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A novel and potent VLA-4 antagonist based on *trans*-4-substituted cyclohexanecarboxylic acid

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ABSTRACT

During the course of our study, it was revealed that the poor pharmacokinetic properties of a series of benzoic acid derivatives such as **1** should be attributed to the diphenylurea moiety. Thus, we replaced the diphenylurea moiety in **1** with a 2-(2-methylphenylamino)benzoxazole moiety which mimics the diphenylurea structure. However, this modification resulted in a significant decrease (**3**, IC_{50} = 19 nM) in VLA-4 inhibitory activity compared to **1** (IC_{50} = 1.6 nM). To address this discrepancy, we worked on optimization of the carboxylic acid moiety in compound **3**. As a result, our efforts have led to the discovery of *trans*-4-substituted cyclohexanecarboxylic acid derivative **11b** (IC_{50} = 2.8 nM) as a novel and potent VLA-4 antagonist. In addition, compound **11b** exhibited favorable pharmacokinetic properties (CL = 3.3 ml/min/kg, *F* = 51%) in rats.

1. Introduction

VLA-4 (very late antigen-4, α4β1 integrin, or CD49d/CD29) is a key cell receptor expressed on most leukocytes.[1] The major counterligands for VLA-4 are VCAM-1 (Vascular Cell Adhesion Molecule-1) expressed on cytokine-stimulated endothelial cells and the alternatively spliced connecting segment-1 domain of fibronectin on the extracellular matrix.[2,3] Through the interaction with these ligands, VLA-4 plays a significant role in the process of adhesion, migration, and activation of inflammatory leukocytes at sites of inflammation. Monoclonal antibodies to the α4 subunit and small molecular VLA-4 antagonists[4] have been used to demonstrate the efficacy in inflammatory animal models of asthma,[5] multiple sclerosis (MS),[6] rheumatoid arthritis,[7] and inflammatory bowel disease (IBD).[8] Natalizumab,[9] a humanized monoclonal antibody to the α4 subunit, has been approved by the FDA for the treatment of MS and Crohn's disease. Because of these excellent proofs of concept, the development of small molecule VLA-4 antagonists with acceptable oral pharmacokinetic profiles is viewed as reasonable approach to a novel class of therapeutic agents.

We recently have reported that benzoic acid derivative **1**[10] (Fig. 1) shows potent VLA-4 inhibitory activity (CHO-K1 cells/human VCAM binding assay, IC_{50} = 1.6 nM) and demonstrates efficacy in a murine asthma model by oral dosing at 15 mg/kg. In addition, during the course of this study it was found that introducing a halogen atom (Cl or Br) into the 3-position on the central benzene in the diphenylurea moiety as in **1** tends to improve the pharmacokinetic properties in rodents and dogs.[11] However, the pharmacokinetic profile of **1** (rat, CL = 6.3 ml/min/kg, *F* = 23%; dog, CL = 5.2 ml/min/kg, *F* = 38%) was not fully pursued. From the result that even the fine tuning in diphenylurea moiety provided the moderate improvement of the poor pharmacokinetic properties, we considered that drastic structural modification of the diphenylurea moiety could lead to a substantial improvement of the pharmacokinetic properties. On the other hand, piperidinylacetic acid derivative **2** was reported to show an IC_{50} of 0.5 μM or less in a Ramos cell assay,[12a] in which diphenylurea moiety was replaced by a 2-(phenylamino)benzoxazole. Therefore, we applied this diphenylurea variant to **1** by replacing the diphenylurea moiety with a 2-(2-methyamino)benzoxazole to see whether the combination maintained the potent activity. Unfortunately, the prepared compound **3** was found to be about 12-fold less potent (IC_{50} = 19 nM) than **1** in the binding assay and this result implied that the benzoic acid moiety as in **3** required optimization. For this reason, we decided to optimize the benzoic acid moiety prior to further investigation of the diphenylurea moiety.

Herein, we report our optimization efforts on the benzoic acid moiety in **3** and the discovery of *trans*-4-substituted cyclohexanecarboxylic acid derivative **11b** showing an IC_{50} value of single digit

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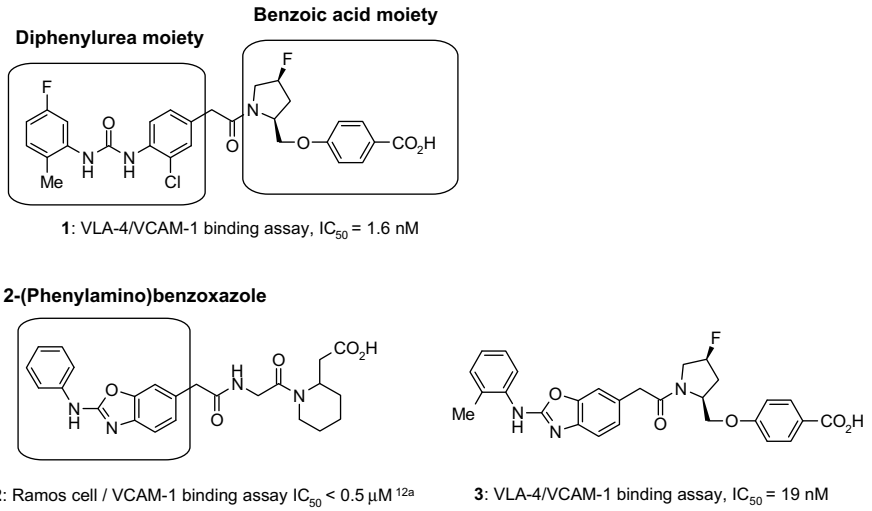


Figure 1.

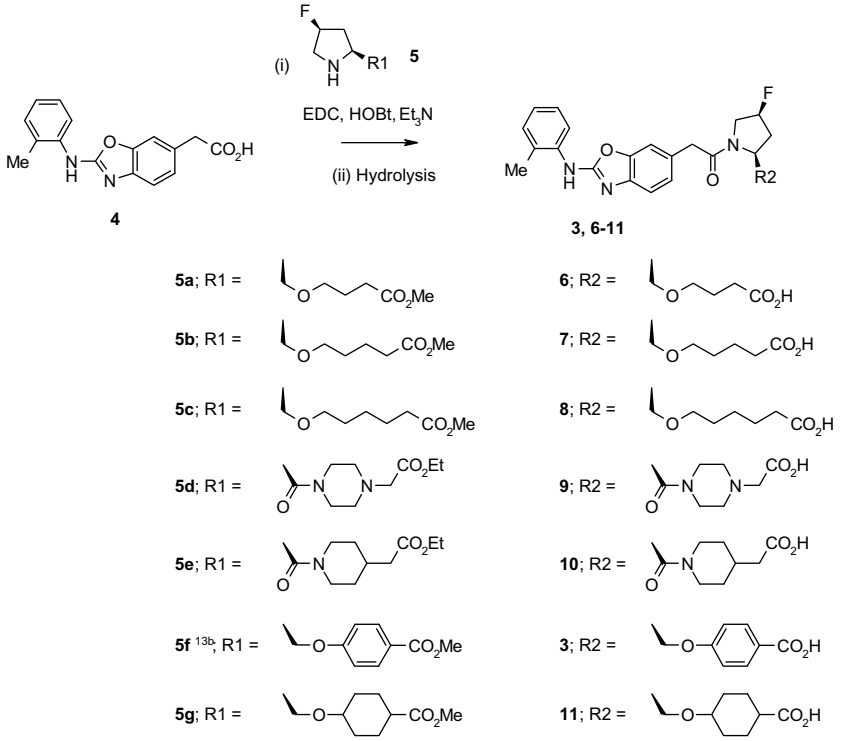
nM in the binding assay. We also report the pharmacokinetics properties of **11b** in rats.

2. Chemistry

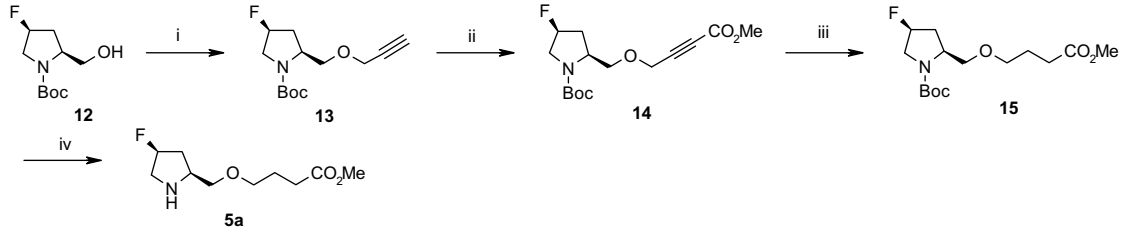
Target compounds were synthesized as depicted in Scheme 1. Thus, 2-(2-methylphenylamino)-6-benzoxazolylacetic acid (**4**) was condensed with (4*S*)-fluoropyrrolidine derivative **5** using EDC and HOBt, followed by basic hydrolysis to afford **3** and **6–11**.

The intermediates **4** and **5** in Scheme 1 were prepared as shown in Schemes 2–7. Treatment of 1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-prolinol (**12**)[13] with propargyl bromide gave the propargyl ether **13**, which was coupled with methyl chloroformate in the presence of *n*-BuLi to provide the methyl 2-butynoate **14.** After hydrogenation of the triple bond in **14**, the Boc group of the resulting methyl butanoate **15** was deprotected by TFA treatment to yield **5a** (Scheme 2).

