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Design and synthesis of DPP-IV inhibitors lacking the electrophilic nitrile group

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Abstract—A series of (4β-substituted)-L-prolylpyrrolidine analogs lacking the electrophilic nitrile function were synthesized and their dipeptidyl peptidase IV (DPP-IV) inhibitory activity and duration of ex vivo activity were evaluated. Structural optimization of a *N*-(3-phenyl-1,2,4-thiadiazol-5-yl)piperazine analog **8**, which was found by high-speed analog synthesis, was carried out to improve the potency and duration of action. A representative compound **26** was evaluated to assess its effect on the plasma glucose level after the oGTT (oral glucose tolerance test) in normal rats. Structure–activity relationships (SAR) are also presented.

1. Introduction

Glucagon-like peptide 1 (GLP-1) is known to act as a mediator of glucose-stimulated insulin secretion, and several clinical studies have shown that this peptide has an antidiabetic action in subjects with type 2 diabetes.[1] The active form of GLP-1 is rapidly inactivated by plasma DPP-IV through cleavage of the dipeptide from the N-terminus, limiting its duration of action.[2,3] Inhibition of DPP-IV results in elevated circulating levels of endogenous GLP-1,[4] which is produced by L-cells of the small intestine in response to food intake.[5] Thus, inhibition of DPP-IV is a new and promising approach for the treatment of type 2 diabetes. Clinical results with P32/98 (**1**) helped to provide proof of principle for the application of DPP-IV inhibitors as glucose lowering agents.[6] Several DPP-IV inhibitors, including LAF237 (**2**),[7] BMS-477118 (**3**),[8] and MK-0431 (**4**)[9] (Fig. 1), are in the late stage of clinical evaluation or have been approved.

The (2*S*)-2-cyanopyrrolidine class of compounds, such as LAF237 (**2**)[7] and BMS-477118 (**3**),[8] includes many potent DPP-IV inhibitors. However, these agents suffer from chemical instability because the basic amine (NH) mounts an intramolecular attack on the electrophilic nitrile of the (2*S*)-2-cyanopyrrolidine moiety that yields cyclic products (Scheme 1). DPP-IV inhibitory activity of dipeptide analogs with (2*S*)-2-cyanopyrrolidine has been reported as well as their chemical stability in aqueous solution (pH 7.4).[10] Although most of the analogs tested have a good half-life, L-prolyl-(2*S*)-2-cyanopyrrolidine itself has a relatively short half-life.

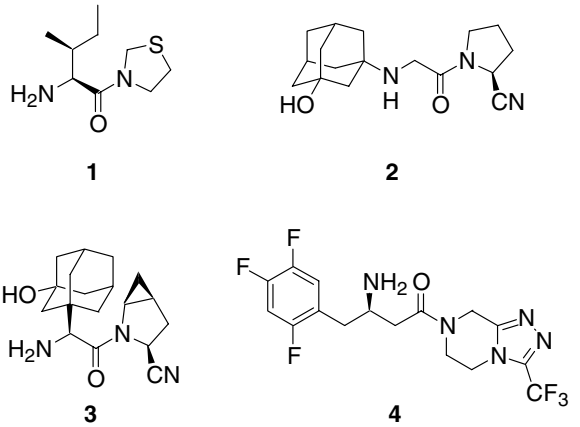

Figure 1. Representative DPP-IV inhibitors under clinical development.

Keywords: DPP-IV inhibitor; (4β-Substituted)-L-prolylpyrrolidine; 1,2,4-Thiadiazol-5-yl-piperazine.
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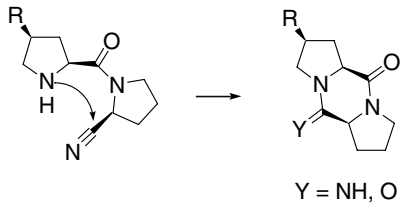


Scheme 1. Intramolecular cyclization of the L-prolyl-(*S*)-2-cyanopyrrolidine analogs.

This intrinsic instability may lead to difficulties in the formulation process.

On the other hand, the nitrile group, which covalently binds to DPP-IV, is not always necessary for DPP-IV inhibition as demonstrated by P32/98 (**1**)[6] and MK-0431 (**4**)[9] (Fig. 1). Because they are free from the intramolecular cyclization described in Scheme 1, these analogs are chemically stable. Analogs **1** and **4** have been shown to improve glucose tolerance in diabetic patients and healthy volunteers despite exhibiting only moderate inhibition.[6,11] Inhibitors lacking the electrophilic nitrile group have also been reported by others. Sakashita et al. reported that [(*S*)-γ-(arylamino)-L-prolyl]thiazolindine compounds with a 4β-(amino)-L-prolyl moiety were a novel series of potent and stable DPP-IV inhibitors,[12] but one of the representative compounds showed only a short duration of plasma DPP-IV inhibition after oral administration. Tsai et al. reported that pyrrolidine-2,4-dicarboxylic acid amides with a 4β-aminocarbonyl-L-prolyl moiety are DPP-IV inhibitors that exhibit a moderate level of activity.[13] One of their representative compounds showed quite a long duration of plasma DPP-IV inhibition after oral administration (10 mg/kg). They also reported that incorporation of the 5-*gem*-dimethyl substituent into the P2 pyrrolidine ring was detrimental to potency compared with that of the unsubstituted analog. However, neither group evaluated their representative compounds with respect to the reduction of plasma glucose during the oGTT in normal rats.

In our previous paper,[14] we reported the discovery of a potent DPP-IV inhibitor **5** (Fig. 2), which has a 5β-methyl-(4β-dimethylaminocarbonyl)-L-prolyl residue as the P2 moiety and (2*S*)-cyanopyrrolidine as the P1 moiety, respectively, and shows a long duration of action but much lower bioavailability (23% in rats) than expected relative to its duration of action. One of the purposes of our research is to identify a DPP-IV inhibitor with more potency and a better pharmacokinetic (PK) profile. Therefore, chemical modification was continued further with regard to the (4β-substituted)-L-prolyl-pyrrolidine analog **6** (Table 1). Another purpose of our research is to avoid safety problems by removing the reactive nitrile group from the P1 moiety. To find a novel chemical lead for further optimization, high-speed analog synthesis of pyrrolidine-2,4-dicarboxylic acid amides was conducted (Scheme 2).

Here we report on the discovery of another series of pyrrolidine-2,4-dicarboxylic acid amides, which have 1-(sulfur-containing hetero-aryl)piperazin-4-ylcarbonyl as a 4β-substituent of the L-prolyl moiety, and are novel and stable DPP-IV inhibitors that show long-lasting and potent ex vivo DPP-IV inhibition in normal rats after oral dosing. Representative compounds **24**–**26** (Fig. 2), which lack electrophilic nitrile group, were found to have strong DPP-IV inhibitory activity and a long duration of action.

2. Chemistry

Synthesis of the compounds listed in Tables 1–5 is outlined in Schemes 3–11. Synthesis of **7**, in which the electrophilic nitrile group was removed from the reported DPP-IV inhibitor,[14] is described in Scheme 3. Condensation of the N-protected (2*S*,4*S*)-pyrrolidine-2,4-dicarboxylic acid half ester **27**[14] with pyrrolidine and thiazolidine in the presence of EDC afforded **28a** and **28b**, respectively. Alkaline hydrolysis of **28a**–**b** provided **29a**–**b**, respectively. Amidation of **29a** with *N*,*N*-dimethylamine produced **30**, while acidic deprotection of **30** gave **7**.

N-(3-Substituted-1,2,4-thiadiazol-5-yl)piperazine analogs **8**–**10** and **18**–**24** were synthesized as described in Scheme 4. Replacement of the 5-chloro residue of 5-chloro-1,2,4-thiadiazoles **32a**–**g** with 1-*N*-*tert*-butoxycarbonylpiperazine **31a** afforded **33a**, **33c**–**h**, respectively. Replacement of the 5-chloro residue of 5-chloro-1,2,4-thiadiazoles **32a** with 1-*N*-*tert*-butoxycarbonylhomopiperazine **31b** afforded **33b**. Then acidic deprotection of **33a**–**h** gave **34a**–**h**, respectively. Amida-

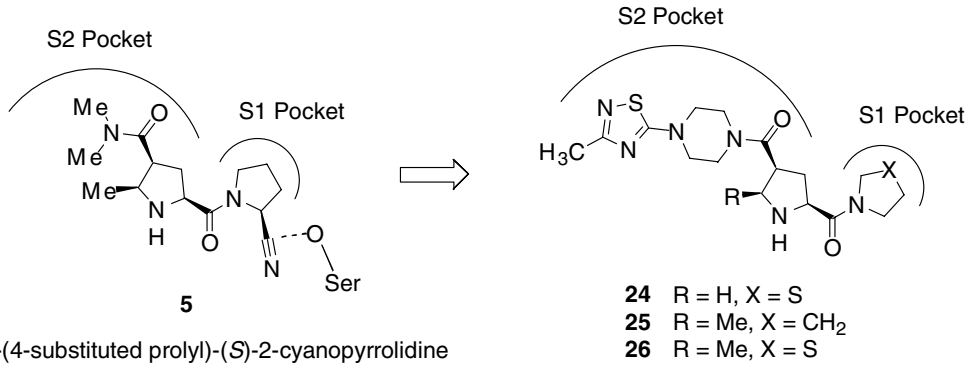
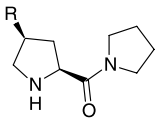
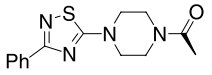
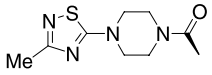


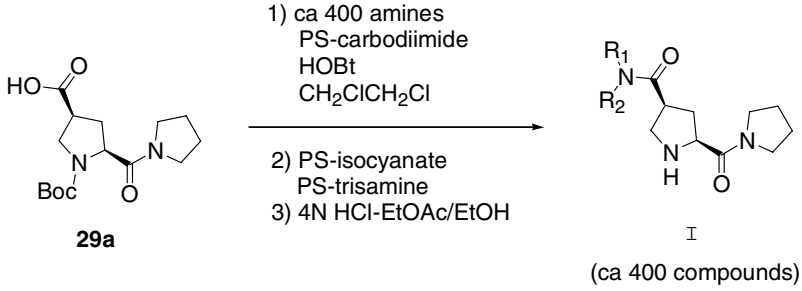
Figure 2. Discovery of new inhibitors.

Table 1. Preliminary SAR study of a newly found hit **8** and related analogs **6**, **7**, and **9**



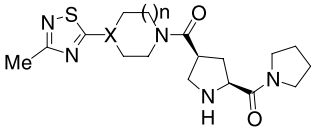
Compound	R	Human DPP-IV IC_{50} (nM)	Human plasma IC_{50} (nM)	Rat plasma IC_{50} (nM)
6	H	9700	NT[a]	NT[a]
7		670	NT[a]	NT[a]
8		20	420	150
9		20	80	58

[a] Not tested.



Scheme 2. High-speed analog synthesis.

Table 2. Effect of structural change of the piperazine moiety of **9** on activity profiles



Compound	X	*n*	Human DPP-IV IC_{50} (nM)	Human plasma IC_{50} (nM)	Rat plasma IC_{50} (nM)
9	N	1	20	80	58
10	N	2	310	970	760
11	C	1	350	970	450

tion of the deprotected amines **34a**–**h** with **29a** resulted in the N-protected analogs **35a**–**h**. Amidation of **29a** with a commercially available *N*-(3-phenyl-1,2,4-thiadiazol-5-yl)piperazine produced **35i**. Amidation of **34a** with **29b**provided **35j**. Acidic deprotection of **35a**–**j** yielded **8**–**10** and **18**–**24**, respectively.

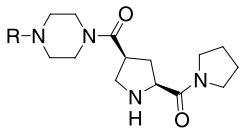
Preparation of 4-(3-methyl-1,2,4-thiadiazol-5-yl)piperidine analog **11** is described in Scheme 5. Replacement of the 5-chloro residue of **32a** with an anion derived from **36** and LDA gave the adduct **37**, after which alkaline hydrolysis followed by decarboxylation afforded **38**. Acidic deprotection of **38** led to the amine **39**. Amidation of **39** with **29a** in the presence of EDC produced a N-protected analog **40**, acidic deprotection of which afforded **11**.

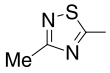
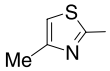
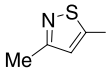
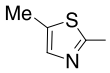
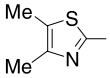
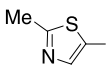
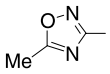
Synthesis of *N*-(4-methylthiazol-2-yl)piperazine analogs **12** and **15** is described in Scheme 6. Reaction of the 1-N-protected piperazine **31a** with 1,1-thiocarbonyldiimidazole (TCDI), followed by aminolysis, gave a thiourea derivative **41**. Reaction of **41** with appropriate α-chlorocarbonyl compounds resulted in the formation of thiazoles **42a**–**b**, after which acidic deprotection provided **43a**–**b**, respectively. Compounds **43a**–**b** were converted to **12** and **15**, respectively, according to the same procedure described for preparation of **11** from **39**.

The *N*-(3-methylisothiazol-5-yl)piperazine analog **13** was prepared from **45**[15] as described in Scheme 7. Aminolysis of **45** with 1-N-protected piperazine **31a** afforded **46**, oxidation of which with iodine gave an isothiazole derivative **47**. Acidic deprotection of **47** gave **48**, which was converted to **13** according to essentially the same procedure as that described for preparation of **11** from **39**.

The *N*-(5-methyl-1,3-thiazol-2-yl)piperazine analog **14** was synthesized as described in Scheme 8. Methylation of a N-protected *N*-(thiazol-2-yl)piperazine **50**, which was prepared by N-protection of 1-thiazol-2-yl-piperazine,[16] with methyl iodide in the presence of *n*-butyl lithium afforded **51**, after which acidic deprotection gave **52**.

Table 3. Effect of structural change of the 1,2,4-thiadiazole moiety of **9** on activity profiles



Compound	R	Human DPP-IV IC_{50} (nM)	Human plasma IC_{50} (nM)	Rat plasma IC_{50} (nM)
9		20	80	58
12		52	150	61
13		69	210	240
14		340	1100	320
15		410	1900	510
16		130	620	340
17		604	1300	960

Compound **52** was converted to **14** according to the procedure described for preparation of **11** from **39**.

Synthesis of *N*-(2-methyl-1,3-thiazol-5-yl)piperazine analog **16** was obtained from **31a** as described in Scheme 9. Sequential reactions (condensation of **31a** with *N*-bezyloxycarbonylglycine in the presence of EDC; catalytic hydrogenation of the resulting peptidic product in the presence of Pd/C; and acetylation with acetic anhydride in the presence of *N*-methyl morpholine) afforded **54**. Then compound **54** was converted to **55** by the reaction with Lawesson's reagent in pyridine. Acidic deprotection of **55** gave **56**, which was transformed to **16** according to essentially the same procedure as that described for preparation of **11** from **39**.

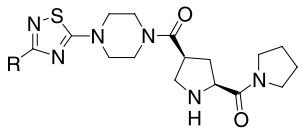
The *N*-(5-methyl-1,2,4-oxadiazol-3-yl)piperazine analog **17** was prepared from **31a** as described in Scheme 10. Cyanation of N-protected piperazine **31a** with cyanogen bromide in the presence of diisopropylethyl amine afforded **58**. Cyclization was carried out by reaction of**58** with hydroxylamine, followed by treatment with acetic anhydride to give **59**, acidic deprotection of which provided **60**. Then compound **60** was converted to **17** according to the procedure described for preparation of **11** from **39**.

