(+)-(*2R,5S*)-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-[6-(trifluoromethyl)pyridin-3-yl]piperazine-1-carboxamide (YM580) as an Orally Potent and Peripherally Selective Nonsteroidal Androgen Receptor Antagonist[1]

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A novel series of trans-*N*-aryl-2,5-dimethylpiperazine-1-carboxamide derivatives was synthesized and their androgen receptor (AR) antagonist activities and in vivo antiandrogenic effects were evaluated. Pharmacological assays indicated that compound **33** was a potent AR antagonist, and subsequent optical resolution provided (+)−(*2R,5S*)-4-[4-cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-[6-(trifluoromethyl)pyridin-3-yl]piperazine-1-carboxamide (**33a**, YM580) which exhibited the most potent antiandrogenic activity. Unlike bicalutamide, compound **33a** decreased the weight of rat ventral prostate in a dose-dependent manner (ED_{50} = 2.2 mg/kg/day), and induced the maximum antiandrogenic effect, comparable to that of surgical castration, without significantly affecting serum testosterone levels. Compound **33a** is a promising clinical candidate for prostate cancer monotherapy.

1. Introduction

With an estimated 220 900 new cases of prostate cancer in the year 2003 in the United States and approximately 28 900 expected prostate cancer-related deaths in the same year, prostate cancer remains the most common cancer in males and the second leading cause of cancer deaths.[2−5] It is well-established that androgens, such as testosterone and 5α-dihydrotestosterone (DHT), play an essential role in stimulating hyperplasia and carcinoma of hormone-sensitive tissue such as that of the prostate.[6] Androgens act through the androgen receptor (AR), which is a member of the superfamily of hormone receptors with a DNA-binding site, two zinc finger domains, and a hormone-binding site.[7−9] AR antagonists have shown clinical benefit as chemotherapeutic agents for prostate cancer and can be divided structurally into two broad categories (Figure 1).[10,11] Steroidal AR antagonists, such as cyproterone acetate (**1**),[12] block androgen action and also have progestational and glucocorticoid activities. However, their overlapping effects with other hormonal systems cause a range of unpleasant side effects, including thrombosis, fluid retention, and loss of libido, which hinder their use as anti-prostate-cancer drugs.[13] A number of nonsteroidal AR antagonists, including flutamide (**2**),[14−16] nilutamide (**3**),[17] and bicalutamide (**4**),[13,18−21] have been reported in the literature.[22−32] Nonsteroidal AR antagonists selectively block androgen action without affecting other hormonal systems and side effects, such as loss of libido and impotence, are therefore less profound. However, they do inhibit the binding of testosterone to ARs in the central nervous system (CNS) which, in turn, interrupts the negative feedback of testosterone on gonadotropin secretion, causing an increased serum testosterone level.[33] The increase in serum testosterone impairs the antiandrogenic activity of AR antagonists and also causes side effects including gynecomastia and breast tenderness. Therefore, potent AR antagonists with fewer adverse effects are highly desirable.

We have previously reported a new series of arylpiperazine derivatives as potent nonsteroidal AR antagonists.[34,35] Among these, compound **5** showed more potent activity than bicalutamide (**4**), both in vitro and in vivo in the testosterone propionate-treated castrated rat model. However, **5** exhibited little activity in the mature intact rat model, which might be a more suitable model to reflect the clinical setting. To identify potent, orally active AR antagonists with less effect on the CNS, we therefore carried out further modification of compound **5**. In this paper, we describe the results of our studies on the synthesis and pharmacological evaluation of *trans*-2,5-dimethylpiperazine derivatives as AR antagonists and the discovery of **33a** as a nonsteroidal AR antagonist with greater oral activity and peripheral selectivity than bicalutamide (**4**).

2. Chemistry

As shown in Scheme 1, two general synthetic procedures were adopted to obtain the target urea derivatives. Our first approach involved the coupling of in situ intermediate (**9**) and the arylpiperazine (**7**). The common intermediate (**7**) was prepared by the ipso substitution of 4-fluoro-2-(trifluoromethyl)benzonitrile (**6**) with *trans*-2,5-dimethylpiperazine. Compound **9** was synthesized by the treatment of the corresponding amine (**8**) with phenyl chloroformate and used without further purification. When substituted nicotinic acid (**10**) was the starting material, the target urea derivatives were prepared by reacting the arylpiperazine (**7**) with the corresponding aryl isocyanate, which was generated by Curtius rearrangement via acyl azide (**11**). Sodium azide allowed the reactions to proceed more cleanly than diphenylphosphoryl azide (DPPA). Compound **34** was obtained by oxidation of the pyridine ring using $NaBO_3$ (Scheme 2).[36] Compound **36** was prepared by hydrolysis of the ester (**30**) followed by Curtius rearrangement using DPPA. Subsequent deprotection of the Boc group of **36** using trifluoroacetic acid (TFA) provided compound **37** (Scheme 3). As shown in Scheme

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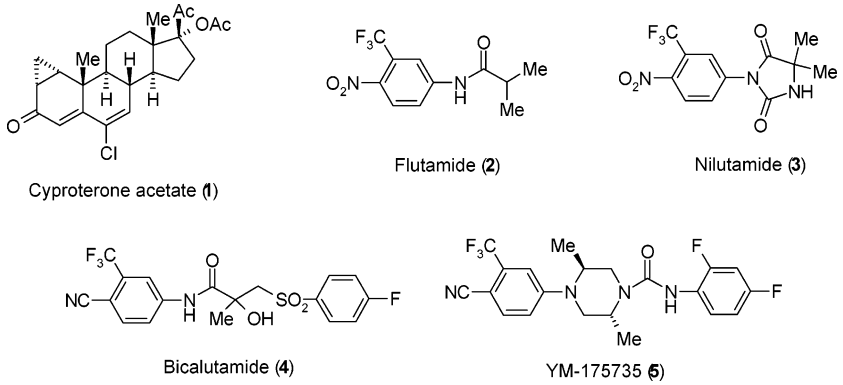
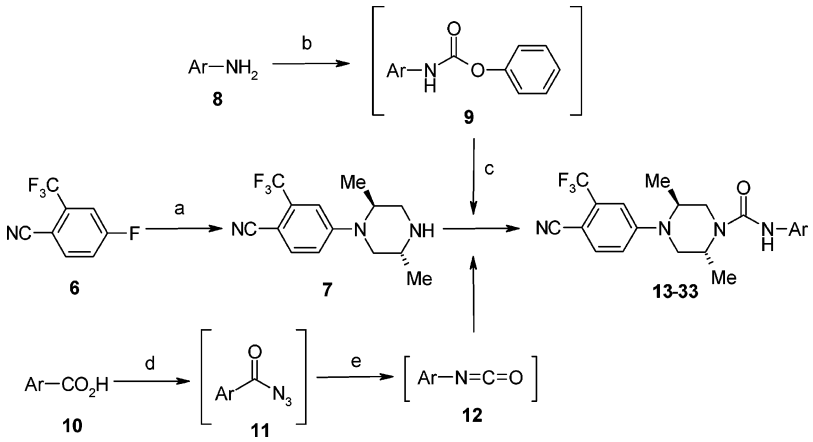


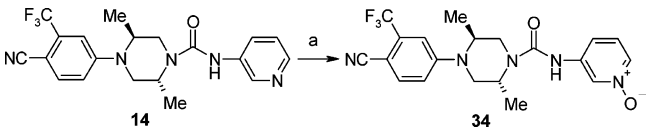
Figure 1. Structures of androgen antagonists.

Scheme 1[a]



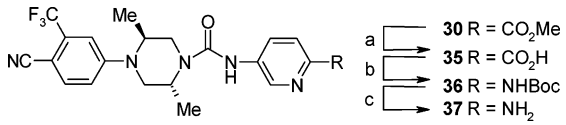
[a] Reagents and conditions: (a) *trans*-2,5-dimethylpiperazine, DMF; (b) PhOCOCl, Et_3N, DMAP, MeCN; (c) pyridine, heat; (d) (i) $(COCl)_2$, cat. DMF, CH_2Cl_2; (ii) NaN_3, Et_3N, MeCN; (e) toluene, heat.

Scheme 2[a]



[a] Reagents: (a) $NaBO_3 \cdot 4H_2O$, AcOH.

Scheme 3[a]



[a] Reagents: (a) 1 M NaOH(aq), MeOH; (b) DPPA, Et_3N, *t*-BuOH; (c) TFA, $CHCl_3$.

