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Lead optimization of [(*S*)-γ-(arylamino)prolyl]thiazolidine focused on γ-substituent: Indoline compounds as potent DPP-IV inhibitors

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Abstract—Dipeptidyl peptidase IV (DPP-IV) inhibitors are looked to as a potential new antidiabetic agent class. A series of [(*S*)-γ-(arylamino)prolyl]thiazolidine compounds in which the electrophilic nitrile is removed are chemically stable DPP-IV inhibitors. To discover a structure for the γ-substituent of the proline moiety more suitable for interacting with the S_2 pocket of DPP-IV, optimization focused on the γ-substituent was carried out. The indoline compound **22e** showed a DPP-IV-inhibitory activity 100-fold more potent than that of the prolylthiazolidine **10** and comparable to that of NVP-DPP728. It also displayed improved inhibitory selectivity for DPP-IV over DPP8 and DPP9 compared to compound **10**. Indoline compounds such as **22e** have a rigid conformation with double restriction of the aromatic moiety by proline and indoline structures to promote interaction with the binding site in the S_2 pocket of DPP-IV. The double restriction effect provides a potent inhibitory activity which compensates for the decrease in activity caused by removing the electrophilic nitrile.

1. Introduction

Dipeptidyl peptidase IV (EC 3.4.14.5, DPP-IV) is a serine protease which recognizes an amino acid sequence having proline or alanine at the second position from the N-terminal and produces dipeptide.[1] DPP-IV is widely distributed in mammalian tissues and plays several physiological roles; in particular its role as a peptidase that rapidly inactivates glucagon-like peptide 1 (GLP-1) has drawn interest.[2] GLP-1 is secreted in response to meal ingestion and stimulates insulin secretion.[3] It has been suggested that potentiation and extension of the action of GLP-1 by DPP-IV inhibition would stimulate insulin secretion after meal ingestion only,[4] and DPP-IV inhibitors have therefore come to be seen as a potential new type of antidiabetic agent free of side effects such as hypoglycemia and exhaustion of pancreatic beta-cells. In particular a potent and long-acting inhibitor might offer advantages in exploiting DPP-IV inhibition. Recent in vivo studies indicate that inhibitory selectivity for DPP-IV over other related prolyl dipeptidases, such as DPP8 and DPP9, is one of the key issues for clinical use since inhibition of DPP8 and/or DPP9 has produced profound toxicity in animal studies.[5]

Several DPP-IV inhibitors have been reported (Fig. 1),[6] a number of which are substrate analogs of the P_2-P_1 fragment. As proline mimics at the P_1 part, (*S*)-2-cyanopyrrolidine and thiazolidine structures are used. (*S*)-2-Cyanopyrrolidine inhibitors, for example NVP-DPP728 (**1**),[7–9] LAF237 (**2**),[9,10] and BMS-477118 (**3**),[11] which

Keywords: DPP-IV inhibitor; Thiazolidine; Indoline.
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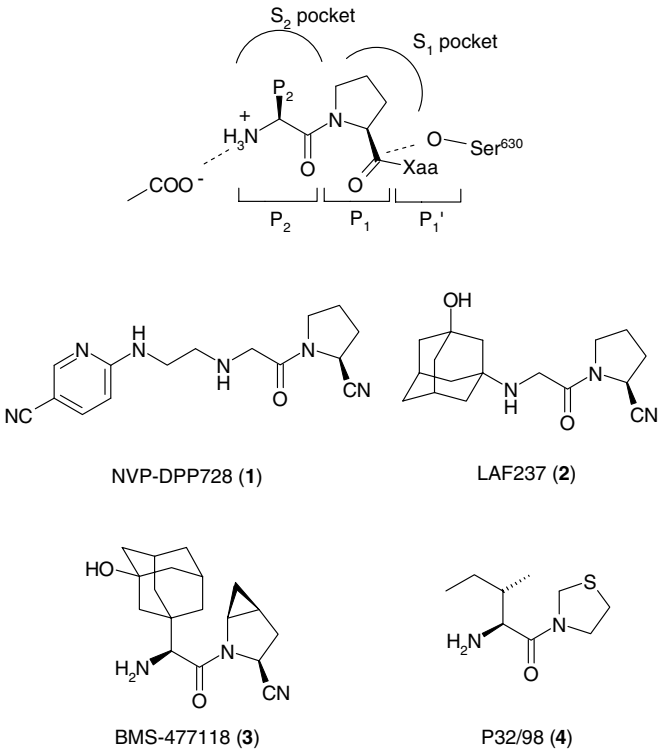
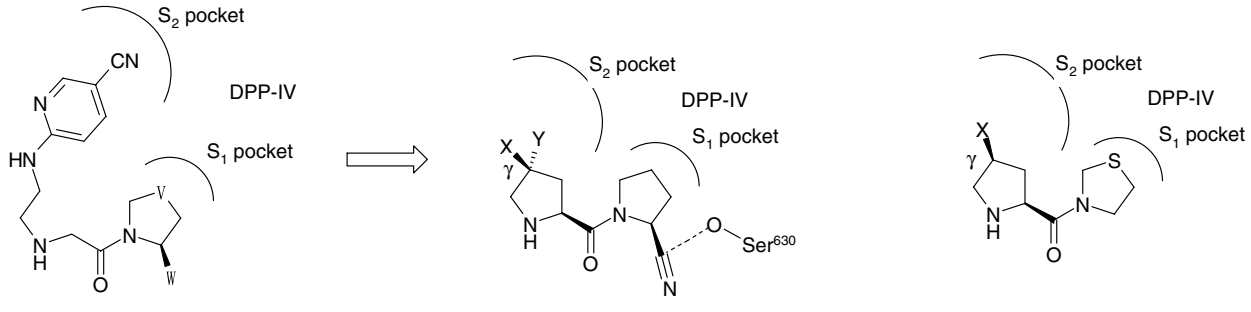


Figure 1.

contain a nitrile group as an electrophilic trap for the Ser^{630} of the catalytic triad, have been reported as potent inhibitors. Thiazolidine inhibitors, which lack an electrophilic nitrile group, are generally of only modest potency. In contrast, P32/98 (**4**) improved glucose tolerance in patients with diabetes and in healthy volunteers despite exhibiting intrinsic moderate inhibitory properties.[12,13]

We previously reported that a series of [(*S*)-γ-(arylamino)prolyl]-(*S*)-2-cyanopyrrolidine compounds had a potent inhibitory activity (Fig. 2b).[14] The representative compound **5** is an analog conformationally restricted using a proline structure and which mimics the folded conformation of the conformationally flexible compound NVP-DPP728 to allow interaction of the (5-cyano-2-pyridyl)amino moiety with the S_2 pocket as shown in Figure 2a. As a result, compound **5** has 5-fold more potent inhibitory activity than NVP-DPP728. In addition, compound **5** with an arylamino group introduced at the γ-position has over 10-fold more potent inhibitory activity than the prolyl-(*S*)-2-cyanopyrrolidine **6**[16] and the (*R*)-isomer **7**.[14]



(a)
(folded conformation)

1 NVP-DPP728 (V = CH, W = CN)
IC_{50} = 1.4 nmol/L
9 (V = S, W = H)
IC_{50} = 270 nmol/L

(b)
[(*S*)-γ-(arylamino)prolyl]-(*S*)-2-cyanopyrrolidine
(X = ArNH-)

5 (X = 5-cyano-2-pyridylamino, Y = H)
IC_{50} = 0.25 nmol/L
6 (X = Y = H)
IC_{50} = 2.9 nmol/L
7 (X= H, Y = 5-cyano-2-pyridylamino)
IC_{50} = 6.6 nmol/L

(c)
[(*S*)-γ-(arylamino)prolyl]thiazolidine
(X = ArNH-)

8 (X = 5-cyano-2-pyridylamino)
IC_{50} = 25.4 nmol/L
10 (X = H)
IC_{50} = 538 nmol/L

Figure 2. [(*S*)-γ-(Arylamino)prolyl]-(*S*)-2-cyanopyrrolidine and [(*S*)-γ-(arylamino)prolyl]thiazolidine. The human DPP-IV-inhibitory activity was measured by fluorescence assay using Gly-Pro-MCA as described in Section 5.

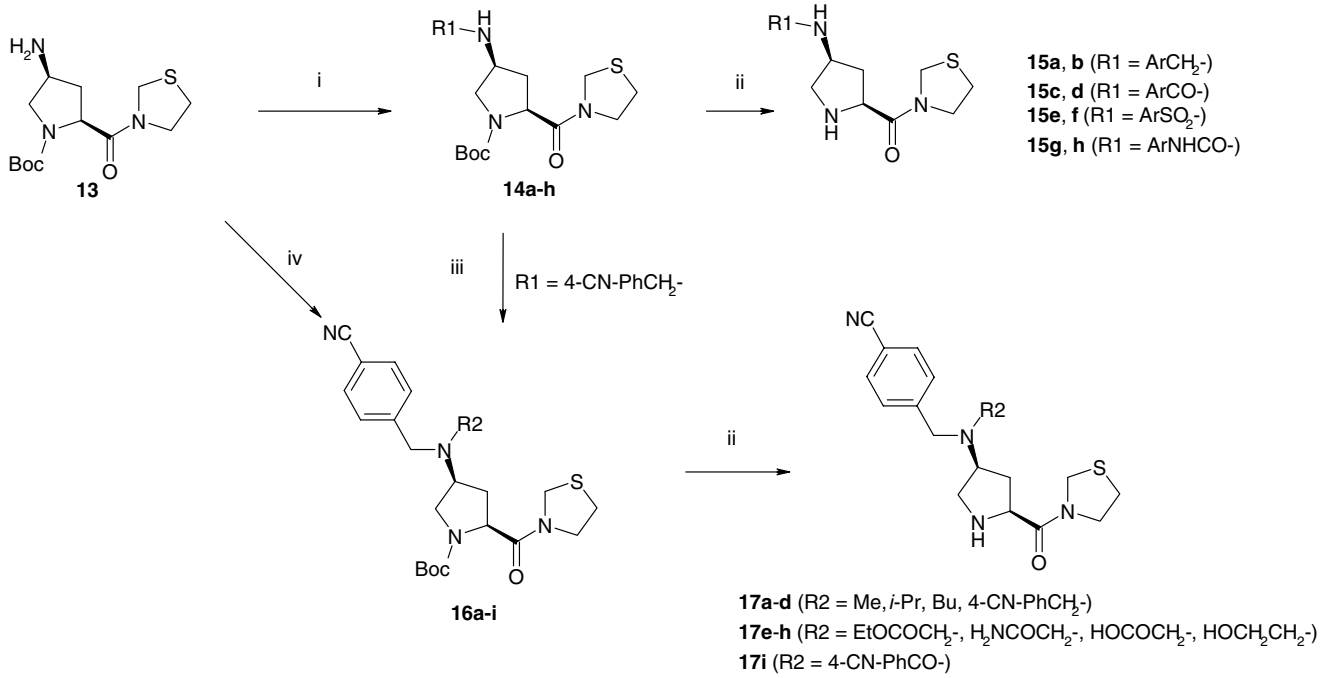
However, the series of [(*S*)-γ-(arylamino)prolyl]-(*S*)-2-cyanopyrrolidine compounds seems to be unstable in neutral aqueous solution (e.g., for compound **5**, $T_{1/2decomp}$ = 1.3 h at pH 6.8 aqueous solution[15]). The instability may result from the intra-molecular cyclization of the nucleophilic amine in the proline moiety and the electrophilic nitrile in the (*S*)-2-cyanopyrrolidine moiety. We previously reported that compound **8**, in which the (*S*)-2-cyanopyrrolidine moiety is converted to a thiazolidine structure, has improved chemical stability and a long half-life in plasma ($t_{1/2}$ = 5.27 h); but compound **8** showed 100-fold decreased inhibitory activity compared to the (*S*)-2-cyanopyrrolidine compound **5** (Fig. 2b and c). This effect demonstrates that the nitrile group of (*S*)-2-cyanopyrrolidine compounds plays a dominant part in the exhibition of inhibitory activity by realizing interaction with the Ser^{630} of the catalytic triad. On the other hand, the thiazolidine analogs have displayed similar features to the (*S*)-2-cyanopyrrolidine compounds; the restricted compound **8** is 10-fold more potent than the flexible compound **9**[17] and 20-fold more potent than the prolylthiazolidine **10**. These results indicate that introducing a substituent at the γ-position of the proline moiety of the prolylthiazolidine core structure provides potent DPP-IV inhibition with improved chemical stability and a good pharmacokinetic profile.[15]

