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Novel non-steroidal/non-anilide type androgen antagonists: discovery of 4-substituted pyrrole-2-carboxamides as a new scaffold for androgen receptor ligands

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Abstract—We designed and synthesized novel pyrrole-2-carboxamide derivatives as androgen antagonists. Compounds **10** and **13** bearing benzylamine or aniline at the 4-position of the pyrrole ring showed moderate androgen antagonistic activity, and inhibited the androgen-dependent growth of Shionogi carcinoma cells (SC-3). Study of the structure–activity relationships of compound **13** led to a potent androgen antagonist **36**, which has higher affinity than flutamide (**4**) for androgen nuclear receptor (AR). Thus, pyrrole-2-carboxamide is a new scaffold for developing AR antagonists.

1. Introduction

Androgen is a steroid hormone required for prostate development and normal prostate function.[1] Androgenic action can be considered to function through an axis including the testicular synthesis of testosterone (**1**, Fig. 1), its transport to target tissues, and its conversion by 5α-reductase to the more active metabolite 5α-dihydrotestosterone (DHT, **2**, Fig. 1). The biological activities of testosterone (**1**), and DHT (**2**) result from the regulation of specific gene expressions through binding to activating androgen receptor (AR). AR is a member of the steroid/thyroid/retinoid/vitamin D_3 nuclear receptor superfamily, and consists of four functional domains, including the DNA-binding domain (DBD) and the ligand-binding domain (LBD).[2,3] As in other nuclear receptors, the structure of the LBD of human AR was elucidated by X-ray crystallography to have 12 α helices and a small β sheet. The 12th helix (H12) plays a critical role in the ligand-dependent activation of the receptor, that is, the binding of androgen to AR induces the active folding of H12, which results in the dissociation of corepressor(s) and the recruitment of coactivator(s).[4] The binding of an AR antagonist to AR induces an inactive folding structure of H12.

Approximately 80–90% of prostate cancers are dependent on androgen at initial diagnosis, and endocrine therapy of prostate cancer is directed toward the reduction of serum androgens and inhibition of AR actions.[5] Thus, androgens can be regarded as promoters of prostate cancers. Consequently, androgen antagonists, which antagonize the biological responses elicited by endogenous and/or exogenous androgens by inhibiting competitively their binding to AR, are expected to be effective for the treatment of these androgen-dependent prostate cancers.[6] Various androgen antagonists have been synthesized for investigation of the AR antagonist strategy for the treatment of prostate cancers. There are two structural types of clinically used androgen antagonists, that is, steroidal type and non-steroidal anilide type. A typical steroidal androgen antagonist is cyproterone acetate (**3**, CPA, Fig. 1), which is the first drug to have been used orally. However, **3** also interacts with progestin and glucocortinoid receptors.[7] Anilide analogues were the first non-steroidal androgen antagonists to be discovered, and have been used for the treatment of prostate cancer. Flutamide (**4**, Fig. 1) was the first non-steroidal androgen antagonist to enter clinical use, but its active metabolite, 2-hydroxyflutamide (**5**, Fig. 1), is excreted so rapidly that frequent dosing (three times a day) is necessary.[8,9] Bicalutamide (**6**, Fig. 1) is marketed as a racemic mixture, and the *R*-isomer of **6** is superior to flutamide (**4**) in terms of its pharmacokinetics and side effect profile.[10] Many of these clinically

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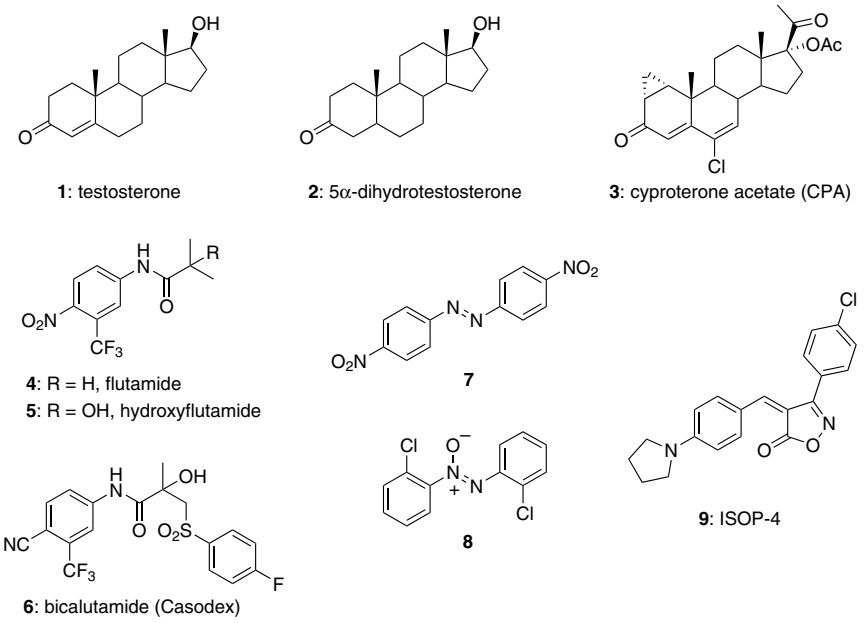


Figure 1. Structures of steroidal and non-steroidal androgen ligands.

employed androgen antagonists are limited by low selectivity across the nuclear hormone receptor superfamily, or by undesirable agonistic activity toward AR mutants such as T877A that can emerge in advanced prostate cancers.[11] Therefore, new types of AR antagonists are needed for clinical application to prostate cancers resistant to the known AR antagonists.

The discovery of the non-steroidal androgen antagonists stimulated researchers to develop other structural types of androgen antagonists, which are effective at mutated androgen receptors. Our previous studies suggested that structures bearing two aromatic groups connected with a two- to four-atom unit mimic as a spacer are suitable in molecular size and shape for AR antagonists.[12] For example, we showed that the azobenzene **7** and azoxybenzene **8**, which possess two atom units as a spacer between two aromatic rings, have high antiandrogen activity, being more potent than **4**.[13] Furthermore, based on these results, we found novel non-steroidal-type AR antagonists with an isoxazolone skeleton, such as ISOP-4 (**9**).[14] ISOP-4 (**9**) consists of two benzene rings connected by methyleneisoxazolone moiety (three-carbon unit as a spacer), and we envisioned that the introduction of a bulky pyrrolidino group might enhance AR-antagonistic activity. Compound **9** and its analogues showed potent growth-inhibitory activity toward androgen-dependent Shionogi carcinoma cells (SC-3),[10,15,16] and high binding affinity to human AR (**9** is more than 200 times more potent than **4**). These compounds have different types of structures from traditional non-steroidal androgen antagonists so far known, and the results encouraged us to develop further a new series of non-steroidal AR antagonists, to enrich the variety of available structures. In this paper, we describe the design, synthesis, and biological activities of novel pyrrole-2-carboxamide derivatives.