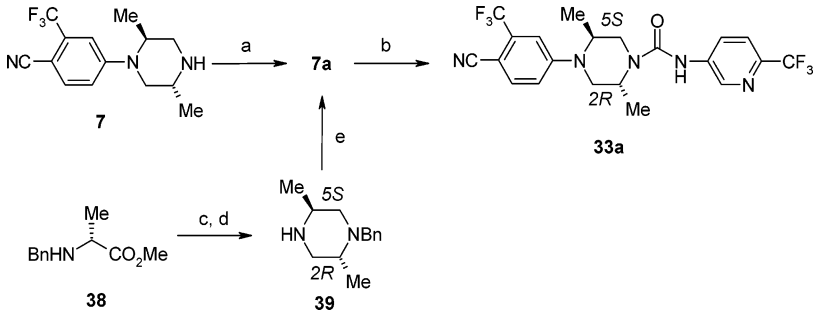
4, the enantiopure **33a** was obtained from 6-(trifluoromethyl)-nicotinic acid and an optically active arylpiperazine (**7a**) resolved by using L-dibenzoyltartaric acid (DIBETA). The absolute configuration of **7a** was determined using a (2*R*,5*S*)-1-benzyl-2,5-dimethylpiperazine prepared from optically active amino acids.[37]

3. Results and Discussion

The in vitro AR antagonist activity of the tested compounds was evaluated using the reporter assay; the resulting IC_{50} values are listed in Tables 1−3. In vivo antiandrogenic activity was tested using the mature intact rat model; the resulting reducing ratios are also listed in Tables 1−3. Although our IC_{50} value of bicalutamide (**4**) against the reporter assay was different from previous literature values,[13,27,28,32] the reason for the discrepancy was probably due to the following differences in the assay conditions: (i) cell line (CHO cells in our experiment), (ii) reporter system (the stably transfected MMTV-luciferase reporter construct in our experiment), and (iii) concentration of DHT (0.3 nM DHT, which was a submaximal concentration, in our experiment). In addition, there were some differences in in vivo experimental methods between ours and that previously reported by ICI and other groups.[13,27,38,39] We thought the reasons for the differences of in vivo activity and serum testosterone concentrations were as follows: (i) the definition of in vivo efficacy (a value of 100% for 0 g of tissues and ca. 95% for castration group in our experiment vs a value of 100% for castration group in ICI's), (ii) the strain of rats (Wistar rats in our experiment vs Sprague−Dawley rats), (iii) the age of the rats (10 weeks age in our experiment vs 15−18 weeks age), and (iv) the blood sampling time after drug treatment (6 h in our experiment vs unknown in ICI's). Furthermore, the tested doses between ICI's and ours were not identical. In view of these difference, we thought our results were not so different from those of ICI and other groups.

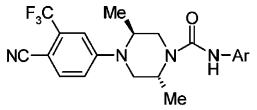
As noted in the Introduction, compound **5** exhibited little activity in the mature intact rats, even at a dose of 30 mg/kg/day. When **5** was administered to mature intact rats, serum testosterone levels were higher than in normal controls, probably due to the inhibition of ARs in the CNS (data not shown). ICI researchers explained that the peripheral selectivity of bicalutamide was due to an inability to penetrate the blood−brain barrier (BBB) as determined by distribution studies using [^{3}H]bicalutamide.[40] Despite substantial uptake of [^{3}H]bicalutamide by the anterior pituitary gland, there was no effect on LH secretion at

Scheme 4[a]



[a] Reagents and conditions: (a) (i) L-DIBETA, EtOH, H_2O; (ii) NaOH(aq); (b) (i) 6-(trifluoromethyl)nicotinic acid, $(COCl)_2$, cat. DMF, CH_2Cl_2; (ii) NaN_3, Et_3N, MeCN; (iii) toluene, heat then **7a**; (c) *N*-Boc-l-alanine, DCC, CH_2Cl_2 then TFA; (d) LAH, THF, reflux; (e) (i) **6**, pyridine, reflux; (ii) $ClCO_2CHClCH_3$, $ClCH_2CH_2Cl$ then MeOH.

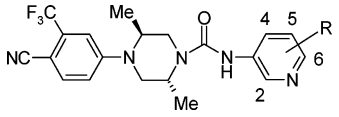
Table 1. In Vitro and in Vivo Activities of the *N*-Arylpiperazine-1-carboxamide Derivatives



Compd	Ar	IC_{50} (μM)[a]	inhibition (%)[b]
5		0.20 (0.16–0.24)	NE[c,d]
13		7.8 (5.6–10.1)	NT[e]
14		0.12 (0.11–0.13)	42 ± 8.2**
15		0.14 (0.12–0.15)	39 ± 10**
16		> 10	NT[e]
17		4.6 (3.8–5.4)	NT[e]
18		1.0 (0.88–1.1)	NT[e]
4		0.89 (0.75–1.0)	45 ± 4.5**

[a] Compounds were tested for their ability to inhibit AR-mediated transcriptional activation using a reporter assay. IC_{50} values were determined by a single experiment run in triplicate (95% confidence intervals). [b] The mean ± SE percent changes from the respective control value of ventral prostate weight after oral administration in 10-week-old male Wistar rats (10 mg/kg/day for 15 days, $n = 5$ or 6). **$p < 0.01$ versus control using Dunnett's multiple comparison test. [c] 30 mg/kg/day. [d] No effect. [e] Not tested.

Table 2. In Vitro and in Vivo Activities of Substituted Pyridin-3-yl Derivatives



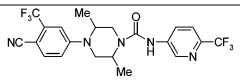
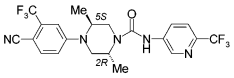
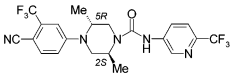
compd	R	IC_{50} (μM)[a]	inhibition (%)[b]
14	H	0.12 (0.11−0.13)	42 ± 2.2**
34	*N*-oxide	>10	NT[d]
19	2-Me	0.68 (0.63−0.73)	NT
20	4-Me	0.33 (0.25−0.41)	11 ± 4.7
21	5-Me	0.25 (0.21−0.28)	11 ± 14
22	6-Me	0.13 (0.11−0.15)	58 ± 3.5**
23	2-OMe	0.19 (0.18−0.20)	NE[c]
24	4-OMe	1.2 (1.0−1.4)	NE
25	5-OMe	0.44 (0.33−0.55)	18 ± 7.7
26	6-OMe	0.17 (0.14−0.20)	52 ± 4.2**
27	6-SMe	0.22 (0.18−0.26)	36 ± 5.2**
28	6-OH	>10	NT
37	6-NH_2	0.37 (0.33−0.41)	39 ± 6.9**
29	6-Cl	0.73 (0.34−1.1)	52 ± 3.1**
30	6-CO_2Me	0.47 (0.42−0.52)	NE
31	6-Ac	0.50 (0.43−0.59)	43 ± 1.8**
32	6-CN	0.20 (0.14−0.26)	53 ± 3.2**
33	6-CF_3	0.21 (0.19−0.23)	64 ± 2.2**
4		0.89 (0.75−1.0)	45 ± 4.5**

[a] Compounds were tested for their ability to inhibit AR-mediated transcriptional activation using a reporter assay. IC_{50} values were determined by a single experiment run in triplicate (95% confidence intervals). [b] The mean ± SE percent changes from the respective control value of ventral prostate weight after oral administration in 10-week-old male Wistar rats (10 mg/kg/day for 15 days, $n = 5$ or 6). **$p < 0.01$ versus control using Dunnett's multiple comparison test. [c] No effect. [d] Not tested.

a dose of 25 mg/kg, suggesting that the pituitary gland was of little importance in the negative feedback effects of androgens in rats. To obtain compounds pharmacologically active in the mature intact rats, we thought that compounds with decreased ability to penetrate the BBB were necessary. It is well-known that the ability to penetrate BBB is correlated with log *D* or polar surface area (PSA).[41] It seemed that compound **5** had high lipophilicity ($PrologD_{7.4} = 4.61$; topological PSA (tPSA) = 50.4),[42] and thus, we first converted the phenyl ring of **5** to other heterocyclic rings in order to decrease the lipophilicity of the compounds (Table 1). Among the pyridine derivatives, both pyridin-3-yl (**14**) and pyridin-4-yl (**15**) derivatives showed potent AR antagonist activity; however, the pyridin-2-yl derivative (**13**) lacked activity. Furthermore, compounds **14** and **15** exhibited in vivo antiandrogenic activity equivalent to that of bicalutamide (**4**). The concentration of **14** in rat hypothalamus was less than that of **5**, suggesting that **14** exerted little negative feedback via inhibition of ARs in the CNS due to less lipophilicity ($PrologD_{7.4} = 3.12$; tPSA = 60.7). Replacement of the phenyl ring with a pyrimidine ring (**16**−**18**) led to a reduction in activity relative to **5**. Among pyrimidine derivatives, compound **18** showed comparatively potent activity, indicating that a nitrogen atom at the ortho-position is unfavorable for AR antagonist activity, and the same tendency was also observed in the pyridine derivatives. Subsequently, we performed substituent optimization on the pyridine ring of **14**.

As shown in Table 2, oxidation of the pyridine ring led to a substantial reduction in activity relative to **14**. Among the methyl derivatives (**19**−**22**), the 6-methyl derivative (**22**) exhibited equivalent AR inhibitory activity to **14** and increased in vivo antiandrogenic activity. Among the methoxy derivatives (**23**−**26**), the 6-methoxy derivative (**26**) also showed potent in vitro and in vivo activity. Therefore, we introduced various substituents at the 6-position on the pyridine ring of **14**. All the 6-position analogues (**27**−**33**), except **28**, exhibited potent AR antagonist activity. The amino derivative (**37**) showed moderate

Table 3. In Vitro and in Vivo Activities of the Enantiomers of **33**

Compd	Structure	IC_{50} (μM)[a]	inhibition (%)[b]
33 (racemate)		0.21 (0.19–0.23)	64 ± 2.2**
33a (2*R*,5*S*)		0.11 (0.092–0.12)	67 ± 4.3**
33b (2*S*,5*R*)		1.3 (1.1–1.5)	NT[c]
4		0.89 (0.75–1.0)	45 ± 4.5**

[a] Compounds were tested for their ability to inhibit AR-mediated transcriptional activation using a reporter assay. IC_{50} values were determined by a single experiment run in triplicate (95% confidence intervals). [b] The mean ± SE percent changes from the respective control value of ventral prostate weight after oral administration in 10-week-old male Wistar rats (10 mg/kg/day for 15 days, $n = 5$ or 6). **$p < 0.01$ versus control using Dunnett's multiple comparison test. [c] Not tested.