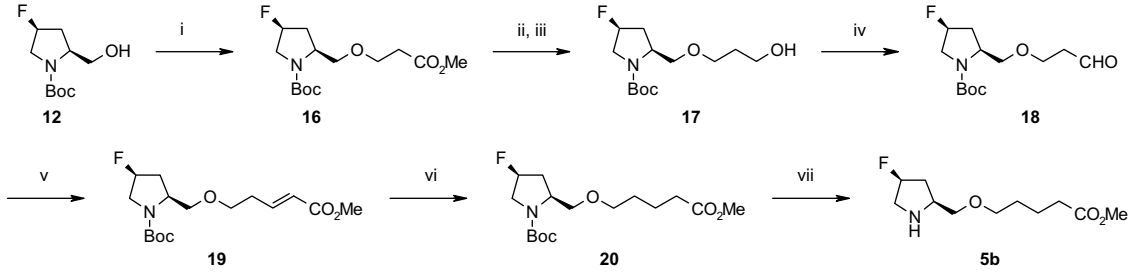
Conversion of the prolinol **12** to methyl 3-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]propionate (**16**) was achieved by means of a Michael addition with methyl acrylate. Basic hydrolysis of the ester group of **16** followed by reduction of the carboxylic acid group with borane–dimethylsulfide complex to afford the alcohol **17**, which was converted to the corresponding aldehyde **18** by oxidation with sulfur trioxide pyridine complex. Homologation by treatment of the aldehyde **18** with Horner–Emmons reagent trimethyl phosphonoacetate, hydrogenation of the



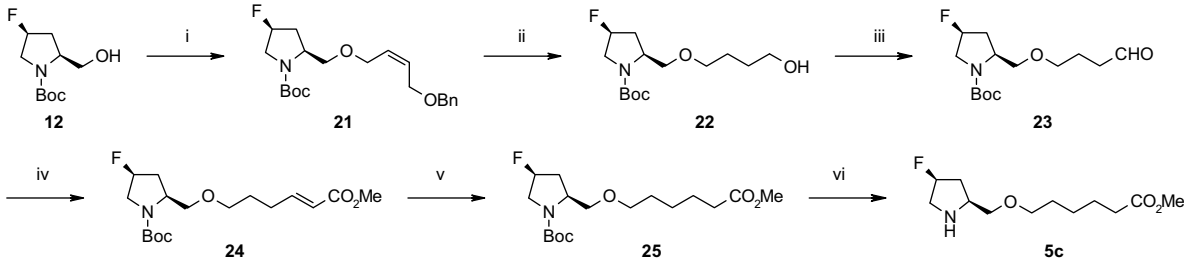
Scheme 1. Reagents and conditions: (i) **5**, EDC, HOBt, DMAP, or Et_3N, DMF; (ii) aq NaOH, THF (21% for **6**, 64% for **7**, 48% for **8**, 42% for **9**, 40% for **10**, 65% for **3**, 48% for **11**).



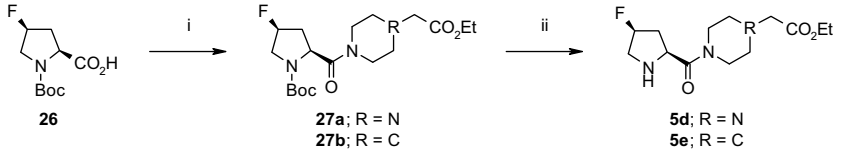
Scheme 2. Reagents and conditions: (i) propargyl bromide, NaH, nBu$_4$NI, THF (85%); (ii) n-BuLi, methyl chloroformate, THF (69%); (iii) H_2, 5% Pd/C, MeOH (61%); (iv) TFA, CH_2Cl_2 (90%).



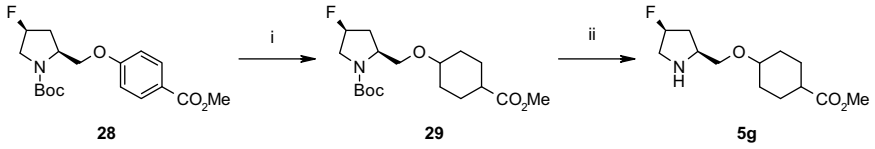
Scheme 3. Reagents and conditions: (i) methyl acrylate, NaH, THF (48%); (ii) aq NaOH, THF; (iii) $BH_3{\cdot}Me_2S$, THF (100% for two steps); (iv) $SO_3{\cdot}Py$, Et_3N, DMSO, CH_2Cl_2 (53%); (v) trimethyl phosphonoacetate, NaH, THF (86%); (vi) 5% Pd/C, H_2, MeOH (78%); (vii) TFA, CH_2Cl_2 (77%).



Scheme 4. Reagents and conditions: (i) *cis*-4-benzyloxy-2-butenyl bromide, NaH, n-Bu$_4$NI, THF (78%); (ii) 20% Pd(OH)$_2$, H_2, EtOH (67%); (iii) $SO_3{\cdot}Py$, Et_3N, DMSO, CH_2Cl_2 (72%); (iv) trimethyl phosphonoacetate, NaH, THF (93%); (v) 5% Pd/C, H_2, MeOH (100%); (vi) TFA, CH_2Cl_2 (85%).



Scheme 5. Reagents and conditions: (i) ethyl 1-piperazinylacetate or ethyl 4-piperidinylacetate, EDC, HOBt, Et_3N, DMF (100% for **27a**, 32% for **27b**); (ii) TFA, CH_2Cl_2 (86% for **5d**, 100% for **5e**).

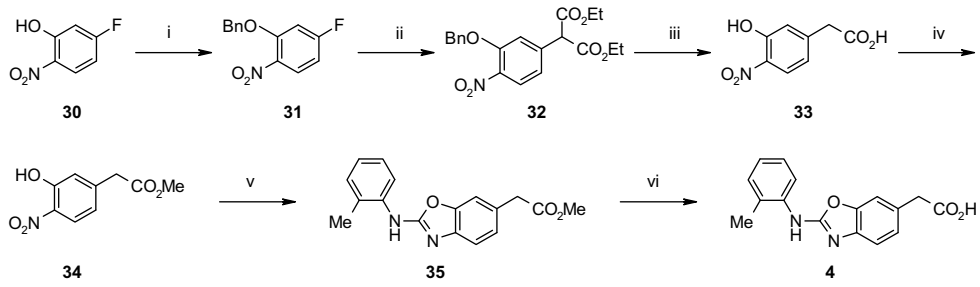


Scheme 6. Reagents and conditions: (i) 5% Rh on alumina, 10 atm H_2, EtOH–AcOH (91%); (ii) TFA, CH_2Cl_2 (84%).

double bond of the methyl 2-pentenoate **19** and deprotection of the Boc group in the methyl pentanoate **20** with TFA to afford **5b** (Scheme 3).

The prolinol **12** was converted to the ether **21** by treatment of *cis*-4-benzyloxy-2-butenyl bromide[14] in the presence of NaH and a catalytic amount of n-Bu$_4$NI. Hydrogenolysis of **21** resulted in reduction of the double bond and removal of the benzyl group at the same time to afford the alcohol **22**, which was converted to methyl 6-[(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]hexanoate (**5c**) following the same procedure as that described for the preparation of **5b** (Scheme 4).

Commercially available ethyl 1-piperazinylacetate or ethyl 4-piperidinylacetate was condensed with 1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-L-proline (**26**)[15] using EDC and HOBt, followed by



Scheme 7. Reagents and conditions: (i) BnBr, K_2CO_3, DMF (91%); (ii) NaH, diethyl malonate, DMF (100%); (iii) concd HCl, AcOH, reflux; (iv) concd H_2SO_4, MeOH, reflux (84%); (v) (1) H_2, Pd/C, EtOH; (2) 2-methylphenylisothiocyanate; (3) HgO, EtOH, reflux (three steps, 84%); (vi) 0.25 N NaOH, THF (77%).

deprotection of the Boc group of the resulting compounds **27a–b** to afford **5d** and **5e** (Scheme 5).

The aromatic ring of methyl 4-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoate (**28**)[13b] was reduced by hydrogenation at 10 atm in the presence of 5% Rh on alumina as a catalyst, followed by deprotection of the Boc group of the cyclohexane **29** to afford **5g** (Scheme 6).

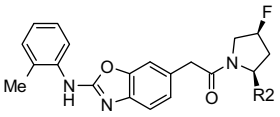
Starting from commercially available 2-nitro-5-fluorophenol (**30**), 2-(2-methylphenylamino)-6-benzoxazolylacetic acid (**4**) was synthesized by following the reported procedure[12] (Scheme 7).

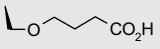
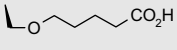
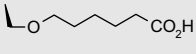
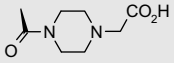
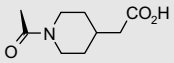
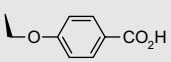
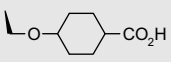
3. Results and discussion

3.1. In vitro activity

Compounds **3** and **6–11** were evaluated for their activities to inhibit the binding of CHO (Chinese hamster ovary) cells expressing VLA-4 to a europium (Eu)-labeled human VCAM-1/Fc chimera. These results are summarized in Table 1.

Table 1
Inhibitory activity of VLA-4 antagonists



Compound	R2	IC_{50} (nM)
6		831
7		198
8		164
9		50
10		19
3		19
11		15

Acylic type derivatives showed a significant loss of potency (**6**, IC_{50} = 831 nM, **7**, IC_{50} = 198 nM, **8**, IC_{50} = 164 nM) compared with **3** (IC_{50} = 19 nM), whereas cyclic type derivatives retained the potency (**10**, IC_{50} = 19 nM and **11**, IC_{50} = 15 nM) except for piperazine derivative (**9**, IC_{50} = 50 nM).

