Bioorganic & Medicinal Chemistry 21 (2013) 70–83

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis, and biological evaluation of 3-aryl-3-hydroxy-1-phenylpyrrolidine derivatives as novel androgen receptor antagonists

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ARTICLE INFO

Article history:
Received 26 September 2012
Revised 30 October 2012
Accepted 1 November 2012
Available online 12 November 2012

Keywords:
Androgen receptor antagonists
Castration-resistant prostate cancers
3-Aryl-3-hydroxy-1-phenylpyrrolidine derivatives

ABSTRACT

We designed and synthesized a series of 3-aryl-3-hydroxy-1-phenylpyrrolidine derivatives **D** and evaluated their potential as novel androgen receptor (AR) antagonists therapeutically effective against castration-resistant prostate cancer (CRPC). Introduction of a methyl group at the 2-position (R^2) of the pyrrolidine ring increased the AR binding affinity. The (2*S*,3*R*) configuration of the pyrrolidine ring was favorable for the AR antagonistic activity. It was found that introduction of an amide substituent (R^1) and a pyridin-3-yl group (Q) was effective for reducing the AR agonistic activity which appeared during the optimization of lead compound **6**. Compound **54** showed potent antitumor effects against a CRPC model of LNCaP-hr cell line in a mouse xenograft, in which bicalutamide exhibited only partial suppression of tumor growth. Thus, the pyrrolidine derivatives such as **54** are novel AR antagonists, and their properties having efficacy against CRPC are distinct from those of a representative first-generation antagonist, bicalutamide.

1. Introduction

Growth of prostate cancer (PC) is primarily stimulated by androgen. Androgen receptor (AR) antagonists, which block androgen signal, have been used for the treatment of PC. Cyproterone acetate,[1] flutamide,[2,3] nilutamide,[4,5] and bicalutamide[6–15] are known as AR antagonists used for clinical purposes (Fig. 1). A combination use of AR antagonists such as bicalutamide and surgical or chemical castration using gonadotropin-releasing hormone analogues, referred to as 'combined androgen blockade (CAB) therapy,' has significant effects against PC. However, prolonged use of an AR antagonist results in development of castration-resistant prostate cancer (CRPC).[16–18] In the cases, the AR antagonists such as bicalutamide become partial agonists,[17] and discontinuation of CAB therapy or change to another AR antagonist should be considered.[19] A microtubule-stabilizing agent, docetaxel,[20] and an androgen biosynthesis inhibitor, abiraterone acetate,[21,22] are currently approved for the treatment of CRPC. A phase 3 study of abiraterone acetate demonstrated the clinical evidence of AR signaling to be an important target in CRPC.[21] Taking into account the numerous unmet medical needs of CRPC, development of novel AR antagonists is an important approach for the treatment of CRPC. Recently, an AR antagonist enzalutamide (MDV-3100) that has been reported to be effective against CRPC[21,23,24] has been approved in the US (Fig. 1). Because of its unique pharmaceutical properties, this agent is categorized as a 'second-generation' AR antagonist in contrast to 'first-generation' antagonists such as bicalutamide or flutamide.

We reported novel 1-arylmethyl-4-phenylpyrrole compounds **A** (represented by compound **1**) and 1-arylmethyl-4-phenylpyrazole and 1-aryloxy-4-phenylpyrazole compounds **B** (represented by compounds **2** and **3**) as orally available AR antagonists effective against PCs, including CRPC (Fig. 2).[25,26] Our structure–activity relationship (SAR) studies for these compounds show that the cyanophenyl group **a** and the arylmethyl/aryloxy moiety **b** are important contributors to the strong AR antagonistic activity.[25,26] Some studies on other AR antagonists having a cyanophenyl group and a second aryl ring were also reported.[27–32]

Taking into account the importance of regions **a** and **b**, we investigated a new series of compounds as novel AR antagonists represented as scaffold **C** by modifying the pyrrole/pyrazole central ring of compounds **A** and **B** (Fig. 2). Scaffold **C** consists of a *p*-cyanophenyl group, a central ring Z, a linker L, and a terminal ring M. Compound **4**, which possessed 3-hydroxypyrrolidine moiety, was

Abbreviations: PC, prostate cancer; AR, androgen receptor; CRPC, castration-resistant prostate cancer; CAB, combined androgen blockade; PSA, prostate-specific antigen; DHT, dihydrotestosterone; SAR, structure–activity relationship; EDC, *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride.

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http://dx.doi.org/10.1016/j.bmc.2012.11.001

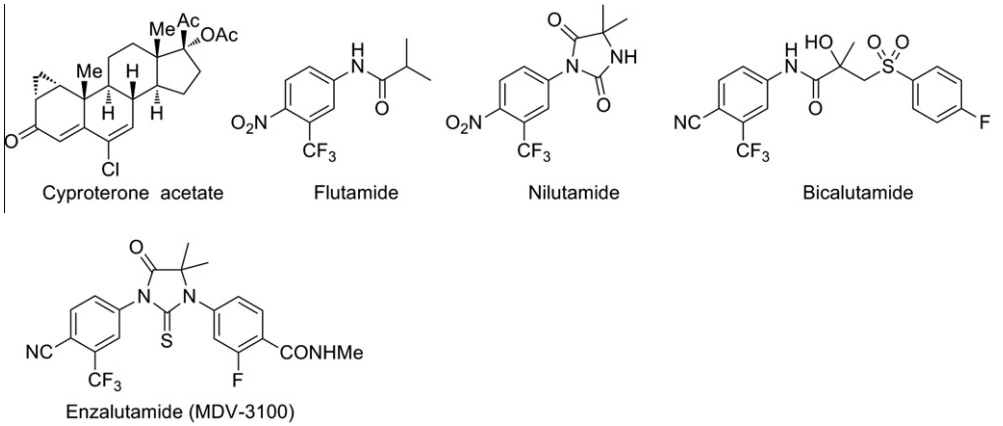


Figure 1. Chemical structure of known androgen receptor (AR) antagonists.

also found to show high AR binding affinity. Thus, an aliphatic ring system possessing a hydroxyl group, such as 3-hydroxypyrrolidine, was employed for the central ring Z. It was anticipated that the Z ring with a hydroxyl moiety would contribute to strong binding affinity to the AR. We set out to explore novel AR antagonists with scaffold **C** and found that 3-benzyl-3-hydroxypyrrolidine derivative **5** and 3-hydroxy-3-phenylpyrrolidine **6** possessed AR antagonistic activity. In addition to the antagonistic activity, compound **5** exhibited AR agonistic activity. On the other hand, compound **6** showed AR antagonistic activity without any significant agonistic activity. This AR activity profile was deemed appropriate for efficacy against CRPC. Thus, we selected compound **6** as a lead for new series of AR antagonists and studied terminal ring Q and substituents R^1–R^3 on Q, central pyrrolidine, and *p*-cyanophenyl group (compound **D**) to obtain compounds with good efficacy and pharmacokinetic (PK) properties.

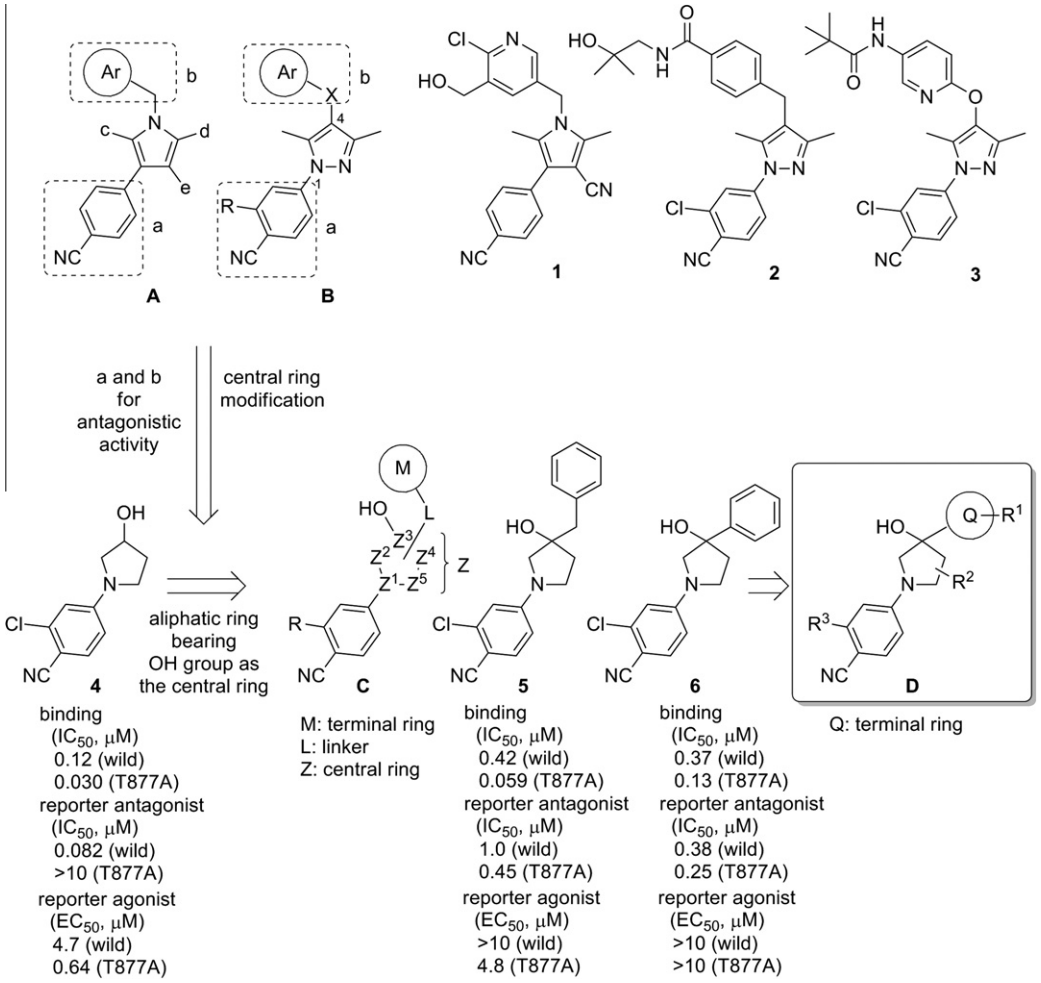
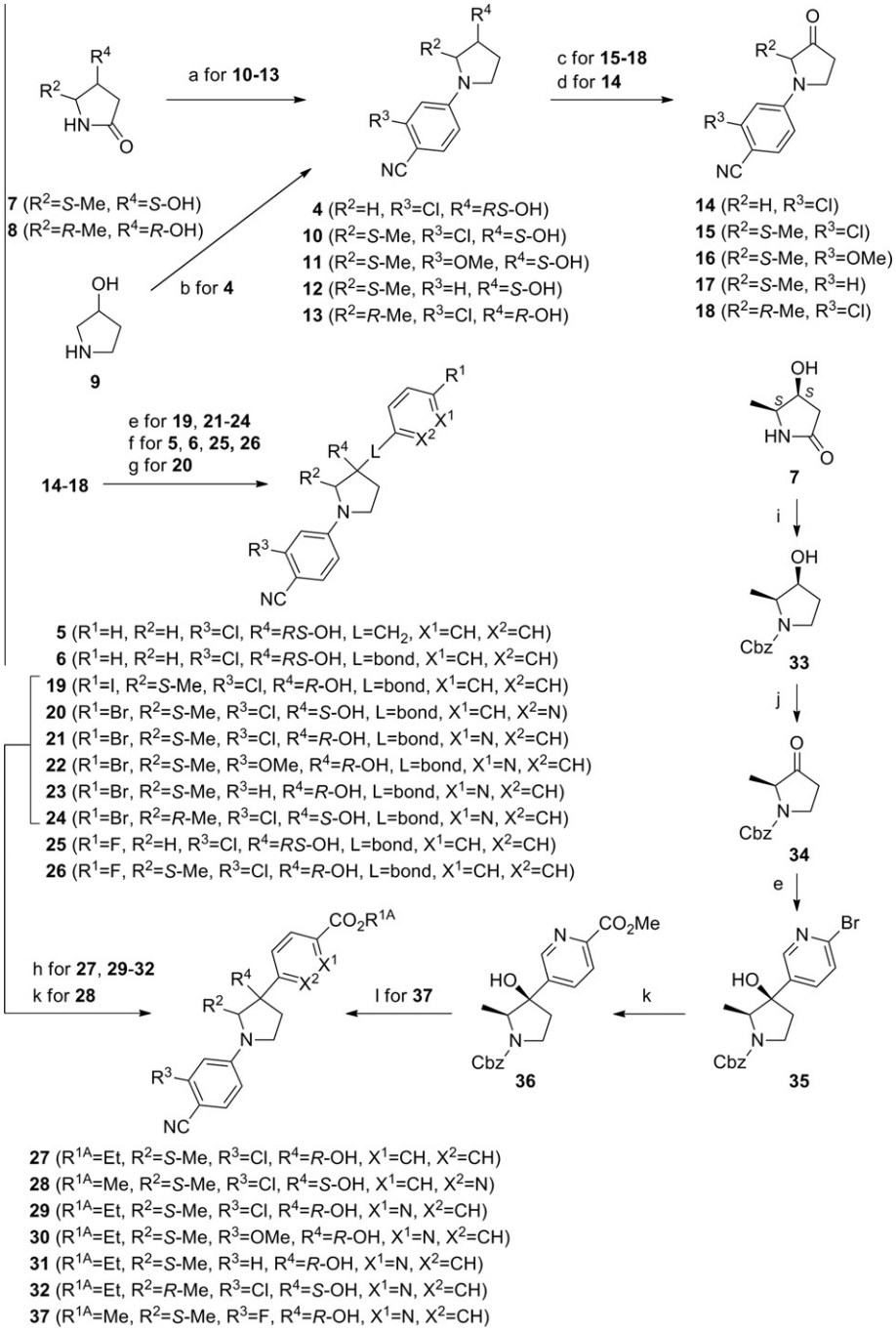


