazol-1-yl}benzonitrile (36)

A mixture of **35** (0.291 g, 0.827 mmol), 5% Pd-C (0.352 g, 0.165 mmol), and EtOAc (7.0 mL) was stirred at room temperature under H_2 atmosphere. After 3 h, Pd-C was removed by filtration and the filtrate was concentrated in vacuo. The residue was purified by preparative HPLC to give **36** (0.101 g, 34%) as a white solid, mp 96–97 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.06 (3H, s), 2.17 (3H, s), 2.61–2.81 (4H, m), 6.91–7.00 (2H, m), 7.00–7.09 (2H, m), 7.43 (1H, dd, J = 8.5 and 2.1 Hz), 7.65–7.74 (2H, m). Anal. Calcd for $C_{20}H_{17}ClFN_3$: C, 67.89; H, 4.84; N, 11.88. Found: C, 67.73; H, 4.80; N, 11.74.

5.1.36. 2-Chloro-4-(4-formyl-3,5-dimethyl-1*H*-pyrazol-1-yl)-benzonitrile (37)

The Vilsmeier reagent was prepared by adding phosphoryl chloride (14.4 mL, 155 mmol) dropwise to ice cold DMF (65 mL) under stirring. The mixture was then stirred for 15 min at 0 °C. To the Vilsmeier reagent was added a solution of **31** (15.0 g, 64.7 mmol) in DMF (90.0 mL). The mixture was heated to 80 °C and stirred for 21 h. The reaction was quenched with water at 0 °C. Insoluble solid was collected by filtration, washed with MeOH and diisopropyl ether, and dried to give **37** as a yellow solid (4.58 g, 27%). The filtrate was extracted twice with EtOAc. The organic layers were combined, washed with saturated aqueous solution of $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and evaporated to give a yellow solid. This solid was diluted with EtOAc and the insoluble solid was separated. This solid was dried to afford **37** (2.64 g, 16%) as a yellow solid. The filtrate was purified by silica gel column chromatography (hexane–EtOAc) to give **37** (2.15 g, 13%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.53 (3H, s), 2.67 (3H, s), 7.51 (1H, d, J = 8.3 Hz), 7.73 (1H, s), 7.81 (1H, d, J = 8.5 Hz), 10.05 (1H, s).

5.1.37. (±)-2-Chloro-4-{4-[(4-fluorophenyl)(hydroxy)methyl]-3,5-dimethyl-1*H*-pyrazol-1-yl}benzonitrile (38)

To an ice-cooled suspension of **37** (0.100 g, 0.385 mmol) in THF (2.0 mL) was added a THF solution of 4-fluorophenylmagnesium bromide (1.0 M, 0.77 mL, 0.77 mmol). After stirring at 0 °C for 2 h, the reaction was quenched with saturated aqueous solution of NH_4Cl, and the mixture was extracted twice with EtOAc. The organic layers were combined, 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 afford **38** (0.109 g, 80%) as a white solid, mp 145–146 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.03 (1H, d, J = 3.6 Hz), 2.18 (3H, s), 2.34 (3H, s), 5.92 (1H, d, J = 2.6 Hz), 7.05 (2H, dd, J = 8.6 and 4.6 Hz), 7.36

(2H, dd, J = 8.2 and 5.4 Hz), 7.48 (1H, dd, J = 8.5 and 2.1 Hz), 7.70 (1H, d, J = 2.1 Hz), 7.74 (1H, d, J = 8.3 Hz). Anal. Calcd for $C_{19}H_{15}ClFN_3O$: C, 64.14; H, 4.25; N, 11.81. Found: C, 64.24; H, 4.29; N, 11.85.

5.1.38. 2-Chloro-4-(4-hydroxy-3,5-dimethyl-1*H*-pyrazol-1-yl)-benzonitrile (39)

To a solution of **37** (2.00 g, 7.70 mmol) in MeCN (42 mL) and EtOAc (26 mL) was added *m*-chloroperbenzoic acid (3.99 g, 23.1 mmol) at room temperature. After stirring at room temperature for 16 h, the mixture was diluted with EtOAc, washed with aqueous solution of $Na_2S_2O_3$, saturated aqueous solution of $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was dissolved in methanol (30 mL), and triethylamine (3.22 mL, 23.1 mmol) was added. The mixture was stirred at room temperature for 1 h and concentrated in vacuo to give a yellow solid. The solid was diluted with toluene/acetone and insoluble solid was collected by filtration to afford **39** (1.10 g, 58%) as a yellow solid. The filtrate was purified by silica gel column chromatography (hexane–EtOAc) to give **39** (0.21 g, 11%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.14 (3H, s), 2.34 (3H, s), 7.69 (1H, d, J = 8.5 Hz), 7.87 (1H, s), 8.02 (1H, d, J = 8.7 Hz), 8.35 (1H, br s).