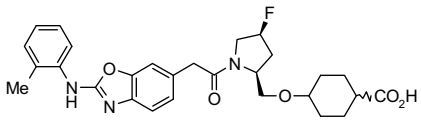
In addition to this structure–activity relationship, during the medicinal chemistry process, we happened to realize from HPLC analysis that the cyclohexanecarboxylic acid derivative **11** contained a small amount of a more polar product (**11b**, ~14%), which was inseparable by conventional column chromatography. Thus, we carried out separation of compound **11** utilizing HPLC to afford the major product **11a** as a less polar fraction and the minor product **11b** as a more polar fraction. From the NMR and MS spectra of both products, it was inferred that **11a** and **11b** could be the *cis*- and *trans*-isomer of the 4-substituted cyclohexanecarboxylic acid. On the other hand, Iguchi et al. have reported that reduction of methyl 4-propylbenzoate using $[RhCl(COD)]_2$ and hydrogen yields the *cis*-isomer (75%) and the *trans*-isomer (14%) of methyl 4-propylcylohexanecarboxylate.[16] From that result, it was considered that the isomers should be produced in the Rh catalyzed hydrogenation reaction of benzoic acid derivative **28** to cyclohexane derivative **29** (Scheme 6).

Next, we evaluated the separated two isomers for their VLA-4 inhibitory activities and the result is shown in Table 2. To our surprise, **11b** (the minor product) exhibited single digit activity (IC_{50} = 2.8 nM), which was 150-fold more potent than that of **11a** (the major product, IC_{50} = 443 nM). This result prompted to us to determine the relative configuration of **11b**.

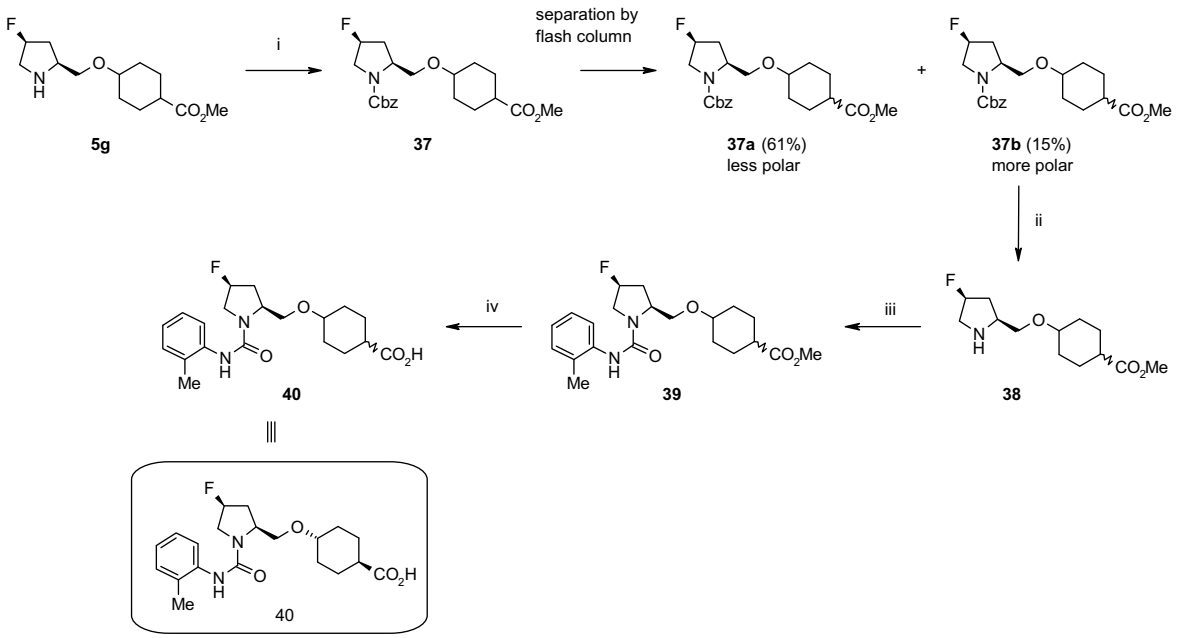
3.2. Determination of relative configuration of 11b

We attempted to determine the relative configuration of **11a,b** through X-ray crystal structure analysis and comparison of ^1H NMR spectra. At first, in consideration of the ease in providing a suitable crystal for X-ray crystal analysis, we prepared urea deriv-

Table 2
Comparison of the inhibitory activity between the separated **11a** and **11b**



Compound	IC_{50} (nM)
11a (the major product, less polar)	443
11b (the minor product, more polar)	2.8



Scheme 8. Reagents and conditions: (i) Z–Cl, aq $NaHCO_3$, CH_2Cl_2 (85%); (ii) H_2, $Pd(OH)_2/C$, MeOH (97%); (iii) 2-methylphenylisocyanate, Et_3N, THF (56%); (iv) aq NaOH, THF, MeOH (93%).

ative **40** utilizing the intermediate **5g** shown in Scheme 8. Thus, after protection of the free amine of **5g** with benzyloxycarbonyl (Cbz) group, we investigated the resulting compound **37** by ^{1}H NMR and HPLC. According to the results, it turned out to be a mixture of the *cis*- and *trans*-isomer, as expected above. Next, the separation of the two isomers was successfully achieved using flash column chromatography, affording **37a** (61%, a less polar fraction) and **37b** (15%, a more polar fraction). After removal of the Cbz group of **37b**, treatment of the resulting amine **38** with 2-methylphenylisocyanate and basic hydrolysis afforded the urea derivative **40**, which was recrystallized from ethyl acetate. X-ray crystal structure analysis of the obtained crystal proved that the relative configuration of the 1 and 4-substituents on the cyclohexane ring was *trans* form (Fig. 2). Furthermore, starting from the methyl *trans*-4-substituted cyclohexanecarboxylate **38**, we synthesized compound **11** according to the procedure shown in Scheme 1, making sure that the ^{1}H NMR and MS data were completely identical with those of **11b**. At the same time, we also synthesized compound **11** starting from methyl *cis*-4-substituted cyclohexanecarboxylate **37a**, finding that the ^{1}H NMR and MS data were completely identical with those of **11a** and no isomerization occured in this basic hydrolysis condition. Consequently, we successfully established the relative configuration of **11b** to be *trans*. On top of that, it was clarified that the *trans*-4-substituted cyclohexanecarboxylic acid skeleton should be the critical pharmacophore responsible for VLA-4 inhibitory activity.

In an effort to elucidate the structure–activity relationship of the carboxylic acid moiety, we examined the structural comparison between benzoic acid derivative (**3**, IC_{50} = 19 nM), *trans*-4-substituted cyclohexanecarboxylic acid (**11b**, IC_{50} = 2.8 nM), and *cis*-4-substituted cyclohexanecarboxylic acid (**11a**, IC_{50} = 443 nM) by using the molecular modeling technique. At first, the lowest energy conformations of these compounds were generated using 'Macromodel version 9.1113'[17] (Fig. 3). We previously reported that the distance between the nitrogen in the pyrrolidine ring and the carbon in the carboxylic acid significantly influences on VLA-4 inhibitory activity.[18] Therefore, we measured the corresponding distance of each conformer. However, we were unable

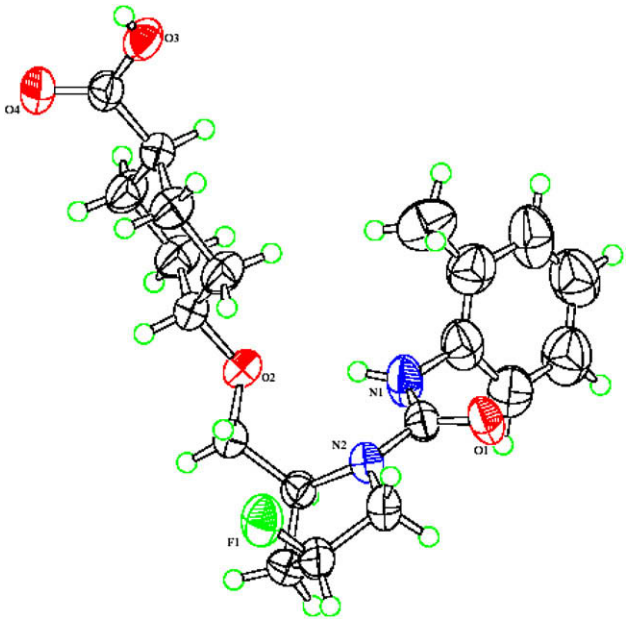


Figure 2. X-ray structure of **40**.

to make the SAR explicit from the results (**3**, 8.108 Å; **11b**, 8.480 Å; **11a**, 8.543 Å). On the other hand, we also attempted to superimpose those carboxylic acid parts using the oxygen at the 4-position in the cyclohexanecarboxylic acid and comparing the direction of the carboxylic acid group from the oxygen (Fig. 4). As a result, compound **3** and **11b** displayed a quite similar direction. But the direction of compound **11a** was obviously different from those of **3** and **11b**, implying that the direction is responsible for the activity. As for the 7-fold more potent activity of **11b** than **3**, we speculate that the higher activity of **11b** could be attributed to structural flexibility of the cyclohexane ring in **11b**, in comparison with the benzene ring in **3**.