Table 4. Binding Affinities of Hormone Receptors for **33a** and **4**

	K_i (nM)[a]			
	AR (human/rat)	PR (rat)	GR (human)	ERα (human)
33a	4.6 (3.6−5.5)/6.2 (5.3−7.1)	3300 (2200−4400)	>100000	>100000
4	19 (16−21)/14 (12−16)	7200 (5600−8700)	>100000	>100000

[a] K_i values were determined by a single experiment study in quadruplicate (95% confidence intervals).

activity; however, the hydroxy derivative (**28**) showed very weak activity, possibly because it might adopt a pyridone form under the assay conditions used. In addition, 6-position analogues that included either an electron-donating group or an electron-withdrawing group also exhibited potent in vivo antiandrogenic activity. Among the pyridin-3-yl derivatives, **33** was the most potent AR antagonist and significantly reduced normal rat ventral prostate to a greater extent than bicalutamide (**4**). Because **33** is a racemate, we evaluated the pharmacological activity of the optically pure enantiomers of **33**.[43] The (2*R*,5*S*)-derivative (**33a**) exhibited greater in vitro and in vivo activity than the racemate (**33**) and the enantiomer (**33b**) (Table 3). In addition, both **33a** and **4** bound strongly to ARs ($K_i = 4.6$ and 19 nM, respectively), and **33a** exhibited good selectivity for ARs over three other steroid receptors (Table 4).

Treatment of mature intact rats with **33a** for 15 days markedly decreased the weight of the ventral prostate in a dose-dependent manner without significant elevation of serum testosterone levels (Figure 2). Compound **33a** exerted its maximum antiandrogenic effect, comparable to that of surgical castration, at a dose of 30 mg/kg/day, and its ED_{50} value was 2.2 (1.5−3.0, 95% confidence intervals) mg/kg/day. In contrast, bicalutamide (**4**) only decreased ventral prostate weight by 55% versus control group at a dose of 100 mg/kg/day, and serum testosterone levels increased in a dose-dependent manner up to this dose. Although PK data of a higher dose of **4** were not obtained at our test, **4** caused a dose-dependent increase of testosterone levels, indicating that oral absorption had not plateaued. On the preliminary study using 25% propylene glycol−25% Tween 80 in water, **4** (100 mg/kg/day) did not show comparable inhibition in the castration group, and its inhibitory activity (63% versus control group) was not so different from that using 0.5% methyl cellulose. Chandolia et al. reported that bicalutamide (**4**) significantly increased LH levels at the dose of 40 mg/kg/day.[44] Their testosterone levels were not significantly increased, but showed the upward tendency. Thus, an increase of LH levels was naturally expected to cause an increase of testosterone levels. We thought that the testosterone levels of bicalutamide showed a significant increase because of less data spread in our experiment. Furthermore, it is well-known that an increase of testosterone levels impairs the antiandrogenic activity of AR antagonists, and we thought that our in vivo data of bicalutamide provided an adequate explanation of this phenomenon. In a comparative study of tissue distribution and pharmacokinetics,

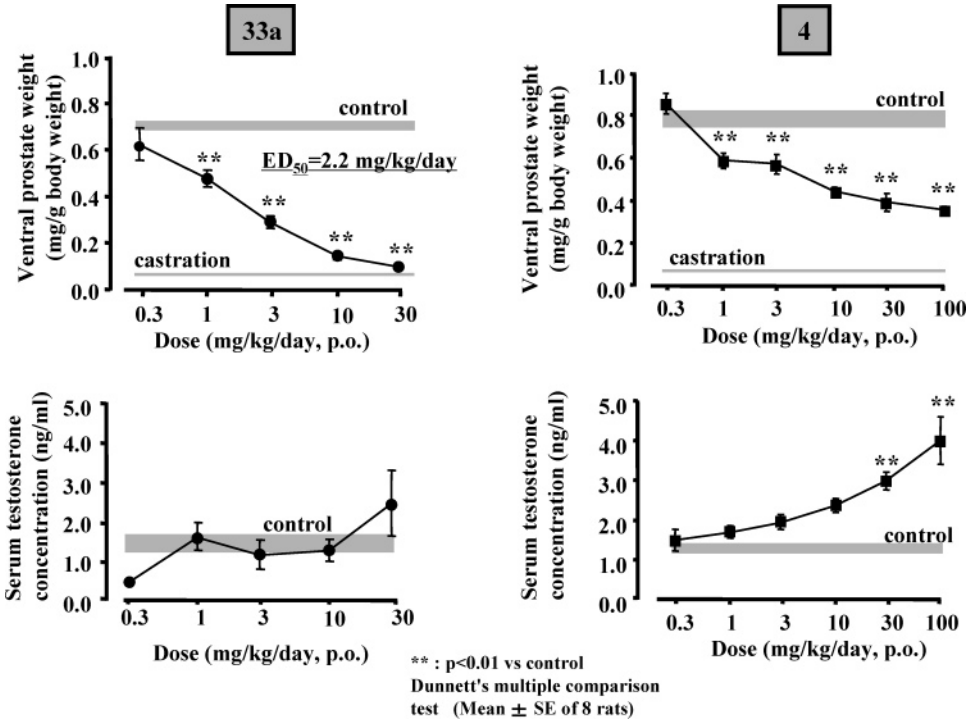


Figure 2. In vivo antiandrogenic activities of **33a** and **4**. Shaded horizontal bars show the range of ventral prostate weights or serum testosterone concentrations for vehicle controls or castration groups. Each point represents the mean ± SE of eight rats. **$p < 0.01$ versus control using Dunnett's multiple comparison test.

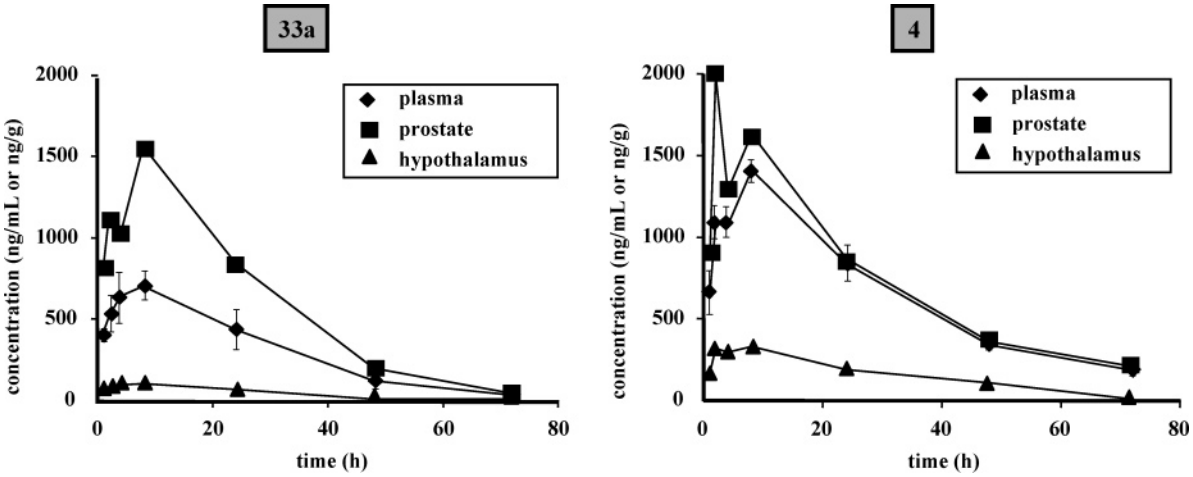


Figure 3. The concentrations of **33a** and **4** in plasma, ventral prostate, and hypothalamus after single administration of 3 mg/kg po in rats. Plasma concentration was the mean ± SD from three rats. Ventral prostate and hypothalamus concentrations were the mean of the pooled tissue of three rats.

Table 5. PK Parameters in Plasma, Ventral Prostate, and Hypothalamus after Single Administration of **33a** and **4** in Rats[a]

	33a			**4**		
	plasma	prostate	hypothalamus	plasma	prostate	hypothalamus
C_{max} (ng/mL or ng/g)	702 ± 85	1561	96	1411 ± 65	1993	321
AUC_0-72h (ng·h/mL or ng·h/g)	21952	43215	2582	46862	52523	10597
ratio to prostate	0.51	1.00	0.06	0.89	1.00	0.20
$t_{1/2}$ (h)	12.5	11.2	ND[b]	22.3	23.4	27.8
CL_{oral} (mL/h/kg)	133	68.3	ND	56.7	50.4	225

[a] Each value represents the average of three rats dosed at 3 mg/kg po. Plasma concentration was mean ± SD from three rats. Ventral prostate and hypothalamus concentrations were the mean of the pooled tissue of three rats. [b] Not determined.

compound **33a** was shown to be more peripherally selective than bicalutamide (**4**). Compound **33a** had a small ratio between prostate (target tissues for main effects) and hypothalamus (target tissues for side effects such as increasing serum testosterone concentrations), probably because of poor penetration across the blood−brain barrier (Figure 3, Table 5). As a result, **33a** has a decreased $PrologD_{7.4}$ and an increased tPSA compared with the lead compound **5** ($PrologD_{7.4}$, 4.39 vs 4.61; tPSA, 60.7 vs 50.4, respectively). The 6-position analogues, which exhibited potent in vivo activity, also had a decreased $PrologD_{7.4}$ and an increased tPSA compared with **5**; however, in vivo activity of the compounds including the other-positioned analogues were not correlated with these physicochemical features, so the details were unknown at the present time.