5.1.39. 2-Chloro-4-{3,5-dimethyl-4-[(5-nitropyridin-2-yl)oxy]-1*H*-pyrazol-1-yl}benzonitrile (40)

To a solution of **39** (0.356 g, 1.44 mmol) in DMF (18 mL) were added 2-bromo-5-nitropyridine (0.438 g, 2.16 mmol) and Cs_2CO_3 (0.703 g, 2.16 mmol). After stirring at 100 °C for 3 h and cooled to room temperature, the mixture was diluted with EtOAc, acidified with saturated aqueous solution of NH_4Cl, and extracted twice with EtOAc. The organic layers were combined, washed with saturated aqueous solution of NH_4Cl and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc). The product was recrystallized from heptane–EtOAc to afford **40** (0.295 g, 56%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.16 (3H, s), 2.32 (3H, s), 7.15 (1H, d, J = 9.1 Hz), 7.57 (1H, dd, J = 8.5 and 2.3 Hz), 7.76 (1H, d, J = 8.5 Hz), 7.80 (1H, d, J = 2.1 Hz), 8.53 (1H, dd, J = 9.1 and 2.6 Hz), 9.05 (1H, d, J = 2.3 Hz).

5.1.40. 2-Chloro-4-{3,5-dimethyl-4-[(6-nitropyridin-3-yl)oxy]-1*H*-pyrazol-1-yl}benzonitrile (41)

Compound **41** was prepared in a manner similar to that described for **40** in 80% yield as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.17 (3H, s), 2.34 (3H, s), 7.43 (1H, dd, J = 9.0 and 2.9 Hz), 7.56 (1H, dd, J = 8.5 and 2.1 Hz), 7.77–7.82 (2H, m), 8.28 (1H, dd, J = 8.9 and 0.4 Hz), 8.37 (1H, dd, J = 2.9 and 0.5 Hz).

5.1.41. 4-{4-[(5-Aminopyridin-2-yl)oxy]-3,5-dimethyl-1*H*-pyrazol-1-yl}-2-chlorobenzonitrile (42)

To a solution of **40** (0.050 g, 0.135 mmol) in acetic acid (0.5 mL) and THF (1 mL) were added zinc powder (0.089 g, 1.35 mmol) and hydrochloric acid (0.023 mL). After stirring at room temperature for 4 h, the mixture was diluted with EtOAc, basified with 25% NH_4OH, and extracted twice with EtOAc. The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was suspended in acetone/toluene and the insoluble solid was filtered off. The filtrate was purified by silica gel column chromatography (hexane–EtOAc) to afford **42** (0.033 g, 72%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.13 (3H, s), 2.33 (3H, s), 3.49 (2H, br s), 6.79 (1H, dd, J = 8.7 and 0.6 Hz), 7.11 (1H, dd, J = 8.7 and 3.0 Hz), 7.56 (1H, dd, J = 8.5 and 2.1 Hz), 7.64 (1H, dd, J = 2.9 and 0.5 Hz), 7.72 (1H, d, J = 8.5 Hz), 7.79 (1H, d, J = 2.1 Hz).

5.1.42. 4-{4-[(6-Aminopyridin-3-yl)oxy]-3,5-dimethyl-1*H*-pyrazol-1-yl}-2-chlorobenzonitrile (43)

Compound **43** was prepared in a manner similar to that described for **42** in 83% yield as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.15 (3H, s), 2.33 (3H, s), 4.40 (2 H, br s), 6.50 (1H, dd, J = 9.0 and 0.7 Hz), 7.13 (1H, dd, J = 9.0 and 2.9 Hz), 7.54 (1H, dd, J = 8.5 and 2.1 Hz), 7.72–7.79 (3H, m).

5.1.43. *N*-(6-{[1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]oxy}pyridin-3-yl)acetamide (44a)

To a solution of **42** (0.087 g, 0.256 mmol) in pyridine (1 mL) was added acetic anhydride (0.036 mL, 0.384 mmol) at 0 °C. The mixture was stirred at 0 °C for 0.5 h and at room temperature for 17 h. The mixture was diluted with EtOAc, neutralized with 1 N HCl, and extracted twice with EtOAc. The organic layers were combined, washed with 1 N HCl, saturated aqueous solution of $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (NH silica gel, hexane–EtOAc). The product was recrystallized from heptane–EtOAc to afford **44a** (0.068 g, 70%) as a yellow solid, mp 195–196 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.14 (3H, s), 2.20 (3H, s), 2.32 (3H, s), 6.97 (1H, dd, J = 8.0 and 1.2 Hz), 7.20 (1H, br s), 7.57 (1H, dd, J = 8.5 and 2.1 Hz), 7.74 (1H, d, J = 8.5 Hz), 7.80 (1H, d, J = 1.9 Hz), 8.06–8.16 (2H, m). Anal. Calcd for $C_{19}H_{16}ClN_5O_2$: C, 59.77; H, 4.22; N, 18.34. Found: C, 59.77; H, 4.21; N, 18.19.