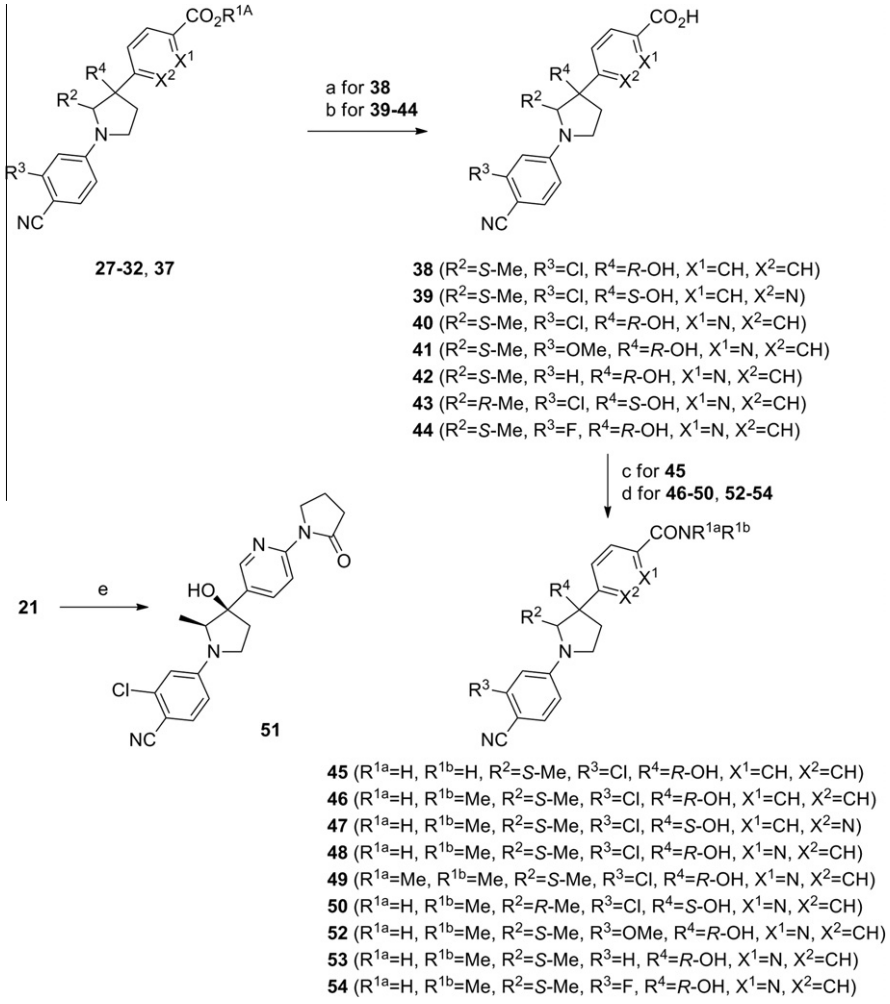
Figure 2. Design of 3-aryl-1-(4-cyanophenyl)-3-hydroxypyrrolidine AR antagonists.



Scheme 1. Synthesis of Compounds **4**–**6**, **25**–**32**, and **37**. Reagents and conditions: (a) (1) sodium bis(2-methoxyethoxy)aluminium dihydride, THF, 70 °C, (2) 2-chloro-4-fluorobenzonitrile (**10** and **13**), 4-fluoro-2-methoxybenzonitrile (**11**), or 4-fluorobenzonitrile (**12**), Li_2CO_3, DMSO, 100 °C, 61–74% for two steps; (b) 2-chloro-4-fluorobenzonitrile, Li_2CO_3, DMSO, 100 °C, 67%; (c) pyridine SO_3 complex, Et_3N, DMSO, room temp, 16–97%; (d) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C to 0 °C, 73%; (e) 1,4-diiodobenzene (**19**) or 2,5-dibromopyridine (**21**–**24** and **35**), *n*BuLi, Et_2O, THF, −78 °C, 36–59%; (f) Grignard reagent, THF, 5 °C to room temp, 9–29%; (g) 2,5-dibromopyridine, *n*BuLi, toluene, −78 °C, 57%; (h) CO, $Pd(OAc)_2$, 1,1′-bis(diphenylphosphino)ferrocene, Et_3N, DMF, EtOH, 70 °C, 53–98% (**29**–**32**) and 6% (**27**, for two steps); (i) (1) sodium bis(2-methoxyethoxy)aluminium dihydride, THF, 70 °C, (2) BnOCOCl, Et_3N, THF, room temp, 79% for two steps; (j) 4-methylmorpholine *N*-oxide, tetrapropylammonium perruthenate, MS-4A, MeCN, room temp, 32%; (k) CO, [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride dichloromethane adduct, Et_3N, MeOH, 60 °C, 86% (**28**) and 66% (**36**); (l) (1) H_2, Pd/C, MeOH, room temp, (2) 2,4-difluorobenzonitrile, Li_2CO_3, DMSO, 100 °C, 8% for two steps.

2. Chemistry

1-(4-Cyanophenyl)-3-hydroxypyrrolidine **4** and 1-(4-cyanophenyl)-3-aryl (or benzyl) pyrrolidines **5**, **6**, **25**–**32**, and **37** were prepared as shown in Scheme 1. Coupling of 2-chloro-4-fluorobenzonitrile and pyrrolidine **9** afforded the 3-hydroxypyrrolidine **4**. Reduction of chiral pyrrolidones **7** and **8**[33] by sodium bis(2-methoxyethoxy)aluminium dihydride followed by the reaction with 4-fluorobenzonitriles or benzyloxycarbonyl chloride resulted in 1-substituted pyrrolidines **10**–**13** or **33**. Oxidation of **4**, **10**–**13** and **33** gave pyrrolidin-3-ones **14**–**18** and **34**. Regioselective lithiation of 2,5-dibromopyridine at the 5-position in diethyl ether[34] and subsequent coupling with **15**–**18** and **34** afforded 3-hydroxy-3-(pyridin-5-yl)pyrrolidines **21**–**24** and **35**, respectively. On the other hand, lithiation of 2,5-dibromopyridine in toluene generated 5-bromo-2-lithiopyridine,[34] which was coupled with **15** to yield



Scheme 2. Synthesis of Compounds **45**–**54**. Reagents and conditions: (a) LiOH, EtOH, THF, H_2O, room temp to 50 °C, 45%; (b) NaOH, EtOH, THF, H_2O, room temp, 60%–quant.; (c) EDC, HOBt ammonium salt, DMF, room temp, 61%; (d) amines ($R^{1a}R^{1b}H$), EDC, HOBt, DMF, THF, room temp, 21–98%; (e) pyrrolidin-2-one, *N*,*N*′-dimethylethylenediamine, K_3PO_4, CuI, toluene, 80 °C, 75%.

3-hydroxy-3-(pyridin-2-yl)pyrrolidine **20**. Compounds **14** and **15** were reacted with Grignard reagents or lithiobenzene to afford 3-hydroxypyrrolidines **5**, **6**, **19**, **25**, and **26**. The reactions to obtain **19**–**24**, **26**, and **35** from chiral 2-methylpyrrolidin-3-ones **15**–**18** and **34** were stereoselective (the detail of the stereochemistry is discussed below). Esters **27**–**32** and **36** were obtained by palladium-catalyzed carbonization reactions of aryl bromide/iodide **19**–**24** and **35** under CO atmosphere in corresponding alcohol, respectively. Compound **37** was synthesized by deprotection of the Cbz group of **36** by hydrogenation reaction in the presence of Pd/C followed by reaction with 2,4-difluorobenzonitrile under basic condition.

Scheme 2 illustrates the synthesis of carboxamides **45**–**50** and **52**–**54** as well as pyrrolidone derivative **51**. Basic hydrolysis of esters **27**–**32** and **37** gave corresponding carboxylic acids **38**–**44**.

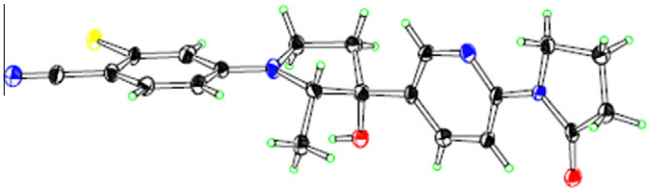


Figure 3. ORTEP drawing of **51**. Ellipsoids are drawn at the 20% probability level.

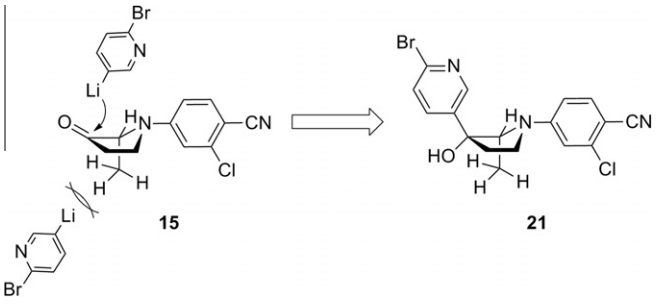
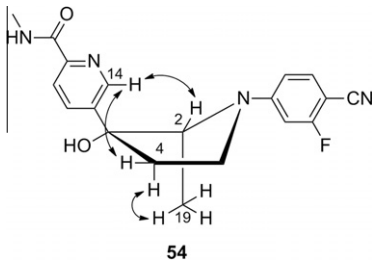


Figure 4. Plausible mechanism of reaction of **15** with 2-bromo-5-lithiopyridine.

Condensation of carboxylic acids **38**–**44** with amines was performed using *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (EDC) to give amides **45**–**50** and **52**–**54**. Copper-catalyzed coupling reaction of bromopyridine **21** with pyrrolidin-2-one in the presence of *N*,*N*′-dimethylethylenediamine and K_3PO_4[35] afforded pyrrolidone derivative **51**.