In the present study, to compensate for the decrease in activity caused by the conversion of the (*S*)-2-cyanopyrrolidine moiety to a thiazolidine structure, we optimized the arylamino structure at the γ-position of the proline moiety. The resulting compound **22e** (IC_{50} = 4.8 nmol/L), with the aromatic moiety at the γ-position restricted using an indoline structure, is 100-fold more potent than the prolylthiazolidine **10** (IC_{50} = 538 nmol/L) and has comparable activity to NVP-DPP728 (IC_{50} = 1.4 nmol/L) despite its lack of an electrophilic nitrile. Its inhibitory selectivity for DPP-IV over DPP8 and DPP9 is ca. 100-fold. The rigid conformation realized by the double restriction with proline and indoline structures is concluded to be effective for improving DPP-IV-inhibitory activity.

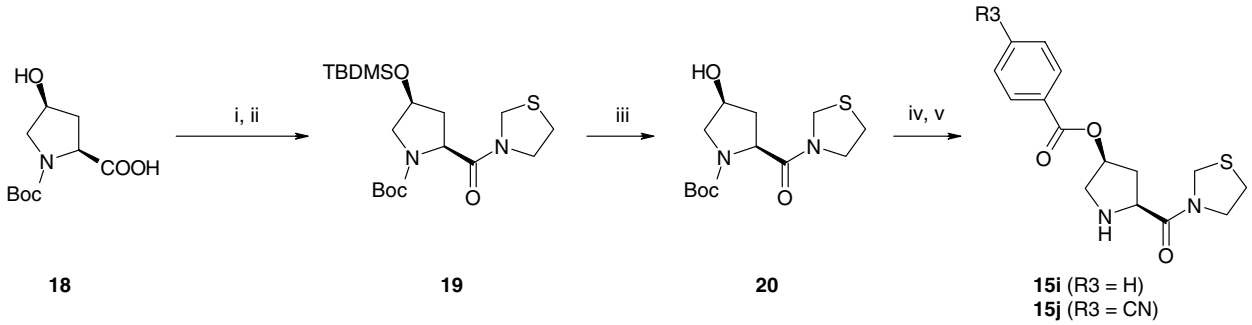
2. Chemistry

Compounds bearing a non-cyclized nitrogen at the γ-position of the proline moiety of the ((*S*)-γ-substituted prolyl)thiazolidine structure (**15a**–**h**, **17a**–**i**) were prepared from ((*S*)-γ-aminoprolyl)thiazolidine **13**[15] (Scheme 1). Introduction of an alkyl, acyl, sulfonyl or carbamoyl group at the γ-amino group of **13**, followed by removal of the Boc group with HCl/AcOEt, afforded the N-mono-introduced compounds **15a**–**h**. The further modified compounds **17a**–**i** were prepared by alkylation or acylation of the γ-amino-compound **13** (for **17d**) or the γ-[(4-cyanobenzyl)amino]-compound **14b** (for **17a**–**c**, **17e**–**i**) and subsequent removal of the Boc group.

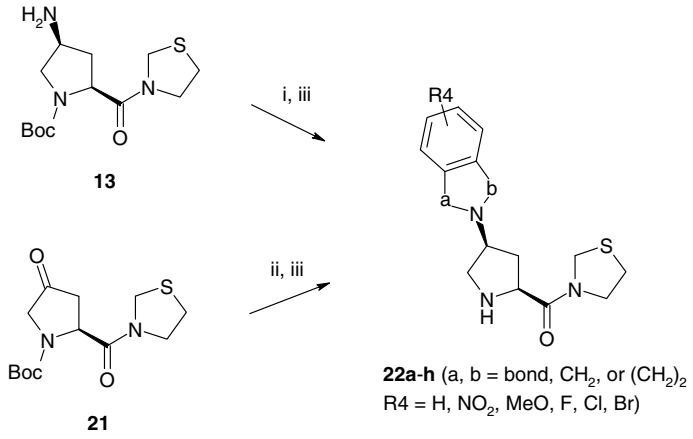
The ester compounds **15i** and **j** were synthesized from the ((*S*)-γ-hydroxyprolyl)thiazolidine **20**, which was prepared from the *cis*-Boc-hydroxyproline **18** by protection of the hydroxyl group as its silylether and amidation with thiazolidine followed by removal of the silyl group. The alcohol **20** was converted to its benzoate followed by deprotection to give the desired esters **15i** and **j** (Scheme 2).



Scheme 1. Reagents: (i) ArCHO, $NaBH_3CN$, AcOH (for **14a**, **b**), ArCOCl, Et_3N (for **14c**, **d**), $ArSO_2Cl$, *N*-methylmorpholine (for **14e**, **f**), or ArNCO (for **14g**, **h**); (ii) H^+; (iii) HCHO, acetone or butyraldehyde, $NaBH_3CN$ or $NaBH(OAc)_3$, AcOH (for **16a**–**c**); R2–Br, *i*-Pr_2NEt (for **16e**–**h**); or 4-CN–PhCOCl, Et_3N (for **16i**); (iv) 4-CN–$PhCH_2Br$, *i*-Pr_2NEt.



Scheme 2. Reagents: (i) TBDMSCl, imidazole; (ii) thiazolidine, WSCI·HCl, HOBt·H_2O, Et_3N; (iii) TBAF; (iv) 4-R3–PhCOCl, Et_3N; (v) H^+.



Scheme 3. Reagents: (i) α,α′-dibromo-*ortho*-xylene; (ii) isoindoline, tetrahydroquinoline or indoline derivatives, $NaBH(OAc)_3$, AcOH; (iii) H^+.

Compounds having an isoindoline, tetrahydroquinoline or indoline structure at the γ-position (**22a–h**) were prepared by alkylation of the amine **13** with α,α′-dibromo-*ortho*-xylene (for **22a**) or reductive amination of the ketone **21**[15] with cyclic amines (for **22b–h**) and subsequent removal of the Boc group (Scheme 3).

3. Results and discussion

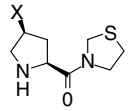
The ((*S*)-γ-substituted prolyl)thiazolidine and related compounds were evaluated for DPP-IV-inhibitory activity in human and rat plasma by fluorescence assay using Gly-Pro-MCA (Tables 1–4).

In a previous study of ((*S*)-γ-substituted prolyl)-(*S*)-2-cyanopyrrolidine, we noted the importance of the aromatic moiety at the γ-position of the proline moiety for DPP-IV inhibition.[14] First, we examined various spacers between this aromatic moiety and the proline moiety. In [(*S*)-γ-(arylamino)prolyl]thiazolidine, since the 4-cyanophenyl compound **12** showed more potent inhibitory activity than the phenyl compound **11**,[15] we selected both phenyl and 4-cyanophenyl groups for the structure of the aromatic moiety (Table 1). Of the resulting compounds, the 4-cyanobenzylamino **15b** and the 4-cyanophenylcarboxamido **15d** had comparable activity to the 4-cyanophenylamino compound **12**. The 4-cyanobenzylamino compound **15b** is prepared easily by reductive alkylation as shown in Scheme 1 and bears a nucleophilic nitrogen at the γ-position of the proline moiety that allows the introduction of further substitutions. We therefore selected this compound as the template for further structure–activity relationship (SAR) study of the γ-substituent of the proline moiety.

We introduced alkyl, aralkyl, or other functionalized groups onto the nitrogen at the γ-position of compound **15b** (Table 2). All of the resulting compounds showed moderately increased activity compared to compound **15b** except the *N*-acyl compound **17i**. Introduction of an alkyl (**17a–c**) or benzyl (**17d**) group both resulted in 3- to 5-fold more potent inhibitory activity. Non-polar groups tended to produce more potency than polar groups (**17a–d** vs **17e–h**). However, no pronounced SAR was evident. This finding suggests that the introduced groups do not interact with the S_2 binding site itself, but allow the aromatic moiety of the flexible 4-cyanobenzyl group to locate the appropriate site for interaction through their steric hindrance effect.