2. Results and discussion

2.1. Molecular design and synthesis

Taking into account the AR ligand structures that we used in the development of azobenzene **7**, azoxybenzene **8**, and isoxazolone **9** derivatives, we designed a new structural framework (general structure shown in Fig. 2). Our design approach was as follows. First, based on the successful introduction of a heterocyclic ring in compound **9**, we replaced one of the benzene rings of **9** with a heterocyclic pyrrole moiety. Second, the pyrrolidino group, a possible functional group for antagonistic activity, was connected through a carbonyl group to the pyrrole ring, since the amide structure is expected to afford superior chemical stability and pharmacological behavior. Finally, the spacer group between the two aromatic rings was varied as shown in –NH–X– (Fig. 2), where X is CO, SO_2, and so on. In this manner, several 4-substituted pyrrole-2-carboxamide derivatives **10**–**13** were designed as AR antagonist candidates (Fig. 3).

Compounds **10**–**13** were prepared from the key intermediate **16** by the method shown in Scheme 1. 4-Nitropy-

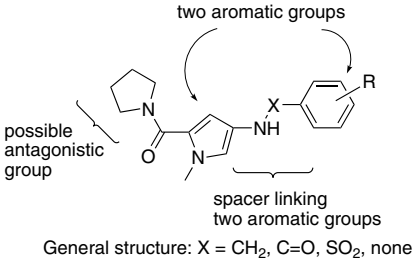


Figure 2. Molecular design of non-anilide types of androgen antagonists.

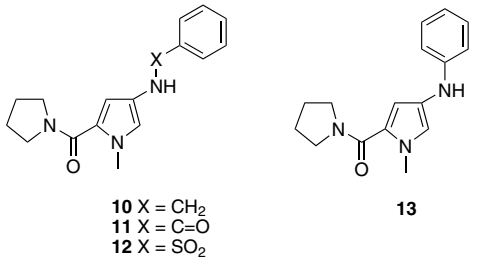
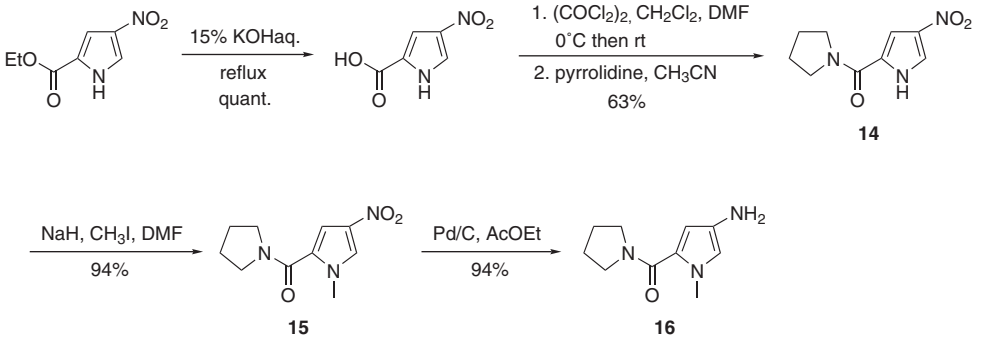
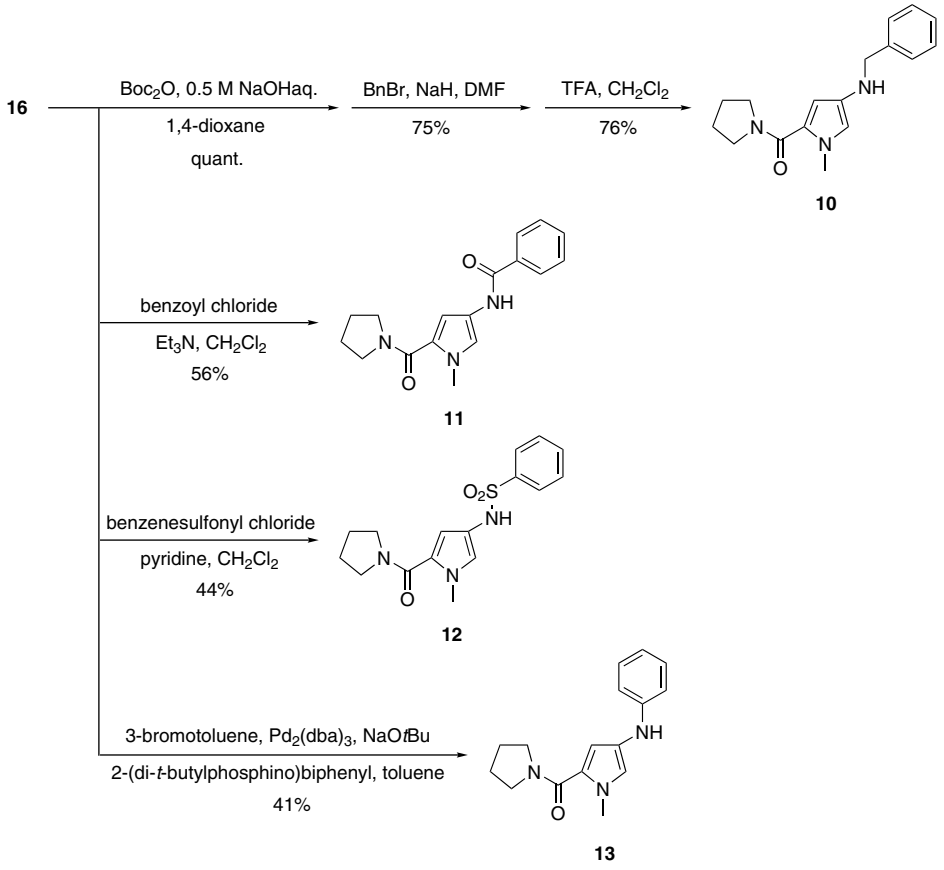


Figure 3. Structures of four types of novel androgen antagonist candidates.

rrole-2-carboxylic acid, obtained from the corresponding ethyl ester by hydrolysis (quant),[17] was then converted to the acid chloride by treatment with oxalyl chloride, followed by condensation with pyrrolidine to afford **14** (63%).[18] *N*-Methylation of **14** and subsequent hydrogenation with Pd–C afforded the aminopyrrole **16**. Starting from **16**, compounds **10**, **11**, **12**, and **13** can be synthesized diversely (Scheme 2). In order to introduce the mono-benzyl group, **16** was first converted to the *tert*-butoxyamide, followed by *N*-benzylation and deprotection of the *tert*-butoxycarbonyl group to afford **10**. Compound **16** was reacted with benzoyl chloride or



Scheme 1. Synthesis of key intermediate (**16**).



