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Bioorganic & Medicinal Chemistry 14 (2006) 2725–2746
Bioorganic & Medicinal Chemistry

Synthesis, biological evaluation, and pharmacokinetic study of prolyl-1-piperazinylacetic acid and prolyl-4-piperidinylacetic acid derivatives as VLA-4 antagonists

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Received 26 September 2005; revised 25 November 2005; accepted 29 November 2005
Available online 27 December 2005

Abstract—A series of prolyl-1-piperazinylacetic acid and prolyl-4-piperidinylacetic acid derivatives were synthesized and evaluated for their activity as VLA-4 antagonists. Of 22 compounds synthesized, 19 compounds showed potent activity with low nanomolar IC_{50} values. In addition, the representative compounds **11o** and **11p** with a hydroxy group in the pyrrolidine ring showed moderate plasma clearance in rats (**11o**, 30 ml/min/kg and **11p**, 21 ml/min/kg) and in dogs (**11o**, 12 ml/min/kg and **11p**, 9 ml/min/kg).

1. Introduction

VLA-4 (very late antigen 4; $\alpha_4\beta_1$ integrin; CD49d/CD29) is a member of the integrin superfamily and is expressed on most leukocytes.[1] It binds a vascular cell adhesion molecule-1 (VCAM-1) expressed on cytokine-stimulated endothelial cells, the alternatively spliced connecting segment-1 domain of fibronectin[2,3] and junctional adhesion molecule 2 on endothelial cells.[4] The VLA-4 plays an important role in the process of inflammation including extravasation of leukocytes and activation of leukocytes at sites of inflammation. *Anti*-VLA-4 antibodies or small molecular VLA-4 antagonists[5] have been reported to inhibit leukocyte infiltration to extravascular tissue and prevent tissue damage in inflammatory disease models of asthma,[6] multiple sclerosis,[7] rheumatoid arthritis,[8] and inflammatory bowel disease.[9] It has recently been reported that a humanized monoclonal *anti*-α_4 antibody, natalizumab,[10] (Elan Pharmaceuticals Inc. and Biogen Idec Inc.) demonstrated promising results in patients with multiple sclerosis, Crohn's disease, and rheumatoid arthritis in clinical trials. Therefore, the development of small molecular VLA-4 antagonist with good oral pharmacokinetic profile is viewed as a reasonable approach to a novel class of therapeutic agents.

We have identified a morpholinyl-4-piperidinylacetic acid derivative **1** with an IC_{50} value of 4.4 nM as a potent VLA-4 antagonist, which demonstrated efficacy in the murine airway inflammation model by oral dosing at 30 mg/kg (Fig. 1).[11] It has been found, however, that **1** showed rapid plasma clearance (69 ml/min/kg) in rats, resulting in low oral bioavailability (<1%). On the other hand, we have also identified a prolyl-1-piperazinylacetic acid derivative **2** with an IC_{50} of 1.6 nM (Fig. 1).[11] Thus, we next chose **2** as a lead compound to investigate the structure and physiochemical properties to improve oral bioavailability while retaining the potent activity. Several substituents were introduced in the pyrrolidine ring, and both one and two methyl groups at the α-carbon of the acetic acid moiety. The piperidine ring was

Keywords: VLA-4; Integrin.
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doi:10.1016/j.bmc.2005.11.058

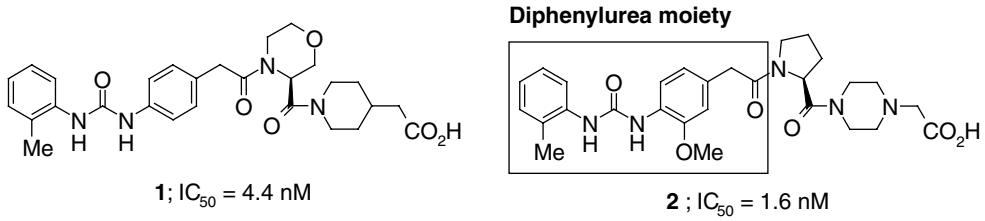


Figure 1.

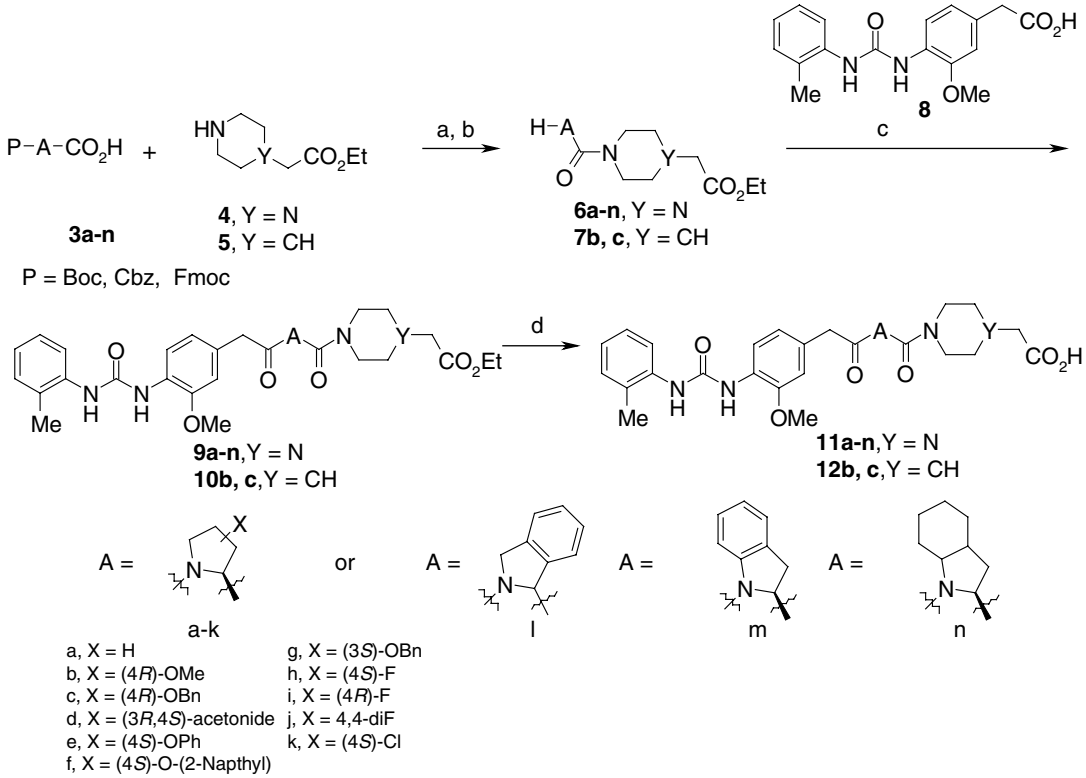
also replaced with a piperazine ring to give a piperazinylacetic acid. The diphenylurea moiety, the 4-(2-methylphenylureido)-3-methoxyphenylacetyl group, was remained fixed, because this moiety has been found to be useful for maintaining potent activity (Fig. 1).[6] Herein we report the synthesis, structure–activity relationships, and physicochemical properties of these compounds. In addition, we examined the pharmacokinetic profiles of some representative compounds and established a relationship between the physicochemical properties and the plasma clearance.

2. Chemistry

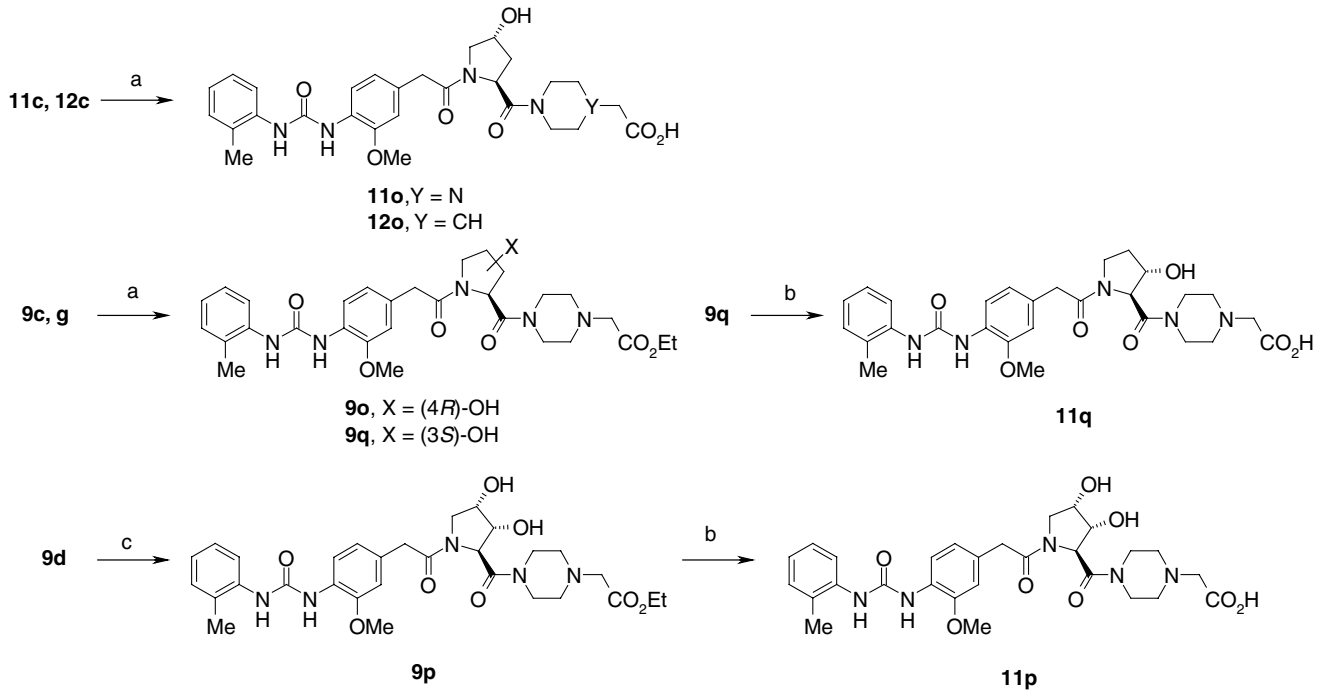
The general synthetic route of prolyl-1-piperazinylacetic acids **11a**–**n** and prolyl-4-piperidinylacetic acids **12b** and **12c** are outlined in Scheme 1. *N*-Protected proline derivatives **3a**–**n** were condensed with ethyl 1-piperazinylacetate (**4**) or ethyl 4-piperidinylacetate (**5**) using standard amide bond-forming conditions followed by deprotection of *N*-protective groups to give **6a**–**n**, **7b**, and **7c**. Coupling of **6a**–**n**, **7b**, and **7c** with 3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetic acid (**8**) and basic hydrolysis afforded **11a**–**n**, **12b**, and **12c**.

Examples **11o** and **12o**, bearing a hydroxyl group in the pyrrolidine ring, were synthesized through *O*-debenzylation of **11c** and **12c** (Scheme 2). In the case of **11p** and **11q**, the *O*-deprotection was conducted before the basic hydrolysis (Scheme 2).

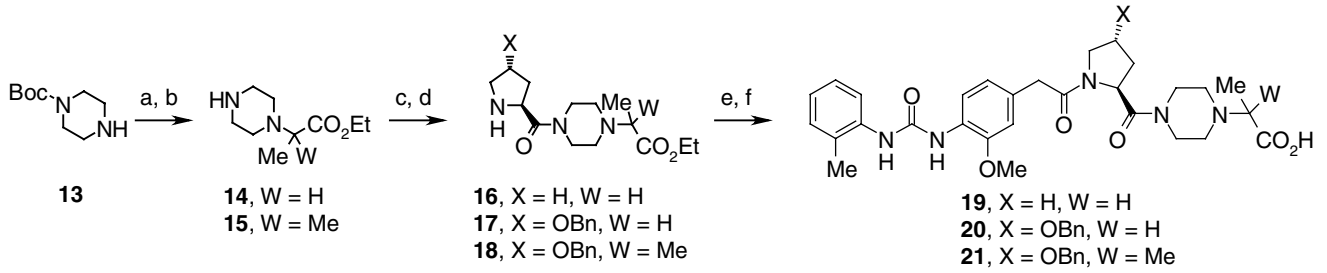
The preparation of compounds having a methyl or dimethyl group at α-position of the acetic acid residue was performed as shown in Scheme 3. Ethyl 2-bromopropionate or ethyl 2-bromo-2-methylpropionate was coupled by substitution reaction with *N*-Boc-piperazine (**13**) in the presence of K_2CO_3, followed by deprotection to give **14** and **15**. Compounds **14** and **15** were converted



Scheme 1. Reagents: (a) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl salt (EDC·HCl), HOBt, Et_3N, THF (CH_2Cl_2 or DMF); (b) TFA, CH_2Cl_2 or H_2, Pd/C, EtOH or TBAF, THF; (c) **8**, EDC·HCl, HOBt, Et_3N, THF (CH_2Cl_2 or DMF); (d) aq NaOH, THF/MeOH.



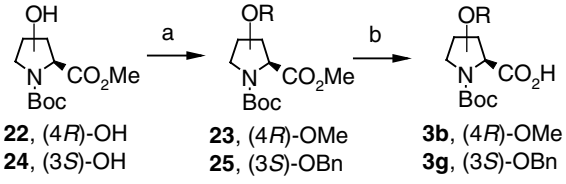
Scheme 2. Reagents: (a) H_2, Pd/C, EtOH (57% for **9o**, 59% for **9q**, 58% for **11o**, 65% for **12o**); (b) aq NaOH, THF or THF/MeOH (90% for **11p**, 95% for **11q**); (c) HCl (*gas*)/MeOH (97%).



Scheme 3. Reagents: (a) $BrCH(CH_3)CO_2Et$ or $BrC(CH_3)_2CO_2Et$, K_2CO_3, DMF; (b) TFA, CH_2Cl_2 [25% for **14** (2 steps), 98% for **15** (2 steps)]; (c) EDC·HCl, DMAP, DMF; (d) TFA, CH_2Cl_2 [26% for **16** (2 steps), 20% for **17** (2 steps), 26% for **18** (2 steps)]; (e) EDC·HCl, HOBt, Et_3N, DMF; (f) aq NaOH, THF/MeOH [12% for **19** (2 steps), 45% for **20** (2 steps), 34% for **21** (2 steps)].

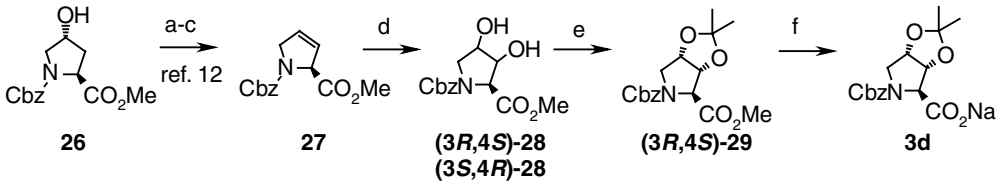
to **19**–**21**, according to the general synthetic route shown in Scheme 1.

Pyrrolidine intermediates **3b** and **3d**–**k** were prepared by using the commercially available hydroxy-L-proline derivatives **22**, **24**, **26**, and **32** as shown in Schemes 4–7. Thus, *N*-Boc-*trans*-4-hydroxy-L-proline methyl ester (**22**) and *N*-Boc-*trans*-3-hydroxy-L-proline methyl ester (**24**) were etherified (MeI or BnBr/NaH) followed by

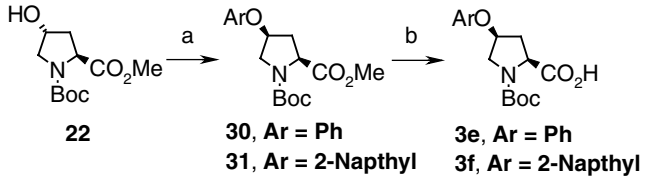


Scheme 4. Reagents: (a) MeI, NaH, DMF (89% for **23**) or BnBr, NaH, tetrabutylammonium iodide, THF (59% for **25**); (b) aq NaOH, THF (quant. for **3b**, 59% for **3g**).

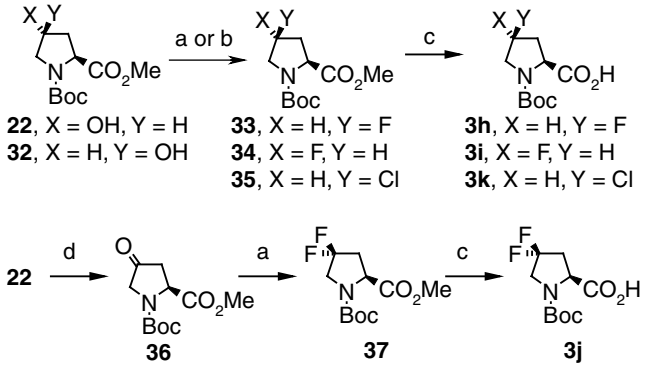
basic hydrolysis to give **3b** and **3g**, respectively (Scheme 4). As for the synthesis of the *N*-*Z*-3,4-isopropylidenedioxy-L-proline derivative **3d**, first, *N*-*Z*-*trans*-4-hydroxy-L-proline methyl ester (**26**) was converted to *N*-*Z*-3,4-dehydro-L-proline methyl ester (**27**) in 3 steps according to a reported procedure[12] (1, tosylation of the hydroxy group of **26**; 2, nucleophilic displacement of the *O*-tosyl group with a phenylselenyl group; and 3, oxidative elimination of the phenylselenyl group). Next, the obtained **27** was dihydroxylated with OsO_4/NMO to give a mixture of diastereomeric isomers [(3*R*,4*S*)-**28**:(3*S*,4*R*)-**28** = ca. 15:1], which were used in the next step without separation of the isomers. After both hydroxy groups of the diols **28** were protected as *O*-isopropylidene ketal, (3*R*,4*S*)-**29** was separated by silica gel column chromatography. Finally, basic hydrolysis of the methyl ester group gave **3d** (Scheme 5). (4*S*)-Aryloxyproline derivatives **3e** and **3f** were prepared through introduction of aryloxy groups by the Mitsunobu reaction and basic hydrolysis (Scheme 6).



