Bioorganic & Medicinal Chemistry 16 (2008) 6799–6812

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

4-(Anilino)pyrrole-2-carboxamides: Novel non-steroidal/non-anilide type androgen antagonists effective upon human prostate tumor LNCaP cells with mutated nuclear androgen receptor

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ARTICLE INFO

Article history:
Received 28 February 2008
Revised 13 May 2008
Accepted 28 May 2008
Available online 18 June 2008

Keywords:
Androgen
Antagonist
Pyrrole-2-carboxamide
Mutated receptor

ABSTRACT

Various 4-(anilino)pyrrole-2-carboxamides were designed and synthesized as novel androgen receptor (AR) antagonists without steroidal or anilide structure, based on our strategy for developing full antagonists of nuclear receptors. Introduction of a bulky *N*-alkyl group, such as a cyclohexylmethyl or benzyl group, increased the binding affinity for wild-type AR and the potency for growth inhibition of androgen-dependent SC-3 cells. Among the compounds obtained, *N*-[4-[(benzyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (**22**) is as potent an AR antagonist as the typical anilide-type AR antagonists hydroxyflutamide and bicalutamide. Further, compound **22** had potent binding affinity for T877A mutated AR, and dose-dependently inhibited the testosterone-induced production of prostate-specific antigen in LNCaP cells bearing T877A AR.

1. Introduction

Androgen receptor (AR) is a member of the nuclear receptor superfamily,[1] and is functionally regulated by the binding of androgens, such as testosterone (**1**, Fig. 1) and its more potent metabolite, dihydrotestosterone (DHT, **2**). These ligands induce sequential conformational changes of the receptor that result in receptor–protein and receptor–DNA interactions. Typical AR antagonists antagonize the biological responses elicited by endogenous and/or exogenous androgens by competitively inhibiting the binding of the latter to AR, and are expected to be effective for the treatment of androgen-dependent prostate cancer.

The AR antagonists so far known can be classified into two structural types, that is, steroidal and non-steroidal compounds.[2–4] Modification of the structures of endogenous androgens, such as **1** and **2**, afforded steroid-type derivatives, including cyproterone acetate (**3**), but these compounds often possess side effects that arise from cross-reactivity with other steroid hormone nuclear receptors, such as progesterone and estrogen receptors, and it is generally difficult to separate effects on androgenic activities from those on anabolic activities. For example, cyproterone acetate (**3**) was originally developed as a progestin that suppresses gonadotropin release,[5] and its AR-antagonistic activity was found as an undesired side effect.

The non-steroidal AR antagonists not only overcome these difficulties, but also achieve tissue selectivity. Moreover, non-steroidal skeletons represent robust scaffolds whose structural modification/development affords wide structural diversity as compared with the steroidal derivatives. For these reasons, various non-steroidal AR antagonists have been synthesized, of which some, such as flutamide (**4**) and bicalutamide (Casodex, **6**), are clinically used to treat prostate cancer (Fig. 1).[6–9] These AR antagonists have a common anilide structure with electron-withdrawing substituents. Although these anilide-type AR antagonists are useful for the treatment of prostate cancer in the early stage, prostate cancer often advances to a 'hormone-refractory' state in which the disease progresses even in the presence of continued androgen ablation or antagonist therapy, suggesting the development of androgen-independent prostate cancer cells.[10] In some cases, these antagonists may act as AR agonists toward the androgen-independent prostate cancer cells, and aggravate the disease. One possible reason for the hormone-refractory state is considered to be the mutation of AR.[11–13] For example, hydroxyflutamide (**5**), an active form of **4**, and **6** act as AR agonists toward T877A and W741L/W741C AR mutants, respectively.[14,15] Thus, new types of non-steroidal AR antagonists are needed to treat prostate cancers resistant to the known AR antagonists.[16–21]

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doi:10.1016/j.bmc.2008.05.063

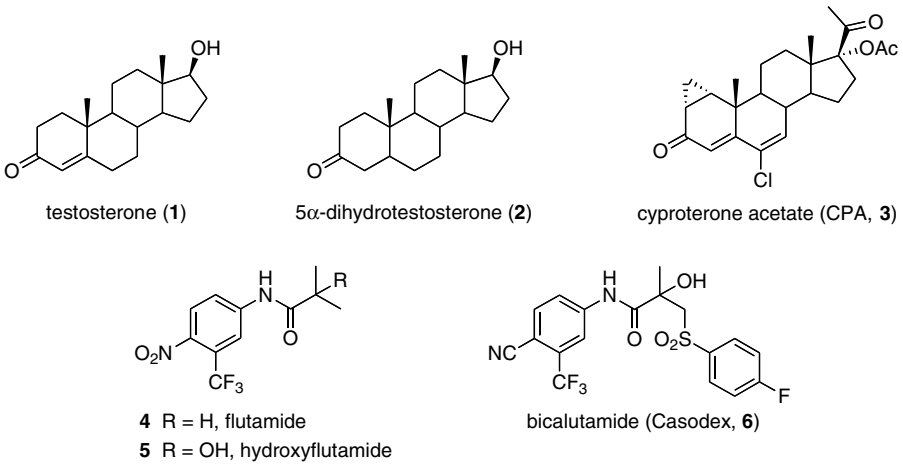


Figure 1. Structures of steroidal and non-steroidal androgen ligands.

Recently, X-ray crystallographic investigations of nuclear receptor–ligand interactions have uncovered possible mechanisms of the agonistic/antagonistic actions.[22–24] Thus, the ligand binding domain (LBD) of nuclear receptors contains 12 α-helices and two short β-turns, and the C-terminal helix (H12) in the LBD plays a critical role in the ligand-dependent activation of the receptor. When the agonist binds to the receptor, H12 is repositioned and serves as a 'lid' on the ligand-binding pocket to stabilize the ligand–receptor complex.[25] This agonist-induced conformational change of the receptor results in the dissociation of a corepressor and the recruitment of a coactivator. On the other hand, the binding of the antagonist to nuclear receptor does not induce the proper folding of H12, and consequently H12 is displaced from the active conformation of the nuclear receptor, and folds over the activation function 2 (AF2) region to disrupt coactivator recruitment or to stabilize the receptor–corepressor complex.[26]

Taking into account these molecular findings, it is one of effective molecular designs of nuclear receptor antagonists to disturb the proper conformation of H12.[27] Based on this idea, we have recently developed novel vitamin D antagonists.[28,29] Computer-assisted docking studies suggested that the bulky substituent on the side chain of vitamin D skeleton inhibits the folding of H12 of vitamin D nuclear receptor to elicit the antagonistic activity. We performed similar structural analyses for AR antagonists, and developed isoxazolone derivatives as novel non-steroidal AR antagonists.[27] That is, an isoxazolone derivative **7** (Fig. 2) was obtained as an AR ligand candidate by virtual screening of a database of commercially available molecules. The structural development of **7** afforded the potent AR antagonist **8** (ISOP-4), bearing a bulky chlorophenyl group that may disturb the H12 folding. Compound **8** possessed high binding affinity for AR and potently suppressed the growth of an androgen-dependent Shionogi carcinoma cell line, SC-3 (IC_{50} = 0.4 μM). In particular, **8** acted as an antagonist toward the mutant AR (T877A) in the LNCaP cell line, although its potency is not high. The results indicated our strategy that H12-folding inhibitor-type AR antagonists might be effective in both wild-type and mutated ARs, and also suggested that the development of non-steroidal/non-anilide type AR antagonists would be feasible. Recently, we reported another type of AR antagonists, 4-(anilino)pyrrole-2-carboxamide derivatives (**9**, and **10**), based on **8** as a lead compound.[30] These compounds showed moderate AR binding affinity and inhibited the growth of SC-3 cells at concentrations of the order of 10 μM, although these compounds are weaker AR antagonists than **5** or **6**. In this paper, we describe the development of novel AR antagonists possessing higher affinity than **5** and **6** for both wild-type and mutant Ars, by using **9** and **10** as lead compounds.

2. Results and discussion

2.1. Design and synthesis of novel AR antagonists

Our previous studies on the SARs of **8** and its derivatives as AR antagonists indicated that a general structural feature for AR-

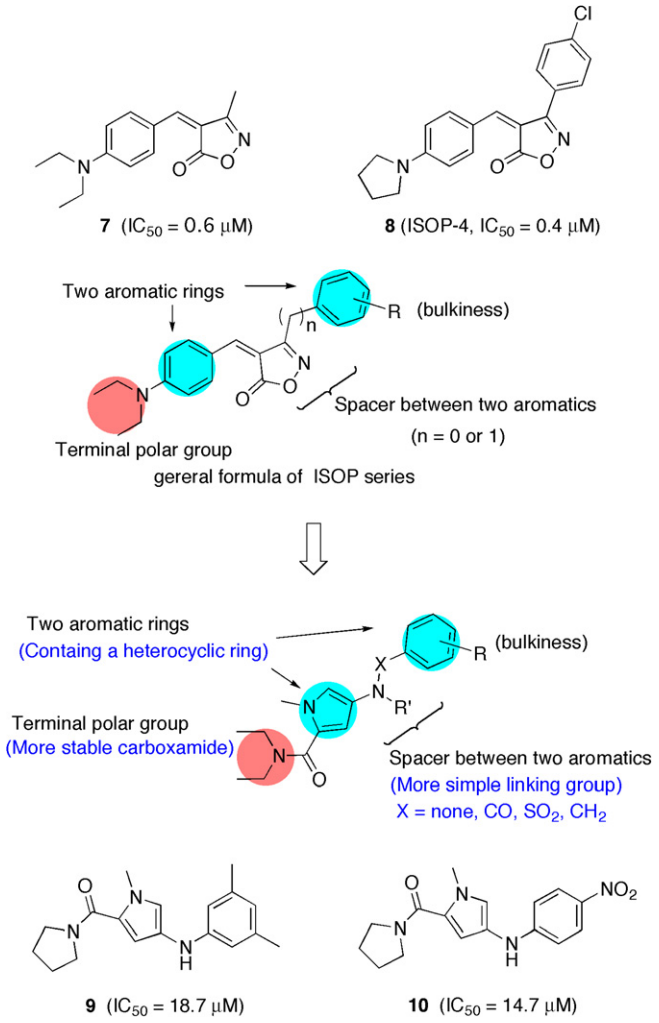
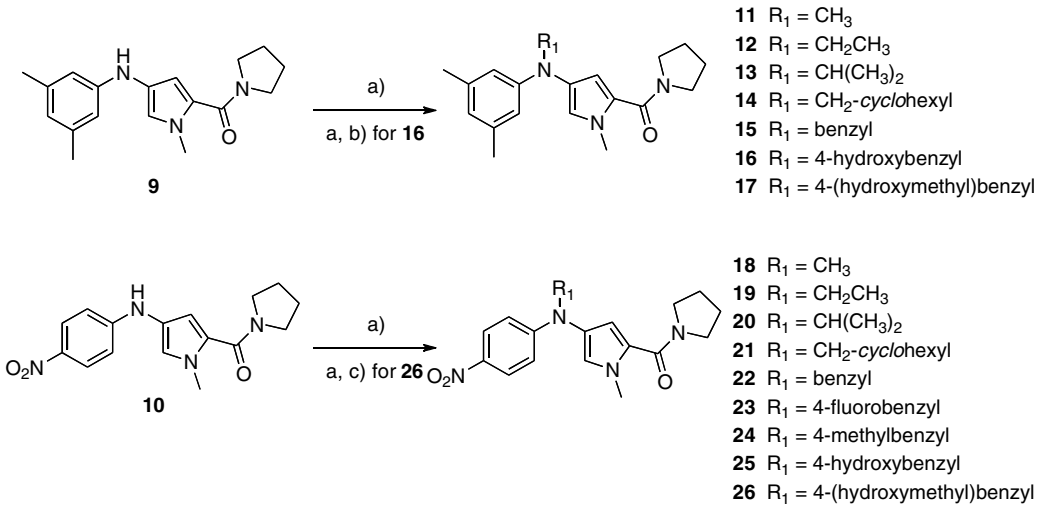


Figure 2. Design of novel non-steroidal/non-anilide type AR antagonists. The IC_{50} values in SC-3 growth-inhibitory assay are shown in parentheses.