N-(3-Methyl-1,2,4-thiadiazol-5-yl)piperazine analogs **25** and **26**, which contain 5β-methylpyrrolidine diamide unit, were synthesized as described in Scheme 11. Condensation of **62**, which was prepared by the previously reported method,[14] with *N*-Cbz-piperazine and *N*-methylpiperazine in the presence of EDC provided **63a** and **63c**, respectively. Catalytic hydrogenation of **63a** in the presence of Pd/C yielded **63b**. Alkaline hydrolysis of **63c** afforded the corresponding carboxylic acid **63d**. Stereoselective hydrogenation of **63b** and **63d** in the presence of PtO_2 resulted in the formation of 2,4,5-*cis* isomer **64a** and **64d**, respectively. N-Protection of **64a** led to **64b**, alkaline hydrolysis of which gave**64c**. Condensation of **64c** with pyrrolidine provided **65a**, catalytic hydrogenation of which afforded **65b**. Condensation of **64d** with thiazolidine afforded **65c**, after which reaction of **65c** with TrocCl gave **65d**.[17] Reductive cleavage of the *N*-Troc with zinc/acetic acid led to production of deprotected **65e**. Aminolysis of **32a** with **65b** and **65e** produced **66a** and **66b**, respectively. Acidic deprotection of **66a** and **66b** resulted in **25** and **26**, respectively.

3. Results and discussion

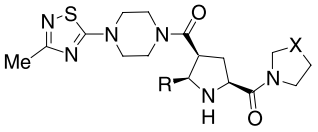
A solution of compounds listed in Tables 1–5 in distilled water was tested in vitro using purified human

Table 4. Effect of the 3-substituent of the 1,2,4-thiadiazole on activity profiles

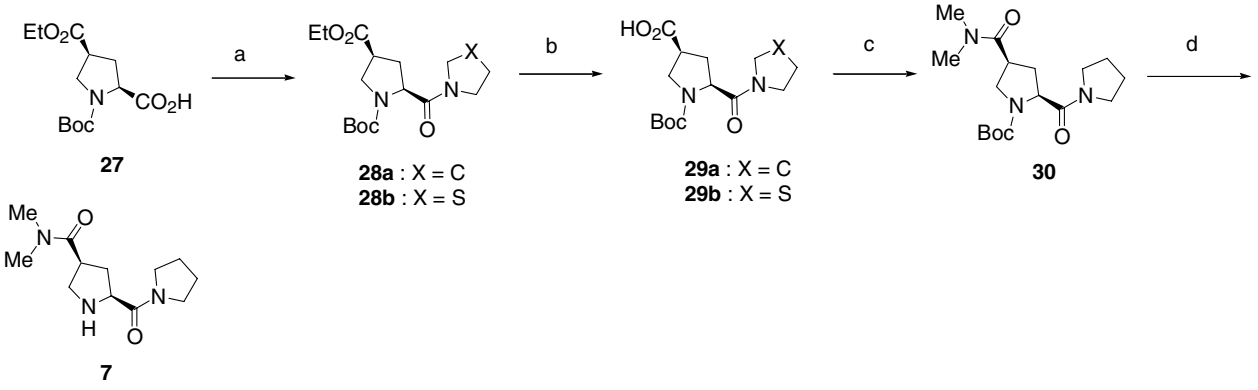


Compound	R	Human DPP-IV IC_{50} (nM)	Human plasma IC_{50} (nM)	Rat plasma IC_{50} (nM)	Plasma DPP-IV inhibition (%) at 3 mg/kg po, normal rats	
					30 min	9 h
8	Ph	20	420	150	64	11
18	H	19	58	45	85	56
9	Me	20	80	58	84	53
19	Et	37	92	120	80	47
20	^{n}Pr	21	86	110	81	48
21	^{i}Pr	52	150	190	85	43
22	$MeOCH_2$	17	40	49	52	41
23	CF_3	23	77	140	91	54

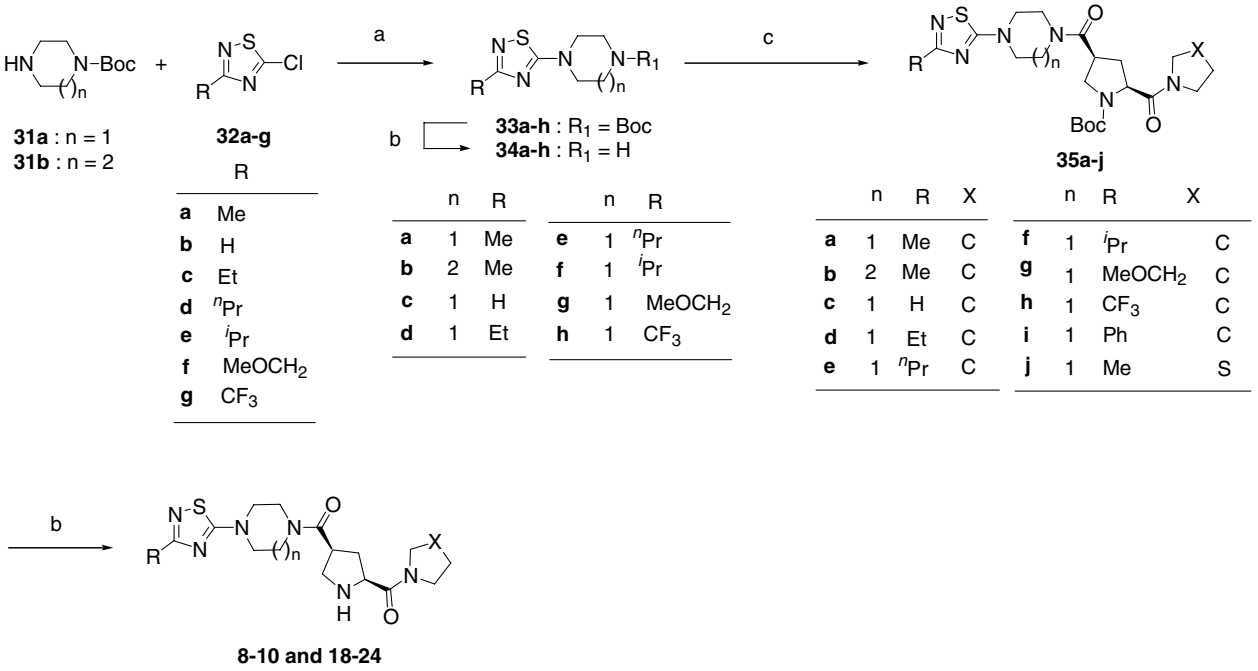
Table 5. Effect of the 5β-methyl residue of the prolyl moiety on activity profiles



Compound	R	X	Human DPP-IV IC_{50} (nM)	Human plasma IC_{50} (nM)	Rat plasma IC_{50} (nM)	Plasma DPP-IV inhibition (%) at 3 mg/kg po, normal rats	
						30 min	9 h
9	H	CH_2	20	80	58	84	53
24	H	S	18	44	76	91	75
25	Me	CH_2	29	35	69	89	74
26	Me	S	14	41	60	95	72



Scheme 3. Synthesis of **7**. Reagents: (a) pyrrolidine or thiazolidine, EDC, HOBt, CH_2Cl_2; (b) NaOH aq, MeOH; (c) Me_2NH, NMM, EDC, HOBt, CH_2Cl_2; (d) *p*-TsOH, EtOH.



	R
a	Me
b	H
c	Et
d	^{n}Pr
e	^{i}Pr
f	$MeOCH_2$
g	CF_3

	n	R
a	1	Me
b	2	Me
c	1	H
d	1	Et

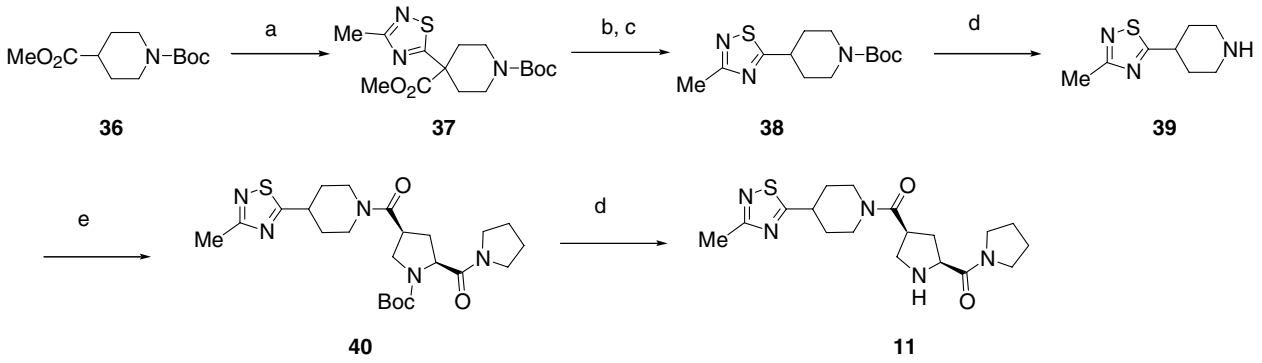
	n	R
e	1	^{n}Pr
f	1	^{i}Pr
g	1	$MeOCH_2$
h	1	CF_3

	n	R	X
a	1	Me	C
b	2	Me	C
c	1	H	C
d	1	Et	C
e	1	^{n}Pr	C

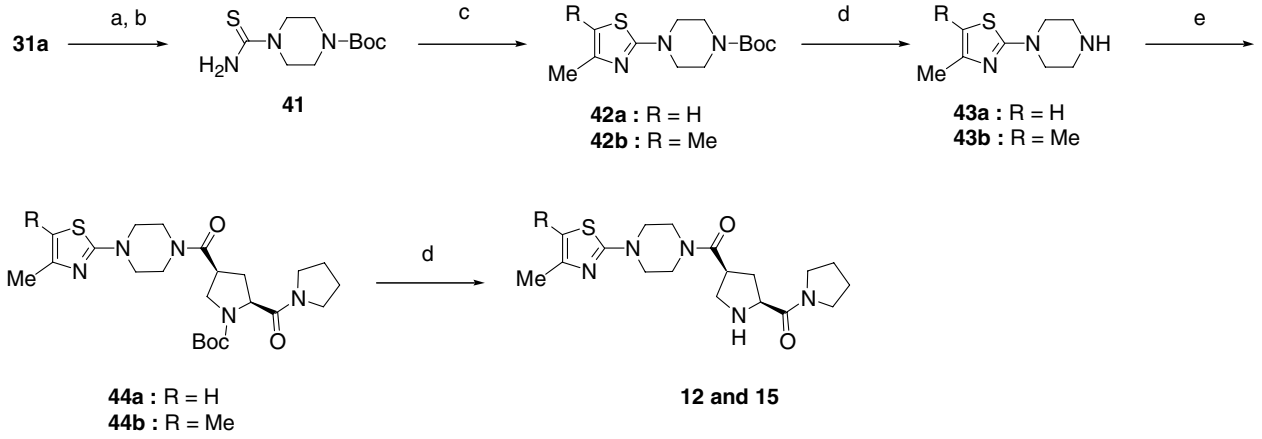
	n	R	X
f	1	^{i}Pr	C
g	1	$MeOCH_2$	C
h	1	CF_3	C
i	1	Ph	C
j	1	Me	S

Scheme 4. Synthesis of **8**–**10** and **18**–**24**. Reagents: (a) Et_3N, EtOH; (b) 4 N HCl/EtOAc; (c) **29a** or **29b**, EDC, HOBt, Et_3N, CH_2Cl_2.

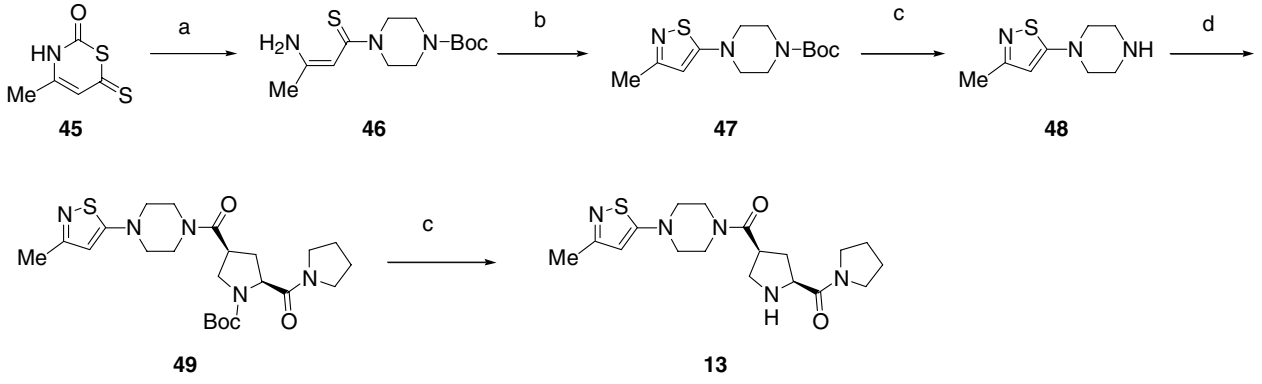
DPP-IV enzyme and intact human and rat plasma (final concentration of plasma was adjusted to 50%). Inhibition was determined using the synthetic substrate H-Gly-Pro-AMC.[18,19] Production of 7-amino-4-methyl coumarin (AMC) was measured over 15 min at 460 nm. Plasma DPP-IV inhibition (%) after oral dos-



Scheme 5. Synthesis of **11**. Reagents: (a) **32a**, LDA, THF; (b) NaOH aq, MeOH; (c) HCl aq, THF; (d) 4 N HCl/1,4-dioxane; (e) **29a**, EDC, HOBt, Et_3N, CH_2Cl_2.



Scheme 6. Synthesis of **12** and **15**. Reagents: (a) TCDI, THF; (b) NH_3, EtOH; (c) MeCOCHRCl, Et_3N, 1,4-dioxane; (d) 4 N HCl/1,4-dioxane; (e) **29a**, EDC, HOBt, Et_3N, CH_2Cl_2.

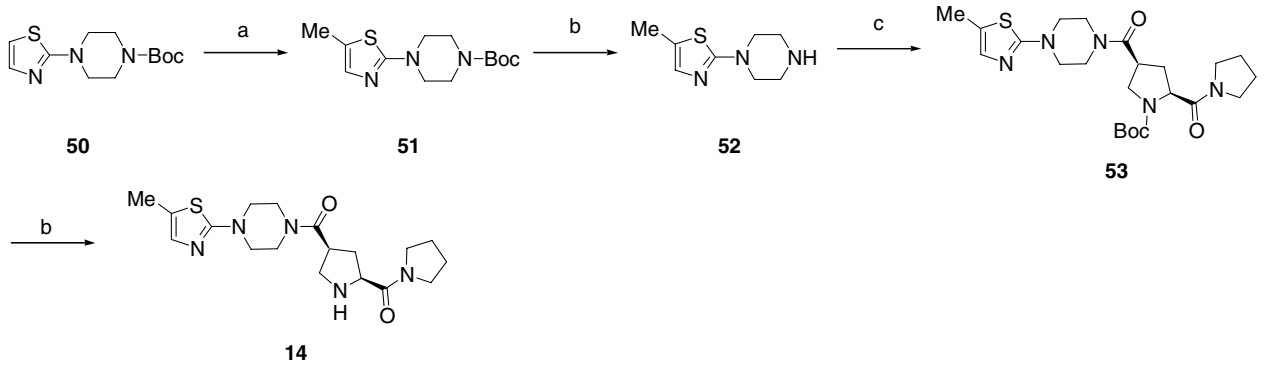


Scheme 7. Synthesis of **13**. Reagents: (a) **31a**, EtOH; (b) I_2, pyridine, EtOH; (c) 4 N HCl/1,4-dioxane; (d) **29a**, EDC, HOBt, NMM, CH_2Cl_2.