Scheme 5. Reagents: (a) 1-(*p*-toluenesulfonyl)imidazole, TfOMe, *N*-methylimidazole, THF (97%); (b) PhSeSePh, $NaBH_4$, MeOH, reflux (82%); (c) H_2O_2, pyridine, CH_2Cl_2 (74%); (d) OsO_4, NMO, acetone/H_2O (1:1, v/v) (95%); (e) 2,2-dimethoxypropane, *p*-TsOH·H_2O, CH_2Cl_2 (cat.) (89%); (f) aq NaOH, THF (91%).



Scheme 6. Reagents: (a) phenol, or 2-naphthol, diisopropyl azodicarboxylate, PPh_3, THF (86% for **30**, 58% for **31**); (b) aq NaOH, THF (58% for **3e**, 100% for **3f**).



Scheme 7. Reagents: (a) DAST, CH_2Cl_2 (10% for **33**, 40% for **34**, 72% for **37**); (b) CCl_4, PPh_3, CH_2Cl_2 (76% for **35**); (c) aq NaOH, THF (85% for **3h**, quant. for **3i**, 92% for **3j**, 83% for **3k**); (d) PDC, CH_2Cl_2 (57%).

4-Fluoroproline derivatives **3h**, **3i**,[13] and (4*S*)-chloroproline derivative **3k** were prepared via fluorination (DAST) or chlorination (PPh_3/CCl_4) of the hydroxyl group in **22** and **32**, respectively. Similarly, 4,4-difluoroproline derivative **3j**[13] was synthesized from *N*-Boc-4-oxo-L-proline methyl ester (**36**) easily prepared by the treatment of **22** with PDC (Scheme 7).

3. Results and discussion

Compounds obtained were evaluated for their activity in the VLA-4/VCAM-1 binding assay and the distribution coefficient [log*D*, *n*-octanol/PBS (pH 7.4)]. The results are summarized in Table 1.

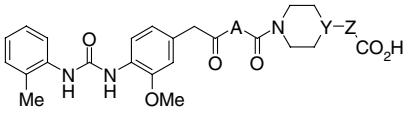
Piperazinylacetic acid derivatives bearing methoxy (**11b**), benzyloxy (**11c**), aryoxy (**11d**–**e**), hydroxy (**11o**), fluoride (**11h**–**j**), and chloride groups (**11k**) at the 4-position in the pyrrolidine ring of **2** showed good potency with IC_{50} values of about 1 nM, suggesting that the bulkiness, configuration, and nature of substituents at this position did not affect the activity. As for the piperidinylacetic acid derivative, introduction of methoxy group (**12b**) and benzyloxy group (**12c**) also retained the activity. The piperidine ring was found to have the same activity as compared with that of the piperazine ring (**12b**, **12c**, and **12o** in comparison with **11b**, **11c**, and **11o**). Similarly, 3,4- and 4,5-di-substituted pyrrolidine derivatives **11d**, **11p**, **11l**, **11m**, and **11n** showed good potency. From these results, it appeared that the 3-, 4-, and 5-positions of the pyrrolidine ring do not participate in the interaction with VLA-4 protein and therefore these positions could be utilized to adjust the physicochemical properties of the compound. The introduction of methyl groups at α-position of the acetic acid residue resulted in a significant decrease in potency of **19**–**21** as compared with **2** and **11c**.

Compounds **11o**, **11p**, **11e**, and **11m** were next evaluated for their pharmacokinetic properties in rats. As a result, **11o** and **11p** bearing a hydroxy group in the pyrrolidine ring showed improved plasma clearance (Cl = 30 ml/min/kg for **11o**, 21 ml/min/kg for **11p**) in comparison with **1**, **11e**, and **11m** (Cl = 69 ml/min/kg for **1**, 77 ml/min/kg for **11e**, 95 ml/min/kg for **11m**) as shown in Table 2. In addition, it was found that both **11o** and **11p** showed moderate plasma clearance in dogs (Cl = 12 ml/min/kg for **11o**, Cl = 9 ml/min/kg for **11p**).

To investigate the reason for the improved plasma clearance of **11o**, we examined how **1** and **11o** were excreted in rats. As a result, it was found that 44% of the unchanged **1** was recovered in bile over 6 h, while 36% of the unchanged **11o** was recovered in urine and 4% in bile over 12 h (Table 3). The results suggested that the change of the excretion pathway from bile to urine is responsible for the improvement in plasma clearance, and significant decrease of lipophilicity ($\log D < 3$) obtained by introduction of the hydroxyl group in the pyrrolidine ring might explain the change in the excretion pathway.

Considering the extremely low lipophilicity of both **11o** and **11p** ($\log D < 3$), these compounds would not be expected to show oral absorption because of poor membrane permeability. Hence, we next attempted a prodrug strategy. We selected the ethyl esters **9o** of **11o** and **9p** of **11p**, which were prepared as intermediates, and examined their oral pharmacokinetic properties in rats. However, as shown in Table 4, **9o** and **9p** showed only 2.7 and 1.5% oral bioavailability, respectively; and it was found that the prodrugs did not result in significant improvement.

Table 1. Inhibition of VLA-4/VCAM-1 by VLA-4 antagonists



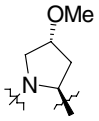
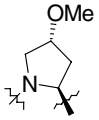
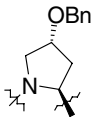
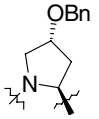
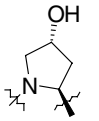
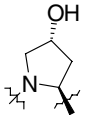
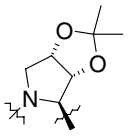
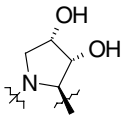
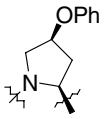
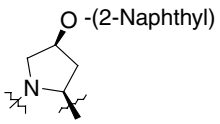
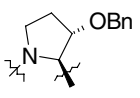
Compound	A	Y	Z	IC_{50} (nM)	log D[a]
1	—	—	—	4.4	−0.7[b]
2		N	–CH_2–	1.6	−1.0
11b		N	–CH_2–	2.0	n.t.
12b		CH	–CH_2–	1.1	n.t.
11c		N	–CH_2–	1.6	0.4
12c		CH	–CH_2–	0.94	0.6
11o		N	–CH_2–	5.1	<−3
12o		CH	–CH_2–	1.1	−1.2
11d		N	–CH_2–	1.6	n.t.
11p		N	–CH_2–	1.4	<−3
11e		N	–CH_2–	1.2	0.1
11f		N	–CH_2–	1.4	1.3
11g		N	–CH_2–	1.3	0.4

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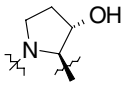
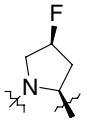
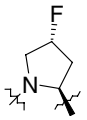
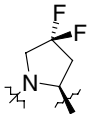
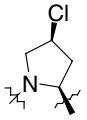
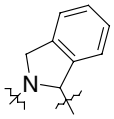
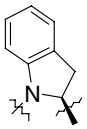
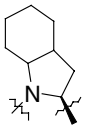
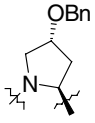
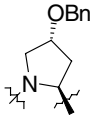
Table 1 (*continued*)

Compound	A	Y	Z	IC_{50} (nM)	log *D*[a]
11q		N	$-CH_2-$	0.96	−1.4
11h		N	$-CH_2-$	1.0	−1.5
11i		N	$-CH_2-$	2.2	−0.8
11j		N	$-CH_2-$	1.4	−0.7
11k		N	$-CH_2-$	1.1	−0.6
11l		N	$-CH_2-$	1.5	0.1
11m		N	$-CH_2-$	2.6	0.2
11n		N	$-CH_2-$	1.0	n.t.
19		N	$-CH(CH_3)-$	66	n.t.
20		N	$-CH(CH_3)-$	231	n.t.
21		N	$-C(CH_3)_2-$	185	n.t.

n.t., not tested.
[a] *n*-Octanol to PBS (pH 7.4) distribution coefficient.
[b] *n*-Octanol to the Japanese Pharmacopoeia Second fluid (pH 6.8) distribution coefficient.

4. Conclusion

In conclusion, we introduced several substituents into the 3-, 4-, and 5-positions of the pyrrolidine ring and methyl group into the α-position of acetic acid and replaced the piperidine ring with a piperazine ring. Except for the introduction of the methyl group at α-position of acetic acid, the other modifications retained low nanomolar potency. Among the derived compounds, we identified **11o** and **11p** which had a hydroxyl group in the pyrrolidine ring, with moderate plasma clearance in rats and dogs. In addition, we found that these compounds were excreted mainly to urine, whereas **1** with high plasma clearance was mainly excreted to bile. Because both compounds had low lipophilicity, we attempted to apply a pro-drug strategy using the ethyl

Table 2. Pharmacokinetic properties of selected VLA-4 antagonists

Compound	Clearance (ml/min/kg)	
	Rat[a]	Dog[b]
1	69	n.t.
11o	30	12
11p	21	9
11e	77	n.t.
11m	95	n.t.

n.t., not tested.
[a] Male Sprague–Dawley rats. Dose: iv infusion (2 h) at 1.2 mg/kg for **1**, 1.0 mg/kg for **11o**, and 0.75 mg/kg for **11p**, **11e**, and **11m**.
[b] Male Beagle dog. Dose: iv infusion (1 h) at 0.75 mg/kg.

esters **9o** of **11o** and **9p** of **11p**. However, the strategy did not result in a significant improvement of oral absorption. Further improvements and structural modifications of these derivatives toward achieving an orally active VLA-4 antagonist will be presented in forthcoming publications.

5. Experimental

5.1. General

Melting points were determined with a YANACO MP-J3 and are uncorrected. Column chromatography was performed with Merck silica gel 60 (particle size 0.060–0.200 or 0.040–0.063) and Daianion HP-20 (MITSUBISHI Chemical Industries, highly porous polymer type synthetic adsorbent). Flash chromatography was performed with Biotage Si and YAMAZEN Hi-Flash packed column. Thin-layer chromatography (TLC) was performed on Merck pre-coated TLC glass sheets with silica gel 60 F_{254}. ^{1}H NMR spectra were recorded on a JEOL JNM-EX-400 spectrometer, and chemical shifts are given in ppm (δ) from tetramethylsilane as the internal standard. IR spectra were recorded on a HITACHI 270-30 spectrometer. ESI mass spectra were recorded on a SCIEX API-150EX spectrometer. FAB mass spectra were recorded on a JEOL JMS-HX110 spectrometer. HR-FAB mass spectra were recorded on a JEOL JMS-700 spectrometer.

5.2. General procedure A: preparation of 4-[4-methoxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11b)

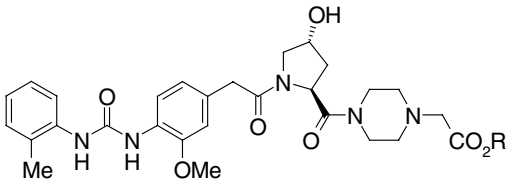
5.2.1. Ethyl 4-[1-(*tert*-butoxycarbonyl)-(4*R*)-methoxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate. A mixture of 1-(*tert*-butoxycarbonyl)-(4*R*)-methoxypyrrolidine-(2*S*)-carboxylic acid (**3b**) (910 mg, 3.71 mmol), ethyl 1-piperazinylacetate (**4**) (640 mg, 3.72 mmol), EDC·HCl (853 mg, 4.45 mmol), HOBt (602 mg, 4.45 mmol), and Et_3N (620 μl, 4.45 mmol) in CH_2Cl_2 (10 ml) was stirred at room temperature for 12 h. The mixture was poured into water and extracted with $CHCl_3$. The combined extracts were washed with brine and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography on silica gel with

Table 3. Excretion of unchanged parent compound in rats

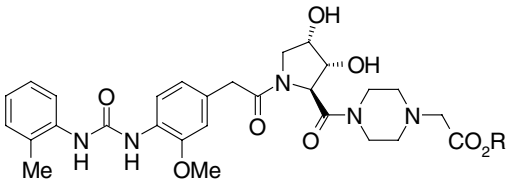
Compound	Cl (iv)[a] (ml/min/kg)	Bile				Urine			
		% Dose			Total	% Dose			Total
		0–2 h	2–4 h	4–6 h	0–6 h	0–3 h	3–6 h	6–12 h	0–12 h
1	69	31[b]	13[b]	nq.	44[b]	n.t.	n.t.	n.t.	n.t.
11o	30	n.t.	n.t.	n.t.	4	8[c]	25[c]	3[c]	36[c]

nq., compound concentration was below the LLOQ of the assay (5 ng/ml).
n.t., not tested.
[a] See also Table 2.
[b] Male Sprague–Dawley rats. Dose: iv infusion (2 h) at 2.4 mg/kg.
[c] Dose: 1.0 mg/kg iv bolus at 0 and 3 h (total 2 mg/kg).

Table 4. Pharmacokinetic properties of **9o** and **9p** in rats



11o, R = H
9o, R = Et



11p, R = H
9p, R = Et

Compound	$F^{a,b}$ (%)
9o	2.7
9p	1.5

[a] Male Sprague–Dawley rats. Dose: p.o. at 2 mg/kg; iv see also Table 2.
[b] Oral plasma concentrations of the parent compounds **9o** and **9p** were measured.

$CHCl_3$/MeOH (50/1, v/v) as an eluent to give the title compound (1.50 g, quant.) as a pale yellow oil. ^1H NMR ($CDCl_3$) δ 1.26–1.30 (3H, m), 1.40 and 1.45 (9H, s, each, amide isomers), 1.97–2.08 (1H, m), 2.14–2.23 (1H, m), 2.55–2.67 (4H, m), 3.24 (2H, s), 3.32 (3H, s), 3.49–3.75 (6H, m), 3.98–4.07 (1H, m), 4.16–4.22 (2H, m), 4.64–4.79 (1H, m); MS (ESI), *m/z* 400 (M^++1).

5.2.2. Ethyl 4-[4-methoxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (6b). To a solution of ethyl 4-[1-(*tert*-butoxycarbonyl)-(4*R*)-methoxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (630 mg, 1.58 mmol) in CH_2Cl_2 (10 ml) was added TFA (10 ml), and the solution was stirred at room temperature for 12 h. The reaction mixture was concentrated in vacuo and made basic with $NaHCO_3$. The mixture was extracted with $CHCl_3$. The combined extracts were washed with brine, dried over Na_2SO_4, and evaporated to give the title compound **6b** (245 mg, 52%) as a reddish brown oil. ^1H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.1 Hz), 1.79–1.86 (4H, m), 2.56–2.61 (4H, m), 3.24 (2H, s), 3.31 (3H, s), 3.50–3.73 (4H, m), 3.93–3.97 (1H, m), 4.04–4.08 (2H, m), 4.19 (2H, q, J = 7.1 Hz); MS (ESI), *m/z* 300 (M^++1).

5.2.3. Ethyl 4-[4-methoxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (9b). A mixture of 3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetic acid (**8**) (116 mg, 0.37 mmol), ethyl 4-[(4*R*)-methoxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (**6b**) (110 mg, 0.37 mmol), EDC·HCl (85 mg, 0.44 mmol), HOBt (60 mg, 0.44 mmol) and Et_3N (60 μl, 0.43 mmol) in THF (10 ml) was stirred at room temperature for 12 h. The reaction mixture was poured into water and extracted with EtOAc. The combined extracts were washed with brine and dried over Na_2SO_4. After removal of the solvent, the residue was purified by TLC with $CHCl_3$/MeOH (10/1, v/v) to give the title compound **9b** (183 mg, 83%) as a colorless amorphous solid. ^1H NMR ($CDCl_3$) δ 1.27 (3H, t, J = 7.1 Hz), 2.01–2.07 (1H, m), 2.13–2.19 (1H, m), 2.29 (3H, s), 2.49–2.65 (4H, m), 3.22 (4H, s), 3.58–3.71 (9H, m), 3.75 (3H, s), 4.08 (1H, m), 4.18 (2H, q, J = 7.1 Hz), 4.93–4.9 (1H, m), 6.26 (1H, s), 6.82 (2H, s), 7.07 (1H, s), 7.13–7.16 (1H, m), 7.22–7.26 (2H, m), 7.50–7.52 (1H, m), 8.03–8.05 (1H, m); MS (FAB), *m/z* 596 (M^++1); Anal. Calcd for $C_{31}H_{41}N_5O_7 \cdot 1.25H_2O$: C, 60.23; H, 7.09; N, 11.33. Found: C, 60.28; H, 7.06; N, 11.15.

5.2.4. 4-[(4*R*)-Methoxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11b). To a stirred solution of ethyl 4-[4-methoxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (**9b**) (460 mg, 0.77 mmol) in THF/MeOH (10 ml, 1/1, v/v) was added 0.5 N NaOH (10 ml) and the reaction mixture was heated under reflux for 5 h. The mixture was acidified with 1 N HCl. After removal of the solvent, the residue was purified by filtration through an ion-exchange resin (DIAION, HP-20) with H_2O to MeOH as an eluent to give a crude substance. The crude substance was purified by TLC with $CHCl_3$/MeOH (10/1, v/v) to give the title compound **11b** (120 mg, 27%) as a pale yellow amorphous solid. ^1H NMR (CD_3OD) δ 1.91–1.95 (1H, m), 2.29 (3H, s), 2.33–2.36 (1H, m), 2.71–2.86 (4H, m), 3.16 (2H, s), 3.23 (3H, s), 3.31 (2H, s), 3.49–3.80 (8H, m), 3.90 (3H, s), 4.05–4.06 (1H, m), 4.92–4.96 (1H, m), 6.80–6.83 (1H, m), 6.93–6.94 (1H, m), 6.99–7.03 (1H, m), 7.13–7.19 (2H, m), 7.56–7.58 (1H, m), 7.98–8.00 (1H, m); MS (FAB), *m/z* 568 (M^++1); HR-MS (FAB) calcd for $C_{29}H_{38}N_5O_7$: 568.2771. Found: 568.2786; IR (ATR) 3225, 3251, 2935, 2829, 1639, 1620, 1599, 1541 cm^{-1}.

Compounds **11c**–**n**, **12b**–**c**, and **19**–**22** were prepared according to procedure A.