4. Conclusion

A novel series of trans-*N*-aryl-2,5-dimethylpiperazine-1-carboxamide derivatives was synthesized, and their AR antagonist activities and in vivo antiandrogenic effects were evaluated. Pharmacological assays indicated that compound **33** was a potent AR antagonist, and subsequent optical resolution provided (+)-(*2R*,*5S*)-4-[4-cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-[6-(trifluoromethyl)pyridin-3-yl]piperazine-1-carboxamide (**33a**, YM580), which exhibited the most potent antiandrogenic activity. Unlike bicalutamide (**4**), **33a** decreased the weight of rat ventral prostate in a dose-dependent manner (ED_{50} = 2.2 mg/kg/day) without significantly affecting serum testosterone levels, and its maximum antiandrogenic effect was comparable to that of surgical castration. On the basis of the findings presented, **33a** has been advanced into extensive preclinical studies, and may be a prime antiandrogen monotherapy candidate for patients with prostate cancer.

5. Experimental Section

5.1. Chemistry. In general, all reagents and solvents were commercial quality and were used without further purification unless otherwise noted. Melting points were determined on a Yanaco MP-500D micro-melting-point apparatus without correction. ^{1}H NMR spectra were measured with a JMN-LA300 or JMN-LA400 spectrometer; chemical shifts are expressed in δ units using tetramethylsilane as the standard (in NMR description, s = singlet, d = doublet, t = triplet, m = multiplet, and br = broad peak). MS spectra were determined with a JEOL JMS-700T or JMS-LX2000 spectrometer. Elemental analysis was performed with a Yanaco MT-5 or MT-6 microanalyzer (C, H, N) and Yokogawa IC-7000S ion chromatographic analyzer (halogens) and were within ±0.4% of theoretical values. The optical purity of the optically active compounds was examined using an analytical chiral column (Daicel Chemical Industries. Ltd. CHIRALCEL OJ-H, 4.6 mm i.d. × 250 mm). The HPLC condition were as follows: mobile phase, *n*-hexane/EtOH(99.5) = 8/1; flow rate, 0.5 mL/min; detection wavelength, 290 nm. Optical rotation measurements were obtained using a JASCO P-1020 polarimeter.

Authentic Material. Bicalutamide (**4**) was prepared at Yamanouchi Pharmaceutical Co., Ltd.

(±)-*trans*-4-(2,5-Dimethylpiperazin-1-yl)-2-(trifluoromethyl)-benzonitrile (7). To a solution of 4-fluoro-2-(trifluoromethyl)-benzonitrile (**6**, 1.0 g, 5.29 mmol) in *N*,*N*-dimethylformamide (DMF, 30 mL) was added *trans*-2,5-dimethylpiperazine (2.4 g, 21.2 mmol) at ambient temperature and the mixture stirred at 80 °C for 21 h. The reaction mixture was diluted with H_2O and extracted with AcOEt. The organic layer was washed with H_2O, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 10/1) to give the title compound (1.3 g, 87%) as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16−1.24 (6H, m), 2.67−2.77 (1H, m), 3.06−3.18 (1H, m), 3.25−3.41 (3H, m), 3.70−3.83 (1H, m), 6.96 (1H, dd, *J* = 8.7 Hz, 2.4 Hz), 7.12 (1H, d, *J* = 2.4 Hz), 7.62 (1H, d, *J* = 8.7 Hz); EI-MS *m*/*z* 283 (M^+).

General Method for the Synthesis of (±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-pyridin-2-ylpiperazine-1-carboxamide Hydrochloride (13). To a solution of 2-aminopyridine (1.0 g, 10.6 mmol) in acetonitrile (50 mL) were added phenyl chloroformate (1.47 mL, 11.7 mmol), triethylamine (1.63 mL, 11.7 mmol), and 4-(dimethylamino)pyridine (DMAP, 130 mg, 1.06 mmol) at 0 °C. After stirring at ambient temperature for 3 h, the reaction mixture was diluted with H_2O and extracted with Et_2O. The organic layer was washed with H_2O, dried over Na_2SO_4, and concentrated in vacuo. To a solution of the residue in MeCN (50 mL) was added **7** (1.5 g, 5.3 mmol) and the mixture refluxed for 2 h. The reaction mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 50/1) to give the free base of the title compound as a colorless form. The free base of **13** was dissolved in AcOEt and the solution was treated with 4 M solution of HCl in AcOEt. The precipitate was recrystallized from EtOH/AcOEt to give the title compound (916 mg, 39%) as a colorless solid: mp 195−197 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.15 (3H, d, J = 6.8 Hz), 1.26 (3H, d, J = 6.3 Hz), 3.34−3.66 (2H, m), 3.72−3.86 (1H, m), 4.05−4.50 (2H, m), 4.74 (1H, br), 7.22−7.45 (3H, m), 7.86 (1H, d, J = 8.8 Hz), 8.12−8.43 (3H, m), 11.43 (1H, br); FAB-MS m/z 404 (M + H$^+$). Anal. ($C_{20}H_{20}N_5OF_3$·HCl) C, H, N, Cl, F.

The following compounds **16**, **17**, **18**, **24**, **26**, and **31** were prepared using a procedure similar to that described for **13** from the corresponding arylamines and the arylpiperazine **7**.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-pyrimidin-2-ylpiperazine-1-carboxamide (16). The title compound was prepared from 2-aminopyrimidine and **7** in 40% yield as a colorless solid: mp 198−200 °C (AcOEt/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 (3H, d, J = 6.3 Hz), 1.19 (3H, d, J = 6.8 Hz), 3.37−3.50 (2H, m), 3.70 (1H, dd, J = 13.2 Hz, 1.9 Hz), 3.76−3.87 (1H, m), 4.25−4.52 (2H, m), 7.03 (1H, t, J = 4.9 Hz), 7.19−7.32 (2H, m), 7.84 (1H, d, J = 8.8 Hz), 8.56 (2H, d, J = 4.9 Hz), 9.58 (1H, br); FAB-MS m/z 405 (M + H$^+$). Anal. ($C_{19}H_{19}N_6OF_3$) C, H, N, F.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-pyrimidin-4-ylpiperazine-1-carboxamide (17). The title compound was prepared from 4-aminopyrimidine and **7** in 21% yield as a colorless solid: mp 151−153 °C (AcOEt/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.09 (3H, d, J = 6.3 Hz), 1.19 (3H, d, J = 6.8 Hz), 3.34−3.49 (2H, m), 3.65−3.76 (1H, m), 3.88−3.99 (1H, m), 4.29−4.40 (1H, m), 4.48−4.60 (1H, m), 7.19−7.34 (2H, m), 7.77−7.88 (2H, m), 8.52 (1H, d, J = 5.9 Hz), 8.78 (1H, br), 9.87 (1H, br); FAB-MS m/z 405 (M + H$^+$). Anal. ($C_{19}H_{19}N_6OF_3$·0.15H_2O) C, H, N, F.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-pyrimidin-5-ylpiperazine-1-carboxamide (18). The title compound was prepared from 5-aminopyrimidine and **7** in 20% yield as a colorless solid: mp 170 °C (AcOEt/*i*-Pr_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (3H, d, J = 6.4 Hz), 1.21 (3H, d, J = 6.3 Hz), 3.36−3.49 (2H, m), 3.70−3.80 (1H, m), 3.84−3.94 (1H, m), 4.33−4.44 (1H, m), 4.46−4.56 (1H, m), 7.23−7.35 (2H, m), 7.86 (1H, d, J = 8.7 Hz), 8.79 (1H, s), 8.92 (2H, s), 8.95 (1H, br); FAB-MS m/z 405 (M + H$^+$). Anal. ($C_{19}H_{19}N_6OF_3$·0.2H_2O) C, H, N, F.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(4-methoxypyridin-3-yl)-2,5-dimethylpiperazine-1-carboxamide Hydrochloride (24). The title compound was prepared from 3-amino-4-methoxypyridine[45] and **7** in 21% yield as a colorless solid: mp 165−167 °C (EtOH/*i*-Pr_2O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (3H, d, J = 6.8 Hz), 1.21 (3H, d, J = 6.8 Hz), 3.31−3.61 (2H, m), 3.70−3.77 (1H, m), 3.82−3.90 (1H, m), 4.14 (3H, s), 4.32−4.41 (1H, m), 4.45−4.53 (1H, m), 7.22−7.32 (2H, m), 7.66 (1H, d, J = 6.3 Hz), 7.86 (1H, d, J = 8.8 Hz), 8.57−8.66 (2H, m), 8.95 (1H, br); FAB-MS m/z 434 (M + H$^+$). Anal. ($C_{21}H_{22}N_5O_2F_3$·HCl·0.25H_2O) C, H, N, Cl, F.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(6-methoxypyridin-3-yl)-2,5-dimethylpiperazine-1-carboxamide (26). The title compound was prepared from 3-amino-6-methoxypyridine and **7** in 43% yield as a colorless solid: mp 159−160 °C (AcOEt/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (3H, d, J = 6.8 Hz), 1.18 (3H, d, J = 6.3 Hz), 3.30−3.45 (2H, m), 3.68−3.77 (1H, m), 3.81 (3H, s), 3.83−3.90 (1H, m), 4.34−4.46 (2H, m), 6.75 (1H, d, J = 8.8 Hz), 7.19−7.36 (2H, m), 7.77 (1H, dd, J = 8.8 Hz, 2.9 Hz), 7.85 (1H, d, J = 8.7 Hz), 8.19 (1H, d, J = 2.9 Hz), 8.56 (1H, br); FAB-MS m/z 434 (M + H$^+$). Anal. ($C_{21}H_{22}N_5O_2F_3$) C, H, N, F.