We determined the absolute stereochemical configuration of **51** by X-ray crystallographic analysis (Fig. 3). The configuration of the substituents on the 2- and 3-positions of the pyrrolidine ring of **51** proved to be (2*S*,3*R*). This result suggested that bromopyridine **21** has (2*S*,3*R*) configuration, which is the same as the stereochemical



54

Figure 5. NOE correlation of **54**.

configuration of **51**. The plausible mechanism for the stereoselective formation of **21** is shown in Figure 4: 2-bromo-5-lithiopyridine can attack pyrrolidin-3-one **15** only from the opposite site of 2*S*-methyl group of **15** because of the steric repulsion with the methyl group.

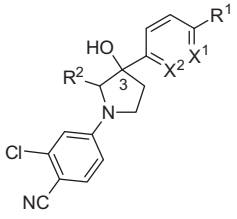
The relative stereochemical configuration of the substituents on the 2- and 3-positions of the pyrrolidine ring of **54** was determined by nuclear magnetic resonance (NMR) analysis (data not shown). Significant nuclear Overhauser effect (NOE) correlations were observed between H-14 and H-2, H-4β as well as H-19 and H-4α (Fig. 5). These observations showed that the methyl group on the 2-position and the pyridine ring on the 3-position of **54** are in trans configuration. On the basis of the results of X-ray crystallographic analysis of **51**, the reaction of pyrrolidin-3-one **34** with 2-bromo-5-lithiopyridine was also expected to give (2*S*,3*R*) isomer **35**. Thus, compound **54** becomes (2*S*,3*R*) isomer having 2-methyl and 3-pyridyl groups with trans configuration.

3. Results and discussion

The binding affinity and antagonistic/agonistic activity of the synthesized compounds to the ARs were measured by the AR binding inhibition test and the AR reporter gene assay, respectively (Tables 1 and 2). In these assays, we used the wild-type AR and a T877A mutant-type AR to estimate the efficacy of our compounds against normal and flutamide-resistant PCs. In addition, to evaluate the potential of the compounds against CRPC, we used LNCaP-hr cell line as a model of CRPC.[26,36] 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 an 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 measured the amount of PSA secreted by LNCaP-hr cells after the addition of the test compound (Table 2). The solubility of the compounds was analyzed in pH 6.8 solution. In these studies, we sought to discover novel AR antagonists that primarily possess strong binding affinity and antagonistic activity without significant agonistic activity.

The SAR trends from the initial synthetic study of compounds **D** are shown in Table 1. Introduction of a methyl group with *S*-configuration at the 2-position (R^2) of the pyrrolidine ring (3-hydroxyl group was *cis* to the 2-methyl) increased the AR binding affinity, although AR agonistic activity was also observed against T877A mutant-type AR (**25** vs **26**). Next, reduction of the AR agonistic activity of **26** was investigated with a focus on folding of helix 12 in the AR ligand-binding domain (LBD)[37–40] on the basis of the SAR information on pyrazole compounds.[26] Because helix 12 folding is an important factor for the expression of AR agonistic activity, inhibition of the folding was supposed to be one of the effective approaches for reduction of the agonistic activity. We hypothesized that terminal moiety Q–R^1 in compound **D** favors inhibition of helix 12 folding, and anticipated that increasing bulkiness of the moiety by replacing the fluoro group in compound **26** with larger R^1 substituents (compound **E**) could result in steric repulsion to reduce the AR agonistic activity (Fig. 6). The SAR study on pyrazole compounds revealed that introduction of amide substituents corresponding to R^1 removed the AR agonistic activity.[26] Thus, we set out to prepare compounds possessing amide substituents at R^1 (**F** in Fig. 6). Although the agonistic activity was notably diminished by replacement with amide substituents, unlike that in pyrazole compounds, residual agonistic activity was still observed (**45** and **46**, EC_{50} = 10 μM). Interestingly, replacement of the

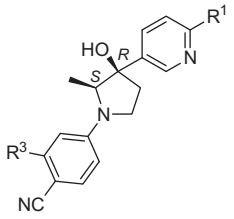
Table 1
Binding inhibitory and antagonistic activities of 3-aryl-3-hydroxy-1-phenylpyrrolidine derivatives



Compd.	R^1	X^1	X^2	R^2	C-3 [a]	Binding IC_{50} [b] (μM)		Reporter: Antagonist IC_{50} [b] (μM)		Reporter: Agonist EC_{50} [b] (μM)	
						Wild	T877A	Wild	T877A	Wild	T877A
6	H	CH	CH	H	*RS*	0.37	0.13	0.38	0.25	>10	>10
25	F	CH	CH	H	*RS*	0.18	0.036	0.068	0.065	>10	>10
26	F	CH	CH	*S*-Me	*R*	0.008	0.0081	0.017	>10	>10	0.02
45	$CONH_2$	CH	CH	*S*-Me	*R*	0.027	0.042	0.017	0.009	>10	10
46	CONHMe	CH	CH	*S*-Me	*R*	0.032	0.018	0.0086	0.01	>10	10
47	CONHMe	CH	N	*S*-Me	*R*	0.068	0.016	0.12	0.10	>10	5.1
48	CONHMe	N	CH	*S*-Me	*R*	0.044	0.10	0.025	0.099	>10	>10
49	$CONMe_2$	N	CH	*S*-Me	*R*	1.1	3.0	1.7	10	>10	>10
50	CONHMe	N	CH	*R*-Me	*S*	1.6	0.097	6.4	1.6	>10	>10
Bicalutamide						0.054	0.12	0.33	0.47	>10	>10
Dihydrotestosterone (DHT)						0.0021	0.0026	>10	>10	0.0062	0.015

[a] Absolute configuration at 3-position of pyrrolidine.
[b] IC_{50} and EC_{50} values shown are the mean values of duplicate measurements.

Table 2
In vitro activities of compounds **48** and **51**–**54**



Compd	R^1	R^3	Binding IC_{50}[b] (μM)		Reporter[a] Antagonist IC_{50}[b] (μM)		LNCaP-hr PSA secretion % Of control[c]	
			Wild	T877A	Wild	T877A	1 μM	10 μM[d]
48	CONHMe	Cl	0.044	0.10	0.025	0.099	39	101
51		Cl	0.013	0.087	0.011	0.16	17	35
52	CONHMe	OMe	0.12	0.24	0.062	0.73	87	NT[e]
53	CONHMe	H	7.6	5.3	0.40	1.4	95	42
54	CONHMe	F	0.83	1.7	0.29	1.7	41	35

[a] Agonistic activities of **48** and **51**–**54** were EC_{50} >10 μM for wild-type or T877A mutant-type AR.
[b] IC_{50} and EC_{50} values shown are the mean values of duplicate measurements.
[c] $n = 3$.
[d] Bicalutamide exhibited more than 100% secretion of control at 10 μM.
[e] Not tested.

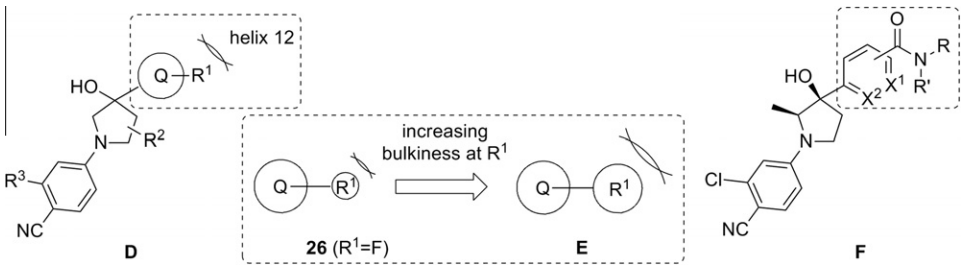


Figure 6. Introduction of an amide group to inhibit folding of helix 12.

benzene ring of **46** ($X^1 = X^2 = CH$) with a pyridine ($X^1 = N$, $X^2 = CH$) reduced the observed agonistic activity (**48**, EC_{50} >10 μM). On the other hand, nicotinamide derivative **47** ($X^1 = CH$, $X^2 = N$) showed weak agonistic activity ($EC_{50} = 5.1$ μM). Thus, we found that the nitrogen atom at X^1 in compounds **F** was sensitive to determine the balance between agonistic and/or antagonistic activity. It was also found that *N*-methylamide group at R^1 could improve the solubility (1.7, 7.0, 42, and 45 μg/mL in pH 6.8 solution for **25**, **45**, **46**, and **48**, respectively). The secondary amide exhibited higher AR antagonistic activity than the tertiary amide (**48** vs **49**). Further, we studied the effect of configuration of the substituents at the 2- and 3-position in the pyrrolidine ring on the antagonistic activity. Compound **50**, an antipode of **48**, showed less potent antagonistic activity than that of **48**. This result suggested that the (2*S*,3*R*) configuration of the methyl and hydroxyl groups in the pyrrolidine compounds was favorable for the antagonistic activity.

Next, we evaluated compound **48**, which possessed strong AR antagonistic activity without any significant agonistic activity, for the inhibitory activity against PSA secretion by LNCaP-hr cells, to estimate the potential activity against CRPC. The compound, however, did not inhibit the PSA secretion at high concentrations. Thus, we sought to enhance the inhibitory activity of compound **48**. In assessing the substituent at R^3 on the cyanophenyl moiety, we found that replacement of the chloro group of compound **48** with a fluoro group increased inhibitory activity against PSA secretion (**54**) (Table 2). Compound **54** did not possess any agonistic activity (EC_{50} >10 μM). On the other hand, methoxy group diminished inhibition of PSA secretion (**52**). Removal of the chloro group (**53**, $R^3 = H$) also decreased PSA secretion inhibition. Conversion of the amide group at R^1 with pyrrolidin-2-one moiety resulted in compound **51**, which failed to show concentration-dependent inhibitory activity. Because bicalutamide exhibited no inhibition against LNCaP-hr PSA secretion (more than 100% secretion of control at 10 μM), it should be noted that pyrrolidine compound such as **54** showed significantly different response in CRPC in vitro model. Compound **54** was orally available in mice (AUC = 231.7 μg h/mL, MRT = 4.07 h at a dose of 30 mg/kg, p.o. ($n = 3$)). On the basis of these biological and physicochemical profiles, we selected **54** for further evaluation.

Compound **54** was evaluated for its in vivo therapeutic efficacy against CRPC in comparison with bicalutamide, using a mouse xenograft model in which LNCaP-hr cells were subcutaneously injected. It was reported that oral administration of bicalutamide showed long duration of plasma concentration in men (mean $t_{1/2}$ of *R*-isomer and *S*-isomer, 4.2 days and 19 h, respectively[41]). Long duration was also observed in mice (data not shown). On the basis of these data for bicalutamide and the PK data for **54** described above, these compounds were orally administered once (bicalutamide) or twice (**54**) daily for 4 weeks. Tumor volume and plasma PSA concentration were measured after the treatment period. Compound **54** showed potent inhibition of tumor growth with T/C values (volume change in a test compound/volume change

in control) of 11% at a dose of 30 mg/kg, bid (Fig. 7). The plasma PSA level in the 30 mg/kg, bid treatment group was also markedly reduced to 18% of that in the vehicle treatment group. In addition, compound **54** demonstrated inhibition of body weight loss caused by the tumor burden. On the other hand, bicalutamide showed only partial suppression of tumor growth (T/C value of 48%) even at a dose of 100 mg/kg, qd. Almost no reduction of plasma PSA level was observed, as anticipated from in vitro studies in an LNCaP-hr PSA secretion system (more than 100% of control at 10 μM). These results suggested that compound **54** was effective against CRPC model and that the property of this compound is different from that of the first-generation antagonist, bicalutamide.

4. Conclusion

To explore novel AR antagonists therapeutically effective against CRPC, we designed, synthesized, and evaluated the efficacies of 3-aryl-3-hydroxy-1-phenylpyrrolidine compounds **D**. The chiral pyrrolidine compounds were synthesized using stereoselective arylation of chiral 2-methylpyrrolidin-3-one derivatives. Introduction of an amide group to the 3-aryl moiety and replacement of the benzene ring with a pyridine notably reduced AR agonistic activity to generate a potent AR antagonist **48**. The (2*S*,3*R*) configuration of the pyrrolidine ring of **48** was favorable for the AR antagonistic activity. Oral administration of compound **54**, discovered by further modification of **48**, showed antitumor effects against LNCaP-hr cell line, a CRPC model, in a mouse xenograft. On the other hand, bicalutamide only showed partial suppression of the growth of LNCaP-hr. Our data suggested that the pyrrolidine compounds **D** such as **54** are novel AR antagonists effective against CRPC model, and their properties are different from those of the first-generation AR antagonist, bicalutamide.