5.3. 1-[(4*R*)-Methoxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-4-piperidinylacetic acid (12b)

5.3.1. Ethyl 1-[1-(*tert*-butoxycarbonyl)-(4*R*)-methoxy-(2*S*)-pyrrolidinylcarbony]-4-piperidinylacetate. Colorless oil (yield, 44%). ^1H NMR ($CDCl_3$) δ 1.25–1.28 (3H, m), 1.42 and 1.45 (total 9H, each s, amide isomers), 1.74–1.86 (2H, m), 1.95–2.05 (2H, m), 2.15–2.26 (4H, m), 2.56–2.64 (1H, m), 3.02–3.16 (1H, m), 3.32 (3H, s), 3.51–3.68 (3H, m), 3.84–4.07 (2H, m), 4.12–4.17 (2H, m), 4.56–4.82 (2H, m); MS (ESI), *m/z* 399 (M^++1).

5.3.2. Ethyl 1-[(4*R*)-methoxy-(2*S*)-pyrrolidinylcarbonyl]-4-piperidinylacetate (7b). Reddish-brown oil (yield, 80%). ^1H NMR ($CDCl_3$) δ 1.24–1.28 (3H, m), 1.74–1.85 (4H, m), 2.01–2.14 (2H, m), 2.23–2.26 (2H, m), 2.60–2.66 (1H, m), 2.90–3.09 (2H, m), 3.26–3.29 (2H, m), 3.32 (3H, s), 3.87–3.95 (2H, m), 4.03–4.11 (1H, m), 4.13–4.15 (2H, m), 4.57–4.60 (1H, m); MS (ESI), *m/z* 299 (M^++1).

5.3.3. Ethyl 1-[(4*R*)-methoxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-4-piperidinylacetate (10b). Colorless amorphous solid (yield, 94%). ^1H NMR ($CDCl_3$) δ 1.05–1.29 (5H, m), 1.68–1.84 (5H, m), 1.99–2.10 (2H, m), 2.13–2.24 (2H, m), 2.26 (3H, s), 2.54–2.62 (1H, m), 3.01–3.18 (1H, m), 3.21–3.23 (3H, m), 3.69 (2H, s), 3.70 (3H, s), 3.93–4.17 (4H, m), 4.46–4.59 (1H, m), 4.93–5.00 (1H, m), 6.63–6.65 (1H, m), 6.78–6.80 (2H, m), 7.08–7.12 (1H, m), 7.20–7.25 (3H, m), 7.55–7.57 (1H, m), 8.03–8.05 (1H, m); MS (FAB), *m/z* 595 (M^++1); Anal. Calcd for $C_{32}H_{42}N_4O_7 \cdot 0.75H_2O$: C, 63.19; H, 7.21; N, 9.21. Found: C, 63.08; H, 7.07; N, 9.01.

5.3.4. 1-[(4*R*)-Methoxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-4-piperidinylacetic acid (12b). Pale brown crystalline powder (yield, 50%), which was recrystallized from $CHCl_3$/Et_2O. Mp 123–127 °C; ^1H NMR (DMSO-d_6) δ 0.83–1.32 (2H, m), 1.52–2.70 (11H, m), 2.83–4.93 (15H, m), 6.58–6.92 (2H, m), 7.78 (1H, d, J = 6.8 Hz), 7.14 (2H, t, J = 7.8 Hz), 8.45 (1H, s), 8.56 (1H, s), 12.12 (1H, br); MS (FAB), *m/z* 567 (M^++1); Anal. Calcd for $C_{30}H_{38}N_4O_7 \cdot 1.5H_2O$: C, 60.69; H, 6.96; N, 9.44. Found: C, 60.70; H, 6.75; N, 9.36; IR (KBr) 3346, 3059, 2935, 1707, 1624, 1531, 1485, 1454 cm^{-1}.

5.4. 4-[(4*R*)-Benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-pipirazinylacetic acid (11c)

5.4.1. Ethyl 1-[(4*R*)-benzyloxy-1-*tert*-butoxycarbonyl-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate. Colorless oil (yield, quant.). ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 6.9 Hz), 1.40 (9H, s), 1.92–2.44 (4H, m), 2.49–2.71 (4H, m), 3.18–3.30 (2H, m), 3.46–3.81 (5H, m), 4.19 (2H, q, J = 7.4 Hz), 4.39–4.86 (3H, m), 7.27–7.39 (5H, m); MS (ESI), m/z 476 (M^++1).

5.4.2. Ethyl 1-[(4*R*)-benzyloxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (6c). Colorless oil (yield, 94%). ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.1 Hz), 1.73–1.90 (3H, m), 2.16 (1H, ddd, J = 13.0, 7.6, 2.7 Hz), 2.53–2.63 (3H, m), 3.03 (1H, dd, J = 12.3, 3.4 Hz), 3.24 (2H, s), 3.32 (1H, dd, J = 12.0, 5.4 Hz), 3.42–3.77 (4H, m), 4.08–4.24 (3H, m), 4.41–4.60 (2H, m), 7.27–7.37 (5H, m); MS (ESI) m/z 376 (M^++1).

5.4.3. Ethyl 4-[(4*R*)-benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-pipirazinylacetate (9c). Colorless amorphous solid (yield, 72%). ^{1}H NMR ($CDCl_3$) δ 1.24–1.30 (4H, m), 2.00–2.08 (1H, m), 2.17–2.25 (1H, m), 2.29 (3H, s), 2.46–2.71 (4H, m), 3.46–3.85 (12H, m), 4.07–4.56 (5H, m), 4.97 (1H, dd, J = 6.9, 8.1 Hz), 6.28 and 6.34 (total 1H, each s, amide isomers), 6.69 and 6.79 (total 2H, each d, J = 7.4 and 8.3 Hz, respectively, amide isomers), 7.02–7.38 (9H, m), 7.49–7.54 (1H, m), 8.05 (1H, d, J = 7.8 Hz); MS (FAB) m/z 672 (M^++1); Anal. Calcd for $C_{35}H_{45}N_5O_7{\cdot}0.75H_2O$: C, 64.85; H, 6.84; N, 10.22. Found: C, 64.93; H, 6.81; N, 10.08; IR (ATR) 3344, 2979, 2935, 2862, 1736, 1705, 1622, 1589, 1527 cm^{-1}.

5.4.4. 4-[(4*R*)-Benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-pipirazinylacetic acid (11c). Colorless amorphous solid (yield, quant.). ^{1}H NMR (DMSO-d_6) δ 1.68–2.12 (2H, m), 2.43–2.80 (8H, m), 3.14–3.67 (16H, m), 3.84 and 3.87 (total 3H, each s, amide isomers), 4.19–4.36 (1H, m), 4.85 (1H, t, J = 7.4 Hz), 4.96–5.20 (1H, m), 6.62–6.79 (1H, m), 6.79–6.97 (2H, m), 7.10 (1H, d, J = 7.8 Hz), 7.15 (1H, d, J = 7.8 Hz), 7.77 (1H, d, J = 8.1 Hz), 7.98 and 7.99 (total 1H, each d, J = 8.3 and 8.1 Hz, respectively, amide isomers), 8.47 (1H, s), 8.54 (1H, s); MS (FAB) m/z 644 (M^++1); HR-MS (FAB) calcd for $C_{35}H_{42}N_5O_7$: 644.3084. Found: 644.3064; Anal. Calcd for $C_{35}H_{41}N_5O_7{\cdot}4H_2O$: C, 58.73; H, 6.90; N, 9.78. Found: C, 58.39; H, 6.55; N, 9.65; IR (ATR) 3334, 3028, 2945, 2873, 1620, 1529, 1485, 1452 cm^{-1}.

5.5. 4-[(4*R*)-Benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-4-piperidinylacetate (12c)

5.5.1. Ethyl 1-[(4*R*)-benzyloxy-1-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinylcarbonyl]-4-piperidinylacetate. Colorless oil (yield, 58%). ^{1}H NMR ($CDCl_3$) δ 1.08–2.95 (23H, m), 3.58–4.81 (10H, m), 7.28–7.36 (5H, m); MS (ESI), m/z 475 (M^++1).

5.5.2. Ethyl 1-[(4*R*)-benzyloxy-(2*S*)-pyrrolidinylcarbonyl]-4-piperidinylacetate (7c). Pale yellow oil (yield, 85%). ^{1}H NMR ($CDCl_3$) δ 1.11–1.28 (5H, m), 1.75–1.82 (3H, m), 2.03–2.25 (6H, m), 2.61–2.63 (1H, m), 2.88–3.04 (2H, m), 3.29–3.34 (1H, m), 3.80–3.90 (1H, m), 4.08–4.16 (4H, m), 4.44–4.59 (2H, m), 7.27–7.34 (5H, m); MS (ESI), m/z 375 (M^++1).

5.5.3. Ethyl 4-[(4*R*)-Benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-4-piperidinylacetate (10c). Colorless amorphous solid (yield, 81%). ^{1}H NMR ($CDCl_3$) δ 1.00–1.12 (1H, m), 1.22–1.27 (3H, m), 1.45–2.60 (13H, m), 1.97–4.50 (14H, m), 4.98–5.00 (1H, m), 6.44 (1H, s), 6.76–6.79 (2H, m), 7.11–7.33 (9H, m), 7.51–7.53 (1H, m), 8.03–8.05 (1H, m); MS (FAB), m/z 671 (M^++1); HR-MS (FAB) calcd for $C_{38}H_{47}N_4O_7$: 671.3445. Found: 671.3474.

5.5.4. 4-[(4*R*)-Benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-4-piperidinylacetic acid (12c). Colorless amorphous solid (yield, 86%). ^{1}H NMR (DMSO-d_6) δ 0.98–1.35 (3H, m), 1.64–2.57 (10H, m), 3.04–4.88 (14H, m), 6.77–6.79 (1H, m), 6.92–6.95 (2H, m), 7.11–7.17 (2H, m), 7.25–7.35 (5H, m), 7.78–7.80 (1H, m), 8.00–8.03 (1H, m), 8.46 (1H, s), 8.55 (1H, s); MS (FAB), m/z 643 (M^++1); Anal. Calcd for $C_{36}H_{42}N_4O_7{\cdot}2.5H_2O$: C, 62.87; H, 6.89; N, 8.15. Found: C, 62.26; H, 6.29; N, 7.74; IR (KBr) 3338, 3060, 3006, 2933, 1724, 1624, 1599, 1533 cm^{-1}.

5.6. 4-[1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(3*R*,4*S*)-isopropylidenedioxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11d)

5.6.1. Ethyl 4-[*N*-benzyloxycarbonyl-(3*R*,4*S*)-isopropylidenedioxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate. Yellow oil (yield, 79%). ^{1}H NMR ($CDCl_3$) δ 1.23–1.30 (3H, m), 1.31 (3H, s), 1.43 and 1.46 (total 3H, each s, amide isomers), 2.24–2.70 (4H, m), 3.12 and 3.23 (total 2H, each s, amide isomers), 3.48–3.84 (5H, m), 3.94 and 3.99 (total 1H, each d, each J = 2.2 Hz, amide isomers), 4.19 (2H, q, J = 7.1 Hz), 4.61 and 4.67 (total each 1H, d, each J = 5.9 Hz, amide isomers), 4.74 and 4.90 (total 1H, each s, amide isomers), 4.81 (1H, m, 1H), 5.04 and 5.10 (total 1H, each d, each J = 12.5 Hz, amide isomers), 5.16 and 5.19 (total 1H, each d, each J = 12.5 Hz, amide isomers), 7.25–7.35 (5H, m); MS (FAB) m/z 476 (M^++1).

5.6.2. Ethyl 4-[(3*R*,4*S*)-isopropylidenedioxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (6d). A suspension of ethyl 4-[*N*-benzyloxycarbonyl-(3*R*,4*S*)-isopropylidenedioxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (1.53 g, 3.22 mmol) and 5% Pd/C (52.2% wet, 1.23 g) in EtOH (30 ml) was hydrogenated under 1 atom hydrogen atmosphere at room temperature for 10 h. After the catalyst was removed by filtration, the filtrate was concentrated to dryness to afford the title compound (**6d**) (0.99 g, 90%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.3 Hz), 1.33 (3H, s), 1.49 (3H, s), 2.00 (1H, s), 2.53-2.64 (4H, m), 3.00 (1H, dd, J = 13.0, 3.9 Hz), 3.05 (1H,

dd, J = 13.0, 1.2 Hz), 3.24 (2H, s), 3.60–3.74 (4H, m), 3.96 (1H, s), 4.19 (2H, q, J = 7.3 Hz), 4.77 (1H, m), 4.87 (1H, dd, J = 5.6, 1.2 Hz); MS (FAB) m/z 342 (M^++1).

5.6.3. Ethyl 4-[1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(3*R*,4*S*)-isopropylidenedioxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acetate (9d). Yellow amorphous solid (yield, 92%). ^{1}H NMR (CD_3OD) δ 1.27 (3H, t, J = 7.1 Hz), 1.29 (3H, s), 1.38 (3H, s), 2.27 (3H, s), 2.45–2.49 (1H, m), 2.56–2.70 (3H, m), 3.20 (2H, s), 3.54 (1H, ddd, J = 13.2, 7.6, 3.2 Hz), 3.61–3.86 (10H, m), 4.18 (2H, q, J = 7.1 Hz), 4.63 (1H, d, J = 6.4 Hz), 4.80–4.84 (1H, m), 5.11 (1H, s), 6.41 (1H, s), 6.76 (1H, d, J = 1.7 Hz), 6.80 (1H, dd, J = 8.3, 1.7 Hz), 7.10–7.15 (2H, m), 7.21–7.24 (2H, m), 7.52 (1H, d, J = 7.6 Hz), 8.05 (1H, d, J = 8.3 Hz); MS (FAB) m/z 638 (M^++1) ; HR-MS (FAB) calcd for $C_{33}H_{44}N_5O_8$: 638.3190. Found: 638.3200; IR (KBr) 3351, 2985, 2937, 1743, 1708, 1631, 1533, 1455 cm^{-1}.

5.6.4. 4-[1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(3*R*,4*S*)-isopropylidenedioxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (9d). Colorless amorphous solid (yield, 37%). ^{1}H NMR (CD_3OD) δ 1.40 (6H, s), 2.13–2.21 (2H, m), 2.89 (3H, s), 2.95–3.20 (4H, m), 3.52 (2H, s), 3.57–3.78 (4H, m), 3.89 and 3.91 (total 3H, each s, amide isomers), 4.00–4.03 (2H, m), 4.74 (1H, m), 4.92 (1H, m), 5.04 (1H, s), 6.75 (1H, m), 6.81–6.93 (2H, m), 7.02 (1H, t, J = 7.8 Hz), 7.14–7.20 (2H, m), 7.56 (1H, m), 8.00 and 8.02 (total 1H, each s, amide isomers), 8.32 and 8.35 (total 1H, each s, amide isomers); MS (FAB) m/z 610 (M^++1); HR-MS (FAB) calcd for $C_{31}H_{40}N_5O_8$: 610.2877. Found: 610.2841; IR (ATR) 3329, 2989, 2937, 2860, 1624, 1531, 1485 cm^{-1}.

5.7. 4-[1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(4*S*)-phenoxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11e)

5.7.1. Ethyl 4-[1-*tert*-butoxycarbonyl-(4*S*)-phenoxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate. Colorless oil (yield, 69%). ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, t, J = 7.1 Hz), 1.42 and 1.47 (total 9H, each s, amide isomers), 2.13 (1H, dd, J = 11.0, 3.9 Hz), 2.40 (1H, s), 2.56 (4H, m), 3.15 (1H, s), 3.20 (1H, s), 3.49 (1H, s), 3.66 (4H, m), 3.96 (1H, m), 4.17 (2H, ABq, J = 1.9 Hz), 4.58–4.89 (2H, m), 6.84 (2H, d, J = 8.1 Hz), 6.96 (1H, t, J = 6.6 Hz), 7.27 (2H, s); MS (ESI) m/z 462 (M^++1).

5.7.2. 4-[(4*S*)-Phenoxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (6e). Yellow amorphous solid (yield, 56%). ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, t, J = 6.8 H), 2.31 (1H, d, J = 13.7 Hz), 2.79 (1H, m), 3.02–3.23 (4H, m), 3.52–3.73 (6H, m), 3.79 (1H, d, J = 11.9 Hz), 4.18 (3H, m), 5.05 (1H, dd, J = 10.5, 3.4 Hz), 5.09 (1H, s), 6.80 (2H, d, J = 8.0 Hz), 7.04 (1H, t, J = 7.6 Hz), 7.30 (2H, t, J = 7.8 Hz); MS (ESI) m/z 362 (M^++1).

5.7.3. Ethyl 4-[1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(4*S*)-phenoxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (9e). Colorless amorphous solid (yield, 86%). ^{1}H NMR ($CDCl_3$) δ 1.26 (3H, t, J = 4.4 Hz), 2.15 (1H, m), 2.29 (3H, s), 2.30–2.81 (8H, m), 2.95–4.30 (12H, m), 4.84–5.05 (2H, m), 6.33 (1H, m), 6.72–7.52 (6H, m), 7.11–7.52 (6H, m), 8.12 (1H, d, J = 7.8 Hz); MS (FAB), m/z 658 (M^++1); Anal. Calcd for $C_{36}H_{43}N_5O_7 \cdot 1.25H_2O$: C, 63.56; H, 6.74; N, 10.29. Found: C, 63.61; H, 6.64; N, 10.29; IR (ATR) 3340, 2983, 2937, 1732, 1701, 1626, 1597, 1587 cm^{-1}.

5.7.4. 4-[1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(4*S*)-phenoxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11e). Colorless amorphous solid (yield, quant.). ^{1}H NMR (CD_3OD) δ 2.10–2.83 (7H, m), 2.92 and 3.02 (total 1H, each s, amide isomers), 3.46–3.88 (10H, m), 3.91 (3H, s), 4.97–5.09 (2H, m), 6.74–7.04 (9H, m), 7.14–7.28 (3H, m), 7.38 and 7.57 (total 1H, each d, each J = 7.8 Hz, amide isomers), 7.95–8.05 (1H, m); MS (FAB), m/z 630 (M^++1); HR-MS (FAB) calcd for $C_{34}H_{40}N_5O_7$: 630.2916. Found: 630.2928; IR (ATR) 3328, 3012, 2941, 1626, 1599, 1529, 1487 cm^{-1}.