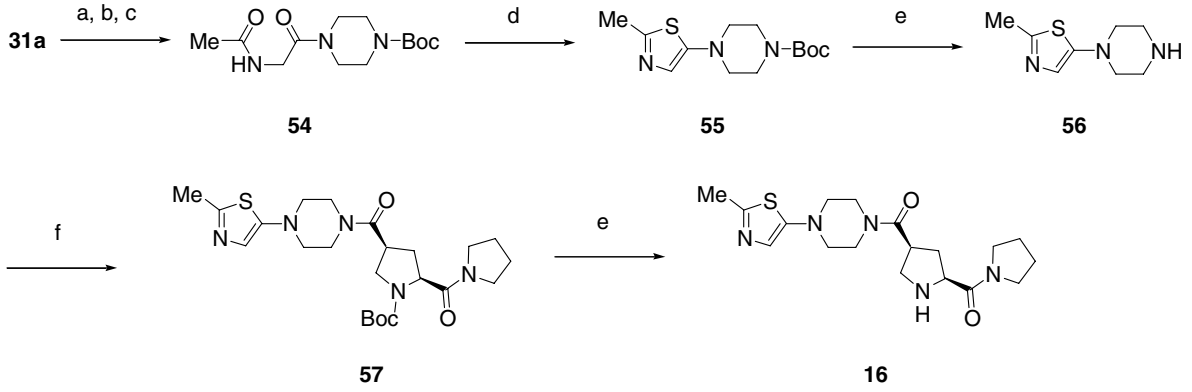
ing (3 mg/kg) was monitored over 30 min and 9 h in normal rats.

In our continuing efforts to develop long-acting inhibitors with once-a-day dosing potential, we previously reported the discovery of compound **5** as a potent DPP-IV inhibitor with a long duration of action.[14] To further explore chemically stable and potent DPP-IV inhibitors with a long duration of action, we next focused on the synthesis and evaluation of a series of 1-[(4β-substituted)-L-prolyl]pyrrolidine analogs lacking the electrophilic nitrile function. By removing the reactive nitrile group from the P1 moiety, the risk of safety problems is expected to be reduced in addition to improvement of the formulation process.

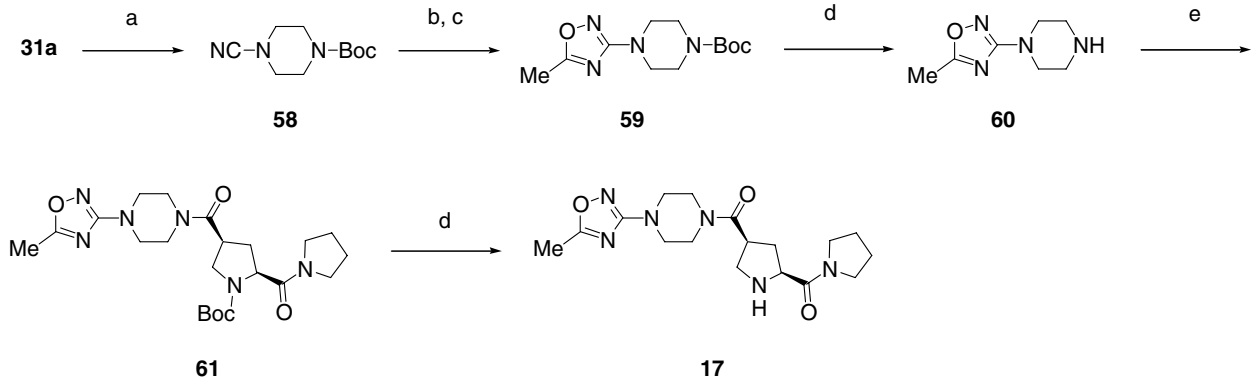
As shown in Table 1, removal of the nitrile group from the reported potent inhibitors with a long duration of activity[14] afforded **7**, which showed a marked reduction of inhibitory activity although its IC_{50} value was more



Scheme 8. Synthesis of **14**. Reagents: (a) MeI, ^{n}BuLi, THF; (b) 4 N HCl/1,4-dioxane; (c) **29a**, EDC, HOBt, Et_3N, CH_2Cl_2.



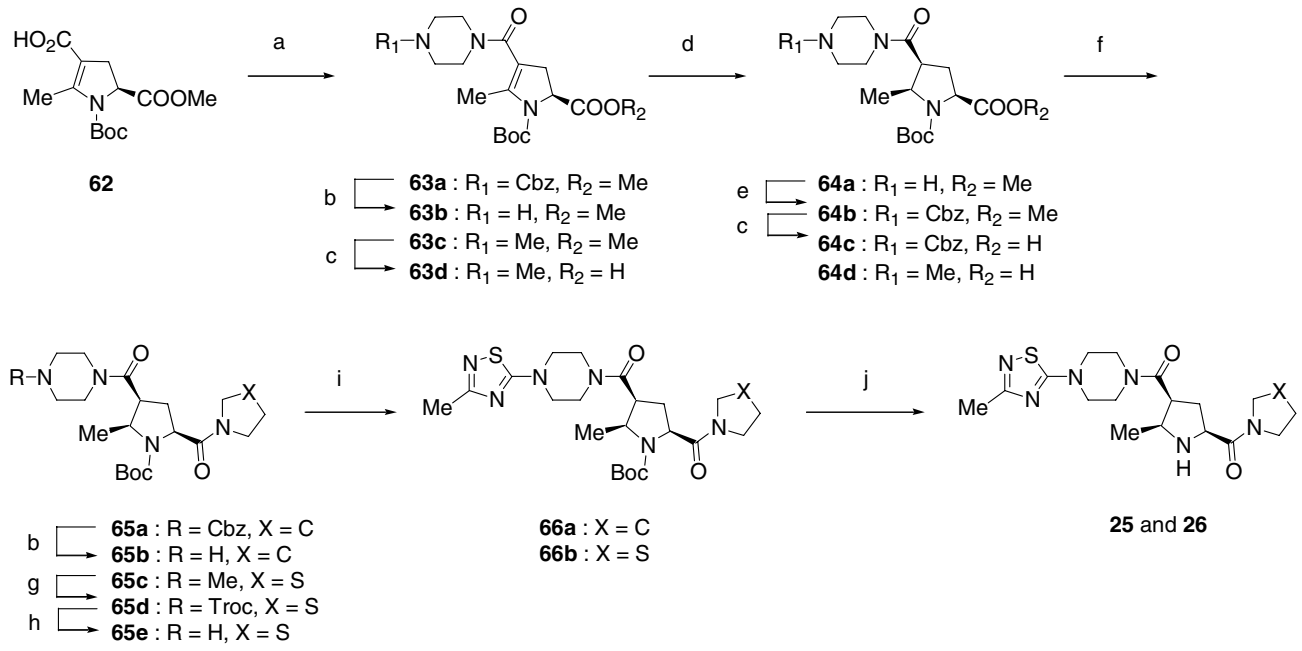
Scheme 9. Synthesis of **16**. Reagents: (a) Cbz-Gly, EDC, HOBt, NMM, CH_2Cl_2; (b) H_2, Pd/C, MeOH; (c) Ac_2O, pyridine, CH_2Cl_2; (d) Lawesson's reagent, pyridine; (e) 4 N HCl/1,4-dioxane; (f) **29a**, EDC, HOBt, NMM, CH_2Cl_2.



Scheme 10. Synthesis of **17**. Reagents: (a) BrCN, $^{i}Pr_2NEt$, CH_2Cl_2; (b) $NH_2OH \cdot HCl$, Et_3N, EtOH; (c) Ac_2O, pyridine; (d) 4 N HCl/1,4-dioxane; (e) **29a**, EDC, HOBt, Et_3N, CH_2Cl_2.

potent than that of the unsubstituted analog **6**. Accordingly, further optimization of the 4β-substituent of **7** was considered to be beneficial to increase DPP-IV inhibition. Based on these considerations, chemical modification was directed to the *N*,*N*-dimethylaminocarbonyl moiety, which is considered to occupy the S2 pocket (Fig. 2).

To find a chemical lead for further optimization, high-speed analog synthesis was conducted. As illustrated in Scheme 2, condensation of compound **29a** with nearly 400 amines in the presence of polymer-supported carbodiimide (PS-carbodiimide), followed by trapping of excess amines with polymer-supported isocyanate (PS-isocyanate), gave nearly 400 amide analogs **I**. This efficient synthetic process resulted in the discovery of 3-phenyl-1,2,4-thiadiazol-5-yl-piperazino analog **8** as a new chemical lead. Compound **8** exhibited much less potent inhibitory activity in both human and rat plasma relative to its potent activity in the enzyme assay, while the corresponding 3-methyl-1,2,4-thiadiazol-5-yl analog **9** showed equipotent activity in the enzyme assay, but



Scheme 11. Synthesis of **25** and **26**. Reagents: (a) *N*-Cbz-piperazine or *N*-methylpiperazine, EDC, HOBt, Et_3N, CH_2Cl_2; (b) H_2, Pd/C, EtOH; (c) NaOH aq, MeOH; (d) H_2, PtO_2, AcOH; (e) Cbz-Cl, $NaHCO_3$ aq, THF; (f) pyrrolidine or thiazolidine, EDC, HOBt, NMM, CH_2Cl_2; (g) TrocCl, CH_3CN; (h) Zn, AcOH; (i) **32a**, Et_3N, CH_2Cl_2; (j) 4 N HCl/1,4-dioxane.

exhibited better plasma inhibitory activity. Strong binding of **8** with plasma proteins was suggested by these results.

To optimize the piperazine moiety of **9**, compounds **10**–**11** were synthesized and evaluated as shown in Table 2. Ring expansion of the piperazine moiety of **9** provided **10**, which had nearly 15-fold less potent inhibitory activity. Replacement of one of the nitrogen atoms of the piperazine moiety of **9** gave **11**, with nearly 18-fold less potent inhibitory activity. In both cases, moderate reduction of plasma inhibitory activity was observed due to a presumed increase of protein binding. Chemical modification of this piperazine moiety seemed to be undesirable based on the above finding.

To further optimize the 3-methyl-1,2,4-thiadiazol-5-yl moiety of **9**, additional heterocyclic analogs were synthesized and evaluated as shown in Table 3. Replacement of the 3-methyl-1,2,4-thiadiazol-5-yl moiety of **9** with 4-methyl-1,3-thiazol-2-yl or 3-methyl-1,2-isothiazol-5-yl afforded **12-13**, respectively, resulting in the reduction of inhibitory activity. Replacement of the 3-methyl-1,2,4-thiadiazol-5-yl of**9** with 5-methyl-1,3-thiazol-2-yl, 4,5-dimethyl-1,3-thiazol-2-yl, or 2-methyl-1,3-thiazol-5-yl afforded **14**–**16**, respectively, which showed a significant decrease of inhibitory activity. Replacement of the 3-methyl-1,2,4-thiadiazol-5-yl moiety of **9** with 5-methyl-1,2,4-oxadiazol-3-yl afforded **17**, which showed a nearly 30-fold decrease of inhibitory activity. Most of the compounds listed in Table 3 had weaker activity in the plasma.

Based on the SAR described above, the sulfur atom of the 1,2,4-thiadiazole of **9** was found to play a very important role in DPP-IV inhibitory activity, as illustrated by the marked reduction of the inhibitory activity of **17**. Second, both the nitrogen atoms of the 1,2,4-thiadiazole nucleus had an auxiliary role in the interaction with the enzyme, as illustrated by the decreased inhibitory activity of the 1,3-thiazole and 1,2-isothiazole analogs **12** and **13**. Introduction of the 5-methyl residue of the 1,3-thiazole moiety of **14** and **15** was found to be unfavorable for inhibitory activity, probably because of steric hindrance of the interaction between the sulfur atom and the enzyme. Decreased activity of the 2-methyl-1,3-thiazole-5-yl analog **16** relative to that of **9** was also considered to occur for the same reason.

To optimize the 3-methyl residue on the 1,2,4-thiadiazole moiety of **9**, the compounds listed in Table 4 were synthesized and evaluated. Removal of the methyl residue of **9** provided **18**, with retention of equipotent inhibitory activity in both of the species tested. Replacement of the methyl residue of **9** with ethyl and *n*-propyl residues afforded **19** and **20**, respectively, which showed substantial retention of inhibitory activity against human DPP-IV in the enzyme assay, but tended to show decreased plasma inhibition in both of the species tested (probably due to an increase of protein binding). Replacement of the methyl residue of the thiadiazole of **9** with an isopropyl residue provided **21** and led to a reduction of activity. Replacement of the hydrophobic methyl residue of **9** with a more hydrophilic methoxymethyl residue gave **22**, which showed equipotent inhibitory activity with little loss of activity in the presence of plasma (due to its predicted lower level of protein binding). Replacement of the methyl residue of **9** with a trifluoromethyl residue provided **23**, which retained inhibitory activity in the enzyme assay but tended to show decreased plasma activity, probably for the increased protein binding. Thus, all of the 3-substituents

on the 1,2,4-thiadiazoles of **8**, **9**, and **19**–**23** were found to be acceptable in the desired enzyme pocket, but their inhibitory activity in plasma decreased along with an increase of their lipophilicity. Inhibition of plasma DPP-IV by **8**–**9** and **18**–**23** after oral dosing (3 mg/kg) was also assessed, as shown in Table 4. Compounds **9**, **18**–**21**, and **23** exhibited nearly the same pattern of ex vivo activity at 30 min and 9 h after oral dosing. They all showed 80–90% inhibition of plasma DPP-IV after 30 min and nearly 50% inhibition after 9 h. The 3-phenyl-1,2,4-thiadiazol-5-yl analog **8** exhibited relatively weaker plasma DPP-IV inhibition, presumably because of an increase in protein binding. The 3-methoxymethyl-1,2,4-thiadiazole analog **22** showed unexpectedly less potent plasma DPP-IV inhibition relative to its in vitro potency. Compound **22** had a good duration of action after it was orally absorbed, so such a result was considered to be due to decreased oral absorption because of the increase in hydrophilicity.

As shown in Table 5, synthesis and evaluation of the thiazolidine analog **24** and the 5β-methyl analogs **25** and **26** was carried out. Analogs **24**–**26** exhibited equipotent inhibition of human DPP-IV in the enzyme assay. These compounds tended to show increased activity relative to **9** in human plasma, while they showed equipotent activity in rat plasma. With regard to plasma DPP-IV inhibition, these compounds showed more potent inhibition and a longer duration of action relative to **9**, indicating improvement of their pharmacokinetics.

Compound **26**, a representative compound of the three compounds, was evaluated with regard to its effect on plasma glucose during the oGTT in normal rats. As shown in Figure 3, it was administered at oral doses of 0.1, 0.3, and 1 mg/kg and its effect on the plasma glucose level was compared with that of the vehicle (control). As a result, compound **26** effectively reduced the plasma glucose level.