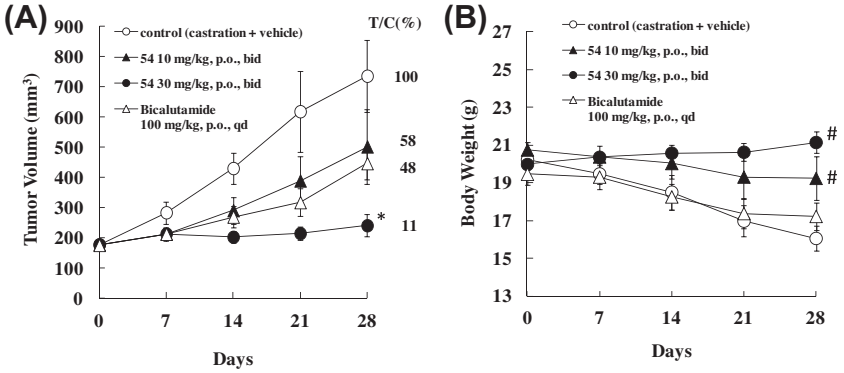
5. Experimental section

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; dt, doublet of triplet; br s, broad singlet; m, multiplet. Specific rotations were measured with a JASCO P-1030 digital polarimeter. 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. Mass spectra (MS) were determined using Waters micromass ZQ or Shimadzu LCMS-2020. High resolution mass spectra (HRMS) were recorded by Shimadzu LCMS-IT-TOF. 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.

5.1.1. 2-Chloro-4-(3-hydroxypyrrolidin-1-yl)benzonitrile (4)

A suspension of 2-chloro-4-fluorobenzonitrile (10.0 g, 64.3 mmol), pyrrolidin-3-ol **9** (5.60 g, 64.3 mmol), and lithium carbonate (7.12 g, 96.4 mmol) in DMSO (100 mL) was stirred at 100 °C



(C)

Compound	Dose (mg/kg, p.o.)	Plasma PSA Mean ± SE (ng / mL) Day 28	T / C (%)
Control (castration + vehicle)	–	23.8 ± 6.1	100
54	10, bid	33.0 ± 14.8	139
54	30, bid	4.3 ± 3.9*	18
Bicalutamide	100, qd	45.6 ± 9.7	191

Figure 7. Antitumor effects of **54** 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 <0.025, Shirley-Williams test vs. control. # P <0.025 Williams test versus control.

for 3 h. The mixture was diluted with H_2O. The precipitated compound was collected by filtration, air-dried, and recrystallized from hexane–EtOAc to give **4** (9.56 g, 67%) as light brown crystals, mp 130.5–131 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.68 (1H, d, J = 4.0 Hz), 2.00–2.30 (2H, m), 3.25–3.36 (1H, m), 3.37–3.49 (1H, m), 3.49–3.64 (2H, m), 4.55–4.80 (1H, m), 6.41 (1H, dd, J = 8.8 and 2.4 Hz), 6.56 (1H, d, J = 2.4 Hz), 7.42 (1H, d, J = 8.8 Hz). MS (ESI) m/z 223 [(M+H)$^+$]. Anal. Calcd for $C_{11}H_{11}ClN_2O$: C, 59.33; H, 4.98; N, 12.58; Cl, 15.92. Found: C, 59.38; H, 4.93; N, 12.53; Cl, 15.78.

5.1.2. 2-Chloro-4-[(2*S*,3*S*)-3-hydroxy-2-methylpyrrolidin-1-yl]benzonitrile (10)

To a solution of (4*S*,5*S*)-4-hydroxy-5-methylpyrrolidin-2-one **7**[33] (7.00 g, 60.8 mmol) in THF (120 mL) was added sodium bis(2-methoxyethoxy)aluminium dihydride (70% in toluene, 59.7 mL, 215 mmol) slowly at 5 °C. After stirring at 70 °C for 3 h, the reaction mixture was cooled to 5 °C followed by addition of sodium carbonate decahydrate (26.1 g, 91.2 mmol). The mixture was stirred at room temperature for 16 h, diluted with THF, and the precipitate was filtered off. The filtrate was concentrated in vacuo, and the residue was dissolved in DMSO (80 mL). To the solution was added lithium carbonate (8.99 g, 122 mmol) and 2-chloro-4-fluorobenzonitrile (9.46 g, 60.9 mmol), and the mixture was stirred at 100 °C for 1 h. The mixture was diluted with EtOAc and H_2O, and the organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **10** (10.7 g, 74%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.18 (3H, d, J = 6.6 Hz), 1.82 (1H, d, J = 5.5 Hz), 2.01–2.14 (1H, m), 2.25–2.35 (1H, m), 3.19–3.28 (1H, m), 3.43–3.51 (1H, m), 3.89–3.98 (1H, m), 4.43–4.52 (1H, m), 6.43 (1H, dd, J = 8.9 and 2.4 Hz), 6.56 (1H, d, J = 2.4 Hz), 7.42 (1H, d, J = 8.9 Hz). MS (ESI) m/z 237 [(M+H)$^+$].

5.1.3. 4-[(2*S*,3*S*)-3-Hydroxy-2-methylpyrrolidin-1-yl]-2-methoxybenzonitrile (11)

Compound **11** was prepared in a manner similar to that described for **10** in 61% yield as a light yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.19 (3H, d, J = 6.6 Hz), 1.91 (1H, d, J = 5.7 Hz), 1.99–2.17 (1H, m), 2.24–2.34 (1H, m), 3.19–3.32 (1H, m), 3.41–3.53 (1H, m), 3.88 (3H, s), 3.91–4.01 (1H, m), 4.40–4.54 (1H, m), 5.98 (1H, d, J = 2.1 Hz), 6.14 (1H, dd, J = 8.7 and 2.3 Hz), 7.32 (1H, d, J = 8.7 Hz). MS (ESI) m/z 233 [(M+H)$^+$].

5.1.4. 4-[(2*S*,3*S*)-3-Hydroxy-2-methylpyrrolidin-1-yl]benzonitrile (12)

Compound **12** was prepared in a manner similar to that described for **10** in 65% yield as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.18 (3H, d, J = 6.3 Hz), 1.96–2.17 (2H, m), 2.22–2.37 (1H, m), 3.16–3.31 (1H, m), 3.40–3.54 (1H, m), 3.88–4.02 (1H, m), 4.39–4.54 (1H, m), 6.50–6.56 (2H, m), 7.42–7.48 (2H, m). MS (ESI) m/z 203 [(M+H)$^+$].

5.1.5. 2-Chloro-4-[(2*R*,3*R*)-3-hydroxy-2-methylpyrrolidin-1-yl]benzonitrile (13)

Compound **13** was prepared in a manner similar to that described for **10** using (4*R*,5*R*)-4-hydroxy-5-methylpyrrolidin-2-one **8**[33] in 67% yield as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.18 (3H, d, J = 6.4 Hz), 1.83 (1H, d, J = 5.5 Hz), 2.02–2.16 (1H, m), 2.32–2.39 (1H, m), 3.16–3.33 (1H, m), 3.39–3.55 (1H, m), 3.87–4.01 (1H, m), 4.39–4.56 (1H, m), 6.43 (1H, dd, J = 8.9 and 2.4 Hz), 6.56 (1H, d, J = 2.4 Hz), 7.41 (1H, d, J = 8.9 Hz).

5.1.6. 2-Chloro-4-(3-oxopyrrolidin-1-yl)benzonitrile (14)

To a solution of oxalyl chloride (2.94 mL, 34.3 mmol) in dichloromethane (80 mL) was added a mixture of DMSO (3.35 mL, 47.2 mmol) and dichloromethane (20 mL) slowly at −78 °C. After stirring at −78 °C for 0.5 h, a solution of **4** (3.00 g, 13.5 mmol) in dichloromethane (100 mL) was added, and the mixture was stirred at −40 °C for 0.5 h. To the mixture was added triethylamine (13.1 mL, 94.0 mmol), and the resulting mixture was stirred at 0 °C for 20 min. Aqueous solution of NH_4Cl was added, and the mixture was diluted with H_2O and EtOAc. The organic layer was washed with H_2O and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was recrystallized from hexane–EtOAc to give **14** (2.18 g, 73%) as light brown crystals, mp 162–163 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.82 (2H, t, J = 7.8 Hz), 3.74–3.85 (4H, m), 6.52 (1H, dd, J = 8.8 and 2.4 Hz), 6.67 (1H, d, J = 2.4 Hz), 7.52 (1H, d, J = 8.8 Hz). MS (ESI) m/z 219 [(M−H)$^-$].

5.1.7. 2-Chloro-4-[(2*S*)-2-methyl-3-oxopyrrolidin-1-yl]benzonitrile (15)

To a solution of **10** (7.00 g, 29.6 mmol) and triethylamine (33.0 mL, 23.7 mmol) in DMSO (100 mL) was added sulfur trioxide pyridine complex (23.5 g, 148 mmol), and the resulting mixture was stirred at room temperature for 1 h. The mixture was diluted with EtOAc and H_2O, and the organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc). The product was recrystallized from hexane–EtOAc to give **15** (2.58 g, 37%) as colorless crystals, mp 97.5–98 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.34 (3H, d, J = 6.8 Hz), 2.68–2.89 (2H, m), 3.63–3.86 (2H, m), 4.03 (1H, q, J = 6.8 Hz), 6.56 (1H, dd, J = 8.8 and 2.4 Hz), 6.70 (1H, d, J = 2.4 Hz), 7.50 (1H, d, J = 8.8 Hz). MS (ESI) m/z 233 [(M−H)$^-$].

5.1.8. 2-Methoxy-4-[(2*S*)-2-methyl-3-oxopyrrolidin-1-yl]benzonitrile (16)

Compound **16** was prepared in a manner similar to that described for **15** in 16% yield as a yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.35 (3H, d, J = 6.6 Hz), 2.73–2.82 (2H, m), 3.68–3.82 (2H, m), 3.92 (3H, s), 4.05 (1H, q, J = 6.9 Hz), 6.11 (1H, d, J = 1.8 Hz), 6.25 (1H, dd, J = 8.7 and 2.4 Hz), 7.40 (1H, d, J = 8.7 Hz). MS (ESI) m/z 231 [(M+H)$^+$].

5.1.9. 4-[(2*S*)-2-Methyl-3-oxopyrrolidin-1-yl]benzonitrile (17)

Compound **17** was prepared in a manner similar to that described for **15** in 97% yield as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.34 (3H, d, J = 6.6 Hz), 2.75–2.80 (2H, m), 3.71–3.77 (2H, m), 4.05 (1H, q, J = 6.6 Hz), 6.67 (2H, d, J = 9.0 Hz), 7.54 (2H, d, J = 9.0 Hz). MS (ESI) m/z 201 [(M+H)$^+$].

5.1.10. 2-Chloro-4-[(2*R*)-2-methyl-3-oxopyrrolidin-1-yl]benzonitrile (18)

Compound **18** was prepared in a manner similar to that described for **15** in 63% yield as a light brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.34 (3H, d, J = 6.8 Hz), 2.68–2.89 (2H, m), 3.63–3.86 (2H, m), 4.03 (1H, q, J = 6.8 Hz), 6.56 (1H, dd, J = 8.8 and 2.4 Hz), 6.70 (1H, d, J = 2.4 Hz), 7.50 (1H, d, J = 8.8 Hz).