5.8. 4-[(2*S*,4*S*)-1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-4-(2-naphthyloxy)-2-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11f)

5.8.1. Ethyl 4-[1-*tert*-butoxycarbonyl-(4*S*)-(2-naphthyloxy)-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate. Yellow oil (yield, 99%). ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, q, J = 7.1 Hz), 1.43 and 1.48 (total 9H, each s, amide isomers), 2.21 (1H, m), 2.36 (1H, m), 2.43–2.80 (4H, m), 3.08 (1H, s), 3.16 (1H, s), 3.42–4.10 (6H, m), 4.18 (2H, t, J = 7.1 Hz), 4.57–5.13 (2H, m), 7.11 (2H, m), 7.37 (1H, t, J = 7.6 Hz), 7.44 (1H, t, J = 7.1 Hz), 7.73 (3H, m); MS (ESI), m/z 512 (M^++1).

5.8.2. 4-[(4*S*)-(2-Naphthyloxy)-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (6f). Yellow oil (yield, 94%). ^{1}H NMR ($CDCl_3$) δ 1.29 (3H, t, J = 7.1 Hz), 2.03 (1H, m), 2.53 (4H, m), 3.01 (1H, dd, J = 13.0, 4.2 Hz), 3.17 (2H, s), 3.42–3.82 (6H, m), 3.96 (1H, dd, J = 9.3, 6.4 Hz), 4.16 (2H, q, J = 9.3, 6.4 Hz), 4.98 (1H, s), 7.06 (1H, d, J = 2.4 Hz), 7.09 (1H, dd, J = 8.8, 2.4 Hz), 7.33 (1H, dt, J = 8.1, 1.2 Hz), 7.42 (1H, dt, J = 8.1, 1.2 Hz), 7.73 (3H, m); MS (ESI), m/z 412 (M^++1).

5.8.3. Ethyl 4-[1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(4*S*)-(2-naphthyloxy)-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate HCl salt (9f). Colorless amorphous solid (yield, 50%). ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, t, J = 6.9 Hz), 2.19 (1H, s), 2.29 (3H, s), 2.30–2.70 (8H, m), 3.11 (2H, s), 3.32–3.98 (8H, m), 4.14 (2H, q, J = 6.9 Hz), 4.99 (2H, m), 6.33 (1H, m), 6.82 (1H, m), 7.00–7.28 (5H, m), 7.35 (1H, t, J = 7.1 Hz), 7.44 (1H, t, J = 7.1 Hz), 7.51 (1H, d, J = 8.1 Hz), 7.67–7.76 (3H, m), 8.06 (1H, d, J = 8.3 Hz); MS (FAB), m/z 708 (M^++1); Anal. Calcd for $C_{40}H_{45}N_5O_7 \cdot HCl \cdot 2.5H_2O$: C, 60.87; H, 6.51; N, 8.87. Found: C, 60.81; H, 6.40; N, 8.79; IR (KBr) 3253, 2978, 1747, 1670, 1628, 1533, 1241 cm^{-1}.

5.8.4. 4-[1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(4*S*)-(2-naphthyloxy)-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11f). Colorless amorphous solid (yield, 80%). ^{1}H NMR (DMSO-d_6) δ 1.74 (1H, s), 1.90 (1H, m), 2.00–3.70 (14H, m), 2.49 (2H, s), 3.85 (3H, s), 3.98–5.20 (2H, m), 6.65–7.85 (14H, m), 8.46 (1H, s), 8.55 (1H, s); MS (FAB), *m*/*z* 680 (M^++1); Anal. Calcd for $C_{38}H_{41}N_5O_7$·EtOH·H_2O: C, 64.59; H, 6.64; N, 9.41. Found: C, 64.34; H, 6.37; N, 9.31; IR (KBr) 3346, 2939, 1628, 1533, 1454, 1257 cm^{-1}.

5.9. 4-[(3*S*)-Benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11g)

5.9.1. Ethyl 4-[(3*S*)-benzyloxy-*N*-*tert*-butoxycarbonyl-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate. Yellow oil (yield, 95%). ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.1 Hz), 1.39 and 1.46 (total 9H, each s, amide isomers), 2.03–2.07 (1H, m), 2.30–3.34 (1H, m), 2.42–2.68 (4H, m), 3.17 (2H, s), 3.41–3.51 (2H, m), 3.53–3.73 (4H, m), 3.97 (1H, m), 4.19 (2H, q, J = 7.1 Hz), 4.41 and 4.43 (total 1H, each d, each J = 11.9 Hz, amide isomers), 4.61 and 4.63 (total 1H, each d, each J = 11.9 Hz, amide isomers), 4.53 and 4.70 (total 1H, each s, amide isomers), 7.23–7.37 (5H, m); MS (FAB) *m*/*z* 476 (M^++1).

5.9.2. Ethyl 4-[(3*S*)-benzyloxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (6g). Yellow oil (yield, 100%). ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.1 Hz), 1.70–1.74 (1H, m), 1.97–2.01 (1H, m), 2.38 (1H, ddd, J = 14.7, 6.8, 3.4 Hz), 2.46 (1H, ddd, J = 14.7, 6.8, 3.4 Hz), 2.49–2.65 (2H, m), 3.02 (1H, ddd, J = 11.2, 9.3, 6.1 Hz), 3.18 (2H, s), 3.23 (1H, m), 3.52 (1H, ddd, J = 13.2, 6.8, 3.4 Hz), 3.60 (1H, ddd, J = 13.2, 6.8, 3.4 Hz), 3.67–3.76 (2H, m), 3.91 (1H, d, J = 3.5 Hz), 4.03 (1H, m), 4.19 (2H, q, J = 7.1 Hz), 4.38 (1H, d, J = 11.5 Hz), 4.60 (1H, d, J = 11.5 Hz), 7.20–7.38 (5H, m); MS (FAB) *m*/*z* 376 (M^++1).

5.9.3. Ethyl 4-[(3*S*)-benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (9g). Yellow amorphous solid (yield, 81%). ^{1}H NMR ($CDCl_3$) δ 1.26 (t, J = 7.3 Hz, 3H), 1.96 (m, 1H), 2.12 (m, 1H), 2.22 (s, 3H), 2.34–2.64 (4H, m), 3.10 (2H, s), 3.47–3.57 (4H, m), 3.60 (2H, s), 3.64 (3H, s), 3.69–3.79 (2H, m), 3.95 (1H, m), 4.17 (2H, q, J = 7.3 Hz), 4.42 (1H, d, J = 12.0 Hz), 4.56 (1H, d, J = 12.0 Hz), 4.92 (1H, s), 6.71–7.00 (2H, m), 7.02–7.06 (2H, m), 7.11–7.35 (7H, m), 7.47 (1H, s), 7.61 (1H, d, J = 7.6 Hz), 8.04 (1H, d, J = 8.3 Hz); MS (FAB) *m*/*z* 672 (M^++1); HR-MS (FAB) calcd for $C_{37}H_{46}N_5O_7$: 672.3397. Found: 672.3386.

5.9.4. 4-[(3*S*)-Benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11g). Yellow amorphous solid (yield, 76%). ^{1}H NMR (DMSO-d_6) δ 1.81–2.09 (m, 2H), 2.25 (s, 3H), 2.35–2.51 (m, 4H), 3.13 (s, 2H), 3.17–3.52 (4H, m), 3.63 (2H, s), 3.66–3.99 (2H, m), 3.86 (3H, s), 3.98 and 4.10 (total 1H, each d, each J = 2.7 Hz, amide isomers), 4.54 (1H, d, J = 11.9 Hz), 4.58 (1H, d, J = 11,9 Hz), 4.83 and 5.01 (total 1H, each s, amide isomers), 6.67 and 6.76 (total 1H, each dd, each J = 8.1, 1.4 Hz, amide isomers), 6.80 and 6.92 (total 1H, each d, each J = 1.4 Hz, amide isomers), 6.93 (1H, t, J = 7.6 Hz), 7.11–7.17 (2H, m), 7.27–7.38 (5H, m), 7.79 (1H, d, J = 7.6 Hz), 8.01 (1H, d, J = 8.1 Hz), 8.46 (1H, s), 8.55 (1H, s); MS (FAB) *m*/*z* 644 (M^++1); Anal. Calcd for $C_{35}H_{41}N_5O_7$·0.5H_2O: C, 62.86; H, 6.33; N, 10.47. Found C, 62.69; H, 6.68; N, 10.58; IR (KBr) 3353, 2938, 1633, 1533, 1454, 1415 cm^{-1}.

5.10. 4-[(4*S*)-Fluoro-1-[3-methoxy-4-[*N*′-(2-methylphenyl)-ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11h)

5.10.1. Ethyl 4-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate. Pale yellow oil (yield, 61%). ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.8 Hz), 1.42 and 1.47 (total 9H, each s, amide isomers), 2.11–2.82 (6H, m), 3.22–3.25 (2H, m), 3.42–3.98 (6H, m), 4.19 (2H, q, J = 7.1 Hz), 4.61 and 4.74 (total 1H, each d, J = 9.3 and 9.6 Hz, respectively, amide isomers), 5.21 (1H, d, J = 53.9 Hz). MS (ESI) *m*/*z*, 388 (M^++1).

5.10.2. Ethyl 4-[(4*S*)-fluoro-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (6h). Yellow oil (yield, 86%). ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.1 Hz), 1.96–2.96 (6H, m), 3.25 (2H, s), 3.46–3.92 (7H, m), 4.19 (2H, q, J = 7.1 Hz), 5.10–5.25 (1H, m); MS (ESI) *m*/*z*, 288 (M^++1).

5.10.3. Ethyl 4-[(4*S*)-fluoro-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (9h). Yellow amorphous solid (yield, 88%). ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, t, J = 7.1 Hz), 2.15–2.71 (9H, m), 3.21 and 3.23 (total 3H, each s, amide isomers), 3.40–4.09 (10H, m), 4.15–4.21 (2H, m), 4.98 (1H, dd, J = 9.6, 2.5 Hz), 5.16–5.29 (1H, m), 6.48–7.27 (7H, m), 7.52–7.54 (1H, m), 8.04-8.05 (1H, m); MS (FAB) *m*/*z*, 584 (M^++1); HR-MS (FAB) calcd for $C_{30}H_{39}FN_5O_6$: 584.2884. Found: 584.2866; IR (ATR) 3344, 2978, 2937, 2829, 1736, 1701, 1630, 1589 cm^{-1}.

5.10.4. 4-[(4*S*)-Fluoro-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11h). Colorless amorphous solid (yield, 33%). ^{1}H NMR (DMSO-d_6) δ 1.96–3.88 (22H, m), 4.91 and 5.07 (total 1H, each d, each J = 8.8 Hz, amide isomers), 5.18–5.38 (1H, m), 6.67–7.17 (5H, m), 7.78 (1H, d, J = 8.1 Hz), 8.00 (1H, d, J = 8.1 Hz), 8.49 (1H, s), 8.57 (1H, s); MS (FAB) *m*/*z*, 556 (M^++1); HR-MS (FAB) calcd for $C_{28}H_{35}FN_5O_6$: 556.2571. Found: 556.2609; IR (ATR) 3330, 2943, 2873, 2810, 1628, 1587, 1529, 1485, 1448 cm^{-1}.

5.11. 4-[(4*R*)-Fluoro-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11i)

5.11.1. Ethyl 4-[1-(*tert*-butoxycarbonyl)-(4*R*)-fluoro-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate. Colorless oil (yield, 66%). ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t,

J = 7.1 Hz), 1.42 and 1.46 (total 9H, each s, amide isomers), 2.02–2.73 (6H, m), 3.25 (2H, d, J = 5.6 Hz), 3.58–3.98 (6H, m), 4.19 (2H, q, J = 7.1 Hz), 4.75 and 4.87 (total 1H, each t, J = 8.2 and 7.8 Hz, respectively, amide isomers), 5.15–5.31 (1H, m); MS (ESI) m/z 388 (M^++1).

5.11.2. Ethyl 4-[(4*R*)-fluoro-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (6i). Yellow oil (yield, 77%). ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.1 Hz), 1.83–1.99 (1H, m), 2.26–2.37 (1H, m), 2.54–2.64 (4H, m), 3.13–3.77 (8H, m), 4.13–4.22 (3H, m), 5.27 (1H, dt, J = 54.9, 4.2 Hz); MS (ESI) m/z 288 (M^++1).

5.11.3. Ethyl 4-[(4*R*)-fluoro-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (9i). Colorless amorphous solid (yield, 97%). ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, t, J = 7.1 Hz), 2.05–2.22 (1H, m), 2.28 (3H, s), 2.36–2.70 (5H, m), 3.21 (2H, s), 3.53–3.90 (11H, m), 4.18 (2H, q, J = 7.1 Hz), 5.05 (1H, t, J = 8.1 Hz), 5.20–5.33 (1H, m), 6.43 (1H, s), 6.79–6.80 (2H, m), 7.11–7.26 (4H, m), 7.52–7.54 (1H, m), 8.06 (1H, d, J = 8.8 Hz); MS (FAB) m/z 584 (M^++1); HR-MS (FAB) calcd for $C_{30}H_{39}FN_5O_6$: 584.2884. Found: 584.2870. Anal. Calcd for $C_{30}H_{38}FN_5O_6 \cdot 1.75H_2O$: C, 58.57; H, 6.80; N, 11.38. Found: C, 58.60; H, 6.41; N, 11.26; IR (ATR) 3346, 2981, 2937, 1739, 1703, 1626, 1589, 1529 cm^{-1}.

5.11.4. 4-[(4*R*)-Fluoro-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11i). Colorless amorphous solid (yield, 39%). ^{1}H NMR (DMSO-d_6) δ 1.91–2.03 (1H, m), 2.24 (3H, s), 2.40–2.60 (5H, m), 2.98 (2H, s), 3.24–3.67 (7H, m), 3.85–4.01 (4H, m), 4.91–5.40 (2H, m), 6.65–7.26 (5H, m), 7.77–7.79 (1H, m), 7.98–8.01 (1H, m), 8.50 (1H, s), 8.58–8.59 (1H, m); MS (FAB) m/z, 556 (M^++1); HR-MS (FAB) calcd for $C_{28}H_{34}FN_5O_6$: 556.2571. Found: 556.2543; IR (KBr) 3338, 2931, 1635, 1600, 1531, 1495, 1487 cm^{-1}.

5.12. 4-[4,4-Difluoro-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11j)

5.12.1. Ethyl 4-[1-(*tert*-butoxycarbonyl)-4,4-difluoro-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate. Yellow oil (yield, 25%). ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.1 Hz), 1.41–1.47 (9H, m), 2.36–2.66 (6H, m), 3.24–3.25 (2H, m), 3.51–3.95 (6H, m), 4.19 (2H, q, J = 7.1 Hz), 4.71–4.88 (1H, m); MS (ESI) m/z, 406 (M^++1).

5.12.2. Ethyl 4-[4,4-difluoro-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (11j). Yellow oil (yield, 45%). ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.1 Hz), 2.24–3.77 (15H, m), 4.12–4.23 (3H, m); MS (ESI), m/z 306 (M^++1).

5.12.3. Ethyl 4-[4,4-difluoro-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (9j). Yellow oil (yield, quant.). ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, t, J = 7.1 Hz), 2.30 (3H, s), 2.51–2.68 (4H, m), 3.23 (2H, s), 3.55–3.92 (13H, m), 4.18 (2H, q, J = 7.1 Hz), 5.05 (1H, dd, J = 8.8, 6.6 Hz), 6.28 (1H, s), 6.78 (1H, s), 6.79 (1H, d, J = 7.8 Hz), 7.11–7.30 (4H, m), 7.50 (1H, d, J = 7.8 Hz), 8.08 (1H, d, J = 7.8 Hz). MS (FAB) m/z 602 (M^++1); HR-MS (FAB) calcd for $C_{30}H_{38}F_2N_5O_6$: 602.2790. Found: 602.2799.

5.12.4. 4-[4,4-Difluoro-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11j). Colorless amorphous solid (yield, 86%). ^{1}H NMR (DMSO-d_6) δ 2.26 (3H, s), 2.30–4.25 (19H, m), 5.09–5.38 (1H, m), 6.75–7.17 (5H, m), 7.75–7.77 (1H, m), 7.99–8.01 (1H, m), 8.66–8.70 (2H, m); MS (FAB) m/z, 574 (M^++1); HR-MS (FAB) calcd for $C_{28}H_{34}F_2N_5O_6$: 574.2477. Found: 574.2488; IR (KBr) 3400, 3392, 2952, 1641, 1539, 1485, 1454, 1417 cm^{-1}.

5.13. 4-[4-Chloro-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (9k)

5.13.1. Ethyl 4-[1-(*tert*-butoxycarbonyl)-(4*S*)-chloro-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate. Colorless oil (yield, 99%). ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.1 Hz), 1.40 and 1.46 (total 9H, each s, amide isomers), 2.04–2.13 (1H, m), 2.58–2.78 (5H, m), 3.23–3.24 (2H, m), 3.51–3.73 (5H, m), 4.04–4.25 (4H, m), 4.52–4.68 (1H, m); MS (FAB), m/z 404 (M^++1).

5.13.2. Ethyl 4-[(4*S*)-chloro-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (6k). Pale yellow oil (yield, 90%). ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.1 Hz), 1.89 (1H, s), 2.01–2.08 (1H, m), 2.54–2.62 (5H, m), 3.09 (1H, dd, J = 4.4, 12.7 Hz), 3.25 (2H, s), 3.34 (1H, d, J = 12.7 Hz), 3.41–3.53 (2H, m), 3.69–3.78 (2H, m), 3.89–3.94 (1H, m), 4.19 (2H, q, J = 7.1 Hz), 4.41–4.43 (1H, m); MS (FAB), m/z 304 (M^++1).