In summary, the design and synthesis of DPP-IV inhibitors lacking an electrophilic nitrile group was performed. High-speed analog synthesis starting from **7**, which was based on **5**, resulted in the discovery of a new chemical lead **8** that possessed 3-phenyl-1,2,4-thiadiazol-5-yl-piperazinylcarbonyl as a 4β-substituent. Detailed SAR studies of (4β-substituted)-L-prolylpyrrolidine analogs, which had 1-*N*-(sulfur-containing hetero-aryl)piperazin-4-ylcarbonyl as the 4β-substituent of the L-prolyl moiety, resulted in the discovery of another structurally new series of inhibitors (represented by **24**–**26**) lacking an electrophilic nitrile group. Their high affinity for DPP-IV was considered to be due to the affinity of the sulfur-containing hetero-aromatic moiety. As a result, the marked reduction of inhibitory activity caused by removal of the reactive nitrile group from **5** was reversed by replacing the 4β-dimethylaminocarbonyl residue with a 4β-[1-*N*-(sulfur-containing hetero-aryl)piperazin-4-ylcarbonyl] residue. These compounds showed potent and long-lasting ex vivo activity. Compound **26** was tested as a representative compound and it demonstrated dose-dependent reduction of the plasma glucose level.

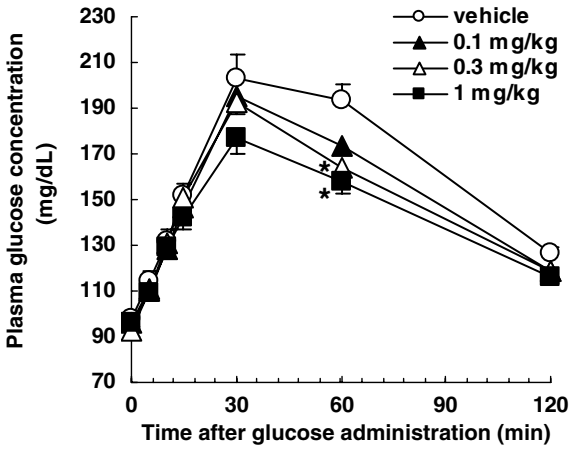


Figure 3. Effects of inhibitor **26** dosed at 0.1, 0.3, 1 mg/kg po versus vehicle control on plasma glucose after the oGTT in normal rats. $^{*}p < 0.05$ versus vehicle by Student's t test. Mean ± SE ($n = 8$).

4. Experimental

4.1. Chemistry

Analytical samples were homogeneous as confirmed by thin layer chromatography (TLC) and afforded spectroscopic results consistent with the assigned structures. Proton nuclear magnetic resonance spectra (^{1}H NMR) were taken on a Varian Mercury 300 spectrometer using deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) as the solvent. The chemical shift values are reported in parts per million (δ) and coupling constants (J) in hertz (Hz). Fast atom bombardment mass spectra (FAB-MS, HRMS) and electron ionization (EI) were obtained on a JEOL JMS-700 spectrometer. Atmospheric pressure chemical ionization (APCI) was determined on a HITACHI M-1200H spectrometer. Infrared spectra (IR) were measured in a JASCO FT/IR-430 spectrometer. Column chromatography was carried out on silica gel [Merck silica gel 60 (0.063–0.200 mm), Wako gel C200, or Fuji Silysia FL60D]. TLC was performed on silica gel (Merck TLC or HPTLC plates, silica gel 60 F254). The following abbreviations for solvents and reagents are used: tetrahydrofuran (THF), diethyl ether (Et_2O), diisopropyl ether ($^iPr_2O$), *tert*-butyl methyl ether (^{t}BuOMe), dimethylsulfoxide (DMSO), ethyl acetate (EtOAc), dimethylformamide (DMF), dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), methanol (MeOH), ethanol (EtOH), isopropyl alcohol (^{i}PrOH), acetic acid (AcOH), hexamethylphosphoric triamide (HMPA), and hydrochloric acid (HCl). Polymer-supported (PS) reagents were purchased from Biotage (PS-carbodiimide: 800371; PS-isocyanate: 800262; PS-trisamine: 800230).

4.1.1. Representative example of the high-speed analog synthesis (Scheme 2) 1-(3-phenyl-1,2,4-thiadiazol-5-yl)-4-{[(3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}piperazine hydrochloride (8). To a solution of **29a** in 1,2-dichloroethane (0.05 M, 200 μL) were added 3-phenyl-5-piperazino-1,2,4-thiadiazole in 1,2-dichloroethane (0.5 M, 60 μL), 1-hydroxybenzotriazole in DMF (0.5 M, 60 μL), and PS-carbodiimide

(1.26 mmol/g, 40 mg). The reaction mixture was shaken for 18 h at room temperature and treated with PS-isocyanate (1.43 mmol/g, 60 mg), PS-trisamine (4.34 mmol/g, 20 mg), and 1,2-dichloroethane (600 μL). After being shaken for 24 h, the reaction mixture was filtered and washed with 1,2-dichloroethane. The combined filtrates were concentrated in vacuo. To a solution of the resulting residue in EtOH (100 μL) was added 4 N hydrogen chloride in EtOAc (200 μL). After being shaken for 4 h, the reaction mixture was evaporated to give the title compound, which was dissolved in DMSO (1 mL) for the in vitro biological assay (10 mM).

According to the same procedures as described above, nearly 400 amide analogs I (Scheme 2) were synthesized.

4.1.2. 1-*tert*-Butyl 3-ethyl (3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-1,3-pyrrolidinedicarboxylate (28a). To a stirred solution of **27** (23.4 g, 81 mmol) in CH_2Cl_2 (160 mL) were added pyrrolidine (7.14 mL, 86 mmol), 1-hydroxybenzotriazole (13.7 g, 90 mmol), *N*-methylmorpholine (10.7 mL, 98 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (18.7 g, 98 mmol) at room temperature. After being stirred for 19 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and evaporated to give **28a** (27 g, 97%) as a pale orange powder. TLC $R_f = 0.38$ (EtOAc/hexane, 4:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.17 (t, $J = 7.0$ Hz, 3H), 1.24–1.43 (m, 9H), 1.61–1.98 (m, 6H), 3.03–3.53 (m, 6H), 3.56–3.76 (m, 1H), 3.98–4.15 (m, 2H), 4.26–4.56 (m, 1H).

4.1.3. 1-*tert*-Butyl 3-ethyl-(3*S*,5*S*)-5-(1,3-thiazolidin-3-ylcarbonyl)-1,3-pyrrolidinedicarboxylate (28b). Compound **28b** was prepared from **27** and thiazolidine in 86% yield as a white powder according to the same procedures as described for the preparation of **28a** from **27** and pyrrolidine. TLC $R_f = 0.34$ (EtOAc/hexane, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (t, $J = 7.1$ Hz, 3H), 1.40 and 1.45 (s, 9H), 2.20–2.37 (m, 1H), 2.40–2.57 (m, 1H), 2.93–3.19 (m, 3H), 3.63–3.74 (m, 1H), 3.79–3.97 (m, 2H), 4.03–4.21 (m, 3H), 4.33–4.78 (m, 3H).

4.1.4. (3*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinecarboxylic acid (29a). To a stirred solution of **28a** (1.56 g, 3.4 mmol) in MeOH (6.8 mL) was added 2 M NaOH (3.4 mL) at 0 °C. After being stirred at 0 °C for 1 h, the reaction was quenched with 2 M HCl (3.4 mL). The organic solvent was removed by evaporation. The resulting residue was diluted with EtOH. Insoluble substance was removed by filtration and the filtrate was evaporated. The resulting solid was washed with EtOAc/hexane (1:2) to yield **29a** (709 mg, 66%) as a white powder. TLC $R_f = 0.11$ (CH_2Cl_2/MeOH, 9:1); MS (APCI, neg. 20 V) *m*/*z* 311 $(M-H)^-$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.28 and 1.37 (s, 9H), 1.61–1.94 (m, 5H), 2.39–2.55 (m, 1H), 2.92–3.80 (m, 7H), 4.30–4.43 (m, 1H), 12.51 (br s, 1H).

4.1.5. (3*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-(1,3-thiazolidin-3-ylcarbonyl)-3-pyrrolidinecarboxylic acid (29b). Compound **29b** was prepared from **28b** in 93% yield as a white powder according to the same procedures as described for the preparation of **29a** from **28a**. $R_f = 0.28$ (CH_2Cl_2/MeOH, 9:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.30 and 1.38 (s, 9H), 1.74–2.03 (m, 1H), 2.39–2.62 (m, 1H), 2.84–3.18 (m, 3H), 3.21–3.49 (m, 1H), 3.53–3.81 (m, 3H), 4.24–4.78 (m, 3H), 12.48 (s, 1H).

4.1.6. *tert*-Butyl (2*S*,4*S*)-4-(dimethylcarbamoyl)-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (30). To a stirred solution of **29a** (100 mg, 0.32 mmol) in CH_2Cl_2 (1 mL) were added dimethylamine hydrochloride (39 mg, 0.48 mmol), 1-hydroxybenzotriazole (43 mg, 0.32 mmol), *N*-methylmorpholine (0.10 mL, 0.96 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (92 mg, 0.48 mmol) at room temperature. After being stirred for 19 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and evaporated to give **30** (98 mg, 90%) as a white powder. TLC $R_f = 0.36$ (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.45 (s, 9H), 1.75–2.07 (m, 4H), 2.25–2.54 (m, 2H), 2.96 (s, 3H), 3.07 (s, 3H), 3.10–3.30 (m, 1H), 3.28–4.03 (m, 6H), 4.28–4.60 (m, 1H).

4.1.7. (3*S*,5*S*)-*N*,*N*-Dimethyl-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinecarboxamide 4-methylbenzenesulfonate (7). A solution of **30** (93 mg, 0.27 mmol) and *p*-toluenesulfonic acid (78 mg, 0.41 mmol) in EtOH (2 mL) was stirred at 90 °C for 5 h. After cooling to room temperature, the reaction mixture was evaporated to give **7** (113 mg, 100%) as a colorless oil. TLC $R_f = 0.21$ (CH_2Cl_2/MeOH, 9:1); MS (APCI, pos.) *m*/*z* 240 $(M+H)^+$; IR (KBr) 3418, 2979, 1645, 1496, 1455, 1172, 1123, 1035, 1011, 685 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.68–1.97 (m, 5H), 2.28 (s, 3H), 2.67–2.80 (m, 1H), 2.82 (s, 3H), 3.00 (s, 3H), 3.27–3.60 (m, 7H), 4.38–4.54 (m, 1H), 7.11 (d, $J = 8.1$ Hz, 2H), 7.47 (d, $J = 8.1$ Hz, 2H), 8.56–8.75 (m, 1H), 9.23–9.44 (m, 1H); HRMS (FAB) calcd for $C_{12}H_{22}N_3O_2$: 240.1712. Found: 240.1715.

4.1.8. *tert*-Butyl 4-(3-methyl-1,2,4-thiadiazol-5-yl)-1-piperazinecarboxylate (33a). To a stirred solution of **31a** (931 mg, 5 mmol) in EtOH (15 mL) were added triethylamine (2.8 mL, 20 mmol) and **32a** (670 mg, 5 mmol). After being refluxed for 2 h, the reaction mixture was evaporated. The resulting residue was diluted with EtOAc. The organic layer was washed with H_2O, brine, then dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (3:2) as an eluant to yield **33a** (1.38 g, 97%) as a white powder. TLC $R_f = 0.33$ (EtOAc/hexane, 3:7); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 9H), 3.63 (s, 8H), 8.62 (d, $J = 2.5$ Hz, 1H), 8.70 (dd, $J = 2.5$, 1.6 Hz, 1H), 9.45 (d, $J = 1.6$ Hz, 1H).

4.1.9. *tert*-Butyl 4-(3-methyl-1,2,4-thiadiazol-5-yl)-1,4-diazepane-1-carboxylate (33b). Compound **33b** was prepared from **31b** and **32a** in 100% yield as a colorless oil according to the same procedures as described for the preparation of **33a** from **31a** and **32a**. TLC $R_f = 0.47$ (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ

1.44 (s, 9H), 1.93–2.05 (m, 2H), 2.41 (s, 3H), 3.30–3.51 (m, 2H), 3.56–3.72 (m, 6H).

According to the same procedures as described for the preparation of **33a** from **31a** and **32a**, compounds **33c–h** were prepared from **31a** and **32b–g**, respectively.

4.1.10. *tert*-Butyl 4-(1,2,4-thiadiazol-5-yl)-1-piperazinecarboxylate (33c). Yield 19%. A colorless oil. TLC $R_f = 0.68$ (EtOAc/hexane, 1:4); MS (APCI, pos. 20 V) *m/z* 271 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 1.49 (s, 9H), 3.48–3.65 (m, 8H), 7.96 (s, 1H).

4.1.11. *tert*-Butyl 4-(3-ethyl-1,2,4-thiadiazol-5-yl)-1-piperazinecarboxylate (33d). Yield 97%. An orange oil. TLC $R_f = 0.52$ (EtOAc/hexane, 1:3); ^1H NMR (300 MHz, $CDCl_3$) δ 1.30 (t, $J = 7.5$ Hz, 3H), 1.48 (s, 9H), 2.74 (q, $J = 7.5$ Hz, 2H), 3.48–3.60 (s, 8H).

4.1.12. *tert*-Butyl 4-(3-propyl-1,2,4-thiadiazol-5-yl)-1-piperazinecarboxylate (33e). Yield 95%. A colorless oil. TLC $R_f = 0.86$ (CH_2Cl_2/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, $J = 7.5$ Hz, 3H), 1.48 (s, 9H), 1.77 (m, 2H), 2.68 (m, 2H), 3.48–3.60 (s, 8H).

4.1.13. *tert*-Butyl 4-(3-isopropyl-1,2,4-thiadiazol-5-yl)-1-piperazinecarboxylate (33f). Yield 78%. A colorless oil. TLC $R_f = 0.83$ (CH_2Cl_2/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.30 (d, $J = 6.9$ Hz, 6H), 1.48 (s, 9H), 3.03 (m, 1H), 3.48–3.60 (s, 8H).

4.1.14. *tert*-Butyl 4-[3-(methoxymethyl)-1,2,4-thiadiazol-5-yl]-1-piperazinecarboxylate (33g). Yield 14%. A colorless oil. TLC $R_f = 0.40$ (EtOAc/hexane, 1:1); MS (APCI, pos. 20 V) *m/z* 315 $(M+H)^+$;^1H NMR (300 MHz, $CDCl_3$) δ 1.49 (s, 9H), 3.49 (s, 3H), 3.50–3.63 (m, 8H), 4.47 (s, 2H).

4.1.15. *tert*-Butyl 4-[3-(trifluoromethyl)-1,2,4-thiadiazol-5-yl]-1-piperazinecarboxylate (33h). Yield 6%. A brown oil. TLC $R_f = 0.32$ (EtOAc/hexane, 1:4); MS (APCI, pos. 20 V) *m/z* 339 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 1.49 (s, 9H), 3.53–3.65 (m, 8H).

4.1.16. *tert*-Butyl (2*S*,4*S*)-4-{[4-(3-methyl-1,2,4-thiadiazol-5-yl)-1-piperazinyl]carbonyl}-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (35a). Compound **33a** (1.37 g, 4.82 mmol) was added to 10% hydrogen chloride in MeOH (40 mL). The resulting suspension was stirred at room temperature for 16 h. The reaction mixture was concentrated in vacuo. The resulting crystalline solid was washed with EtOH to yield **34a** (1.05 g, 85%) as a white powder. To a stirred suspension of **34a** (213 mg, 0.83 mmol), in CH_2Cl_2 (3 mL) were added **29a** (200 mg, 0.64 mmol), *N*-methylmorpholine (0.37 mL, 3.36 mmol), 1-hydroxybenzotriazole (127 mg, 0.83 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (160 mg, 0.83 mmol). After being stirred for 17 h at room temperature, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 5% $KHSO_4$, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using CH_2Cl_2/MeOH (9:1) as an eluant to yield **35a** (235 mg, 76%) as a white powder. TLC $R_f = 0.44$ (CH_2Cl_2/MeOH, 9:1);^1H NMR (300 MHz, DMSO-d_6) δ 1.29 and 1.37 (s, 9H), 1.59–2.00 (m, 5H), 2.28 (s, 3H), 2.36–2.46 (m, 1H), 3.13–3.79 (m, 15H), 4.25–4.45 (m, 1H).