(±)-*trans*-*N*-(6-Acetylpyridin-3-yl)-4-[4-cyano-3-(trifluoromethyl)phenyl]-2,5-dimethylpiperazine-1-carboxamide (31). The title compound was prepared from 1-(5-aminopyridin-2-yl)ethanone trifluoroacetate[46] and **7** in 40% yield as a colorless solid: mp 141−143 °C (AcOEt/*i*-Pr_2O/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (3H, d, J = 6.8 Hz), 1.21 (3H, d, J = 6.9 Hz), 2.58 (3H, s), 3.37−3.54 (2H, m), 3.69−3.82 (1H, m), 3.86−3.97 (1H, m), 4.33−4.44 (1H, m), 4.48−4.59 (1H, m), 7.22−7.34 (2H, m), 7.86 (1H, d, J = 8.8 Hz), 7.92 (1H, d, J = 8.8 Hz), 8.11 (1H, dd, J = 8.8 Hz, 2.5 Hz), 8.81 (1H, d, J = 2.5 Hz), 9.17 (1H, br); FAB-MS m/z 446 (M + H$^+$). Anal. ($C_{22}H_{22}N_5O_2F_3$) C, H, N, F.

General Method for the Synthesis of (±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-pyridin-3-ylpiperazine-1-carboxamide Hydrochloride (14). To a solution of nicotinoyl chloride hydrochloride (1.5 g, 8.43 mmol) in MeCN (30 mL) were added sodium azide (932 mg, 14.3 mmol) and triethylamine (2.47 mL, 17.7 mmol). The mixture was stirred at 5 °C for 40 min. After addition of ice−water (100 mL), the mixture was extracted with Et_2O, and the combined organic layer was washed with brine, dried, and concentrated to obtain nicotinoyl azide (823 mg, 66%) as a yellow crystal. A solution of nicotinoyl azide (823 mg, 5.56 mmol) in toluene (20 mL) was heated at 100 °C for 1.5 h. After cooling, to the reaction mixture was added a solution of *trans*-4-(2,5-dimethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (1.1 g, 3.89 mmol) in toluene (5 mL). The mixture was stirred at ambient temperature for 10 min. After concentration of the solvent, the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 20/1). The resulting solid was dissolved in AcOEt and the solution was treated with a 4 M solution of HCl in AcOEt. The precipitate was further purified by recrystallization from EtOH to give the title compound (1276 mg, 34%) as a colorless crystalline solid: mp 208−212 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.13 (3H, d, J = 6.3 Hz), 1.23 (3H, d, J = 6.8 Hz), 3.33−3.59 (2H, m), 3.70−3.78 (1H, m), 4.02−4.17 (1H, m), 4.34−4.45 (1H, m), 4.55−4.72 (1H, m), 7.23−7.36 (2H, m), 7.86 (1H, d, J = 9.3 Hz), 7.91−7.99 (1H, m), 8.51 (1H, d, J = 5.4 Hz), 8.68−8.78 (1H, m), 9.22 (1H, d, J = 2.4 Hz), 10.05 (1H, br); FAB-MS m/z 404 (M + H$^+$). Anal. ($C_{20}H_{20}N_5OF_3$·HCl) C, H, N, Cl, F.

The following compounds **15**, **19**−**23**, **25**, **27**−**30**, **32**, and **33** were prepared using a procedure similar to that described for **14** from the corresponding nicotinic acid and the arylpiperazine **7**.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-pyridin-4-ylpiperazine-1-carboxamide Hydrochloride (15). The title compound was prepared from isonicotinic acid and **7** in 76% yield as a colorless solid: mp 169−171 °C (EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (3H, d, J = 6.6 Hz), 1.23 (3H, d, J = 6.5 Hz), 3.10−3.65 (2H, m), 3.70−3.84 (1H, m), 3.96−4.20 (1H, m), 4.30−4.49 (1H, m), 4.65 (1H, br), 7.20−7.37 (2H, m), 7.86 (1H, d, J = 8.8 Hz), 8.14 (2H, d, J = 7.3 Hz), 8.59 (2H, d, J = 7.3 Hz), 10.74 (1H, br), 14.82 (1H, br); FAB-MS m/z 404 (M + H$^+$). Anal. ($C_{20}H_{20}N_5OF_3$·HCl·2H_2O) C, H, N, Cl, F.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-(2-methylpyridin-3-yl)piperazine-1-carboxamide (19). The title compound was prepared from 2-methylnicotinic acid and **7** in 53% yield as a colorless solid: mp 236−238 °C (EtOH/AcOEt); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.14 (3H, d, J = 6.9 Hz), 1.21 (3H, d, J = 6.9 Hz), 2.38 (3H, s), 3.36−3.50 (2H, m), 3.66−3.79 (1H, m), 3.81−3.92 (1H, m), 4.29−4.54 (2H, m), 7.14−7.35 (3H, m), 7.50−7.59 (1H, m), 7.85 (1H, d, J = 8.8 Hz), 8.24 (1H, dd, J = 4.4 Hz, 1.4 Hz), 8.31 (1H, br); FAB-MS m/z 418 (M + H$^+$). Anal. ($C_{21}H_{22}N_5OF_3$) C, H, N, F.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-(4-methylpyridin-3-yl)piperazine-1-carboxamide (20). The title compound was prepared from 4-methylnicotinic acid hydro-

chloride and **7** in 26% yield as a colorless solid: mp 225 °C (AcOEt); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.14 (3H, d, J = 6.9 Hz), 1.21 (3H, d, J = 6.9 Hz), 3.36−3.50 (2H, m), 3.73 (1H, dd, J = 13.2 Hz, 2.0 Hz), 3.81−3.91 (1H, m), 4.30−4.54 (2H, m), 7.20−7.34 (3H, m), 7.85 (1H, d, J = 8.8 Hz), 8.22 (1H, d, J = 4.9 Hz), 8.30 (1H, s), 8.38 (1H, br); FAB-MS m/z 418 (M + H$^+$). Anal. ($C_{21}H_{22}N_5OF_3$) C, H, N, F.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-(5-methylpyridin-3-yl)piperazine-1-carboxamide hydrochloride (21). The title compound was prepared from 5-methylnicotinic acid hydrochloride and **7** in 65% yield as a colorless solid: mp 230−233 °C (EtOH/1,4-dioxane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (3H, d, J = 6.3 Hz), 1.22 (3H, d, J = 6.3 Hz), 2.45 (3H, s), 3.40 (1H, dd, J = 13.2 Hz, 3.9 Hz), 3.43−3.53 (1H, m), 3.70−3.82 (1H, m), 3.98−4.10 (1H, m), 4.33−4.45 (1H, m), 4.53−4.67 (1H, m), 7.24−7.34 (2H, m), 7.86 (1H, d, J = 9.2 Hz), 8.40 (1H, br), 8.53 (1H, br), 9.03 (1H, d, J = 1.9 Hz), 9.86 (1H, br); FAB-MS m/z 418 (M + H$^+$). Anal. ($C_{21}H_{22}N_5OF_3$·HCl) C, H, N, Cl, F.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-(6-methylpyridin-3-yl)piperazine-1-carboxamide (22). The title compound was prepared from 6-methylnicotinic acid and **7** in 21% yield as a colorless solid: mp 233−234 °C (AcOEt); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 (3H, d, J = 6.3 Hz), 1.18 (3H, d, J = 6.9 Hz), 2.39 (3H, s), 3.34−3.46 (2H, m), 3.68−3.78 (1H, m), 3.83−3.93 (1H, m), 4.31−4.42 (1H, m), 4.44−4.55 (1H, m), 7.13 (1H, d, J = 8.3 Hz), 7.23−7.33 (2H, m), 7.78 (1H, dd, J = 8.3 Hz, 2.5 Hz), 7.85 (1H, d, J = 8.8 Hz), 8.51 (1H, d, J = 2.5 Hz), 8.65 (1H, br); FAB-MS m/z 418 (M + H$^+$). Anal. ($C_{21}H_{22}N_5OF_3$) C, H, N, F.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(2-methoxypyridin-3-yl)-2,5-dimethylpiperazine-1-carboxamide (23). The title compound was prepared from 2-methoxynicotinic acid and **7** in 52% yield as a colorless solid: mp 174−176 °C (AcOEt); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (3H, d, J = 6.9 Hz), 1.19 (3H, d, J = 6.4 Hz), 3.38−3.49 (2H, m), 3.68−3.75 (1H, m), 3.79−3.85 (1H, m), 3.91 (3H, s), 4.28−4.49 (2H, m), 6.92−6.98 (1H, m), 7.25 (1H, dd, J = 8.8 Hz, 2.4 Hz), 7.29 (1H, d, J = 2.4 Hz), 7.81−7.95 (4H, m); FAB-MS m/z 434 (M + H$^+$). Anal. ($C_{21}H_{22}N_5O_2F_3$) C, H, N, F.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(5-methoxypyridin-3-yl)-2,5-dimethylpiperazine-1-carboxamide (25). The title compound was prepared from 5-methoxynicotinic acid hydrochloride[47] and **7** in 70% yield as a colorless solid: mp 198−201 °C (AcOEt/Et$_2$O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (3H, d, J = 6.4 Hz), 1.20 (3H, d, J = 6.8 Hz), 3.35−3.50 (2H, m), 3.75 (1H, dd, J = 13.2 Hz, 1.5 Hz), 3.80 (3H, s), 3.86−3.94 (1H, m), 4.31−4.43 (1H, m), 4.41−4.57 (1H, m), 7.28 (1H, dd, J = 8.8 Hz, 2.4 Hz), 7.31 (1H, d, J = 2.4 Hz), 7.59−7.64 (1H, m), 7.86 (1H, d, J = 8.8 Hz), 7.91 (1H, d, J = 2.9 Hz), 8.32 (1H, d, J = 2.0 Hz), 8.77 (1H, br); FAB-MS m/z 434 (M + H$^+$). Anal. ($C_{21}H_{22}N_5O_2F_3$) C, H, N, F.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-[6-(methylthio)pyridin-3-yl]piperazine-1-carboxamide (27). The title compound was prepared from 6-(methylsulfanyl)nicotinic acid and **7** in 63% yield as a colorless solid: mp 175−177 °C (AcOEt/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (3H, d, J = 6.3 Hz), 1.19 (3H, d, J = 6.8 Hz), 2.48 (3H, s), 3.35−3.47 (2H, m), 3.67−3.79 (1H, m), 3.81−3.94 (1H, m), 4.27−4.56 (2H, m), 7.21 (1H, d, J = 8.8 Hz), 7.25−7.33 (2H, m), 7.79 (1H, dd, J = 8.8 Hz, 2.4 Hz), 7.85 (1H, d, J = 8.8 Hz), 8.55 (1H, d, J = 2.4 Hz), 8.70 (1H, br); FAB-MS m/z 450 (M + H$^+$). Anal. ($C_{21}H_{22}N_5OF_3S$) C, H, N, F, S.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-*N*-(6-hydroxypyridin-3-yl)-2,5-dimethylpiperazine-1-carboxamide (28). The title compound was prepared from 6-hydroxynicotinic acid and **7** in 5% yield as a gray solid: mp 160−164 °C (AcOEt/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.09 (3H, d, J = 6.4 Hz), 1.15 (3H, d, J = 6.9 Hz), 3.27−3.40 (2H, m), 3.64−3.85 (2H, m), 4.28−4.47 (2H, m), 6.26−6.33 (1H, m) 7.21−7.32 (2H, m), 7.46 (1H, d, J = 2.9 Hz), 7.48 (1H, s), 8.19 (1H, d, J = 8.8 Hz), 8.24 (1H, br), 11.28 (1H, br); FAB-MS m/z 420 (M + H$^+$). Anal. ($C_{20}H_{20}N_5O_2F_3$·0.5H$_2$O) C, H, N, F.