3.3. Pharmacokinetic properties of 11b

In order to make sure how the replacement of the diphenylurea moiety with 2-(2-methylphenylamino)benzoxazole moiety affects pharmacokinetic properties, we determined the pharmacokinetic profile of **11b** in rats. As can be seen in Table 3, compound **11b** showed excellent exposure (AUC = 14,932 ng h/ml), plasma clearance (CL = 3.3 ml/min/kg) and oral bioavailability (F = 51%), finding that these profiles were preferable compared to those of compound **1** (AUC = 1438 ng h/ml at 2 mg/kg oral dosing, CL = 6.3 ml/min/kg, F = 23%).[10]

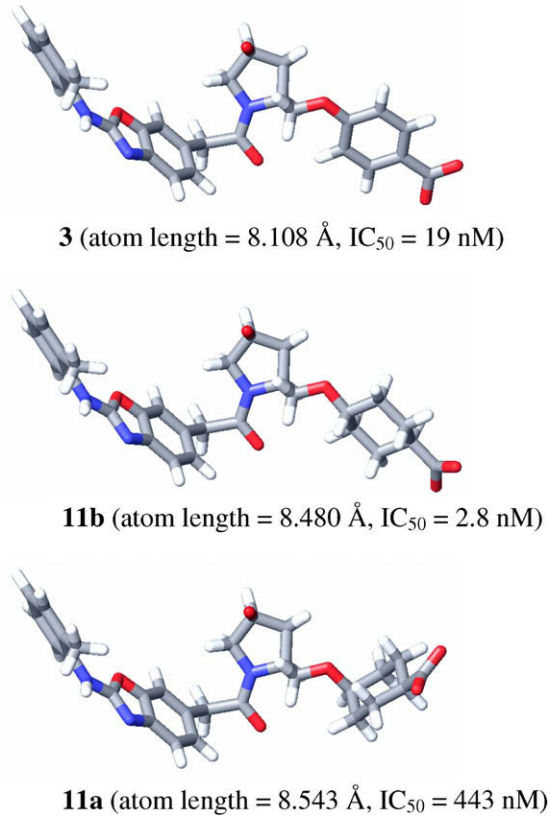


Figure 3. Stable conformations of **3**, **11b** and **11a**.

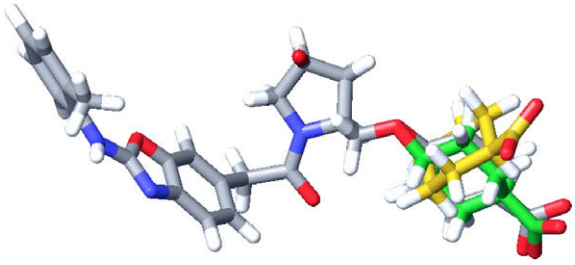


Figure 4. Comparison of the direction of the carboxylic acid moiety (gray: **3**, green: **11b**, yellow: **11a**).

4. Conclusion

To improve the pharmacokinetic properties of the potent VLA-4 antagonist **1** (IC_{50} = 1.6 nM), we firstly replaced the diphenylurea moiety in **1** with a 2-(phenylamino)benzoxazole moiety. This modification resulted in compound **3** showing less potent activity (IC_{50} = 19 nM) than **1**. Therefore, we focused on optimization of the benzoic acid moiety of **3** prior to getting around to the modification of the diphenylurea moiety. Consequently, we discovered the *trans*-4-substituted cyclohexanecarboxylic acid **11b**, which exhibits comparable activity with an IC_{50} value of 2.8 nM to **1**. On top of that, compound **11b** shows preferable pharmacokinetic properties (CL = 3.3 ml/min/kg, F = 51%) compared to those of **1** in rats, indicating that the structural modification of the diphenylurea moiety should lead to improvement of pharmacokinetic properties. Further biological evaluation of **11b** and structural modification of the 2-(phenylamino)benzoxazole moiety, while keeping the *trans*-4-substituted cyclohexanecarboxylic acid skeleton as a valuable pharmacophore to retain VLA-4 inhibitory activity, will be reported in a forthcoming publication.

5. Experimental

5.1. General

The melting points were determined on a YANACO MP-J3 and were uncorrected. Column chromatography was performed with a Merck Silica Gel 60 (particle size 0.060–0.200 or 0.040–0.063). Flash column chromatography was performed with Biotage FLASH Si or YAMAZEN Hi-Flash packed columns. Thin-layer chromatography (TLC) was performed on Merck pre-coated TLC glass sheets with Silica Gel 60 F254. The ^{1}H NMR spectra were recorded on a JEOL JNM-EX-400 spectrometer, and chemical shifts are given in ppm (δ) from tetramethylsilane as an internal standard. The spectral splitting patterns are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiple. The IR spectra were recorded on a HORIBA FT-720 spectrometer. The mass spectra were recorded on a SCIEX API-150EX spectrometer (ESI) or a JEOL JMS-HX110 spectrometer (FAB). The HRMS spectra were recorded on a JEOL JMS-100LP spectrometer. Elemental analysis was performed using a Perkin–Elmer CHNS/O 2400II, a Leco CHNS-932 and a YOKOKAWA analysis IC7000RS.

5.2. General procedure A: preparation of 4-[(4*S*)-fluoro-1-[2-(2-methylphenylamino)-6-benzoxazolylacetyl]-(2*S*)-pyrrolidinylmethoxy]benzoic acid (3)

A mixture of [2-(2-methylphenylamino)-6-benzoxazolyl]acetic acid (**4**) (300 mg, 1.06 mmol), methyl 4-[(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoate[13b] (**5f**) (269 mg, 1.06 mmol), EDC·HCl (305 mg, 1.59 mmol), HOBt (5 mg, 0.04 mmol) and DMAP (5 mg, 0.04 mmol) in DMF (10 ml) was stirred at room temperature for 15 h. The mixture was diluted with H_2O and extracted with EtOAc.

Table 3
Pharmacokinetic properties of **11b** in rats (n = 4)

F (%)	PO (5 mg/kg)			IV (1 mg/kg)			
	AUC (ng h/ml)	C_{max} (ng/ml)	$T_{1/2}$ (h)	AUC (ng h/ml)	CL (ml/min/kg)	Vdss (l/kg)	$T_{1/2}$ (h)
51	14,932	10,441	1.5	5804	3.3	0.16	1.1

The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with $CHCl_3$–EtOAc (9:1, v/v) as an eluent to give methyl 4-[(4*S*)-fluoro-1-[2-(2-methylphenylamino)-6-benzoxazolylacetyl]-(2*S*)-pyrrolidinylmethoxy]benzoate (573 mg, 100%) as a pale yellow foam. ^{1}H NMR ($CDCl_3$) δ 2.06–2.22 (1H, m), 2.36 (3H, s), 2.43–2.59 (1H, m), 3.69–4.15 (8H, m), 4.52–4.65 (2H, m), 5.24–5.37 (1H, m), 6.86–7.10 (4H, m), 7.21–7.32 (4H, m), 7.39–7.42 (1H, m), 7.95–8.01 (2H, m), 8.05–8.07 (1H, d, J = 8.1 Hz); MS (ESI) m/z 518 $[M+H]^+$.

To a stirred solution of methyl 4-[(4*S*)-fluoro-1-[2-(2-methylphenylamino)-6-benzoxazolylacetyl]-(2*S*)-pyrrolidinylmethoxy]benzoate (573 mg, 1.11 mmol) in THF (10 ml) was added 0.25 N NaOH (8.8 ml, 2.22 mmol) and the reaction mixture was stirred at room temperature for 15 h. The mixture was acidified with 1 N HCl and extracted with $CHCl_3$–MeOH (10:1, v/v). The combined extracts were dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH (20:1 to 10:1, v/v) as an eluent to give the title compound (365 mg, 65%) as a colorless amorphous solid. ^{1}H NMR (DMSO-d_6) δ 2.25–2.51 (2H, m), 2.30 (3H, s), 3.70–4.67 (7H, m), 5.30–5.50 (1H, m), 7.03–7.09 (4H, m), 7.24–7.34 (4H, m), 7.81–7.91 (3H, m), 9.61 (1H, br s); MS (ESI) m/z 504 $[M+H]^+$; HRMS (ESI) Calcd for $C_{28}H_{26}FN_3O_5$+H: 504.19347. Found: 504.19076. Anal. Calcd for $C_{28}H_{26}FN_3O_5 \cdot 0.5H_2O$: C, 65.62; H, 5.31; N, 8.20. Found: C, 65.97; H, 5.61; N, 7.73.

Compound **9**–**11** were prepared according to general procedure A.

5.3. 4-[(4*S*)-Fluoro-1-[2-(2-methylphenylamino)-6-benzoxazolylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetic acid (9)

Yield 42% (two steps). Colorless amorphous solid. IR (ATR) 1633, 1571, 1434, 1242, 1223, 982, 752 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.90–2.22 (2H, m), 2.30 (3H, s), 2.46–2.67 (4H, m), 3.14 (2H, d, J = 5.9 Hz), 3.37–4.04 (8H, m), 4.90–5.12 (1H, m), 5.18–5.38 (1H, m), 6.97–7.10 (2H, m), 7.22–7.28 (3H, m), 7.40 (1H, d, J = 1.2 Hz), 7.83 (1H, t, J = 7.1 Hz), 9.62 (1H, br s); MS (ESI) m/z 524 $[M+H]^+$; HRMS (ESI) Calcd for $C_{27}H_{30}FN_5O_5$+H: 524.23092. Found: 524.22953. Anal. Calcd for $C_{27}H_{30}FN_5O_5 \cdot 2H_2O$: C, 57.95; H, 6.12; N, 12.52. Found: C, 58.01; H, 5.84; N, 12.46.

5.4. 1-[(4*S*)-Fluoro-1-[2-(2-methylphenylamino)-6-benzoxazolylacetyl]-(2*S*)-pyrrolidinylcarbonyl]-4-piperidinylacetic acid (10)

Yield 40% (two steps). Colorless amorphous solid. IR (ATR) 1712, 1639, 1573 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.21–1.26 (2H, m), 1.79–1.87 (2H, m), 2.04–2.09 (1H, m), 2.28–2.30 (3H, m), 2.35 (3H, s), 2.44–2.64 (3H, m), 3.06–3.15 (1H, m), 3.75–4.01 (4H, m), 4.58–4.61 (1H, m), 5.02–5.04 (1H, m), 5.17–5.31 (1H, m), 7.06–7.12 (2H, m), 7.21–7.35 (5H, m), 7.88 (1H, d, J = 8.8 Hz); MS (ESI) m/z 523 $[M+H]^+$; HRMS (ESI) Calcd for $C_{28}H_{31}FN_4O_5$+H: 523.23567. Found: 523.23313.