5.13.3. Ethyl 4-[(4*S*)-chloro-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (9k). Colorless amorphous solid (yield, 91%). ^{1}H NMR ($CDCl_3$) δ 1.25–1.29 (3H, m), 2.04–2.12 (1H, m), 2.25 (3H, s), 2.55–2.75 (5H, m), 3.21 (2H, s), 3.51–3.80 (11H, m), 4.02–4.07 (1H, m), 4.15–4.21 (2H, m), 4.81–4.85 (1H, m), 6.74–6.83 (3H, m), 7.08–7.34 (4H, m), 7.54–7.56 (1H, m), 8.05–8.07 (1H, m); MS (FAB), m/z 600 (M^++1); HR-MS (FAB) calcd for $C_{30}H_{39}ClN_5O_6$: 600.2589. Found: 600.2591; IR (KBr) 3346, 2979, 2937, 2862, 1743, 1703, 1635, 1599 cm^{-1}.

5.13.4. 4-[(4*S*)-Chloro-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11k). Colorless amorphous solid (yield, 97%). ^{1}H NMR (DMSO-d_6) δ 2.29 (3H, s), 2.39–2.66 (4H, m), 2.80–3.72 (12H, m), 3.85 and 3.88 (total 3H, each s, amide isomers), 4.09–4.58 (2H, m), 4.78–5.06 (1H, m), 6.66 and 6.76 (total 1H, each d, each J = 8.0 Hz, amide isomers), 6.90–7.23 (4H, m), 7.77 (1H, d, J = 9.6 Hz), 8.02 (1H, d, J = 8.0 Hz), 8.45 (1H, s), 8.54 (1H, s); MS (FAB), m/z 572 (M^++1); HR-MS (FAB) calcd for $C_{28}H_{35}ClN_5O_6$: 572.2276. Found: 572.2281; IR (KBr) 3346, 3022, 2939, 1641, 1600, 1589, 1531, 1495 cm^{-1}.

5.14. (±)-4-[2-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-1-isoindolinylcarbonyl]-1-piperazinylacetic acid (11l)

5.14.1. (±)-Ethyl 4-[2-(9-fluorenylmethoxycarbonyl)-1-isoindolinylcarbony]-1-piperazinylacetate. A mixture of (±)-2-(9-fluorenylmethoxycarbonyl)-1-isoindolinecarboxylic acid (958 mg, 2.49 mmol), ethyl 1-piperazinylacetate (**4**) (428 mg, 2.49 mmol), EDC·HCl (572 mg, 2.98 mmol), HOBt (403 mg, 2.98 mmol), and Et_3N (410 ml, 2.94 mmol) in CH_2Cl_2 (10 ml) was stirred at room temperature for 12 h. The mixture was poured into water and extracted with $CHCl_3$. The combined extracts were washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with $CHCl_3$/MeOH (100:1, v/v) as an eluent to give the title compound (1.07 g, 80%) as a colorless amorphous solid. ^{1}H NMR ($CDCl_3$) δ 1.22–1.30 (3H, m), 2.51–2.80 (4H, m), 3.15 and 3.27 (total 2H, each s, amide isomers), 3.59–3.74 (2H, m), 3.88–4.00 (2H, m), 4.11–4.59 (5H, m), 4.83–5.06 (2H, m), 5.88 and 6.01 (total 1H, each d, each J = 2.2 Hz, amide isomers), 6.99–7.78 (12H, m); FAB-MS, *m*/*z* 540 (M^++1).

5.14.2. (±)-Ethyl 4-[2-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-1-isoindolinylcarbonyl]-1-piperazinylacetate (9l). To a stirred solution of (±)-ethyl 4-[2-(9-fluorenylmethoxycarbonyl)-1-isoindolinylcarbony]-1-piperazinylacetate (410 mg, 0.77 mmol) in DMF (8 ml) was added TBAF (1 M solution in THF, 0.8 ml, 0.80 mmol), and the reaction mixture was stirred at room temperature for 1 h. To the reaction mixture were added 3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetic acid (239 mg, 0.76 mmol), EDC·HCl (175 mg, 0.91 mmol), HOBt (123 mg, 0.91 mmol), and Et_3N (160 μl, 1.14 mmol), and the reaction mixture was stirred at room temperature for 12 h. The mixture was poured into water and extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography on silica gel with $CHCl_3$/MeOH (100:1 to 30:1, v/v) as an eluent to give the title compound **9l** [392 mg, 84% (2 steps)] as a pale yellow amorphous solid. ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.1 Hz), 2.19 (3H, s), 2.49–2.77 (4H, m), 3.22 (2H, s), 3.50–3.53 (2H, m), 3.62 (3H, s), 3.73 (2H, s), 3.80–3.84 (2H, m), 4.01–4.15 (1H, m), 4.18 (2H, q, J = 7.1 Hz), 4.81–4.84 (1H, m), 4.99–5.02 (1H, m), 6.12 (1H, s), 6.80 (1H, s), 6.82–6.84 (1H, m), 7.05–7.39 (8H, m), 7.56–7.58 (1H, m), 8.07–8.09 (1H, m); MS (FAB), *m*/*z* 614 (M^++1); Anal. Calcd for $C_{34}H_{39}N_5O_6 \cdot 1.25H_2O$: C, 64.19; H, 6.57; N, 11.01. Found: C, 64.27; H, 6.44; N, 10.76.

5.14.3. (±)-4-[2-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-1-isoindolinylcarbonyl]-1-piperazinylacetic acid (11l). Prepared from **9l** according to the basic hydrolysis steps of general procedure A, the title compound **11l** was obtained as a colorless crystalline powder (48%). Mp 167–171 °C; ^{1}H NMR (DMSO-d_6) δ 2.42 (3H, s), 2.50–2.75 (4H, m), 3.19 (2H, s), 3.22–4.10 (10H, m), 4.71–4.99 (2H, m), 6.17 (1H, s), 6.80–7.41 (8H, m), 7.78—7.80 (1H, m), 8.02–8.04 (1H, m), 8.48 (1H, s), 8.57 (1H, s); MS (FAB), *m*/*z* 586 (M^++1); Anal. Calcd for $C_{32}H_{35}N_5O_6 \cdot 4.5H_2O$: C, 57.65; H, 6.65; N, 10.50. Found: C, 57.84; H, 6.44; N, 10.39; IR (KBr) 3354, 3016, 2931, 1705, 1633, 1589, 1531, 1486 cm^{-1}.

5.15. 4-[1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-2,3-dihydro-1H-(2*S*)-indolylcarbonyl]-1-piperazinylacetic acid (11m)

5.15.1. Ethyl [1-*tert*-butoxycarbonyl-2,3-dihydro-1H-(2*S*)-indolylcarbonyl]-1-piperazinylacetate. Yellow oil. (yield, 54%). ^{1}H NMR ($CDCl_3$) δ 1.23–1.31 (4H, m), 1.43–1.66 (8H, m), 2.04 (1H, s), 2.53–2.89 (4H, m), 2.91–3.03 (1H, m), 3.26 (2H, s), 3.70–3.76 (5H, m), 4.12 and 4.20 (total 2H, each q, J = 7.4 and 7.1 Hz, respectively, amide isomers), 5.01–5.29 (1H, m), 6.91 (1H, t, J = 7.5 Hz), 7.08 (1H, d, J = 7.4 Hz), 7.18 (1H, s); MS (FAB) *m*/*z* 417 (M^++1).

5.15.2. Ethyl [2,3-Dihydro-1H-(2*S*)-indolylcarbonyl]-1-piperazinylacetate (6m). Brown oil (yield, quant.). ^{1}H NMR ($CDCl_3$) δ 1.29 (3H, t, J = 7.1 Hz), 2.53–2.72 (4H, m), 3.14 (1H, dd, J = 15.7, 5.4 Hz), 3.27 (2H, s), 3.42–3.73 (5H, m), 4.20 (2H, q, J = 7.1 Hz), 4.54 (1H, dd, J = 10.5, 5.6 Hz), 4.62 (1H, s), 6.72–6.80 (2H, m), 7.04 (2H, d, J = 7.6 Hz).; MS (FAB) *m*/*z* 317 (M^++1).

5.15.3. Ethyl 4-[1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-2,3-dihydro-1H-(2*S*)-indolylcarbonyl]-1-piperazinylacetate (9m). Colorless amorphous solid (yield, 85%). ^{1}H NMR ($CDCl_3$) δ 1.24–1.32 (5H, m), 2.30 and 2.31 (total 3H, each s, amide isomers), 2.53–2.80 (5H, m), 2.88–3.85 (14H, m), 3.97–4.25 (3H, m), 5.12 and 5.54 (total 1H, each d, J = 8.1 and 7.6 Hz, respectively, amide isomers), 6.18 (1H, s), 6.66–7.30 (4H, m), 7.46–7.53 (1H, m), 8.09 (1H, t, J = 9.3 Hz), 8.31 (1H, d, J = 7.8 Hz); MS (FAB) *m*/*z* 614 (M^++1); Anal. Calcd for $C_{34}H_{39}N_5O_6 \cdot 0.75H_2O$: C, 65.11; H, 6.51; N, 11.17. Found: C, 65.18; H, 6.61; N, 11.06; IR (ATR) 3351, 2979, 2935, 2823, 1732, 1655, 1589, 1527, 1481 cm^{-1}.

5.15.4. 4-[1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-2,3-dihydro-1H-(2*S*)-indolylcarbonyl]-1-piperazinylacetic acid (11m). Colorless amorphous solid (yield, 84%). ^{1}H NMR (DMSO-d_6) δ 2.42–2.76 (6H, m), 3.85–2.93 (10H, m), 3.86 (3H, s), 4.61 (1H, q, J = 5.5 Hz), 5.63 (1H, dd, J = 11.3, 2.9 Hz), 6.54 (1H, dd, J = 13.6, 7.2 Hz), 6.69–7.27 (5H, m), 7.59 (1H, d, J = 7.8 Hz), 8.02 and 8.09 (total 1H, each d, each J = 8.1 Hz, amide isomers), 8.49 (1H, s), 8.59 (1H, s); MS (FAB) *m*/*z* 586 (M^++1); HR-MS (FAB) calcd for $C_{32}H_{35}N_5O_6$: 586.2666. Found: 86.2652; IR (ATR) 3342, 3016, 1631, 1601, 1529, 1481 cm^{-1}.

5.16. 4-[1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-octahydroindolylcarbonyl]-1-piperazinylacetic acid (11n)

5.16.1. Ethyl 4-[1-(*tert*-butoxycarbonyl)-(2*S*)-octahydroindolylcarbonyl]-1-piperazinylacetate. Colorless oil (yield, 99%). ^{1}H NMR ($CDCl_3$) δ 1.12–1.30 (6H, m), 1.38 and

1.44 (total 9H, each s, amide isomers), 1.55–1.66 (7H, m), 1.91–1.98 (2H, m), 2.12–2.30 (1H, m), 2.52–2.69 (3H, m), 3.23–3.24 (2H, m), 3.56–3.61 (2H, m), 3.74–3.86 (2H, m), 4.19 (2H, q, J = 7.1 Hz), 4.50–4.62 (1H, m); ESI, m/z 424 (M^++1).

5.16.2. Ethyl 4-[(2*S*)-octahydroindolylcarbonyl]-1-piperazinylacetate (6n). Pale yellow oil (yield, 86%). ^{1}H NMR ($CDCl_3$) δ 1.20-1.30 (5H, m), 1.39–2.02 (9H, m), 2.12–2.19 (1H, m), 2.53–2.63 (4H, m), 3.07–3.11 (1H, m), 3.24 (2H, s), 3.43–3.54 (2H, m), 3.66–3.77 (2H, m), 3.91–3.94 (1H, m), 4.19 (2H, q, J = 7.1 Hz); ESI, m/z 324 (M^++1).

5.16.3. Ethyl 4-[1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-octahydroindolylcarbonyl]-1-piperazinylacetate (9n). Colorless amorphous solid (yield, 52%). ^{1}H NMR ($CDCl_3$) δ 1.10–1.35 (5H, m), 1.47–1.50 (1H, m), 1.61–2.02 (7H, m), 2.24 and 2.25 (total 3H, each s, amide isomers), 2.32–3.26 (1H, m), 2.51–2.64 (4H, m), 3.19 (2H, s), 3.44–3.69 (9H, m), 3.85–3.91 (1H, m), 4.17 (2H, q, J = 7.1 Hz), 4.78 (1H, t, J = 9.0 Hz), 6.62–6.83 (3H, m), 7.10–7.11 (1H, m), 7.19–7.24 (3H, m), 7.56–7.58 (1H, m), 8.00–8.04 (1H, m); MS (FAB), m/z 620 (M^++1); HR-MS (FAB) calcd for $C_{34}H_{46}N_5O_6$: 620.3448. Found: 620.3433.

5.16.4. 4-[1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-octahydroindolylcarbonyl]-1-piperazinylacetic acid (11n). Colorless amorphous solid (yield, 81%). ^{1}H NMR (DMSO-d_6) δ 1.07–1.27 (3H, m), 1.36–1.40 (1H, m), 1.59–1.86 (6H, m), 2.05–2.30 (4H, m), 2.43–2.63 (4H, m), 3.17 (2H, s), 3.25–3.40 (1H, m), 3.52–3.73 (4H, m), 3.83–4.03 (5H, m), 4.71–4.91 (1H, m), 6.64–6.75 (1H, m), 6.91–6.94 (2H, m), 7.10–7.26 (2H, m), 7.78–7.81 (1H, m), 7.97–8.00 (1H, m), 8.46 (1H, s), 8.55 (1H, s); ESI, m/z 592 (M^++1); HR-MS (FAB) calcd for $C_{32}H_{42}N_5O_6$: 592.3135. Found: 592.3154; IR (ATR) 3346, 3016, 2927, 2854, 1612, 1529, 1485, 1452 cm^{-1}.

5.17. 4-[4-Hydroxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)-ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-pipirazinylacetic acid (11o)

A suspension of 4-[(4*R*)-benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-pipirazinylacetic acid (**11c**) (400 mg, 0.62 mmol) and 20% $Pd(OH)_2$/C (0.40 g) in EtOH (20 ml) was hydrogenated under 1 atom hydrogen atmosphere at room temperature for 3 days. After the catalyst was removed by filtration, the filtrate was concentrated to give the title compound **11o** (200 mg, 58%) as a colorless crystalline powder. ^{1}H NMR (DMSO-d_6) δ 2.25–2.31 (1H, m), 2.40–2.69 (5H, m), 3.05–3.66 (7H, m), 3.71-3.79 (1H, m), 3.81 and 3.84 (total 3H, each s, amide isomers), 4.07–4.29 (1H, m), 4.35–4.49 (2H, m), 4.78–5.08 (1H, m), 6.66 and 6.77 (total 1H, each dd, J = 7.8, 1.7 and 8.1, 1.7 Hz, respectively, amide isomers), 6.79 and 6.91 (total 1H, each d, J = 1.7 and 1.2 Hz, respectively, amide isomers), 6.92–6.96 (2H, m), 7.11 (1H, d, J = 10.0 Hz), 7.15 (1H, d, J = 7.8 Hz), 7.24–7.36 (4H, m), 7.78 (1H, dd, J = 8.1, 1.0 Hz), 8.00 and 8.01 (total 1H, each d, J = 8.3 and 8.1 Hz, respectively, amide isomers), 8.47 (1H, s), 8.54 (1H, s). MS (FAB) m/z 554 (M^++1); HR-MS (FAB) calcd for $C_{28}H_{36}N_5O_7$: 554.2615. Found: 554.2644; IR (KBr) 3319, 3018, 2945, 2843, 1612, 1529, 1483 cm^{-1}.

5.18. 1-[1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-4-hydroxy-(2*S*)-pyrrolidinylcarbonyl]-4-piperidinyl]acetic acid (12o)

By the procedure described for compound **11o** but with 4-[(4*R*)-benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-pipirazinylacetic acid (**12c**) in place of **11c**, the title compound **12o** was obtained as a colorless amorphous solid (65%). ^{1}H NMR (DMSO-d_6) δ 1.66–2.55 (11H, m), 3.01–4.34 (12H, m), 4.83–5.08 (2H, m), 6.74–6.80 (1H, m), 6.89–6.93 (2H, m), 7.09–7.15 (2H, m), 7.76–7.78 (1H, m), 7.97–7.99 (1H, m), 8.44 (1H, s), 8.53 (1H, s); MS (FAB), m/z 553 (M^++1); Anal. Calcd for $C_{29}H_{36}N_4O_7{\cdot}2\ H_2O{\cdot}HCl$: C, 55.72; H, 6.61; N, 8.96. Found: C, 55.70; H, 6.12; N, 8.49; IR (KBr) 3346, 3006, 2937, 1709, 1537, 1485 cm^{-1}.

5.19. Ethyl 4-[4-hydroxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-pipirazinylacetate (9o)

By the procedure described for compound (**11o**), but with ethyl 4-[(4*R*)-benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-pipirazinylacetate in place of **11c**, the title compound **9o** was obtained as a colorless oil (57%). ^{1}H NMR ($CDCl_3$) δ 1.27 (3H, t, J = 7.4 Hz), 1.93–2.18 (2H, m), 2.25 (3H, s), 2.44–2.67 (4H, m), 3.19 (2H, s), 3.44–3.90 (10H, m), 4.17 (2H, q, J = 7.1 Hz), 4.45–4.52 (1H, m), 4.96 (1H, t, J = 7.7 Hz), 6.62–6.83 (3H, m), 7.10 (2H, t, J = 7.6 Hz), 7.20 (2H, t, J = 8.3 Hz), 7.31 (2H, s), 7.95 and 7.99 (total 1H, each d, each J = 8.1 Hz, amide isomers); MS (ESI) m/z 582 (M^++1); HR-MS (FAB) calcd for $C_{30}H_{40}N_5O_7$: 582.2928. Found: 582.2936. Anal. Calcd for $C_{30}H_{39}N_5O_7{\cdot}H_2O$: C, 60.09; H, 6.89; N, 11.68. Found: C, 59.73; H, 6.92; N, 11.37; IR (ATR) 3340, 2983, 2831, 1620, 1529, 1485, 1542 cm^{-1}.