(±)-trans-*N*-(6-Chloropyridin-3-yl)-4-[4-cyano-3-(trifluoromethyl)phenyl]-2,5-dimethylpiperazine-1-carboxamide (29). The title compound was prepared from 6-chloronicotinic acid and **7** in 44% yield as a colorless solid: mp 197−199 °C (AcOEt); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 (3H, d, J = 6.3 Hz), 1.20 (3H, d, J = 6.8 Hz), 3.35−3.49 (2H, m), 3.68−3.79 (1H, m), 3.83−3.94 (1H, m), 4.30−4.42 (1H, m), 4.44−4.56 (1H, m), 7.23−7.33 (2H, m), 7.40 (1H, d, J = 8.8 Hz), 7.86 (1H, d, J = 8.8 Hz), 7.99 (1H, dd, J = 8.8 Hz, 3.0 Hz), 8.53 (1H, d, J = 3.0 Hz), 8.90 (1H, br); FAB-MS m/z 438 (M + H$^+$). Anal. ($C_{20}H_{19}N_5OClF_3$) C, H, N, Cl, F.

(±)-Methyl 5-[({*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethylpiperazin-1-yl}carbonyl)amino]pyridine-2-carboxylate (30). The title compound was prepared from 6-(methoxycarbonyl)nicotinic acid[48] and **7** in 39% yield as a colorless solid: mp 259−260 °C (MeOH/AcOEt/*i*-Pr$_2$O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (3H, d, J = 6.4 Hz), 1.21 (3H, d, J = 6.3 Hz), 3.36−3.52 (2H, m), 3.71−3.79 (1H, m), 3.85 (3H, s), 3.89−3.96 (1H, m), 4.32−4.43 (1H, m), 4.47−4.59 (1H, m), 7.23−7.34 (2H, m), 7.86 (1H, d, J = 8.8 Hz), 8.00 (1H, d, J = 8.8 Hz), 8.13 (1H, dd, J = 8.8 Hz, 2.5 Hz), 8.81 (1H, d, J = 2.5 Hz), 9.15 (1H, br); FAB-MS m/z 462 (M + H$^+$). Anal. ($C_{22}H_{22}N_5O_3F_3$) C, H, N, F.

(±)-*trans*-*N*-(6-Cyanopyridin-3-yl)-4-[4-cyano-3-(trifluoromethyl)phenyl]-2,5-dimethylpiperazine-1-carboxamide (32). The title compound was prepared from 6-cyanonicotinic acid and **7** in 31% yield as a beige solid: mp 182−184 °C (AcOEt/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.11 (3H, d, J = 6.8 Hz), 1.22 (3H, d, J = 6.4 Hz), 3.41 (1H, dd, J = 13.2 Hz, 3.9 Hz), 3.44−3.53 (1H, m), 3.69−3.81 (1H, m), 3.86−3.98 (1H, m), 4.31−4.44 (1H, m), 4.47−4.58 (1H, m), 7.22−7.36 (2H, m), 7.86 (1H, d, J = 8.8 Hz), 7.91 (1H, d, J = 8.7 Hz), 8.15 (1H, dd, J = 8.8 Hz, 2.4 Hz), 8.85 (1H, d, J = 2.4 Hz), 9.26 (1H, br); FAB-MS m/z 429 (M + H$^+$). Anal. ($C_{21}H_{19}N_6OF_3$) C, H, N, F.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-[6-(trifluoromethyl)pyridin-3-yl]piperazine-1-carboxamide (33). The title compound was prepared from 6-(trifluoromethyl)-nicotinic acid and **7** in 82% yield as a colorless solid: mp 148−149 °C (AcOEt/*i*-Pr$_2$O/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (3H, d, J = 6.4 Hz), 1.22 (3H, d, J = 6.4 Hz), 3.35−3.55 (2H, m), 3.68−3.83 (1H, m), 3.86−4.00 (1H, m), 4.30−4.62 (2H, m), 7.21−7.39 (2H, m), 7.80 (1H, d, J = 8.8 Hz), 7.86 (1H, d, J = 9.3 Hz), 8.20 (1H, dd, J = 8.8 Hz, 2.4 Hz), 8.86 (1H, d, J = 2.4 Hz), 9.18 (1H, br); FAB-MS m/z 472 (M + H$^+$). Anal. ($C_{21}H_{19}N_5OF_6$) C, H, N, F.

(±)-*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-(1-oxidopyridin-3-yl)piperazine-1-carboxamide (34). To a solution of *trans*-4-[4-cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-pyridin-3-ylpiperazine-1-carboxamide (605 mg, 1.5 mmol) in AcOH (3 mL) was added $NaBO_3$·4H$_2$O (507 mg, 3.3 mmol) at 40 °C.[36] After stirring at 40 °C for 3 days, the reaction mixture was cooled to ambient temperature, diluted by H$_2$O, and extracted with $CHCl_3$. The organic layer was washed with saturated, aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 7/1) and recrystallized from MeOH/AcOEt to give the title compound (202 mg, 32%) as a pale yellow powder: mp 238 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.10 (3H, d, J = 6.6 Hz), 1.19 (3H, d, J = 6.6 Hz), 3.33−3.49 (2H, m), 3.68−3.79 (1H, m), 3.82−3.92 (1H, m), 4.30−4.55 (2H, m), 7.22−7.33 (3H, m), 7.40−7.47 (1H, m), 7.81−7.88 (2H, m), 8.53−8.58 (1H, m), 8.90 (1H, br); FAB-MS m/z 420 (M + H$^+$). Anal. ($C_{20}H_{20}N_5O_2F_3$) C, H, N, F.

(±)-5-[({*trans*-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethylpiperazin-1-yl}carbonyl)amino]pyridine-2-carboxylic Acid (35). A mixture of (±)-methyl 5-[({*trans*-4-[4-cyano-3-(trifluoromethyl)phenyl]-2,5-dimethylpiperazin-1-yl}carbonyl)amino]-pyridine-2-carboxylate (**30**, 5.0 g, 9.73 mmol) and 1 M NaOH. (10.2 mL, 10.2 mmol) in MeOH (100 mL) was stirred at ambient temperature for 6 h. The reaction mixture was acidified with 1 M

HCl, and the precipitate was filtered to give the title compound (3.9 g, 90%) as a colorless solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.11 (3H, d, J = 6.8 Hz), 1.21 (3H, d, J = 6.6 Hz), 3.30−3.47 (2H, m), 3.70−3.80 (1H, m), 3.86−3.97 (1H, m), 4.30−4.45 (1H, m), 4.50−4.60 (1H, m), 7.24−7.33 (2H, m), 7.85 (1H, d, J = 9.2 Hz), 7.97 (1H, d, J = 8.6 Hz), 8.10 (1H, dd, J = 8.6 Hz, 2.4 Hz), 8.80 (1H, d, J = 2.4 Hz), 9.12 (1H, br); FAB-MS m/z 448 (M + H$^+$).