Scheme 2. Synthetic methods for pyrrole derivatives (**10**–**13**).

benzenesulfonyl chloride under usual conditions to afford **11** (56%) and **12** (44%), respectively. Buchwald's amination reaction of **16** using $Pd_2(dba)_3$, 2-(di-*t*-butylphosphino)biphenyl, and sodium *tert*-butoxide afforded the *N*-phenylated compound **13** (41%).[19,20] Compounds **17**–**36** (Tables 2 and 3) were prepared similarly by Buchwald's amination, using the key intermediate **16** and the substituted aromatic compounds. All compounds were characterized by ^{1}H NMR, and mass spectroscopy, and elemental analysis.

2.2. Biological activities

Androgenic and anti-androgenic activities of the compounds were assessed by using a combination of two assay systems.[14] The ability of the compounds as AR ligands was assessed by competitive receptor-binding assay using [^{3}H]testosterone and recombinant human AR extracted from cytosol of hAR-LBD (human AR ligand-binding domain) transformed *E. coli*.[14,21,22] The growth promotion/inhibition assay using androgen-dependent SC-3 cells was performed to determine whether the compounds are androgen agonists or antagonists. None of the compounds prepared showed growth-promoting activity toward SC-3 cells, suggesting that none was an androgen agonist. Therefore, only growth-inhibitory activities of the compounds (IC_{50} values) toward testosterone-promoted growth of SC-3 cells are presented in this paper. Briefly, SC-3 cells were incubated under standard conditions with 10 nM testosterone in the presence of various concentrations of a test compound. The increase of cell number in the absence of test compound was defined as 100%, and relative/expedient anti-androgenic activity of each test compound was presented as the IC_{50} value, the concentration at which the testosterone-promoted increase of cell number was reduced to 50%. The IC_{50} values thus measured showed some deviation from experiment to experiment. However, the results were basically reproducible. Each experiment was performed in triplicate and repeated at least three times, and typical sets of data (mean value of the triplicate) are presented in this paper. The values presented/compared in each table were obtained from experiments performed at the same time. In our assay system, the IC_{50} value of flutamide (**4**) was determined to be 4–8 μM.[14]

In the general structure shown in Figure 2, we first examined the compounds with various spacer groups (–NH–X–) between the pyrrole and unsubstituted benzene ring. The growth-inhibitory activities toward SC-3 cells of compounds **10**–**13** are shown in Table 1. The amide and sulfonamide derivatives **11** and **12** were inactive below 100 μM. On the other hand, the benzylamine derivative **10** and aniline derivative **13** showed growth-inhibitory activity, with IC_{50} values (52.3 μM and 43.7 μM for **10** and **13**, respectively), about one-tenth as potent as that of flutamide (IC_{50} = 4.93 μM in this experiment: Table 1). This tendency in the activity is consistent with the receptor (AR)-binding activity of these compounds, and among the four pyrrole derivatives, the amine derivatives **10** and **11** exhibited AR binding affinity (Fig. 4).

Considering the synthetic convenience and chemical properties of **13**, compared with the benzylamine derivative **10**, we next synthesized 18 analogs of **13** (**17**–**34**) with various substituents on the 2-, 3-, or 4-position of the phenyl ring, and examined their growth-inhibitory activities toward SC-3. As shown in Table 2, all the compounds examined showed similar growth-inhibitory activity to compound **13**, and some of them were more potent than **13**, though there was no clear overall tendency in the substituent effect. Among them, compounds having a chlorine atom exhibited the most potent inhibition, and the IC_{50} of the *meta* derivative **28** (5.92 μM) is comparable to that of flutamide (IC_{50} = 7.96 μM in this experiment: Table 3). We focused our attention on two potent compounds, the halogenated compound **28** (R = 3-Cl; IC_{50} = 5.92 μM) and the non-halogenated compound **23** (R = 3-Me; IC_{50} = 30.1 μM) as the next lead compounds for further structural modification. Based on our concept of the structure–activity relationships of androgen antagonists,[23] we expected that introduction of a second substituent at the *meta* position would increase the potency of antagonistic activity, and thus *m, m*-disubstituted derivatives **35** and **36** were synthesized. As shown in Table 3,

Table 1. Growth-inhibitory activities toward SC-3 cells

Compd	IC_{50} value (μM)
Flutamide (**4**)	4.93
10	52.3
11	>100
12	>100
13	43.7

IC_{50} value of compounds for testosterone (10 nM)-induced increase of cell growth.

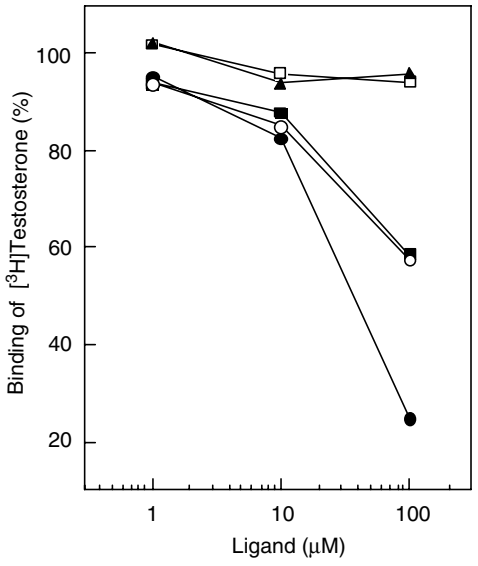
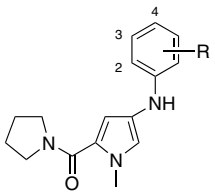


Figure 4. Receptor-binding assay using human AR. Competitive binding assay using 10 nM [^{3}H]testosterone and recombinant human AR-LBD with compounds: ● (flutamide, **4**), ■ (**10**), □ (**11**), ▲ (**12**), ○ (**13**).

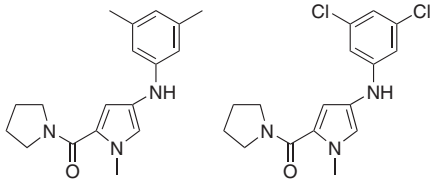
Table 2. Growth-inhibitory activities toward SC-3 cells



Compd	IC_{50} value (μM)
17: R = 4-CH_3	32.2
18: R = 4-OCH_3	46.9
19: R = 4-NO_2	14.7
20: R = 4-CN	>50
21: R = 4-F	32.6
22: R = 4-Cl	13.6
23: R = 3-CH_3	30.1
24: R = 3-OCH_3	45
25: R = 3-NO_2	31.5
26: R = 3-CN	>50
27: R = 3-F	24.8
28: R = 3-Cl	5.92
29: R = 2-CH_3	>50
30: R = 2-OCH_3	>50
31: R = 2-NO_2	28.2
32: R = 2-CN	>50
33: R = 2-F	24.4
34: R = 2-Cl	14.4

IC_{50} value of compounds for testosterone (10 nM)-induced increase of cell growth.