We were therefore interested in the effect of conformational restriction of the aromatic moiety of the γ-substituent of the proline moiety (Tables 3, 4). The aromatic moiety of compounds **11** and **15a** was restricted by a carbon chain, formed isoindoline, tetrahydroquinoline or indoline structure (**22a–c**, Table 3). All the restricted analogs **22a–c** showed more potent activity than the

Table 1. DPP-IV inhibition by ((*S*)-γ-substituted prolyl)thiazolidines



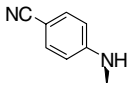
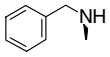
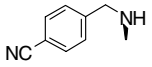
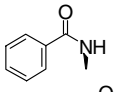
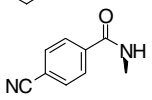
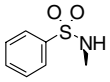
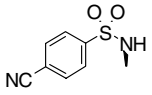
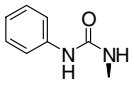
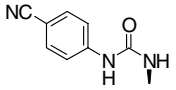
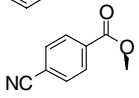
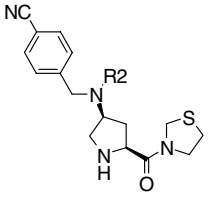
Compound	X	DPP-IV inhibition, IC_{50} (nmol/L)	
		Human	Rat
11		147	190
12		25.2	33.6
15a		181	171
15b		34.0	34.3
15c		>300	236
15d		37.2	34.1
15e		>300	>300
15f		>300	>300
15g		154	151
15h		85.9	76.2
15i		>300	>300
15j		>300	>300

Table 2. DPP-IV inhibition by ((*S*)-γ-substituted prolyl)thiazolidines



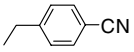
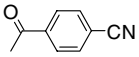
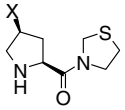
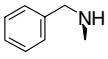
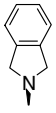
Compound	R2	DPP-IV inhibition, IC_{50} (nmol/L)	
		Human	Rat
15b	H	34.0	34.3
17a	Me	9.6	13.6
17b	*i*-Pr	7.1	12.5
17c	*n*-Bu	9.1	5.0
17d		6.4	9.3
17e	CH_2COOEt	13.6	15.3
17f	CH_2CONH_2	13.9	16.5
17g	CH_2COOH	16.8	16.3
17h	CH_2CH_2OH	17.0	23.0
17i		228	168

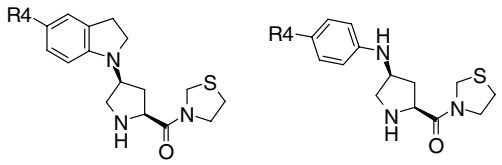
Table 3. DPP-IV inhibition by ((*S*)-γ-substituted prolyl)thiazolidines



Compound	X	DPP-IV inhibition, IC_{50} (nmol/L)	
		Human	Rat
11		147	190
15a		181	171
22a		26.4	39.0
22b		112	99.8
22c		11.0	19.5

phenylamino (**11**) or benzyl (**15a**) derivatives, with the indoline **22c** being the most potent. It appeared thus that an indoline structure is more suited to DPP-IV inhibition than a phenylamino or benzylamino structure. Next, compound **22c** was modified by introducing a number of substituents at the 5-position of the indoline moiety [**22d**–**h**, Table 4(A)] and the results were compared with the corresponding phenylamino compounds [**23-25**, Table 4(B)]. Of the indoline compounds (A), although all the compounds with substituents introduced (**22d**–**h**) showed increased activity compared to compound **22c**, no influence of electronic properties was observed. For example, substitution with an electron-withdrawing nitro group (**22d**) and an electron-donating methoxy group (**22e**) both resulted in more potent activity. This result is similar to that previously obtained in phenylamino compounds (**23**–**25**). On the other hand, in comparison of compounds bearing the same substituent on the aromatic moiety, indoline com-

Table 4. DPP-IV inhibition by indolinyl compounds (A) and phenylamino compounds (B)



(A) (B)

Compound	R4		DPP-IV inhibition, IC_{50} (nmol/L)	
			Human	Rat
22c	H	A	11.0	19.5
11	H	B	147	190
22d	NO_2	A	7.9	10.3
23	NO_2	B	19.2	21.0
22e	MeO	A	4.8	6.1
24	MeO	B	51.5	54.9
22f	F	A	5.8	10.1
22g	Cl	A	7.0	13.4
25	Cl	B	45.4	55.1
22h	Br	A	6.5	10.7

pounds (A) showed 2- to 10-fold more potent inhibitory activity than the corresponding phenylamino compounds (B). This result indicates that, although the aromatic moiety of the indoline compounds (A) interacts with the same binding site as does that of the phenylamino compounds (B), conformational restriction of the aromatic moiety with an indoline structure produces more potent activity.

To further understand the mechanism of the inhibition, a modeling study was carried out to investigate the possible interaction between the (γ-substituted prolyl)thiazolidine compounds and DPP-IV. The predicted binding modes for the 5-methoxyindolinyl compound **22e** and the 4-methoxyphenylamino compound **24** at the active site of DPP-IV are shown in Figure 3. The models suggest that the aromatic moiety of both the indolinyl group of **22e** and the phenylamino group of **24** achieves an aromatic π–π interaction with the side chain of the Phe^{357} in the S_2 pocket. The aromatic moiety of **22e** seems to be potentially more suited to aromatic π–π interaction with the side chain of Phe^{357} due to the restricting effect of the indoline structure, which may provide potent inhibitory activity.

The indoline compounds presented in this paper have a rigid conformation based on the flexible compounds **9** or NVP-DPP728 but doubly restricted by proline and indoline structures which allows interaction of their aromatic moiety with the binding site in the S_2 pocket of DPP-IV. The double restriction produces potent inhibitory activity which compensates for the decreased activity caused by removing the electrophilic nitrile. For example, compound **22e** (IC_{50} = 4.8 nmol/L) has 50-fold more potent activity than the flexible compound **9** (IC_{50} = 270 nmol/L) and 100-fold more potent activity than the prolylthiazolidine **10** (IC_{50} = 538 nmol/L).

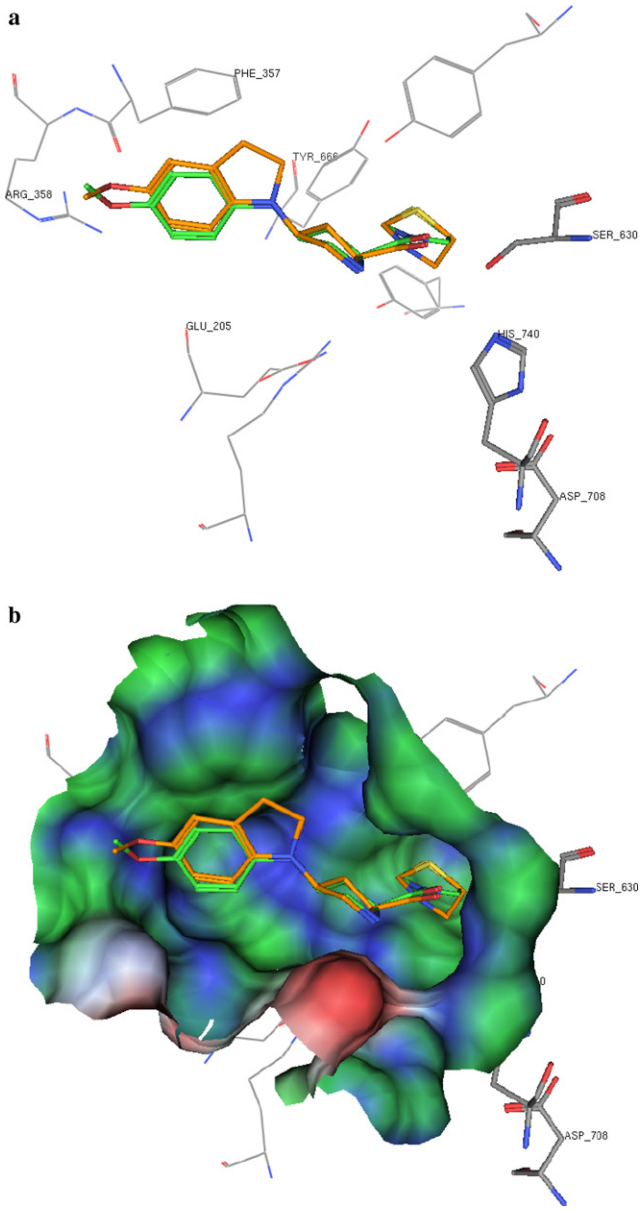
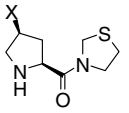
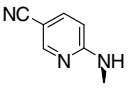
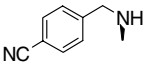
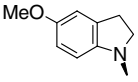
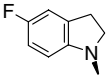


Figure 3. (a) Compounds **22e** and **24** bound to DPP-IV (PDB 1N1M, Ref. 18) are docked by ASEDock (Refs. 19 and 20) with MOE (MOE, Chemical Computing Group). Carbon atoms of the enzyme are in gray and, carbon atoms of ligand compounds **22e** and **24** are orange and green, respectively. Oxygen and nitrogen atoms of both enzyme and ligands are in red and blue, respectively. (b) Molecular surface representation showing the interaction of compounds and DPP-IV (green, hydrophobic, blue, hydrophilic, and red, exposed).

The inhibitory activity of **22e** is comparable to that of NVP-DPP728 (IC_{50} = 1.4 nmol/L) despite its lack of an electrophilic nitrile. Of the P_2–P_1 fragment substrate analog inhibitors, compounds bearing (*S*)-γ-substituted proline at the P_2 site seem to represent a suitable structure to produce interaction of their γ-substituents with the side chain of the amino acid residues in the S_2 pocket of DPP-IV.[14,15] Compounds optimized by further restriction with an indoline structure at the γ-position therefore have potent inhibitory activity compensating for the decrease caused by removing the electrophilic nitrile at the P_1 site.

Table 5. Selectivity of representative DPP-IV inhibitors



Compound	X	IC_{50} (nmol/L)		
		DPP-IV	DPP8	DPP9
10	H	558	4687	6129
8		25.4	4756	3735
15b		34.0	678.9	748.7
22c		11.0	736.5	501.1
22e		4.8	378.8	710.7
22f		5.8	562.8	518.9

Since inhibition of DPP8 and/or DPP9 has shown profound toxicity in in vivo studies,[5] it is important for clinical use to develop a selective DPP-IV inhibitor. The results for selectivity are shown in Table 5. The prolylthiazolidine **10** showed weak selectivity for DPP-IV over DPP8 and DPP9 (both ca. 10-fold), while the γ-substituted prolyl thiazolidine compounds **8**, **15b**, **22c**, **e**, **f** exhibited improved selectivity for DPP-IV over DPP8 and DPP9 arising from the increased DPP-IV-inhibitory activity. For example, the indoline compounds **22e**, **f** had ca. 100-fold selectivity. This finding of improved selectivity of γ-substituted prolylthiazolidine compounds may indicate that a DPP-IV-specific binding site exists in the S_2 pocket, as suggested in the above modeling study, and that it was binding with this site that increased the selectivity of DPP-IV inhibition.