(±)-*tert*-Butyl {5-[({*trans*-4-[4-Cyano-3-(trifluoromethyl)-phenyl]-2,5-dimethylpiperazin-1-yl}carbonyl)amino]pyridin-2-yl}-carbamate (36). To a mixture of (±)-5-[({*trans*-4-[4-cyano-3-(trifluoromethyl)phenyl]-2,5-dimethylpiperazin-1-yl}carbonyl)-amino]pyridine-2-carboxylic acid (**35**, 930 mg, 2.08 mmol) and *tert*-BuOH (20 mL) were added DPPA (0.58 mL, 2.7 mmol) and triethylamine (0.38 mL, 2.7 mmol). The mixture was heated at 100 °C for 4 h. After concentration of the solvent, the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 50/1) to give the title compound (800 mg, 74%) as a pale yellow powder: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.11 (3H, d, J = 6.6 Hz), 1.18 (3H, d, J = 6.6 Hz), 1.47 (9H, s), 3.33−3.46 (2H, m), 3.68−3.80 (1H, m), 3.82−3.92 (1H, m), 4.30−4.60 (2H, m), 7.23−7.34 (2H, m), 7.68 (1H, d, J = 9.2 Hz), 7.80 (1H, dd, J = 9.0 Hz, 2.0 Hz), 7.85 (1H, d, J = 9.0 Hz), 8.33 (1H, d, J = 2.8 Hz), 8.61 (1H, br), 9.54 (1H, br); FAB-MS m/z 519 (M + H$^+$).

(±)-*trans*-*N*-(6-Aminopyridin-3-yl)-4-[4-cyano-3-(trifluoro-methyl)phenyl]-2,5-dimethylpiperazine-1-carboxamide (37). A mixture of (±)-*tert*-butyl {5-[({*trans*-4-[4-cyano-3-(trifluoromethyl)phenyl]-2,5-dimethylpiperazin-1-yl}carbonyl)amino]pyridin-2-yl}carbamate (**36**, 800 mg, 1.54 mmol), $CHCl_3$ (20 mL), and TFA (20 mL) was stirred at 0 °C for 13 h and the solution was concentrated in vacuo. The residue was diluted with saturated aqueous $NaHCO_3$ and extracted with AcOEt. The organic layer was dried and concentrated under reduced pressure, and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 50/1) to give the title compound (378 mg, 59%) as a colorless solid: mp 193−195 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.10 (3H, d, J = 6.8 Hz), 1.16 (3H, d, J = 6.3 Hz), 3.30−3.42 (2H, m), 3.64−3.76 (1H, m), 3.78−3.89 (1H, m), 4.28−4.50 (2H, m), 5.60 (2H, br), 6.39 (1H, d, J = 8.8 Hz), 7.26 (1H, dd, J = 8.8 Hz, 2.5 Hz), 7.30 (1H, d, J = 2.5 Hz), 7.41 (1H, dd, J = 8.8 Hz, 2.5 Hz), 7.84 (1H, d, J = 8.8 Hz), 7.89 (1H, d, J = 2.5 Hz), 8.24 (1H, br); FAB-MS m/z 419 (M + H$^+$). Anal. ($C_{20}H_{21}N_6OF_3$) C, H, N, F.

(+)-(2*R*,5*S*)-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-[6-(trifluoromethyl)pyridin-3-yl]piperazine-1-carboxamide (33a). Route A to Optically Active 7a. **Optical Resolution of Racemic 7.** (±)-*trans*-4-(2,5-Dimethylpiperazin-1-yl)-2-(trifluoromethyl)benzonitrile (**7**, 20 g, 70.6 mmol) was converted to the corresponding hemi-L-DIBETA salt in EtOH (400 mL) and H_2O (40 mL) followed by recrystallization from EtOH (180 mL) and H_2O (20 mL) to give the hemi-L-DIBETA salt of **7a** (6.2 g, 20%) as colorless crystals: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.07 (3H, d, J = 6.8 Hz), 1.13 (3H, d, J = 6.4 Hz), 2.83 (1H, dd, J = 13.2, 3.2 Hz), 3.14−3.38 (2H, m), 3.41−3.60 (2H, m), 4.06−4.22 (1H, m), 5.65 (1H, s), 7.11 (1H, dd, J = 9.2, 2.4 Hz), 7.17 (1H, d, J = 2.0 Hz), 7.43−7.63 (3H, m), 7.82 (1H, d, J = 8.8 Hz), 7.88−8.00 (2H, m). The hemi-L-DIBETA salt of **7a** (11.7 g, 25.3 mmol) was neutralized with aqueous NaOH solution and then extracted with toluene. The organic layer was washed with H_2O, dried over $MgSO_4$, and concentrated in vacuo to give **7a** (7.2 g, quant.) as a pale yellow oil. ^{1}H NMR and MS spectra were similar to those of **7a**. $[\alpha]^{25}_D$ = +100.6° (c = 1.018, EtOH).

Route B to Optically Active 7a. The absolute configuration of **7a** was determined using (2*R*,5*S*)-1-benzyl-2,5-dimethylpiperazine (**39**) prepared from optically active amino acids. A 0.98 g portion of *N*-Boc-L-alanine was added to 50 mL of 0 °C-cooled CH_2Cl_2 solution containing 1.07 g of *N*,*N*-dicyclohexylcarbodiimide (DCC), and the mixture was stirred for 5 min. This solution was mixed with 10 mL of CH_2Cl_2 solution containing 1.0 g of *N*-benzyl-D-alanine methyl ester[49] (**38**) and stirred at ambient temperature for 2 days. White precipitate was separated by filtration and washed with Et_2O, and then the filtrate was concentrated in vacuo. The residue was dried under reduced pressure, dissolved with 30 mL of CH_2Cl_2, cooled to 0 °C, mixed with 5 mL of TFA, and then stirred at ambient temperature for 3 h. The reaction mixture was neutralized with saturated aqueous Na_2CO_3, extracted with $CHCl_3$, dried over $MgSO_4$, filtered, and then concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/AcOEt = 1/5) to obtain 1.0 g of (3*S*,6*R*)-1-benzyl-3,6-dimethylpiperazine-2,5-dione as a colorless oil. In an atmosphere of argon, 0.57 g of lithium aluminum hydride was added at 0 °C to 30 mL of THF solution containing 1.0 g of (3*S*,6*R*)-1-benzyl-3,6-dimethylpiperazine-2,5-dione, and the mixture was stirred overnight under reflux. To the reaction mixture cooled at 0 °C was added 1.0 mL of water dropwise and 1.0 mL of 10% aqueous NaOH dropwise, and then the mixture was further mixed with 1.0 mL of water and stirred for 30 min. The precipitate was separated by filtration and washed with AcOEt, and the filtrate was washed with 10% aqueous K_2CO_3 and saturated brine, dried over $MgSO_4$, filtered, and then concentrated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/NH_4OH = 25/1/0.1) to obtain 0.75 g of (2*R*,5*S*)-1-benzyl-2,5-dimethylpiperazine (**39**) as a yellow oil:[37] ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (3H, d, J = 6.2 Hz), 1.14 (3H, d, J = 6.0 Hz), 1.49 (1H, br), 1.63 (1H, dd, J = 11.0, 10.3 Hz), 2.17−2.28 (1H, m), 2.60−2.70 (2H, m), 2.74−2.83 (1H, m), 2.91 (1H, dd, J = 12.1, 3.1 Hz), 3.09 (1H, d, J = 13.4 Hz), 4.10 (1H, d, J = 13.5 Hz), 7.22−7.32 (5H, m); FAB-MS m/z 205 (M + H$^+$); $[\alpha]^{25}_D$ = −133.5° (c = 1.0, $CHCl_3$). A 15 mL portion of pyridine solution containing 0.63 g of **39** was mixed with 0.7 g of 4-fluoro-2-trifluoromethylbenzonitrile (**6**) and stirred at 90 °C for 2 days. The reaction mixture was concentrated in vacuo and the residue was purified by silica gel column chromatography (*n*-hexane/AcOEt = 9/2) to obtain (2*S*,5*R*)-4-(4-benzyl-2,5-dimethylpiperazin-1-yl)-2-trifluoromethylbenzonitrile (0.32 g, 28%) as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (3H, d, J = 6.6 Hz), 1.24 (3H, d, J = 6.6 Hz), 2.33−2.42 (1H, m), 2.85−2.93 (1H, m), 3.10−3.25 (1H, m), 3.34−3.41 (1H, m), 3.44−3.53 (1H, m), 3.57 (1H, d, J = 13.5 Hz), 3.67 (1H, d, J = 13.5 Hz), 3.90−4.10 (1H, m), 6.85−6.92 (1H, m), 7.03−7.07 (1H, m), 7.25−7.41 (5H, m), 7.58 (1H, d, J = 8.8 Hz); FAB-MS m/z 374 (M + H$^+$). A 0.92 mL portion of 1-chloroethyl chloroformate was added to 20 mL of CH_2Cl_2 solution containing 0.31 g of (2*S*,5*R*)-4-(4-benzyl-2,5-dimethylpiperazin-1-yl)-2-trifluoromethylbenzonitrile, and the mixture was stirred for 2 days with heating under reflux. The reaction mixture was concentrated in vacuo, mixed with 20 mL of MeOH, and then stirred for 1 day with heating under reflux. The reaction mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 9/1) to obtain 0.19 g of (2*S*,5*R*)-4-(2,5-dimethylpiperazin-1-yl)-2-trifluoromethylbenzonitrile (**7a**) as a yellow oil. Optical rotation and ^{1}H NMR and MS spectra were similar to those of **7a** derived from route A. The title compound was prepared using a procedure similar to that described for racemic **33** from the 6-(trifluoromethyl)nicotinic acid and **7a** in 83% yield as a colorless solid (t_R = 35.1 min, > 99.9% ee): mp 180−182 °C (EtOH); $[\alpha]^{20}_D$ = +30.1° (c = 1.00, EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (3H, d, J = 6.3 Hz), 1.22 (3H, d, J = 6.4 Hz), 3.42 (1H, dd, J = 13.2, 3.9 Hz), 3.48 (1H, d, J = 11.2 Hz), 3.77 (1H, d, J = 12.7 Hz), 3.93 (1H, d, J = 13.6 Hz), 4.40 (1H, m) 4.54 (1H, m), 7.28 (1H, d, J = 8.8 Hz), 7.32 (1H, s), 7.80 (1H, d, J = 8.8 Hz), 7.86 (1H, d, J = 8.8 Hz), 8.20 (1H, dd, J = 8.8, 2.0 Hz), 8.86 (1H, d, J = 2.0 Hz), 9.19 (1H, s); FAB-MS m/z 472 (M + H$^+$). Anal. ($C_{21}H_{19}N_5OF_6$) C, H, N, F.