Table 3. Growth-inhibitory activities toward SC-3 cells



35 **36**

Compd	IC_{50}(μM)
Flutamide	7.96
35	18.7
36	2.68

IC_{50} value of compounds for testosterone (10 nM)-induced increase of cell growth.

35 showed more potent activity (IC_{50} = 18.7 μM) than the corresponding mono-substituted compound (**23**) in the inhibition of SC-3 cell growth. Similarly the dichloro-substituted compound (**36**) is more potent (IC_{50} = 2.68 μM) than the mono-substituted compound (**28**) and is also more potent than flutamide (IC_{50} = 7.96 μM in this experiment: Table 3). Figure 5 shows that the AR binding activity is parallel with the IC_{50} values in SC-3 assay. Compound **36** has a stronger affinity than flutamide (**4**). These results indicated that compound **36**, based on the new pyrrole-2-carboxamide scaffold, is a potent AR antagonist, which inhibits the growth of androgen-dependent prostate cancer cells SC-3.

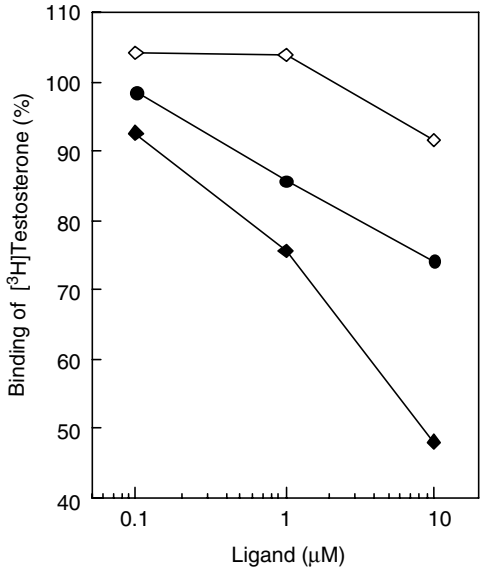


Figure 5. Receptor-binding assay using human AR. Competitive binding assay using 10 nM [^{3}H]testosterone and recombinant human AR-LBD with compounds: ● (flutamide, **4**), ◊ (**35**), ♦ (**36**).

3. Conclusion

We have designed androgen antagonists of a new non-steroidal/non-anilide type, based on our data and concepts about the structures of nuclear receptor agonists/antagonists. The molecular design and the structural modification studies successfully led to potent androgen antagonists with unique 4-anilino-pyrrole-2-carboxamide structures. Among them, **36** possesses potent AR-binding affinity, higher than that of flutamide (**4**) in our assays. The effect of the bulkiness of the second substituent on the nitrogen atom of aniline ring may greatly influence the AR antagonist activity. These compounds are also expected to be useful as lead compounds for developing growth inhibitors of androgen-resistant prostate cancer cells with mutated AR. Further investigations on 4-anilino-pyrrole-2-carboxamides derivatives, especially on the detailed structure–activity relationships and the biological activities toward various prostate cancer cells, are under way.

4. Experimental

4.1. Chemicals

[1,2,6,7-^{3}H]Testosterone ([^{3}H]testosterone, 101 Ci/mmol) and isopropyl β-D-thiogalactoside were purchased from Amersham Pharmacia Biotech (England). Tris-base, EDTA, glycerol, DTT, sodium chloride, potassium chloride, sodium molybdate, and testosterone were purchased from Wako Pure Chemical, Ltd (Japan). Sources of other reagents were as follows: Bacto® Tryptone and Bacto® Yeast Extract, Difco Laboratories (USA); Coomassie Protein Assay Reagent and Albumin Standard, Pierce (USA); Atomlight scintillation cocktail, Packard Bioscience (Netherlands); MEM (modified Eagle's medium), and flutamide (**4**), Sigma–Aldrich

Japan K. K. (Japan); FBS (fetal bovine serum), Gibco (USA); Cell Counting Kit, Dojindo (Japan). All other chemicals were purchased from Tokyo Kasei Kogyo Co., Ltd (Japan), Kanto Chemical Co., Inc. (Japan), Sigma–Aldrich Japan K. K. (Japan), or Lancaster Synthesis (UK).

4.2. General synthetic methods

Commercially available reagents were used as supplied. Routine thin layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck, Germany). Column chromatography was done using silica gel 60 spherical or silica gel 60 N spherical (Kanto Chemical Co., Inc., Japan). Melting points were determined on a MP-J3 melting point apparatus (Yanaco, Japan) and are uncorrected. Fast atom bombardment mass spectra (FAB-MS) were measured by a MS-JEOL JMS-HX110 mass spectrometer using a nitrobenzyl alcohol matrix as appropriate. Proton nuclear magnetic resonance (NMR) spectra data were obtained on a JEOL ALPHA500 spectrometer (500 MHz) and were consistent with assigned structures. Chemical shifts are given in parts per million (ppm) downfield from internal reference TMS in δ units, and coupling constants (J values) are given in hertz (Hz). Elemental analysis was performed on a MT-6 elemental analyzer (Yanagimoto, Tokyo).

4.2.1. Preparation of 4-nitropyrrole-2-carboxylic acid. A suspension of ethyl 4-nitropyrrole-2-carboxylate (10 g, 54 mmol) in 15% KOH aq (140 mL) was heated at 100 °C for 19 h. The solution was cooled and acidified to about pH 1 with 2 M HCl and was extracted with ether. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated to give 4-nitropyrrole-2-carboxylic acid as a pale yellow solid (9.3 g, quant). ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.18 (s, 1H), 12.95 (s, 1H), 7.99 (d, 1H, J = 1.4 Hz), 7.19 (d, 1H, J = 1.9 Hz).

4.2.2. *N*-(4-Nitropyrrole-2-carbonyl)pyrrolidine (14). Oxalyl chloride was added to a solution of 4-nitropyrrole-2-carboxylic acid (10 g, 64 mmol) in dichloromethane (150 mL) and DMF (25 mL) at 0 °C and stirred at room temperature for 4.5 h. Removal of the solvent under reduced pressure gave a colorless liquid. Pyrrolidine (11 mL, 128 mmol) was added to a solution of the liquid in dry acetonitrile at 0 °C and stirred for 30 min. Further stirring for 1.5 h at room temperature afforded a pale yellow precipitate, which was collected by filtration in vacuo to provide **14** (8.4 g, 63%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.6 (s, 1H), 7.91 (s, 1H), 7.13 (s, 1H), 3.71 (m, 2H), 3.47 (m, 2H), 1.94 (m, 2H), 1.82 (m, 2H); MS (FAB) m/z 209 (M^+).