4. Conclusion

A series of [(*S*)-γ-(arylamino)prolyl]thiazolidine compounds with the electrophilic nitrile removed are chemically stable DPP-IV inhibitors.[15] In the present study, we carried out optimization focused on the γ-substituent of these compounds. The resulting compounds bearing an indoline structure at the γ-position showed potent DPP-IV inhibitory activity. The representative compound **22e** (IC_{50} = 4.8 nmol/L) had 50-fold more potent activity than the flexible compound **9** (IC_{50} = 270 nmol/L) and 100-fold more potent activity than the prolylthiazolidine **10** (IC_{50} = 538 nmol/L). The inhibitory selectivity of **22e** for DPP-IV over DPP8 and DPP9 was ca. 100-fold. The indoline compounds presented in this paper have a rigid conformation with an aromatic moiety doubly restricted by proline and indoline structures to allow interaction with the binding site in the S_2 pocket of DPP-IV. The double restriction provides potent inhibitory activity that compensates for the decrease in activity caused by removing the electrophilic nitrile.

5. Experimental

5.1. Chemistry

^{1}H NMR spectra were measured on a Bruker DPX-300 instrument or on a Bruker AMX-500 with tetramethylsilane as the internal standard; chemical shifts are reported in parts per million (ppm, δ units). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; br s, broad singlet. Mass spectra (MS) were recorded on a JEOL JMS-700 instrument operating in the chemical ionization (CI) mode. Electron analysis for carbon, hydrogen, and nitrogen was performed with a Yanagimoto CHN CORDER MT-6. Chromatography refers to flash chromatography conducted on silica gel BW-300 (Fuji Silysia). All chemicals and solvents were of reagent grade unless otherwise specified. For drying organic solutions in extraction, anhydrous sodium sulfate or anhydrous magnesium sulfate was used unless otherwise indicated. The following abbreviations are used: DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; EDC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOBT, 3-hydroxybenztriazole hydrate.

5.1.1. 3-((2*S*,4*S*)-4-Benzylamino-2-pyrrolidinylcarbonyl)-1,3-thiazolidine dihydrochloride (15a). (1) To a solution of 3-((2*S*,4*S*)-4-amino-1-*tert*-butoxycarbonyl-2-pyrrolidinylcarbonyl)-1,3-thiazolidine[14] (**13**, 904 mg, 3 mmol) in methanol (16 mL) was added benzaldehyde (318 mg, 3 mmol), and the mixture was stirred at room temperature for 30 min. Sodium cyanoborohydride (189 mg, 3 mmol) and acetic acid (5 drops) were added to the reaction mixture, and the mixture was stirred for 6 h. The reaction mixture was evaporated under reduced pressure. Saturated aqueous sodium hydrogencarbonate solution was added to the residue and the mixture was extracted with ethyl acetate. The extract was dried and the solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography to give 3-[(2*S*,4*S*)-4-benzylamino-1-*tert*-butoxycarbonyl-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (**14a**, 742 mg, 63%) as a colorless transparent oil.

(2) Compound **14a** (742 mg, 1.90 mmol) was dissolved in ethyl acetate (3.8 mL) and 4 mol/L hydrochloric acid–ethyl acetate (2.4 mL) was added to the solution. The mixture was stirred at room temperature for 18 h and the precipitated solid was collected to give the title compound (540 mg, 74%) as a white powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 2.10–2.32 (1H, m), 2.86–3.20 (3H, m), 3.49–4.03 (5H, m), 4.21 (2H, s), 4.39–4.80 (3H, m), 7.31–7.52 (3H, m), 7.52–7.72 (2H, m), 10.17 (4H, br s). Anal. Calcd for $C_{15}H_{21}N_3OS \cdot 13/$

5HCl: C, 46.65; H, 6.16; N, 10.88. Found: C, 46.44; H, 6.07; N, 10.89.

5.1.2. 3-[(2*S*,4*S*)-4-(4-Cyanophenylmethyl)amino-2-pyrrolidinylcarbonyl]-1,3-thiazolidine dihydrochloride (15b). (1) From compound **13** (904 mg, 3 mmol) and 4-cyanobenzaldehyde (393 mg, 3 mmol) with addition of sodium cyanoborohydride (189 mg, 3 mmol) and acetic acid (5 drops) using a procedure analogous to synthesis of **14a**, the compound 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(4-cyanophenylmethyl)amino-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (**14b**, 389 mg, 31%) was prepared as a colorless transparent oil.

(2) Compound **14b** (389 mg, 0.943 mmol) was dissolved in ethyl acetate (0.9 mL) and 4 mol/L hydrochloric acid–ethyl acetate (1.2 mL) was added to the solution. The mixture was stood at room temperature for 18 h. The precipitated solid was collected to give the title compound (286 mg, 74%) as a white powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 2.06–2.34 (1H, m), 2.85–3.01 (1H, m), 3.01–3.20 (2H, m), 3.50–4.06 (5H, m), 4.30 (2H, s), 4.41–4.79 (3H, m), 7.80 (2H, d, J = 8.1 Hz), 7.95 (2H, d, J = 8.4 Hz), 9.05 (1H, br s), 10.30 (3H, br s).

Anal. Calcd for $C_{16}H_{20}N_4OS{\cdot}2HCl$: C, 49.36; H, 5.70; N, 14.39. Found: C, 49.13; H, 5.89; N, 14.23.

5.1.3. 3-((2*S*,4*S*)-4-Benzoylamino-2-pyrrolidinylcarbonyl)-1,3-thiazolidine (15c). (1) To a solution of 3-((2*S*,4*S*)-4-amino-1-*tert*-butoxycarbonyl-2-pyrrolidinylcarbonyl)-1,3-thiazolidine[15] (**13**, 499 mg, 1.65 mmol) in dichloromethane (10 mL) were added 4-methylmorpholine (218 μL, 1.98 mmol) and benzoyl chloride (202 μL, 1.74 mmol) and the mixture stirred at room temperature for 22 h. To the reaction mixture was added 10% citric acid solution, and the mixture was extracted with ethyl acetate. The extract was washed successively with saturated aqueous sodium hydrogencarbonate solution and brine, and dried. The solvent was evaporated under reduced pressure to give 3-((2*S*,4*S*)-4-benzoylamino-1-*tert*-butoxycarbonyl-2-pyrrolidinylcarbonyl)-1,3-thiazolidine (**14c**, 652 mg, 97%) as a white powder.

(2) Compound **14c** (648 mg, 1.60 mmol) was dissolved in ethyl acetate (4 mL) and 4 mol/L hydrochloric acid–ethyl acetate (2 mL) was added to the solution. The mixture was stirred at room temperature for 19 h. The precipitated solid was collected and added sodium hydrogencarbonate solution, and the mixture was extracted with chloroform. The extract was washed with brine. The solvent was evaporated under reduced pressure to give the title compound (250 mg, 51%) as a white powder.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.76–1.81 (1H, m), 2.33–2.39 (1H, m), 2.91–3.09 (5H, m), 3.63–3.95 (3H, m), 4.34–4.70 (3H, m), 7.44–7.53 (3H, m), 7.80–7.82 (2H, m), 8.38 (1H, br s).

Anal. Calcd for $C_{15}H_{19}N_3O2S{\cdot}1/3H_2O$: C, 57.85; H, 6.37; N, 13.49. Found: C, 57.75; H, 6.11; N, 13.44.

5.1.4. 3-[(2*S*,4*S*)-4-(4-Cyanobenzoyl)amino-2-pyrrolidinylcarbonyl]-1,3-thiazolidine hydrochloride (15d). (1) From compound **13** (543 mg, 1.80 mmol), 4-methylmorpholine (238 μL, 2.16 mmol), and 4-cyanobenzoyl chloride (313 mg, 1.89 mmol) using a procedure analogous to synthesis of **14c**, the compound 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(4-cyanobenzoyl)amino-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (**14d**, 804 mg) was prepared as a white powder.

(2) Compound **14d** (798 mg) was dissolved in ethyl acetate (0.5 mL) and 4 mol/L hydrochloric acid–ethyl acetate (2.3 mL) was added to the solution. The mixture was stirred at room temperature for 19 h. The precipitated solid was collected to give the title compound (513 mg, 67%) as a white powder.

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.01–2.06 (1H, m), 2.81–2.86 (1H, m), 3.03–3.14 (2H, m), 3.36–3.50 (2H, m), 3.65–3.94 (2H, m), 4.45–4.75 (4H, m), 7.98–8.06 (4H, m), 8.86 (1H, br s), 9.07–9.12 (1H, m), 10.49 (1H, br s).

Anal. Calcd for $C_{16}H_{18}N_4\ O_2S{\cdot}HCl{\cdot}3/10C_4H_8O_2$: C, 50.23; H, 5.73; N, 13.62. Found: C, 50.21; H, 5.75; N, 13.70.

5.1.5. 3-((2*S*,4*S*)-4-Phenylsulfonylamino-2-pyrrolidinylcarbonyl)-1,3-thiazolidine hydrochloride (15e). (1) From compound **13** (543 mg, 1.80 mmol), 4-methylmorpholine (240 μL, 2.18 mmol), and benzenesulfonyl chloride (240 μL, 1.88 mmol) using a procedure analogous to synthesis of **14c**, the compound 3-((2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-phenylsulfonylamino-2-pyrrolidinylcarbonyl)-1,3-thiazolidine (**14e**, 644 mg, 81%) was prepared as a white powder.

(2) Compound **14e** (634 mg, 1.44 mmol) was dissolved in ethyl acetate (4 mL) and 4 mol/L hydrochloric acid–ethyl acetate (1.8 mL) was added to the solution. The mixture was stirred at room temperature for 67 h. The precipitate was collected by filtration to give the title compound (437 mg, 72%) as a pale-yellow powder.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.68–1.74 (1H, m), 2.50–2.58 (1H, m), 3.00–3.08 (3H, m), 3.17–3.20 (1H, m), 3.53–3.87 (3H, m), 4.38–4.63 (3H, m), 7.62–7.71 (3H, m), 7.84–7.85 (2H, m), 8.24–8.27 (1H, m) 9.50 (2H, br s).