(−)-(2*S*,5*R*)-4-[4-Cyano-3-(trifluoromethyl)phenyl]-2,5-dimethyl-*N*-[6-(trifluoromethyl)pyridin-3-yl]piperazine-1-carboxamide (33b). The title compound was prepared using a procedure similar to described for **33a** from 6-(trifluoromethyl)nicotinic acid and **7b**, which was resolved as the hemi-D-DIBETA salt, in 49% yield as a colorless solid (t_R = 29.3 min, > 99.9% ee): mp 151−153 °C (AcOEt/*n*-hexane); $[\alpha]^{20}_D$ = −28.2° (c = 1.002, EtOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.12 (3H, d, J = 6.3 Hz), 1.22 (3H, d, J = 6.3 Hz), 3.41 (1H, dd, J = 13.6, 3.9 Hz), 3.44−3.53 (1H, m), 3.69−3.83 (1H, m), 3.87−3.98 (1H, m), 4.32−4.45 (1H,

m) 4.47−4.61 (1H, m), 7.23−7.35 (2H, m), 7.80 (1H, d, $J = 8.8$ Hz), 7.86 (1H, d, $J = 8.8$ Hz), 8.20 (1H, dd, $J = 8.8$, 2.0 Hz), 8.86 (1H, d, $J = 2.0$ Hz), 9.18 (1H, s); FAB-MS m/z 472 ($M + H^+$). Anal. ($C_{21}H_{19}N_5OF_6$) C, H, N, F.

5.2. Pharmacology. 5.2.1. Evaluation of Transcriptional Activity for Human Androgen Receptor. (a) Establishment of CHO Cells Stably Transfected with Human Androgen Receptor Gene and MMTV-Luciferase Reporter Gene or SV40-Luciferase Gene. Chinese hamster ovary (CHO) cells were maintained in α-modified Eagle's medium supplemented with 10% fetal bovine serum (FBS). The culture medium of neomycin-resistant clone cells was supplemented with 10% dextran-coated charcoal-stripped FBS (DCC−FBS) and 500 μg/mL of neomycin. The CHO cells were transfected at 40−70% confluence in 10-cm Petri dishes with a total of 20 μg of DNA (pMAMneoLUC; MMTV-luciferase reporter plasmid and pSG5-hAR; human androgen receptor expression plasmid, or SV40-LUC; SV40-luciferase reporter plasmid containing the neomycin resistant gene) by calcium phosphate mediated transfection. The stable transfected cells were selected in the culture medium supplemented with neomycin. The selected clone was designated as AR/CHO#3 (human AR gene and MMTV-luciferase reporter gene integrated CHO cell) or SV/CHO#10 (SV-40-luciferase reporter gene integrated CHO cell), respectively.

(b) Activities of the Test Compounds To Inhibit Androgen Receptor Mediated Transcription Induced by DHT (AR Antagonist Activity). The stable transfected AR/CHO#3 or SV/CHO#10 cells were plated onto 96-well luminoplates (Packard) at a density of 2×10^4 cells/well, respectively. Four to 8 h later, the medium was changed to the medium containing DMSO, 0.3 nM of DHT, or 0.3 nM of DHT and the test compound. At the end of incubation, the medium was removed and then cells were lysed with 20 μL of lysis buffer [25 mM Tris-HCl (pH 7.8), 2 mM dithiothreitol, 2 mM 1,2-cyclohexanediaminetetraacetic acid, 10% glycerol, and 1% TritonX-100]. Luciferase substrate [20 mM Tris-HCl (pH 7.8), 1.07 mM $(MgCO_3)_4Mg(OH)_2{\cdot}5H_2O$, 2.67 mM $MgSO_4{\cdot}7H_2O$, 0.1 mM EDTA, 33.3 mM dithiothreitol, 0.27 mM coenzyme A, 0.47 mM luciferin, 0.53 mM ATP] was added and luciferase activity was measured with a ML3000 luminometer (Dynatech Laboratories). AR antagonist activities were calculated using the formula below:

$$\text{AR antagonist activity (\%)} = 100(I - X)/(I - B)$$

where I is (luciferase activity of AR/CHO#3)/(luciferase activity of SV/CHO#10), in the presence of 0.3 nM of DHT; B is (luciferase activity of AR/CHO#3)/(luciferase activity of SV/CHO#10), in the presence of DMSO; X is (luciferase activity of AR/CHO#3)/(luciferase activity of SV/CHO#10), in the presence of 0.3 nM of DHT and the test compound.

The concentration of compounds showing 50% AR antagonist activity, the IC_{50} values, were obtained through nonlinear analysis using the statistical analysis system (SAS).

5.2.2. Evaluation of Antiandrogenic Activities and Serum Testosterone Concentration in Mature Intact Male Rats. In Tables 1−3, the test compounds were suspended in 0.5% methyl cellulose solution and orally administered to male Wister rats at 10 weeks of age, once a day for 15 days. After 6 h of the final administration, the rats were sacrificed by decapitation and blood samples were collected for testosterone assay. Serum was prepared from each blood sample and was frozen at −30 °C until assay. Then the wet weight of the ventral prostate gland was measured to evaluate the prostate gland reducing action of the test compound. The prostate gland reducing action of the test compound was calculated on the basis of the below formula, using a test group (in which the test compound was administered) and a control group (in which methyl cellulose alone was administered).

$$\text{reducing ratio (\%)} = 100(B - A)/B$$

where A is the wet weight of the ventral prostate gland in the test group and B is the wet weight of the ventral prostate gland in the control group. The reducing ratio was based on the control group, which meant 100% was defined as 0 g weight of tissues and ca. 95% as the castration group.

In Figure 2, compound **33a** was dissolved in 25% propylene glycol−25% Tween 80 and 50% water, and compound **4** was suspended in 0.5% methyl cellulose solution. Each control group was corresponded to the treatment of each vehicle. The evaluation of reducing ratio was similarly carried out as above. In the preliminary study using 25% propylene glycol−25% Tween 80 in water, **4** (100 mg/kg/day) did not show comparable inhibition to the castration group, and its inhibitory activity (63% versus control group) was not so different from that using 0.5% methyl cellulose (55% versus control group). The ED_{50} value of **33a** was calculated from the thus obtained reducing ratio using linear regression analysis, but it was impossible to calculate the statistically significant ED_{50} value for **4** due to its plateaued in vivo efficacy at higher dose.

Serum concentrations of testosterone were determined by radioimmunoassay using a DPC Total Testosterone Kit.

The results were analyzed using Dunnett's multiple comparison test versus the control group. Differences with $p < 0.05$ were considered statistically significant.

5.2.3. Binding Assay for Hormone Receptors. Binding assay of **33a** and bicalutamide (**4**) against hormone receptors (AR, PR, GR, and ERα) were performed in the reported methods.[50−52] hGR and hERα were purchased from PanVera Corp.

5.2.4. Pharmacokinetic Studies. Compounds **33a** and **4** were formulated in 25% propylene glycol−25% Tween 80 and 50% water, and a single oral dose of 3 mg/kg was given to rats. The animals were sacrificed at various times up to 72 h after drug administration, and blood, ventral prostate, and hypothalamus were collected. Each plasma sample was harvested from blood by centrifugation. The tissues of ventral prostate and hypothalamus of three rats were pooled and homogenized in order to ensure a measurable quantity. All samples were stored at −20 °C or below until analysis. Chromatographic separations and quantification of the drugs and the appropriate internal standards were achieved by a reverse phase HPLC method on a Develosil ODS−UG-33 μM column (75 × 3.0 mm i.d.) for **33a** or on a TSK-gel ODS-80Ts column (150 × 4.6 mm i.d.) for **4**. The HPLC system used in this study consisted of a Waters 2690 separation module with column heater (Milford, MA), coupled to a Waters 2487 dual λ UV/vis detector operated at 297 nm for **33a** or at 270 nm for **4**. The mobile phase composition was 0.05 M aqueous Na_2HPO_4/aqueous 0.05M K_2HPO_4/MeCN (1:1:2, v/v/v) at a flow rate of 0.5 mL/min for **33a** or 0.05M phosphate salt buffer (pH 7.4)/MeCN (1:1, v/v) at a flow rate of 1.0 mL/min for **4**. The HPLC analysis was performed at 35 °C for **33a** or at 40 °C for **4**, and the data acquisition and management were achieved with a Waters millennium chromatography manager. The lower limit of quantification for the analytical method was 20 ng/mL of test article in plasma, 33.3 ng/g in ventral prostate, and 100 ng/g in hypothalamus, respectively. The mean and SD of the plasma concentrations of a drug at each time point was calculated using Microsoft Excel for Windows 95, version 7.0. All pharmacokinetic parameters were calculated from noncompartmental models using WinNonlin program, version 3.1 (Scientific Consulting Inc.).

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Supporting Information Available: Elemental analysis data of **13**−**34**, **33a**, **33b**, and **37**. This material is available free of charge via the Internet at http://pubs.acs.org.

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