According to the same procedures as described for the preparation of **35a** from **33a**, compounds **35b–h** were prepared from **33b–h**, respectively.

4.1.17. *tert*-Butyl (2*S*,4*S*)-4-{[4-(3-methyl-1,2,4-thiadiazol-5-yl)-1,4-diazepan-1-yl]carbonyl}-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (35b). Yield 90%. A white powder. TLC $R_f = 0.42$ (CH_2Cl_2/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.45 (s, 9H), 1.73–2.09 (m, 6H), 2.17–2.39 (m, 2H), 2.41 (s, 3H), 2.99–3.23 (m, 1H), 3.27–3.98 (m, 14H), 4.30–4.51 (m, 1H).

4.1.18. *tert*-Butyl (2*S*,4*S*)-2-(1-pyrrolidinylcarbonyl)-4-{[4-(1,2,4-thiadiazol-5-yl)-1-piperazinyl]carbonyl}-1-pyrrolidinecarboxylate (35c). Yield 76%. A white powder. TLC $R_f = 0.36$ (CH_2Cl_2/MeOH, 9:1); MS (APCI, pos. 20 V) *m/z* 465 $(M+H)^+$; ^1H NMR (300 MHz, DMSO-d_6) δ 1.29 and 1.37 (s, 9H), 1.62–1.97 (m, 5H), 2.34–2.46 (m, 1H), 3.19–3.79 (m, 15H), 4.26–4.46 (m, 1H), 8.05 (s, 1H).

4.1.19. *tert*-Butyl (2*S*,4*S*)-4-{[4-(3-ethyl-1,2,4-thiadiazol-5-yl)-1-piperazinyl]carbonyl}-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (35d). Yield 59%. A white powder. TLC $R_f = 0.55$ (CH_2Cl_2/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.31 (t, $J = 7.5$ Hz, 3H), 1.40 and 1.45 (s, 9H), 1.80–2.22 (m, 4H), 2.30–2.56 (m, 2H), 2.74 (q, $J = 7.5$ Hz, 2H), 3.10–3.79 (m, 15H), 4.26–4.46 (m, 1H).

4.1.20. *tert*-Butyl (2*S*,4*S*)-4-{[4-(3-propyl-1,2,4-thiadiazol-5-yl)-1-piperazinyl]carbonyl}-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (35e). Yield 54%. A white powder. TLC $R_f = 0.38$ (CH_2Cl_2/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, $J = 7.4$ Hz, 3H), 1.40 and 1.45 (s, 9H), 1.71–2.11 (m, 6H), 2.26–2.59 (m, 2H), 2.66–2.77 (m, 2H), 3.09–3.29 (m, 1H), 3.29–4.10 (m, 14H), 4.34–4.63 (m, 1H).

4.1.21. *tert*-Butyl (2*S*,4*S*)-4-{[4-(3-isopropyl-1,2,4-thiadiazol-5-yl)-1-piperazinyl]carbonyl}-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (35f). Yield 66%. A white powder. TLC $R_f = 0.33$ (CH_2Cl_2/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.31 (d, $J = 7.0$ Hz, 6H), 1.40 and 1.45 (s, 9H), 1.75–2.09 (m, 4H), 2.27–2.64 (m, 2H), 2.95–3.11 (m, 1H), 3.11–3.28 (m, 1H), 3.29–4.03 (m, 14H), 4.32–4.59 (m, 1H).

4.1.22. *tert*-Butyl (2*S*,4*S*)-4-({4-[3-(methoxymethyl)-1,2,4-thiadiazol-5-yl]-1-piperazinyl}carbonyl)-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (35g). Yield 77%. A white powder. TLC $R_f = 0.43$ (CHCl3/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.45 (s, 9H), 1.68–2.13 (m, 4H), 2.27–2.62 (m, 2H), 3.07–3.27 (m, 1H), 3.31–4.02 (m, 14H), 3.49 (s, 3H), 4.33–4.60 (m, 1H), 4.47–4.48 (m, 2H).

4.1.23. *tert*-Butyl (2*S*,4*S*)-2-(1-pyrrolidinylcarbonyl)-4-({4-[3-(trifluoromethyl)-1,2,4-thiadiazol-5-yl]-1-piperazinyl}carbonyl)-1-pyrrolidinecarboxylate (35h). Yield 99%. A brown powder. TLC $R_f = 0.35$ (CH_2Cl_2/MeOH, 9:1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.45 (s, 9H), 1.78–2.06 (m, 4H), 2.30–2.59 (m, 2H), 3.07–4.02 (m, 15H), 4.34–4.58 (m, 1H).

4.1.24. *tert*-Butyl (2*S*,4*S*)-4-{[4-(3-phenyl-1,2,4-thiadiazol-5-yl)-1-piperazinyl]carbonyl}-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (35i). To a stirred solution of **29a** (200 mg, 0.32 mmol) in CH_2Cl_2 (1 mL) were added 3-phenyl-5-piperazino-1,2,4-thiadiazole in 1,2-dichloroethane (0.5 M, 1.4 mL), 1-hydroxybenzotriazole (95 mg, 0.70 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (134 mg, 0.70 mmol) at room temperature. After being stirred for 19 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and evaporated to give **35i** (255 mg, 74%) as a white powder. TLC $R_f = 0.75$ (EtOAc/MeOH, 5:1); ^1H NMR (300 MHz, DMSO-d_6) δ 1.30 and 1.38 (s, 9H), 1.71–2.00 (m, 4H), 2.40–2.55 (m, 2H), 3.20–3.84 (m, 15H), 4.30–4.42 (m, 1H), 7.42–7.50 (m, 3H), 8.05–8.14 (m, 2H).

According to the same procedures as described for the preparation of **35a** from **33a** and **29a**, compounds **35j** were prepared from **33a** and **29b**.

4.1.25. *tert*-Butyl (2*S*,4*S*)-4-{[4-(3-methyl-1,2,4-thiadiazol-5-yl)-1-piperazinyl]carbonyl}-2-(1,3-thiazolidin-3-ylcarbonyl)-1-pyrrolidinecarboxylate (35j). Yield 91%. A colorless oil. TLC $R_f = 0.40$ (EtOAc/MeOH, 5:1); MS (ESI, pos.) *m*/*z* 497 $(M+H)^+$; ^1H NMR (300 MHz, DMSO-d_6) δ 1.30 and 1.38 (s, 9H), 1.71–2.09 (m, 2H), 2.29 (s, 3H), 2.89–3.18 (m, 2H), 3.33–3.84 (m, 13H), 4.30–4.70 (m, 3H).

4.1.26. 1-(3-Phenyl-1,2,4-thiadiazol-5-yl)-4-{[(3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}piperazine hydrochloride (8). To a stirred solution of **35i** (255 mg, 0.47 mmol) in EtOH (10 mL) was added 4 N hydrogen chloride in EtOAc (1 mL). The resulting suspension was stirred at room temperature for 16 h. The reaction mixture was concentrated in vacuo. The resulting solid was washed with EtOAc to yield **8** (234 mg, 100%) as an ivory powder. TLC $R_f = 0.30$ ($CHCl_3$/MeOH, 9:1); MS (FAB, pos.) *m*/*z* 441 $(M+H)^+$; IR (KBr) 3400, 3053, 1647, 1553, 1466, 1434, 1354, 1298, 1236, 1170 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.71–1.98 (m, 5H), 2.65–2.87 (m, 1H), 3.22–3.82 (m, 15H), 4.35–4.55 (m, 1H), 7.40–7.52 (m, 3H), 8.03–8.17 (m, 2H), 8.69 (s, 1H), 9.97 (s, 1H).

According to the same procedures as described for the preparation of **8** from **35i**, compounds **9**–**10**, **18**–**24** were prepared from **35a**–**h**, **35j**, respectively.

4.1.27. 1-(3-Methyl-1,2,4-thiadiazol-5-yl)-4-{[(3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}piperazine hydrochloride (9). Yield 87%. A white powder. TLC $R_f = 0.52$ ($CHCl_3$/MeOH/AcOH, 75:20:5); MS (FAB, pos.) *m*/*z* 379 $(M+H)^+$; IR (KBr) 3400, 3053, 1647, 1553, 1466, 1434, 1354, 1298, 1236, 1170 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.72–1.96 (m, 5H), 2.29 (s, 3H), 2.62–2.84 (m, 1H), 3.14–3.77 (m, 15H), 4.33–4.56 (m, 1H), 8.66 (s, 1H), 10.38 (s, 1H); HRMS (FAB) calcd for $C_{17}H_{26}N_6O_2S$: 379.1916. Found: 379.1917.

4.1.28. 1-(3-Methyl-1,2,4-thiadiazol-5-yl)-4-{[(3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}-1,4-diazepane hydrochloride (10). Yield 90%. A white powder. TLC $R_f = 0.25$ (CH_2Cl_2/MeOH/AcOH, 8:1:1); MS (APCI, pos.) *m*/*z* 393 $(M+H)^+$; IR (KBr) 3378, 2925, 2878, 2711, 1645, 1455, 1361, 1254, 1153, 1040 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.70–2.03 (m, 7H), 2.28 (s, 3H), 2.58–2.75 (m, 1H), 3.20–3.94 (m, 15H), 4.36–4.56 (m, 1H), 7.85–9.00 (m, 1H), 10.17–11.25 (m, 1H); HRMS (FAB) calcd for $C_{18}H_{28}N_6O_2S$: 393.2073. Found: 393.2079.

4.1.29. 1-{[(3*S*,5*S*)-5-(1-Pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}-4-(1,2,4-thiadiazol-5-yl)piperazine hydrochloride (18). Yield 100%. A white powder. TLC $R_f = 0.17$ ($CHCl_3$/MeOH, 9:1); MS (ESI, pos.) *m*/*z* 365 $(M+H)^+$; IR (KBr) 3410, 2973, 2881, 1646, 1553, 1448, 1375, 1349, 1284, 1239 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.74–1.96 (m, 5H), 2.66–2.82 (m, 1H), 3.25–3.77 (m, 15H), 4.32–4.53 (m, 1H), 8.06 (s, 1H), 8.53–8.80 (m, 1H), 10.25–10.48 (m, 1H); HRMS (FAB) calcd for $C_{16}H_{24}N_6O_2S$: 365.176. Found: 365.175.

4.1.30. 1-(3-Ethyl-1,2,4-thiadiazol-5-yl)-4-{[(3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}piperazine hydrochloride (19). Yield 69%. A white powder. TLC $R_f = 0.31$ ($CHCl_3$/MeOH, 9:1); MS (APCI, pos.) *m*/*z* 393 $(M+H)^+$; IR (KBr) 3409, 1648, 1552, 1452, 1376, 1354, 1240, 1033, 1008 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.19 (t, 3H), 1.71–2.00 (m, 5H), 2.62 (q, $J = 7.6$ Hz, 2H), 2.68–2.82 (m, 1H), 3.25–3.62 (m, 15H), 4.34–4.54 (m, 1H), 8.56–8.78 (m, 1H), 10.06–10.29 (m, 1H); HRMS (FAB) calcd for $C_{18}H_{28}N_6O_2S$: 393.2073. Found: 393.2076.

4.1.31. 1-(3-Propyl-1,2,4-thiadiazol-5-yl)-4-{[(3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}piperazine hydrochloride (20). Yield 100%. A white powder. TLC $R_f = 0.58$ ($CHCl_3$/MeOH, 5:1); MS (FAB, pos.) *m*/*z* 407 $(M+H)^+$; IR (KBr) 3410, 2966, 2877, 1645, 1555, 1451, 1371, 1285, 1239 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 0.89 (t, $J = 7.4$ Hz, 3H), 1.58–1.75 (m, 2H), 1.75–1.97 (m, 5H), 2.58 (t, $J = 7.4$ Hz, 2H), 2.67–2.83 (m, 1H), 3.16–3.85 (m, 15H), 4.30–4.58 (m, 1H), 8.67 (s, 1H), 10.32 (s, 1H); HRMS (FAB) calcd for $C_{19}H_{30}N_6O_2S$: 407.2229. Found: 407.2232.

4.1.32. 1-(3-Isopropyl-1,2,4-thiadiazol-5-yl)-4-{[(3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}piperazine hydrochloride (21). Yield 86%. A white powder. TLC $R_f = 0.58$ ($CHCl_3$/MeOH, 5:1); MS (FAB, pos.) *m*/*z* 407 $(M+H)^+$; IR (KBr) 3409, 2974, 2879, 2735, 1644, 1555, 1451, 1385, 1349, 1284 cm^{-1}; ^1H NMR

(300 MHz, DMSO-d_6) δ 1.20 (d, J = 7.0 Hz, 6H), 1.71–1.96 (m, 5H), 2.65–2.81 (m, 1H), 2.84–3.01 (m, 1H), 3.16–3.86 (m, 15H), 4.33–4.53 (m, 1H), 8.66 (s, 1H), 10.31 (s, 1H); HRMS (FAB) calcd for $C_{19}H_{30}N_6O_2S$: 407.2229. Found: 407.2233.

4.1.33. 1-[3-(Methoxymethyl)-1,2,4-thiadiazol-5-yl]-4-{[(3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}piperazine hydrochloride (22). Yield 75%. A white powder. TLC R_f = 0.57 ($CHCl_3$/MeOH, 4:1); MS (FAB, pos.) m/z 409 $(M+H)^+$; IR (KBr) 3421, 2977, 2885, 1647, 1558, 1472, 1450, 1375, 1342, 1238 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.68–2.01 (m, 5H), 2.64–2.86 (m, 1H), 3.30 (s, 3H), 3.33–3.73 (m, 15H), 4.32 (s, 2H), 4.37–4.52 (m, 1H), 8.67 (s, 1H), 10.25 (s, 1H); HRMS (FAB) calcd for $C_{18}H_{28}N_6O_3S$: 409.2022. Found: 409.2021.

4.1.34. 1-{[(3*S*,5*S*)-5-(1-Pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}-4-[3-(trifluoromethyl)-1,2,4-thiadiazol-5-yl]-piperazine hydrochloride (23). Yield 57%. An orange powder. TLC R_f = 0.35 (CH_2Cl_2/MeOH, 10:1); MS (FAB, pos.) m/z 433 $(M+H)^+$; IR (KBr) 3363, 2924, 2852, 1656, 1649, 1637, 1569, 1561, 1450, 1193 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.70–2.03 (m, 5H), 2.64–2.84 (m, 1H), 3.18–3.85 (m, 15H), 4.36–4.54 (m, 1H), 8.41–8.96 (m, 1H), 9.80–10.47 (m, 1H); HRMS (FAB) calcd for $C_{17}H_{23}F_3N_6O_2S$: 433.1634. Found: 433.1631.

4.1.35. 1-(3-Methyl-1,2,4-thiadiazol-5-yl)-4-{[(3*S*,5*S*)-5-(1,3-thiazolidin-3-ylcarbonyl)-3-pyrrolidinyl]carbonyl}piperazine hydrochloride (24). Yield 98%. A white powder. TLC R_f = 0.31($CHCl_3$/MeOH, 9:1); MS (ESI, pos.) m/z 397 $(M+H)^+$; IR (KBr) 3405, 2925, 2857, 1652, 1556, 1442, 1383, 1334, 1287, 1119 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.76–1.99 (m, 1H), 2.29 (s, 3H), 2.66–2.86 (m, 1H), 2.92–3.20 (m, 2H), 3.30–3.97 (m, 13H), 4.34–4.77 (m, 3H), 8.59–8.95 (m, 1H), 10.20–10.55 (m, 1H); HRMS (FAB) calcd for $C_{16}H_{24}N_6O_2S_2$: 397.148. Found: 397.1483.