4.2.3. *N*-(1-Methyl-4-nitropyrrole-2-carbonyl)pyrrolidine (15). Iodomethane (2.2 mL, 35 mmol) was added to a suspension of sodium hydride (700 mg, 29 mmol) and **14** (2.9 g, 14 mmol) in DMF (50 mL) at 0 °C, and the mixture was stirred for 30 min at 0 °C and then at room temperature for 4.5 h. The reaction was quenched with water, and the mixture was extracted with dichloromethane. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated to give **15** as a pale yellow solid (3.0 g, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (d, 1H, J = 2.1 Hz), 6.99 (d, 1H, J = 2.1 Hz), 3.90 (s, 3H), 3.64 (br s, 4H), 1.95 (m, 4H); MS (FAB) 224 (MH^+).

4.2.4. *N*-(4-Amino-1-methylpyrrole-2-carbonyl)pyrrolidine (16). 10% Pd/C was added to a solution of **15** (3.0 g, 13 mmol) in ethyl acetate (80 mL) at room temperature. The reaction mixture was vigorously stirred under positive pressure of hydrogen for 36 h at room temperature and then filtered through Celite. The Celite was rinsed with ethyl acetate and the combined filtrates were evaporated. The residue was purified by silica gel column chromatography (chloroform then chloroform–methanol = 20:1) to give **16** as a reddish brown liquid (2.4 g, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.25 (d, 1H, J = 2.1 Hz), 6.09 (d, 1H, J = 2.1 Hz), 3.75 (s, 3H), 3.61 (br, 4H), 1.90 (m, 4H); MS (FAB) m/z 193 (M^+).

4.2.5. *N*-(4-Benzylamino-1-methylpyrrole-2-carbonyl)pyrrolidine (10). Di-*t*-butyl dicarbonate (290 mg, 1.3 mmol) was added to a solution of 0.5 M NaOH aq (2 mL) and **16** (217 g, 1.1 mmol) in 1,4-dioxane (2 mL) at 0 °C. The reaction mixture was stirred at room temperature for 5 min, then the reaction was quenched with 2 M HCl aq. The mixture was extracted with ethyl acetate. The extracts were washed with brine, dried over $MgSO_4$, and evaporated to give *N*-(4-*t*-butoxycarbonylamino-1-methylpyrrole-2-carbonyl)pyrrolidine as a white solid (332 mg, quant). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.85 (s, 1H), 6.30 (s, 1H), 6.16 (s, 1H), 3.80 (s, 3H), 3.63–3.62 (m, 4H), 1.92–1.89 (m, 4H), 1.49 (s, 9H); MS (FAB) 294 (MH^+), 293 (M^+).

Benzyl bromide (90 μL, 0.76 mmol) was added to a suspension of *N*-(4-*t*-butoxycarbonylamino-1-methylpyrrole-2-carbonyl)pyrrolidine (148 mg, 0.51 mmol) and sodium hydride (30 g, 1.3 mmol) in DMF (2 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h and then the reaction was quenched with water. The mixture was extracted with dichloromethane. The extracts were washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane–ethyl acetate = 1:4) to give *N*-[4-[*N*-benzyl-*N*-(*t*-butoxycarbonyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine as a white solid (145 mg, 75%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33–7.24 (m, 6H), 6.36 (s, 1H), 4.79 (s, 2H), 3.78 (s, 3H), 3.58–3.55 (m, 4H), 1.94–1.86 (m, 4H), 1.49 (s, 9H); MS (FAB) 383 (M^+).

TFA (2 mL) was added to a solution of *N*-[4-[*N*-benzyl-*N*-(*t*-butoxycarbonyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (8 mg, 0.021 mmol) in dichloromethane (2 mL) at room temperature. The reaction mixture was left for 15 min, then evaporated, and the residue was dissolved in 1 M NaOH aq. This solution was extracted with ethyl acetate. The extracts were washed by brine, dried over $MgSO_4$, and evaporated to give **10** as a yellow solid (8 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37

(d, 2H, J = 7.2 Hz), 7.32 (t, 2H, J = 7.2 Hz), 7.26 (t, 1H, J = 7.2 Hz), 6.19 (d, 1H, J = 1.7 Hz), 6.08 (d, 1H, J = 1.7 Hz), 4.14 (s, 2H), 3.74 (s, 3H), 3.59 (br s, 4H), 1.92–1.88 (m, 4H); MS (FAB) 283 (M^+), 284 (MH^+); HRMS (FAB) $C_{17}H_{21}N_3O$, calcd for 283.1685, found 283.1717.

4.2.6. *N*-(4-Benzoylamino-1-methylpyrrole-2-carbonyl)-pyrrolidine (11). Benzoyl chloride (28 μL, 0.24 mmol) was added to a solution of **16** (52 mg, 0.27 mmol) and triethylamine (41 μL, 0.29 mmol) in dichloromethane (1 mL) at 0 °C and stirred at room temperature for 1 h. The reaction was quenched with 2 M HCl, then the mixture was extracted with dichloromethane. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane–ethyl acetate = 4:1; 2:1; 1:4; then ethyl acetate only) and the product was recrystallized with *n*-hexane/ethyl acetate to afford **11** as a white solid (16 mg, 20%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.86 (d, 2H, J = 7.2 Hz), 7.51 (t, 1H, J = 7.2 Hz), 7.44 (t, 2H, J = 7.3 Hz), 7.27 (d, 1H, J = 1.2 Hz), 6.51 (d, 2H, J = 2.1 Hz), 3.83 (s, 3H), 3.63 (br, 4H), 1.90 (m, 4H); MS 298 (MH^+), 297 (M^+); HRMS (FAB, MH^+) $C_{17}H_{20}N_3O_2$, calcd for 298.1556, found 298.1593.

4.2.7. *N*-(4-Benzenesulfonylamino-1-methylpyrrole-2- carbonyl)pyrrolidine (12). Benzenesulfonyl chloride (56 μL, 0.44 mmol) was added to a solution of **16** (93 mg, 0.48 mmol) and pyridine (43 μL, 0.53 mmol) in dichloromethane (1 mL) at -20 °C and stirred at room temperature for 4.5 h. The reaction was quenched with 2 MHCl aq, then the mixture was extracted with dichloromethane. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane–ethyl acetate = 1:1; 1:2; 1:4) and the product was recrystallized from *n*-hexane/ethyl acetate to afford **12** as a pale yellow solid (71 mg, 44%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (dd, 2H, J = 8.5, 1.2 Hz), 7.55 (dd, 1H, J = 7.2, 1.2 Hz), 7.46 (m, 2H), 6.52 (d, 1 H, J = 1.7 Hz), 6.08 (d, 1H, J = 2.1 Hz), 5.96 (s, 1 H), 3.75 (s, 3H), 3.49 (br s, 4H), 1.89–1.84 (m, 4H); MS (FAB) 334 (MH^+), 333 (M^+); HRMS (FAB, MH^+) $C_{16}H_{20}N_3O_3S$, calcd for 334.1225, found 334.1245.