5.20. 4-[(3*S*)-Hydroxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11q)

5.20.1. Ethyl 4-[(3*S*)-hydroxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (9q). By the procedure described for compound **11o**, but with ethyl 4-[(3*S*)-benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (**9g**) in place of **11c**, the title compound **9q** was obtained as a colorless amorphous solid (59%). ^{1}H NMR (CD_3OD) δ 1.26 (3H, t, J = 7.1 Hz), 1.97 (1H, m), 2.15 (1H, m), 2.29 (3H, s), 2.54–2.74 (4H, m), 3.28 (3H, m), 3.47–3.71 (4H, m), 3.73 (2H, s), 3.75–3.81 (2H, m), 3.91 (3H, s), 4.17 (2H, q, J = 7.1 Hz), 4.27–4.33 (total 1H, m), 4.73 and 4.77 (total 1H, each s, amide isomers),

6.73 and 6.81 (total 1H, each dd, each $J = 8.3$, 1.3 Hz, amide isomers), 6.84 and 6.95 (total 1H, each d, each $J = 1.3$ Hz, amide isomers), 7.02 (1H, dt, $J = 7.6$, 1.3 Hz), 7.13–7.20 (2H, m), 7.57 (1H, dd, $J = 8.3$, 1.3 Hz), 7.98 (1H, d, $J = 8.3$ Hz); MS (FAB) *m/z* 582 (M^++1).

5.20.2. 4-[(3*S*)-Hydroxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11q). Prepared from **9q** according to the basic hydrolysis steps of general procedure A, the title compound **11q** was obtained as a yellow amorphous solid (95%). ^{1}H NMR (DMSO-d_6) δ 1.80 (1H, m), 1.96 (1H, m), 2.25 (3H, s), 2.32–2.64 (4H, m), 2.89 (2H, s), 3.27 (1H, m), 3.48–3.71 (7H, m), 3.85 and 3.87 (total 3H, each s, amide isomers), 4.11 and 4.24 (total 1H, each d, each $J = 2.9$ Hz, amide isomers), 4.66 and 4.80 (total 1H, each s, amide isomers), 6.66 and 6.76 (total 1H, each d, each $J = 8.3$ Hz, amide isomers), 6.92 (1H, t, $J = 7.3$ Hz), 6.81 and 6.94 (1H, s), 7.10–7.16 (2H, m), 7.79 (1H, d, $J = 8.3$ Hz), 8.00 (1H, d, $J = 8.3$ Hz), 8.56 (1H, s), 8.61 (1H, s); MS (FAB) *m/z* 554 (M^++1); Anal. Calcd for $C_{28}H_{35}N_5O_7 \cdot 2.5H_2O$: C, 56.18; H, 6.73; N, 11.70. Found: C, 56.13; H, 7.05; N, 11.58; IR (KBr) 3345, 2927, 1627, 1536, 1454, 1415 cm^{-1}.

5.21. 4-[1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(3*R*,4*R*)-dioxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11p)

5.21.1. Ethyl 4-[1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(3*R*,4*R*)-dioxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (9p). A solution of ethyl 4-[1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(3*R*,4*R*)-isopropylidenedioxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acetate (**9d**) (168 mg, 0.26 mmol) in satd HCl (gas)/MeOH (10 ml) was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure. The residue was diluted with sat. $NaHCO_3$ solution and extracted with $CHCl_3$/MeOH (5:1, v/v). The extracts were washed with brine, dried over Na_2SO_4, and concentrated to dryness. The residue was purified by chromatography on silica gel with $CHCl_3$/MeOH (5:1, v/v) as an eluent to give the title compound **9p** (151 mg, 97%) as a yellow amorphous solid. ^{1}H NMR ($CDCl_3$) δ 1.26 (3H, t, $J = 7.1$ Hz), 2.22 (3H, s), 2.39–2.66 (4H, m), 3.10–3.76 (13H, m), 4.03–4.28 (5H, m), 4.58 and 4.72 (total 1H, each d, $J = 3.4$ and 4.4 Hz, respectively, amide isomers), 6.59 and 6.69 (total 1H, each d, $J = 8.3$ and 7.4 Hz, respectively, amide isomers), 6.74 (1H, s), 7.01–7.24 (4H, m), 7.46–7.61 (2H, m), 7.91 and 7.96 (total 1H, each d, each $J = 8.1$ Hz, amide isomers); MS (FAB) *m/z* 598 (M^++1); HR-MS (FAB) calcd for $C_{30}H_{40}N_5O_8$: 598.2877. Found: 598.2838; IR (ATR) 3340, 2937, 2835, 1736, 1620, 1529, 1485 cm^{-1}.

5.21.2. 4-[1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(3*R*,4*R*)-dioxy-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (11p). Prepared from **9p** according to the basic hydrolysis steps of general procedure A, the title compound **11p** was obtained as a colorless amorphous solid (yield, 90%). ^{1}H NMR (DMSO-d_6) δ 2.31–2.68 (5H, m), 2.92 (3H, s), 3.23–3.86 (10H, m), 3.85 and 3.87 (total 3H, each s, amide isomers), 3.92 (1H, t, $J = 3.4$ Hz), 3.95–4.12 (1H, m), 4.60 (1H, d, $J = 2.7$ Hz), 4.80 (1H, s), 6.65 and 6.76 (total 1H, each d, $J = 8.5$ and 8.1 Hz, respectively, amide isomers), 6.93 (2H, s), 7.11 (1H, d, $J = 7.8$ Hz), 7.15 (1H, d, $J = 7.8$ Hz), 7.79 (1H, d, $J = 7.8$ Hz), 8.00 (1H, d, $J = 8.3$ Hz), 8.57 (1H, s), 8.62 (1H, s); MS (FAB) *m/z* 570 (M^++1); Anal. Calcd for $C_{28}H_{35}N_5O_8 \cdot 2.75H_2O$: C, 54.32; H, 6.59; N, 11.37; Found: C, 54.45; H, 6.44; N, 10.96; IR (KBr) 3300, 2937, 2819, 1628, 1601, 1535, 1485, 1454 cm^{-1}.

5.22. 2-[4-[1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]-phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinyl]propionic acid (19)

5.22.1. Ethyl 2-(4-*tert*-butoxycarbonyl-1-piperazinyl)propionate. A mixture of *tert*-butyl-1-piperazinylcarboxylate (**13**) (24.22 g, 130 mmol), ethyl 2-bromopropionate (23.54 g, 130 mmol), and K_2CO_3 (27.09 g, 196 mmol) in DMF (500 ml) was stirred at room temperature for 12 h. The reaction mixture was poured into ice water and extracted with EtOAc. The combined extracts were washed with brine and dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography on silica gel with $CHCl_3$/EtOAc (5:1, v/v) as an eluent to give the title compound (20.7 g, 56%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.23–1.26 (6H, m), 1.42 (9H, s), 2.52–2.57 (4H, m), 3.23–3.29 (1H, m), 3.39–3.45 (4H, m), 4.10–4.18 (2H, m).

5.22.2. Ethyl 2-(1-piperazinyl)propionate (14). To the solution of ethyl 2-(4-*tert*-butoxycarbonyl-1-piperazinyl)propionate (20.70 g, 72.3 mmol) in CH_2Cl_2 (50 ml), TFA (50 ml) was added and the solution was stirred for 1 h. The reaction mixture was concentrated in vacuo and made basic with $NaHCO_3$. The mixture was extracted with $CHCl_3$/MeOH. The extracts were washed with brine, dried over Na_2SO_4, and evaporated to give the title compound **14** (5.90 g, 44%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.23–1.27 (6H, m), 2.85–2.94 (8H, m), 3.20–3.24 (1H, m), 4.12–4.17 (2H, m).

5.22.3. Ethyl 2-[4-[1-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinyl]propionate. Pale yellow oil (yield, 58%). ^{1}H NMR ($CDCl_3$) δ 1.22–1.29 (6H, m), 1.37–1.42 (9H, m), 1.69–2.14 (4H, m), 2.51–2.70 (4H, m), 3.25–3.69 (7H, m), 4.10–4.16 (2H, m), 4.48–4.64 (1H, m); MS (ESI), *m/z* 384 (M^++1).

5.22.4. Ethyl 2-[4-((2*S*)-pyrrolidinylcarbonyl)-1-piperazinyl]propionate (16). Pale yellow oil (yield, 45%). ^{1}H NMR ($CDCl_3$) δ 1.18–1.25 (6H, m), 1.58–3.60 (15H, m), 3.93–3.96 (1H, m), 4.07–4.13 (2H, m); MS (ESI), *m/z* 284 (M^++1).

5.22.5. Ethyl 2-[4-[1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinyl]propionate. Pale yellow oil (yield, 27%). ^{1}H NMR ($CDCl_3$) δ 1.21–1.31 (6H, m), 1.67–2.71 (13H, m), 3.23–3.70 (10H, m), 4.10–4.16 (2H, m), 4.84–4.86 (1H, m), 6.43 (1H, s), 6.75–6.83 (2H, m), 7.07–7.24 (4H, m), 7.50–7.52 (1H, m), 7.99–8.01 (1H, m); MS

(FAB), *m*/*z* 580 (M^++1); HR-MS (FAB) calcd for $C_{31}H_{42}N_5O_6$: 580.3135. Found: 580.3112.

5.22.6. 2-[4-[1-[3-Methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinyl]propionic acid (19). Pale yellow needles (yield, 44%), which were crystallized from *n*-hexane/ether. Mp 165–168 °C; ^{1}H NMR (DMSO-d_6) δ 1.10–1.14 (3H, m), 1.66–4.81 (24H, m), 6.74–6.79 (1H, m), 6.90–6.94 (2H, m), 7.09–7.16 (2H, m), 7.77–7.79 (1H, m), 7.96–8.00 (1H, m), 8.50 (1H, s), 8.57–8.58 (1H, m); MS (FAB), *m*/*z* 552 (M^++1) ; HR-MS (FAB) calcd for $C_{29}H_{38}N_5O_6$: 552.2822. Found: 552.2855; IR (KBr) 3330, 2970, 2945, 2875, 1616, 1589, 1529 cm^{-1}.

5.23. 2-[4-[(4*R*)-Benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinyl]propionic acid (20)

5.23.1. Ethyl 2-[4-[(4*R*)-benzyloxy-1-(*tert*-butoxycarbonyl)-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinyl]propionate. Pale yellow oil (yield, 58%). ^{1}H NMR ($CDCl_3$) δ 1.22–1.29 (6H, m), 1.37–1.42 (9H, m), 1.68–2.28 (4H, m), 2.51–2.59 (4H, m), 3.26–3.72 (6H, m), 4.10–4.65 (5H, m), 7.26–7.34 (5H, m); MS (ESI), *m*/*z* 490 (M^++1).

5.23.2. Ethyl 2-[(4*R*)-(4-benzyloxy-(2*S*)-pyrrolidinylcarbonyl)-1-piperazinyl]propionate (17). Pale yellow oil (yield, 45%). ^{1}H NMR ($CDCl_3$) δ 1.18–1.24 (6H, m), 1.74–3.57 (13H, m), 4.02–4.12 (4H, m), 4.38–4.48 (2H, m), 7.19–7.29 (5H, m); MS (ESI), *m*/*z* 389 (M^++1).

5.23.3. Ethyl 2-[4-[(4*R*)-benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinyl]propionate. Pale yellow amorphous solid (yield, 81%). ^{1}H NMR ($CDCl_3$) δ 1.21–1.29 (6H, m), 1.97–2.68 (9H, m), 3.22–4.40 (17H, m), 4.92–4.96 (1H, m), 6.44–6.48 (1H, m), 6.74–6.76 (2H, m), 7.08–7.32 (9H, m), 7.49–7.51 (1H, m), 8.01–8.05 (1H, m). MS (FAB) *m*/*z* 686 (M^++1); HR-MS (FAB) calcd for $C_{38}H_{48}N_5O_7$: 686.3554. Found: 686.3586.

5.23.4. 2-[4-[(4*R*)-Benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinyl]propionic acid (20). Pale yellow crystalline powder (yield, 55%), which was crystallized from *n*-hexane/ether. Mp 147–150 °C ^{1}H NMR (DMSO-d_6) δ 1.13–1.15 (3H, m), 1.93–4.85 (25H, m), 6.58–6.66 (2H, m), 6.93–6.97 (1H, m), 7.06–7.09 (2H, m), 7.17–7.29 (5H, m), 7.56–7.58 (2H, m), 7.79 (1H, s), 7.96–7.98 (1H, m); MS (FAB) *m*/*z* 658 (M^++1); HR-MS (FAB) calcd for $C_{36}H_{44}N_5O_7$: 658.3241. Found: 658.3226. Anal. Calcd for $C_{36}H_{43}N_5O_7{\cdot}2H_2O$: C, 62.32; H, 6.83; N, 10.09. Found: C, 61.86; H, 6.62; N, 9.83; ; IR (ATR) 3342, 2941, 1620, 1589, 1529, 1485 cm^{-1}.

5.24. 2-[4-[(4*R*)-Benzyloxy-1-[3-methoxy-4-[*N*′-2-(methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinyl]-2-methylpropionic acid (21)

5.24.1. Ethyl 2-[4-(*tert*-butoxycarbonyl)-1-piperazinyl]-2-methylpropionate. By the procedure described for ethyl 2-(4-*tert*-butoxycarbonyl-1-piperazinyl)propionate, but with 2-bromo-2-methylpropionate in place of ethyl 2-bromopropionate, the title compound was obtained as a yellow oil (yield, 99%). ^{1}H NMR δ 1.27 (3H, t, *J* = 7.1 Hz), 1.31 (6H, s), 1.45 (9H, s), 2.52–2.55 (4H, m), 3.40–3.43 (4H, m), 4.17 (2H, q, *J* = 7.1 Hz); EI-MS *m*/*z* 300 (M^+).

5.24.2. Ethyl 2-(1-piperazinyl)-2-methylpropionate (15). By the procedure described for compound **14**, but with ethyl 2-[4-(*tert*-butoxycarbonyl)-1-piperazinyl]-2-methylpropionate in place of ethyl 2-(4-*tert*-butoxycarbonyl-1-piperazinyl)propionate, the title compound was obtained as a yellow oil (yield, 99%). ^{1}H NMR δ 1.28 (3H, t, *J* = 7.3 Hz), 1.31 (6H, s), 2.64–2.68 (4H, m), 2.98–3.00 (4H, m), 3.71 (1H, s), 4.17 (2H, q, *J* = 7.3 Hz); EI-MS *m*/*z* 200 (M^+).

5.24.3. Ethyl 2-[4-[(4*R*)-benzyloxy-1-*tert*-butoxycarbonyl-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinyl]-2-methylpropionate. Colorless oil (yield, 87%). ^{1}H NMR δ 1.27 (3H, t, *J* = 7.1 Hz), 1.31 (3H, s), 1.32 (3H, s), 1.40 and 1.45 (total 9H, each s, amide isomers), 1.98–2.08 (1H, m), 2.19–2.29 (1H, m), 2.47–2.74 (4H, m), 3.46–3.78 (6H, m), 4.16 (2H, q, *J* = 7.1 Hz), 4.19–4.28 (1H, m), 4.44 and 4.47 (total 1H, each d, each *J* = 12.0 Hz, amide isomers), 4.52 and 4.56 (total 1H, each d, each *J* = 12.0 Hz, amide isomers), 4.73–4.82 (1H, m), 7.28–7.36 (5H, m); MS (FAB) *m*/*z* 504 (M^++1).

5.24.4. Ethyl 2-[4-[(4*R*)-benzyloxy-2-pyrrolidinyl]-1-piperazinyl]-2-methylpropionate (18). Yellow oil (yield, 87%). ^{1}H NMR δ 1.27 (3H, t, *J* = 7.1 Hz), 1.32 (6H, s), 1.82 (1H, ddd, *J* = 13.4, 8.5, 6.3 Hz), 2.15 (1H, ddd, *J* = 13.4, 7.6, 2.7 Hz), 2.27 (1H, s), 2.54–2.64 (4H, m), 3.01 (1H, dd, *J* = 12.2, 3.4 Hz), 3.33 (1H, dd, *J* = 12.2, 5.1 Hz), 3.39–3.57 (4H, m), 4.09 (1H, t, *J* = 7.8 Hz), 4.11–4.21 (1H, m), 4.17 (2H, q, *J* = 7.1 Hz), 4.47 (1H, d, *J* = 11.7 Hz), 4.54 (1H, d, *J* = 11.7 Hz), 7.28–7.38 (5H, m); MS (FAB) *m*/*z* 404 (M^++1).

5.24.5. Ethyl 2-[4-[(4*R*)-benzyloxy-1-[3-methoxy-4-[*N*′-(2-methylphenyl)ureido]phenylacetyl]-2-pyrrolidinylcarbonyl]-1-piperazinyl]-2-methylpropionate. Colorless oil (yield, 98%). ^{1}H NMR δ 1.25 (3H, t, *J* = 7.1 Hz), 1.29 (6H, s), 2.03 (1H, ddd, *J* = 13.2, 7.8, 5.4 Hz), 2.21 (1H, ddd, *J* = 13.2, 9.0, 3.9 Hz), 2.27 (3H, s), 2.43–2.54 (2H, m), 2.63 (1H, m), 2.73 (1H, m), 3.49–3.55 (2H, m), 3.58–3.66 (6H, m), 3.68 (3H, s), 4.15 (2H, q, *J* = 7.1 Hz), 4.28 (1H, m), 4.35 (1H, d, *J* = 12.0 Hz), 4.42 (1H, d, *J* = 12.0 Hz), 4.97 (1H, dd, *J* = 8.3, 6.8 Hz), 6.56 (1H, s), 6.76–6.79 (2H, m), 7.11–7.17 (2H, m), 7.21–7.24 (2H, m), 7.27–7.35 (5H, m), 7.53 (1H, d, *J* = 7.8 Hz), 8.05 (1H, d, *J* = 8.3 Hz); MS (FAB) *m*/*z* 700 (M^++1); HR-MS (FAB) calcd for $C_{39}H_{50}N_5O_7$: 700.3710. Found: 700.3676.