5.1.11. 4-(3-Benzyl-3-hydroxypyrrolidin-1-yl)-2-chlorobenzonitrile (5)

To a solution of **14** (200 mg, 0.906 mmol) in THF (10 mL) was added 1.03 M benzylmagnesium bromide in THF (3.52 mL, 3.63 mmol) at 5 °C, and the mixture was stirred at room temperature for 0.5 h. The mixture was diluted with aqueous solution of NH_4Cl and EtOAc. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc). The product was recrystallized from hexane–EtOAc to give **5** (26 mg, 9%) as colorless crystals, mp 149.5–151 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.65 (1H, s), 1.93–2.07 (1H, m), 2.07–2.33 (1H, m), 2.94–3.10 (2H, m), 3.22 (1H, dd, J = 10.6 and 1.2 Hz), 3.36–3.86 (3H, m), 6.37 (1H, dd,

J = 8.9 and 2.4 Hz), 6.51 (1H, d, J = 2.4 Hz), 7.22–7.46 (6H, m). MS (ESI) m/z 313 [(M+H)$^+$]. HRMS Calcd for $C_{18}H_{17}ClN_2O$ 313.1102. Found 313.1088. Analytical HPLC showed 100% purity.

5.1.12. 2-Chloro-4-(3-hydroxy-3-phenylpyrrolidin-1-yl)benzonitrile (6)

To a solution of **14** (208 mg, 0.943 mmol) in THF (10 mL) was added 1.0 M phenylmagnesium bromide in THF (3.52 mL, 3.52 mmol) at 5 °C, and the mixture was stirred at room temperature for 0.5 h. The reaction mixture was diluted with H_2O and EtOAc. The organic layer was 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 **6** (76 mg, 27%) as colorless crystals, mp 122–126 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.99 (1H, s), 2.33–2.45 (1H, m), 2.46–2.61 (1H, m), 3.50–3.83 (4H, m), 6.44 (1H, dd, J = 8.8 and 2.4 Hz), 6.59 (1H, d, J = 2.3 Hz), 7.29–7.47 (4H, m), 7.48–7.56 (2H, m). MS (ESI) m/z 299 [(M+H)$^+$]. Anal. Calcd for $C_{17}H_{15}ClN_2O$: C, 68.34; H, 5.06; N, 9.38; Cl, 11.87. Found: C, 68.35; H, 5.20; N, 9.28; Cl, 11.78.

5.1.13. 4-[(2*S*,3*S*)-3-(5-Bromopyridin-2-yl)-3-hydroxy-2-methylpyrrolidin-1-yl]-2-chlorobenzonitrile (20)

To a solution 2,5-dibromopyridine (1.21 g, 5.11 mmol) in toluene (8.5 mL) was added 1.6 M butyllithium in hexane (3.84 mL, 6.14 mmol) dropwise at −78 °C under argon atmosphere. After stirring at −78 °C for 1 h, was added a solution of **15** (1.00 g, 4.26 mmol) in toluene (10 mL). The mixture was stirred at −78 °C for 0.5 h, and allowed to warm to room temperature. The mixture was diluted with H_2O, and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **20** (0.949 g, 57%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.29 (3H, d, J = 6.4 Hz), 2.22–2.36 (1H, m), 2.42–2.53 (1H, m), 3.34–3.46 (1H, m), 3.65–3.78 (1H, m), 3.91 (1H, q, J = 6.3 Hz), 4.99 (1H, s), 6.49 (1H, dd, J = 8.9 and 2.4 Hz), 6.62 (1H, d, J = 2.4 Hz), 7.02 (1H, dd, J = 8.5 and 0.8 Hz), 7.45 (1H, d, J = 8.9 Hz), 7.83 (1H, dd, J = 8.5 and 2.3 Hz), 8.63 (1H, dd, J = 2.3 and 0.8 Hz). MS (ESI) m/z 392 [(M+H)$^+$].

5.1.14. 4-[(2*S*,3*R*)-3-(6-Bromopyridin-3-yl)-3-hydroxy-2-methylpyrrolidin-1-yl]-2-chlorobenzonitrile (21)

To a solution 2,5-dibromopyridine (656 mg, 2.77 mmol) in Et_2O (40 mL) was added 1.6 M butyllithium in hexane (1.73 mL, 2.77 mmol) dropwise at −78 °C under argon atmosphere. After stirring at −78 °C for 0.5 h, was added a solution of **15** (500 mg, 2.13 mmol) in THF (5 mL). The mixture was stirred at −78 °C for 0.5 h, and allowed to warm to room temperature. The mixture was diluted with aqueous solution of NH_4Cl and EtOAc. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc). The product was recrystallized from hexane–EtOAc to give **21** (415 mg, 50%) as light yellow crystals, mp 201 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33 (3H, d, J = 6.4 Hz), 2.19 (1H, s), 2.29–2.40 (1H, m), 2.41–2.54 (1H, m), 3.21–3.35 (1H, m), 3.62–3.73 (1H, m), 4.01 (1H, q, J = 6.4 Hz), 6.49 (1H, dd, J = 8.9 and 2.4 Hz), 6.62 (1H, d, J = 2.4 Hz), 7.44–7.51 (2H, m), 7.54–7.60 (1H, m), 8.38–8.41 (1H, m). MS (ESI) m/z 392 [(M+H)$^+$].

5.1.15. 4-[(2*S*,3*R*)-3-(6-Bromopyridin-3-yl)-3-hydroxy-2-methylpyrrolidin-1-yl]-2-methoxybenzonitrile (22)

Compound **22** was prepared in a manner similar to that described for **21** in 59% yield as a light yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33 (3H, d, J = 6.4 Hz), 2.24 (1H, s), 2.27–2.37 (1H, m), 2.41–2.54 (1H, m), 3.24–3.33 (1H, m), 3.63–3.73 (1H, m), 3.89 (3H, s), 4.02 (1H, q, J = 6.5 Hz), 6.02 (1H, d, J = 2.3 Hz), 6.19 (1H, dd, J = 8.7 and 2.3 Hz), 7.37 (1H, d, J = 8.7 Hz), 7.43–7.49 (1H, m), 7.54–7.61 (1H, m), 8.41 (1H, dd, J = 2.6 and 0.8 Hz). MS (ESI) m/z 388 [(M+H)$^+$].

5.1.16. 4-[(2*S*,3*R*)-3-(6-Bromopyridin-3-yl)-3-hydroxy-2-methylpyrrolidin-1-yl]benzonitrile (23)

Compound **23** was prepared in a manner similar to that described for **21** in 24% yield as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.32 (3H, d, J = 6.3 Hz), 2.25 (1H, s), 2.27–2.37 (1H, m), 2.42–2.53 (1H, m), 3.22–3.32 (1H, m), 3.62–3.72 (1H, m), 4.02 (1H, q, J = 6.3 Hz), 6.59 (2H, d, J = 9.0 Hz), 7.46 (1H, dd, J = 8.4 and 0.6 Hz), 7.50 (2H, d, J = 9.0 Hz), 7.57 (1H, dd, J = 8.4 and 2.7 Hz), 8.39 (1H, dd, J = 2.7 and 0.6 Hz). MS (ESI) m/z 358 [(M+H)$^+$].

5.1.17. 4-[(2*R*,3*S*)-3-(6-Bromopyridin-3-yl)-3-hydroxy-2-methylpyrrolidin-1-yl]-2-chlorobenzonitrile (24)

Compound **24** was prepared in a manner similar to that described for **21** in 18% yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33 (3H, d, J = 6.4 Hz), 2.19 (1H, s), 2.29–2.40 (1H, m), 2.41–2.54 (1H, m), 3.21–3.35 (1H, m), 3.62–3.73 (1H, m), 4.01 (1H, q, J = 6.4 Hz), 6.49 (1H, dd, J = 8.9 and 2.4 Hz), 6.62 (1H, d, J = 2.4 Hz), 7.44–7.51 (2H, m), 7.54–7.60 (1H, m), 8.38–8.41 (1H, m). MS (ESI) m/z 392 [(M+H)$^+$].

5.1.18. 2-Chloro-4-[3-(4-fluorophenyl)-3-hydroxypyrrolidin-1-yl)benzonitrile (25)

Compound **25** was prepared in a manner similar to that described for **6** in 28% yield as a colorless solid, mp 163–164.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.04 (1H, s), 2.29–2.60 (2H, m), 3.53–3.87 (4H, m), 6.44 (1H, dd, J = 8.7 and 2.3 Hz), 6.58 (1H, d, J = 2.3 Hz), 7.05–7.17 (2H, m), 7.43 (1H, d, J = 8.7 Hz), 7.46–7.55 (2H, m). MS (ESI) m/z 317 [(M+H)$^+$]. Anal. Calcd for $C_{17}H_{14}ClFN_2O$: C, 64.46; H, 4.45; N, 8.84; Cl, 11.19; F, 6.00. Found: C, 64.61; H, 4.61; N, 8.76; Cl, 11.08; F, 5.78.

5.1.19. 2-Chloro-4-[(2*S*,3*R*)-3-hydroxy-3-(4-fluorophenyl)-2-methylpyrrolidin-1-yl]benzonitrile (26)

Compound **26** was prepared in a manner similar to that described for **6** in 29% yield as a colorless solid, mp 95.5–97 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.32 (3H, d, J = 6.4 Hz), 1.99 (1H, s), 2.26–2.51 (2H, m), 3.15–3.30 (1H, m), 3.56–3.69 (1H, m), 4.04 (1H, q, J = 6.4 Hz), 6.49 (1H, dd, J = 8.8 and 2.4 Hz), 6.62 (1H, d, J = 2.4 Hz), 6.97–7.10 (2H, m), 7.31–7.41 (2H, m), 7.45 (1H, d, J = 8.8 Hz). MS (ESI) m/z 331 [(M+H)$^+$]. Anal. Calcd for $C_{18}H_{16}ClFN_2O$: C, 65.36; H, 4.88; N, 8.47; Cl, 10.72; F, 5.74. Found: C, 65.45; H, 5.07; N, 8.33; Cl, 10.64; F, 5.55. $[\alpha]_D^{25}$ −118.9 (c 0.17, MeOH).

5.1.20. Ethyl 4-[(2*S*,3*R*)-1-(3-chloro-4-cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]benzoate (27)

To a suspension of 1,4-diiodobenzene (330 mg, 1.00 mmol) in Et_2O (5.0 mL) was added 1.6 M butyllithium in hexane (0.75 mL, 1.20 mmol) at -78 °C under argon atmosphere. After stirring at −78 °C for 0.5 h, was added a solution of **15** (235 mg, 1.00 mmol) in THF (2.0 mL). The mixture was stirred at −78 °C for 0.5 h and then at room temperature for 16 h. The mixture was diluted with aqueous solution of NH_4Cl and 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 2-chloro-4-[(2*S*,3*R*)-3-hydroxy-3-(4-iodophenyl)-2-methylpyrrolidin-1-yl]benzonitrile **19** (120 mg) as a yellow solid.

A mixture of the compound (120 mg), palladium(II) acetate (12.0 mg, 0.053 mmol), 1,1′-bis(diphenylphosphino)ferrocene (30.0 mg, 0.054 mmol), triethylamine (0.075 mL, 0.54 mmol), DMF (2.0 mL), and EtOH (2.0 mL) was stirred at 70 °C for 4 h under carbon monoxide atmosphere. The mixture was diluted with H_2O, and extracted with EtOAc. The organic layer was washed with 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 **27** (23 mg, 6%) as colorless crystals, mp 129–130 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.32 (3H, d, J = 6.3 Hz), 1.38 (3H, t, J = 7.2 Hz), 2.05 (1H, s), 2.32–2.50 (2H, m), 3.22–3.32 (1H, m), 3.62–3.70 (1H, m), 4.07 (1H, q, J = 6.3 Hz), 4.37 (2H, q, J = 7.2 Hz), 6.49 (1H, dd, J = 8.7 and 2.4 Hz), 6.63 (1H, d, J = 2.4 Hz), 7.42–7.50 (3H, m), 8.03 (2H, d, J = 8.7 Hz). MS (ESI) m/z 385 [(M+H)$^+$].