5.5. 4-[[2-(2-Methylphenylamino)-6-benzoxazolylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (11)

Yield 48% (two steps). Colorless amorphous solid. ^{1}H NMR ($CDCl_3$) δ 1.24–2.28 (9H, m), 2.35 (3H, s), 2.38–2.51 (2H, m), 3.34–4.16 (7H, m), 4.22–4.41 (1H, m), 5.14–5.30 (1H, m), 7.06–7.16 (2H, m), 7.21–7.39 (5H, m), 7.79–7.94 (1H, m); MS (ESI) m/z 510 $[M+H]^+$; HRMS (ESI) Calcd for $C_{28}H_{32}FN_3O_5$+H: 510.24042. Found: 510.24033. Anal. Calcd for $C_{28}H_{32}FN_3O_5$: C, 66.00; H, 6.33; N, 8.25; F, 3.73. Found: C, 65.66; H, 6.43; N, 8.02; F, 3.59; Major (1,4-*cis* isomer): minor (1,4-*trans* isomer) = 6:1.

5.6. Purification of 11 by HPLC

HPLC analysis and purification of **11** were performed on a SHIMADZU 10A series with a Waters Symmetry C_{18} column (4.6 × 250 mm) using MeCN–0.02 N NaOAc buffer (1:1, v/v) as an eluent.

5.6.1. *cis*-4-[[2-(2-Methylphenylamino)-6-benzoxazolylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (11a)

HPLC retention time: 4.4 min (less polar); IR (KBr) 2933, 1693, 1641, 1575 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.45–1.55 (2H, m), 1.68–2.02 (6H, m), 2.11–2.52 (3H, m), 2.34 (3H, s), 3.30–4.11 (7H, m), 4.22–4.38 (1H, m), 5.14–5.29 (1H, m), 7.05–7.12 (2H, m), 7.20–7.33 (5H, m), 7.72 (1H, dd, J = 8.0, 10.6 Hz); MS (FAB) m/z 510 $[M^+ + H]^+$; HRMS (ESI) Calcd for $C_{28}H_{32}FN_3O_5$+H: 510.24042. Found: 510.23849.

5.6.2. *trans*-4-[[2-(2-Methylphenylamino)-6-benzoxazolylacetyl]-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (11b)

HPLC retention time: 3.3 min (more polar); IR (KBr) 2937, 1699, 1641, 1574 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26–1.30 (2H, m), 1.45–1.52 (2H, m), 2.05–2.10 (5H, m), 2.21–2.50 (2H, m), 2.36 (3H, s), 3.22–3.26 (1H, m), 3.32–3.98 (6H, m), 4.20–4.42 (1H, m), 5.17–5.31 (1H, m), 7.09–7.11 (2H, m), 7.22–7.36 (5H, m), 7.81–7.82 (1H, m); MS (FAB) m/z 510 $[M^+ + H]^+$; Anal. Calcd for $C_{28}H_{32}FN_3O_5$: C, 66.00; H, 6.33; N, 8.25. Found: C, 65.71; H, 6.42; N, 8.08.

5.7. General procedure B: preparation of 4-[(4*S*)-fluoro-1-[2-(2-methylphenylamino)-6-benzoxazolylacetyl]-(2*S*)-pyrrolidinylmethoxy]butanoic acid (6)

A mixture of [2-(2-methylphenylamino)-6-benzoxazolyl]acetic acid (**4**) (282 mg, 1.00 mmol), methyl 4-[(4*S*)-fluoro-(2*S*)-pyrrolidinyl]methoxybutanoate (**5a**) (219 mg, 1.00 mmol), EDC·HCl (288 mg, 1.50 mmol), HOBt (203 mg, 1.50 mmol) and Et_3N (0.70 ml, 5.00 mmol) in DMF (4 ml) was stirred at room temperature for 24 h. The mixture was diluted with H_2O and extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by preparative TLC ($CHCl_3$–acetone = 10:1, v/v) to give methyl 4-[(4*S*)-fluoro-1-[2-(2-methylphenylamino)-6-benzoxazolylacetyl]-(2*S*)-pyrrolidinylmethoxy]butanoate (378 mg, 78%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.88–2.30 (5H, m), 2.35 (3H, s), 2.37–2.45 (3H, m), 3.28–4.00 (9H, m), 4.12–4.48 (1H, m), 5.15–5.37 (1H, m), 7.06–7.10 (2H, m), 7.22–7.32 (4H, m), 7.38 (1H, dd, J = 8.1, 4.9 Hz), 8.05 (1H, d, J = 8.1 Hz); MS (ESI) m/z 484 $[M+H]^+$.

To a solution of methyl 4-[(4*S*)-fluoro-1-[2-(2-methylphenylamino)-6-benzoxazolylacetyl]-(2*S*)-pyrrolidinylmethoxy]butanoate (378 mg, 0.78 mmol) in THF (20 ml) was added 0.25 N NaOH (20 ml) and the reaction mixture was stirred at 50 °C for 17 h. After being cooled to room temperature, the mixture was acidified with 1 N HCl and extracted with $CHCl_3$–MeOH (10:1, v/v). The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by preparative TLC ($CHCl_3$–MeOH = 10:1, v/v) to give the title compound (99 mg, 27%) as a yellow oil. To a solution of the compound (99 mg, 0.21 mmol) in EtOH (3 ml) was added 1 N NaOH (0.21 ml, 0.21 mmol) and stirred. The solution was concentrated in vacuo and triturated with diethyl ether to give the title compound as a pale yellow amorphous solid. Sodium salt. ^{1}H NMR ($CDCl_3$) δ 1.68–2.30 (5H, m), 2.24 (3H, s), 2.41–2.46 (2H, m), 3.30–4.00 (7H, m), 4.24–4.40 (1H, m), 5.14–5.28 (1H, m), 7.07–7.16 (2H, m),

7.20–7.34 (5H, m), 7.69–7.75 (1H, m); MS (ESI), *m/z* 470 $[M+H]^+$; HRMS (ESI) Calcd for $C_{25}H_{28}FN_3O_5$+H: 470.20912. Found: 470.20604. Anal. Calcd for $C_{25}H_{27}FN_3O_5Na\cdot 3H_2O$: C, 55.04; H, 6.10; N, 7.70. Found: C, 55.26; H, 5.94; N, 7.38.

Compound **7** and **8** were prepared according to general procedure B.

5.8. 5-[(4*S*)-Fluoro-1-[2-(2-methylphenyl)amino-6-benzoxazolylacetyl]-(2*S*)-pyrrolidinylmethoxy]pentanoic acid (7)

Yield 64% (two steps). Light brown amorphous solid. Sodium salt. IR (KBr) 3425, 3251, 2939, 2868, 1643, 1574, 1439 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.01–1.30 (3H, m), 1.45–1.55 (4H, m), 1.83–1.92 (2H, m), 2.03–2.23 (2H, m), 2.28 (3H, s), 3.15–4.43 (5H, m), 4.18–4.36 (1H, m), 5.23–5.41 (1H, m), 6.99–7.03 (2H, m), 7.18–7.30 (5H, m), 7.82–7.86 (1H, m); MS (ESI) *m/z* 484 $[M+H]^+$, 506 $[M+Na]^+$; HRMS (ESI) Calcd for $C_{26}H_{30}FN_3O_5$+H: 484.22477. Found: 484.22139. Anal. Calcd for $C_{26}H_{29}FN_3O_5\cdot Na\cdot 2H_2O$: C, 57.66; H, 6.14; N, 7.76; F, 3.51. Found: C, 57.61; H, 6.20; N, 7.26; F, 3.34.

5.9. 6-[(4*S*)-Fluoro-1-[2-(2-methylphenyl)amino-6-benzoxazolylacetyl]-(2*S*)-pyrrolidinylmethoxy]hexanoic acid (8)

Yield 48% (two steps). Pale yellow amorphous solid. Sodium salt. IR (KBr) 3423, 3211, 2937, 2864, 1643, 1574, 1485, 1574 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.36–1.72 (6H, m), 1.92–2.28 (4H, m), 2.34 (3H, s), 3.26–4.00 (8H, m), 4.20–4.43 (1H, m), 5.13–5.28 (1H, m), 7.04–7.11 (2H, m), 7.21–7.34 (5H, m), 7.76 (1H, t, *J* = 7.3 Hz); MS (ESI) *m/z* 498 $[M+H]^+$; HRMS (ESI) Calcd for $C_{27}H_{32}FN_3O_5$+H: 498.24042. Found: 498.23922. Anal. Calcd for $C_{27}H_{31}FN_3O_5Na\cdot 1.0H_2O$: C, 60.33; H, 6.19; N, 7.82. Found: C, 60.11; H, 6.19; N, 7.41.

5.10. 1-(*tert*-Butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-propargyloxymethylpyrrolidine (13)

To a solution of 1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-prolinol[13] (**12**) (660 mg, 3.01 mmol) in THF (20 ml) were added NaH (181 mg, 4.52 mmol, 60% in oil), *n*-Bu_4NI (cat.) and propargyl bromide (0.40 ml, 3.62 mmol) at 0 °C under an atmosphere of nitrogen. The stirring mixture was allowed to warm up to room temperature for 7 h. The reaction mixture was cooled in an ice bath, poured into ice water and extracted with EtOAc. The combined extracts were washed with satd $NaHCO_3$ and brine. After being dried over Na_2SO_4, the extracts were concentrated in vacuo. The residue was purified by column chromatography on silica gel with *n*-hexane–EtOAc (4:1, v/v) as an eluent to give the title compound (660 mg, 85%) as a yellow oil. ^1H NMR ($CDCl_3$) δ 1.48 (9H, s), 1.99–2.17 (1H, m), 2.37–2.44 (2H, m), 3.44 (1H, br s), 3.50–3.72 (2H, m), 3.83 (1H, br s), 4.05 (1H, br s), 4.17 (2H, s), 5.14–5.30 (1H, m); MS (ESI) *m/z* 258 $[M+H]^+$.