4.3. General procedure for *N*-aryl compounds (13–36)

A well-dried flask was evacuated and backfilled with argon. The flask was charged with $Pd_2(dba)_3$ (1–10 mmol%Pd), NaO^{t}Bu (1.4 equiv), BINAP or 2-(di-*t*-butylphosphino)biphenyl (1–10 mmol%), aryl bromide (0.9 equiv), and amine (1.0 equiv) in toluene (1–2 mL), then evacuated and backfilled with argon. The reaction mixture was heated at 80 °C until the aryl bromide was consumed. The mixture was diluted with ethyl ester, washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography.

4.3.1. *N*-[1-Methyl-4-(phenylamino)pyrrole-2-carbonyl]-pyrrolidine (13). Yield: 41%; khaki crystals (*n*-hexane–ethyl acetate); mp 121 °C; ^{1}H NMR (500 MHz, $CDCl_3$) 7.17 (t, 2 H, J = 7.7 Hz), 6.78–6.72 (m, 3H), 6.60 (S, 1H), 6.39 (s, 1 H), 3.84 (s, 3H), 3.63 (br, 4H), 1.93–1.91 (m, 4H). MS (FAB) 269 (M^+). Anal. Calcd for $C_{16}H_{19}N_3O$: C, 71.35; H, 7.11; N, 15.60. Found: C, 71.09; H, 7.11; N, 15.46.

4.3.2. *N*-[1-Methyl-4-(4-tolylamino)pyrrole-2-carbonyl]-pyrrolidine (17). Yield: 22%; khaki crystals (*n*-hexane–ethyl acetate); mp 154 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.98 (d, 2H, J = 8.1 Hz), 6.70 (d, 2H, J = 8.1 Hz), 6.57 (d, 1H, J = 1.2 Hz), 6.37 (d, 1H, J = 1.7 Hz), 3.83 (s, 3H), 3.62 (br s, 4H), 2.04 (s, 3H), 1.91 (m, 4H). MS (FAB) 283 (M^+); HRMS (FAB, M) $C_{17}H_{21}N_3O$, calcd for 283.1685, found 283.1734.

4.3.3. *N*-[4-(4-Methoxyphenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (18). Yield: 53%; pale yellow needles; mp 157 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76 (m, 4H), 6.54 (d, 1H, J = 1.7 Hz), 6.34 (d, 1H, J = 1.7 Hz), 3.82 (s, 3H), 3.74 (s, 3H), 3.63 (br s, 4H), 1.91 (m, 4H). MS; *m*/*z* 299 (M^+); HRMS (FAB, M) $C_{17}H_{21}N_3O_2$, calcd for 299.1634, found 299.1680.

4.3.4. *N*-[1-Methyl-4-(4-nitrophenylamino)pyrrole-2-carbonyl]pyrrolidine (19). Yield: 34%; vermillion crystals (*n*-hexane–ethyl acetate); mp 218 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (d, 2H, J = 9.4 Hz), 6.70 (d, 2H, J = 9.4 Hz), 6.65 (d, 1H, J = 1.7 Hz), 6.39 (d, 1H, J = 1.7 Hz), 5.77 (s, 1H), 3.87 (s, 3H), 3.64 (br s, 4H), 1.94 (m, 4H). MS (FAB) 314 (M^+); HRMS (FAB, M) $C_{16}H_{18}N_4O_3$, calcd for 314.1379, found 314.1396.

4.3.5. *N*-[4-(4-Cyanophenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (20). Yield: 32%; pale yellow crystals (*n*-hexane–ethyl acetate); mp 198 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 (d, 2H, J = 8.9 Hz), 6.72 (d, 2H, J = 8.5 Hz), 6.62 (d, 1H, J = 1.7 Hz), 6.37 (d, 1H, J = 1.7 Hz), 5.50 (s, 1H), 3.86 (s, 3H), 3.63 (br s, 4H), 1.95–1.92 (m, 4H). MS (FAB) 294 (M^+). Anal. Calcd for $C_{17}H_{18}N_4O$: C, 69.37; H, 6.16; N, 19.03. Found: C, 69.33; H, 6.19; N, 19.06.

4.3.6. *N*-[4-(4-Fluorophenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (21). Yield: 36%; pale yellow crystals (*n*-hexane–ethyl acetate); mp 164 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.86 (dd, 2H, J = 8.5, 8.9 Hz), 6.70 (dd, 2H, J = 8.9, 4.2 Hz), 6.57 (d, 1H, J = 1.7 Hz), 6.35 (d, 1H, J = 1.7 Hz), 3.83 (s, 3H), 3.63 (br s, 4H), 1.92 (m, 4H). MS; *m*/*z* 287 (M^+). Anal. Calcd for $C_{16}H_{18}N_3OF$: C, 66.88; H, 6.31; N, 14.62. Found: C, 66.88; H, 6.42; N, 14.49.

4.3.7. *N*-[4-(4-Chlorophenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (22). Yield: 40%; white crystals (*n*-hexane–ethyl acetate); mp 185 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.10 (d, 2H, J = 8.5 Hz), 6.68 (d, 2H, J = 8.5 Hz), 6.58 (d, 1H, J = 2.1 Hz), 6.36 (d, 1H, J = 2.1 Hz), 3.84 (s, 3H), 3.62 (br s, 4H), 1.92 (m, 4H). MS; *m*/*z* 303, 305 (M^+), 304, 306 (MH^+). Anal. Calcd for $C_{16}H_{18}N_3OCl$: C, 63.26; H, 5.97; N, 13.83. Found: C, 63.07; H, 6.02; N, 13.78.

4.3.8. *N*-[1-Methyl-4-(3-tolylamino)pyrrole-2-carbonyl]-pyrrolidine (23). Yield: 21%; pale yellow crystals (*n*-hexane–ethyl acetate); mp 165 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.05 (dd, 1H, J = 8.5, 7.6 Hz), 6.59–6.55 (m, 4H), 6.38 (d, 1H, J = 1.7 Hz), 3.84 (s, 3H), 3.63 (br s, 4H), 2.26 (s, 3H), 1.92 (m, 4H). MS; *m/z* 283 (M^+). Anal. Calcd for $C_{17}H_{21}N_3O$: C, 72.06; H, 7.47; N, 14.83. Found: C, 71.76; H, 7.49; N, 14.62.