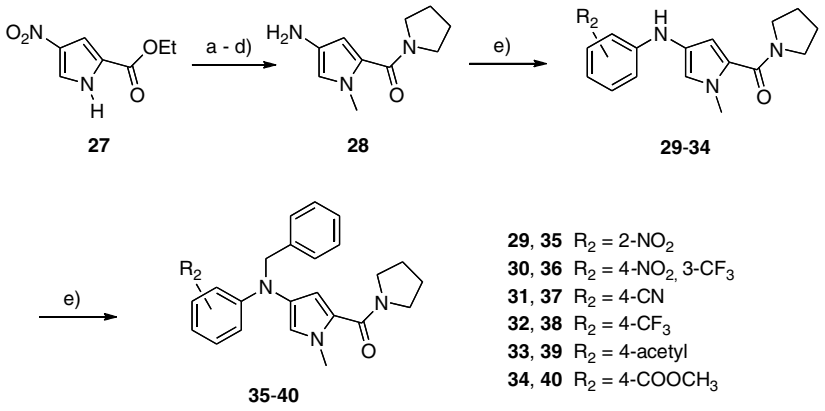
antagonistic activity consists of two aromatic rings connected by a suitable linking group, with one benzene ring bearing a dialkylamino group, that is, such structures meet the requirements for binding to the receptor (Fig. 2).[27] Comparison of the partial agonistic activity of **7** and the antagonistic activity of **8** showed that the chlorophenyl group of **8** is thought to disturb the folding of H12 of AR. Similarly, the pyrrolecarboxamides, **9** and **10**, have two aromatic rings linked by a nitrogen atom.[30] In these compounds, the terminal pyrrolidine ring is attached to the aromatic ring through the carbonyl group, affording a more stable amide structure. When we compare the molecular structures of **8** and **9**/**10**, the pyrrolecarboxamides are smaller in size than the corresponding moiety of **8**, and this might reduce the AR binding affinity. In other words, the *N*-(pyrrolyl)aniline skeleton of **9** and **10** may correspond to the phenylmethanoisoxazolone structure of **8**. Therefore, introduction of a bulky substituent corresponding to the chlorophenyl group of **8** into **9**/**10** should be effective for the development of novel AR antagonists. We thus designed and synthesized novel (*N, N*-disubstituted amino)pyrrolecarboxamide derivatives as shown in Schemes 1–3.

The *N,N*-disubstituted 4-(anilino)pyrrole-2-carboxamides **11**–**17** and **18**–**24** were synthesized from **9** and **10**, respectively, by treatment with NaH, followed by addition of alkyl halide (Scheme 1). In the case of an alkyl halide bearing a hydroxyl group, direct N-alkylation proceeded in low yield (31% for compound **17**). Therefore, the hydroxyl group was protected with an acetyl (for compound **16**) or *tert*-butyldimethylsilyl group (for compounds **25** and **26**), and then deprotected after the N-alkylation. In order to evaluate substituent effects on the phenyl ring of **22**, various *N*-aryl-*N*-benzylaminopyrrolecarboxamides, **35**–**40**, were synthesized (Scheme 2). Ethyl 4-nitropyrrole-2-carboxylate (**27**) was converted to the aminopyrrolecarboxamide **28** in four steps.[30] Compound **28** was treated with substituted bromobenzene in the presence of $Pd_2(dba)_3$, BINAP, and Cs_2CO_3 to afford compounds **29**–**34**,[31] which were *N*-benzylated to give compounds **35**–**40**.

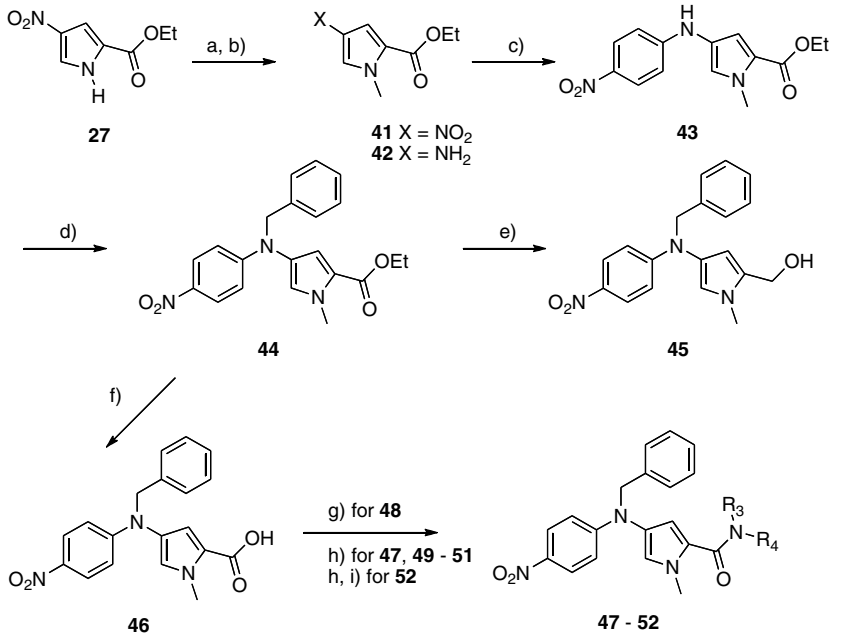
The pyrrolidine ring of **8** is important for the AR-antagonistic activity. Therefore, several modifications of the pyrrolidine ring of **22** were performed, that is, introduction of ester (**44**), hydroxymethyl (**45**), carboxylate (**46**), and various carboxamides (**47**–**52**) (Scheme 3). N-Methylation followed by catalytic hydrogenation of ethyl 4-nitropyrrole-2-carboxylate (**27**) afforded **42**. Condensation of **42** and 1-bromo-4-nitrobenzene in the presence of $Pd_2(dba)_3$, BINAP, and Cs_2CO_3 afforded **43** in 56% yield. In this reaction, when we used *t*-BuONa instead of Cs_2CO_3,**43** was obtained in lower yield. Benzylation of **43** gave **44** in 94% yield, and **44** was reduced with $LiAlH_4$ to afford **45** in 68% yield, or saponified with



Scheme 1. Syntheses of (*N, N*-disubstituted amino)pyrrolecarboxamides. Reagents: (a) Alkyl halide, NaH, DMF; (b) K_2CO_3, MeOH; (c) TBAF, DMF.



Scheme 2. Syntheses of *N*-aryl-*N*-benzylaminopyrrolecarboxamides. Reagents and conditions: (a) 15% KOH, reflux; (b) $(COCl_2)_2$, CH_2Cl_2, DMF then pyrrolidine, CH_3CN; (c) NaH, CH_3I, DMF; (d) 10% Pd/C, H_2, AcOEt; (e) R_3-PhBr, BINAP, $Pd_2(dba)_3$, Cs_2CO_3, toluene, 80 °C; (f) BnBr, NaH, DMF.



Scheme 3. Syntheses of *N*-aryl-*N*-benzylaminopyrrole derivatives. Reagents and conditions: (a) NaH, CH_3I, DMF; (b) 10% Pd/C, H_2, AcOEt; (c) 1-bromo-4-nitrobenzene, BINAP, $Pd_2(dba)_3$, Cs_2CO_3, toluene, 80 °C; (d) BnCl, NaH, DMF; (e) $LiAlH_4$, THF, 0 °C; (f) 30% KOH, MeOH, THF; (g) diethylamine, HOBt, EDCI, CH_2Cl_2; (h) MsCl, Et_3N, THF, 0 °C then R_1R_2NH; (i) concd HCl, MeOH, 65 °C.

potassium hydroxide to produce **46** in 96% yield. The key intermediate **46** was converted to various amides **47**–**52** by using HOBt/EDCI or MsCl/Et_3N as coupling reagents.

2.2. Biological activities

The test compounds were first evaluated by using a combination of two in vitro assay systems, that is, a competitive binding assay of wild-type AR (wtAR) using [^{3}H]testosterone and a growth inhibition assay using androgen-dependent SC-3 cells.[32] Activity of the selected compounds toward mutated AR was analyzed by the use of LNCaP cells, which express T877A point-mutated AR.[33–35] Quantitative assessment of androgenic and anti-androgenic activities of the compounds was performed by measurement of the amount of prostate-specific antigen (PSA) produced by LNCaP cells. PSA is a marker molecule of prostate tumor malignancy and its production is known to be induced by androgens.[36,37]

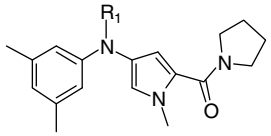
None of the pyrrolecarboxamides exhibited AR-agonistic activity in our assay systems. The effect of the introduction of N-substituents into compound **9** on the AR-antagonistic activities is shown in Table 1. Introduction of a small N-substituent (compounds **11**–**13**) did not affect the activity, while introduction of a bulky substituent such as a cyclohexylmethyl or a benzyl group caused an increase of the activity: the wtAR binding affinities of **14** (K_i = 0.49 μM) and **15** (K_i = 0.35 μM) are more potent than that of **9** by one order of magnitude, and these compounds are as active as **5** (K_i = 0.43 μM). However, they showed high cytotoxicity in SC-3 cell assay at concentrations above 1 μM. Compounds **16** and **17**, bearing a polar hydroxyl group, showed weaker wtAR affinity than the corresponding derivative **15**, but these compounds are less toxic, and they inhibited SC-3 cell growth with similar potency to **4**. Similar SARs were observed among the derivatives of compound **10** bearing a polar nitro group on the anilino moiety (Table 2). Among the synthesized compounds, the *N*-cyclohexylmethyl (**21**, K_i = 0.52 μM) and *N*-benzyl analogs (**22**, K_i = 0.11 μM) showed much greater wtAR affinity and more potent SC-3 cell growth inhibition. Unlike **14** and **15**, compounds **21** (IC_{50} = 0.88 μM) and **22** (IC_{50} = 0.44 μM) did not show apparent cytotoxicity under the assay conditions, and showed similar inhibitory activity to **5** (IC_{50} = 0.35 μM) and **6** (IC_{50} = 0.27 μM). In this case, also, the introduction of a further substituent, such as a fluoro, methyl, or hydroxyl group, on the benzyl group of **22** decreased the potency in either of the assays.

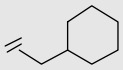
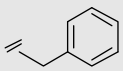
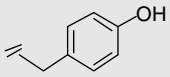
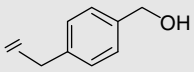
Since some compounds bearing the xylidine moiety, such as **14** and **15**, exhibited significant cytotoxicity, we examined further structural modification, using **22** as the lead compound. For this purpose, we synthesized several analogs of **22** having an electron-withdrawing group other than a nitro group on the benzene ring, and determined their activities (Table 3). Among the synthesized compounds, **22** was the most potent. Interestingly compound **36**, having the same substituents (3-CF_3, 4-NO_2) as flutamide (**4**), showed weaker activity than **22**. Compound **37**, having a 4-cyano group, showed similar potency to **22**, while replacement of the nitro group of **22** with a trifluoromethyl, an acetyl, or an ester group resulted in relatively strong binding affinity, but weaker antagonistic activity as compared with **22** in SC-3 cell assay.

Next, we examined the modification of the terminal carboxamide group. Except for compounds **49**,**51**, and **52**, all the derivatives shown in Table 4 exhibited potent wtAR binding affinity, but the potency in SC-3 cell growth inhibition assay did not correlate with the binding affinity. The ester derivative **44** (K_i = 0.18 μM, IC_{50} = 0.95 μM) was nearly as active as compound **22** in both assays, while a carboxylic acid derivative **46** showed higher wtAR binding affinity (K_i = 0.075 μM) and exhibited partial agonistic activity in SC-3 growth inhibition assay (data not shown). Based on these results, the amide derivatives seem to be more effective as AR antagonists than the corresponding ester or carboxylic acid derivatives, and compound **22** was the most potent AR antagonist among the synthesized compounds, at least in our two assay systems.