5.1.21. Methyl 6-[(2*S*,3*S*)-1-(3-chloro-4-cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]pyridine-3-carboxylate (28)

To a solution of **20** (800 mg, 2.04 mmol) and triethylamine (0.34 mL, 2.44 mmol) in MeOH (20 mL) was added [1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloride dichloromethane adduct (166 mg, 0.203 mmol), and the resulting mixture was stirred at 60 °C overnight under carbon monoxide atmosphere. The mixture was diluted with H_2O, and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **28** (653 mg, 86%) as a brown amorphous. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.30 (3H, d, J = 6.4 Hz), 2.26–2.38 (1H, m), 2.45–2.57 (1H, m), 3.38–3.50 (1H, m), 3.68–3.80 (1H, m), 3.89–4.00 (4H, m), 5.28 (1H, s), 6.50 (1H, dd, J = 8.7 and 2.3 Hz), 6.63 (1H, d, J = 2.7 Hz), 7.19 (1H, dd, J = 8.1 and 0.9 Hz), 7.45 (1H, d, J = 9.1 Hz), 8.31 (1H, dd, J = 8.3 and 2.3 Hz), 9.16 (1H, d, J = 2.3 Hz). MS (ESI) m/z 372 [(M+H)$^+$].

5.1.22. Ethyl 5-[(2*S*,3*R*)-1-(3-chloro-4-cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]pyridine-2-carboxylate (29)

A mixture of **21** (250 mg, 0.637 mmol), palladium(II) acetate (29.0 mg, 0.129 mmol), 1,1′-bis(diphenylphosphino)ferrocene (71.0 mg, 0.128 mmol), triethylamine (0.18 mL, 1.29 mmol), DMF (5.0 mL), and EtOH (2.0 mL) was stirred at 70 °C for 16 h under carbon monoxide atmosphere. The mixture was diluted with aqueous solution of $NaHCO_3$ and EtOAc. The organic layer was washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **29** (130 mg, 53%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.35 (3H, d, J = 6.4 Hz), 1.44 (3H, t, J = 7.1 Hz), 2.24 (1H, s), 2.31–2.44 (1H, m), 2.46–2.59 (1H, m), 3.22–3.36 (1H, m), 3.61–3.74 (1H, m), 4.07 (1H, q, J = 6.4 Hz), 4.47 (2H, q, J = 7.1 Hz), 6.50 (1H, dd, J = 8.8 and 2.4 Hz), 6.63 (1H, d, J = 2.4 Hz), 7.47 (1H, d, J = 8.8 Hz), 7.88 (1H, dd, J = 8.3 and 2.4 Hz), 8.13 (1H, d, J = 8.3 Hz), 8.72 (1H, d, J = 2.4 Hz). MS (ESI) m/z 386 [(M+H)$^+$].

5.1.23. Ethyl 5-[(2*S*,3*R*)-1-(4-cyano-3-methoxyphenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]pyridine-2-carboxylate (30)

Compound **30** was prepared in a manner similar to that described for **29** in 66% yield as a colorless powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.35 (3H, d, J = 6.4 Hz), 1.43 (3H, t, J = 7.2 Hz), 2.31–2.43 (2H, m), 2.48–2.60 (1H, m), 3.24–3.38 (5H, m), 3.62–3.75 (1H, m), 4.08 (1H, q, J = 6.2 Hz), 4.47 (2H, q, J = 7.1 Hz), 6.03 (1H, d, J = 2.3 Hz), 6.20 (1H, dd, J = 8.7 and 2.1 Hz), 7.37 (1H, d, J = 8.5 Hz), 7.89 (1H, dd, J = 8.2 and 2.4 Hz), 8.12 (1H, dd, J = 8.3 and 0.8 Hz), 8.73–8.76 (1H, m). MS (ESI) m/z 382 [(M+H)$^+$].

5.1.24. Ethyl 5-[(2*S*,3*R*)-1-(4-cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]pyridine-2-carboxylate (31)

Compound **31** was prepared in a manner similar to that described for **29** in 97% yield as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.34 (3H, d, J = 6.6 Hz), 1.43 (3H, t, J = 7.2 Hz), 2.21 (1H, s), 2.30–2.40 (1H, m), 2.45–2.57 (1H, m), 3.22–3.33 (1H, m), 3.62–3.73 (1H, m), 4.05–4.15 (1H, m), 4.47 (2H, q, J = 7.2 Hz), 6.60 (2H, d, J = 9.0 Hz), 7.51 (2H, d, J = 9.0 Hz), 7.89 (1H, dd, J = 8.4 and 2.4 Hz), 8.12 (1H, dd, J = 8.4 and 0.6 Hz), 8.72 (1H, dd, J = 2.4 and 0.6 Hz). MS (ESI) m/z 352 [(M+H)$^+$].

5.1.25. Ethyl 5-[(2*R*,3*S*)-1-(3-chloro-4-cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]pyridine-2-carboxylate (32)

Compound **32** was prepared in a manner similar to that described for **29** in 98% yield as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.35 (3H, d, J = 6.4 Hz), 1.44 (3H, t, J = 7.1 Hz), 2.24 (1H, s), 2.31–2.44 (1H, m), 2.46–2.59 (1H, m), 3.22–3.36 (1H, m), 3.61–3.74 (1H, m), 4.07 (1H, q, J = 6.4 Hz), 4.47 (2H, q, J = 7.1 Hz), 6.50 (1H, dd, J = 8.8 and 2.4 Hz), 6.63 (1H, d, J = 2.4 Hz), 7.47 (1H, d, J = 8.8 Hz), 7.88 (1H, dd, J = 8.3 and 2.4 Hz), 8.13 (1H, d, J = 8.3 Hz), 8.72 (1H, d, J = 2.4 Hz). MS (ESI) m/z 386 [(M+H)$^+$].

5.1.26. Benzyl (2*S*,3*S*)-3-hydroxy-2-methylpyrrolidine-1-carboxylate (33)

To a suspension of **7** (7.00 g, 60.8 mmol) in THF (120 mL) was added sodium bis(2-methoxyethoxy)aluminium dihydride (70% in toluene, 60 mL, 216 mmol) at 0 °C. After stirring at 70 °C for 4 h, the reaction mixture was cooled to 0 °C followed by addition of sodium carbonate decahydrate (52.2 g, 182 mmol). The mixture was stirred at room temperature overnight, and the precipitate was filtered off. The filtrate was concentrated in vacuo, and the residue was dissolved in THF (120 mL). To the solution was added triethylamine (12.7 mL, 91.1 mmol) and benzyl chloroformate (11.0 mL, 78.0 mmol) at 0 °C. After stirring at room temperature for 18 h, H_2O was added and extracted with EtOAc. The organic layer was washed with diluted hydrochloric acid and brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **33** (11.3 g, 79%) as a light brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.15–1.30 (3H, m), 1.66 (1H, d, J = 5.1 Hz), 1.82–1.97 (1H, m), 2.00–2.15 (1H, m), 3.40–3.55 (2H, m), 3.90–4.00 (1H, m), 4.29–4.39 (1H, m), 5.05–5.22 (2H, m), 7.30–7.40 (5H, m). MS (ESI) m/z 236 [(M+H)$^+$].

5.1.27. Benzyl (2*S*)-2-methyl-3-oxopyrrolidine-1-carboxylate (34)

To a solution of **33** (13.5 g, 117 mmol) in MeCN (100 mL) was added molecular sieves 4A (13.3 g), 4-methylmorpholine *N*-oxide (10.0 g, 85.4 mmol), and tetrapropylammonium perruthenate (0.40 g, 1.14 mmol). After stirring at room temperature for 20 h, the mixture was concentrated in vacuo. To the residue was added EtOAc, and the precipitate was filtered off. The filtrate was concentrated in vacuo, and the residue was purified by silica gel column chromatography (hexane–EtOAc) to give **34** (8.74 g, 32%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.34 (3H, d, J = 6.6 Hz), 2.50–2.70 (2H, m), 3.60–3.75 (1H, m), 3.90–4.10 (2H, m), 5.15 (1H, d, J = 12.3 Hz), 5.20 (1H, d, J = 12.3 Hz), 7.30–7.40 (5H, m). MS (ESI) m/z 234 [(M+H)$^+$].

5.1.28. Benzyl (2*S*,3*R*)-3-(6-bromopyridin-3-yl)-3-hydroxy-2-methylpyrrolidine-1-carboxylate (35)

To a solution of 2,5-dibromopyridine (2.64 g, 11.1 mmol) in Et_2O (165 mL) was added 1.6 M butyllithium in hexane (7.0 mL, 11.2 mmol) at -78 °C under argon atmosphere. After stirring at -78 °C for 0.5 h, was added a solution of **34** (2.00 g, 8.57 mmol) in THF (30 mL) dropwise. The mixture was stirred at −78 °C for

0.5 h, and allowed to warm to room temperature. The mixture was diluted with aqueous solution of NH_4Cl and EtOAc. The organic layer was dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **35** (1.57 g, 36%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.27–1.33 (3H, m), 2.13–2.26 (3H, m), 3.60–3.76 (2H, m), 3.91–4.06 (1H, m), 5.08–5.22 (2H, m), 7.29–7.39 (5H, m), 7.44–7.49 (1H, m), 7.63 (1H, dd, J = 8.3 and 2.6 Hz), 8.50 (1H, d, J = 2.1 Hz). MS (ESI) m/z 391 [(M+H)$^+$].

5.1.29. Methyl 5-{(2*S*,3*R*)-1-[(benzyloxy)carbonyl]-3-hydroxy-2-methylpyrrolidin-3-yl}pyridine-2-carboxylate (36)

Compound **36** was prepared in a manner similar to that described for **28** in 66% yield as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.30 (3H, d, J = 5.7 Hz), 2.17–2.31 (2H, m), 2.62 (1H, s), 3.63–3.76 (1H, m), 4.00 (3H, s), 4.01–4.10 (1H, m), 5.09–5.21 (2H, m), 7.30–7.39 (5H, m), 7.92 (1H, dd, J = 8.2 and 2.4 Hz), 8.10 (2H, d, J = 8.3 Hz), 8.85 (1H, dd, J = 2.5 and 0.8 Hz). MS (ESI) m/z 371 [(M+H)$^+$].

5.1.30. Methyl 5-[(2*S*,3*R*)-1-(4-cyano-3-fluorophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]pyridine-2-carboxylate (37)

A mixture of **36** (13.86 g, 35.4 mmol), 10% Pd/C (50% wet, 0.50 g), and MeOH (60 mL) was stirred at room temperature under H_2 atmosphere for 3 h. After filtration through a pad of Celite, the filtrate was concentrated in vacuo, and the residue was dissolved in DMSO (120 mL). To the solution was added lithium carbonate (6.63 g, 89.7 mmol) and 2,4-difluorobenzonitrile (7.29 g, 52.4 mmol), and the resulting mixture was stirred at 100 °C for 2 h. The mixture was diluted with H_2O and EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc) to give **37** (1.04 g, 8%) as a light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.35 (3H, d, J = 6.4 Hz), 2.33–2.43 (1H, m), 2.47 (1H, s), 2.49–2.58 (1H, m), 3.28 (1H, dt, J = 10.1 and 7.7 Hz), 3.61–3.72 (1H, m), 4.00 (3H, s), 4.01–4.10 (1H, m), 6.30–6.43 (2H, m), 7.41 (1H, dd, J = 8.7 and 7.6 Hz), 7.88 (1H, dd, J = 8.2 and 2.4 Hz), 8.10–8.15 (1H, m), 8.71–8.75 (1H, m). MS (ESI) m/z 356 [(M+H)$^+$].