4.3.9. *N*-[4-(3-Methoxyphenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (24). Yield: 67%; khaki needles (*n*-hexane–ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.02 (d, 1H, J = 8.1 Hz), 6.59 (d, 1H, J = 1.7 Hz), 6.39 (d, 1H, J = 2.1 Hz), 6.38 (dd, 1H, J = 8.1, 2.1 Hz), 6.34 (t, 1H, J = 2.1 Hz), 6.31 (dd, 1H, J = 8.1, 2.1 Hz), 3.83 (s, 3H), 3.74 (s, 3H), 3.63 (br s, 4H), 1.91 (m, 4H). MS; *m/z* 299 (M^+). Anal. Calcd for $C_{17}H_{21}N_3O_2$: C, 68.20; H, 7.07; N, 14.04. Found: C, 68.36; H, 7.19; N, 13.90.

4.3.10. *N*-[1-Methyl-4-(3-nitrophenylamino)pyrrole-2-carbonyl]pyrrolidine (25). Yield: 9.6%; vermillion oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (dd, 1H, J = 2.1, 2.3 Hz), 7.53 (dd, 1H, J = 8.1, 2.1 Hz), 7.26 (t, 1H, J = 8.1 Hz), 7.00 (dd, 1H, J = 8.1, 2.1 Hz), 6.63 (d, 1H, J = 1.7 Hz), 6.39 (d, 1H, J = 1.7 Hz), 5.41 (s, 1H), 3.86 (s, 3H), 3.64 (br s, 4H), 1.93 (m, 4H). MS; *m/z* 314 (M^+), 315 (MH^+); HRMS (FAB, M) $C_{16}H_{18}N_4O_3$, calcd for 314.1379, found 314.1348.

4.3.11. *N*-[4-(3-Cyanophenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (26). Yield: 9.3%; yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) 7.20 (dd, 1H, J = 8.1, 7.7 Hz), 6.97–6.90 (m, 3H), 6.60 (d, 1H, J = 1.7 Hz), 6.35 (d, 1H, J = 1.7 Hz), 5.32 (s, 1H), 3.85 (s, 3H), 3.63 (br s, 4H), 1.93 (m, 4H). MS; *m/z* 294 (M^+), 295 ($M-H^+$); HRMS (FAB, M) $C_{17}H_{18}N_4O$, calcd for 294.1481, found 294.1491.

4.3.12. *N*-[4-(3-Fluorophenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (27). Yield: 32%; khaki crystals (*n*-hexane–ethyl acetate); mp 117 °C; ^{1}H NMR (500 MHz, $CDCl_3$) 7.08 (ddd, 1H, J = 8.1, 8.1, 6.0 Hz), 6.60 (d, 1H, J = 1.7 Hz), 6.50 (dd, 1H, J = 8.1, 2.1 Hz), 6.46–6.37 (m, 4H), 3.85 (s, 3H), 3.63 (br s, 4H), 1.92 (m, 4H). MS (FAB) *m/z* 287 (M^+). Anal. Calcd for $C_{16}H_{18}N_3OF$: C, 66.88; H, 6.31; N, 14.62. Found: C, 66.82; H, 6.50; N, 14.36.

4.3.13. *N*-[4-(3-Clorophenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (28). Yield: 18%; khaki crystals (*n*-hexane–ethyl acetate); mp 157 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.04 (dd, 1H, J = 8.14, 7.93 Hz), 6.72 (dd, 1H, J = 2.1, 2.1 Hz), 6.67 (ddd, 1H, J = 7.9, 1.8, 0.9 Hz), 6.60 (ddd, 1H, J = 8.2, 2.1, 0.9 Hz), 6.58 (d, 1H, J = 1.8 Hz), 6.36 (d, 1H, J = 2.1 Hz), 3.84 (s, 3H), 3.62 (br s, 4H), 1.92 (m, 4H). MS (FAB) *m/z* 303, 305 (M^+), 304, 306 ($M-H^+$); HRMS (FAB, M) $C_{16}H_{18}N_3OCl$, calcd for 303.1138, found 303.1151.

4.3.14. *N*-[1-Methyl-4-(2-tolylamino)pyrrole-2-carbonyl]-pyrrolidine (29). Yield: 6.0%; yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) 7.07 (d, 1H, J = 7.7 Hz), 7.04 (t, 1H, J = 8.1 Hz), 6.84 (d, 1H, J = 8.1 Hz), 6.68 (t, 1H, J = 7.2 Hz), 6.59 (d, 1H, J = 1.7 Hz), 6.39 (d, 1H, J = 1.7 Hz), 4.91 (s, 1H), 3.85 (s, 3H), 3.63 (br s, 4H), 2.23 (s, 3H), 1.92 (m, 4H). MS (FAB) *m/z* 283 (M^+); HRMS (FAB, M) $C_{16}H_{21}N_3O$, calcd for 283.1685, found 283.1695.

4.3.15. *N*-[4-(2-Methoxyphenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (30). Yield: 58%; yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) 6.87 (dd, 1H, J = 7.6, 2.1 Hz), 6.87–6.79 (m, 2H), 6.69 (dt, 1H, J = 7.6, 1.7 Hz), 6.61 (d, 1H, J = 1.7 Hz), 6.41 (d, 1H, J = 1.7 Hz), 5.65 (s, 1H), 3.88 (s, 3H), 3.63 (br s, 4H), 1.91 (m, 4H). MS (FAB) *m/z* 299 (M^+); HRMS (FAB, M) $C_{17}H_{21}N_3O_2$, calcd for 299.1634, found 299.1591.

4.3.16. *N*-[1-Methyl-4-(2-nitrophenylamino)pyrrole-2-carbonyl]pyrrolidine (31). Yield: 39%; vermillion oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.12 (s, 1H), 8.16 (dd, 1H, J = 8.5, 1.2 Hz), 7.33 (t, 1H, J = 7.2 Hz), 7.09 (dd, 1H, J = 8.5, 1.2 Hz), 6.69–6.67 (m, 2H), 6.66 (d, 1H, J = 1.2 Hz), 6.42 (d, 1H, J = 1.7 Hz), 3.88 (s, 3H), 3.64 (br, 4H), 1.94 (m, 4H). MS 314 (M^+), 315 (MH^+); HRMS (FAB, M) $C_{16}H_{18}N_4O_3$, calcd for:314.1379, found 314.1343.

4.3.17. *N*-[4-(2-Cyanophenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (32). Yield: 31% yellow oil; ^{1}H NMR (500 MHz, $CDCl_3$) 7.40 (d, 1H, J = 6.8 Hz), 7.29 (t, 1H, J = 8.1 Hz), 6.84 (d, 1H, J = 8.4 Hz), 6.69 (t, 1H, J = 7.6 Hz), 6.64 (d, 1H, J = 1.9 Hz), 6.39 (d, 1H, J = 1.7 Hz), 5.87 (s, 1H), 3.86 (s, 3H), 3.63 (br s, 4H), 1.93 (m, 4H). MS (FAB) *m/z* 294 (M^+), 295 (MH^+),HRMS (FAB, M); $C_{17}H_{18}N_4O$, calcd for: 294.1481, found 294.1489.