Anal. Calcd for $C_{14}H_{19}N_3O_3S_2{\cdot}HCl{\cdot}3/10C_4H_8O_2{\cdot}H_2O$: C, 43.22; H, 5.82; N, 9.95. Found: C, 43.21; H, 5.64; N, 10.19.

5.1.6. 3-[(2*S*,4*S*)-4-(4-Cyanophenylsulfonyl)amino-2-pyrrolidinylcarbonyl]-1,3-thiazolidine hydrochloride (15f). (1) From compound **13** (1.09 g, 3.63 mmol), 4-methylmorpholine (480 μL, 4.37 mmol), and 4-cyanobenzenesulfonyl chloride (0.780 g, 3.87 mmol) using a procedure analogous to synthesis of **14c**, the compound 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(4-cyanophenylsulfonyl)amino-2-pyrrolidinylcarbonyl]-1,

3-thiazolidine (**14f**, 1.67 g, 99%) was prepared as a white powder.

(2) Compound **14f** (798 mg, 1.71 mmol) was dissolved in ethyl acetate (5 mL) and 4 mol/L hydrochloric acid–ethyl acetate (2.2 mL) was added to the solution. The mixture was stirred at room temperature for 68 h. The precipitate was collected by filtration to give the title compound (544 mg, 70%) as a pale-yellow powder.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.68–1.75 (1H, m), 2.53–2.59 (1H, m), 3.02–3.09 (3H, m), 3.23–3.28 (1H, m), 3.54–3.90 (3H, m), 4.40–4.64 (3H, m), 8.01 (2H, d, J = 8.4 Hz), 8.13 (2H, d, J = 8.4 Hz), 8.62–8.65 (1H, m), 9.93 (2H, br s).

Anal. Calcd for $C_{15}H_{18}N_4O_3S2{\cdot}HCl{\cdot}2/5C_4H_8O2{\cdot}H_2O$: C, 43.71; H, 5.35; N, 12.28. Found: C, 43.69; H, 5.26; N, 12.48.

5.1.7. 3-[(2*S*,4*S*)-4-(3-Phenylureido)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine trifluoroacetate (15g). (1) To a solution of 3-((2*S*,4*S*)-4-amino-1-*tert*-butoxycarbonyl-2-pyrrolidinylcarbonyl)-1,3-thiazolidine[15] (**13**, 401 mg, 1.33 mmol) in tetrahydrofuran (10 mL) was added phenyl isocyanate (167 mg, 1.40 mmol) and the mixture stirred at room temperature for 18 h. To the reaction mixture was added 10% citric acid solution and the mixture was extracted with ethyl acetate. The extract was washed successively with saturated aqueous sodium hydrogencarbonate solution and brine. The solvent was evaporated under reduced pressure to give 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(3-phenylureido)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (**14g**, 560 mg, quant.) as a white solid.

(2) To compound **14g** (532 mg, 1.27 mmol) was added trifluoroacetic acid (2 mL) and the mixture was stirred at room temperature for 3 h. The mixture was evaporated under reduced pressure to give the title compound (363 mg, 63%) as a brown powder.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.75–1.80 (1H, m), 2.75–2.80 (1H, m), 3.04–3.20 (3H, m), 3.43–3.47 (1H, m), 3.68–3.89 (2H, m), 4.40–4.71 (4H, m), 6.72–6.75 (1H,m), 6.91 (1H, t, J = 7.4 Hz), 7.21–7.24 (2H, m), 7.39 (2H, d, J = 7.8 Hz), 8.85 (1H, br s), 8.89–8.90 (1H, m), 9.60 (1H, br s).

Anal. Calcd for $C_{15}H_{20}N_4O_2S{\cdot}C_2HF_3O_2{\cdot}H_2O$: C, 45.13; H, 5.12; N, 12.38. Found: C, 45.24; H, 5.11; N, 12.18.

5.1.8. 3-{[2*S*,4*S*]-4-[3-(4-Cyanophenyl)ureido]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine (15h). (1) From compound **13** (640 mg, 2.12 mmol) and 4-cyanophenyl isocyanate (321 mg, 2.23 mmol) using a procedure analogous to synthesis of **14g**, the compound 3-{(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-[3-(4-cyanophenyl)ureido]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine (**14h**, 992 mg, quant.) was prepared as a white powder.

(2) To a solution of compound **14h** (978 mg, 2.20 mmol) in chloroform (5 mL) was added trifluoroacetic acid (3 mL), and the mixture was stirred at room temperature for 8 h. The solvent was evaporated under reduced pressure, and to the residue was added saturated aqueous sodium hydrogencarbonate solution. The mixture was extracted with chloroform. The extract was concentrated under reduced pressure to give the title compound (140 mg, 18%) as a white powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.60–1.68 (1H, m), 2.22–2.32 (1H, m), 2.67–2.72 (1H, m), 2.91–3.11 (4H, m), 3.65–3.93 (3H, m), 4.13–4.16 (1H, m), 4.43–4.72 (2H, m), 6.47 (1H, d, J = 7.2 Hz), 7.56 (2H, d, J = 8.7 Hz), 7.65 (2H, d, J = 8.7 Hz), 9.11 (1H, s).

Anal. Calcd for $C_{16}H_{19}N_5O_2S{\cdot}9/13H_2O$: C, 53.70; H, 5.74; N, 19.57. Found: C, 54.09; H, 5.63; N, 19.17.

5.1.9. 3-{(2*S*,4*S*)-4-[*N*-(4-Cyanophenylmethyl)-*N*-methylamino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine dihydrochloride (17a). (1) To a solution of 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(4-cyanophenylmethyl)amino-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (**14b**, 1.35 g) in acetonitrile (20 mL) was added 37% formaldehyde solution (0.788 mL), and the mixture was stirred at room temperature for 30 min. Sodium cyanoborohydride (0.305 g) and acetic acid (5 drops) were added to the reaction mixture, and the reaction mixture was stirred for 1 h. The reaction mixture was evaporated under reduced pressure. Saturated aqueous sodium hydrogencarbonate solution was added to the residue and the mixture was extracted with ethyl acetate. The extract was dried and the solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography to give 3-{(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-[*N*-(4-cyanophenylmethyl)-*N*-methylamino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine (**16a**, 0.953 g, 68%) as white amorphous.

(2) Compound **16a** (0.818 g, 1.90 mmol) was dissolved in ethyl acetate (3.8 mL) and 4 mol/L hydrochloric acid–ethyl acetate (2.4 mL) was added to the solution. The mixture was stirred at room temperature for 5 h. The precipitated solid was collected to give the title compound (0.683 g, 77%) as a white powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 2.20–2.48 (1H, m), 2.57 (3H, s), 2.80–3.20 (3H, m), 3.57–4.17 (5H, m), 4.20–4.85 (5H, m), 7.88 (2H, d, J = 7.8 Hz), 7.96 (2H, d, J = 8.4 Hz), 9.12 (1H, br s).

Anal. Calcd for $C_{17}H_{22}N_4OS{\cdot}2HCl{\cdot}1/2C_4H_8O_2{\cdot}H_2O$: C, 49.03; H, 6.50; N, 12.04. Found: C, 49.07; H, 6.62; N, 12.08.

5.1.10. 3-{(2*S*,4*S*)-4-[*N*-(4-Cyanophenylmethyl)-*N*-isopropylamino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine dihydrochloride (17b). (1) To a solution of 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(4-cyanophenylmethyl)amino-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (**14b**, 833 mg, 2 mmol) in 1,2-dichloroethane (10 mL) were added acetone (5.73 mL), acetic acid (0.229 mL, 4 mmol), and sodium triacetoxyborohydride (1.70 g, 8 mmol), and the mixture was stirred at room temperature for 3 days.

Saturated aqueous sodium hydrogencarbonate solution was added to the reaction mixture and the mixture was extracted with ethyl acetate. The extract was evaporated under reduced pressure.The residue was purified by silica gel chromatography to give 3-{(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-[*N*-(4-cyanophenylmethyl)-*N*-isopropylamino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine (**16b**, 818 mg, 90%).

(2) Compound **16b** (792 mg, 1.73 mmol) was dissolved in ethyl acetate (3.47 mL) and 4 mol/L hydrochloric acid–ethyl acetate (2.16 mL) was added to the solution. The mixture was stirred at room temperature for 18 h and the precipitated solid was collected to give the title compound (637 mg, 72%) as a white powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 0.90–1.60 (6H, m), 1.95–2.45 (1H, m), 2.65–3.20 (3H, m), 3.40–4.90 (11H, m), 7.50–8.30 (4H, m).

Anal. Calcd for $C_{19}H_{26}N_4OS{\cdot}2HCl{\cdot}3/5C_4H_8O_2{\cdot}H_2O$: C, 50.53; H, 6.90; N, 11.01. Found: C, 50.49; H, 7.05; N, 11.26.

5.1.11. 3-{(2*S*,4*S*)-4-[*N*-Butyl-*N*-(4-cyanophenylmethyl)amino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine dihydrochloride (17c). (1) From compound **14b** (833 mg, 2 mmol), *n*-butyraldehyde (216 mg, 3 mmol), acetic acid (0.229 mL, 4 mmol), and sodium triacetoxyborohydride (0.848 g, 4 mmol) using a procedure analogous to synthesis of **16b**, the compound 3-{(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-[*N*-butyl-*N*-(4-cyanophenylmethyl)amino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine (**16c**, 837 mg, 89%) was prepared.

(2) Compound **16c** (830 mg, 1.76 mmol) was dissolved in ethyl acetate (3.51 mL) and 4 mol/L hydrochloric acid–ethyl acetate (2.20 mL) was added to the solution. The mixture was stirred at room temperature for 18 h and the precipitated solid was collected to give the title compound (607 mg, 72%) as a pale-yellow powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 0.80 (3H, t, J = 7.2 Hz), 1.18 (2H, quint, J = 6.9 Hz), 1.30–1.90 (2H, m), 2.10–2.50 (1H, m), 2.60–3.24 (5H, m), 3.54–4.87 (9H, m), 7.60–8.20 (4H, m).