5.1.31. 4-[(2*S*,3*R*)-1-(3-Chloro-4-cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]benzoic acid (38)

A mixture of **27** (740 mg, 1.92 mmol), lithium hydroxide monohydrate (120 mg, 2.85 mmol), EtOH (5.0 mL), THF (5.0 mL), and H_2O (10 mL) was stirred at room temperature for 1.5 h and then at 50 °C for 2 h. The mixture was diluted with aqueous NaOH solution, washed with EtOAc, acidified with hydrochloric acid, and extracted with a mixture of EtOAc and MeOH. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was recrystallized from MeOH–Et_2O to give **38** (308 mg, 45%) as colorless crystals, mp 293–295 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.16 (3H, d, J = 6.0 Hz), 2.22–2.40 (2H, m), 3.15–3.35 (1H, m), 3.55–3.70 (1H, m), 4.07 (1H, q, J = 6.0 Hz), 5.79 (1H, s), 6.67 (1H, dd, J = 9.0 and 2.4 Hz), 6.81 (1H, d, J = 2.4 Hz), 7.53 (2H, d, J = 8.4 Hz), 7.61 (1H, d, J = 9.0 Hz), 7.89 (2H, d, J = 8.4 Hz), 12.88 (1H, br s). MS (ESI) m/z 357 [(M+H)$^+$].

5.1.32. 6-[(2*S*,3*S*)-1-(3-Chloro-4-cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]pyridine-3-carboxylic acid (39)

To a solution of **28** (550 mg, 1.48 mmol) in THF (13 mL) and EtOH (13 mL) was added 1 N NaOH solution (7.4 mL, 7.40 mmol), and the resulting mixture was stirred at room temperature for 5 h. The mixture was diluted with H_2O, neutralized with 1 N HCl, and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was washed with hexane–diisopropyl ether to give **39** (464 mg, 88%) as a light brown powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.16 (3H, d, J = 6.2 Hz), 2.21–2.34 (1H, m), 2.44–2.57 (1H, m), 3.25–3.43 (1H, m), 3.56–3.69 (1H, m), 4.24 (1H, q, J = 6.2 Hz), 5.98 (1H, s), 6.63 (1H, dd, J = 8.9 and 2.4 Hz), 6.75 (1H, d, J = 2.3 Hz), 7.57 (2H, d, J = 8.9 Hz), 7.84 (1H, dd, J = 8.3 and 0.8 Hz), 8.29 (1H, dd, J = 8.3 and 2.3 Hz), 8.93 (1H, dd, J = 2.1 and 0.8 Hz), 13.33 (1H, br s). MS (ESI) m/z 358 [(M+H)$^+$].

5.1.33. 5-[(2*S*,3*R*)-1-(3-Chloro-4-cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]pyridine-2-carboxylic acid (40)

To a solution of **29** (95 mg, 0.246 mmol) in THF (3.0 mL) and EtOH (3.0 mL) was added 1 N NaOH solution (0.99 mL, 0.99 mmol), and the resulting mixture was stirred at room temperature for 2 h. The mixture was neutralized with 1 N HCl and concentrated in vacuo (not to driness). The precipitated compound was collected by filtration and washed with H_2O to give **40** (78 mg, 89%) as a colorless powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.18 (3H, d, J = 6.4 Hz), 2.24–2.47 (2H, m), 3.15–3.34 (1H, m), 3.56–3.72 (1H, m), 4.15 (1H, q, J = 6.2 Hz), 6.03 (1H, br s), 6.70 (1H, dd, J = 8.9 and 2.3 Hz), 6.84 (1H, d, J = 2.3 Hz), 7.63 (1H, d, J = 8.9 Hz), 7.91–7.96 (1H, m), 8.01 (1H, d, J = 8.1 Hz), 8.70 (1H, d, J = 1.7 Hz). MS (ESI) m/z 358 [(M+H)$^+$].

5.1.34. 5-[(2*S*,3*R*)-1-(4-Cyano-3-methoxyphenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]pyridine-2-carboxylic acid (41)

Compound **41** was prepared in a manner similar to that described for **40** in 95% yield as a colorless powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.21 (3H, d, J = 6.5 Hz), 2.30–2.45 (2H, m), 3.23–3.32 (1H, m), 3.55–3.70 (1H, m), 3.87 (3H, s), 4.14 (1H, q, J = 6.5 Hz), 6.00 (1H, br s), 6.22 (1H, d, J = 1.7 Hz), 6.30 (1H, dd, J = 8.7 and 1.8 Hz), 7.40 (1H, d, J = 8.7 Hz), 7.93–8.05 (2H, m), 8.68 (1H, d, J = 1.5 Hz). MS (ESI) m/z 354 [(M+H)$^+$].

5.1.35. 5-[(2*S*,3*R*)-1-(4-Cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]pyridine-2-carboxylic acid (42)

Compound **42** was prepared in a manner similar to that described for **40** in 60% yield as a light brown solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.18 (3H, d, J = 6.0 Hz), 2.28–2.48 (2H, m), 3.18–3.40 (1H, m), 3.55–3.65 (1H, m), 4.08 (1H, q, J = 6.0 Hz), 5.97 (1H, br s), 6.71 (2H, d, J = 9.0 Hz), 7.55 (2H, d, J = 9.0 Hz), 7.90 (1H, dd, J = 8.1 and 2.1 Hz), 7.98 (1H, d, J = 8.1 Hz), 8.64 (1H, d, J = 2.1 Hz). MS (ESI) m/z 324 [(M+H)$^+$].

5.1.36. 5-[(2*R*, 3*S*)-1-(3-Chloro-4-cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]pyridine-2-carboxylic acid (43)

Compound **43** was prepared in a manner similar to that described for **40** in quantitative yield as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.18 (3H, d, J = 6.4 Hz), 2.24–2.47 (2H, m), 3.15–3.34 (1H, m), 3.56–3.72 (1H, m), 4.15 (1H, q, J = 6.2 Hz), 6.03 (1H, br s), 6.70 (1H, dd, J = 8.9 and 2.3 Hz), 6.84 (1H, d, J = 2.3 Hz), 7.63 (1H, d, J = 8.9 Hz), 7.91–7.96 (1H, m), 8.01 (1H, d, J = 8.1 Hz), 8.70 (1H, d, J = 1.7 Hz). MS (ESI) m/z 358 [(M+H)$^+$].

5.1.37. 5-[(2*S*,3*R*)-1-(4-Cyano-3-fluorophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]pyridine-2-carboxylic acid (44)

Compound **44** was prepared in a manner similar to that described for **40** in 90% yield as a colorless solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.19 (3H, d, J = 6.2 Hz), 2.31–2.45 (2H, m), 3.19–3.32 (1H, m), 3.57–3.67 (1H, m), 4.08–4.19 (1H, m), 6.03 (1H, s), 6.55–6.68 (2H, m), 7.54–7.61 (1H, m), 7.93–8.04 (2H, m), 8.69 (1H, dd, J = 2.3 and 0.8 Hz). MS (ESI) m/z 342 [(M+H)$^+$].

5.1.38. 4-[(2*S*,3*R*)-1-(3-Chloro-4-cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]benzamide (45)

A solution of **38** (120 mg, 0.336 mmol), *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (97.0 mg, 0.504 mmol), and 1-hydroxy-1*H*-benzotriazole ammonium salt (77.0 mg, 0.504 mmol) in DMF (5.0 mL) was stirred at room temperature over night. The reaction mixture was diluted with H_2O, and extracted with EtOAc. The organic layer was washed with 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 (MeOH–EtOAc). The product was recrystallized from hexane–EtOAc to give **45** (73 mg, 61%) as colorless crystals, mp 265–266 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.16 (3H, d, J = 6.3 Hz), 2.25–2.40 (2H, m), 3.15–3.35 (1H, m), 3.55–3.65 (1H, m), 4.08 (1H, q, J = 6.3 Hz), 5.73 (1H, s), 6.68 (1H, d, J = 8.7 Hz), 6.81 (1H, d, J = 2.4 Hz), 7.31 (1H, br s), 7.47 (2H, d, J = 2.4 Hz), 7.61 (1H, d, J = 8.4 Hz), 7.82 (2H, d, J = 8.7 Hz), 7.89 (1H, br s). MS (ESI) m/z 356 [(M+H)$^+$]. Anal. Calcd for $C_{19}H_{18}ClN_3O_2$: C, 64.13; H, 5.10; N, 11.81; Cl, 9.96. Found: C, 63.91; H, 5.21; N, 11.70; Cl, 9.86. $[\alpha]_D^{25}$ −157.1 (c 0.19, MeOH).

5.1.39. 5-[(2*S*,3*R*)-1-(4-Cyano-3-fluorophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]-*N*-methylpyridine-2-carboxamide (54)

A mixture of **44** (2.82 g, 8.26 mmol), 2.0 M methylamine in THF (12.4 mL, 24.8 mmol), *N*-[3-(dimethylamino)propyl]-*N*′-ethylcarbodiimide hydrochloride (1.90 g, 9.91 mmol), 1-hydroxybenzotriazole (1.34 g, 9.92 mmol), and DMF (40 mL) was stirred at room temperature for 14 h. The reaction mixture was diluted with H_2O and EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc). The product (2.57 g) was combined with another lot (1.00 g), and the whole was recrystallized from hexane–EtOAc to give **54** (2.67 g, 66%, 100% ee) as colorless crystals, mp 196–197 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.37 (3H, d, J = 6.4 Hz), 2.29–2.42 (1H, m), 2.47–2.62 (1H, m), 3.01 (3H, d, J = 5.1 Hz), 3.10 (1H, s), 3.18–3.31 (1H, m), 3.60–3.72 (1H, m), 4.05 (1H, q, J = 6.3 Hz), 6.32 (1H, dd, J = 12.4 and 2.3 Hz), 6.39 (1H, dd, J = 8.9 and 2.3 Hz), 7.40 (1H, dd, J = 8.7 and 7.5 Hz), 7.79 (1H, dd, J = 8.2 and 2.4 Hz), 7.88–7.99 (2H, m), 8.45–8.49 (1H, m). MS (ESI) m/z 355 [(M+H)$^+$]. Anal. Calcd for $C_{19}H_{19}FN_4O_2$: C, 64.40; H, 5.40; N, 15.81; F, 5.36. Found: C, 64.39; H, 5.45; N, 15.84; F, 5.31. $[\alpha]_D^{25}$ −172.0 (c 0.84, MeOH).

5.1.40. 4-[(2*S*,3*R*)-1-(3-Chloro-4-cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]-*N*-methylbenzamide (46)

Compound **46** was prepared in a manner similar to that described for **54** in 69% yield as colorless crystals, mp 215–216 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.16 (3H, d, J = 6.3 Hz), 2.25–2.35 (2H, m), 2.76 (3H, d, J = 4.5 Hz), 3.28–3.38 (1H, m), 3.55–3.65 (1H, m), 4.07 (1H, q, J = 6.3 Hz), 5.73 (1H, s), 6.67 (1H, dd, J = 9.0 and 2.1 Hz), 6.81 (1H, d, J = 2.1 Hz), 7.48 (2H, d, J = 8.4 Hz), 7.60 (1H, d, J = 9.0 Hz), 7.77 (2H, d, J = 8.4 Hz), 8.35 (1H, q, J = 4.5 Hz). MS (ESI) m/z 370 [(M+H)$^+$]. Anal. Calcd for $C_{20}H_{20}ClN_3O_2$: C, 64.95; H, 5.45; N, 11.36; Cl, 9.59. Found: C, 64.90; H, 5.53; N, 11.24, Cl, 9.54. $[\alpha]_D^{25}$ −168.8 (c 0.16, MeOH).

5.1.41. 6-[(2*S*,3*S*)-1-(3-Chloro-4-cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]-*N*-methylnicotinamide (47)

Compound **47** was prepared in a manner similar to that described for **54** in 98% yield as an orange amorphous. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.29 (3H, d, J = 6.1 Hz), 2.26–2.37 (1H, m), 2.44–2.56 (1H, m), 3.05 (3H, d, J = 4.9 Hz), 3.37–3.50 (1H, m), 3.67–3.78 (1H, m), 3.93 (1H, q, J = 6.7 Hz), 5.22 (1H, s), 6.11 (1H, br s), 6.49 (1H, dd, J = 8.7 and 2.3 Hz), 6.63 (1H, d, J = 2.7 Hz), 7.19 (1H, d, J = 8.3 Hz), 7.45 (1H, d, J = 8.7 Hz), 8.10 (1H, dd, J = 8.3 and 2.3 Hz), 8.91 (1H, s). MS (ESI) m/z 371 [(M+H)$^+$]. HRMS Calcd for $C_{19}H_{19}ClN_4O_2$ 371.1269. Found 371.1247. Analytical HPLC showed 99.0% purity. $[\alpha]_D^{25}$ −109.5 (c 0.20, MeOH).