5.24.6. 2-[4-[(4*R*)-Benzyloxy-1-[3-methoxy-4-[*N*′-2-(methylphenyl)ureido]phenylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinyl]-2-methylpropionic acid (21). Pale yellow amorphous solid (yield, 35%). ^{1}H NMR δ 1.16 (6H, s), 1.84–1.93 (1H, m), 2.25 (3H, s), 2.24–2.30 (1H, m), 2.41–2.61 (3H, m), 2.62–2.66 (1H, m), 3.23–3.60 (6H, m), 3.74–3.82 (2H, m), 3.85 (3H, s), 4.19–4.23 (1H, m),

4.40 (1H, d, J = 11.7 Hz), 4.46 (1H, d, J = 11.7 Hz), 4.84 (1H, t, J = 7.3 Hz), 6.78 (1H, d, J = 8.3 Hz), 6.91–6.95 (2H, m), 7.11–7.17 (2H, m), 7.26–7.35 (5H, m), 7.78 (1H, d, J = 8.3 Hz), 8.01 (1H, d, J = 8.3 Hz), 8.51 (1H, s), 8.58 (1H, s); MS (FAB) m/z 672 (M^++1); HR-MS (FAB) calcd for $C_{37}H_{46}N_5O_7$: 672.3397. Found: 672.3414. Anal. Calcd for $C_{37}H_{45}N_5O_7 \cdot 3.5H_2O$: C, 60.48; H, 7.13; N, 9.53. Found: C, 60.71; H, 6.46; N, 9.21; IR (KBr) 3345, 2935, 1702, 1631, 1598, 1531, 1454 cm^{-1}.

5.25. 1-(*tert*-Butoxycarbonyl)-(4*R*)-methoxypyrrolidine-(2*S*)-carboxylic acid (3b)

5.25.1. Methyl 1-(*tert*-butoxycarbonyl)-(4*R*)-methoxypyrrolidine-(2*S*)-carboxylate (23). To a stirred solution of *N*-Boc-*trans*-4-hydroxy-L-proline methyl ester (**22**) (1.98 g, 8.1 mmol) and MeI (1.22 ml, 19.6 mmol) in DMF (15 ml) was added 60% oily NaH (0.39 g, 16.2 mmol) at −10 °C, and the reaction mixture was stirred at room temperature for 3 h. The mixture was poured into ice water and extracted with EtOAc. The combined extracts were washed with brine and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography on silica gel with $CHCl_3$/MeOH (50:1, v/v) as an eluent to give the title compound **23** (1.87 g, 89%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.41 (9H, s), 2.01–2.08 (1H, m), 2.31–2.38 (1H, m), 3.32 (3H, s), 3.48–3.67 (2H, m), 3.73 (s, 3 H), 3.96–3.97 (1H, m), 4.30-4.40 (1H, m); MS (FAB), m/z 260 (M^++1).

5.25.2. 1-(*tert*-Butoxycarbonyl)-(4*R*)-methoxypyrrolidine-(2*S*)-carboxylic acid (3b). To a stirred solution of methyl 1-(*tert*-butoxycarbonyl)-(4*R*)-methoxypyrrolidine-2-carboxylate (**23**) (1.87 g, 7.2 mmol) in THF (15 ml) was added 0.25 N NaOH (15 ml) and the reaction mixture was stirred at room temperature for 12 h. After that, 0.5 N NaOH (3 ml) was added to the reaction mixture and the mixture was heated under reflux for 3 h. The mixture was made acidic with 1 N HCl and extracted with $CHCl_3$. The combined extracts were washed with brine, dried over Na_2SO_4, and evaporated to give the title compound **3b** (1.80 g, quant.) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.49 (9H, s), 2.10–2.43 (2H, m), 3.33 (3H, s), 3.47–3.73 (2H, m), 3.97–3.98 (1H, m), 4.31–4.45 (1H, m); MS (FAB), m/z 246 (M^++1).

5.26. (3*S*)-Benzyloxy-1-(*tert*-butoxycarbonyl)pyrrolidine-(2*S*)-carboxylic acid (3g)

5.26.1. Methyl (3*S*)-benzyloxy-1-(*tert*-butoxycarbonyl)pyrrolidine-(2*S*)-carboxylate (25). To a stirred solution of methyl 1-(*tert*-butoxycarbonyl)-(3*S*)-hydroxypyrrolidine-2-carboxylate (**24**) [14] (2.50 g, 10.2 mmol), benzyl bromide (1.50 ml, 12.2 mmol), and tetrabutylammonium iodide (1.13 g, 3.06 mmol) in THF (70 ml) was added 60% oily NaH (0.45 g, 11.2 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 3 h. The mixture was poured into ice water and extracted with EtOAc. The combined extracts were washed with brine and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography on silica gel with *n*-hexane/EtOAc (2:1, v/v) as an eluent to give the title compound **25** (2.14 g, 59%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.41 and 1.47 (total 9H, each s, amide isomers), 1.62 (1H, m), 2.04 (1H, m), 3.50–3.69 (2H, m), 3.73 and 3.74 (total 3H, each s, amide isomers), 4.12 (1H, m), 4.34–4.71 (3H, m), 7.27–7.39 (5H, m); MS (FAB) m/z 336 (M^++1).

5.26.2. (3*S*)-Benzyloxy-1-(*tert*-butoxycarbonyl)pyrrolidine-(2*S*)-carboxylic acid (3g). By the procedure described for compound **3b**, but with methyl 1-(*tert*-butoxycarbonyl)-(3*S*)-hydroxypyrrolidine-2-carboxylate (**25**) in place of **23**, the title compound **3g** was obtained as a yellow oil (100%). ^{1}H NMR ($CDCl_3$) δ 1.49 (9H, s), 1.99–2.12 (2H, m), 3.45–3.62 (2H, m), 4.37–4.66 (4H, m), 7.27–7.39 (5H, m); MS (FAB) m/z 322 (M^++1).

5.27. 1-Benzyloxycarbonyl-(3*R*,4*R*)-isopropylidenedioxy-(2*S*)-pyrrolidinylcarboxylic acid sodium salt (3d)

5.27.1. Methyl 1-benzyloxycarbonyl-(4*R*)-*p*-toluenesulfonyloxy-(2*S*)-pyrrolidinylcarboxylate. To a solution of *p*-toluenesulfonylimidazole (10.0 g, 45.0 mmol) in THF (55 ml) at 0 °C was added methyl triflate (5.60 ml, 49.5 mmol). The mixture was stirred for 1h. A solution of *N*-*Z*-*trans*-(4*R*)-hydroxy-L-proline methyl ester (**26**) (8.41 g, 30.1 mmol) and *N*-methylimidazole (3.60 ml, 44.9 mmol) in THF (20 ml) was added to the reaction mixture, and the resulting mixture was allowed to rise to room temperature and was stirred for 24 h. The mixture was poured into water and extracted with EtOAc. The combined extracts were washed with satd. $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated to dryness. The residue was purified by chromatography on silica gel with *n*-hexane/EtOAc (3:1, v/v) as an eluent to give the title compound (12.7 g, 97%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.15–2.29 (1H, m), 2.44 and 2.46 (total 3H, each s, amide isomers), 2.50–2.55 (1H, m), 3.63–3.71 (2H, m), 3.52 and 3.74 (total 3H, each s, amide isomers), 4.44 and 4.47 (total 1H, each t, each J = 7.8 Hz, amide isomers), 4.99–5.18 (3H, m), 7.27–7.37 (7H, m), 7.75–7.79 (2H, m).

5.27.2. Methyl 1-benzyloxycarbonyl-(4*S*)-phenylseleno-(2*S*)-pyrrolidinylcarboxylate. To a solution of diphenyldiselenide (12.7 g, 29.2 mmol) in MeOH (100 ml) was added $NaBH_4$ (2.87 g, 75.9 mmol). The reaction mixture was heated under reflux for 24 h and cooled to room temperature. To the reaction mixture was added a solution of methyl 1-benzyloxycarbonyl-(4*R*)-*p*-toluenesulfonyloxy-(2*S*)-pyrrolidinylcarboxylate (12.7 g, 29.2 mmol) in MeOH (50 ml), and the mixture was refluxed for 5 h. The mixture was concentrated and diluted with Et_2O, washed with water and brine, dried over Na_2SO_4, and concentrated to dryness. The residue was purified by chromatography on silica gel with *n*-hexane:EtOAc (1:1, v/v) as an eluent to give the title compound (10.0 g, 82%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 2.07–2.11 (1H, m), 2.66–2.70 (1H, m), 3.47–3.64 (2H, m), 3.57 and 3.75 (total 3H, each s, amide isomers), 4.05–4.09 (1H, m), 4.34–4.38 (1H, m), 5.02 and 5.09 (total 1H, each d, each J = 12.2 Hz, amide isomers), 5.17

(1H, d, J = 12.2 Hz), 7.26–7.36 (8H, m), 7.53–7.55 (2H, m).

5.27.3. Methyl 1-benzyloxycarbonyl-3,4-dehydro-(2*S*)-pyrrolidinylcarboxylate (27). To a stirred solution of methyl 1-benzyloxycarbonyl-(4*S*)-phenylseleno-(2*S*)-pyrrolidinylcarboxylate (10.0 g, 23.9 mmol) and pyridine (3.0 ml, 48.8 mmol) in CH_2Cl_2 (120 ml) at 0 °C was added dropwise H_2O_2 (31%, 6.80 g, 59.8 mmol). The reaction mixture was allowed to warm to room temperature and was stirred for 24 h. The reaction mixture was diluted with CH_2Cl_2 and washed with 0.5 N HCl solution, satd $NaHCO_3$ solution, 5% Na_2SO_3 solution, and brine. The separated organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by chromatography on silica gel with *n*-hexane:EtOAc (2:1, v/v) as an eluent to give the title compound **27** (4.62 g, 74%) as a dark brown oil. ^{1}H NMR ($CDCl_3$) δ 3.60 and 3.76 (total 3H, each s, amide isomers), 4.24–4.39 (2H, m), 5.07–5.11 (1H, m), 5.14 (1H, d, J = 12.2 Hz), 5.22 (1H, d, J = 12.2 Hz), 5.73–5.78 (1H, m), 5.96–6.01 (1H, m), 7.28–7.40 (5H, m); MS (ESI) m/z 284 (M^++Na).

5.27.4. Methyl 1-benzyloxycarbonyl-3,4-dihydroxy-(2*S*)-pyrrolidinylcarboxylate (28). To a solution of methyl 1-benzyloxycarbonyl-3,4-dehydro-(2*S*)-pyrrolidinylcarboxylate (**27**) (4.62 g, 17.7 mmol) and *N*-methylmorpholine-*N*-oxide (50% in H_2O, 6.51 g, 27.8 mmol) in acetone (25 ml) was added a catalytic amount of OsO_4 at 0 °C. The reaction mixture was stirred at room temperature for 24 h and quenched by the addition of 10% Na_2SO_3 solution. The mixture was stirred for 3 h and extracted with EtOAc. The extracts were washed with brine, dried over Na_2SO_4, and concentrated to dryness. The residue was purified on chromatography by silica gel with $CHCl_3$:MeOH (10/1, v/v) as an eluent to give the title compound **28** (4.98 g, 95%) as a dark brown oil. ^{1}H NMR ($CDCl_3$) δ 3.55–3.59 (1H, m), 3.68–3.80 (3H, m), 3.56 and 3.75 (total 3H, each s, amide isomers), 4.22–4.29 (3H, m), 4.98 and 5.09 (total 1H, each d, J = 12.5 and 12.4 Hz, respectively, amide isomers), 5.14 and 5.17 (total 1H, each d, J = 12.4 and 12.5 Hz, respectively, amide isomers), 7.28–7.35 (5H, m); MS (ESI) m/z 296 (M^++1).

5.27.5. Methyl 1-benzyloxycarbonyl-(3*R*,4*R*)-isopropylidenedioxy-(2*S*)-pyrrolidinylcarboxylate [(3*R*,4*S*)-29]. To a solution of methyl 1-benzyloxycarbonyl-3,4-dihydroxy-(2*S*)-pyrrolidinylcarboxylate (**28**) (1.41 g, 4.77 mmol) and 2,2-dimethoxypropane (6.0 ml, 48.8 mmol) in CH_2Cl_2 (20 ml) was added *p*-TsOH·H_2O (164 mg, 0.86 mmol). After being stirred at room temperature for 1 h, the mixture was diluted with $CHCl_3$ and washed with satd $NaHCO_3$ solution and brine. The separated organic layer was dried over Na_2SO_4 and concentrated to dryness. The residue was purified on chromatography by silica gel with *n*-hexane:EtOAc (2/1, v/v) as an eluent to give the title compound (3*R*,4*S*)-**29** (1.42 g, 89%) as a colorless oil. And further elution gave a stereoisomer of the title compound methyl 1-benzyloxycarbonyl-(3*S*,4*R*)-isopropylidenedioxy-(2*S*)-pyrrolidinylcarboxylate [(3*S*,4*R*)-**29**] (0.10 g, 6%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.31 (3H, s), 1.46 (3H, s), 3.61–3.65 (1H, m), 3.64 and 3.77 (total 3H, each s, amide isomers), 3.91 and 3.96 (total 1H, each d, each J = 12.7 Hz, amide isomers), 4.54 and 4.63 (total 1H, each s, amide isomers), 4.75–4.77 (2H, m), 5.07 and 5.13 (total 1H, each d, J = 12.5 and 12.4 Hz, respectively, amide isomers), 5.18 and 5.20 (total 1H, each d, J = 12.4 and 12.5 Hz, respectively, amide isomers), 7.28–7.37 (5H, m).

5.27.6. 1-Benzyloxycarbonyl-(3*R*,4*S*)-isopropylidenedioxy-(2*S*)-pyrrolidinylcarboxylic acid sodium salt (3d). To a solution of methyl 1-benzyloxycarbonyl-(3*R*,4*R*)-isopropylidenedioxy-(2*S*)-pyrrolidinylcarboxylate [(3*R*,4*S*)-**29**] (1.42 g, 4.23 mmol) in THF/MeOH (4:1, v/v, 45 ml) was added 0.25 N NaOH (25 ml, 6.35 mmol). After being stirred at room temperature for 24 h, the reaction mixture was concentrated to dryness to afford the title compound **3d** (1.32 g, 91%) as a colorless solid. ^{1}H NMR (DMSO-d_6) δ 1.22 (3H, s), 1.31 (3H, s), 3.45 (1H, d, J = 2.9 Hz), 3.52 (1H, d, J = 3.9 Hz), 4.00 (1H, d, J = 4.4 Hz), 4.60 (1H, m), 4.65 and 4.70 (total 1H, each d, each J = 6.1 Hz, amide isomers), 4.96 and 5.01 (total 1H, each d, each J = 13.2 Hz, amide isomers), 5.05 and 5.08 (total 1H, each d, each J = 13.2 Hz, amide isomers), 7.25–7.35 (5H, m); MS (FAB) m/z 344 (M^++1); IR (KBr) 1617, 1673 cm^{-1}.

5.28. 1-(*tert*-Butoxycarbonyl)-(4*S*)-phenoxy-(2*S*)-pyrrolidinylcarboxylic acid (3e)

5.28.1. Methyl (1-*tert*-butoxycarbonyl)-(4*S*)-phenoxy-(2*S*)-pyrrolidinylcarboxylate (30). To a stirred mixture of *N*-Boc-*trans*-4-hydroxy-L-proline methyl ester (**22**) (4.69 g, 19.1 mmol), phenol (1.98 g, 21.0 mmol), and PPh_3 (5.51 g, 21.0 mmol) in THF (80 ml) was added diisopropyl azodicarboxylate (4.13 ml, 21.0 mmol) at room temperature under a nitrogen atmosphere. The mixture was stirred for 12 h. After removal of the solvent, the resulting residue was chromatographed on silica gel with $CHCl_3$/EtOAc (10:1, v/v) as an eluent to give the title compound **30** (5.31 g, 86%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.43 and 1.48 (total 9H, each s, amide isomers), 2.48 (1H, m), 3.75 (6H, s), 4.42–4.96 (2H, m), 6.88–7.35 (5H, m); MS (ESI), m/z 322 (M^++1).

5.28.2. 1-(*tert*-Butoxycarbonyl)-(4*S*)-phenoxy-(2*S*)-pyrrolidinylcarboxylic acid (3e). To a stirred solution of methyl (1-*tert*-butoxycarbonyl)-(4*S*)-phenoxy-(2*S*)-pyrrolidinylcarboxylate (**30**) (5.31 g, 16.5 mmol) in THF (132 ml) was added 0.25 N NaOH (132 ml, 33.0 mmol) at room temperature. The resulting mixture was stirred for 12 h. After removal of the solvent, the mixture was acidified by the addition of 1 N HCl and extracted with $CHCl_3$. The combined extracts were washed with brine, dried over Na_2SO_4, and evaporated. The residue was recrystallized from *n*-hexane/$CHCl_3$, to give the title compound (**3e**) (2.96 g, 58%) as a colorless powder. ^{1}H NMR (DMSO-d_6) δ 1.36 (9H, s), 2.16 (1H, d, J = 13.2 Hz), 2.46–2.64 (1H, m), 3.40–3.50 (1H, m), 3.71 (1H, dt, J = 12.0, 5.4 Hz), 4.26 (1H, dt, J = 9.5, 7.1 Hz), 4.95–5.04 (m, 1H), 6.80–6.90 (2H, m), 6.94 (1H, t, J = 7.3 Hz), 7.28 (2H, t, J = 7.3 Hz); MS (ESI), m/z 308 (M^++1).

5.29. 1-(*tert*-Butoxycarbonyl)-(4*S*)-(2-naphthyloxy)-(2*S*)-pyrrolidinylcarboxylic acid (3f)

5.29.1. Methyl 1-(*tert*-butoxycarbonyl)-(4*S*)-(2-naphthyloxy)-(2*S*)-pyrrolidinylcarboxylate (31). To a stirred mixture of *N*-Boc-*trans*-4-hydroxy-L-proline methyl ester (**22**) (4.22 g, 17.2 mmol), 2-naphthol (2.73 g, 18.9 mmol), and PPh_3 (4.96 g, 18.9 mmol) in THF (80 ml) was added diisopropyl azodicarboxylate (3.72 ml, 18.9 mmol) at room temperature under an atmosphere of nitrogen. After stirring for 12 h, the mixture was concentrated in vacuo. The residue was chromatographed on silica gel with $CHCl_3$/EtOAc (10:1, v/v) as an eluent to give the title compound **31** (5.37 g), which included an inseparable substrate and was used without further purification. MS (ESI), *m*/*z* 372 (M^++1).