In the structural development of **8** into pyrrolecarboxamides, we introduced a pyrrole ring instead of one of the two benzene rings of **8**. In order to confirm the significance of the pyrrolecarboxamide skeleton, the biological activities of the benzenecarboxa-

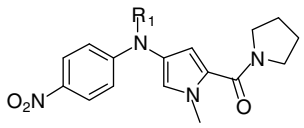
Table 1
wtAR binding affinity and antagonistic activity of compounds **9** and **11–17**



Compound	R_1	wtAR binding affinity[a] (K_i, μM)	SC-3 growth-inhibitory activity[b] (IC_{50}, μM)
4	Flutamide	3.7 ± 0.9	4.2 ± 0.5
5	Hydroxyflutamide	0.43 ± 0.03	0.35 ± 0.02
6	(*R*)-Bicalutamide	0.049 ± 0.012	0.27 ± 0.05
9	H	3.1 ± 1.0	15.0 ± 0.2
11	CH_3	6.1 ± 0.3	26
12	CH_2CH_3	2.7 ± 0.8	22.5 ± 2.1
13	$CH(CH_3)_2$	2.7 ± 1.6	10
14		0.49 ± 0.54	—[c]
15		0.35 ± 0.13	—[c]
16		0.69 ± 0.05	6.6 ± 4.8
17		2.2 ± 1.8	5.8 ± 0.2

[a] Competitive binding assay using 10 nM [^{3}H]testosterone and wtAR with test compounds. The conditions were the same as for other tables and Figure 3.
[b] The antagonistic activity was assessed in terms of IC_{50} for the growth of SC-3 cells induced by 10 nM testosterone. The conditions were the same as for other tables and Figure 3.
[c] Toxicity was observed at 1 μM test compound.

Table 2
wtAR binding affinity and antagonistic activity of compounds **10** and **18–26**



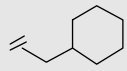
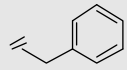
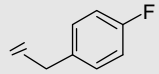
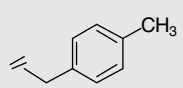
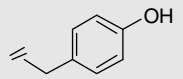
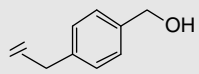
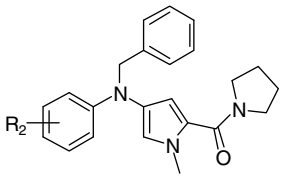
Compound	R_1	wtAR binding affinity (K_i, μM)	SC-3 growth-inhibitory activity (IC_{50}, μM)
10	H	4.9 ± 0.7	>30
18	CH_3	3.6 ± 0.6	>30
19	CH_2CH_3	2.0 ± 0.5	15.5 ± 3.5
20	$CH(CH_3)_2$	3.4 ± 0.9	14.5 ± 2.1
21		0.52 ± 0.42	0.88 ± 0.18
22		0.11 ± 0.02	0.44 ± 0.08
23		0.80 ± 0.08	4.0 ± 0.5
24		0.81 ± 0.22	4.7 ± 0.4
25		0.73 ± 0.02	4.8 ± 0.7
26		1.8 ± 0.5	5.5 ± 2.6

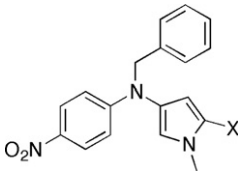
Table 3
wtAR binding affinity and antagonistic activity of compounds **35**–**40**

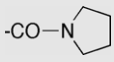
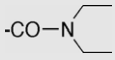
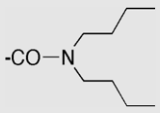
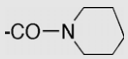
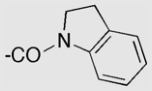
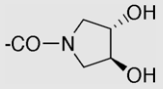


Compound	R_2	wtAR binding affinity (K_i, μM)	SC-3 growth-inhibitory activity (IC_{50}, μM)
22	4-NO_2	0.11 ± 0.03	0.44 ± 0.08
35	2-NO_2	1.3 ± 0.1	—[b]
36	3-CF_3, 4-NO_2	—[a]	2.5 ± 0.7
37	4-CN	0.13 ± 0.06	0.61 ± 0.23
38	4-CF_3	0.27 ± 0.03	1.7 ± 0.6
39	4-Acetyl	0.14 ± 0.05	2.0 ± 0.8
40	4-$COOCH_3$	0.18 ± 0.04	6.1 ± 3.1

[a] 52% inhibition at 30 μM test compound.
[b] 27% inhibition at 1 μM test compound.

Table 4
wtAR binding affinity and antagonistic activity of compounds **44**–**52**

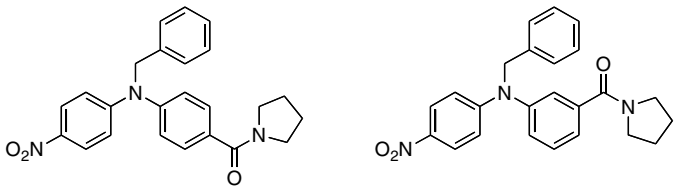


Compound	X (-CO-NR_3R_4)	wtAR binding affinity (K_j, μM)	SC-3 growth-inhibitory activity (IC_{50}, μM)
22		0.11 ± 0.03	0.44 ± 0.08
44	–COOEt	0.18 ± 0.15	0.95 ± 0.28
45	–CH_2OH	0.17 ± 0.06	2.1 ± 0.1
46	–COOH	0.075 ± 0.042	—[d]
47	–$CONH_2$	0.22	1.95
48		0.10 ± 0.01	0.99 ± 0.59
49		—[a]	6.1 ± 3.8
50		0.11 ± 0.01	1.7 ± 0.1
51		—[b]	1.0 ± 0.8
52		—[c]	13.7 ± 6.1

[a] 25% inhibition at 1 μM test compound.
[b] 25% inhibition at 3 μM test compound.
[c] 42% inhibition at 1 μM test compound.
[d] Compound **46** showed partial agonistic activity alone, and weak antagonistic activity toward 10 nM testosterone (IC_{50}: 6.2 μM).

mides **53** and **54** were examined. The activities of **53** and **54** were considerably decreased, especially in the SC-3 cell growth inhibition assay (Fig. 3), which confirms that the pyrrole ring is important for potent AR-antagonistic activity.

AR gene mutations in the LBD, which alter the ligand specificity and/or functional activity, are thought to contribute to the ability of some antagonists of wtAR to exhibit agonistic activity toward mutated ARs. LNCaP prostate cancer cells express AR with a T877A point mutation on helix 11, and wtAR antagonists such as **5** promoted the growth of these cells,[33–35] while **6** inhibited their proliferation. Therefore, we examined the binding affinity of selected compounds for T877A AR from homogenized LNCaP cells (Table 5). Under our experimental conditions, both **5** (K_i = 0.003 μM) and **6** (K_i = 0.010 μM) had potent affinities for T877A AR, and compound **22** (K_i = 0.005 μM) showed higher affinity for LNCaP than did **6** (Table 5). It should be noted that direct comparison of K_i values themselves determined for T877A AR in this paper with those for wtAR determined in this paper is not



53
K_i, 0.34 μM[a]; IC_{50}, 3.7 μM[b]

54
K_i, 0.37 μM[a]; IC_{50}, 4.9 μM[b]

[a]wtAR binding affinity, and [b]SC-3 growth inhibition activity

Figure 3. Structures and activities of benzenecarboxamides **53** and **54**.

appropriate, because the former and the latter were assessed by using full-size intact T877A AR extracted from human LNCaP cells and recombinant human AR ligand binding domain, respectively. Concerning the cell assay, species difference between murine SC-3 and human LNCaP cells might not be excluded. Even though, it is interesting that compound **15**, which has a wtAR affinity comparable with that of **22**, exhibited only very weak binding affinity for T877A AR. Other compounds bearing electron-withdrawing substituents (compounds **37**–**40**) showed similar binding affinity toward both wt and T877A ARs. On the other hand, compound **51**, which lacks affinity for wtAR, showed a T877A AR affinity as potent as those of **5** and **6**. This result suggests that the binding domain of T877A AR may have a larger pocket near the residues that interact with the carboxamide moiety of **22** as a result of the mutation. It has been reported that other steroid hormones also can bind the mutated T877A receptor, promoting the growth of mutated prostate cancer cells.[33–35]

Then, we determined the AR-agonistic and -antagonistic activities of selected compounds by measuring with an ELISA the amount of prostate-specific antigen (PSA) produced by LNCaP cells (Fig. 4). Agonistic activity can be assessed in terms of enhanced production of PSA and antagonistic activity can be detected in terms of inhibition of PSA production induced by **1**. Compound **5** promoted PSA production as well as **1**. A carboxyl derivative **46** with wtAR partial agonistic activity also increased the PSA production, while **6** and all the carboxamides examined showed no agonistic activity, except for **48** at high concentration. Among them,

Table 5
Binding affinity for T877A mutated AR

Compound	K_i, μM[a]
Hydroxyflutamide (**5**)	0.003 ± 0.001 (0.43)
(*R*)-Bicalutamide (**6**)	0.010 ± 0.004 (0.051)
15	(0.35)[b]
22	0.005 ± 0.001 (0.11)
23	0.14 ((0.80)
24	0.13 (0.81)
25	0.33 (0.73)
26	1.4 (1.8)
36	0.049 ± 0.007 (—)
37	0.006 ± 0.003 (0.13)
38	0.036 ± 0.009 (0.27)
39	0.13 ± 0.06 (0.14)
40	0.18 ± 0.01 (0.18)
44	0.007 ± 0.001 (0.18)
46	0.10 ± 0.07 (0.075)
48	0.003 ± 0.001 (0.10)
50	0.027 ± 0.011 (0.11)
51	0.009 ± 0.007 (—)

[a] Competitive binding assay using 1 nM [^{3}H]testosterone and soluble fraction of homogenized LNCaP cells with test compounds. K_i values toward wtAR are shown in parentheses.
[b] 20% inhibition at 3 μM test compound.

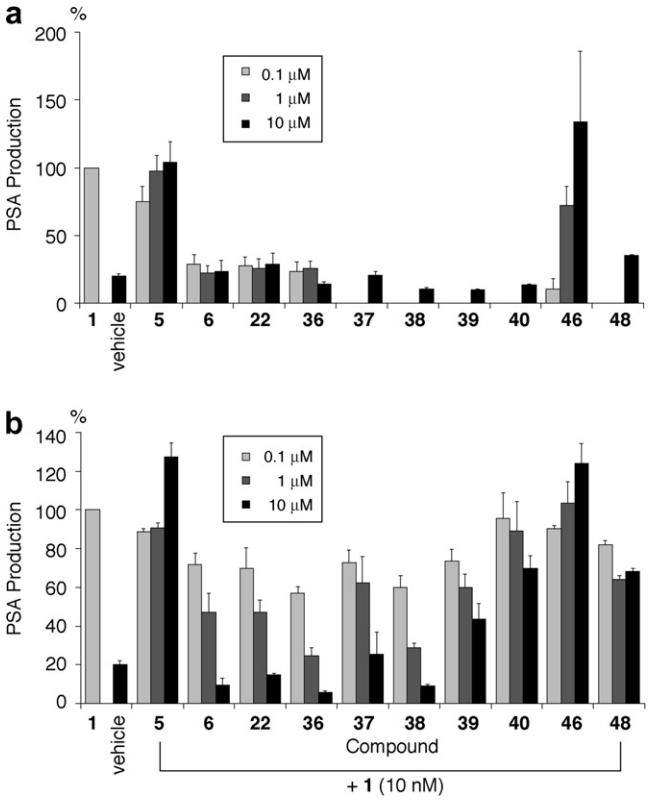


Figure 4. The effect on PSA production of test compounds (a) alone and (b) in the presence of 10 nM testosterone (**1**) in LNCaP cells. The amount of PSA produced in the presence of test compounds was normalized to that produced in the presence of 10 nM testosterone (**1**) alone, taken as 100%.

bicalutamide (**6**) and the carboxamides **22**, **36**–**38** showed AR-antagonistic activity in LNCaP cells (Fig. 4b). Compounds **39** and **40** have relatively weak binding ability for T877A AR (25–35 times weaker binding than compound **22**), and consistently with this, they did not show AR-antagonistic activity. Compound **48**, which has the most potent binding ability to T877A AR (K_i = 0.003 μM), did not inhibit PSA production. Compound **22**, as well as **6**, tended to suppress the proliferation of LNCaP cells, while **5** increased LNCaP cell growth (*data not shown*). Thus, compound **22** acted as a potent AR antagonist toward both wtAR and T877A AR.