4.1.36. 1-*tert*-Butyl 4-methyl 4-(3-methyl-1,2,4-thiadiazol-5-yl)-1,4-piperidinedicarboxylate (37). To a stirred solution of diisopropylamine (499 mg, 4.93 mmol) in THF (3 mL) was added butyllithium in hexane (1.54 M, 3.2 mL) at 0 °C. After being stirred for 30 min, the reaction mixture was cooled to −78 °C. To the reaction mixture was added a solution of **36** (1.0 g, 4.11 mmol) in THF (3 mL). After being stirred at −78 °C for 1 h, a solution of **32a** (664 mg, 4.93 mmol) in THF (3 mL) was added. After being stirred at −78 °C for 1 h, the reaction mixture was warmed up to room temperature, poured into H_2O, and extracted with EtOAc. The organic layer was washed with H_2O, brine, dried over $MgSO_4$, and evaporated to give **37** (1.51 g), which was used for the next reaction without further purification.

4.1.37. *tert*-Butyl 4-(3-methyl-1,2,4-thiadiazol-5-yl)-1-piperidinecarboxylate (38). To a stirred solution of **37** (570 mg, 1.67 mmol) in MeOH (6 mL) was added 2 M NaOH (2 mL) at room temperature. After being stirred for 1 h, the reaction mixture was evaporated. The resulting residue was diluted with THF (4 mL) and treated with 2 M HCl (3 mL). After being stirred for 3 h, the reaction was quenched with aqueous $NaHCO_3$. The reaction mixture was extracted with EtOAc. The organic layer was washed with H_2O, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (2:3) as an eluant to yield **38** (470 mg, 99%) as a colorless oil. TLC R_f = 0.45 (EtOAc/hexane, 1:1); MS (APCI, pos. 20 V) m/z 284 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 1.47 (s, 9H), 1.63–1.83 (m, 2H), 2.04–2.16 (m, 2H), 2.65 (s, 3H), 2.81–2.97 (m, 2H), 3.21 (tt, J = 11.6, 3.8 Hz, 1H), 4.12–4.28 (m, 2H).

4.1.38. 4-(3-Methyl-1,2,4-thiadiazol-5-yl)piperidine hydrochloride (39). To a stirred solution of **38** (470 mg, 1.66 mmol) in dioxane (2 mL) was added 4 N hydrogen chloride in dioxane (2 mL) at room temperature. After being stirred for 17 h, the reaction mixture was evaporated to give **39** (404 mg), which was used for the next reaction without further purification.

4.1.39. *tert*-Butyl (2*S*,4*S*)-4-{[4-(3-methyl-1,2,4-thiadiazol-5-yl)-1-piperidinyl]carbonyl}-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (40). Compound **40** was prepared from **29a** and **39** in 86% yield as a white powder according to the same procedures as described for the preparation of **30** from **29a** and dimethylamine. TLC R_f = 0.36 (EtOAc/MeOH, 9:1); ^1H NMR (300 MHz, DMSO-d_6) δ 1.29 and 1.37 (s, 9H), 1.44–1.95 (m, 7H), 1.97–2.19 (m, 2H), 2.37–2.45 (m, 1H), 2.54 (s, 3H), 2.68–2.83 (m, 1H), 3.11–3.55 (m, 8H), 3.57–3.71 (m, 1H), 3.99–4.14 (m, 1H), 4.26–4.49 (m, 2H).

4.1.40. 4-(3-methyl-1,2,4-thiadiazol-5-yl)-1-{[(3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}piperidine hydrochloride (11). Compound **11** was prepared from **40** in 98% yield as a white powder according to the same procedures as described for the preparation of **8** from **35i**. TLC R_f = 0.50 ($CHCl_3$/MeOH/AcOH, 5:1:0.1); MS (FAB, pos.) m/z 378 $(M+H)^+$; IR (KBr) 3411, 2926, 2722, 1645, 1498, 1454, 1369, 1294, 1227, 1005 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.43–1.96 (m, 7H), 1.99–2.19 (m, 2H), 2.55 (s, 3H), 2.64–2.89 (m, 3H), 3.16–3.60 (m, 8H), 3.92–4.06 (m, 1H), 4.28–4.53 (m, 2H), 8.65 (s, 1H), 9.91 (s, 1H); HRMS (FAB) calcd for $C_{18}H_{27}N_5O_2S$: 378.1964. Found: 378.1966.

4.1.41. *tert*-Butyl 4-thiocarbamoyl-1-piperazinecarboxylate (41). To a stirred solution of 1,1′-thiocarbonyldiimidazole (1.51 g, 8.47 mmol) in THF (4 mL) was added a solution of **31a** (1.43 g, 7.70 mmol) in EtOH (4 mL) at room temperature. After being stirred for 30 min, the reaction mixture was diluted with EtOH (12 mL). Ammonia gas was bubbled for 5 min. After being stirred for 2 days, the reaction mixture was concentrated in vacuo. The resulting solid was washed with EtOAc to yield **41** (948 mg, 50%) as a white powder. TLC R_f = 0.55 (CH_2Cl_2/MeOH, 10:1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 9H), 3.51–3.60 (m, 4H), 3.77–3.93 (m, 4H), 5.76–5.86 (m, 2H).

4.1.42. *tert*-Butyl 4-(4-methyl-1,3-thiazol-2-yl)-1-piperazinecarboxylate (42a). To a stirred solution of **41** (500 mg, 2.0 mmol) in dioxane (2 mL) were added triethylamine (0.34 mL, 2.5 mmol) and chloroacetone (0.20 mL, 2.5 mmol). After being stirred for 15 h at 90 °C, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, then dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1:4) as an eluant to yield **42a** (540 mg, 94%) as a yellow oil. TLC $R_f = 0.30$ (EtOAc/hexane, 1:4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 9H), 2.25 (d, $J = 1.1$ Hz, 3H), 3.39–3.47 (m, 4H), 3.51–3.59 (m, 4H), 6.15 (q, $J = 1.0$ Hz, 1H).

4.1.43. *tert*-Butyl 4-(4,5-dimethyl-1,3-thiazol-2-yl)-1-piperazinecarboxylate (42b). To a stirred solution of **41** (500 mg, 2.0 mmol) in dioxane (4 mL) were added triethylamine (0.57 mL, 4.0 mmol) and 3-chloro-2-butanone (0.21 mL, 4.0 mmol). After being refluxed for 17 h, the reaction mixture was concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1:4) as an eluant to yield **42b** (134 mg, 22%) as a white powder. TLC $R_f = 0.27$ (EtOAc/hexane, 1:4); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.47 (s, 9H), 2.14 (s, 3H), 2.20 (s, 3H), 3.31–3.42 (m, 4H), 3.49–3.57 (m, 4H).

4.1.44. *tert*-Butyl (2*S*,4*S*)-4-{[4-(4-methyl-1,3-thiazol-2-yl)-1-piperazinyl]carbonyl}-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (44a). To a stirred solution of compound **42a** (535 mg, 1.89 mmol) in dioxane (2 mL) was added 4 N hydrogen chloride in dioxane (4.7 mL). The resulting suspension was stirred at room temperature for 3 h. The reaction mixture was evaporated to give **43a** (465 mg). To a stirred suspension of **43a** (465 mg, 1.89 mmol) in CH_2Cl_2 (8 mL) were added **29a** (491 mg, 1.58 mmol), *N*-methylmorpholine (0.44 mL, 4.0 mmol), 1-hydroxybenzotriazole (289 mg, 1.89 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (362 mg, 1.89 mmol). After being stirred for 6 h at room temperature, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 5% $KHSO_4$, aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using CH_2Cl_2/MeOH (9:1) as an eluant to yield **44a** (711 mg, 94%) as a white powder. TLC $R_f = 0.30$ (CH_2Cl_2/MeOH, 9:1);^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.45 (s, 9H), 1.78–2.07 (m, 4H), 2.26 (s, 3H), 2.30–2.60 (m, 2H), 3.10–3.25 (m, 1H), 3.31–4.05 (m, 14H), 4.32–4.58 (m, 1H), 6.18 (s, 1H).

4.1.45. *tert*-Butyl (2*S*,4*S*)-4-{[4-(4,5-dimethyl-1,3-thiazol-2-yl)-1-piperazinyl]carbonyl}-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (44b). To a stirred solution of compound **42b** (130 mg, 0.44 mmol) in EtOH (2 mL) was added to 4 N hydrogen chloride in EtOAc (1 mL). The resulting suspension was stirred at room temperature for 2 h. The reaction mixture was evaporated to give **43b** (123 mg). To a stirred suspension of **43b** (120 mg, 0.44 mmol) in CH_2Cl_2 (1 mL) were added **29a** (137 mg, 0.44 mmol), *N*-methylmorpholine (0.12 mL, 1.0 mmol), 1-hydroxybenzotriazole (81 mg, 0.53 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (101 mg, 0.53 mmol). After being stirred for 17 h at room temperature, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with aqueous $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/MeOH (4:1) as an eluant to yield **44b** (171 mg, 79%) as a white powder. TLC $R_f = 0.57$ (CH_2Cl_2/MeOH, 9:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 and 1.37 (s, 9H), 1.66–1.95 (m, 5H), 2.03 (s, 3H), 2.15 (s, 3H), 2.33–2.47 (m, 1H), 3.13–3.73 (m, 15H), 4.26–4.45 (m, 1H).

4.1.46. 1-(4-Methyl-1,3-thiazol-2-yl)-4-{[(3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}piperazine hydrochloride (12). Compound **12** was prepared in 97% yield as a white powder from **44a** according to the same procedures as described for the preparation of **8** from **35i**. TLC $R_f = 0.49$ (CH_2Cl_2/MeOH, 10:1); MS (FAB, pos.) *m*/*z* 378 $(M+H)^+$; IR (KBr) 3387, 2967, 2925, 2877, 1727, 1647, 1448, 1376, 1348, 1241 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.70–1.98 (m, 5H), 2.25 (s, 3H), 2.63–2.83 (m, 1H), 3.19–3.93 (m, 15H), 4.34–4.54 (m, 1H), 6.67 (s, 1H), 8.54–8.78 (m, 1H), 10.42–10.65 (m, 1H); HRMS (FAB) calcd for $C_{18}H_{27}N_5O_2S$: 378.1964. Found: 378.1963.

4.1.47. 1-(4,5-Dimethyl-1,3-thiazol-2-yl)-4-{[(3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}piperazine hydrochloride (15). Compound **15** was prepared in 99% yield as a white powder from **44b** according to the same procedures as described for the preparation of **8** from **35i**. TLC $R_f = 0.32$ ($CHCl_3$/MeOH, 9:1); MS (ESI, pos.) *m*/*z* 392 $(M+H)^+$; IR (KBr) 3398, 2955, 2883, 1646, 1614, 1446, 1377, 1349, 1288, 1241 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.73–1.96 (m, 5H), 2.11–2.20 (m, 6H), 2.65–2.83 (m, 1H), 3.25–3.77 (m, 15H), 4.28–4.58 (m, 1H), 8.58–8.75 (m, 1H), 10.05–10.36 (m, 1H); HRMS (FAB) calcd for $C_{19}H_{29}N_5O_2S$: 392.212. Found: 392.2122.

4.1.48. *tert*-Butyl 4-[(2*Z*)-3-amino-2-butenethioyl]-1-piperazinecarboxylate (46). To a stirred solution of **45** (159 mg, 1 mmol) in ^{i}PrOH (1 mL) was added **31a** (186 mg, 1 mmol). After being refluxed for 10 h, the reaction mixture was evaporated to give **46** (288 mg), which was used for the next reaction without further purification.

4.1.49. *tert*-Butyl 4-(3-methyl-5-isothiazolyl)-1-piperazinecarboxylate (47). To a stirred solution of **46** (288 mg, 1 mmol) and pyridine (0.17 mL, 2.1 mmol) in EtOH (3 mL) was added iodine (254 mg, 1 mmol) in EtOH (4 mL) at 0 °C. After being stirred for 2 h, the reaction mixture was evaporated. The resulting residue was diluted with EtOAc. Insoluble substance was removed by filtration and the filtrate was evaporated. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (4:6) as an eluant to yield **47** (99 mg, 34% from **45**) as a white powder. TLC $R_f = 0.44$ (CH_2Cl_2/MeOH, 9:1);^{1}H NMR (300 MHz,

$CDCl_3$) δ 1.47 (s, 9H), 2.33 (s, 3H), 3.16 (t, J = 5.4 Hz, 2H), 3.57 (t, J = 5.4 Hz, 2H), 6.05 (s, 1H).

4.1.50. 1-(3-Methyl-5-isothiazolyl)piperazine hydrochloride (48). To a stirred solution of **47** (94 mg, 0.33 mmol) in dioxane (2 mL) was added 4 N hydrogen chloride in dioxane (2 mL) at room temperature. After being stirred for 1 h, the reaction mixture was evaporated to give **48** (77 mg), which was used for the next reaction without further purification.

4.1.51. *tert*-Butyl (2*S*,4*S*)-4-{[4-(3-methyl-5-isothiazolyl)-1-piperazinyl]carbonyl}-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (49). Compound **49** was prepared from **29a** and **48** in 83% yield as a white powder according to the same procedures as described for the preparation of **30** from **29a** and dimethylamine. TLC R_f = 0.45 (CH_2Cl_2/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.45 (s, 9H), 1.78–2.07 (m, 4H), 2.35 (s, 3H), 2.30–2.60 (m, 2H), 3.10–3.25 (m, 1H), 3.31–4.05 (m, 14H), 4.32–4.58 (m, 1H), 6.07 (s, 1H).

4.1.52. 1-(3-Methyl-5-isothiazolyl)-4-{[(3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}piperazine hydrochloride (13). Compound **13** was prepared from **49** in 98% yield as a pale yellow powder according to the same procedures as described for the preparation of **8** from **35i**. TLC R_f = 0.29 ($CHCl_3$/MeOH, 9:1); MS (MALDI, pos.) m/z 378 $(M+H)^+$; IR (KBr) 3410, 3100, 2955, 2881, 1646, 1553, 1444, 1381, 1283, 1236 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.61–2.07 (m, 5H), 2.22 (s, 3H), 2.59–2.85 (m, 1H), 2.93–3.87 (m, 15H), 4.26–4.62 (m, 1H), 6.33 (s, 1H), 8.46–8.89 (m, 1H), 10.17–10.59 (m, 1H); HRMS (FAB) calcd for $C_{18}H_{27}N_5O_2S$: 378.1964. Found: 378.1966.

4.1.53. *tert*-Butyl 4-(5-methyl-1,3-thiazol-2-yl)-1-piperazinecarboxylate (51). To a stirred solution of **50** (1.0 g, 3.71 mmol) in THF (10 mL) was added butyllithium in hexane (1.54 M, 2.7 mL) at −78 °C. The reaction mixture was warmed up to −30 °C and then cooled to −78 °C. The reaction mixture was treated with methyl iodide (0.35 mL, 5.57 mmol) and warmed up to room temperature. The reaction was quenched with aqueous NH_4Cl. The reaction mixture was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporatd to give **51** (1.0 g, 95%) as a pale yellow powder. TLC R_f = 0.24 (EtOAc/hexane, 1:4);^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 9H), 2.29–2.32 (m, 3H), 3.36–3.43 (m, 4H), 3.51–3.58 (m, 4H), 6.80–6.85 (m, 1H).