5.11. Methyl 4-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]-2-butynoate (14)

To a solution of 1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-propargyloxymethylpyrrolidine (177 mg, 0.688 mmol) in THF (6 ml) was added *n*-BuLi (0.76 ml, 1.17 mol, 1.54 M solution in *n*-hexane) at −50 °C under a nitrogen atmosphere. After 20 min stirring at the same temperature, methyl chloroformate (80 μl, 1.03 mmol) was added to the reaction mixture at −40 °C and allowed to −20 °C for 1 h. To the reaction mixture was added satd NH_4Cl, and the mixture was extracted with EtOAc. The combined extracts were washed with satd $NaHCO_3$ and brine. After being dried over Na_2SO_4, the extracts were concentrated in vacuo. The residue was purified by preparative TLC (*n*-hexane–EtOAc = 2:1, v/v) to give the title compound (149 mg, 69%) as a yellow oil. ^1H NMR ($CDCl_3$) δ 1.48 (9H, s), 2.01–2.28 (1H, m), 2.36–2.41 (1H, m), 3.45–3.73 (3H, m), 3.78 (3H, s), 3.83 (1H, dd, *J* = 8.5, 4.6 Hz), 3.98–4.34 (3H, m), 5.14–5.27 (1H, m); MS (ESI) *m/z* 316 $[M+H]^+$.

5.12. Methyl 4-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]butanoate (15)

A suspension of methyl 4-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]-2-butynoate (195 mg, 0.618 mmol) and 5% Pd/C (wet) (200 mg) in MeOH (4 ml) was stirred at room temperature under a hydrogen atmosphere for 8 h. After removing the catalyst by filtration, the filtrate was concentrated in vacuo. 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 (120 mg, 61%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.89 (2H, t, *J* = 6.8 Hz), 1.93–2.17 (1H, m), 2.3–2.42 (3H, m), 3.32 (1H, t, *J* = 8.5 Hz), 3.42–3.60 (5H, m), 3.67 (3H, s), 3.94–4.12 (1H, m), 5.13–5.27 (1H, m); MS (ESI) *m/z* 320 $[M+H]^+$.

5.13. Methyl 4-[(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]butanoate (5a)

To a solution of methyl 4-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]butanoate (120 mg, 0.376 mmol) in CH_2Cl_2 (2 ml) was added TFA (0.5 ml) at room temperature. After stirring for 1 h, the mixture was concentrated in vacuo. The residue was diluted with CH_2Cl_2 and 1 N NaOH, and extracted with CH_2Cl_2. The combined extracts were washed with brine. After being dried over Na_2SO_4, the extract was concentrated in vacuo to give the title compound (74 mg, 90%) as a yellow oil. ^1H NMR ($CDCl_3$) δ 1.73–1.86 (1H, m), 1.90–1.94 (2H, m), 2.18–2,23 (2H, m), 2.41 (2H, t, *J* = 7.3 Hz), 2.82–2.95 (1H, m), 3.27–3.35 (2H, m), 3.43–3.53 (4H, m), 3.67 (3H, s), 5.11–5.25 (1H, m).

5.14. General procedure C: preparation of methyl 5-[(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]pentanoate (5b)

5.14.1. Methyl 3-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]propionate (16)

To a cooled (−50 °C), stirred suspension of NaH (60% in oil, 365 mg, 9.12 mmol) in THF (20 ml) was added 1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-prolinol (1.00 g, 4.56 mmol) in THF (15 ml) for 5 min. After 15 min stirring, methyl acrylate (0.95 ml, 10.5 mmol) was added to the reaction mixture at −50 °C and stirred for 15 min. The reaction mixture was warmed up to −40 °C and kept for 14 h. The reaction mixture was acidified with AcOH (1 ml), diluted with ice water and extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. 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 (670 mg, 48%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.98–2.15 (1H, m), 2.36 (1H, dd, *J* = 20.5, 5.6 Hz), 2.58 (2H, t, *J* = 6.6 Hz), 3.35 (1H, t, *J* = 9.0 Hz), 3.48–3.80 (8H, m), 3.94–4.17 (1H, m), 5.13–5.26 (1H, m); MS (ESI) *m/z* 306 $[M+H]^+$.

5.14.2. 3-[1-(*tert*-Butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]propanol (17)

To a stirred solution of methyl 3-[1-(*tert*-butox-ycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]propionate (2.84 g, 9.30 mmol) in THF (40 ml) was added 0.25 N NaOH (40 ml) and the reaction mixture was stirred at room temperature for 19 h. After removal of the solvent, the mixture was acidified with 1 N HCl and ex-

tracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give 3-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]-propionic acid (2.48 g, 92%) as a colorless oil. To a stirred solution of the product in THF (100 ml) was added 10.0 M $BH_3{\cdot}Me_2S$ (2.79 ml, 27.9 mmol) at room temperature and the reaction mixture was stirred at 60 °C for 30 min. After being cooled to room temperature, the reaction mixture was poured into ice water and extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH (20:1, v/v) as an eluent to give the title compound (2.84 g, 100%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.64–1.73 (1H, m), 1.83 (2H, br s), 2.00–2.41 (2H, m), 3.37 (1H, t, J = 9.1 Hz), 3.50–3.85 (6H, m), 4.02–4.18 (1H, m), 5.14–5.28 (1H, m); MS (ESI) *m/z* 278 $[M+H]^+$.

5.14.3. 3-[1-(*tert*-Butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]propanal (18)

To a stirred solution of 3-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]propanol (2.84 g, 9.30 mmol), Et_3N (7.78 ml, 55.8 mmol) and DMSO (6.61 ml, 93.7 mmol) in CH_2Cl_2 (43 ml) was added $SO_3{\cdot}$pyridine (4.44 g, 27.9 mmol) at 0 °C and the reaction mixture was stirred at 0 °C to room temperature for 2 h. After removal of the solvent, the mixture was diluted with water and extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, and condensed in vacuo. The residue was purified by column chromatography on silica gel with *n*-hexane–EtOAc (4:1, v/v) as an eluent to give the title compound (1.35 g, 53%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.95–2.16 (1H, m), 2.30–2.39 (1H, m), 2.65 (2H, br s), 3.36 (1H, t, J = 9.8 Hz), 3.52–3.87 (5H, m), 4.00–4.13 (1H, m), 5.13–5.26 (1H, m), 9.79 (1H, s).

5.14.4. Methyl (*E*)-5-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]-2-pentenoate (19)

To a cooled (0 °C), stirred solution of trimethyl phosphonoacetate (0.95 ml, 5.88 mmol) in THF (40 ml) was added NaH (60% in oil, 235 mg, 5.88 mmol) under a nitrogen atmosphere and the resulting mixture was stirred for 10 min. To the resulting mixture was added 3-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]propanal (1.35 g, 4.90 mmol) in THF (40 ml) and the reaction mixture was stirred at 0 °C for 2 h. The mixture was poured into the ice water and extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel with $CHCl_3$–acetone (10:1, v/v) as an eluent to give the title compound (1.40 g, 86%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 2.02–2.21 (1H, m), 2.30–2.58 (2H, m), 3.34 (1H, t, J = 10.0 Hz), 3.45–3.71 (7H, m), 3.73 (3H, s), 5.13–5.28 (1H, m), 5.89 (1H, d, J = 15.9 Hz), 6.95 (1H, dt, J = 15.9, 7.1 Hz).

5.14.5. Methyl 5-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]pentanoate (20)

A suspension of (*E*)-5-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]-2-pentenoate (1.40 g, 4.22 mmol) and 5% Pd/C (wet) (700 mg) in MeOH (50 ml) was stirred at room temperature under hydrogen atmosphere for 20 h. After removing the catalyst by filtration, the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel with *n*-hexane–EtOAc (4:1, v/v) as an eluent to give the title compound (1.09 g, 78%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.61–1.74 (3H, m), 1.98–2.18 (2H, m), 2.31–2.42 (3H, m), 3.31 (1H, dt, J = 7.9, 1.5 Hz), 3.40–3.73 (5H, m), 3.67 (3H, s), 4.09 (1H, br s), 5.13–5.27 (1H, m).

5.14.6. Methyl 5-[(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]pentanoate (5b)

To a solution of methyl 5-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]pentanoate (1.09 g, 3.27 mmol) in CH_2Cl_2 (50 ml) was added TFA (10 ml) and the reaction mixture was stirred at room temperature for 3.5 h. After removal of the solvent, the residue was diluted with CH_2Cl_2 and 1 N NaOH, and extracted with CH_2Cl_2. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give the title compound (590 mg, 77%) as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.59–1.96 (5H, m), 2.09–2.20 (1H, m), 2.34 (2H, t, J = 7.1 Hz), 2.81–2.94 (1H, m), 3.27–3.35 (2H, m), 3.42–3.53 (4H, m), 3.67 (3H, s), 5.11–5.25 (1H, m); MS (ESI) *m/z* 234 $[M+H]^+$.

5.15. (*Z*)-(2*S*)-(4-Benzyloxy-2-butenyloxy)methyl-1-(*tert*-butoxycarbonyl)-(4*S*)-fluoropyrrolidine (21)

To a solution of 1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-prolinol (2.46 g, 11.2 mmol) in THF (100 ml) was added NaH (896 mg, 22.4 mmol, 60% in oil) at 0 °C under an atmosphere of nitrogen. After 20 min stirring, 4-benzyloxy-2-butenyl bromide (2.76 g, 11.2 mmol) in THF (100 ml) was added to the reaction mixture for 10 min and after 5 min, *n*-Bu_4NI (100 mg, 0.27 mmol) was added and stirred for 15 min at the same temperature. The reaction mixture was allowed to warm up to room and stirred for 19 h. The reaction mixture was poured into ice water and the mixture was extracted with EtOAc. The combined extracts were washed with brine. After being dried over Na_2SO_4, the extracts were concentrated in vacuo. The residue was purified by column chromatography on silica gel with *n*-hexane–EtOAc (4:1, v/v) as an eluent to give the title compound (3.30 g, 78%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.98–2.25 (1H, m), 2.35–2.43 (1H, m), 3.31 (1H, t, J = 9.3 Hz), 3.48–3.81 (3H, m), 3.95–4.18 (5H, m), 4.51 (2H, s), 5.13–5.26 (1H, m), 5.70–5.84 (2H, m), 7.27–7.53 (5H, m); MS (ESI) *m/z* 380 $[M+H]^+$.