The SARs of this series of AR ligands indicate that our strategy is useful for the design of novel AR antagonists. The introduction of a bulky N-substituent into the weak AR antagonists **9** and **10** increased both the AR affinity and the antagonistic activity. A xylidine derivative **15** did not bind to T877A AR, which means that the xylene moiety of compound **15** seems to be important for selective binding to wtAR. The aromatic ring with an electron-withdrawing group is favorable for enhancing the binding affinity of the compound to the mutated AR. Another significant structural feature is the carboxamide group. The carboxyl derivative **46** has a high binding affinity for wtAR and acted as a partial agonist toward wtAR. The diethylamide **48** is a potent antagonist for wtAR, but not for T877A AR. Introduction of the bulky substituent on the polar functional group (compounds **49, 51**, and **52**) decreased the wt AR binding affinity, while compound **51** showed potent binding affinity for T877A AR. Thus, the effect of the polar substituent on the 2-position of the pyrrole ring is also significant for both binding affinity and activation/inactivation of wild-type and mutated ARs. We examined computer-assisted docking calculations of our novel compounds using the reported crystal structures of AR LBD. However, only the crystal structures of AR LBD-agonist complexes

(active conformations) have been reported so far, including T877A AR-bicyclic isoindoledione derivative[38] and W741L AR-**6**[15] (agonist for this mutant) complexes. In our computational study using the active form of wtAR, several structures were picked up as possible stable receptor–ligand complexes. More detailed analyses on the interaction of novel AR antagonists with AR LBDs, both wild-type and mutants, including theoretical and X-ray crystallographical analyses, are ongoing.

3. Conclusion

In line with our strategy for developing full antagonists of nuclear receptors, we have designed and synthesized a series of AR antagonists with a pyrrolecarboxamide scaffold. Among them, compound **22** displayed high binding affinity for wtAR and potent growth-inhibitory activity against SC-3 cells. Further, compound **22** exhibited antagonistic activity against T877A AR, having a potency comparable with that of (*R*)-bicalutamide (**6**). Although it is unknown whether compound **22** antagonizes other mutated ARs, including W741L/W741C AR mutants, for which bicalutamide (**6**) acts as an agonist, compound **22** is considered to be promising as a scaffold for the development of novel agents for anti-AR therapy of prostate cancer.

4. Experimental

4.1. General information

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. Elemental analyses were carried out in the Microanalytical Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo, and were within ±0.3% of the theoretical values. Concerning oily/non-crystalline compounds, their purity was confirmed by thin layer chromatography and proton nuclear magnetic resonance (NMR) spectroscopy. Fast atom bombardment mass spectra (FAB-MS) were measured with a MS-JEOL JMS-HX110 mass spectrometer using a nitrobenzyl alcohol matrix as appropriate. NMR spectra data were obtained on a JEOL ALPHA500 spectrometer (500 MHz). 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). Compounds **9**, **10**, and **28** were prepared by the methods previously reported.[30]

4.2. Synthesis

4.2.1. General synthetic procedure A for compounds 11–26

Alkyl halide (1.1–1.5 equiv) was added to a suspension of **9** or **10** (1 equiv) and sodium hydride (1.5–2 equiv) in DMF at 0 °C. The reaction mixture was stirred until **9** or **10** was consumed, then poured into water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography or preparative TLC.

4.2.2. *N*-[4-[(3,5-Dimethylphenyl)(methyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (11)

The title compound was prepared from compound **9** and methyl iodide by procedure A (73%). Yellow oil: ^{1}H NMR ($CDCl_3$) δ 6.54 (d, 1H, J = 1.7 Hz), 6.49 (s, 2H), 6.42 (s, 1H), 6.36 (d, 1H, J = 1.7 Hz), 3.86 (s, 3H), 3.63 (br, 4H), 3.18 (s, 3H), 2.24 (s, 6H), 1.93–1.90 (m, 4 H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.6, 149.8, 138.3, 131.7, 124.6, 121.0, 119.6, 112.2, 109.9, 49.3, 46.2, 41.0, 36.5, 26.5, 24.0, 21.5. MS *m/z* 311 (M^+), 312 (MH^+); HRMS calcd for $C_{17}H_{21}N_3O$ (M) 311.1988, found 311.2017.

4.2.3. *N*-[4-[(3,5-Dimethylphenyl)(ethyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (12)

The title compound was prepared from compound **9** and ethyl iodide by procedure A (27%). Colorless crystals: mp 86–87 °C (*n*-hexane–ethyl acetate): ^{1}H NMR ($CDCl_3$) δ 6.57 (s, 1H), 6.48 (s, 2H), 6.41 (s, 1H), 6.36 (s, 1H), 3.86 (s, 3H), 3.62 (br, 4H), 3.58 (q, 2H, J = 7.2 Hz), 2.22 (s, 6H), 1.92–1.90 (m, 4H), 1.19 (t, 3H, J = 7.2 Hz).^{13}C NMR (125 MHz, $CDCl_3$) δ 161.6, 148.5, 138.6, 129.1, 124.9, 122.4, 119.5, 112.2, 111.2, 49.4, 47.3, 46.2, 36.6, 26.6, 24.1, 21.6, 12.4. MS *m/z* 325 (M^+), 326 (MH^+). Anal. Calcd for $C_{20}H_{27}N_3O$: C, 73.81; H, 8.36; N, 12.91. Found: C, 73.93; H, 8.27; N, 12.96.

4.2.4. *N*-[4-[(3,5-Dimethylphenyl)(*iso*-propyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (13)

The title compound was prepared from compound **9** and isopropyl iodide by procedure A at 100 °C for 17 h (13%). Yellow oil: ^{1}H NMR ($CDCl_3$) δ 6.49 (s, 1H), 6.34 (s, 3H), 6.28 (d, 1H, J = 1.7 Hz), 4.19 (sept, 1H, J = 6.4 Hz), 3.89 (s, 3H), 3.62 (br, 4H), 2.21 (s, 6H), 1.92 (m, 4H), 1.10 (d, 6H, J = 6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.7, 149.9, 138.3, 138.3, 125.9, 124.9, 123.6, 118.5, 115.1, 111.4, 49.5, 47.0, 46.3, 36.7, 26.6, 24.1, 21.7, 20.7; MS *m/z* 339 (M^+), 340 (MH^+); HRMS calcd for $C_{21}H_{29}N_3O$ (M) 339.2311, found 339.2310.

4.2.5. *N*-[4-[(Cyclohexylmethyl)(3,5-dimethylphenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (14)

The title compound was prepared from compound **9** and cyclohexylmethyl bromide by procedure A at 100 °C for 17 h (13%). Yellow oil; ^{1}H NMR ($CDCl_3$) δ 6.51 (s, 1H), 6.42 (s, 2H), 6.36 (s, 1H), 6.33 (s, 1H), 3.86 (s, 3H), 3.62 (br, 4H), 3.26 (d, 2H, J = 6.8 Hz), 2.22 (s, 6H), 1.93–1.90 (m, 4H), 1.81 (d, 2H, J = 6.8 Hz), 1.74–1.68 (m, 4H), 1.22–1.12 (m, 3H), 0.97–0.88 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.6, 150.0, 138.3, 130.8, 124.7, 122.3, 119.0, 112.1, 111.2, 59.9, 49.4, 46.2, 36.6, 31.2, 26.6, 25.9, 24.1, 21.6, 21.6; MS *m/z* 393 (M^+); HRMS calcd for $C_{25}H_{35}N_3O$ (M) 393.2624, found 393.2626.

4.2.6. *N*-[4-[(Benzyl)(3,5-dimethylphenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (15)

The title compound was prepared from compound **9** and benzyl bromide by procedure A (58%). White crystals: mp 130 °C (*n*-hexane–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 7.34–7.27 (m, 4H), 7.22 (t, 1H, J = 6.8 Hz), 6.54 (s, 3H), 6.43 (s, 1H), 6.37 (s, 1H), 4.80 (s, 2H), 3.80 (s, 3H), 3.59–3.57 (m, 4H), 2.19 (s, 6H), 1.91–1.88 (m, 4H);^{13}C NMR (125 MHz, $CDCl_3$) δ 161.5, 148.8, 138.8, 129.0, 126.7, 125.8, 121.6, 120.4, 112.7, 110.4, 57.4, 48.0, 47.3, 36.5, 25.2, 21.6; MS *m/z* 387 (M^+), 388 (MH^+); Anal. Calcd for $C_{25}H_{29}N_3O$: C, 77.48; H, 7.54; N, 10.84. Found: C, 77.42; H, 7.59; N, 10.82.

4.2.7. *N*-[4-[(3,5-Dimethylphenyl)(4-hydroxyphenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (16)

4-(Chloromethyl)phenyl acetate (104 μL, 0.67 mmol) was added to a suspension of **9** (100 mg, 0.34 mmol), triethylamine (94 μL, 0.67 mmol), and KI (112 mg, 0.67 mmol) in DMF (2 mL) at 0 °C. The reaction mixture was stirred at room temperature for 16.5 h, then poured into water, and extracted with ethyl acetate. 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 = 3:2; 1:1) and preparative TLC (*n*-hexane–ethyl acetate = 1:4) to give *N*-[4-[(4-acetoxyl-

phenyl)(3,5-dimethylphenyl)amino]-1-methylpyrrole-2-carbonyl] pyrrolidine as a colorless oil (110 mg, 76%); ^{1}H NMR ($CDCl_3$) δ 7.31 (d, 2H, J = 8.5 Hz), 7.01 (d, 2H, J = 8.5 Hz), 6.51 (d, 1H, J = 2.1 Hz), 6.42 (s, 2H), 6.41 (s, 1H), 6.35 (d, 1H, J = 2.1 Hz), 4.78 (s, 2H), 3.81 (s, 3H), 3.62–3.56 (m, 4H), 2.29 (s, 3H), 2.20 (s, 6H), 1.94–1.88 (br, 4H); MS m/z 445 (M^+), 446 (MH^+), HRMS calcd for $C_{27}H_{31}N_3O_3$(M) 445.2365, found 445.2364. K_2CO_3 (18 mg, 0.13 mmol) was added to a solution of *N*-[4-[(4-acetoxylphenyl)(3,5-dimethylphenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (58 mg, 0.13 mmol) in methanol (500 μL) and water (50 μL) at room temperature, and the mixture was stirred for 4.5 h. The solution was acidified to about pH 1 with 2 M HCl aq and extracted with ether. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by preparative TLC (*n*-hexane–ethyl acetate = 1:4) to give **16** (35 mg, 67%). White powder: mp 208.5–210 °C (acetone):^{1}H NMR (DMSO-d_6) δ 9.22 (s, 1H), 7.06 (d, 2H, J = 8.5 Hz), 6.76 (d, 1H, J = 1.7 Hz), 6.66 (d, 2H, J = 8.5 Hz), 6.39 (s, 2H), 6.34 (d, 1H, J = 1.7 Hz), 6.25 (s, 1H), 4.63 (s, 2H), 3.67 (s, 3H), 3.55–3.38 (br, 4H), 2.08 (s, 6H), 1.82–1.79 (m, 4H);^{13}C NMR (125 MHz, DMSO-d_6) δ 160.6, 155.9, 148.9, 137.4, 129.6, 129.2, 127.7, 124.4, 121.8, 118.8, 115.0, 111.8, 109.8, 55.5, 48.7, 45.9, 35.7, 26.0, 23.6, 21.3; MS m/z 403 (M^+), 404 (MH^+), HRMS calcd for $C_{25}H_{29}N_3O_2$ (M) 403.2260, found 403.2282.

4.2.8. *N*-[4-[(3,5-Dimethylphenyl)(4-hydroxymethylbenzyl) amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (17)

4-Hydroxymethylbenzyl bromide (34 mg, 0.17 mmol) was added to a suspension of **9** (25 mg, 0.084 mmol), triethylamine (23 μL, 0.17 mmol), and KI (28 mg, 0.17 mmol) in DMF (1 mL) at 0 °C. The reaction mixture was stirred at 80 °C for 2.5 h, then was poured into water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by preparative TLC (*n*-hexane–ethyl acetate = 1:1) to give **17** (11 mg, 31%). Colorless oil; ^{1}H NMR (CD_3OD) δ 7.25 (s, 4H), 6.64 (s, 1H), 6.45 (s, 2H), 6.37 (s, 1H), 6.32 (s, 1H), 4.74 (s, 2H), 4.54 (s, 2H), 3.70 (s, 3H), 3.58–3.48 (m, 4H), 2.10 (s, 6H), 1.91–1.86 (m, 4H);^{13}C NMR (125 MHz, CD_3OD) δ 163.7, 150.6, 141.0, 140.0, 139.2, 132.3, 128.1, 128.0, 125.6, 122.9, 120.7, 113.7, 111.8, 65.0, 57.5, 50.8, 47.4, 36.3, 27.3, 25.0, 21.7; MS m/z 417 (M^+), 418 (MH^+), HRMS calcd for $C_{26}H_{31}N_3O_2$(M) 417.2416, found 417.2414.