Anal. Calcd for $C_{20}H_{28}N_4OS{\cdot}2HCl{\cdot}4/5H_2O$: C, 52.24; H, 6.93; N, 12.18. Found: C, 52.31; H, 7.21; N, 11.94.

5.1.12. 3-{(2*S*,4*S*)-4-[*N*-(4-Cyanophenylmethyl)-*N*-(ethoxycarbonylmethyl)amino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine dihydrochloride (17e). (1) To a solution of 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(4-cyanophenylmethyl)amino-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (**14b**, 0.833 g, 2 mmol) in *N*-methyl-2-pyrrolidone (6 mL) were added ethyl bromoacetate (0.333 mL, 3 mmol) and diisopropylethylamine (1.05 mL, 6 mmol) and the mixture was stirred at room temperature for 18 h. The reaction mixture was added to saturated aqueous sodium hydrogencarbonate solution, and the mixture was extracted with ethyl acetate. The extract was evaporated under reduced pressure. The residue was purified by silica gel chromatography to give 3-{(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-[*N*-(4-cyanophenylmethyl)-*N*-(ethoxycarbonylmethyl)amino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine (**16e**, 1.01 g, quant.) as an oil.

(2) Compound **16e** (976 mg, 1.94 mmol) was dissolved in ethyl acetate (3.88 mL) and 4 mol/L hydrochloric acid–ethyl acetate (2.43 mL) was added to the solution. The mixture was stirred at room temperature for 18 h and the precipitated solid was collected to give the title compound (630 mg, 68%) as a white powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.18 (3H, t, J = 7.1Hz), 1.67–1.90 (1H, m), 2.56–2.75 (1H, m), 2.94–3.22 (3H, m), 3.25–4.00 (8H, m), 4.07 (2H, q, J = 7.1 Hz), 4.34–4.78 (3H, m), 7.54 (2H, d, J = 8.2 Hz), 7.81 (2H, d, J = 8.2 Hz), 8.86 (1H, br s), 10.40 (1H, br s).

Anal. Calcd for $C_{20}H_{26}N_4O_3S{\cdot}2HCl{\cdot}1/10H_2O$: C, 50.33; H, 5.96; N, 11.74. Found: C, 50.72; H, 6.36; N, 11.75.

5.1.13. 3-{(2*S*,4*S*)-4-[*N*-(Carbamoylmethyl)-*N*-(4-cyanophenylmethyl) amino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine dihydrochloride (17f). (1) From compound **14b** (0.833 g, 2 mmol), 2-bromoacetamide (0.276 mL, 2 mmol), and diisopropylethylamine (1.05 mL, 6 mmol) using a procedure analogous to synthesis of **16e**, the compound 3-{(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-[*N*-(carbamoylmethyl)-*N*-(4-cyanophenylmethyl)amino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine (**16f**, 0.599 g, 63%) was prepared.

(2) Compound **16f** (599 mg, 1.27 mmol) was dissolved in ethyl acetate (2.53 mL) and 4 mol/L hydrochloric acid–ethyl acetate (1.27 mL) was added to the solution. The mixture was stirred at room temperature for 6 h and the precipitated solid was collected to give the title compound (416 mg, 69%) as a white powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.76–1.99 (1H, m), 2.62–2.83 (1H, m), 2.90–4.10 (11H, m), 4.25–4.80 (3H, m), 7.22 (1H, br s), 7.44 (1H, br s), 7.64 (2H, d, J = 8.1 Hz), 7.84 (2H, d, J = 8.4 Hz), 8.82 (1H, br s), 10.35 (1H, br s).

Anal. Calcd for $C_{18}H_{23}N_5O_2S{\cdot}2HCl{\cdot}1/5C_4H_8O_2{\cdot}4/5H_2O$: C, 47.20; H, 5.94; N, 14.64. Found: C, 47.49; H, 6.01; N, 14.27.

5.1.14. 3-{(2*S*,4*S*)-4-[*N*-(Carboxymethyl)-*N*-(4-cyanophenylmethyl)amino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine 2 trifluoroacetate (17g). (1) From compound **14b** (0.833 g, 2 mmol), *tert*-butyl bromoacetate (0.443 mL, 3 mmol), and diisopropylethylamine (1.05 mL, 6 mmol) using a procedure analogous to synthesis of **16e**, the compound 3-{(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-[*N*-(*tert*-butoxycarbonylmethyl)-*N*-(4-cyanophenylmethyl)amino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine (**16g**, 0.990 g, 93%) was prepared.

(2) Compound **16g** (881 mg, 1.53 mmol) was dissolved in ethyl acetate (3.06 mL) and 4 mol/L hydrochloric

acid–ethyl acetate (6.91 mL) was added to the solution. The mixture was stirred at room temperature for 3 days. The precipitated solid was purified by HPLC to give the title compound (141 mg, 17%) as a white powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.65–1.84 (1H, m), 2.57–2.74 (1H, m), 2.96–3.19 (3H, m), 3.22–4.00 (8H, m), 4.37–4.72 (3H, m), 7.53 (2H, d, J = 8.4 Hz), 7.81 (2H, d, J = 8.1 Hz), 8.77 (1H, br s), 9.63 (1H, br s).

Anal. Calcd for $C_{18}H_{22}N_4O_3S{\cdot}8/5C_2HF_3O_2$: C, 45.72; H, 4.27; N, 10.06. Found: C, 45.74; H, 4.53; N, 10.07.

5.1.15. 3-{(2*S*,4*S*)-4-[*N*-(4-Cyanophenylmethyl)-*N*-(2-hydroxyethyl)amino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine dihydrochloride (17h). (1) To a solution of 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(4-cyanophenylmethyl)amino-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (**14b**, 1.67 g, 4 mmol) in *N*-methyl-2-pyrrolidone (12 mL) were added 2-bromoethanol (1.42 mL, 20 mmol) and diisopropylethylamine (2.09 mL, 12 mmol), and the mixture was stirred at 80 °C for 2 days. The reaction mixture was added to saturated aqueous sodium hydrogencarbonate solution and the mixture was extracted with ethyl acetate. The extract was dried and the solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography to give 3-{(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-[*N*-(4-cyanophenylmethyl)-*N*-(2-hydroxyethyl)amino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine (**16h**, 0.480 g, 26%).

(2) Compound **16h** (480 mg, 1.04 mmol) was dissolved in ethyl acetate (2.08 mL) and 4 mol/L hydrochloric acid–ethyl acetate (1.04 mL) was added to the solution. The mixture was stirred at room temperature for 8 h and the precipitated solid was collected to give the title compound (351 mg, 65%) as a brown powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.85–1.97 (1H, m), 2.02–2.33 (2H, m), 2.70–4.80 (14H, m), 7.60–8.00 (4H, m), 9.00 (1H, br s), 10.50 (1H, br s).

Anal. Calcd for $C_{18}H_{24}N_4O_2S{\cdot}2HCl{\cdot}\ 7/10C_4H_8O_2{\cdot}7/5H_2O$: C, 48.02; H, 6.66; N, 10.77. Found: C, 48.09; H, 6.81; N, 10.47.

5.1.16. 3-{(2*S*,4*S*)-4-[*N*-(4-Cyanobenzoyl)-*N*-(4-cyanophenylmethyl)amino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine hydrochloride (17i). (1) 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(4-cyanophenylmethyl)amino-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (**14b**, 0.833 g) was dissolved in dichloromethane (10 mL), and triethylamine (0.418 mL) and 4-cyanobenzoyl chloride (0.331 g) were added to the solution. The mixture was stirred at room temperature for 18 h. The reaction mixture was added to saturated aqueous sodium hydrogencarbonate solution, and the mixture was extracted with ethyl acetate. The extract was dried and the solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography to give 3-{(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-[*N*-(4-cyanobenzoyl)-*N*-(4-cyanophenylmethyl)amino]-2-pyrrolidinylcarbonyl}-1,3-thiazolidine (**16i**, 0.956 g, 87%).

(2) Compound **16i** (514 mg, 0.941 mmol) was dissolved in ethyl acetate (1.88 mL) and 4 mol/L hydrochloric acid–ethyl acetate (1.18 mL) was added to the solution. The mixture was stirred at room temperature for 18 h and the precipitated solid was collected to give the title compound (320 g, 66%) as a white powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.62–2.39 (1H, m), 2.45–2.82 (1H, m), 2.90–3.25 (2H, m), 3.30–3.95 (4H, m), 4.25–5.00 (6H, m), 7.30–8.20 (8H, m).

Anal. Calcd for $C_{24}H_{23}N_5O_2S{\cdot}HCl{\cdot}1/5C_4H_8O_2{\cdot}4/5H_2O$: C, 57.95; H, 5.33; N, 13.62. Found: C, 57.92; H, 5.25; N, 13.47.

5.1.17. 3-{(2*S*,4*S*)-4-[Bis(4-cyanophenylmethyl)]amino-2-pyrrolidinylcarbonyl}-1,3-thiazolidine dihydrochloride (17d). (1) To a solution of 3-((2*S*,4*S*)-4-amino-1-*tert*-butoxycarbonyl-2-pyrrolidinylcarbonyl)-1,3-thiazolidine[15] (**13**, 0.904 g, 3 mmol) in *N*-methyl-2-pyrrolidone (9 mL) were added 4-cyanobenzyl bromide (1.29 g, 6.6 mmol) and diisopropylethylamine (1.57 mL, 9 mmol), and the mixture was stirred at room temperature for 18 h. The reaction mixture was added to saturated aqueous sodium hydrogencarbonate solution, and the mixture was extracted with ethyl acetate. The extract was dried and the solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography to give 3-{(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-[bis(4-cyanophenylmethyl)]amino-2-pyrrolidinylcarbonyl}-1,3-thiazolidine (**16d**, 1.27 g, 80%).

(2) Compound **16d** (1.13 g, 2.12 mmol) was dissolved in ethyl acetate (4.24 mL) and 4 mol/L hydrochloric acid–ethyl acetate (2.65 mL) was added to the solution. The mixture was stirred at room temperature for 18 h and the precipitated solid was collected to give the title compound (444 mg, 39%) as a white powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.81–1.94 (1H, m), 2.57–2.79 (1H, m), 3.00–3.95 (11H, m), 4.39–4.75 (3H, m), 7.55 (4H, d, J = 8.1Hz), 7.79 (4H, d, J = 8.1 Hz), 8.78 (1H, br s), 10.19 (1H, br s).