4.1.54. 1-(5-Methyl-1,3-thiazol-2-yl)piperazine hydrochloride (52). To a stirred solution of **51** (1.0 g, 3.53 mmol) in EtOAc (5 mL) was added 4 N hydrogen chloride in dioxane (9 mL) at room temperature. After being stirred for 2 h, the reaction mixture was evaporated to give **52** (739 mg), which was used for the next reaction without further purification.

4.1.55. *tert*-Butyl (2*S*,4*S*)-4-{[4-(5-methyl-1,3-thiazol-2-yl)-1-piperazinyl]carbonyl}-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (53). Compound **53** was prepared from **29a** and **52** in 86% yield as a white powder according to the same procedures as described for the preparation of **30** from **29a** and dimethylamine. TLC R_f = 0.50 (CH_2Cl_2/MeOH, 10:1);^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40 and 1.45 (s, 9H), 1.77–2.04 (m, 4H), 2.32 (d, J = 1.3 Hz, 3H), 2.33–2.56 (m, 2H), 3.08–3.27 (m, 1H), 3.31–4.01 (m, 14H), 4.32–4.55 (m, 1H), 6.81–6.86 (m, 1H).

4.1.56. 1-(5-Methyl-1,3-thiazol-2-yl)-4-{[(3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}piperazine hydrochloride (14). Compound **14** was prepared from **53** in 87% yield as a white powder according to the same procedures as described for the preparation of **8** from **35i**. TLC R_f = 0.44 (CH_2Cl_2/MeOH, 5:1); MS (FAB, pos.) m/z 378 $(M+H)^+$; IR (KBr) 3387, 3077, 2925, 2761, 1647, 1444, 1376, 1286, 1238, 1157 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.70–1.97 (m, 5H), 2.21–2.34 (m, 3H), 2.65–2.84 (m, 1H), 3.21–3.82 (m, 15H), 4.33–4.54 (m, 1H), 7.10–7.20 (m, 1H), 8.56–8.78 (m, 1H), 10.27–10.49 (m, 1H); HRMS (FAB) calcd for $C_{18}H_{27}N_5O_2S$: 378.1964. Found: 378.1964.

4.1.57. *tert*-Butyl 4-(acetamidoacetyl)-1-piperazinecarboxylate (54). To a stirred solution of **31a** (1.87 g, 10 mmol) in CH_2Cl_2 (20 mL) were added *N*-benzyloxycarbonyl glycine (2.0 g, 9.56 mmol), 1-hydroxybenzotriazole (1.61 g, 10.5 mmol), *N*-methylmorpholine (1.2 mL, 10.5 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.01 g, 10.5 mmol) at room temperature. After being stirred for 6 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and evaporated. To a solution of the resulting residue in MeOH (32 mL) was added 10% palladium on carbon (364 mg). The reaction mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 2 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. To a solution of the resulting residue in CH_2Cl_2 (4.8 mL) were added pyridine (1.2 mL, 14 mmol) and acetic anhydride (0.68 mL, 7.21 mmol) at room temperature. After being stirred for 2 h, the reaction mixture was evaporated. The resulting residue was washed with EtOAc/hexane (1:1) to give **54** (1.25 g, 90%) as a white powder. TLC R_f = 0.32 (EtOAc/MeOH, 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 9H), 2.05 (s, 3H), 3.31–3.49 (m, 6H), 3.57–3.68 (m, 2H), 4.07 (d, J = 4.0 Hz, 2H), 6.57 (s, 1H).

4.1.58. *tert*-Butyl 4-(2-methyl-1,3-thiazol-5-yl)-1-piperazinecarboxylate (55). To a stirred solution of **54** (1.24 g, 4.35 mmol) in pyridine (9 mL) was added Lawesson's reagent (1.76 g, 4.35 mmol) at room temperature. After being stirred for 8 h at 100 °C, the reaction mixture was poured into aqueous $NaHCO_3$ and extracted with EtOAc/hexane (5:1). The organic layer was washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and evaporated. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (3:2) as an eluant to yield **55** (165 mg, 13%) as a colorless oil. TLC R_f = 0.41 (EtOAc/hexane, 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 9H), 2.58

(s, 3H), 2.94–3.06 (m, 4H), 3.50–3.60 (m, 4H), 6.81 (s, 1H).

4.1.59. 1-(2-Methyl-1,3-thiazol-5-yl)piperazine hydrochloride (56). To a stirred solution of **55** (130 mg, 0.46 mmol) in MeOH (3 mL) was added 10% hydrogen chloride in MeOH (3 mL) at room temperature. After being stirred for 2 h at 40 °C, the reaction mixture was evaporated to give **56** (91 mg), which was used for the next reaction without further purification.

4.1.60. *tert*-Butyl (2*S*,4*S*)-4-{[4-(2-methyl-1,3-thiazol-5-yl)-1-piperazinyl]carbonyl}-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (57). Compound **57** was prepared from **29a** and **56** in 70% yield as a white powder according to the same procedures as described for the preparation of **30** from **29a** and dimethylamine. TLC $R_f = 0.26$ (EtOAc/MeOH, 9:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 and 1.37 (s, 9H), 1.63–1.94 (m, 5H), 2.35–2.45 (m, 1H), 2.48–2.48 (m, 3H), 2.88–3.06 (m, 4H), 3.15–3.74 (m, 11H), 4.28–4.45 (m, 1H), 6.83 (s, 1H).

4.1.61. 1-(2-methyl-1,3-thiazol-5-yl)-4-{[(3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}piperazine hydrochloride (16). Compound **16** was prepared from **57** in 77% yield as a white powder according to the same procedures as described for the preparation of **8** from **35i**. TLC $R_f = 0.19$ ($CHCl_3$/MeOH, 4:1); MS (APCI, pos.) *m*/*z* 378 $(M+H)^+$; IR (KBr) 3378, 3071, 2959, 2876, 2378, 1642, 1586, 1536, 1446, 1235 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.69–2.01 (m, 5H), 2.58 (s, 3H), 2.64–2.81 (m, 1H), 2.92–3.20 (m, 4H), 3.22–3.76 (m, 11H), 4.31–4.54 (m, 1H), 7.05 (s, 1H), 8.56–8.76 (m, 1H), 10.14–10.35 (m, 1H); HRMS (FAB) calcd for $C_{18}H_{27}N_5O_2S$: 378.1964. Found: 378.1965.

4.1.62. *tert*-Butyl 4-cyano-1-piperazinecarboxylate (58). To a stirred solution of **31a** (3.0 g, 16.1 mmol) in CH_2Cl_2 (15 mL) were added diisopropylethylamine (3.1 mL, 18 mmol) and cyanogen bromide (1.88 g, 17.8 mmol) at 0 °C. After being stirred for 30 min, the reaction mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with H_2O, brine, then dried over $MgSO_4$, and evaporated. The resulting residue was washed with hexane to give **58** (3.35 g, 99%) as a white powder. TLC $R_f = 0.43$ (EtOAc/hexane, 1:2); MS (APCI, pos. 20 V) *m*/*z* 212 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.46 (s, 9H), 3.11–3.30 (m, 4H), 3.45–3.59 (m, 4H).

4.1.63. *tert*-Butyl 4-(5-methyl-1,2,4-oxadiazol-3-yl)-1-piperazinecarboxylate (59). To a stirred solution of **58** (300 mg, 1.23 mmol) in EtOH (2 mL) were added triethylamine (0.18 mL, 1.3 mmol) and hydroxylamine hydrochloride (90 mg, 1.3 mmol) at room temperature. After being stirred for 30 min at 80 °C, the reaction mixture was cooled to room temperature and diluted with EtOAc (5 mL). The insoluble substance was removed by filtration and the filtrate was evaporated. To a stirred solution of the resulting residue in pyridine (2 mL) was added acetic anhydride (0.12 mL, 1.3 mmol) at room temperature. After being stirred for 3 h at 80 °C, the reaction mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with H_2O, brine, then dried over $MgSO_4$, and evaporated. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1:2) as an eluant to yield **59** (186 mg, 56%) as a white powder. TLC $R_f = 0.48$ (EtOAc/hexane, 1:2); MS (APCI, pos. 20 V) *m*/*z* 269 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 9H), 2.46 (s, 3H), 3.35–3.46 (m, 4H), 3.46–3.57 (m, 4H).

4.1.64. 1-(5-Methyl-1,2,4-oxadiazol-3-yl)piperazine hydrochloride (60). To a stirred solution of **59** (495 mg, 1.84 mmol) in dioxane (2 mL) was added 4 N hydrogen chloride in dioxane (2 mL) at room temperature. After being stirred for 17 h, the reaction mixture was evaporated to give **60** (358 mg), which was used for the next reaction without further purification.

4.1.65. *tert*-Butyl (2*S*,4*S*)-4-{[4-(5-methyl-1,2,4-oxadiazol-3-yl)-1-piperazinyl]carbonyl}-2-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (61). Compound **61** was prepared from **29a** and **60** in 82% yield as a white powder according to the same procedures as described for the preparation of **30** from **29a** and dimethylamine. TLC $R_f = 0.46$ (EtOAc/MeOH, 9:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 and 1.37 (s, 3H), 1.67–1.95 (m, 5H), 2.36–2.43 (m, 1H), 2.44 (s, 3H), 3.19–3.72 (m, 15H), 4.27–4.44 (m, 1H).

4.1.66. 1-(5-Methyl-1,2,4-oxadiazol-3-yl)-4-{[(3*S*,5*S*)-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}piperazine hydrochloride (17). Compound **17** was prepared from **61** in 98% yield as a white powder according to the same procedures as described for the preparation of **8** from **35i**. TLC $R_f = 0.40$ ($CHCl_3$/MeOH/AcOH, 5:1:0.1); MS (FAB, pos.) *m*/*z* 363 $(M+H)^+$; IR (KBr) 3407, 1648, 1596, 1560, 1450, 1413, 1353, 1269, 1241, 1164 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.72–1.96 (m, 5H), 2.45 (s, 3H), 2.62–2.83 (m, 1H), 3.19–3.72 (m, 15H), 4.31–4.55 (m, 1H), 8.66 (s, 1H), 10.14 (s, 1H); HRMS (FAB) calcd for $C_{17}H_{26}N_6O_3$: 363.2145. Found: 363.2145.

4.1.67. 1-*tert*-Butyl 2-methyl (2*S*)-4-({4-[(benzyloxy)carbonyl]-1-piperazinyl}carbonyl)-5-methyl-2,3-dihydro-1H-pyrrole-1,2-dicarboxylate (63a). To a stirred solution of **62** (10.7 g, 37.6 mmol) in CH_2Cl_2 (40 mL) were added benzy 1-piperazinecarboxylate (9.93 mmol), 1-hydroxybenzotriazole (5.07 g, 37.6 mmol), *N*-methylmorpholine (10.3 mL, 93.8 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (10.8 g, 56.3 mmol) at room temperature. After being stirred for 19 h, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 10% aqueous citric acid, aqueous $NaHCO_3$, brine, then dried over $MgSO_4$, and evaporated. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1:2) as an eluant to yield **63a** (17.5 g, 90%) as a white powder. TLC $R_f = 0.27$ (EtOAc/hexane, 2:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 9H), 2.18 (s, 3H), 2.56–2.67 (m, 1H), 3.10–3.25 (m, 1H), 3.45–3.61 (m,

8H), 3.76 (s, 3H), 4.69 (dd, J = 12.1, 4.8 Hz, 1H), 5.15 (s, 2H), 7.30–7.43 (m, 5H).

4.1.68. 1-*tert*-Butyl 2-methyl (2*S*)-5-methyl-4-(1-piperazinylcarbonyl)-2,3-dihydro-1H-pyrrole-1,2-dicarboxylate (63b). To a solution of **63a** (16.5 g, 33.8 mmol) in EtOH (200 mL) was added 10% palladium on carbon (3.3 g). The reaction mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 1 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo to yield **63b** (12.7 g, 100%) as a white powder. TLC R_f = 0.10 (CH_2Cl_2/MeOH, 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.18 (s, 3H), 2.56–2.68 (m, 1H), 2.81–2.88 (m, 4H), 3.11–3.25 (m, 1H), 3.47–3.58 (m, 4H), 3.76 (s, 3H), 4.69 (dd, J = 12.2, 5.0 Hz, 1H).

4.1.69. 1-*tert*-Butyl 2-methyl (2*S*)-5-methyl-4-[(4-methyl-1-piperazinyl)carbonyl]-2,3-dihydro-1H-pyrrole-1,2-dicarboxylate (63c). Compound **63c** was prepared from **62** and 1-methylpiperazine in 99% yield as a yellow oil according to the same procedures as described for the preparation of **63a** from **62** and benzy 1-piperazinecarboxylate. TLC R_f = 0.10 (CH_2Cl_2/MeOH, 5:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.15–2.20 (m, 3H), 2.31 (s, 3H), 2.35–2.42 (m, 4H), 2.56–2.68 (m, 1H), 3.10–3.25 (m, 1H), 3.50–3.64 (m, 4H), 3.76 (s, 3H), 4.69 (dd, J = 12.0, 4.8 Hz, 1H).

4.1.70. (2*S*)-1-(*tert*-Butoxycarbonyl)-5-methyl-4-[(4-methyl-1-piperazinyl)carbonyl]-2,3-dihydro-1H-pyrrole-2-carboxylic acid (63d). To a stirred solution of **63c** (6.6 g, 17.5 mmol) in MeOH (50 mL) was added 2 M NaOH (17.5 mL) at room temperature. After being stirred for 1 h, the reaction was quenched with 2 M HCl (18 mL). The reaction mixture was evaporated. The resulting residue was diluted with EtOH. The insoluble substance was removed by filtration and the filtrate was evaporated to give **63d** (6.18 g), which was used for the next reaction without further purification.

4.1.71. 1-*tert*-Butyl 2-methyl (2*S*,4*S*,5*S*)-4-({4-[(benzyloxy)carbonyl]-1-piperazinyl}carbonyl)-5-methyl-1,2-pyrrolidinedicarboxylate (64b). To a solution of **63b** (12.7 g, 35.9 mmol) in AcOH (180 mL) was added platinum(IV) oxide (2.5 g, 11 mmol). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 29 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. To a stirred solution of the resulting residue in H_2O (30 mL) and ^{i}Pr$_2$O (30 mL) were added $NaHCO_3$ (28.4 g, 338 mmol) and benzyloxycarbonyl chloride (5.4 mL, 37.2 mmol) at room temperature. After being stirred for 18 h, the reaction mixture was diluted with EtOAc. The organic layer was separated, washed with brine, then dried over $MgSO_4$, and evaporated. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (1:1) as an eluant to yield **64b** (9.87 g, 60%) as a white powder. TLC R_f = 0.31 (EtOAc/hexane, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (d, J = 6.6 Hz, 3H), 1.40 and 1.46 (s, 3H), 2.22–2.40 (m, 1H), 2.56–2.76 (m, 1H), 3.11–3.68 (m, 9H), 3.74 (s, 3H), 4.04–4.40 (m, 2H), 5.16 (s, 2H), 7.31–7.43 (m, 5H).