5.1.42. 5-[(2*S*,3*R*)-1-(3-Chloro-4-cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]-*N*-methylpyridine-2-carboxamide (48)

Compound **48** was prepared in a manner similar to that described for **54** in 21% yield as colorless crystals, mp 205–205.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.36 (3H, d, J = 6.4 Hz), 2.30–2.43 (1H, m), 2.45–2.60 (1H, m), 2.78 (1H, s), 3.02 (3H, d, J = 5.1 Hz), 3.20–3.34 (1H, m), 3.59–3.75 (1H, m), 4.06 (1H, q, J = 6.4 Hz), 6.50 (1H, dd, J = 8.8 and 2.4 Hz), 6.63 (1H, d, J = 2.4 Hz), 7.47 (1H, d, J = 8.8 Hz), 7.83 (1H, dd, J = 8.2 and 2.4 Hz), 7.94 (1H, br s), 8.01–8.06 (1H, m), 8.50 (1H, d, J = 1.5 Hz). MS (ESI) m/z 371 [(M+H)$^+$]. Anal. Calcd for $C_{19}H_{19}ClN_4O_2$: C, 61.54; H, 5.16; N, 15.11; Cl, 9.56. Found: C, 61.50; H, 5.19; N, 14.97; Cl, 9.50. $[\alpha]_D^{25}$ −158.0 (c 0.15, MeOH).

5.1.43. 5-[(2*S*,3*R*)-1-(3-Chloro-4-cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]-*N*,*N*-dimethylpyridine-2-carboxamide (49)

Compound **49** was prepared in a manner similar to that described for **54** in 51% yield as colorless crystals, mp 150–151 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33 (3H, d, J = 6.3 Hz), 2.25–2.35 (1H, m), 2.45–2.55 (1H, m), 3.04 (3H, s), 3.13 (3H, s), 3.19–3.29 (1H, m), 3.60 (1H, s), 3.60–3.70 (1H, m), 4.03 (1H, q, J = 6.3 Hz), 6.49 (1H, dd, J = 8.3 and 2.4 Hz), 6.62 (1H, d, J = 2.4 Hz), 7.40–7.50 (2H, m), 7.72 (1H, dd, J = 8.1 and 2.4 Hz), 8.45 (1H, d, J = 2.4 Hz). MS (ESI) m/z 385 [(M+H)$^+$]. HRMS Calcd for $C_{20}H_{21}ClN_4O_2$ 385.1426. Found 385.1401. Anal. Calcd for $C_{20}H_{21}ClN_4O_2$: C, 62.42; H, 5.50; N, 14.56. Found: C, 62.28; H, 5.48; N, 14.56. $[\alpha]_D^{25}$ −168.8 (c 0.16, MeOH).

5.1.44. 5-[(2*R*,3*S*)-1-(3-Chloro-4-cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]-*N*-methylpyridine-2-carboxamide (50)

Compound **50** was prepared in a manner similar to that described for **54** in 32% yield as colorless crystals, mp 202–203 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.18 (3H, d, J = 6.3 Hz), 2.25–2.45 (2H, m), 2.79 (3H, d, J = 4.8 Hz), 3.18–3.28 (1H, m), 3.55–3.70 (1H, m), 4.16 (1H, q, J = 6.3 Hz), 6.01 (1H, s), 6.70 (1H, dd, J = 9.0 and 2.1 Hz), 6.85 (1H, d, J = 2.1 Hz), 7.63 (1H, d, J = 9.0 Hz), 7.90–8.00 (2H, m), 8.56 (1H, s), 8.75 (1H, d, J = 4.8 Hz). MS (ESI) m/z 371 [(M+H)$^+$]. Anal. Calcd for $C_{19}H_{19}ClN_4O_2$: C, 61.54; H, 5.16; N, 15.11; Cl, 9.56. Found: C, 61.40; H, 5.22; N, 14.79, Cl, 9.40. $[\alpha]_D^{25}$ +142.8 (c 0.16, MeOH).

5.1.45. 5-[(2*S*,3*R*)-1-(4-Cyano-3-methoxyphenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]-*N*-methylpyridine-2-carboxamide (52)

Compound **52** was prepared in a manner similar to that described for **54** in 46% yield as colorless crystals, mp 221–223 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.36 (3H, d, J = 6.2 Hz), 2.30–2.41 (1H, m), 2.46–2.56 (2H, m), 3.02 (3H, d, J = 5.1 Hz), 3.25–3.34 (1H, m), 3.63–3.76 (1H, m), 3.89 (3H, s), 4.05–4.13 (1H, m), 6.04 (1H, d, J = 2.1 Hz), 6.21 (1H, dd, J = 8.7 and 2.1 Hz), 7.38 (1H, d, J = 8.7 Hz), 7.86 (1H, dd, J = 8.2 and 2.4 Hz), 7.96 (1H, s), 8.07–8.13 (1H, m), 8.53 (1H, d, J = 1.5 Hz). MS (ESI) m/z 367 [(M+H)$^+$]. Anal. Calcd for $C_{20}H_{22}N_4O_3$: C, 65.56; H, 6.05; N, 15.29. Found: C, 65.28; H, 6.17; N, 15.17. $[\alpha]_D^{25}$ −151.3 (c 0.18, MeOH).

5.1.46. 5-[(2*S*,3*R*)-1-(4-Cyanophenyl)-3-hydroxy-2-methylpyrrolidin-3-yl]-*N*-methylpyridine-2-carboxamide (53)

Compound **53** was prepared in a manner similar to that described for **54** in 54% yield as colorless crystals, mp 207–208 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.19 (3H, d, J = 6.0 Hz), 2.22–2.48 (2H, m), 2.78 (3H, d, J = 4.8 Hz), 3.10–3.25 (1H, m), 3.53–3.63 (1H, m), 4.09 (1H, q, J = 6.0 Hz), 5.98 (1H, s), 6.72 (2H, d,

J = 8.7 Hz), 7.56 (2H, d, J = 8.7 Hz), 7.95–8.00 (2H, m), 8.50 (1H, s), 8.75 (1H, q, J = 4.8 Hz). MS (ESI) m/z 337 [$(M+H)^+$]. Anal. Calcd for $C_{19}H_{22}N_4O_2$: C, 67.84; H, 5.99; N, 16.66. Found: C, 67.79; H, 5.89; N, 16.67. $[\alpha]_D^{25}$ −184.4 (c 0.20, MeOH).

5.1.47. 2-Chloro-4-{(2*S*,3*R*)-3-hydroxy-2-methyl-3-[6-(2-oxopyrrolidin-1-yl)pyridin-3-yl]pyrrolidin-1-yl}benzonitrile (51)

A suspension of **21** (204 mg, 0.520 mmol), pyrrolidin-2-one (0.047 mL, 0.619 mmol), *N*,*N*′-dimethylethylenediamine (0.011 mL, 0.102 mmol), tripotassium phosphate (220 mg, 1.04 mmol), and copper(I) iodide (10.0 mg, 0.0525 mmol) in toluene (10 mL) was stirred at 80 °C for 3 h. The reaction mixture was diluted with H_2O and EtOAc. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane–EtOAc). The product was recrystallized from hexane–EtOAc to give **51** (155 mg, 75%) as colorless crystals, mp 171–174 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33 (3H, d, J = 6.4 Hz), 2.03–2.20 (2H, m), 2.26 (1H, s), 2.30–2.39 (1H, m), 2.42–2.53 (1H, m), 2.66 (2H, t, J = 8.0 Hz), 3.20–3.29 (1H, m), 3.63 (1H, ddd, J = 10.2, 8.3 and 4.4 Hz), 4.02–4.11 (3H, m), 6.48 (1H, dd, J = 8.8 and 2.4 Hz), 6.62 (1H, d, J = 2.3 Hz), 7.45 (1H, d, J = 8.7 Hz), 7.76 (1H, dd, J = 8.8 and 2.6 Hz), 8.31 (1H, d, J = 2.6 Hz), 8.37 (1H, d, J = 8.9 Hz). MS (ESI) m/z 397 [$(M+H)^+$]. Anal. Calcd for $C_{21}H_{21}ClN_4O_2$: C, 63.55; H, 5.33; N, 14.12; Cl, 8.93. Found: C, 63.46; H, 5.30; N, 13.96; Cl, 8.84. $[\alpha]_D^{25}$ −152.5 (c 0.79, MeOH).

5.2. Determination of the absolute configuration of 51

A colorless platelet (0.25 × 0.20 × 0.10 mm^3) of **51** was analyzed with a Rigaku RAXIS RAPID diffractometer using graphite monochromated Cu Kα radiation to obtain the following crystal data: $C_{21}H_{21}ClN_4O_2$, crystal system orthorhombic, space group $P2_12_12_1$ (#19), lattice parameters a = 9.476(5) Å, b = 10.592(6) Å, c = 18.483(9) Å, α = 90°, β = 90°, γ = 90°, V = 1855(6) Å^3, Z = 4, T = 100 K. Of the 16,594 reflections collected, 3304 were unique (R_{int} = 0.166). The refinement converged with R_1 = 0.072 and wR_2 = 0.243 for 642 reflections with $I > 2\sigma(I)$. The absolute configuration of **51** was established as (2*S*,3*R*) based on the Flack parameter,[42] 0.11(5). Further details of the X-ray structure data are available on request from the Cambridge Crystallographic Data Centre (deposition number CCDC 902808).

5.3. Solubility determination

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

5.4. Biology

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

After FreeStyle293F (Invitrogen) cells were transfected with pcDNA3.1 containing an androgen receptor (AR) gene (wild-type AR or T877A mutant-type AR) by using 293fectin transfection reagent (Invitrogen), these cells were seeded into an Erlenmeyer flask (Corning, 1L, 430518) at 1.1 × 10^6 cells/mL in FreeStyle293 Expression Medium (Invitrogen). After 48 h shaking incubation (125 rpm) at 37 °C in a 8% CO_2 atmosphere, these cells were washed with TEG Buffer (10 mM Tris–HCl (pH 7.2), 50 mM EDTA, 10% Glycerol), and suspended with TEGM Buffer (10 mM Tris–HCl (pH 7.2), 1 mM EDTA, 10% glycerol, 10 mM Na_2MoO_4, 1 mM DTT, 1 mM 2-ME, 1× Complete protease inhibitor tablet (Roche)). After freezing and thawing to lyse cells, lysate was centrifuged at 228,000×g at 4 °C for 20 min. The supernatant was stored at −80 °C as AR cell lysate. To cell lysate solution containing an AR or a T877A mutant-type AR were added [17-α-methyl-^{3}H] mibolerone (final 3 nM, PerkinElmer NET-919) and a compound, and the mixture was incubated at 4 °C for 3 h. B (Bound)/F (Free) were separated by the dextran/charcoal method.[43] The label count of B was measured, and the inhibitory rate of the compound was calculated.

5.4.2. 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.

5.4.3. 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.

5.4.4. 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 microliters 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, **54** at a dose of 10, 30 mg/kg, twice daily (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 × a × 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).

Acknowledgments

We thank Dr. Keiji Kamiyama, Dr. Tomoyasu Ishikawa, and Dr. Yumi N. Imai for helpful discussions; Ms. Keiko Higashikawa for the help in X-ray crystallographic analysis; Mr. Motoo Iida for the help in NMR analysis; Mr. Masaharu Nakayama, Mr. Takashi Santou, Mr. Masahiko Hattori, Mr. Hideo Araki, Ms. Mayumi Imai,

and Ms. Kazuyo Nakamura for the technical assistance for conducting biological assays.

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