5.1.44. *N*-(6-{[1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]oxy}pyridin-3-yl)-2,2-dimethylpropanamide (44b)

To a solution of **42** (1.39 g, 4.09 mmol) in THF (100 mL) were added 2,2-dimethylpropanoyl chloride (0.55 mL, 4.50 mmol) and triethylamine (1.25 mL, 9.00 mmol) at 0 °C. After stirring at 0 °C for 2 h, the mixture was diluted with EtOAc, neutralized with 1 N HCl, and extracted twice with EtOAc. The organic layers were combined, washed with saturated aqueous solution of NH_4Cl and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc). The product was recrystallized from heptane–EtOAc to afford **44b** (1.59 g, 92%) as a yellow solid, mp 191–192 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33 (9H, s), 2.14 (3H, s), 2.32 (3H, s), 6.95 (1H, d, J = 9.3 Hz), 7.27 (1H, br s), 7.57 (1H, dd, J = 8.5 and 2.1 Hz), 7.74 (1H, d, J = 8.7 Hz), 7.80 (1H, d, J = 2.1 Hz), 8.07–8.17 (2H, m). Anal. Calcd for $C_{22}H_{22}ClN_5O_2$: C, 62.34; H, 5.23; N, 16.52. Found: C, 62.60; H, 5.21; N, 16.63.

5.1.45. *N*-(5-{[1-(3-Chloro-4-cyanophenyl)-3,5-dimethyl-1*H*-pyrazol-4-yl]oxy}pyridin-2-yl)acetamide (45)

Compound **45** was prepared in a manner similar to that described for **44a** in 63% yield as a yellow solid, mp 186–187 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.15 (3H, s), 2.20 (3H, s), 2.33 (3H, s), 7.23 (1H, d, J = 2.8 Hz), 7.55 (1H, dd, J = 8.5 and 2.1 Hz), 7.72–7.80 (2H, m), 7.83 (1H, br s), 8.00 (1H, d, J = 3.0 Hz), 8.15 (1H, d, J = 9.1 Hz). Anal. Calcd for $C_{19}H_{16}ClN_5O_2$: C, 59.77; H, 4.22; N, 18.34. Found: C, 59.54; H, 4.23; N, 18.16.

5.2. Cassette dosing in mice

All experiments were conducted in accordance with the regulations of Animal Care and Use Committee of the Takeda Pharmaceutical Company Ltd. Test compounds were administered as a cassette dosing to mice. After oral administration, blood samples were collected. The blood samples were centrifuged to obtain the plasma fraction. The plasma samples were deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant was diluted with LC mobile phase and centrifuged again. The compound concentrations in the supernatant were measured by LC/MS/MS.

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 293 fectin 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.[42] 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)

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), 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 transfect ion 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. PSA secretion assay in LNCaP-hr cells

LNCaP-hr cells were seeded into 24-well plates at 40,000 cells/well in phenol red-free RPMI1640 containing 10% DCC-FBS, and on the next day, test compounds or bicalutamide of 1–10 μM were added. The PSA levels in the conditioned media were determined with an enzyme-immunoassay kit (Dainippon Pharmaceutical Co., Ltd) 3 days after the treatment with the compounds.

5.3.4. Antitumor effects against LNCaP-hr cell line in mouse xenograft model

Five-week-old male BALB/c athymic nude mice were purchased from Charles River Japan (Kanagawa, Japan) and maintained on a 12/12 h light/dark cycle (light on at 8 am) with constant temperature (25 °C) and given free access to food and water. One hundred microliter of LNCaP-hr cell suspension in PBS/Matrigel (BD Biosciences; 1:1) at a cell density of 5×10^7 cells/mL was inoculated into the flank region of each mouse after castration on the same day. When the average tumor volume reached to approximately 100–300 mm^3, grouping was made to give the similar average tumor volume ($n = 6$ for each group). From the next day of the grouping, **28h** at a dose of 20, 40 mg/kg, twice daily (bid), **44b** at a dose of 50 mg/kg, bid, bicalutamide at a dose of 100 mg/kg, once daily or vehicle (0.5% methylcellulose), twice daily was orally administered to the mice for 28 days. The tumor size was measured with a caliper and expressed in mm^3 using the formula $0.5 \times a \times b^2$, where *a* is the largest diameter and *b* is largest diameter perpendicular to *a*. Body weight was measured weekly. At the end of experiments, blood samples were collected to measure the serum PSA levels by ELISA (Markit M PA; Dainippon Sumitomo Pharma, Japan). Antitumor activity was 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).

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