Anal. Calcd for $C_{24}H_{25}N_5OS{\cdot}2HCl{\cdot}1/4C_4H_8O_2{\cdot}2/5H_2O$: C, 56.26; H, 5.63; N, 13.12. Found: C, 56.42; H, 5.84; N, 13.09.

5.1.18. 3-((2*S*,4*S*)-1-*tert*-Butoxycarbonyl-4-hydroxy-2-pyrrolidinylcarbonyl)-1,3-thiazolidine (20). (1) To a solution of *N*-*tert*-butoxycarbonyl-L-*cis*-4-hydroxyproline (**18**, 23.1 g, 100 mmol) and imidazole (30.0 g, 441 mmol) in DMF (300 mL) was added *tert*-butyldimethylsilyl chloride (33.3 g, 221 mmol). After stirring at room temperature for 16 h, water (300 mL) was gradually added under ice-cooling. The reaction solution was acidified with 10% aqueous citric acid solution and extracted with ethyl acetate. The extract was washed 3 times with water and with brine, and dried. The solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography to give *N*-*tert*-butoxycarbonyl-L-*cis*-4-*tert*-butyldimethylsilyloxyproline (27.4 g, 79%) as a white solid.

(2) To a solution of *N*-*tert*-butoxycarbonyl-L-*cis*-4-*tert*-butyldimethylsilyloxyproline (5.55 g, 16.1 mmol) and thiazolidine (1.4 mL, 18.8 mmol) in DMF (55 mL) were added triethylamine (2.24 mL, 16.1 mmol), HOBT (2.96 g, 19.3 mmol), and EDC (3.70 g, 19.3 mmol), and the mixture was stirred at room temperature for 13 h. The reaction mixture was added to saturated aqueous sodium hydrogencarbonate solution and extracted with ethyl acetate. The extract was washed with water and brine, and dried. The solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography to give 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-*tert*-butyldimethylsilyloxy-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (**19**, 3.41 g, 51%) as a white solid.

(3) Compound **19** (3.36 g, 8.06 mmol) was dissolved in tetrahydrofuran (50 mL), and a 1.0 mol/L solution (9 mL) of tetrabutylammonium fluoride in tetrahydrofuran was added dropwise under ice-cooling. The mixture was stirred at room temperature for 18 h, and the solvent was evaporated under reduced pressure. The residue was added to brine and extracted with ethyl acetate. The extract was dried and the solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography to give the title compound (2.44 g, 100%) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.25–1.45 (9H, m), 1.52–1.70 (1H, m), 2.35–2.50 (1H, m), 2.95–3.20 (3H, m), 3.50–3.80 (3H, m), 4.10–4.25 (1H, m), 4.37–4.78 (3H, m), 5.18 (1H, br s).

5.1.19. 3-((2*S*,4*S*)-4-Benzoyloxy-2-pyrrolidinylcarbonyl)-1,3-thiazolidine (15i). (1) To a solution of compound **20** (643 mg, 2.13 mmol) in dichloromethane (10 mL) were added triethylamine (0.63 mL, 4.52 mmol), benzoyl chloride (0.44 mL, 3.79 mmol), and 4-(dimethylamino)pyridine (23 mg), and the mixture was stirred at room temperature for 16 h. Saturated aqueous sodium hydrogencarbonate solution was added to the reaction mixture and the mixture was extracted with chloroform. The extract was evaporated under reduced pressure. The residue was purified by silica gel chromatography to give 3-((2*S*,4*S*)-4-benzoyloxy-1-*tert*-butoxycarbonyl-2-pyrrolidinylcarbonyl)-1,3-thiazolidine (515 mg, 60%) as an oil.

(2) The compound thus prepared (413 mg, 1.02 mmol) was dissolved in ethyl acetate (4 mL) and 4 mol/L hydrochloric acid–ethyl acetate (1.3 mL) was added to the solution. The mixture was stirred at room temperature for 14 h and the solvent was evaporated under reduced pressure. The residue was added to aqueous sodium hydrogencarbonate solution and extracted with ethyl acetate. The extract was washed with brine and dried. The solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography to give the title compound (315 mg, quant.) as an oil.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.83–1.93 (1H, m), 2.45–2.57 (1H, m), 2.87 (1H, dd, J = 12.8, 4.2 Hz), 2.99 (1H, t, J = 6.3 Hz), 3.08 (1H, t, J = 6.3 Hz), 3.19 (1H, d, J = 12.8 Hz), 3.57–3.77 (1.5H, m), 3.83–3.98 (1.5H, m), 4.42 (0.5H, d, J = 9.5 Hz), 4.48–4.58 (1H, m), 4.72 (0.5H, d, J = 9.5 Hz), 5.28–5.36 (1H, m), 7.52 (2H, t, J = 7.4 Hz), 7.65 (1H, t, J = 7.4 Hz), 7.93 (2H, d, J = 7.4 Hz).

HRMS (CI) calcd for $C_{15}H_{19}N_2O_3S$ ($M+H^+$) *m*/*e* 307.1116; found *m*/*e* 307.1111.

5.1.20. 3-[(2*S*,4*S*)-4-(4-Cyanobenzoyl)oxy-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (15j). (1) From compound **20** (445 mg, 1.50 mmol), triethylamine (0.63 mL, 4.52 mmol), and 4-cyanobenzoyl chloride (371 mg, 2.24 mmol) using a procedure analogous to step (1) of synthesis of **15i**, the compound 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(4-cyanobenzoyl)oxy-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (519 mg, 80%) was prepared as a pale-brown solid.

(2) From the compound thus prepared (386 mg, 0.895 mmol) using a procedure analogous to step (2) of synthesis of **15i**, the title compound (280 mg, 94%) was prepared as a pale-yellow solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.88–1.97 (1H, m), 2.46–2.58 (1H, m), 2.88 (1H, dd, J = 12.9, 4.0 Hz), 2.99 (1H, t, J = 6.3 Hz), 3.09 (1H, t, J = 6.3 Hz), 3.23 (1H, d, J = 12.9 Hz), 3.57–3.76 (1.5H, m), 3.84–3.99 (1.5H, m), 4.42 (0.5H, d, J = 9.5 Hz), 4.48–4.57 (1H, m), 4.72 (0.5H, d, J = 9.5 Hz), 5.33–5.38 (1H, m), 8.01 (2H, d, J = 8.3 Hz), 8.07 (2H, d, J = 8.3 Hz).

HRMS (CI) calcd for $C_{16}H_{18}N_3O_3S$ ($M+H^+$) *m*/*e* 332.1069; found *m*/*e* 332.1071.

5.1.21. 3-[(2*S*,4*S*)-4-(2-Isoindolinyl)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine 2 trifluoroacetate (22a). (1) To a solution of 3-((2*S*,4*S*)-4-amino-1-*tert*-butoxycarbonyl-2-pyrrolidinylcarbonyl)-1,3-thiazolidine[14] (**13**, 1.49 g) in DMF (50 mL) were added potassium carbonate (2.04 g) and α,α′-dibromo-*ortho*-xylene (1.37 g), and the mixture was stirred at room temperature for 18 h. To the reaction mixture was added water and the mixture was extracted with chloroform. The extract was washed with brine and dried. The solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography to give 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(2-isoindolinyl)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (1.26 g, 63%) as a pale-brown solid.

(2) The compound thus prepared (730 mg, 1.81 mmol) was dissolved in dichloromethane (5 mL) and trifluoroacetic acid (2 mL) was added to the solution. The mixture was stirred at room temperature for 15 h and the solvent was evaporated under reduced pressure. Ether was added to the residue and the unsolved solid was collected to give the title compound (910 mg, 90%) as a brown powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 2.05–2.14 (1H, m), 2.88–2.96 (1H, m), 3.05–3.17 (2H, m), 3.42–4.02 (5H, m), 4.44–4.75 (7H, m), 7.31–7.37 (4H,m).

Anal. Calcd for $C_{16}H_{21}N_3OS{\cdot}2C_2HF_3O_2{\cdot}3/2H_2O$: C, 43.01; H, 4.69; N, 7.52. Found: C, 43.07; H, 4.43; N, 7.33.

5.1.22. 3-[(2*S*,4*S*)-4-(1,2,3,4-Tetrahydro-1-quinolyl)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine hydrochloride (22b). (1) To a solution of 3-((*S*)-1-*tert*-butoxycarbonyl-4-oxo-2-pyrrolidinylcarbonyl)-1,3-thiazolidine[15] (**21**, 450 mg, 1.50 mmol), 1,2,3,4-tetrahydroquinoline (0.23 mL, 1.83 mmol), and acetic acid (0.09 mL, 1.57 mmol) was added sodium triacetoxyborohydride (0.636 g), and the mixture was stirred at room temperature for 24 h. To the reaction mixture was added aqueous sodium hydrogencarbonate solution and extracted with ethyl acetate. The extract was washed with brine and dried. The solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography to give 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(1,2,3,4-tetrahydro-1-quinolyl)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (100 mg, 16%) as an oil.

(2) The compound thus prepared (100 mg, 0.239 mmol) was dissolved in ethyl acetate (3 mL) and 4 mol/L hydrochloric acid–ethyl acetate (0.3 mL) was added to the solution. The mixture was stirred at room temperature for 17 h and the precipitated solid was collected to give the title compound (60 mg, 65%) as a pale-brown-reddish powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.76–1.97 (3H, m), 2.59–2.73 (3H, m), 3.02–3.5 (6H, m), 3.62–3.94 (2H, m), 4.42–4.86 (4H, m), 6.57 (1H, d, J = 7.2 Hz), 6.80 (1H, d, J = 8.3 Hz), 6.92 (1H, d, J = 7.2 Hz), 6.97–7.07 (1H, m), 8.84 (1H, br s), 10.04 (1H, br s).

Anal. Calcd for $C_{17}H_{23}N_3OS{\cdot}7/5HCl{\cdot}1/5C_4H_8O_2$: C, 55.37; H, 6.79; N, 10.88. Found: C, 55.36; H, 7.16; N, 10.58.

5.1.23. 3-[(2*S*,4*S*)-4-(1-Indolinyl)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine dihydrochloride (22c). (1) From compound **21** (601 mg, 2.00 mmol), indoline (0.27 mL, 2.41 mmol), acetic acid (0.11 mL, 1.92 mmol), and sodium triacetoxyborohydride (850 mg, 4.01 mmol) using a procedure analogous to step (1) of synthesis of **22b**, the compound 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(1-indolinyl)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (460 mg, 57%) was obtained as a white solid.