4.2.9. *N*-[4-[(Methyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (18)

The title compound was prepared from compound **10** and methyl iodide by procedure A (90%). Yellow powder; mp 158–159 °C (*n*-hexane–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 8.02 (d, 2H, J = 9.4 Hz), 6.69 (d, 1H, J = 9.4 Hz), 6.63 (d, 1H, J = 2.1 Hz), 6.35 (d, 1H, J = 2.1 Hz), 3.86 (s, 3H), 3.62 (br, 4H), 3.30 (s, 3H), 1.94–1.91 (m, 4H);^{13}C NMR (125 MHz, $CDCl_3$) δ 161.2, 154.5, 137.6, 128.8, 125.8, 125.7, 122.1, 111.7, 110.2, 49.4, 46.3, 41.3, 36.7, 26.6, 24.1; MS m/z 328 (M^+), 329 (MH^+), HRMS calcd for $C_{17}H_{20}N_4O_3$ (M) 328.1535, found 328.1537; Anal. Calcd for $C_{17}H_{20}N_4O_3$: C, 62.18; H, 6.14; N, 17.06. Found: C, 62.26; H, 6.10; N, 17.20.

4.2.10. *N*-[4-[(Ethyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (19)

The title compound was prepared from compound **10** and ethyl iodide by procedure A (92%). Orange oil: ^{1}H NMR ($CDCl_3$) δ 7.98 (d, 2H, J = 9.4 Hz), 6.63 (d, 2H, J = 9.4 Hz), 6.61 (d, 1H, J = 1.7 Hz), 6.33 (d, 1H, J = 1.7 Hz), 3.87 (s, 3H), 3.65 (q, 2H, J = 6.8 Hz), 3.62 (br, 4H), 1.93–1.90 (m, 4H), 1.22 (t, 3H, J = 6.8 Hz);^{13}C NMR (125 MHz, $CDCl_3$) δ 161.1, 153.9, 137.2, 126.7, 125.9, 125.8, 122.8, 111.5, 111.1, 49.4, 47.6, 46.3, 36.7, 26.5, 24.0, 12.2; MS m/z 342 (M^+), 343 (MH^+), HRMS calcd for $C_{18}H_{22}N_4O_3$ (M) 342.1692, found 342.1683.

4.2.11. *N*-[4-[(4-Nitrophenyl)(*iso*-propyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (20)

The title compound was prepared from compound **10** and isopropyl bromide by procedure A at 50 °C for 2 d (83%). Yellow oil; ^{1}H NMR ($CDCl_3$) δ 7.96 (d, 2H, J = 9.4 Hz), 6.59 (d, 2H, J = 9.4 Hz), 6.51 (d, 1H, J = 1.7 Hz), 6.23 (d, 1H, J = 1.7 Hz), 4.25 (sept, 1H, J = 6.4 Hz), 3.88 (s, 3H), 3.61 (br, 4H), 1.92–1.89 (m, 4H), 1.13 (d, 6H, J = 6.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.2, 154.8, 137.1, 125.9, 125.7, 125.1, 121.1, 113.8, 111.8, 49.4, 48.3, 46.3, 36.7, 26.5, 24.0, 20.6; MS m/z 356 (M^+), 357 (MH^+), HRMS calcd for $C_{19}H_{24}N_4O_3$ (M) 356.1848, found 356.1851.

4.2.12. *N*-[4-[(Cyclohexylmethyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (21)

The title compound was prepared from compound **10** and cyclohexylmethyl bromide by procedure A at 70 °C for 2 d (84%). Orange foam; ^{1}H NMR ($CDCl_3$) δ 8.00 (d, 2H, J = 9.4 Hz), 6.67 (d, 2H, J = 9.4 Hz), 6.59 (d, 1H, J = 1.3 Hz), 6.32 (d, 1H, J = 1.3 Hz), 3.88 (s, 3 H), 3.63 (br, 4H), 3.45 (d, 2H, J = 6.8 Hz), 1.94–1.92 (m, 4H), 1.77–1.65 (m, 6H), 1.25–1.17 (m, 3H), 0.99–0.95 (m, 2H);^{13}C NMR (125 MHz, $CDCl_3$) δ 161.2, 154.8, 137.2, 128.0, 125.8, 125.7, 122.7, 111.9, 110.9, 59.8, 49.4, 46.3, 36.7, 36.6, 31.0, 29.5, 26.5, 26.3, 25.8, 24.0; MS m/z 410 (M^+), 411 (MH^+), HRMS calcd for $C_{23}H_{30}N_4O_3$ (M) 410.2318, found 410.2301.

4.2.13. *N*-[4-[(Benzyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (22)

The title compound was prepared from compound **10** and benzyl bromide by procedure A (84%). Orange crystals: mp 116–116.5 °C (acetone–methanol); ^{1}H NMR ($CDCl_3$) δ 8.01 (d, 2H, J = 9.4 Hz), 7.34 (t, 2H, J = 7.2 Hz), 7.28 (t, 1H, J = 7.2 Hz), 7.26 (d, 2H, J = 7.2 Hz), 6.74 (d, 2H, J = 9.4 Hz), 6.63 (d, 1H, J = 2.1 Hz), 6.39 (d, 1H, J = 2.1 Hz), 4.90 (s, 2H), 3.84 (s, 3H), 3.62–3.59 (m, 4H), 1.94–1.86 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.1, 154.1, 137.9, 137.0, 128.8, 127.9, 127.3, 126.3, 125.8, 125.7, 122.3, 112.2, 110.5, 57.3, 49.4, 46.3, 36.7, 26.5, 24.1; MS m/z 404 (M^+), 405 (MH^+); HRMS calcd for $C_{23}H_{24}N_4O_3$ (M) 404.1848, found 404.1875; Anal. Calcd for $C_{23}H_{24}N_4O_3$: C, 68.30; H, 5.98; N, 13.85. Found: C, 68.35; H, 6.05; N, 13.86.

4.2.14. *N*-[4-[(4-Fluorobenzyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (23)

The title compound was prepared from compound **10** and 4-fluorobenzyl chloride by procedure A at 70 °C for 1 h (46%). Orange oil; ^{1}H NMR ($CDCl_3$) δ 7.98 (d, 2H, J = 9.4 Hz), 7.21 (dd, 2H, J = 8.5, 5.5 Hz), 7.00 (t, 2H, J = 8.5 Hz), 6.71 (d, 2H, J = 9.4 Hz), 6.60 (d, 1H, J = 2.1 Hz), 6.34 (d, 1H, J = 2.1 Hz), 4.85 (s, 2H), 3.84 (s, 3H), 3.61–3.57 (m, 4H), 1.94–1.90 (m, 4H);^{13}C NMR (125 MHz, $CDCl_3$) δ 160.2 (d, J = 246 Hz), 154.0, 138.1, 132.7, 128.1 (d, J = 7 Hz), 127.7, 126.0, 125.8, 122.3, 115.6 (d, J = 20 Hz), 112.2, 110.5, 56.6, 49.4, 46.3, 36.7, 26.5, 24.1; MS m/z 422 (M^+), 423 (MH^+), HRMS calcd for $C_{23}H_{23}N_4O_3$ (M) 422.1754, found 422.1761.

4.2.15. *N*-[4-[(4-Methylbenzyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (24)

The title compound was prepared from compound **10** and 4-methylbenzyl bromide by procedure A (79%). Orange oil; ^{1}H NMR ($CDCl_3$) δ 7.99 (d, 2H, J = 9.4 Hz), 7.13 (s, 4H), 6.73 (d, 2H, J = 9.4 Hz), 6.62 (d, 1H, J = 2.1 Hz), 6.37 (d, 1H, J = 2.1 Hz), 4.85 (s, 2H), 3.84 (s, 3H), 3.61–3.58 (m, 4H), 2.33 (s, 3H), 1.91–1.89 (m, 4H);^{13}C NMR (125 MHz, $CDCl_3$) δ 161.2, 154.1, 137.9, 137.0, 133.9, 129.4, 128.0, 126.3, 125.8, 125.7, 122.4, 112.2, 110.6, 49.4,

46.3, 36.7, 26.6, 24.1, 21.0; MS *m/z 418* (M^+), 419 (MH^+), HRMS calcd for $C_{24}H_{26}N_4O_3$ (M) 418.2005, found 418.2005.

4.2.16. *N*-[4-[(4-Hydroxybenzyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (25)

A solution of 4-*tert*-butyl-dimethylsilyloxybenzyl bromide (74 mg, 0.25 mmol) in DMF (1 mL) was added to a suspension of **10** (70 mg, 0.23 mmol) and sodium hydride (14 mg, 0.35 mmol) in DMF (1 mL) at 0 °C. The reaction mixture was stirred at room temperature for 20 min, then poured into water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (chloroform–methanol = 20:1) to give **25** (79 mg, 66%) as orange powder: mp 223–224 °C (*n*-hexane–ethyl acetate); ^{1}H NMR (DMSO-d_6) δ 9.29 (s, 1H), 7.97 (d, 2H, J = 9.4 Hz), 7.07 (d, 2H, J = 8.5 Hz), 6.69 (d, 1H, J = 2.1 Hz), 6.80 (d, 2H, J = 9.4 Hz), 6.69 (d, 2H, J = 8.5 Hz), 6.46 (d, 1H, J = 2.1), 4.81 (s, 2H), 3.71 (s, 3H), 3.54 (br, 2H), 3.41 (br, 2H), 1.82–1.79 (m, 4H);^{13}C NMR (125 MHz, DMSO-d_6) δ 160.3, 156.4, 154.1, 136.6, 127.8, 127.1, 127.0, 125.7, 125.4, 122.5, 115.3, 112.3, 110.1, 79.1, 56.0, 48.7, 45.9, 36.0, 26.0, 23.6; MS *m/z* 420 (M^+), 421 (MH^+), HRMS calcd for $C_{23}H_{24}N_4O_3$ (M) 420.1798, found 420.1800.

4.2.17. *N*-[4-[(4-Hydroxymethylbenzyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (26)

A solution of 4-(*tert*-butyl-dimethylsilyloxymethyl)benzyl bromide (71 mg, 0.23 mmol) in DMF (1 mL) was added to a suspension of **10** (64 mg, 0.20 mmol) and sodium hydride (14 mg, 0.35 mmol) in DMF (1 mL) at 0 °C. The reaction mixture was stirred at 80 °C for 1 h, then poured into water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by preparative TLC (*n*-hexane–ethyl acetate = 1:2) to give *N*-[4-[[(4-*tert*-butyl-dimethylsilyloxymethyl)benzyl](4-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine as an orange oil (88 mg, 79%). ^{1}H NMR ($CDCl_3$) δ 8.00 (d, 2H, J = 9.4 Hz), 7.29 (d, 2H, J = 8.1 Hz), 7.21 (d, 2H, J = 8.1 Hz), 6.73 (d, 2H, J = 9.4 Hz), 6.62 (d, 1H, J = 1.7 Hz), 6.38 (d, 1H, J = 1.7 Hz), 4.88 (s, 2H), 4.72 (s, 2H), 3.84 (s, 3H), 3.62–3.59 (m, 4H), 1.94–1.91 (m, 4H), 0.94 (s, 9H), 0.09 (s, 6H);^{13}C NMR (125 MHz, $CDCl_3$) δ 161.1, 154.1, 140.6, 137.8, 135.5, 127.9, 126.4, 126.3, 125.8, 125.7, 122.4, 112.2, 110.6, 64.5, 57.1, 49.4, 46.3, 36.7, 26.5, 25.9, 24.0, 18.3, -5.3; MS *m/z* 548 (M^+), 549 (MH^+), HRMS calcd for $C_{30}H_{40}N_4O_4Si$ (M) 548.2819, found 548.2793. A solution of TBAF in THF was added to a solution of *N*-[4-[[(4-*tert*-butyl-dimethylsilyloxymethyl)benzyl](4-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (78 mg, 0.14 mmol) in THF (3 ml) at room temperature. The reaction mixture was stirred for 3 h, then diluted with ethyl acetate, and washed with water and saturated NH_4Cl aq The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by preparative TLC (*n*-hexane–ethyl acetate = 1:2) to give **26** (60 mg, 97%). Orange foam:^{1}H NMR ($CDCl_3$) δ 7.97 (d, 2H, J = 9.4 Hz), 7.30 (d, 2H, J = 8.1 Hz), 7.20 (d, 2H, J = 8.1 Hz), 6.69 (d, 2H, J = 9.4 Hz), 6.61 (d, 1H, J = 2.1 Hz), 6.37 (d, 1H, J = 2.1 Hz), 4.87 (s, 2H), 4.63 (s, 2H), 3.79 (s, 3H), 3.57 (m, 4H), 1.91–1.89 (m, 4H);^{13}C NMR (125 MHz, $CDCl_3$) δ 161.1, 154.0, 140.3, 137.8, 136.1, 127.9, 127.3, 126.4, 125.7, 125.6, 122.3, 112.1, 110.5, 64.5, 57.0, 49.4, 46.3, 36.6, 26.4, 24.0; MS *m/z* 434 (M^+), 435 (MH^+), HRMS calcd for $C_{24}H_{26}N_4O_4$ (M) 434.1954, found 434.1984.