5.16. 4-[1-(*tert*-Butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]-1-butanol (22)

A suspension of (*Z*)-(2*S*)-(4-benzyloxy-2-butenyloxy)methyl-1-(*tert*-butoxycarbonyl)-(4*S*)-fluoropyrrolidine (4.58 g, 12.1 mmol) and 20% $Pd(OH)_2$ (dry) (4.0 g) in EtOH (200 ml) was stirred at room temperature under a hydrogen atmosphere for 14 h. After removing the catalyst by filtration, the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel with $CHCl_3$–acetone (10:1, v/v) as an eluent to give the title compound (2.42 g, 67%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.62 (2H, s), 1.98–2.23 (2H, m), 2.32–2.40 (1H, m), 3.33–3.38 (1H, m), 3.52–3.82 (8H, m), 4.01–4.13 (1H, m), 5.14–5.27 (1H, m); MS (ESI) *m/z* 292 $[M+H]^+$.

Compounds **23–25** and **5c** were prepared according to general procedure C.

5.17. 4-[1-(*tert*-Butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]butyraldehyde (23)

Yield 72%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.91 (2H, t, J = 6.6 Hz), 1.96–2.17 (1H, m), 2.31–2.40 (1H, m), 2.52 (2H, t, J = 7.1 Hz), 3.32 (1H, t, J = 9.8 Hz), 3.49–4.14 (6H, m), 5.13–5.27 (1H, m), 9.77 (1H, s).

5.18. Methyl (*E*)-6-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]-2-hexenoate (24)

Yield 93%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.73 (1H, br s), 1.99–2.51 (4H, m), 3.29–3.34 (1H, m), 3.41–3.71 (6H, m), 3,73

(3H, s), 3.97–4.20 (1H, m), 5.14–5.27 (1H, m), 5.83 (1H, d, J = 15.4 Hz), 6.94–7.01 (1H, m); MS (ESI) m/z 346 $[M+H]^+$.

5.19. Methyl 6-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]hexanoate (25)

Yield 100%. Colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.37–1.39 (2H, m), 1.47 (9H, s), 1.57–1.70 (4H, m), 1.98–2.18 (1H, m), 2.31 (3H, t, J = 7.3 Hz), 2.35–2.44 (1H, m), 3.31 (1H, t, J = 8.8 Hz), 3.41–3.60 (3H, m), 3.67 (3H, s), 3.68–4.18 (2H, m), 5.13–5.26 (1H, m).

5.20. Methyl 6-[(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]hexanoate (5c)

Yield 85%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.38–1.70 (6H, m), 2.21–2.30 (1H, m), 2.34 (2H, t, J = 6.8, Hz), 2.46–2.61 (1H, m), 3.50–3.74 (9H, m), 4.15 (1H, m), 5.30–5.45 (1H, m); MS (ESI) m/z 248 $[M+H]^+$.

5.21. General procedure D: preparation of ethyl 4-[(4*S*)-fluoro-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (5d)

5.21.1. Ethyl 4-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (27a)

To a stirred solution of ethyl 1-piperazinylacetate (222 mg, 1.29 mmol) and 1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylcarboxylic acid[15] (**26**) (300 mg, 1.29 mmol) in THF (10 ml) was added EDC·HCl (370 mg, 1.93 mmol), HOBt (198 mg, 1.29 mmol), and Et_3N (540 μg, 3.87 mmol), and the resulting mixture was stirred at room temperature for 16 h. After removal of the solvent, the residue was purified by flash column chromatography (Biotage 25M) with $CHCl_3$–MeOH (95:5, v/v) as an eluent to give the title compound (500 mg, 100%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.28 (3H, t, J = 7.2 Hz), 1.42–1.47 (9H, m), 2.15–2.26 (1H, m), 2.39–2.67 (5H, m), 3.23–3.24 (2H, m), 3.46–3.91 (6H, m), 4.19 (2H, q, J = 7.2 Hz), 4.60–4.75 (1H, m), 5.13–5.29 (1H, m); MS (ESI) m/z 388 $[M+H]^+$.

5.21.2. Ethyl 4-[(4*S*)-fluoro-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (5d)

To a stirred solution of ethyl 4-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylcarbonyl]-1-piperazinylacetate (670 mg, 1.73 mmol) in CH_2Cl_2 (5 ml) was added TFA (5 ml) and the reaction mixture was stirred at room temperature for 15 h. The mixture was condensed in vacuo and the residue was made basic with satd $NaHCO_3$. The mixture was extracted with $CHCl_3$–MeOH (10:1, v/v). The combined extracts were washed with brine, dried over K_2CO_3, and evaporated to give the title compound (427 mg, 86%) as a yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.25 (3H, t, J = 7.2 Hz), 2.22–2.33 (1H, m), 2.67–2.81 (1H, m), 3.17–3.50 (5H, m), 3.57–3.71 (6H, m), 4.13 (2H, br s), 4.22 (2H, q, J = 7.2 Hz), 4.78 (1H, br s), 5.37–5.50 (1H, m); MS (ESI) m/z 288 $[M+H]^+$.

Compounds **27b** and **5e** were prepared according to general procedure D.

5.22. Ethyl 1-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylcarbonyl]-4-piperidinylacetate (27b)

Yield 32%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.13–1.22 (2H, m), 1.26 (3H, t, J = 7.2 Hz), 1.47 (9H, s), 1.70 (2H, s), 1.75–1.81 (2H, m), 2.03–2.24 (4H, m), 2.43–2.57 (2H, m), 3.02–3.10 (1H, m), 3.75–3.83 (2H, m), 4.14 (2H, q, J = 7.2 Hz), 4.60 (1H, d, J = 9.6 Hz), 5.13–5.27 (1H, m).

5.23. Ethyl 1-[(4*S*)-fluoro-(2*S*)-pyrrolidinylcarbonyl]-4-piperidinylacetate (5e)

Yield 100%. Yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.13–1.22 (2H, m), 1.26 (3H, t, J = 7.2 Hz), 1.76–1.82 (2H, m), 1.87–2.02 (3H, m), 2.24–2.38 (3H, m), 2.66–2.78 (2H, m), 3.03–3.06 (1H, m), 3.46–3.51 (1H, m), 3.74–3.80 (1H, m), 3.90–3.91 (1H, m), 4.14 (2H, q, J = 7.2 Hz), 4.60–4.62 (1H, m), 5.10–5.24 (1H, m).

5.24. Methyl 4-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (29)

A mixture of methyl 4-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]benzoate[13b] (**28**) (550 mg, 1.56 mmol) and 5% Rh on alumina (300 mg) in EtOH–AcOH (11 ml, 10:1, v/v) was hydrogenated at room temperature at 10 atm for 24 h. After removing the catalyst by filtration, the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel with *n*-hexane–EtOAc (4:1, v/v) as an eluent to give the title compound (510 mg, 91%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.49–1.67 (4H, m), 1.78–2.13 (5H, m), 2.28–2.48 (2H, m), 3.26–3.33 (1H, m), 3.47–3.75 (7H, m), 3.96–4.13 (1H, m), 5.13–5.26 (1H, m); MS (ESI) m/z 382 $[M+Na]^+$.

5.25. Methyl 4-[(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (5g)

To a stirred solution of 4-[1-(*tert*-butoxycarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (200 mg, 0.56 mmol) in CH_2Cl_2 (5 ml) was added TFA (5 ml) and the reaction mixture was stirred at room temperature for 16 h. The mixture was made basic by satd $NaHCO_3$ and extracted with $CHCl_3$. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give the title compound (121 mg, 84%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.26–2.38 (11H, m), 2.82–2.95 (1H, m), 3.25–3.57 (6H, m), 3.67 (3H, s), 5.11–5.25 (1H, m); MS (ESI) m/z 260 $[M+H]^+$.

5.26. Methyl 2-(2-methylphenylamino)-6-benzoxazolylacetate (35)

A solution of methyl 3-hydroxy-4-nitrophenylacetate[12] (**34**) (1.00 g, 4.74 mmol) in EtOH (20 ml) was hydrogenated over 5% Pd/C (500 mg) for 15 h. The mixture was filtered to remove the catalyst and filtrate was added *o*-tolylisothiocyanate (765 μl, 5.69 mmol) and the mixture was stirred for 15 h. HgO (1.72 g, 7.94 mmol) was added to this mixture and the mixture was heated under reflux for 4 h. After being cooled to room temperature, the mixture was filtered through a Celite pad. The filtrate was concentrated in vacuo and the residue was purified by column chromatography on silica gel with $CHCl_3$–EtOAc (10:1, v/v) as an eluent to give the title compound (1.18 g, 84%) as a colorless solid. Mp 78–80 °C; ^{1}H NMR ($CDCl_3$) δ 2.35 (3H, s), 3.69 (2H, s), 3.70 (3H, s), 7.07–7.33 (6H, m), 7.40 (1H, d, J = 8.1 Hz), 8.06 (1H, d, J = 8.1 Hz); MS (ESI) m/z 297 $[M+H]^+$; HRMS (ESI) Calcd for $C_{17}H_{16}N_2O_3$+H: 297.12392. Found: 297.12028.