(2) From the compound thus prepared (436 mg, 1.08 mmol) using a procedure analogous to step (2) of synthesis of **22b**, the title compound (293 mg, 74%) was obtained as a white powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.83–1.97 (1H, m), 2.62–2.77 (1H, m), 2.88 (2H, t, J = 8.2 Hz), 3.04 (1H, t, J = 7.0 Hz), 3.12 (1H, t, J = 6.2 Hz), 3.20–3.95 (6H, m), 4.40–4.78 (4H, m), 6.55–6.68 (2H, m), 6.98–7.09 (2H, m), 8.84 (1H, br s), 10.31 (1H, br s).

Anal. Calcd for $C_{16}H_{21}N_3OS{\cdot}17/10HCl$: C, 52.59; H, 6.26; N, 11.50. Found: C, 52.67; H, 6.54; N, 11.45.

5.1.24. 3-[(2*S*,4*S*)-4-(5-Nitro-1-indolinyl)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine hydrochloride (22d). (1) From compound **21** (450 mg, 1.50 mmol), 5-nitroindoline (295 mg, 1.80 mmol), acetic acid (0.090 mL, 1.57 mmol), and sodium triacetoxyborohydride (636 mg, 3.00 mmol) using a procedure analogous to step (1) of synthesis of **22b**, the compound 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(5-nitro-1-indolinyl)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (153 mg, 23%) was obtained as an oil.

(2) From the compound thus prepared (153 mg, 0.341 mmol) using a procedure analogous to step (2) of synthesis of **22b**, the title compound (116 mg, 88%) was obtained as a yellow powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.92–2.06 (1H, m), 2.67–2.80 (1H, m), 3.00–3.17 (4H, m), 3.27–3.94 (6H, m), 4.42–4.78 (4H, m), 6.62 (1H, d, J = 8.9 Hz), 7.87 (1H, d, J = 2,3 Hz), 8.04 (1H, dd, J = 8.9, 2.3 Hz), 9.1 (1H, br s), 10.2 (1H, br s).

Anal. Calcd for $C_{16}H_{20}N_4O_3S{\cdot}11/10HCl$: C, 49.46; H, 5.47; N, 14.42. Found: C, 49.60; H, 5.61; N, 14.03.

5.1.25. 3-[(2*S*,4*S*)-4-(5-Methoxy-1-indolinyl)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine dihydrochloride (22e). (1) From compound **21** (751 mg, 2.50 mmol), 5-methoxyindoline (410 mg, 2.75 mmol), acetic acid (0.143 mL, 2.50 mmol), and sodium triacetoxyborohydride (1.06 g, 5.00 mmol) using a procedure analogous to step (1) of synthesis of **22b**, the compound 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(5-methoxy-1-indolinyl)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (1010 mg, 93%) was obtained as a white solid.

(2) From the compound thus prepared (326 mg, 0.752 mmol) using a procedure analogous to step (2) of synthesis of **22b**, the title compound (262 mg, 78%) was obtained as a white powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80–1.95 (1H, m), 2.62–2.75 (1H, m), 2.86 (2H, t, J = 7.9 Hz), 3.04 (1H, t, J = 7.0 Hz), 3.12 (1H, t, J = 6.2 Hz), 3.17–3.52 (4H, m), 3.65 (3H, s), 3.66–4.08 (6H, m), 4.28–4.77 (4H, m), 6.54 (1H, d, J = 8.5 Hz), 6.63 (1H, dd, J = 8.5, 2.4 Hz), 6.75 (1H, d, J = 2.4 Hz), 8.83 (1H, br s), 10.40 (1H, br s).

Anal. Calcd for $C_{17}H_{23}N_3O_2S{\cdot}19/10HCl{\cdot}1/2C_4H_8O_2$: C, 51.08; H, 6.52; N, 9.41. Found: C, 51.46; H, 6.78; N, 9.07.

5.1.26. 3-[(2*S*,4*S*)-4-(5-Fluoro-1-indolinyl)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine dihydrochloride (22f). (1) From compound **21** (496 mg, 1.65 mmol), 5-fluoroindoline (200 mg, 1.46 mmol), acetic acid (0.084 mL, 1.47 mmol), and sodium triacetoxyborohydride (0.618 g, 2.92 mmol) using a procedure analogous to step (1) of synthesis of **22b**, the compound 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(5-fluoro-1-indolinyl)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (492 mg, 80%) was obtained as a pale-brown solid.

(2) From the compound thus prepared (487 mg, 1.16 mmol) using a procedure analogous to step (2) of synthesis of **22b**, the title compound (357 mg, 79%) was obtained as a white powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.80–1.95 (1H, m), 2.62–2.75 (1H, m), 2.88 (2H, t, J = 8.2 Hz), 3.04 (1H, t, J = 7.0 Hz), 3.11 (1H, t, J = 6.2 Hz), 3.18–3.52 (4H, m), 3.60–3.94 (2H, m), 4.35–4.78 (4H, m), 6.55 (1H, dd, J = 8.8, 4.3 Hz), 6.85 (1H, td, J = 8.8, 2.6 Hz), 6.94 (1H, dd, J = 8.5, 2.6 Hz), 8.90 (1H, br s), 10.44 (1H, br s).

Anal. Calcd for $C_{16}H_{20}FN_3OS{\cdot}19/10HCl$: C, 49.19; H, 5.65; N, 10.76. Found: C, 49.18; H, 5.94; N, 10.54.

5.1.27. 3-[(2*S*,4*S*)-4-(5-Chloro-1-indolinyl)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine hydrochloride (22g). (1) From compound **21** (665 mg, 2.21 mmol), 5-chloroindoline (340 mg, 2.21 mmol), acetic acid (0.127 mL, 2.22 mmol), and sodium triacetoxyborohydride (0.937 g, 4.42 mmol) using a procedure analogous to step (1) of synthesis of **22b**, the compound 3-[(2*S*,4*S*)-1-*tert*-butoxycarbonyl-4-(5-chloro-1-indolinyl)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (393 mg, 41%) was obtained as a white solid.

(2) From the compound thus prepared (389 mg, 0.888 mmol) using a procedure analogous to step (2) of synthesis of **22b**, the title compound (242 mg, 72%) was obtained as a white powder.

1H NMR (300 MHz, DMSO-d_6) delta 1.81–1.95 (1H, m), 2.62–2.74 (1H, m), 2.90 (2H, t, J = 8.3 Hz), 3.04 (1H, t, J = 7.1 Hz), 3.12 (1H, t, J = 6.2 Hz), 3.18-3.52 (4H, m), 3.60–3.94 (2H, m), 4.38–4.77 (4H, m), 6.57 (1H, d, J = 8.3 Hz), 7.03–7.11 (2H, m), 8.86 (1H, br s), 10.38 (1H, br s).

Anal. Calcd for $C_{16}H_{20}ClN_3OS{\cdot}HCl{\cdot}3/10H_2O$: C, 50.61; H, 5.73; N, 11.07. Found: C, 50.65; H, 5.62; N, 11.00.

5.1.28. 3-[(2*S*,4*S*)-4-(5-Bromo-1-indolinyl)-2-pyrrolidinylcarbonyl]-1,3-thiazolidine hydrochloride (22h). (1) From compound **21** (0.901 g, 3.00 mmol), 5-bromoindoline (0.713 g, 3.60 mmol), acetic acid (0.180 mL, 3.14 mmol), and sodium triacetoxyborohydride (1.27 g, 5.99 mmol) using a procedure analogous to step (1) of synthesis of **22b**, the compound 3-[(2*S*,4*S*)-4-(5-bromo-1-indolinyl)-1-*tert*-butoxycarbonyl-2-pyrrolidinylcarbonyl]-1,3-thiazolidine (1.31 g, 91%) was obtained as a white solid.

(2) From the compound thus prepared (340 mg, 0.705 mmol) using a procedure analogous to step (2) of synthesis of **22b**, the title compound (251 mg, 84%) was obtained as a pale-red powder.

^{1}H NMR (300 MHz, DMSO-d_6) δ 1.82–1.96 (1H, m), 2.62–2.74 (1H, m), 2.91 (2H, t, J = 8.3 Hz), 3.04 (1H, t, J = 7.0 Hz), 3.12 (1H, t, J = 6.2 Hz), 3.18–3.54 (4H, m), 3.62–3.93 (2H, m), 4.37–4.77 (4H, m), 6.53 (1H, d, J = 8.1 Hz), 7.15–7.24 (2H, m), 8.91 (1H, br s), 10.27 (1H, br s).

Anal. Calcd for $C_{16}H_{20}BrN_3OS{\cdot}HCl{\cdot}2/5H_2O$: C, 45.11; H, 5.16; N, 9.86. Found: C, 45.04; H, 5.31; N, 9.69.

5.2. Biological methods

5.2.1. DPP-IV inhibitory activity. The DPP-IV inhibitory activity of human plasma and rat plasma was measured by fluorescence assay using Gly-Pro-MCA (Peptide Institute Inc.) as a DPP-IV-specific fluorescent substrate. Reaction solutions containing 20 μL of human or rat plasma (10-fold diluted solution), 20 μL of fluorescent substrate (100 μmol/L), 140 μL of buffer (0.003% Brij-35 containing PBS), and 20 μL of test substrate (of various concentrations) were incubated at room temperature for 60 min using a 96-well flat-bottomed microtiter plate. The measured fluorescent intensity (excitation 360 nm/emission 465 nm, SPECTRA FLUOR, TECAN) was taken as the DPP-IV activity. The inhibitory rate relative to the solvent addition group was calculated and IC_{50} values were determined by logistic analysis.

5.2.2. DPP8, 9-inhibitory activity. DPP8 and DPP9 enzymes were prepared from cytoplasmic fractions of cells expressing recombinant human DPP8 or DPP9, respectively. Reaction solutions containing 20 μL of test compounds of various concentrations, 20 μL of enzyme preparations, 140 μL of buffer (0.003% Brij-35 containing PBS), and 20 μL of Gly-Pro-MCA (50 μmol/L, Peptide Institute Inc.) were incubated at 37 °C for 30 min using a 96-well flat-bottomed microtiter plate. The measured fluorescence intensity of AMC was taken as the enzyme activity. IC_{50} value was calculated as described above.

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