4.1.72. (2*S*,4*S*,5*S*)-4-({4-[(Benzyloxy)carbonyl]-1-piperazinyl}carbonyl)-1-(*tert*-butoxycarbonyl)-5-methyl-2-pyrrolidinecarboxylic acid (64c). Compound **64c** was prepared from **64b** in 96% yield as a white powder according to the same procedures as described for the preparation of **63d** from **63c**. TLC R_f = 0.28 (CH_2Cl_2/MeOH, 10:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14 (d, J = 5.9 Hz, 3H), 1.43 (s, 9H), 2.28–2.49 (m, 1H), 2.62–2.88 (m, 1H), 3.14–3.84 (m, 9H), 4.08–4.51 (m, 2H), 5.16 (s, 2H), 7.28–7.46 (m, 5H).

4.1.73. (2*S*,4*S*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-methyl-4-[(4-methyl-1-piperazinyl)carbonyl]-2-pyrrolidinecarboxylic acid (64d). To a solution of **63d** (6.18 g, 17.5 mmol) in AcOH (80 mL) was added platinum(IV) oxide (1.5 g, 6.6 mmol). The mixture was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 29 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The resulting residue was diluted with dioxane and treated with 4 N hydrogen chloride in dioxane (5 mL). The reaction mixture was evaporated. The resulting residue was washed with EtOAc to give **64d** (4.40 g, 64%) as a white powder. TLC R_f = 0.10 (CH_2Cl_2/MeOH, 4:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.80–1.06 (m, 3H), 1.32 and 1.39 (s, 9H), 2.07–2.38 (m, 2H), 2.60–3.60 (m, 11H), 3.90–4.45 (m, 3H), 11.60 (br s, 1H), 12.47 (br s, 1H).

4.1.74. Benzyl 4-{[(2*S*,3*S*,5*S*)-1-(*tert*-butoxycarbonyl)-2-methyl-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}-1-piperazinecarboxylate (65a). Compound **65a** was prepared from **64c** in 45% yield as a white powder according to the same procedures as described for the preparation of **28a** from **27**. TLC R_f = 0.45 (EtOAc/MeOH, 20:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17 (d, J = 6.6 Hz, 1H), 1.21 (d, J = 6.6 Hz, 2H), 1.39 (s, 6H), 1.46 (s, 3H), 1.77–2.02 (m, 4H), 2.13–2.29 (m, 1H), 2.58–2.87 (m, 1H), 3.10–3.86 (m, 13H), 4.20–4.51 (m, 2H), 5.15 (s, 2H), 7.32–7.42 (m, 5H).

4.1.75. *tert*-Butyl (2*S*,3*S*,5*S*)-2-methyl-3-(1-piperazinylcarbonyl)-5-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (65b). Compound **65b** was prepared from **65a** in 79% yield as a white powder according to the same procedures as described for the preparation of **63b** from **63a**. TLC R_f = 0.65 (EtOAc/MeOH, 10:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (d, J = 6.6 Hz, 2 H), 1.23 (d, J = 6.6 Hz, 1H), 1.39 (s, 6H), 1.46 (s, 3H), 1.74–2.09 (m, 7H), 2.12–2.30 (m, 1H), 2.55–3.01 (m, 4H), 3.10–3.29 (m, 1H), 3.31–3.89 (m, 7H), 4.07–4.44 (m, 2H).

4.1.76. *tert*-Butyl (2*S*,3*S*,5*S*)-2-methyl-3-[(4-methyl-1-piperazinyl)carbonyl]-5-(1,3-thiazolidin-3-ylcarbonyl)-1-pyrrolidinecarboxylate (65c). Compound **65c** was prepared from **64d** and thiazolidine in 77% yield as a white powder according to the same procedures as described for the preparation of **28b** from **27** and thiazolidine. TLC R_f = 0.45 (EtOAc/MeOH, 20:1); MS (APCI, pos. 20 V) m/z 427 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (d, J = 6.8 Hz, 3H), 1.40 (s, 6H), 1.47 (s, 3H), 1.98–2.55 (m, 5H), 2.60–3.32 (m, 3H), 3.37–3.98 (m, 6H), 3.95–4.74 (m, 8H).

4.1.77. 2,2,2-Trichloroethyl 4-{[(2*S*,3*S*,5*S*)-1-(*tert*-butoxycarbonyl)-2-methyl-5-(1,3-thiazolidin-3-ylcarbonyl)-3-pyrrolidinyl]carbonyl}-1-piperazinecarboxylate (65d). To a stirred solution of **65c** (2.18 g, 5.11 mmol) in CH_3CN (17 mL) was added 2,2,2-trichloroethyl chloroformate (1.4 mL, 10.2 mmol) at room temperature. After being stirred for 2 h, the reaction mixture was evaporated. The resulting residue was purified by silica gel chromatography using EtOAc/hexane (4:1) as an eluant to yield **65d** (1.50 g, 41%) as a white powder. TLC $R_f = 0.37$ (EtOAc/hexane, 4:1); MS (APCI, pos. 20 V) *m*/*z* 587 $(M+H)^+$; ^1H NMR (300 MHz, $CDCl_3$) δ 1.13–1.24 (m, 3H), 1.37–1.50 (m, 9H), 2.16–2.36 (m, 1H), 2.60–2.83 (m, 1H), 2.92–4.00 (m, 13H), 4.23–4.52 (m, 2H), 4.53–4.73 (m, 2H), 4.75–4.83 (m, 2H).

4.1.78. *tert*-Butyl (2*S*,3*S*,5*S*)-2-methyl-3-(1-piperazinylcarbonyl)-5-(1,3-thiazolidin-3-ylcarbonyl)-1-pyrrolidinecarboxylate (65e). To a stirred solution of **65d** (1.22 g, 2.08 mmol) in AcOH (10 mL) was added zinc powder (1.36 g, 20.8 mmol) at room temperature. After being stirred for 15 h, insoluble substance was removed by filtration and the filtrate was evaporated. The resulting residue was diluted with dioxane (20 mL) and treated with 4 N hydrogen chloride in dioxane (0.6 mL). The reaction mixture was evaporated to give **65e** (880 mg), which was used for the next reaction without further purification.

4.1.79. *tert*-Butyl (2*S*,3*S*,5*S*)-2-methyl-3-{[4-(3-methyl-1,2,4-thiadiazol-5-yl)-1-piperazinyl]carbonyl}-5-(1-pyrrolidinylcarbonyl)-1-pyrrolidinecarboxylate (66a). To a stirred solution of **65b** (200 mg, 0.51 mmol) in CH_2Cl_2 (0.5 mL) were added triethylamine (0.080 mL, 0.57 mmol) and **32a** (69 mg, 0.51 mmol) at room temperature. After being stirred for 17 h, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with H_2O, brine, then dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by silica gel chromatography using EtOAc/MeOH (4:1) as an eluant to yield **66a** (247 mg, 98%) as a white powder. TLC $R_f = 0.40$ (EtOAc/MeOH, 4:1); MS (APCI, pos. 20 V) *m*/*z* 492 $(M+H)^+$; ^1H NMR (300 MHz, DMSO-d_6) δ 0.97 (d, $J = 6.4$ Hz, 3H), 1.29 (s, 6H), 1.37 (s, 3H), 1.64–1.93 (m, 4H), 2.08–2.27 (m, 2H), 2.29 (s, 3H), 3.19–3.36 (m, 7H), 3.37–3.78 (m, 6H), 4.12 (d, $J = 7.0$ Hz, 1H), 4.21–4.34 (m, 1H).

4.1.80. *tert*-Butyl (2*S*,3*S*,5*S*)-2-methyl-3-{[4-(3-methyl-1,2,4-thiadiazol-5-yl)-1-piperazinyl]carbonyl}-5-(1,3-thiazolidin-3-ylcarbonyl)-1-pyrrolidinecarboxylate (66b). Compound **66b** was prepared from **65e** and **32a** in 25% yield as a white powder according to the same procedures as described for the preparation of **66a** from **65b** and **32a**. TLC $R_f = 0.31$ (EtOAc/MeOH, 20:1); ^1H NMR (300 MHz, $CDCl_3$) δ 1.15–1.32 (m, 3H), 1.40 (s, 6H), 1.46 (s, 3H), 2.17–2.35 (m, 1H), 2.43 (s, 3H), 2.55–2.85 (m, 1H), 2.90–3.97 (m, 13H), 4.25–4.81 (m, 4H).

4.1.81. 1-{[(2*S*,3*S*,5*S*)-2-Methyl-5-(1-pyrrolidinylcarbonyl)-3-pyrrolidinyl]carbonyl}-4-(3-methyl-1,2,4-thiadiazol-5-yl)piperazine hydrochloride (25). Compound **25** was prepared from **66a** in 89% yield as a white powder according to the same procedures as described for the preparation of **8** from **35i**. TLC $R_f = 0.32$ ($CHCl_3$/MeOH, 9:1); MS (ESI, pos.) *m*/*z* 393 $(M+H)^+$; IR (KBr) 3393, 2974, 1643, 1556, 1449, 1384, 1345, 1281, 1231, 995 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.15 (d, $J = 7.0$ Hz, 3H), 1.68–2.00 (m, 4H), 2.12–2.27 (m, 1H), 2.29 (s, 3H), 2.52–2.67 (m, 1H), 3.18–3.75 (m, 13H), 3.80–4.02 (m, 1H), 4.34–4.55 (m, 1H), 7.89–8.34 (m, 1H), 10.26–10.72 (m, 1H); HRMS (FAB) calcd for $C_{18}H_{28}N_6O_2S$: 393.2073. Found: 393.2072.

4.1.82. 1-(3-Methyl-1,2,4-thiadiazol-5-yl)-4-{[(2*S*,3*S*,5*S*)-2-methyl-5-(1,3-thiazolidin-3-ylcarbonyl)-3-pyrrolidinyl]carbonyl}piperazine hydrochloride (26). Compound **26** was prepared from **66b** in 99% yield as a white powder according to the same procedures as described for the preparation of **8** from **35i**. TLC $R_f = 0.55$ (CH_2Cl_2/MeOH, 5:1); MS (APCI, pos.) *m*/*z* 411 $(M+H)^+$; IR (KBr) 3370, 2924, 1634, 1552, 1442, 1371, 1228, 1147, 1118, 1041 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.11–1.24 (m, 3H), 2.15–2.30 (m, 1H), 2.29 (s, 3H), 2.54–2.70 (m, 1H), 2.99–3.17 (m, 2H), 3.38–3.80 (m, 11H), 3.82–4.00 (m, 1H), 4.38–4.77 (m, 3H), 8.03–8.23 (m, 1H), 10.37–10.60 (m, 1H); HRMS (FAB) calcd for $C_{17}H_{26}N_6O_2S_2$: 411.1637. Found: 411.1638.

4.2. Biological method

4.2.1. Purification of human DPP-IV. Human DPP-IV was purified according to the published procedure with some modifications.[18] Briefly, the enzyme was prepared from pooled plasma obtained from healthy volunteers by ammonium sulfate precipitation (50–70%). After extensive dialysis against 25 mM Tris–HCl (pH 7.4), the resulting material was mixed with DEAE cellulose and DE52 (Whatman Chemical Separation, Inc., USA) for 60 min, and eluted with buffer containing 100 mM NaCl. Fractions (10 mL) were collected, and the fraction showing maximum DPP-IV activity was dialyzed against 25 mM MES–NaOH (pH 6.0). DPP-IV activity in the fractions was detected from the hydrolysis of Gly-Pro-7-amido-4-methyl-coumarin (Gly-Pro-AMC) (Sigma–Aldrich, USA), using the standard method described below. The DE52 elute was loaded onto an SP Sepharose Fast Flow column (GE Healthcare, Sweden), and the flow-through fraction-containing DPP-IV was then applied to a DEAE cellulose column (Whatman DE52). Bound proteins were eluted with 25 mM Tris–HCl (pH 7.8) containing 150 mM NaCl. Fractions (10 mL) were collected, and the fraction with the maximum DPP-IV activity was concentrated using polyethylene glycol 20000 (PEG20000). The concentrate was applied to a Sephacryl S-300 High Resolution 26/60 column (GE Healthcare, Sweden), which was eluted at a flow rate of 0.1 mL/min. Fractions (1 mL) were collected, and the fractions-containing DPP-IV activity were pooled.

4.2.2. Enzyme assays. Enzymatic activity was determined at 37 °C by assessing the cleavage rate of a substrate, Gly-Pro-AMC (30 μM) (Sigma–Aldrich, USA).[19] Briefly, 10 μL of DPP-IV solution was added to each well of a 96-well flat-bottomed microtiter plate,

followed by the addition of 50 μL of 60 μM Gly-Pro-AMC, 10 μL of 500 mM Tris–HCl (pH 7.4), 20 μL of distilled water, and 10 μL of a test compound. Then the change of fluorescence was monitored at 37 °C using a spectrofluorometer (excitation at 355 nm/emission at 460 nm) (f_{max}, Molecular Devices, USA). Initial DPP-IV activity was calculated over the first 15 min of the reaction from the rate of increase in the fluorescence intensity (arbitrary units 1 mL). The percent inhibition by each test compound was calculated relative to the effect of the solvent alone and IC_{50} values were determined by logistic regression analysis.

4.2.3. DPP-IV inhibitory activity in human plasma and rat plasma. The DPP-IV inhibitory activity in human plasma and rat plasma was measured by a fluorescence assay using Gly-Pro-AMC (Sigma–Aldrich, USA) as a specific fluorescent substrate of DPP-IV. Reaction solutions containing 50 μL of human or rat plasma, 25 μL of fluorescent substrate (120 μM), 15 μL of distilled water, and 10 μL of a test compound (at various concentrations) were incubated at 37 °C for 15 min in a 96-well flat-bottomed microtiter plate. The fluorescence intensity measured using a spectrofluorometer (excitation at 355 nm/emission at 460 nm) (f_{max}, Molecular Devices, USA) was used to define the DPP-IV activity. The percent inhibition of DPP-IV relative to the solvent alone was calculated and IC_{50} values were determined by logistic regression analysis.

4.2.4. DPP-IV inhibition in rats. Male Sprague–Dawley (SD) rats were purchased from Charles River Laboratories, Japan. The rats were housed in an air-conditioned animal room with controlled temperature (24 ± 2 °C), humidity (55 ± 5%), and lighting (12:12 h light/dark cycle), and were provided with standard pellet food for rodents (CRF-1, Oriental Yeast, Japan) and water ad libitum. All procedures were conducted according to the ONO Pharmaceutical Animal Care Committee guidelines. After fasting for at least 8 h, rats (6–7 weeks old) were orally administrated a test compound dissolved in 0.5% methyl cellulose as a single dose of 3 mg/kg. Blood samples were collected from the jugular vein before administration, and 0.25, 0.5, 1, 2, 4, 6, and 9 h after administration. Each blood sample was immediately centrifuged to obtain plasma and the DPP-IV activity was determined. Briefly, 50 μL of plasma was added to each well of a 96-well flat-bottomed microtiter plate, followed by the addition of 50 μL of 60 μM substrate. Then the initial rate of DPP-IV activity was measured using the method described above, and the percent inhibition was calculated relative to basal DPP-IV activity.

4.2.5. Oral glucose tolerance test in rats. The effect of compound **26** on the plasma glucose profile was assessed in male SD rats (400–460 g). After the animals were fasted for at least 20 h, they were dosed orally with the vehicle (0.5% methyl cellulose) or compound **26** (0.1, 0.3, or 1 mg/kg) at −30 min. Blood samples (75 μL) were collected from the tail vein into heparinized tubes at −5 min. Glucose (1 g/kg) was administered orally at 0 min and additional blood samples (75 μL) were collected at 5, 10, 15, 30, 60, and 120 min. Plasma was extracted after centrifugation and stored at −80 °C until the determination of glucose levels by the glucose oxidase peroxidase dye method (Diacolor GC, Toyobo, Japan).

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