5.29.2. 1-(*tert*-Butoxycarbonyl)-(4*S*)-(2-naphthyloxy)-(2*S*)-pyrrolidinylcarboxylic acid (3f). To a stirred solution of methyl 1-(*tert*-butoxycarbonyl)-(4*S*)-(2-naphthyloxy)-(2*S*)-pyrrolidinylcarboxylate (**31**) (5.37 g) in THF (116 ml) was added 0.25 N NaOH (116 ml, 29.0 mmol) at room temperature. The resulting mixture was stirred for 12 h. After removal of the solvent, the mixture was acidified by the addition of 1 N HCl and extracted with $CHCl_3$. The combined extracts were washed with brine, dried over Na_2SO_4, and evaporated. The residue was recrystallized from *n*-hexane/$CHCl_3$, to give the title compound **3f** [4.44 g, 85% (2 steps)] as a colorless powder. ^{1}H NMR (DMSO) δ 1.37 and 1.41 (total 9H, each s, amide isomers), 2.26 (1H, d, J = 13.9 Hz), 2.65 (1H, m), 3.47 (1H, d, J = 11.5 Hz), 3.81 (1H, m), 4.30 (1H, m), 5.14 (1H, m), 7.02–7.86 (7H, m); MS (ESI), *m*/*z* 358 (M^++1).

5.30. 1-(*tert*-Butoxycarbonyl)-(4*S*)-fluoropyrrolidine-(2*S*)-carboxylic acid (3h)

5.30.1. Methyl 1-(*tert*-butoxycarbonyl)-(4*S*)-fluoropyrrolidine-(2*S*)-carboxylate (33). To a stirred solution of *N*-Boc-*trans*-4-hydroxy-L-proline methyl ester (**22**) (2.0 g, 8.15 mmol) in CH_2Cl_2 (16 ml) was added DAST (1.2 ml, 12.2 mmol) at −78 °C. The mixture was allowed to warm to room temperature, and the stirring was continued for 5 h. The mixture was poured into water and extracted with EtOAc. The combined extracts were washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel with $CHCl_3$/EtOAc (20:1, v/v) as an eluent to give the title compound **33** (830 mg, 41%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.44 and 1.49 (total 9H, s, each, amide isomers), 2.28–2.53 (2H, m), 3.56–3.90 (5H, m), 4.43 and 4.55 (total 1H, each d, each J = 9.3 Hz, amide isomers), 5.20 (1H, d, J = 9.3 Hz); MS (ESI) *m*/*z*, 248 (M^++1).

5.30.2. 1-(*tert*-Butoxycarbonyl)-(4*S*)-fluoropyrrolidine-(2*S*)-carboxylic acid (3h). To a stirred solution of methyl 1-*tert*-butoxycarbonyl-(4*S*)-fluoropyrrolidine-(2*S*)-carboxylate (**33**) (830 mg, 3.36 mmol) in THF (25 ml) was added 0.25 N NaOH (27 ml, 6.71 mmol). The mixture was stirred for 3 h. The mixture was poured into cold 1 N HCl and extracted with $CHCl_3$. The combined extracts were dried over $MgSO_4$ and evaporated to give the title compound **3h** (669 mg, 85%) as a colorless crystalline powder. Mp 160–162 °C; ^{1}H NMR (DMSO-d_6) δ 1.36 and 1.41 (total 9H, each s, amide isomers), 2.17–2.58 (2H, m), 3.16–3.66 (2H, m), 4.27 (1H, t, J = 11.3 Hz), 5.25 (1H, dd, J = 53.6, 3.4 Hz), 12.54 (1H, s); MS (FAB) *m*/*z*, 234 (M^++1).

5.31. 1-(*tert*-Butoxycarbonyl)-(4*R*)-fluoropyrrolidine-(2*S*)-carboxylic acid (3i)

5.31.1. Methyl 1-(*tert*-butoxycarbonyl)-(4*R*)-fluoropyrrolidine-(2*S*)-carboxylate (34). By the procedure described for compound **33**, but with **32** in place of **22**, the title compound **34** was obtained as a pale yellow oil (yield, 40%). ^{1}H NMR ($CDCl_3$) δ 1.42 and 1.47 (total 9H, each s, amide isomers), 2.02–2.19 (1H, m), 2.52–2.64 (1H, m), 3.56–3.96 (5H, m), 4.40–4.47 (1H, m), 5.21 (1H, dt, J = 52.4, 3.4 Hz); MS (ESI) *m*/*z* 270 (M^++Na).

5.31.2. 1-(*tert*-Butoxycarbonyl)-(4*R*)-fluoropyrrolidine-(2*S*)-carboxylic acid (3i). A mixture of methyl 1-(*tert*-butoxycarbonyl)-(4*R*)-fluoropyrrolidine-(2*S*)-carboxylate (**34**) (345 mg, 1.40 mmol), 0.25 N NaOH (11 ml, 2.79 mmol), and THF (10 ml) was stirred for 12 h. The mixture was acidified by the addition of 1 N HCl (3 ml) and extracted with $CHCl_3$. The combined extracts were dried over $MgSO_4$ and evaporated to give the title compound **3i** (336 mg, quant) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.44–1.58 (9H, m), 2.44–2.56 (2H, m), 3.42–3.97 (2H, m), 4.43 and 4.45 (total 1H, each t, each, J = 8.3 Hz, amide isomers), 5.13–5.26 (1H, m); MS (ESI) *m*/*z* 234 (M^++1).

5.32. 1-(*tert*-Butoxycarbonyl)-4,4-difluoropyrrolidine-(2*S*)-carboxylic acid (3j)

5.32.1. Methyl 1-(*tert*-butoxycarbonyl)-4-oxopyrrolidine-(2*S*)-carboxylate (36). To a stirred solution of methyl *N*-Boc-*trans*-4-hydroxy-L-proline methyl ester (**22**) (2.0 g, 8.15 mmol) in CH_2Cl_2 were added 3 Å molecular sieves (2 g) and PDC (4.60 g, 12.2 mmol). The mixture was stirred for 3 days. The mixture was filtered through a Celite pad, and the filtrate was evaporated. The residue was chromatographed on silica gel with $CHCl_3$/MeOH (10:1, v/v) as an eluent to give the title compound **36** (1.13 g, 57%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.46–1.48 (9H, m), 2.56–2.61 (1H, m), 2.88–3.00 (1H, m), 3.77 (3H, s), 3.82–3.88 (2H, m), 4.71–4.83 (1H, m); MS (ESI) *m*/*z*, 266 (M^++Na).

5.32.2. Methyl 1-(*tert*-butoxycarbonyl)-4,4-difluoropyrrolidine-(2*S*)-carboxylate (37). To a stirred solution of methyl 1-(*tert*-butoxycarbonyl)-4-oxopyrrolidine-(2*S*)-carboxylate (**36**) (1.13 g, 4.65 mmol) in CH_2Cl_2 (20 ml) was added DAST (1.1 ml, 11.6 mmol) at −78 °C. The mixture was allowed to warm to room temperature. After 15 h stirring, the mixture was poured into water and extracted with EtOAc. The combined extracts were washed with brine, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel with $CHCl_3$/EtOAc (20:1, v/v) as an eluent to give the title compound **37** (885 mg, 72%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.42 and 1.47 (total 9H, each s, amide iso-

mers), 2.46 (1H, ddd, d = 26.9, 13.7, 5.1 Hz), 2.62–2.78 (1H, m), 3.75–3.95 (5H, m), 4.43–4.57 (1H, m); MS (ESI) m/z, 288 (M^++Na).

5.32.3. 1-(*tert*-Butoxycarbonyl)-4,4-difluoropyrrolidine-(2*S*)-carboxylic acid (3j). To a stirred solution of methyl 1-(*tert*-butoxycarbonyl)-4,4-difluoropyrrolidine-(2*S*)-carboxylate (**37**) (885 mg, 3.34 mmol) in THF (25 ml) was added 0.25 N NaOH (26.7 ml, 6.67 mmol), and the stirring was continued for 1 h. The mixture was poured into 1 N HCl (100 ml) and extracted with $CHCl_3$. The combined extracts were washed with brine, dried over $MgSO_4$, and evaporated to give the title compound **3j** (775 mg, 92%) as a yellow crystalline solid. Mp 113–117 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 and 1.49 (total 9H, each s, each, amide isomers), 2.53–2.80 (2H, m), 3.71–3.90 (2H, m), 4.20-4.61 (1H, m); MS (FAB) m/z, 252 (M^++1); Anal. Calcd for $C_{10}H_{15}F_2O_4$: C, 47.81; H, 6.02; N, 5.58. Found: C, 48.06; H, 6.05; N, 5.45.

5.33. 1-(*tert*-Butoxycarbonyl)-(4*S*)-chloropyrrolidine-(2*S*)-carboxylic acid (3k)

5.33.1. Methyl 1-(*tert*-butoxycarbonyl)-(4*S*)-chloropyrrolidine-(2*S*)-carboxylate (35). To a stirred solution of methyl *N*-Boc-*trans*-4-hydroxy-L-proline methyl ester (**22**) (3.15 g, 12.8 mmol) in CH_2Cl_2/CCl_4 (50 ml, 1:1, v/v) was added Ph_3P (7.11 g, 27.1 mmol), and the reaction mixture was stirred at room temperature for 2.5 h. To the mixture was added EtOH (10 ml), and the reaction mixture was stirred for 12 h. After removal of the solvent, the residue was purified by column chromatography on silica gel with *n*-hexane/EtOAc (3:1, v/v) as an eluent to give the title compound **35** (2.57 g, 76%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.42 (9H, s), 2.33–2.39 (1H, m), 2.67–2.77 (1H, m), 3.60–3.67 (1H, m), 3.76 (3H, s), 3.80–4.00 (1H, m), 4.32–4.46 (2H, m); FAB-MS, m/z 264 (M^++1).

5.33.2. 1-(*tert*-Butoxycarbonyl)-(4*S*)-chloropyrrolidine-(2*S*)-carboxylic acid (3k). To a stirred solution of methyl 1-(*tert*-butoxycarbonyl)-(4*S*)-chloropyrrolidine-(2*S*)-carboxylate (**35**) (2.57 g, 9.8 mmol) in THF (20 ml) was added 0.5 N NaOH (20 ml), and the reaction mixture was heated under reflux for 2 h. The mixture was poured into 1 N HCl and extracted with $CHCl_3$. The combined extracts were washed with brine, dried over Na_2SO_4, and evaporated to give the title compound **3k** (2.02 g, 83%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.44 and 1.48 (total 9H, each s, amide isomers), 2.44–2.76 (2H, m), 3.64 (1H, m), 3.91–3.97 (1H, m), 4.38–4.46 (2H, m), 8.17 (1H, s); MS (FAB), m/z 250 (M^++1).

5.34. VLA-4/VCAM-1 binding assay

A human VLA-4-expressing cell line, 4B4, was established at Pharmacopeia (New Jersey, USA), by transfecting both the α4 gene and β1 gene of VLA-4 into CHO-K1 cells. The 4B4 cells were maintained in F-12 medium (Ham's F-12) supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (Invitrogen Corporation, Carlsbad, USA) and 1 mg/ml G-418 (Geneticin, Invitrogen Corporation, Carlsbad, USA).

Human VCAM-1/Fc Chimera (R&D Systems Inc., Minneapolis, USA) was labeled with Eu as follows. The protein was reconstituted in labeling buffer (150 mM NaCl, 50 mM sodium carbonate, pH 8.5). Eu-Labelling Reagent (Perkin-Elmer Inc., Wellesley, USA) reconstituted with the labeling buffer was added to the protein solution. The mixture was then incubated at room temperature for 4 days. The Eu-labeled protein was purified with a PD-10 column (Amersham Biosciences KK, Tokyo, Japan) and stored at −80 °C until use.

All assays were performed in duplicate. Prior to the assay, the 4B4 cells were suspended at 3×10^5 cells/ml in F-12 medium (Ham's F-12) supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (Invitrogen Corporation, Carlsbad, USA). One hundred microliters of the 4B4 cell suspension at 3×10^5 cells/ml was distributed into each well of 96-well culture plates (Costar, 3599, USA). The plates were incubated in a 5% CO_2 humidified incubator at 37 °C (Themo Forma, model: 3120, Forma Scientific, Inc. USA) for 2 days.

The medium was discarded, and each well was washed twice with 300 μl of chilled wash buffer (25 mM Hepes (pH 7.5), 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 4 mM $MnCl_2$). Then 50 μl compound solution was distributed to the wells, followed by 50 μl of 2 nM of Eu-labelled Human VCAM-1/Fc Chimera diluted with the wash buffer (final concentration: 1 nM). The plates were incubated for 60 min at room temperature, and the wells were washed 4 times with 300 μl of chilled wash buffer. Finally, 100 μl of the enhancement solution (Perkin-Elmer Inc., Wellesley, USA) was added to each well. The plates were placed on a shaker for 5 min. Eu fluorescence was then measured in a time-resolved fluorometer (DELFIA Research fluorometer, model: 1234-001, Perkin-Elmer Inc., Wellesley, USA).

The concentration of compound required for 50% inhibition in the assay was determined.

5.35. Distribution coefficient

The distribution coefficients ($\log D$) were determined by the well-known shake-flask method.[15] Four hundred micromolar of compound solution in *n*-octanol (2 ml)/PBS (2 ml, pH 7.4) or the Japanese Pharmacopoeia Second fluid (2 ml, pH 6.8) was placed on a shaker for 30 min. After centrifuging at 3000 rpm for 10 min, each layer was assayed using LC–MS methodologies (LC–Mass spectrometer: 1100 Series LC/MSD, Agilent; Analytical Column: X Terra® MSC18 3.5 μm, 3.0 × 30 mm, Waters; Mobile Phase: 10 mM ammonium acetate buffer (pH 4.5)/0.05% acetic acid in acetonitrile = 95:5 to 10:90 v/v). The values of $\log D$ were analyzed using Analyst software program (version 1.4, Applied Bio. Systems).

5.36. Pharmacokinetic studies on rats

Male Sprague–Dawley rats (280–360 g, obtained from Hilltop Laboratories and Charles River Laboratories) were used for the study. Animals were surgically implant-

ed with catheters in the right and left jugular veins. The jugular catheters were composed of silastic tubing (i.d. 0.02 in. and o.d. 0.037 in.). The lock solution for the catheter contained streptokinase (Kabikinase®, 18750 U/ml) and heparin (500 U/ml) in 25% dextrose. Care was taken to apply only the catheter volume (≈0.05–0.07 ml) of lock solution in order to minimize systemic exposure to this mixture. Animals were housed in clear PVC boxes with lids in unidirectional airflow rooms with controlled temperature (20 ± 4 °C) and relative humidity (50 ± 20%) and a 12 h light/dark cycle. Animals were provided food and tap water ad libitum, except for the 12 h fasting (food withdrawn) period prior to dosing.

Intravenous administration: each compound was dissolved in sterile 0.9% saline (pH adjusted to 8.0 with sodium bicarbonate) for a target concentration of 0.5 or 1.0 mg of each compound per milliliter and administered by 2 h intravenous infusion at a targeted dose rate of 0.75, 1.0, or 1.2 mg/kg, respectively. Oral administration: each compound was suspended in 0.5% (w/v) aqueous methylcellulose for a target concentration of 0.2 mg of each compound per milliliter and administeredby oral gavage at a targeted dose rate of 2.0 mg/kg.

Plasma samples were assayed by using LC–MS/MS methodologies (Mass spectrometer: Micro Quattro II, Micromass, Inc. or TSQ 7000 Triple Stage Quadrupole, Finnigan; HPLC System: HP 1100 Binary Pump, Hewlett–Packard Company or 2690 Separations Module, Waters; Analytical Column: Columbus, C8, 2 mm × 100 mm, 5 μm Phenomenex or XDB-C18, 2.1 × 150 mm, 5 μm, Zorbax; Mobile Phase: 2% formic acid in water/acetonitrile = 90:10 to 10:90 v/v or 0.2% acetic acid in water/acetonitrile = 76:24 to 55:45 v/v). Plasma concentrations were analyzed using WinNolin software program (version 1.1, Scientific Consulting, Inc.).

5.37. Excretion studies on rats

The test system, husbandry, dose preparation (1 mg/ml), and analytical conditions obeyed the Pharmacokinetic Studies' method (Section 5.36).

Compound **1** was administered by 2 h intravenous infusion at a targeted dose rate of 2.4 mg/kg. A bile sample was collected from each animal during and after the iv infusion at 0–2, 2–4, and 4–6 h time intervals.

Compound **9o** was administered by intravenous bolus injection at times 0 and 3 h (1.0 mg/kg per injection, total 2.0 mg/kg). Bile samples were collected at 0–3 h (prior to second dose) and 3–6 h time intervals. Urine samples were collected at 0–3, 3–6, and 6–12 h time intervals.

5.38. Pharmacokinetic studies on dogs

Male Beagle dogs (10.4–12.2 kg, obtained from Marshall Animals Inc.) were used for the study. Animals were implanted with a catheter in the cephalic vein of the foreleg for blood sampling and in the hind leg (saphenous vein) for iv infusion. Animals were housed in stainless steel cages in a controlled environment (72 ± 4 °F) and relative humidity (50 ± 20%) and a 12 h light/dark cycle. Animals were provided food and tap water ad libitum. During dosing and sampling, the dogs were restrained in Pavlov slings for ≈2 h.

The dosing solution was freshly prepared in a vehicle comprising 2.9% ethanol, 0.1% DMSO, and 97% sterile saline (0.9% w/v, pH adjusted to 8.4 with 8% sodium bicarbonate). The final compound concentration in the dosing mixture was 0.5 mg of each compound/ml. Each animal received the dosing mixture as a constant 1 h intravenous infusion via the implanted catheter at a targeted dose rate of 0.75 mg of each compound/kg/h.

Plasma samples were assayed by using LC–MS/MS methodologies (Mass spectrometer: TSQ 7000 Triple Stage Quadrupole, Finnigan; HPLC System: 2690 Separations Module, Waters; Analytical Column: XDB-C18, 2.1 × 150 mm, 5 μm, Zorbax; Mobile Phase: 0.2% formic acid in water/acetonitrile = 67:33 v/v). Plasma concentrations were analyzed by using the WinNolin software program (version 1.1, Scientific Consulting, Inc.).

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