5.27. 2-(2-Methylphenylamino)-6-benzoxazolylacetic acid (4)

To a stirred solution of methyl 2-(2-methylphenylamino)-6-bezoxazolylacetate (1.18 g, 3.98 mmol) in THF (30 ml) was added 0.25 N NaOH (32 ml, 7.96 mmol) and the reaction mixture was stirred at room temperature for 15 h. The mixture was acidified with 1 N HCl and extracted with $CHCl_3$–MeOH (5:1, v/v). The combined extracts were dried over $MgSO_4$, and concentrated in vacuo to give the title compound (867 mg, 77%) as a pale yellow solid. Mp 198–200 °C; ^{1}H NMR (DMSO-d_6) δ 2.31 (3H, s), 3.64 (2H, s), 7.09–7.13 (2H, m), 7.24–7.30 (3H, m), 7.39 (1H, d, J = 1.0 Hz), 7.80 (1H, dd, J = 7.3, 1.5 Hz); MS (ESI) m/z 283 $[M+H]^+$; HRMS (ESI) Calcd for $C_{16}H_{14}N_2O_3$+H: 283.10823. Found: 283.10425.

5.28. Methyl 4-[1-benzyloxycarbonyl-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (37)

To a stirred solution of methyl 4-[(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (529 mg, 2.04 mmol) and carbobenzyloxychloride (30–35% in toluene, 1.21 ml, 2.04 mmol) in CH_2Cl_2 (20 ml) was added saturated $NaHCO_3$ (5 ml) at room temperature, and the resulting mixture was stirred for 5.5 h. The mixture was poured into water and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on silica-gel with *n*-hexane–EtOAc (2:1, v/v) as an eluent to give a diastereomeric mixture of methyl 4-[1-benzyloxycarbonyl-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (**37**, 684 mg, 85%) as a colorless oil. The two diastereomers were separated by middle pressure silica gel column chromatography (YAMAZEN YFLC-5404-FC, $\Phi 26 \times 300$ mm) with *n*-hexane–EtOAc (3:1, v/v) as an eluent to give *cis*-isomer (**37a**, 489 mg, 61%) as a colorless oil and *trans*-isomer (**37b**, 123 mg, 15%) as a colorless oil.

First fraction (**37a**) (*less* polar, *cis*-isomer): ^{1}H NMR ($CDCl_3$) δ 1.45–1.52 (2H, m), 1.60–1.65 (2H, m), 1.80–1.90 (4H, m), 2.01–2.18 (1H, m), 2.31–2.37 (1H, m), 2.42–2.51 (1H, m), 3.33–3.51 (2H, m), 3.60–3.75 (3H, m), 3.67 (3H, s), 4.11–4.15 (1H, m), 5.10–5.29 (3H, m), 7.30–7.37 (5H, m); MS (ESI) *m/z* 394 $[M+H]^+$.

Second fraction (**37b**) (*more* polar, *trans*-isomer): ^{1}H NMR ($CDCl_3$) δ 1.15–1.30 (2H, m), 1.40–1.50 (2H, m), 1.99–2.13 (5H, m), 2.25 (1H, br s), 2.39–2.48 (1H, m), 3.18–3.36 (2H, m), 3.61–3.86 (3H, m), 3.67 (3H, s), 4.05–4.14 (1H, m), 5.10–5.29 (3H, m), 7.31–7.37 (5H, m); MS (ESI) *m/z* 394 $[M+H]^+$.

5.29. Methyl *trans*-4-[(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (38)

A mixture of methyl *trans*-4-[1-benzyloxycarbonyl-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (121 mg, 0.31 mmol) and $Pd(OH)_2$ (20% on carbon, 19 mg) in MeOH (5 ml) was stirred under a hydrogen atmosphere (1 atm) at room temperature overnight. The mixture was filtered to remove the catalyst. The filtrate was concentrated in vacuo to give the title compound (77 mg, 97%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.21–1.31 (2H, m), 1.41–1.51 (2H, m), 1.74–1.87 (1H, m), 1.98–2.09 (5H, m), 2.11–2.30 (2H, m), 2.48 (1H, br s), 2.85–2.98 (1H, m), 3.22–3.59 (4H, m), 3.66 (3H, s), 5.12–5.26 (1H, m); MS (ESI) *m/z* 260 $[M+H]^+$.

5.30. Methyl *trans*-4-[1-(2-methylphenylaminocarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (39)

To a stirred solution of methyl *trans*-4-[(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (1.33 g, 5.13 mmol) and *o*-tolyl isocyanate (636 μl, 5.13 mmol) in THF (50 ml) was added Et_3N (143 μl, 1.03 mmol) at room temperature and the resulting mixture was stirred for 15 h. The mixture was concentrated in vacuo and the residue was poured into 1 N HCl. The mixture was extracted with EtOAc, dried over Na_2SO_4, and evaporated. The residue was purified by recrystallization from EtOAc–*n*-hexane to give the title compound (1.13 g, 56%) as a pale yellow powder. ^{1}H NMR ($CDCl_3$) δ 1.21–1.31 (2H, m), 1.39–1.48 (2H, m), 1.98–2.39 (8H, m), 2.26 (3H, s), 3.32–3.37 (1H, m), 3.60–3.77 (2H, m), 3.65 (3H, s), 3.90–4.04 (1H, m), 4.17–4.21 (1H, m), 5.17–5.32 (1H, m), 7.00 (1H, t, J = 6.8 Hz), 7.14–7.22 (2H, m), 7.77 (1H, d, J = 7.6 Hz), 8.03 (1H, s); MS (ESI) *m/z* 393 $[M+H]^+$.

5.31. *trans*-4-[1-(2-Methylphenylaminocarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid (40)

To a stirred solution of methyl *trans*-4-[1-(2-methylphenylaminocarbonyl)-(4*S*)-fluoro-(2*S*)-pyrrolidinylmethoxy]cyclohexanecarboxylate (200 mg, 0.51 mmol) in THF–MeOH (10 ml, 1:1, v/v) was added 0.25 N NaOH (10.2 ml, 2.55 mmol) and the reaction mixture was stirred at room temperature for 5 h. The mixture was poured into 1 N HCl and extracted with $CHCl_3$–MeOH (10:1, v/v). The extract was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by recrystallization from EtOAc–*n*-hexane to give the title compound (179 mg, 93%) as a colorless powder. ^{1}H NMR ($CDCl_3$) δ 1.21–1.31 (2H, m), 1.39–1.51 (2H, m), 2.00–2.13 (5H, m), 2.20–2.41 (2H, m), 2.26 (3H, s), 3.31–3.38 (1H, m), 3.60–3.63 (1H, m), 3.68–3.77 (2H, m), 3.90–4.04 (1H, m), 4.17–4.22 (1H, m), 5.16–5.32 (1H, m), 7.00 (1H, t, J = 7.6 Hz), 7.14–7.19 (2H, m), 7.75 (1H, d, J = 8.4 Hz), 8.03 (1H, s); MS (ESI) *m/z* 379 $[M+H]^+$.

5.32. X-ray crystallography

Crystal of **40** suitable for X-ray analysis was obtained by recrystallization from EtOAc. The diffraction data were collected on AFC7R diffractometer with Cu Kα radiation (λ = 1.54178 Å). The structure of **40** was solved by direct methods. The refinement was made with anisotropic displacement factors for all non-hydrogen atoms. All the hydrogen atoms were refined isotropically. The hydrogen atoms bound to carbon atoms were generated geometrically and their coordinates were fixed while they were refined. On the other hand, coordinates of hydrogen atoms bound to nitrogen or oxygen atoms were altered while they were refined.

CCDC 707340 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

5.33. Molecular modeling

3D structure building and molecular modeling were performed using Ligprep 2.0113.[17] All the conformation searches were performed using Macromodel 9.1113.[17] A total of 10,000 conformers were generated for each molecule using the serial torsional-low mode sampling method and subsequent single-cycle minimization with truncated Newton conjugate gradient was performed for each conformer. The OPLS2005 forcefield was used for the energy calculation. All the calculations were carried out on a Hewlett-Packard workstation xw8200 running Red Hat Linux 9 and equipped with two 3.2 GHz Xeon processors and 2GB RAM.

5.34. VLA-4/VCAM-1 binding assay

The inhibitory activity of the compounds against VLA-4 was determined by the method described in a reported publication.[10] Briefly, CHO cells expressing VLA-4 were used. The binding of the human VCAM-1/Fc chimeric antibody (R&D Systems Inc.) labeled with europium was measured using a time-resolved fluorometer (DELFIA Research fluorometer, model 1234-001; Perkin–Elmer Inc.). The concentration of the compound required for 50% inhibition in the assay was determined.

5.35. Pharmacokinetic studies on rats

Male Sprague-Dawley rats [Crj: CD(SD) IGS, 7 weeks old, Charles River Laboratories] were used. The animals were fasted for 18 h prior to dosing. Each group consisted of 4 animals. The rats were orally administered compound **11b** at the doses of 5 mg/kg

dissolved in 0.5% (w/v) MC with 3 equiv NaOH aqueous solution. The rats were intravenously administered compound **11b** at a dose of 1 mg/kg dissolved in saline with 3 equiv NaOH solution. Blood samples (0.4 ml) were collected at 0.08 (or 0.25 for p.o.), 0.5, 1, 2, and 6 h after the administration. These analytical samples were stood at room temperature, followed by centrifugation at 15,000 rpm for 10 min at 4 °C. The plasma fractions were subsequently stored in a −20 °C freezer until being analyzed. The concentrations of compounds **11b** were determined by an LC/MS/MS method, comprised of an Alliance 2695 HPLC (Waters), Symmetry Shield RP8, 2.1 × 50 mm, 3.5 μm column (Waters), and TSQ-700 (Thermo Electron, Waltham, MA). The mobile phase consisted of 10 mM $HCOONH_4$ in water/methanol; the gradient condition was 90/10 to 10/90. The plasma concentrations versus the time data were analyzed by non-compartmental approaches using the WINNONLIN software program (version 1.13.1 Pharsight, Mountain View, CA).

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