4.2.18. General procedure B for compounds 29–34

A well-dried flask was evacuated and backfilled with argon. The flask was charged with $Pd_2(dba)_3$ (1–10 mol%Pd), NaO-*t*-Bu or Cs_2CO_3 (1.4 equiv), BINAP or 2-(di-*tert*-butylphosphino)biphenyl (1–10 mol%), aryl bromide (0.9 equiv), and **28**[30] (1.0 equiv) in toluene, 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 acetate, washed with water and brine, dried over $MgSO_4$, and evaporated. Otherwise, the mixture was filtered through Celite and eluted with dichloromethane, and the filtrate was evaporated. The residue was purified by silica gel column chromatography.

4.2.19. *N*-[4-(2-Nitrophenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (29)

The title compound was prepared from **28** and 2-bromonitrobenzene by procedure B (39%). Vermillion oil; ^{1}H NMR ($CDCl_3$) 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, 1 H, 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 *m/z* 314 (M^+), 315 (MH^+); HRMS calcd for $C_{16}H_{18}N_4O_3$ (M) 314.1379, found 314.1343.

4.2.20. *N*-[4-(4-Nitro-3-trifluoromethylphenylamino)-1-methyl pyrrole-2-carbonyl]pyrrolidine (30)

The title compound was prepared from **28** and 1-bromo-4-nitro-3-trifluoromethylbenzene by procedure B (70%). Pale yellow powder: mp 202–203 °C (*n*-hexane–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 7.95 (d, 1H, J = 9.0 Hz), 7.08 (d, 1H, J = 2.1 Hz), 6.83 (dd, 1H, J = 9.0, 2.6 Hz), 6.62 (d, 1H, J = 1.7 Hz), 6.36 (d, 1H, J = 1.7 Hz), 6.23 (s, 1H), 3.85 (s, 3H), 3.63 (br, 4H), 1.96–1.93 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.3, 151.7, 137.0, 126.6 (q, J = 33.3 Hz), 125.9, 122.4 (q, J = 273 Hz), 121.5, 120.9, 113.8, 111.8 (q, J = 7.4 Hz), 109.8, 49.5, 46.4, 36.6, 26.5, 24.1; MS *m/z* 382 (M^+), 383 (MH^+); HRMS calcd for $C_{17}H_{17}F_3N_4O_3$ (M) 382.1253, found 382.1263.

4.2.21. *N*-[4-(4-Cyanophenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (31)

The title compound was prepared from **28** and 4-bromobenzonitrile by procedure B (32%). Pale yellow crystals: mp 294 °C (*n*-hexane–ethyl acetate); ^{1}H NMR ($CDCl_3$) 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, 4H), 2.40 (s, 3H), 1.95–1.92 (m, 4H); MS *m/z* 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.

4.2.22. *N*-[4-(4-Trifluoromethylphenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (32)

The title compound was prepared from **28** and 4-bromotrifluoromethylbenzene by procedure B (51%). Pale yellow foam; ^{1}H NMR ($CDCl_3$) δ 7.37 (d, 2H, J = 8.5 Hz), 6.76 (d, 2H, J = 8.5 Hz), 6.60 (d, 1H, J = 1.7 Hz), 6.37 (d, 1H, J = 1.7 Hz), 5.50 (s, 1H), 3.84 (s, 3H), 3.63 (br, 4H), 1.93–1.91 (m, 4H); MS *m/z* 382 (M^+), 383 (MH^+); HRMS calcd for $C_{17}H_{17}F_3N_4O_3$ (M) 382.1253, found 382.1263.

4.2.23. *N*-[4-(4-Acetylphenylamino)-1-methylpyrrole-2-carbonyl] pyrrolidine (33)

The title compound was prepared from **28** and 4-bromoacetophenone by procedure B (56%). Pale yellow powder: mp 181–182 °C (*n*-hexane–dichloromethane); ^{1}H NMR ($CDCl_3$) δ 7.79 (d, 2H, J = 9.0 Hz), 6.72 (d, 2H, J = 9.0 Hz), 6.61 (d, 1H, J = 1.7 Hz), 6.38 (d, 1H, J = 1.7 Hz), 5.71 (s, 1H), 3.84 (s, 3H), 3.63 (br, 4H), 2.48 (s, 3H), 1.93–1.90 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.3, 161.4, 151.9, 130.7, 127.4, 125.3, 122.6, 121.3, 112.2, 110.0, 60.3, 49.5, 46.4, 36.6, 26.6, 26.0, 24.1, 14.1; MS *m/z* 311 (M^+), 312 (MH^+); HRMS calcd for $C_{18}H_{21}N_3O_2$ (M) 311.1634, found 311.1605.

4.2.24. *N*-[4-(4-Methoxycarbonylphenylamino)-1-methylpyrrole-2-carbonyl]pyrrolidine (34)

The title compound was prepared from **28** and methyl 4-bromobenzoate by procedure B (66%). White powder, mp 186–

187 °C (*n*-hexane–dichloromethane); ^{1}H NMR ($CDCl_3$) δ 7.83 (d, 2H, J = 8.5 Hz), 6.71 (d, 2H, J = 8.5 Hz), 6.59 (d, 1H, J = 1.7 Hz), 6.37 (d, 1H, J = 1.7 Hz), 5.73 (s, 1H), 3.83 (s, 6H) 3.61 (br, 4H), 1.92–1.89 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.2, 161.4, 151.4, 131.4, 125.1, 122.8, 121.1, 119.0, 112.2, 110.0, 51.5, 49.4, 46.3, 36.5, 26.5, 24.0; MS *m/z* 327 (M^+), 328 (MH^+); HRMS calcd for $C_{18}H_{21}N_3O_3$ (M) 327.1583, found 327.1601.

4.2.25. General procedure C for compounds 35–40

Benzyl bromide (1.1–1.5 equiv) was added to a suspension of **29**–**34** (1 equiv) and sodium hydride (1.5–2 equiv) in DMF at 0 °C. The reaction mixture was stirred at 0 °C until the starting material was consumed, then poured into water, and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by silica gel column chromatography and preparative TLC.

4.2.26. *N*-[4-[(Benzyl)(2-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (35)

The title compound was prepared from **29** and benzyl bromide by procedure C (17%). Vermilion oil; ^{1}H NMR ($CDCl_3$) δ 7.66 (dd, 1H, J = 1.7, 8.1 Hz), 7.38–7.34 (m, 3H), 7.30 (t, 2H, J = 7.7 Hz), 7.24–7.20 (m, 2H), 6.95 (dt, 1H, J = 1.3, 8.1 Hz), 6.26 (d, 1H, J = 2.1 Hz), 6.01 (d, 1H, J = 2.1 Hz), 4.80 (s, 2H), 3.69 (s, 3H), 3.51 (br, 4H), 1.88–1.85 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.6, 142.6, 138.0, 133.0, 131.6, 128.6, 127.1, 127.1, 126.0, 124.6, 123.1, 120.9, 117.4, 106.0, 58.4, 49.3, 46.2, 36.3, 26.6, 24.1; MS *m/z* 404 (M^+), 405 (MH^+); HRMS calcd for $C_{25}H_{27}N_3O_3$ (M) 404.1848, found 404.1845.

4.2.27. *N*-[4-[(Benzyl)(4-nitro-3-trifluoromethylphenyl) amino]-1-methylpyrrole-2-carbonyl]pyrrolidine (36)

The title compound was prepared from **30** and benzyl bromide by procedure C (71%). Yellow needles. mp 158–159 °C (*n*-hexane–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 7.92 (d, 1H, J = 9.4 Hz), 7.35 (t, 2H, J = 7.7 Hz), 7.29 (t, 1H, J = 7.7 Hz), 7.24 (d, 2H, J = 7.7 Hz), 7.18 (d, 1H, J = 3.0 Hz), 6.82 (dd, 1H, J = 9.4, 3.0 Hz), 6.63 (d, 1H, J = 2.1 Hz), 6.37 (d, 1H, J = 2.1 Hz), 4.90 (s, 2 H), 3.85 (s, 3H), 3.60 (br, 4H), 1.95–1.92 (m, 4 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.0, 152.5, 136.5, 136.3, 128.9, 128.5, 127.6, 127.4, 126.4, 126.2 (q, J = 31.4 Hz), 126.1, 122.3 (q, J = 274 Hz), 122.0, 114.0, 111.9 (q, J = 7.4 Hz), 110.1, 57.5, 49.4, 46.3, 36.7, 26.5, 24.1; MS *m/z* 472 (M^+), 473 (MH^+); HRMS calcd for $C_{24}H_{23}F_3N_4O_3$ (M) 472.1722, found 472.1712; Anal. Calcd for $C_{24}H_{23}F_3N_4O_3$: C, 61.01; H, 4.91; N, 11.86. Found: C, 60.93; H, 5.00; N, 11.70.

4.2.28. *N*-{4-[(Benzyl)(4-cyanophenyl)amino]-1-methylpyrrole-2-carbonyl}pyrrolidine (37)

The title compound was prepared from **31** and benzyl bromide by procedure C (36%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.36 (d, 2H, J = 9.4 Hz), 7.33 (t, 2H, J = 7.2 Hz), 7.28–7.24 (m, 3H), 6.75 (d, 2H, J = 9.4 Hz), 6.37 (d, 1H, J = 2.1 Hz), 4.85 (s, 2H), 3.83 (s, 3H), 3.61–3.59 (m, 4H), 1.94–1.91 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.2, 152.1, 137.4, 133.2, 128.7, 128.1, 127.1, 126.3, 125.6, 122.4, 120.4, 113.1, 110.7, 98.6, 56.9, 49.4, 46.3, 36.7, 26.5, 24.0; MS *m/z* 384 (M^+), 385 (MH^+); HRMS calcd for $C_{24}H_{24}N_4O$ (M) 384.1950, found 384.1975.

4.2.29. *N*-{4-[(Benzyl)(4-trifluoromethylphenyl)amino]-1-methylpyrrole-2-carbonyl}pyrrolidine (38)

The title compound was prepared from **32** and benzyl bromide by procedure C (30%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.35–7.23 (m, 7H), 6.80 (d, 2H, J = 9.0 Hz), 6.60 (d, 1H, J = 1.7 Hz), 6.39 (d, 1H, J = 1.7 Hz), 4.85 (s, 2H), 3.83 (s, 3H), 3.62–3.59 (m, 4H), 1.93–1.90 (m, 4H); MS *m/z* 427 (M^+), 428 (MH^+); HRMS calcd for $C_{24}H_{24}F_3N_3O$ (M) 427.1871, found 427.1880.

4.2.30. *N*-{4-[(4-Acetylphenyl)(benzyl)amino]-1-methylpyrrole-2-carbonyl}pyrrolidine (39)

The title compound was prepared from **33** and benzyl bromide by procedure C (55%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.76 (d, 2H, J = 9.2 Hz), 7.33–7.23 (m, 5H), 6.77 (d, 2H, J = 9.2 Hz), 6.60 (d, 1H, J = 1.8 Hz), 6.39 (d, 1H, J = 1.8 Hz), 4.88 (s, 2H), 3.83 (s, 3H), 3.61–3.58 (m, 4H), 2.47 (s, 3H), 1.93–1.90 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.3, 161.4, 152.9, 137.9, 130.3, 128.7, 128.6, 127.1, 126.6, 126.5, 125.5, 122.4, 112.5, 110.9, 57.0, 49.5, 46.3, 36.7, 26.6, 26.0, 24.1; MS *m/z* 401 (M^+), 402 (MH^+); HRMS calcd for $C_{25}H_{27}N_3O_2$ (M) 401.2103, found 401.2147.