4.3.18. *N*-[4-(2-Fluorophenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (33). Yield: 22%; khaki crystals (*n*-hexane–ethyl acetate); mp 105 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.01–6.97 (m, 1H), 6.94–6.88 (m, 2H), 6.67–6.63 (m, 1H), 6.62 (d, 1H, J = 1.7 Hz), 6.40 (d, 1H, J = 2.1 Hz), 5.30 (s, 1H), 3.85 (s, 3H), 3.64 (br s, 4H), 1.92 (m, 4H). MS (FAB) *m/z* 287 (M^+); HRMS (FAB, M); $C_{16}H_{18}N_3OF$, calcd for: 287.1434, found 287.1400.

4.3.19. *N*-[4-(2-Clorophenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (34). Yield: 27%; khaki crystals (*n*-hexane–ethyl acetate); mp 124 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (dd, 1H, J = 7.2, 1.2 Hz), 7.04 (dt, 1H, J = 8.1, 1.2 Hz), 6.85 (dd, 1H, J = 8.5, 1.2 Hz), 6.66–6.63 (m, 2H), 6.40 (d, 1H, J = 2.1 Hz), 5.64 (s, 1H), 3.85 (s, 3H), 3.63 (br s, 4H), 1.92 (m, 4H). MS (FAB) *m/z* 303, 305 (M^+), 304, 306 ($M-H^+$). Anal. Calcd for $C_{16}H_{18}N_3OCl$: C, 63.26; H, 5.97; N, 13.83. Found: C, 62.99; H, 5.98; N, 13.78.

4.3.20. *N*-[4-(3,5-Dimethylphenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (35). Yield: 23%; white crystals (*n*-hexane–ethyl acetate); mp 134 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.91 (s, 1H), 6.50–6.47 (m, 3H), 6.40 (s, 1H), 3.84 (s, 3H), 3.68 (br s, 4H), 2.22 (s, 3H), 1.91 (br s, 4H). MS (FAB) *m/z* 297 (M^+), 298 ($M-H^+$). Anal.

Calcd for $C_{18}H_{23}N_3O$: C, 72.70; H, 7.80; N, 14.13. Found: C, 72.50; H, 7.70; N, 14.15.

4.3.21. *N*-[4-(3,5-Dichlorophenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (36). Yield: 23%; pale yellow crystals (*n*-hexane–ethyl acetate); mp 149 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.69–6.59 (m, 4H), 6.34 (s, 1H), 3.85 (s, 3H), 3.63 (br, 4H), 1.95–1.92 (m, 4H). MS (FAB) *m*/*z* 337,339 (M^+), 338,340 (MH^+); HRMS (FAB, M); $C_{16}H_{17}N_3OCl_2$, calcd for: 337.0749, found 337.0741.

4.4. Receptor binding assay

Binding affinities of test compounds for hAR (human androgen receptor) were measured in competition experiments using [^{3}H]testosterone and cytosolic fraction of hAR-LBD (hAR ligand-binding domain)-transformed *E. coli* as described previously.[14] A hAR-LBD expression plasmid vector which codes GST-hAR-LBD (627–919 aa, EF domain) fusion protein under the *lac* promoter (provided by Professor S. Kato, Univ. of Tokyo) was transfected into *E. coli* strain *HB-101*. The bacteria (10 mL) were cultured for two nights, then added to 1 L of LB medium and incubated at 27 °C until the OD_{600} reached 0.5–0.6. Following the addition of IPTG to a concentration of 1 mM, incubation was continued for an additional 4.5 h. Cells were harvested by centrifugation at 4000*g* at 4 °C for 15 min and stored at −80 °C until use. All subsequent operations were performed at 4 °C. The bacterial pellet obtained from 40 mL of culture was resuspended in 1 mL of ice-cold TEGDM buffer (10 mM Tris, 1 mM EDTA, 10% glycerol, 10 mM DTT, 10 mM sodium molybdate). This suspension was subjected to sonication using 7 s × 12 bursts on ice (USP-600A sonicator, Shimadzu, Japan) and crude GST-hAR-LBD fraction was prepared by centrifugation of the suspension at 12,000*g* for 30 min at 4 °C. This supernatant protein, crude receptor fraction, was diluted to a protein concentration of 0.3–0.5 mg/mL and used in binding assays as GST-hAR-LBD fraction. Total protein was determined by using a Coomassie Protein Assay Reagent and Albumin Standard (Pierce). Binding studies were performed by incubating increasing concentrations (100 nM to 100 μM) of test compound (dissolved in DMSO) with the GST-hAR-LBD fraction in the presence of a saturating concentration of [^{3}H]testosterone (10 nM) at 4 °C for 12–18 h. Non-specific binding was assessed by addition of a 1000-fold excess of non-radioactive testosterone. Separation of bound and free radioactivity was achieved by the charcoal method. After incubation was completed, 500 μL of 1.5% (w/v) dextran-coated charcoal (Sigma) was added to each reaction solution. After 20 min, the solution was centrifuged at 550*g* for 10 min (high-speed refrigerated microcentrifuge, TOMY MX-15). The protein fraction (350 μL) was collected and the radioactivity was determined with a liquid scintillation counter. All experiments were performed in duplicate.

4.5. SC-3 growth inhibition assay

Shionogi Carcinoma-3 (SC-3) cells were cloned from Shionogi Carcinoma 115 cells, which were established from a mouse breast cancer. SC-3 shows androgen-dependent growth.[15,16] In this assay, androgenic and anti-androgenic activities of test compounds were determined in terms of SC-3 growth promotion by test compound alone and inhibition of testosterone-induced cell growth, respectively. SC-3 cells were cultured in the presence of MEM supplemented with 2% FBS and 10 nM testosterone at 37 °C 5%CO_2. All experiments were performed in triplicate or more. For SC-3 cell growth-inhibition assay, the cells were trypsinized, and seeded into 96-well plates at 2.0×10^4 cell/mL in MEM containing 2% DCC-FBS. Various concentrations of test compound (from 0.1 μM to 100 μM DMSO solution) and/or testosterone in DMSO solution (final concentration 10 nM) were added on the next day. Then the plates were incubated at 37 °C 5% CO_2 for 3 days, and the cell number was determined using the WST-1 method with a Cell Counting Kit (Dojindo) and an MPR-A4i2 micro plate reader (Tosoh, Japan). The number of cells on wells with testosterone alone was defined as 100%. The concentration of test compounds that inhibited the increase of the cell number induced by 10 nM testosterone by 50% was quantified (IC_{50}) after log-logit transformation.

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