4.2.31. *N*-{4-[(Benzyl)(4-methoxycarbonylphenyl)amino]-1-methylpyrrole-2-carbonyl}pyrrolidine (40)

The title compound was prepared from **34** and benzyl bromide by procedure C (35%). Colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.78 (d, 2H, J = 9.0 Hz), 7.30 (t, 2H, J = 7.7 Hz), 7.25 (d, 2H, J = 7.7 Hz), 7.22 (t, 1H, J = 7.7 Hz), 6.75 (d, 2H, J = 9.0 Hz), 6.58 (d, 1H, J = 1.7 Hz), 6.38 (d, 1H, J = 1.7 Hz), 4.85 (s, 2H), 3.81 (s, 3H), 3.81 (s, 3H), 3.60–3.57 (m, 4H), 1.91–1.88 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.2, 161.4, 152.8, 138.1, 131.0, 128.8, 128.6, 127.0, 126.5, 125.4, 122.4, 118.3, 112.5, 110.9, 57.0, 51.5, 49.4, 46.3, 36.7, 26.6, 24.1; MS *m/z* 417 (M^+), 418 (MH^+); HRMS calcd for $C_{25}H_{27}N_3O_3$ (M) 417.2052, found 417.2070.

4.2.32. Ethyl 1-methyl-4-nitropyrrole-2-carboxylate (41)

Methyl iodide (2 mL, 32 mmol) was added to a suspension of ethyl 4-nitropyrrole-2-carboxylate (5.3 g, 29 mmol) and sodium hydride (1 g, 43 mmol) in DMF (50 mL) at 0 °C. The reaction mixture was stirred at room temperature for 30 min, then poured into water at 0 °C, and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was recrystallized from *n*-hexane–ethyl acetate to give **41** (5.5 g, 28 mmol, 97%) as white crystals. ^{1}H NMR ($CDCl_3$) δ 7.58 (d, 1H, J = 1.7 Hz), 7.41 (t, 1H, J = 1.7 Hz), 4.31 (q, 2H, J = 6.8 Hz), 3.98 (s, 3 H), 1.37 (t, 1H, J = 6.8 Hz);^{13}C NMR (125 MHz, $CDCl_3$) δ 160.2, 135.2, 127.5, 123.2, 112.6, 60.9, 37.9, 14.2; MS *m/z* 199 (MH^+).

4.2.33. Ethyl 4-amino-1-methylpyrrole-2-carboxylate (42)

10% Pd/C was added to a solution of **41** (1.0 g, 5.1 mmol) in ethyl acetate (20 mL) at room temperature. The reaction mixture was vigorously stirred under positive pressure of hydrogen for 1 h at room temperature and then filtered through Celite. The Celite was rinsed with ethyl acetate, and the combined filtrates were evaporated to give **42** as colorless oil (0.85 g, 99%). ^{1}H NMR ($CDCl_3$) δ 6.43 (d, 1H, J = 1.7 Hz), 6.31 (d, 1H, J = 1.7 Hz), 4.21 (q, 2H, J = 7.3 Hz), 3.77 (s, 3H), 2.94 (s, 2H), 1.29 (t, 3H,J = 7.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.0, 130.7, 120.3, 117.4, 108.0, 59.5, 36.2, 14.3; MS *m/z* 199 (MH^+).

4.2.34. Ethyl 1-methyl-4-(4-nitrophenyl)aminopyrrole-2-carboxylate (43)

A well-dried flask was evacuated and backfilled with argon. The flask was charged with **42** (0.85 g, 5.1 mmol), toluene (20 mL), BINAP (0.31 g, 10 mol%), Cs_2CO_3 (2.3 g, 7.1 mmol), $Pd_2(dba)_3$ (230 mg, 10 mol%Pd), and 1-bromo-4-nitrobenzene (1.0 g, 5.0 mmol), then evacuated and backfilled with argon. The reaction mixture was stirred at 80 °C for 6 h, then diluted with ethyl acetate, washed with water and brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (*n*-hexane–ethyl acetate = 5:1; 4:1; 3:1; 2:1; 1:1) to give **43** as a yellow powder (0.81 g, 56%). ^{1}H NMR ($CDCl_3$) δ 8.05 (d, 2 H, J = 9.4 Hz), 6.82 (d, 1H, J = 1.7 Hz), 6.75 (d, 1H, J = 1.7 Hz), 6.72 (d, 2H, J = 9.4 Hz), 5.93 (s, 1H), 4.28 (q, 2H, J = 7.3 Hz), 3.93 (s, 3 H), 1.35 (t, 3H, J = 7.3 Hz);^{13}C NMR (125 MHz, $CDCl_3$) δ 160.8, 152.5,

138.8, 126.3, 124.3, 122.4, 121.9, 113.7, 112.1, 60.2, 37.0, 14.4; MS *m/z* 289 (M^+), 290 (MH^+).

4.2.35. Ethyl 4-[(benzyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carboxylate (44)

Benzyl bromide (0.39 mL, 3.4 mmol) and potassium iodide (0.47 g, 2.8 mmol) were added to a suspension of **43** (0.81 g, 2.8 mmol) and sodium hydride (0.10 g, 4.2 mmol) in DMF (25 mL) at 0 °C, under argon. The reaction mixture was stirred at room temperature for 4.5 h, then poured into water, and extracted with ethyl acetate. 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 = 5:1; 4:1; 3:1) to give **44** as a yellow foam (1.0 g, 94%). ^{1}H NMR ($CDCl_3$) δ 7.98 (d, 2H, *J* = 9.4 Hz), 7.31 (t, 2H, *J* = 7.3 Hz), 7.24–7.21 (m, 3H), 6.84 (d, 1H, *J* = 2.1 Hz), 6.71 (d, 1H, *J* = 2.1 Hz), 6.70 (d, 2H, *J* = 9.4 Hz), 4.88 (s, 2H), 4.26 (q, 2H, *J* = 7.2 Hz), 3.88 (s, 3 H), 1.31 (t, 3H, *J* = 7.2 Hz);^{13}C NMR (125 MHz, $CDCl_3$) δ 160.7, 153.8, 138.1, 136.8, 128.8, 128.7, 127.3, 126.1, 125.7, 125.2, 122.1, 114.4, 112.3, 60.1, 57.2, 37.0, 14.3; MS *m/z* 379 (M^+), 380 (MH^+); HRMS calcd for $C_{21}H_{21}N_3O_4$ (M) 379.1532, found 379.1520.

4.2.36. 4-[(Benzyl)(4-nitrophenyl)amino]-2-hydroxymethyl-1-methylpyrrole (45)

A solution of **44** (53 μL, 0.14 mmol) in THF (1.2 mL) was added to a suspension of lithium aluminum hydride (8.0 mg, 0.21 mmol) in THF (0.3 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1.5 h, and then quenched with water (10 μL) and 2 M NaOH aq (10 μL) at 0 °C. $MgSO_4$ was added to the solution, and the whole solution was filtered. The filtrate was evaporated, and the residue was purified by preparative TLC (*n*-hexane–ethyl acetate = 1:1) to give **45** as an orange oil (32 mg, 68%). ^{1}H NMR ($CDCl_3$) δ 7.97 (d, 2H, *J* = 9.4 Hz), 7.33–7.23 (m 5H), 6.71 (d, 2H, *J* = 9.4 Hz), 6.56 (d, 1H, *J* = 1.7 Hz), 6.03 (d, 1H, *J* = 1.7 Hz), 4.89 (s, 2H), 4.57 (s, 2H), 3.65 (s, 3H);^{13}C NMR (125 MHz, $CDCl_3$) δ 154.2, 137.6, 137.1, 131.4, 128.7, 128.3, 127.2, 126.1, 125.7, 119.0, 112.1, 106.5, 57.4, 56.6, 33.9; MS *m/z* 337 (M^+), 338 (MH^+); HRMS calcd for $C_{19}H_{19}N_3O_3$ (M) 337.1426, found 337.1440.

4.2.37. 4-[(Benzyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carboxylic acid (46)

A suspension of **44** (1.0 g, 2.6 mmol) in 30% KOH aq (15 mL), methanol (15 mL), and tetrahydrofuran (7 mL) was heated at 110 °C for 2 h. The solution was cooled and acidified to about pH 1 with 2 M HCl aq and then extracted with ether. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated to give **46** (0.89 g, 96%). Yellow powder. mp 185.5–186.5 °C (*n*-hexane–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 8.02 (d, 2 H, *J* = 9.4 Hz), 7.35 (t, 2 H, *J* = 7.3 Hz), 7.28 (t, 1H, *J* = 7.3 Hz), 7.25 (d, 2H, *J* = 7.3 Hz), 7.00 (d, 1H, *J* = 2.1 Hz), 6.80 (d, 1H, *J* = 2.1 Hz), 6.75 (d, 2H, *J* = 9.4 Hz), 4.92 (s, 2H), 3.92 (s, 3 H);^{13}C NMR (125 MHz, $CDCl_3$) δ 164.1, 153.7, 138.4, 136.8, 129.2, 128.9, 127.5, 126.7, 126.3, 125.8, 120.9, 116.5, 112.4, 57.1, 37.3; MS *m/z* 351 (M^+), 352 (MH^+). Anal. Calcd for $C_{19}H_{17}N_3O_4$: C, 64.95; H, 4.88; N, 11.96. Found: C, 64.84; H, 4.90; N, 12.01.

4.2.38. General procedure D for compounds 47, 49–52

Methanesulfonyl chloride (1.1 equiv) was added to a solution of **46** (1 equiv) and triethylamine (3 equiv) in THF at 0 °C, under argon. The reaction mixture was stirred at 0 °C for 30 min. Amine (1.5 equiv) was then added, and the reaction mixture was stirred at 0 °C until **46** was consumed. The mixture was poured into saturated NH_4Cl, and extracted with ethyl ester. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography or preparative TLC.

4.2.39. 4-[(Benzyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carboxamide (47)

The title compound was prepared from **46** and ammonia by procedure D (78%). Yellow powder. mp 208–209 °C (*n*-hexane–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 8.02 (d, 2H, *J* = 9.4 Hz), 7.35 (t, 2H, *J* = 7.3 Hz), 7.29 (t, 1H, *J* = 7.3 Hz), 7.25 (d, 2H, *J* = 7.3 Hz), 6.74 (d, 2H, *J* = 9.4 Hz), 6.71 (d, 1H, *J* = 1.7 Hz), 6.51 (d, 1H, *J* = 1.7 Hz), 5.45 (br, 2H), 4.91 (s, 2H), 3.94 (s, 3H);^{13}C NMR (125 MHz, $CDCl_3$) δ 162.6, 153.9, 138.3, 136.9, 128.9, 128.4, 127.5, 126.3, 125.8, 124.6, 124.0, 112.3, 110.1, 57.3, 37.1; MS *m/z* 350 (M^+), 351 (MH^+), HRMS calcd for $C_{19}H_{18}N_4O_3$ (M) 350.1379, found 350.1334.

4.2.40. *N*,*N*-Diethyl 4-[(4-nitrophenyl)(benzyl)amino]-1-methylpyrrole-2-carboxamide (48)

Diethylamine (58 μL, 0.57 mmol) was added to a solution of **46** (50 mg, 0.14 mmol), HOBt (19 mg, 0.14 mmol), and EDCI (27 mg, 0.14 mmol) in dichloromethane (1.0 mL) at room temperature, under argon. The reaction mixture was stirred at room temperature for 16 h, then poured into water. The mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by preparative TLC (*n*-hexane–ethyl acetate = 2:1; two times) to give **48** as an orange oil (52 mg, 92%). ^{1}H NMR (CD_3OD) δ 7.91 (d, 2H, *J* = 9.4 Hz), 7.29–7.25 (m, 4H), 7.20 (t, 1H, *J* = 6.4 Hz), 6.83 (d, 1H, *J* = 2.1 Hz), 6.77 (d, 2H, *J* = 9.4 Hz), 6.22 (d, 1H, *J* = 2.1 Hz), 4.90 (s, 2H), 3.65 (s, 3 H), 3.47 (q, 4H, *J* = 6.8 Hz), 1.16 (t, 6H, *J* = 6.8 Hz);^{13}C NMR (125 MHz, CD_3OD) δ 165.2, 155.8, 138.9, 138.8, 129.7, 129.4, 128.3, 127.9, 127.0, 126.6, 123.0, 113.4, 109.3, 58.0 44.7, 41.4, 35.7, 13.8; MS *m/z* 406 (M^+), 407 (MH^+); HRMS calcd for $C_{23}H_{26}N_4O_3$ (M) 406.2005, found 406.2015.

4.2.41. *N*,*N*-Di-*n*-butyl-4-[(benzyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carboxamide (49)

The title compound was prepared from **46** and di-*n*-butylamine by procedure D (77%). Orange oil; ^{1}H NMR ($CDCl_3$) δ 7.98 (d, 2H, *J* = 9.4 Hz), 7.32 (t, 2H, *J* = 7.3 Hz), 7.26 (t, 1H, *J* = 7.3 Hz), 7.24 (d, 2H, *J* = 7.3 Hz), 6.73 (d, 2H, *J* = 9.4 Hz), 6.62 (d, 1H, *J* = 2.1 Hz), 6.18 (d, 1H, *J* = 2.1 Hz), 4.90 (s, 2H), 3.70 (s, 3H), 3.44 (t, 4H, *J* = 7.7 Hz), 1.56 (br, 4H), 1.28 (br, 4H), 0.90 (br, 6H);^{13}C NMR (125 MHz, $CDCl_3$) δ 163.3, 154.1, 137.8, 137.0, 128.7, 128.0, 127.3, 126.3, 126.1, 125.7, 120.9, 112.1, 108.0, 57.3, 48.9, 45.2, 35.6, 30.3, 20.0, 13.7; MS *m/z* 462 (M^+), 463 (MH^+), HRMS calcd for $C_{27}H_{34}N_4O_3$ (M) 462.2631, found 462.2587.

4.2.42. *N*-[4-[(Benzyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]piperidine (50)

The title compound was prepared from **46** and piperidine by procedure D (91%). Yellow powder. Mp 111–112 °C (*n*-hexane–ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 8.01 (d, 2H, *J* = 9.4 Hz), 7.33 (t, 2H, *J* = 7.3 Hz), 7.27 (t, 1H, *J* = 7.3 Hz), 7.25 (d, 2H, *J* = 7.3 Hz), 6.75 (d, 2H, *J* = 9.4 Hz), 6.62 (d, 1H, *J* = 1.7 Hz), 6.17 (d, 1H, *J* = 1.7 Hz), 4.90 (s, 2H), 3.73 (s, 3 H), 3.64–3.61 (m, 4H), 1.71–1.68 (m, 2H), 1.61–1.56 (m, 4H);^{13}C NMR (125 MHz, $CDCl_3$) δ 162.0, 154.1, 137.9, 137.0, 128.7, 128.0, 127.3, 126.4, 125.7, 125.4, 121.5, 112.2, 109.1, 57.2, 46.2, 35.7, 26.1, 24.6; MS *m/z* 418 (M^+), 419 (MH^+), HRMS calcd for $C_{24}H_{26}N_4O_3$ (M) 418.2005, found 406.2009.

4.2.43. *N*-[4-[(Benzyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]indoline (51)

The title compound was prepared from **46** and indoline by procedure D (53%). Yellow powder. mp 203–208 °C (chloroform–ethyl acetate); ^{1}H NMR (DMSO-d_6) δ 8.00 (d, 2H, *J* = 9.4 Hz), 7.99 (br, 1H), 7.35–7.29 (m, 4H), 7.24 (t, 2H, *J* = 6.8 Hz), 7.15 (d, 1H, *J* = 2.1 Hz), 7.14 (d, 1H, *J* = 7.3 Hz), 6.99 (t, 1H, *J* = 7.3 Hz), 6.84 (d, 2H, *J* = 9.4 Hz), 6.70 (d, 1H, *J* = 2.1 Hz), 4.99 (s, 2H), 4.23 (t, 2H, *J* = 8.1 Hz), 3.73 (s, 3H), 3.07 (t, 2H, *J* = 8.1 Hz);^{13}C NMR

(125 MHz, DMSO-d_6) δ 160.1, 154.0, 142.9, 137.2, 136.9, 132.6, 128.6, 127.4, 127.0, 126.7, 126.5, 125.7, 125.3, 124.9, 123.6, 123.3, 116.6, 112.4, 110.6, 56.5, 50.5, 35.9, 28.0; MS *m*/*z* 452 (M^+), 453 (MH^+), HRMS calcd for $C_{27}H_{24}N_4O_3$ (M) 452.1848, found 452.1845; Anal. Calcd for $C_{27}H_{24}N_4O_3$: C, 71.67; H, 5.35; N, 12.38. Found: C, 71.39; H, 5.45; N, 12.27.

4.2.44. (3*S*,4*S*)-*N*-[4-[(Benzyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]-3,4-dihydroxypyrrolidine (52)

(3*S*,4*S*)-*N*-[4-[(Benzyl)(4-nitrophenyl)amino]-1-methylpyrrole-2-carbonyl]-3,4-di(methoxymethoxy)pyrrolidine was prepared from **46** and (3*S*,4*S*)-di(methoxymethoxy)pyrrolidine by procedure D, and hydrolyzed (46 mg, 0.088 mmol) in methanol (1.5 mL) and 2 M HCl aq at 65 °C. After 1 h, the mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (chloroform–methanol = 40:1; 20:1) to give **52** (28 mg, 73%) as a yellow powder. mp 212.5–213 °C (*n*-hexane–ethanol); ^{1}H NMR (CD_3OD) δ 8.00 (d, 2H, *J* = 9.4 Hz), 7.33–7.29 (m, 4H), 7.25–7.24 (m, 1H), 6.89 (d, 1H, *J* = 1.7 Hz), 6.84 (d, 2H, *J* = 9.4 Hz), 6.49 (d, 1H, *J* = 1.7 Hz), 4.99 (d, 1H, *J* = 17.7 Hz), 4.93 (d, 1H, *J* = 17.7 Hz), 4.10 (d, 2H, *J* = 14.1 Hz), 3.86–3.81 (m, 2H), 3.78 (s, 3H), 3.56 (d, 1H, *J* = 11.5 Hz), 3.47 (d, 1H, *J* = 11.5 Hz);^{13}C NMR (125 MHz, CD_3OD) δ 164.1, 155.9, 139.1, 138.9, 129.8, 129.5, 128.3, 127.9, 126.6, 126.6, 124.6, 113.6, 112.5, 76.3, 74.6, 58.1, 56.1, 53.4, 36.6; MS *m*/*z* 436 (M^+), 437 (MH^+); HRMS calcd for $C_{23}H_{24}N_4O_5$(M) 436.1747, found 436.1761.

4.2.45. *N*-[4-[(Benzyl)(4-nitrophenyl)amino]phenylcarbonyl] pyrrolidine (53)

The title compound was prepared from ethyl 4-(4-nitrophenyl)aminobenzoate by the procedure shown in Scheme 3. Yellow powder. Mp 81–82 °C (*n*-hexane–dichloromethane); ^{1}H NMR ($CDCl_3$) δ 8.04 (d, 2H, *J* = 9.4 Hz), 7.60 (t, 2H, *J* = 8.6 Hz), 7.31 (m, 5H), 7.33 (d, 2H, *J* = 8.5 Hz), 6.81 (d, 2H, *J* = 9.4 Hz), 5.13 (s, 2H), 3.66 (t, 2H, *J* = 6.8 Hz), 3.49 (t, 2H, *J* = 6. 8 Hz), 1.98 (quint, 2H, *J* = 6.8 Hz), 1.91 (quint, 2H, *J* = 6.8 Hz);^{13}C NMR (125 MHz, $CDCl_3$) δ 168.7, 152.7, 147.2, 139.3, 136.7, 134.9, 129.3, 129.0, 127.6, 125.7, 125.6, 114.4, 56.5, 53.4, 49.7, 46.3, 26.5, 24.4; MS *m*/*z* 401 (M^+), 402 (MH^+), HRMS calcd for $C_{24}H_{24}N_3O_3$(MH^+) 402.1818, found 402.1858.

4.2.46. *N*-[3-[(Benzyl)(4-nitrophenyl)amino]phenylcarbonyl] pyrrolidine (54)

The title compound was prepared from ethyl 3-(4-nitrophenyl)aminobenzoate by the procedure shown in Scheme 3. Orange oil; ^{1}H NMR ($CDCl_3$) δ 8.01 (d, 2H, *J* = 9.4 Hz), 7.38 (m, 9H), 6.74 (d, 2H, *J* = 9.4 Hz), 5.05 (s, 2H), 3.61 (t, 2H, *J* = 6.8 Hz), 3.33 (t, 2H, *J* = 6.8 Hz), 1.95 (quint, 2H, *J* = 6.8 Hz), 1.87 (quint, 2H, *J* = 6.8 Hz);^{13}C NMR (125 MHz, $CDCl_3$) δ 168.4, 152.9, 145.7, 139.7, 136.7, 130.0, 128.9, 127.9, 127.5, 126.4, 125.7, 125.4, 125.2, 113.7, 56.6, 49.5, 46.2, 26.4, 24.3; MS *m*/*z* 401 (M^+), 402 (MH^+), HRMS calcd for $C_{24}H_{24}N_3O_3$ (MH^+) 402.1818, found 402.1810.

4.3. 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 *Escherichia coli*, as described previously.[27] A hAR-LBD expression plasmid vector, which encodes GST-hAR-LBD (627–919 aa, EF domain) fusion protein under the *lac* promoter (provided by Prof. 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 micro centrifuge, 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.

4.4. 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.[32] In this assay, androgenic and anti-androgenic activities of test compounds were determined in terms of SC-3 growth promotion by the test compound alone and inhibition of testosterone-induced cell growth by the test compound, 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 in wells with testosterone alone was defined as 100%. The concentration of the test compound that inhibited the increase of the cell number induced by 10 nM testosterone by 50% was quantified (IC_{50}) after log-logit transformation.

4.5. Receptor binding assay with LNCaP cells

LNCaP cells were cultured in the presence of RPMI1640 supplemented with 10% FBS at 37 °C under an atmosphere of 5% CO_2 in air. Cells were harvested with a cell scraper and by centrifugation at 800*g* at 4 °C for 5 min, and stored at −80 °C until use. The collected cells (ca. 1.2 g) were suspended in 21 mL of the cell extraction buffer (10 mM Tris, 1.5 mM EDTA, 0.25 M sucrose, 10 mM sodium molybdate, 1 mM PMSF). This suspension was subjected to homogenization (0 °C, 1000 rpm) and a crude fraction was

prepared by centrifugation of the suspension at 105,000*g* for 1 h for 4 °C. This supernatant protein, crude receptor fraction, was diluted to a protein concentration of 0.25 mg/mL with the cell extraction buffer. Binding studies were performed by incubating increasing concentrations of test compound (dissolved in DMSO) with receptor fraction in the presence of a saturating concentration of [^{3}H]testosterone (1 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 500*g* for 10 min. The protein fraction (350 μL) was collected and the radioactivity was determined with a liquid scintillation counter. All experiments were performed in duplicate.

4.6. PSA production assay of LNCaP by ELISA

LNCaP cells maintained in RPMI1640 medium supplemented with 10% FBS were plated at the concentration of 3×10^5 cell/mL with an appropriate concentration (0–10 μM) of a test compound and incubated in the same medium for 48 h at 37 °C under an atmosphere containing 5% CO_2 in the presence or absence of 10 nM testosterone. After the incubation, the supernatant was collected and the amount of PSA contained in the supernatant was quantified by the use of ELISA PSA Assay Kit (Cayman Co. Ltd) according to the protocol recommended by the supplier. The amount of PSA produced in the presence of 10 nM testosterone alone was defined as 100% (Fig. 4).

Acknowledgments

This study was supported by Kato Memorial Bioscience Foundation, and Grants-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and the Japan Society for the Promotion of Science. K.W. acknowledges the support of the Japan Society for the Promotion of Science in the form of a Fellowship for Japanese Junior Scientists.

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